

Temporal Change in Community Composition, Ecological Niche, and Phenotypic
Selection in Minnesota's Rare Arctic Relict Plants

A THESIS

SUBMITTED TO THE FACULTY OF THE
UNIVERSITY OF MINNESOTA

BY

John P. Vallez

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Briana L. Gross

July 2021

Acknowledgements

I would like to thank Dr. Briana Gross for serving as my advisor. My graduate experience has been defined by her dedication, passion, and patience. I am forever grateful for her time and guidance throughout this project. I would also like to thank Dr. Julie Etterson for her leadership, counsel, and for co-proposing this wonderful project. I would like to thank my committee members, Dr. Amanda Grusz and Dr. Jessica Savage, for their instruction and input on this project.

The collection of these data would not have been possible without Matt Jahnke, Sophie Levi, Ryan Carlson, and Joe Rose. I would like to thank the Integrated Biosciences Program at the University of Minnesota Duluth and the members of the Gross Lab for always enriching the conversation, providing unrelenting support and true comradery throughout my time at UMD. Funding for this project was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR). Additional thanks to the countless individuals from the Minnesota Department of Natural Resources (MN-DNR) who assisted in various ways throughout the project.

Thank you to my parents for always supporting my endeavors and valuing education. Lastly, thank you to my wife, Brittany, for her support, passion for life, and willingness to pursue the countless adventures that I propose.

Abstract

Rare, arctic relict plant populations of Minnesota, found in the cool and moist microclimate produced along the cold waters of Lake Superior, face an uncertain future due to anthropogenic climate change. In this study, we used a variety of approaches to examine the contemporary impacts of climate change and to predict how populations will respond in coming decades. To investigate how these arctic relict communities have already changed, we surveyed population occurrence based on historical herbarium records, and compared community composition after nearly 20 years of climate change across a latitudinal gradient that mimics a range of different degrees of warming. Large temporal dissimilarity in community composition occurred across all sites surveyed, and southern sites showed an overall mean decrease in species richness. Ecological niche modeling based on Minnesota populations of *Primula mistassinica*, *Pinguicula vulgaris*, and *Euphrasia hudsoniana* show a high probability of drastic reduction in suitable habitat under a conservative climate model forecast. To understand the current patterns of selection in Minnesota arctic relict populations, we conducted a phenotypic selection analysis on the same three species across the latitudinal gradient. All species sampled showed reduced flowering in the most southern populations, where average summer temperatures were nearly +2 °C warmer than the northernmost plots. Phenotypic selection analysis of *Pinguicula vulgaris*, the species estimated to be most vulnerable to climatic changes according to ecological niche models, revealed differences in the direction of selection on flowering date between northern and southern populations. Preliminary estimates of population viability yielded mixed results and additional data is needed to forecast the future of these arctic relict communities in Minnesota.

Table of Contents

Acknowledgements.....	i
Abstract.....	ii
List of Tables.....	iv
List of Figures.....	v
Introduction.....	1
Methods.....	4
Results.....	13
Discussion.....	17
References.....	49

List of Tables

Table 1: Relevé Abundance and Cover Code Conversion	24
Table 2: Herbarium Record Locations.....	25, 26,27
Table 3: Ecological Niche model statistics.....	28
Table 4: ENM Site Suitability	29
Table 5: Analysis of Phenotypic Trait Means.....	30, 31, 32
Table 6: Phenotypic Selection Analysis	33, 34, 35
Table 7: Population Viability Analysis for <i>Primula</i>	36
Table 8: Population Viability Analysis for <i>Pinguicula</i>	37

List of Figures

Figure 1: Studied Arctic Relict Species	38
Figure 2: Locations of Relevé Surveys.....	39
Figure 3: Locations of Sites for Phenotypic Selection Analysis.....	40
Figure 4: Range Contraction in <i>Pinguicula</i> and <i>Euphrasia</i>	41
Figure 5: Heat Map of Relevé Bray-Curtis Dissimilarity	42
Figure 6: Bray-Curtis Dissimilarity Across Latitude.....	43
Figure 7: Bray-Curtis Dissimilarity in Arctic Relicts Across Latitude	44
Figure 8: Temporal Change in Species Diversity.....	45
Figure 9: Cluster Analysis of Relevé Sites.....	46
Figure 10: Ecological Niche Models.....	47
Figure 11: Selection on Flowering Date in <i>Pinguicula</i>	48

Introduction

Anthropogenic climate change is greatly altering the living landscape, and understanding how populations will respond to projected changes is crucial for efforts to conserve biodiversity in the face of major predicted declines (IPBES 2019). The optimal place to examine the potential impacts of climate change on the distribution of species is on range margins, where populations often experience extreme conditions relative to the rest of the range (Nadeau & Urban 2019). While it is well established that the cool range margin is often expanded as the result of ecological and evolutionary processes driven by climate change (Thomas et al. 2001, Mason et al. 2015), it is at the warm range margin where the potential negative impacts of climate change will first be observed. This is especially true for arctic plants that are adapted to cool temperatures, yet are predicted to experience the steepest temperature increases under current predictions of climate change (Hampe & Petit 2005, Cohen et al. 2014, Ballinger et al. 2020).

Populations of plants studied in the field have displayed various responses to climate change (Kremers et al. 2015, reviewed by Parmesan & Hanley 2016), and one possible response is a range shift through expansion or contraction. Ecological methods measuring community composition and range changes that result from changing climate are particularly important for predicting overall plant diversity in space in time (Harrison et al. 2017). Indeed, field studies of plants have exposed various responses to changing climate (Kremers et al. 2015, reviewed by Parmesan & Hanley 2016), including range shifts through expansion or contraction. These studies provide strong evidence for large-scale, poleward, cool range margin expansions due to climate change (Feeley et al. 2020). However, the main abiotic drivers of range shifts, the limits of range contraction, and the

ability of populations to persist at warm range margins remain understudied (reviewed by Cahill et al. 2014). Understanding impacts of climate change is especially critical for warm range margin populations that must ultimately either adapt or face local extirpation, thereby driving further range contraction.

Adaptation is one way for populations to respond to a changing climate, but it is most effective in large populations with high genetic diversity. Thus, marginal populations face a challenge because they are often (though not always) small and spatially isolated (Vucetich & Waite 2003). For example, even if gene flow can mitigate the impacts of climate change by improving fitness at the range edge (Bontrager & Angert 2018), it is unlikely that small, fragmented populations will experience adequate gene flow to drive adaptive evolution. Small populations have reduced effective population sizes, with limited genetic variation for selection to act upon (Person et al. 2009, reviewed by Brindle & Vines 2006). This can potentially limit both gene flow and the amount of standing variation, the two major sources of variation, in which selection can act upon.

Interestingly, even when facing climatic stress, populations at the warm range margin might not decline as quickly as predicted due to several mechanisms that allow populations to effectively “stall” and remain on the landscape (Sheth & Angert 2018). This stall can either prolong a population’s existence long enough for adaptation to occur, or simply prolong the time until local extirpation. One possible mechanism of stalling is demographic compensation, where some phenotypic traits associated with fitness can temporarily offset the overall population and individual fitness decline (Doak & Morris 2012). Demographic compensation has been documented across climate gradients in

several plant species (Villellas et al. 2015). While demographic compensation may temporarily prolong a population's ability to endure climate change, at least one study shows that it does not, ultimately, rescue populations at the warm range margin (Sheth & Angert 2018). Another potential stall mechanism for populations to remain on the warm range margin, is phenotypic plasticity, where single genotypes result in a multitude of phenotypes induced by unique environments (Schlichting 1986). While there is evidence that plasticity can both be both adaptive and maladaptive margins (Van Kleunen & Fischer 2005, reviewed by Zettlemyer & Peterson 2021), the degree to which plasticity may extend population survival at the warm range margin, remains unresolved (Gratani 2014). Ultimately, these delays could allow sufficient time for adaptive mechanisms to evolve and, thus, maintain a population at the warm range margin.

In Minnesota, rare, arctic relict plant populations persist at the southern limit of their ranges because of their proximity to the cold waters of Lake Superior, which generate cool microclimates along the lake shore throughout the year (Given & Soper 1981). These floristic elements have existed in Minnesota since glaciers retreated nearly 11,000 years ago (Dyke et al. 2003). Currently, Lake Superior is one of the fastest warming lakes in the world, with surface temperatures increasing at a rate of +1.2°C per decade, over three times the global mean in surface temperature rise (O'Reilly et al. 2015). This warming hotspot will have major ramifications for the relict populations that rely on a cool microclimate being produced by the lake, as they are already at the limit of their climate tolerance (Hampe & Jump 2011). Within Minnesota, these fragmented populations are distributed across a latitudinal gradient (47°N–48°N), where mean summer temperatures vary by over 2°C. This gradient provides a kind of natural

experiment that can be used to understand and predict how warming temperatures will impact these, and other, rare plant communities.

We used a variety of approaches to explore how arctic plant communities are changing in response to a warming climate. By leveraging historical community surveys and herbarium records, we examined how the range and community composition of these relict assemblages have changed through time. In addition to examining broad community change and possible range shifts of individual species, we used ecological niche models for three representative relict species, *Primula mistassinica* (Michx. 1803), *Pinguicula vulgaris* (L. 1753), and *Euphrasia hudsoniana* (Fernald & Wiegand 1915), to better understand the climate variables that define these communities and to make predictions about how niche-defining climate metrics may drive range shifts over the next 20–40 years. To investigate how arctic relict populations are responding to changing conditions at the warm range margin, we performed a phenotypic selection analysis across a latitudinal gradient. Using these approaches, we examine the response of threatened arctic relict plant communities of Minnesota to a changing climate.

Materials and Methods

Arctic Relicts

Primula mistassinica (Figure 1a) is not strictly an arctic relict species but is often found along the shores of Lake Superior in relict plant assemblages. It is also found in northwestern Minnesota in calcareous fen habitats and in the southern St. Croix River Valley, on sandstone bluffs with well-maintained seepage throughout the summer. This species has a much greater distribution in North America compared to true relict species and is distributed from the Great Lakes region to the northeast, as well as throughout

Canada, with multiple ecotypes present across the range (Guggisberg et al. 2006). The genus *Primula* has a long history of evolutionary study due to early observations of heterostyly by Darwin (reviewed by Gilmartin 2015, Darwin 1862). *Primula mistassinica* is a perennial with leaves in a basal rosette (width 1–10 cm) surrounding a central inflorescence (height ≥ 15 cm); flowers lavender (1–10), blooming in May (Chadde 2019). It reproduces sexually ($2n = 18$), with each fruit generating 20–50 seeds (Kelso 2020).

Pinguicula vulgaris (Figure 1b) is a small carnivorous plant. In Minnesota, it is restricted to the shores of Lake Superior where it is confined to wet habitats in small pools of water that persist throughout the summer. Populations are fragmented, occurring only in Lake and Cook counties, and are dependent on constant moisture, which is rarely found on the sun-exposed, basalt shorelines of Lake Superior. *Pinguicula vulgaris* is a perennial with a rosette up to 15 cm wide. It is distinguished by leaves that are flat-laying, yellowish-green, and produce a sticky, sweet secretion that captures small insects. Excreted digestive enzymes break down the insect into a nutrient slurry that is eventually absorbed (Legendre 2000). It reproduces clonally and sexually ($2n = 64$) (Casper & Stimper 2009) and produces 1–3 inflorescences that can reach a height of 15 cm, each with a single tubular, purple flower that blooms in June or July (Chadde 2019). In Minnesota, *Pinguicula vulgaris* was listed as a special species of concern by the Minnesota Department of Natural Resources (MN-DNR) in 1984 due to limited and fragmented high-quality habitat.

Euphrasia hudsoniana (Fernald and Wiegand 1915) (Figure 1c), like most arctic relict plants in Minnesota, is restricted to the microclimate produced along the shores of

Lake Superior. Its range also extends into the Canadian provinces of Ontario and Manitoba. The species is often found in damp cracks, crevices, and seepages along the shoreline or in bryophyte mats where moisture can be retained. *Euphrasia hudsoniana* is an annual, growing up to 35 cm tall (Chadde 2019). Despite evidence of cross-pollination, *E. hudsoniana* is mainly self-pollinating due to its smaller flower size (Sell & Yeo 1970, French et al. 2005). Stems branch intermittently and it produces multiple small white flowers in July and August. This species is a hemi-parasite which can greatly increase the size of individual plants depending on the presence and type of host. It is assumed to be a tetraploid, like most *Euphrasia* species (Becher et al. 2020). According to the MN-DNR, *Euphrasia hudsoniana* is an arctic relict of special concern and has recently been exposed to additional pressures from the European invasive, *Euphrasia stricta* (D. Wolff ex J.F. Lehm 1809). A recent study has shown that *E. hudsoniana* is hybridizing with this invader (Zlonis & Gross 2018), suggesting the threat of genetic swamping (Levin et al. 1996).

Historical Range and Occurrence Records

Herbarium records for the relict species *Primula mistassinica*, *Pinguicula vulgaris*, and *Euphrasia hudsoniana* collected within Minnesota were gathered from the Bell Museum Herbarium at the University of Minnesota Twin Cities (MIN) and the Olga Lakela Herbarium at the University of Minnesota Duluth (DUL). A small number of records were also obtained using digital data from the Flora of Wisconsin Database (WIS-VP, NC, ML-VP) and the MN-DNR. Precise localities were compiled from these records for multiple analyses. Historical occurrence sites were revisited in the summers of 2020 and 2021 to check for site occupancy. Visits were mostly made while plants were

flowering, to increase visibility and aid in locating individuals. Island sites and private property were deemed inaccessible and were not included in this study.

Temporal and Spatial Community Level Change

Historical Relevé surveys containing at least one of the three relict species of interest were obtained from the Minnesota Biological Survey (MBS). A total of 28 historical surveys meeting this requirement were found, ranging from the years 1998–2012. Of the 28 surveyed sites, 17 sites found in northwestern Minnesota contained disjunct populations of *Primula mistassinica*. These plant communities, not in close proximity to Lake Superior, were determined to not be relevant to the arctic relict community assemblages of interest. Of the remaining 11 historical surveys, two were removed from resurvey: one was on private property, and the other was found too far inland to harbor the same plant community as the others. The remaining nine sites, originally surveyed in 2000 or 2001, were targeted for resurvey. These nine selected lakeshore sites were across a latitudinal gradient ranging from 47°00'51"N to 47°50'59"N and varied in size from 25 m² to 100 m². Final sites for resurvey were located in Two Harbors (TH), Gooseberry Falls State Park (GF1 and GF2), Split Rock Lighthouse State Park (SR1 and SR2), Tettegouche State Park (TT), Temperance River State Park (TR1 and TR2), and Horseshoe Bay in Hovland, MN (HB) (Figure 2). In 2019, MBS contracted to have four of these sites resurveyed. The remaining five sites were resurveyed in 2020 adhering to protocols outlined in *A Handbook for Collecting Vegetation Plot Data in Minnesota: The Relevé Method* (Minnesota Department of Natural Resources 2013). The final survey data set contained nine sites with both

historical and modern plant community and abundance data as well as the associated physical site data.

Species lists and abundance data used for analyses were restricted to species categorized by the Relevé Method as broadleaf deciduous, needleleaf evergreen, graminoids, and forbs. All other species listed in the remaining Relevé life form categories were not included in analyses due to poor identification confidence. Relevé surveys contain mixed abundance and cover group codes, therefore species abundance, generated using cover category designation based on a modified Braun-Banquet scale (Mueller-Dombois & Ellenberg 1974, Channon & Heard 1997), was used in downstream analyses (Table 1).

Historical and modern plant community assemblages were compared with both Jaccard and Bray-Curtis dissimilarity measures using the R package “vegan” version 2.5-7 (Oksanen et al. 2020) to identify pairwise temporal and spatial diversity between sites. Jaccard and Bray-Curtis dissimilarity values range from 0 to 1, and a dissimilarity value of 0 indicates identical community assemblage between the compared groups, whereas a value of 1 signifies no common species between the compared groups. Linear regression was used to identify differences in Bray-Curtis dissimilarity between resurveyed sites across a latitudinal gradient. Furthermore, we isolated *Primula*, *Pinguicula*, and *Euphrasia* from Relevé data and used linear regression to test for differences in Bray-Curtis dissimilarity between resurveyed sites across a latitudinal gradient for these species. Cluster analysis using complete linkage in the R package “vegan” version 2.5-7 (Oksanen et al. 2020) was performed in order to identify any natural groupings among Relevé sites based on community composition as calculated by Bray-Curtis. Change in

species richness and Shannon Diversity Index (Shannon 1948) between historical and modern Relevé surveys were calculated. A two-sample, paired t-test was used to determine whether mean values for these metrics differed significantly between historical and modern surveys. All analyses were performed using R version 3.6.1 (R Core Team 2019).

Ecological Niche Modeling

Ecological niche modeling (ENM), implemented in Maxent (version 3.4.4; Phillips et al. 2004, 2006), was performed for *Primula mistassinica*, *Pinguicula vulgaris*, and *Euphrasia hudsoniana*. Locality data from herbarium records were used to generate the species presence record for all models. Presence records were restricted to Minnesota's Lake Superior shoreline, to capture the microclimate generated by the lake. Bioclimatic data (yearly averages) were extracted for 1970–2000 from WorldClim2 (Fick & Hijmans 2017) at 30-arc-second, or roughly 1 km², spatial resolution. The R package “virtualspecies” (Leroy et al. 2015) was used to identify correlated climate variables using Pearson's correlation coefficient. Variables with a Pearson's correlation coefficient of greater than 0.7 were grouped. From each of these correlated groups, one representative variable was selected, based on its contribution to generating the summer microclimate. The selected variables annual mean temperature, temperature seasonality, isothermality, mean temperature of warmest quarter, precipitation seasonality, and precipitation of wettest quarter were then used to shape predictive parameters for all final models. Future climate ENMs were constructed using the bioclimatic variables generated by the Canadian Earth System Model Version 5 (Swart et al. 2019). Data from the Shared Socio-economic Pathway 245 (SSP 245) model, which simulates moderate warming for

2041–2060 (yearly averages), were used for niche predictions at 2.5-arc-second spatial resolution.

Phenotypic Variation Across the Latitudinal Gradient

Three to four sites across the sampled latitudinal gradient, where mean summer temperatures varied 2°C, of each species were selected for monitoring individual plants and gathering demographic and phenotypic data for selection and population viability analyses. *Pinguicula*, *Primula*, and *Euphrasia* were surveyed at three sites: Gooseberry Falls State Park (47°08'05.2"N 91°27'41.0"W), Temperance River State Park (47°33'13.9"N 90°52'13.5"W), and Artist Point in Grand Marais (47°44'42.5"N 90°20'07.0"W); *Euphrasia* was sampled at an additional southern site, located at Two Harbors Lighthouse (47°00'51.3"N 91°39'42.4"W) (Figure 3). Three 1×1 m plots were established at each site. Due to the clumped distribution of these species amongst the cracks and crevices of the exposed bedrock, a random plot design could not be used. Instead, plots were established in the early growing season based on the presence of the target species, and final plots contained between 31 and 88 individuals. All plots were marked to ensure relocation between years. Individuals were assigned an ID using a novel grid system that broke the plot into 10-centimeter rows, labeled A through J, and each plant's position in the row was recorded in centimeters from the west side of the plot. Mean summer temperatures were recorded using iButton temperature and humidity data loggers placed near the median plot at each site. Measurements were taken weekly during times of major phenological change and monthly, from May until late September. We collected phenotypic data for all individuals, including plant height, rosette width, leaf number, flower number, seed number, and fruit diameter. For *Euphrasia*, we also

recorded the number of branches and whether leaves had bristle tips (i.e., were hirsute), which are traits that distinguish *E. hudsoniana* from the invasive congener, *E. stricta*. We also collected phenological data, including date of first flower and date of fruit set. Total individuals across all sites numbered 551 (*Primula mistassinica*), 458 (*Pinguicula vulgaris*), and 623 (*Euphrasia hudsoniana*).

Both raw and transformed phenotypic data failed to meet the assumptions of normality and equal variance needed for parametric one-way ANOVA. Therefore, we analyzed trait means using Kruskal-Wallis one-way ANOVA on ranks. Kruskal-Wallis effect size, measuring the amount of variance in the dependent variable explained by the independent variable, was also calculated. We used Dunn's Test for post-hoc pairwise analysis and calculated Bonferroni adjusted p-values to identify significant pairwise differences between population means.

Phenotypic Selection Analysis

We performed phenotypic selection analysis for *Primula*, *Pinguicula*, and *Euphrasia* at each location. Selection analyses require a measure of individual fitness, usually fecundity. However, seeds could not be collected from individuals within plots without sacrificing the accuracy of multi-year population viability analysis. Therefore, linear regression was used to predict individual plant fecundity based on measurements and seed counts from plants outside of the plots. Fruit width was used as a predictor variable for seed number in *Primula* ($R^2 = 0.10$, $p = 0.22$, $b = 8.25$) and *Pinguicula* ($R^2 = 0.25$, $p = 0.005$, $b = 48.8$). In *Euphrasia*, flower number was used as a proxy for fecundity. Relative fitness of each individual, calculated as the individual's predicted seed or flower number divided by the maximum predicted seed or flower number found

at each location, was calculated independently for each site. All phenotypic measurements were standardized to a mean value of 0 and a standard deviation of 1.

Multiple linear regression using backward elimination was used to identify traits with a significant linear relationship when regressed with fitness (Lande & Arnold 1983). This analysis was completed for all sites and significant variables found in at least one site were retained in all other site models, regardless of significance. Direct selection (β_i), which measures the effect of a trait on relative fitness when other traits are held constant, was calculated as the regression coefficient for the multiple linear regression model. We also calculated total selection (S), which includes the effects of direct and indirect selection as the result of trait correlations (Brodie et al. 1995).

Population Viability Analysis

We performed preliminary population viability analyses in *Primula mistassinica* and *Pinguicula vulgaris* using deterministic matrix models based on population size and structure (Caswell 2001). Population structure was divided into seedling and reproducing groups for demographic data from 2020 and 2021. Mortality between years was also included in the transition matrix. Demographic analyses were performed using the R package “popbio” (Stubben and Milligan 2007) to examine if populations at different latitudinal sites varied in projected growth or decline in population size. Due to having only two consecutive years of demographic data, only count-based metrics were reported to avoid inflating the impact of climate variability within this limited time frame.

Therefore, the reported population growth rate, or lambda (λ), represents the one-year change between sites from 2020 to 2021. Values above 1 indicate a growing population

whereas values below 1 show a declining population. Additionally, year-to-year mortality for the two perennial species, *Primula* and *Pinguicula*, was also calculated.

Results

Historical Range and Occurrence Record

Historical records representing North Shore populations of *Primula mistassinica*, including 115 herbarium specimens spanning 1878–2012, were identified for this study (Table 2a). During the summer of 2020, 14 of these historical localities were revisited; *Primula* was not found at five of these sites (Table 2a). For *P. vulgaris*, 87 herbarium specimens spanning 1879–2001, were found and 10 of these geographic locations were revisited (Table 2b). *Pinguicula* was not found at two of the locations revisited in the summer of 2020, including the southernmost site, Knife River, collected in 1948 (Figure 4). A total of 118 herbarium specimens spanning 1878–2014, were identified for *E. hudsoniana*. In total, 14 previously sampled sites were revisited in 2020 and *Euphrasia* was found at all but two of these locations (Table 2c). It was not found at the southernmost site, Stony Point, where a specimen was collected in 1944 (Figure 4). However, it should be noted that resolving current *Euphrasia* occurrence records remains a challenge due to the recent spread of the invasive *Euphrasia stricta* and the difficulty in accurately differentiating between the two species.

Temporal and Spatial Community Dissimilarity

Overall, there was a large community dissimilarity between historical (2000/2001) and modern (2019/2020) survey sites (Figure 5). Bray-Curtis dissimilarity values ranged from 0.14 to 0.60. The southernmost sites (TH, GF, and SR) represented the extremes of this variability, with dissimilarity values of 0.14 to 0.60, and two of the

southern sites (SR1 and TH) displayed the largest temporal change (0.60). Despite these large changes found in southern sites, linear regression showed that latitude was not significant in predicting Bray-Curtis dissimilarity scores across all sites ($R = 0.04$, $p = 0.93$, Figure 6). As expected, Jaccard dissimilarity values, which only account for presence/absence data, showed similar trends with values ranging from 0.24 (GF1) to 0.75 (TH and SR1) (Figure 5, below diagonal). Examining Bray-Curtis dissimilarity measures of *Primula*, *Pinguicula*, and *Euphrasia* between historical and modern sites shows significant temporal dissimilarity ($t(8) = 5.354$, $p < 0.001$) and dissimilarity measures range from 0.07 to 0.30. Linear regression showed that latitude did not significantly predict Bray-Curtis dissimilarity among these three species ($R = -0.58$, $p = 0.10$) (Figure 7). Shannon diversity index values decreased over time in all resurveyed sites, with the exception of TR1 (Figure 8). A paired t-test showed no significant reduction in Shannon diversity over time in these communities ($t(8) = 1.887$, $p = 0.09$). However, when TR1 was removed from this analysis, the reduction in diversity was significant ($t(7) = 3.725$, $p = 0.007$). Cluster analysis (Figure 9) of Bray-Curtis dissimilarity values identified two large groups consisting of northern and southern communities. The southernmost, Two Harbors site grouped separately and was the most dissimilar from all other sites. As expected, within groups almost all sites were clustered together with their corresponding temporal match with the exception of SR1. Modern SR1 pairs with both historical and modern GB2 communities and this unique grouping falls within a broader cluster containing all the northern sites (TR1, TR2, HB).

Ecological Niche Modeling

As expected, ecological niche models for all three species highlight the microclimate habitat currently found along the North Shore of Lake Superior (Figure 10). All models identified isothermality as having the largest permutation importance. As a result, isothermality contributed the most to all three of the final models selected (Table 3). All other selected climate variables contributed between 0.1% and 20.3%. Current models identify decreased habitat suitability at the southern range for each species (Table 4). *Pinguicula* showed the greatest current variability in habitat suitability (Figure 10b). Conservative future climate models for 2040–2060 yearly means (CanESM5, SSP 245) show a drastic reduction in overall habitat suitability in Minnesota for all three species. *Pinguicula* is the most impacted by these predictive climate models with habitat suitability most reduced in Minnesota (Figure 10e).

Phenotypic Variation Across a Latitudinal Gradient

All three species showed significant differences in population trait means across the latitudinal gradient, where mean summer temperatures ranged from 15.8°C at Artist Point to 18.7°C at Two Harbors Lighthouse (Table 4). There were significant differences between at least one site and the others for all measured traits, with the exception of fruit number in *Pinguicula* (Table 5). Overall, the warmer southern populations were smaller and less likely to flower compared to the northern populations sampled. Populations of *Primula* at the southernmost Gooseberry site were smaller, both in inflorescence height and rosette width, and were less likely to flower compared to the Artist Point population, with the flowering individuals producing fewer estimated seeds (Table 5a). Patterns were similar for *Pinguicula*, as the southern populations were smaller in size for both inflorescence height and rosette width; they also produced fewer estimated seeds than the

plot to the north and were less likely to flower, compared to Artist Point populations (Table 5b). Populations of *Euphrasia* exhibited similar patterns (Table 5c). At the southernmost site, *Euphrasia* was smaller and produced fewer flowers. However, Artist Point populations, where interspecific hybridization has been documented (Zlonis & Gross 2018), had a noticeable decline in growth and fecundity metrics compared to the next most northern site, Temperance.

Phenotypic Selection Analysis

Patterns of selection for *Primula* were similar across all sites (Table 6a). Taller inflorescences were under strong, positive direct and total selection in all populations. At northern sites, positive total selection for larger plants that were wider and had more leaves was detected. The southern Gooseberry populations showed no significant total selection for rosette width or leaf number. Date of first flower was not under significant direct or total selection for any of the populations sampled and was therefore not included in the model. All populations show evidence of total selection for increased rosette width, despite direct selection for smaller rosette width (Table 6a). However, this conflicting pattern is only significant for both direct and total selection in the Temperance population.

Evidence for selective forces acting on *Pinguicula* varied the most among sites for all studied species (Table 6b). Most notably, date of first flower exposed opposing patterns of selection between the northern and southern sites. Gooseberry populations were under strong, significant total selection for earlier flowering, whereas the Artist Point population is under significant total selection for later flowering. Total linear

selection on date of first flower only also supports this pattern of selection (Figure 11). Positive total selection for larger plants with more leaves was significant across all sites.

Patterns of selection among *Euphrasia* populations were the most similar of any species studied (Table 6c). In all populations, plants experienced strong total selection for plant height, stem thickness, and an increase in leaf number. However, there was an inverse pattern in the southern population, where significant direct selection slightly favored shorter plants. In the Temperance population, total selection for earlier flowering was significantly favored. In Two Harbors, significant direct selection for earlier flowering was also detected.

Preliminary Population Viability Analysis

Preliminary population viability analyses show a surprising increase in year-to-year population sizes of *Primula mistassinica* and *Pinguicula vulgaris* at all sites between 2020 and 2021 (Table 7) (Table 8). However, despite this broad population increase, there is still a noticeable latitudinal gradient in population growth rates, with southern sites having a smaller λ . This pattern is also true for annual mortality in *Primula*, with the southernmost Gooseberry site having the largest 2020 mortality (0.13) and the northernmost site, Artist Point, having the smallest 2020 mortality (0.05) (Table 8). There was no latitudinal pattern in the 2020 mortality of *Pinguicula vulgaris*.

Discussion

Arctic relict plant communities have changed over the last 20 years and, of the relict species studied, two have likely experienced a southern range contraction. Understanding how surviving relict populations will respond to accelerated climate warming will be important in projecting whether these iconic species continue to inhabit

the shores of Lake Superior. Furthermore, changes in these species could signal broader potential changes for similar plant communities. Using a combination of approaches, we compared 9 populations across a latitudinal gradient on the shores of Lake Superior in Minnesota, and leveraged the warmer, southernmost sites to predict how northern populations might respond to future warming.

Range Contraction and Climate Stress at the Warm Range Margin

We documented no occurrence in the southernmost populations of *Euphrasia hudsoniana* and *Pinguicula vulgaris* in 2020 (Figure 4). Although confirming the absence of a population and identifying general range limits is challenging, the discrete nature of arctic relict plant populations and their restricted, near-shore distributions lead us to conclude that the southern ranges of these two relict species have contracted. This is consistent with other findings showing an overall decline in arctic plant populations, including *Pinguicula*, at their warm range margin (Lesica & McCune 2004). *Primula mistassinica* was found at the southernmost site, Stony Point, in 2020 (Table 2). Unlike *Pinguicula* and *Euphrasia*, *Primula* has several other disjunct populations throughout the state and it is clear that further work is needed to disentangle the many factors that shape its distribution. In all three species, the degree of landscape fragmentation appears to be increasing across their range in Minnesota. This fragmentation could result from climate change but could also be due to increased development along the lake shore (Moore 2000) or other unknown factors. Whatever the cause, increased fragmentation will ultimately have the same effect, as a meta-analysis of the genetic impacts of fragmentation predicts reduced gene flow, further hindering the potential for the spread of adaptive alleles into these populations (Schlaepfer et al. 2018).

We found large changes in dissimilarity across all sites, which could be caused by either an increase or a decrease in species diversity. However, we did not identify an abundance of new species, and invasive species are not an apparent factor, as only one new invasive (a *Sedum* with only a few individuals in SR1) was found in the modern Relevé plots. The harsh conditions along the lake in which these communities exist can be inhospitable to invasive species, making them less likely to invade (Zefferman et al. 2015). Instead, the large change in temporal dissimilarity across all sites likely stems from a decrease in species diversity—with the exception of the TR1 site, where species diversity increased (Figure 8). This local trend is consistent with global trends in measured decreases in biodiversity (Ceballos et al. 2015).

While our approach does not speak to the cause of these diversity changes, our findings are consistent with an experimental study of arctic plant communities where various treatments, including changes in precipitation and temperature mimicking future climate conditions, led to overall reductions in community diversity over a period of nine years (Chapin et al. 1995), roughly half the time of our resurvey. One intriguing result of our cluster analysis (Figure 9) was that it identified the modern SR1 site as being most similar to both modern and historical southern GB2 surveys, which are located to the south. This pairing is most likely due to a large reduction of species diversity at the SR1 site, making it more similar to the GB2 site. This anomalous grouping, found near the southern range limit, further highlights changes occurring in these communities.

Ecological niche models (ENMs) identified that *Pinguicula vulgaris* is facing the largest degree of climatic stress at the southern range limit, with greatly reduced suitable habitat (Figure 10b, 10e) (Table 4). Ecological niche models (ENMs) also show that all

three species are facing extreme predicted climate stress due to future changes in temperature and precipitation (Table 4). This is consistent with other studies examining the main abiotic drivers of climate stress at warm range margins (reviewed by Cahill et al. 2014). Interestingly, the southernmost sites, under the greatest climatic stress for arctic relict communities as outlined by ENMs, also show two of the largest Bray-Curtis dissimilarity values. This reduction in diversity and further predicted climate stress, coupled with a possible range contraction based on herbarium occurrence data, provide strong evidence that these species' ranges are shifting and suggest that they will likely continue to do so. Range shifts at the warm range margin, like those observed here, have already occurred in great numbers, resulting in local extinction driven by climate stress (Wiens et al. 2016).

Selection Across a Climate Gradient

All three species studied showed significant reductions in fecundity, flowering, and plant size metrics at the southernmost studied sites in 2020 (Table 5). While annual climate variability among sampled sites could be driving these differences, early 2021 data (not shown) point to similar reductions, consistent with observations globally (Harrison et al. 2020). Decreased flowering is particularly detrimental for annual *Euphrasia*, where phenotypic variation between sites in just one year could potentially result in rapid demographic and evolutionary changes (Franks et al. 2007). Interestingly, the northernmost, Artist Point, population of *Euphrasia* also shows reduced growth and fecundity when compared to the other northern site. It should be noted, again, that *Euphrasia* at the Artist Point site is hybridizing with the invasive congener *E. stricta* (Zlonis & Gross 2018), and we are currently examining genetic resolution to determine if

rate of hybridization has increased. If hybridization between species within our plots is confirmed, phenotypic differences outlined between hybrid and native populations could be evidence of reduced fecundity in hybrid individuals, and could accelerate the extirpation of native *Euphrasia hudsoniana* in these locations (Levin et al. 1996).

Quantitative genetic approaches are particularly powerful for understanding how rare plants are potentially adapting to climate change (reviewed by Edwards 2015), and our selection analyses highlight the impacts of varied climatic conditions across the surveyed sites. Most of the traits with evidence for significant direct and total selection exhibited similar patterns, with several traits showing opposing selection, as measured by direct and total selection (Table 6). The population of *Primula* at Temperance is an example of this, with direct selection on rosette width being negative and total selection being positive. In this case, strong selection for increased inflorescence height could be driving increased rosette width, despite direct selection being slightly negative.

Interestingly, the date of first flower in *Pinguicula* shows opposing selection between northern and southern sites (Table 6; Figure 11). Earlier flowering is favored in the warm southern site, while later flowering is favored in the cooler northern site. This is consistent with well-studied phenological advance driving earlier flowering in warmer, southern populations (Post et al. 2018). Determining whether this opposing pattern of selection is the result of annual differences in climate, drift, or is a constant selective pressure is yet to be determine and will require several more years of data. Surprisingly, the impacts of this selection pattern are not expressed in the population phenotypic means (Table 5b), as there is no significant difference in date of first flower between the southern and northern populations. This could be a signal that selection pressures are

fluctuating from year to year or that selective pressures are recent and have not yet impacted this perennial species. Alternatively, this could signal that there is not enough standing variation to facilitate a response to selection in this isolated, southern population (Bell 2013).

We observed an unexpected increase in the number of individuals at all sites for *Primula* (Table 7) and *Pinguicula* (Table 8). This increase could be the result of permissive climatic conditions in 2020, or it might be a signal of demographic compensation, where population metrics show growth even as phenotypic traits related to size and fecundity decline overall (Villellas et al 2015). However, the count-based method used, due to the limited timeframe of this study, though informative, does not include important demographic categories, such as seedlings and reproducing individuals (Lotts et al. 2004). Multiple years of demographic data are needed to support these findings, to determine if populations are undergoing demographic compensation, and to ensure population viability analyses and extinction predictions are reliable in these perennial species (Menges 2000). Despite the overall population increase in 2021, *Primula* exhibited higher mortality, which was correlated with increased site temperatures across the latitudinal gradient. This is consistent with an experimental warming study in finding a positive correlated between increased mortality and rising temperatures in arctic plant seedlings (Milbau et al. 2007).

Future Population Viability

It is clear that the arctic relict plant communities of Minnesota face a challenging future as the climate rapidly changes. The collective support from the methods explored here raises serious concerns about the future of these arctic relict plant communities. The

southern range of two of these species appears to have contracted within the last century, and ecological niche models highlight the extreme climate stress that is likely in the future. Furthermore, populations found along the shore are becoming more fragmented, reducing the potential for gene flow. While adaptation from standing variation could still potentially allow these populations to survive into the future (Chevin et al. 2010), it is not clear that the populations are responding to selection, at least based on one year of data collection. Moreover, populations at the warm range margin show signs of stress, with reduced fecundity, reduced size, and increased mortality. These southern populations signal an uncertain future for these species in Minnesota that needs to be further examined. Additionally, clarifying the degree of hybridization in *Euphrasia hudsoniana* is needed to understand whether hybrid individuals might hold an adaptive advantage, or will lead to accelerated decline resulting from reduced fitness. Only through continued and comprehensive monitoring of these populations and multi-year population viability analyses can we make confident predictions about the trajectory of these populations, and understand whether they will be able to adapt to the pressures of changing climate.

Table 1. Relevé abundance and cover codes with values based on a Braun-Blanquet scale used for MN-DNR Relevé data. A modified Braun-Banquet values (Heard & Channon 1997) was used for abundance analyses.

Braun-Blanquet Based Code from MN-DNR Relevé	Abundance/Cover Values	Modified Braun- Banquet Scale
r	<5% cover, single individual	0.1
+	<5% cover, few (2-20) individuals	0.5
1	<5% cover, many individuals	1
2	5-25% cover	2
3	25-50% cover	3
4	50-75% cover	4
5	75-100% cover	5

Table 2. Occurrence record from herbarium vouchers for *Primula* (2a), *Pinguicula* (2b), and *Euphrasia* (2c). Locations for each population are listed from southern to northern sites; X in far-right column indicates species was found in 2020.

Table 2a.

<i>Primula</i> Location Description	County	Collector & Number	Institution Code	Latitude	Longitude	<i>Primula</i> Found in 2020
Stony Point	St. Louis	Lakela 7476	UMD	46.92549	-91.81601	X
Knife River	Lake	Lakela 27878	MIN	46.94743	-91.78709	X
Two Harbors	Lake	Sheldon S13054	UMD	47.014242	-91.662216	X
Gooseberry State Park	Lake	Lakela 27878	UMD	47.21048	-91.35351	X
Split Rock State Park	Lake	Reschke -	DNR	47.210480	-91.353510	X
East Beaver Bay	Lake	Lakela 27878	UMD	47.266998	-91.281901	
Tettegouche State Park	Lake	Nielsen 3084a	MIN	47.33641	-91.197129	
Tettegouche State Park	Lake	Kellner -	MIN	47.339644	-91.184761	X
Temperance River	Cook	Schuster A5007	MIN	47.550759	-90.876452	
Temperance River	Cook	Reschke -	DNR	47.55379	-90.87099	X
Cascade River State Park	Cook	Smith 32120	MIN	47.707602	-90.516483	
Artist Point East	Cook	Monson 4420	UMD	47.7452	-90.329912	
Artist Point West	Cook	Rosendahl 4695	MIN	47.745201	-90.334752	X
Horseshoe Bay	Cook	Reschke -	MIN	47.84989	-89.93343	X

Table 2b.

<i>Pinguicula</i> Location Description	County	Collector & Number	Institution Code	Latitude	Longitude	<i>Pinguicula</i> Found in 2020
Knife River	Lake	Lakela 7476	MIN	46.94743	-91.78709	
Two Harbors	Lake	Sheldon S4807	MIN	47.014242	-91.662216	X
Gooseberry State Park	Lake	Moore 18204	MIN	47.21048	-91.35351	X
East Beaver Bay	Lake	Lakela 6088	MIN	47.266998	-91.281901	
Father Baraga's Cross	Cook	Lee 3040	MIN	47.550759	-90.876452	X
Temperance River	Cook	Reschke 284367	DNR	47.55379	-90.87099	X
Tofte Park	Cook	Lakela 4782	MIN	47.573211	-90.835916	X
Artist Point East	Cook	Slark -	MIN	47.7452	-90.329912	X
Artist Point West	Cook	Wheeler 4504	MIN	47.745201	-90.334752	X
Horseshoe Bay	Cook	Reschke 284730	DNR	47.84989	-89.93343	X

Table 2c.

<i>Euphrasia</i> Location Description	County	Collector & Number	Institution Code	Latitude	Longitude	<i>Euphrasia</i> Found in 2020
Stony Point	St. Louis	Lakela 4856	UMD	46.92549	-91.81601	
Two Harbors	Lake	Roberts -	MIN	47.014242	-91.662216	X
Gooseberry State Park	Lake	Lakela 2730	UMD	47.13965	-91.45573	X
Split Rock State Park	Lake	Lee 2644	UMD	47.210480	-91.353510	X
Tettegouche	Lake	Smith 6902	MIN	47.33641	-91.197129	
Tettegouche	Lake	Reschke 284479	DNR	47.347504	-91.180956	X
Sugarloaf Cove	Cook	Monson 6359	UMD	47.486659	-90.980587	X
Temperance River	Cook	Smith 1143	UMD	47.550759	-90.876452	X
Temperance River	Cook	Lakela 4792	MIN	47.55379	-90.87099	X
Tofte Park	Cook	Lakela 4777	UMD	47.573211	-90.835916	X
Cascade River	Cook	Briggs -	MIN	47.707602	-90.516483	X
Artist Point East	Cook	Cheney -	MIN	47.7452	-90.329912	X
Artist Point West	Cook	Clemants 762	MIN	47.745201	-90.334752	X
Horseshoe Bay	Cook	Scott 802	MIN	47.84989	-89.93343	X

Table 3. Ecological Niche Model (ENM) statistics for *Primula*, *Pinguicula*, and *Euphrasia*. Models were constructed using selected WorldClim bioclimatic variables and the contribution of each variable is listed in order of importance. Model AUC is listed for each species model.

Model	Variable and Percent Contribution to Model						
	Isothermality	Temperature Seasonality	Precipitation of Wettest Quarter	Precipitation Seasonality	Mean Temp of Warmest Quarter	Annual Mean Temp	Model AUC
<i>Primula</i>	52.6	20.3	13.3	9.9	3.9	0.1	0.993
<i>Pinguicula</i>	42	14.8	14	13.5	11.8	3.9	0.996
<i>Euphrasia</i>	52.2	18.4	17.6	7.4	3.8	0.6	0.994

Table 4. Model temperature data for selected population study sites from WorldClim 1970–2000 bioclimatic variables, 2020 temperature sensor field data, and future forecast estimates from CIMP6 2040–2060. Suitability values, ranging from 0 to 1, were extracted from the constructed ecological niche model (Figure 10) for each species.

Site	Warmest Quarter Mean Temperature in °C			Historical ENM Habitat Suitability WorldClim 1970–2000			Future ENM Habitat Suitability CIMP6 2040–2060		
	1970-2000	Summer 2020	2040-2060	<i>Primula</i>	<i>Pinguicula</i>	<i>Euphrasia</i>	<i>Primula</i>	<i>Pinguicula</i>	<i>Euphrasia</i>
Two Harbors	17.6	18.7	18.8	0.46	0.08	0.85	5.5×10^{-6}	5.4×10^{-8}	7.5×10^{-5}
Gooseberry	17.0	17.7	18.5	0.77	0.34	0.94	5.8×10^{-6}	6.0×10^{-8}	5.1×10^{-5}
Temperance	16.5	17.5	17.9	0.91	0.90	0.97	1.3×10^{-5}	1.3×10^{-7}	1.3×10^{-4}
Artist Point	15.9	15.8	16.7	0.82	0.59	0.97	2.6×10^{-5}	2.2×10^{-7}	3.5×10^{-4}

Table 5. Kruskal-Wallis one-way ANOVA on ranks of mean trait measurements for *Primula* (5a), *Pinguicula* (5b), and *Euphrasia* (5c) across sites. Effect sizes are interpreted as 0.01– <0.06 (small), 0.06– <0.14 (moderate) and ≥ 0.14 (large). Post-Hoc Dunn’s Test with Bonferroni adjusted p-value with pairwise comparisons between Artist Point (A), Temperance (T), Gooseberry (G), and, for *Euphrasia*, Two Harbors (TH). Significant terms are bolded.

Table 5a.

<i>Primula</i> Trait	Location	Mean	Std. Dev.	Kruskal-Wallis Effect Size	p	Dunn’s Test with adj-p
Stalk Height (cm) n=551	Gooseberry	1.70	1.48	0.130	<0.001	A-G <0.001
	Temperance	2.97	2.90			A-T 0.006
	Artist Point	3.06	2.24			G-T <0.001
Rosette Width (cm) n=551	Gooseberry	1.74	0.81	0.176	<0.001	A-G <0.001
	Temperance	3.16	1.98			A-T 0.141
	Artist Point	3.02	1.32			G-T <0.001
Leaf Number n=551	Gooseberry	6.84	1.88	0.014	0.008	A-G 0.077
	Temperance	6.50	1.40			A-T 0.007
	Artist Point	7.08	1.65			G-T 0.308
Percent Flowered n=551	Gooseberry	0.08	0.27	0.026	<0.001	A-G <0.001
	Temperance	0.19	0.39			A-T 0.233
	Artist Point	0.24	0.42			G-T 0.125
Date of First Flower n=93	Gooseberry	138.0	7.75	0.302	<0.001	A-G 0.387
	Temperance	145.3	5.03			A-T <0.001
	Artist Point	138.6	3.46			G-T <0.001
Produced Fruit n=551	Gooseberry	0.08	0.28	0.016	0.004	A-G 0.008
	Temperance	0.18	0.39			A-T 0.734
	Artist Point	0.20	0.40			G-T 0.014
Date of First Fruit n=81	Gooseberry	156.0	4.00	0.128	0.002	A-G 0.171
	Temperance	157.3	3.55			A-T 0.031
	Artist Point	154.8	2.26			G-T 0.003
Fruit Number Per Plant n=81	Gooseberry	1.53	1.01	0.103	0.007	A-G 0.929
	Temperance	2.12	1.04			A-T 0.016
	Artist Point	1.45	0.63			G-T 0.027
Estimated Seeds Per Plant n=79	Gooseberry	42.08	8.07	0.163	<0.001	A-G 0.169
	Temperance	57.24	18.02			A-T 0.020
	Artist Point	46.19	12.00			G-T 0.001

Table 5b.

<i>Pinguicula</i> Trait	Location	Mean	Std. Dev.	Kruskal-Wallis Effect Size	p	Dunn's Test with adj-p	
Stalk Height (cm) n=458	Gooseberry	2.64	2.69	0.056	<0.001	A-G	<0.001
	Temperance	2.35	2.18			A-T	<0.001
	Artist Point	3.90	3.11			G-T	0.427
Rosette Width (cm) n=458	Gooseberry	3.37	1.25	0.033	<0.001	A-G	0.003
	Temperance	3.22	1.01			A-T	<0.001
	Artist Point	3.90	1.47			G-T	0.406
Leaf Number n=458	Gooseberry	4.66	1.38	0.009	0.043	A-G	0.335
	Temperance	5.01	1.35			A-T	0.335
	Artist Point	4.84	1.29			G-T	0.039
Percent Flowered n=458	Gooseberry	0.37	0.48	0.009	0.003	A-G	0.003
	Temperance	0.49	0.50			A-T	0.220
	Artist Point	0.56	0.50			G-T	0.074
Date of First Flower n=214	Gooseberry	158.1	5.31	0.26	<0.001	A-G	0.199
	Temperance	164.1	4.40			A-T	<0.001
	Artist Point	157.8	5.23			G-T	<0.001
Produced Fruit n=458	Gooseberry	0.35	0.47	0.021	0.003	A-G	1
	Temperance	0.45	0.50			A-T	1
	Artist Point	0.54	0.50			G-T	1
Date of First Fruit n=204	Gooseberry	170.0	23.12	0.164	<0.001	A-G	0.002
	Temperance	174.4	2.80			A-T	<0.001
	Artist Point	170.6	4.31			G-T	0.020
Fruit Number Per Plant n=204	Gooseberry	1.20	0.41	0.021	0.058	A-G	0.087
	Temperance	1.28	0.49			A-T	0.182
	Artist Point	1.42	0.55			G-T	0.509
Estimated Seeds Per Plant n=170	Gooseberry	128.1	76.52	0.066	0.001	A-G	0.247
	Temperance	120.2	84.80			A-T	<0.001
	Artist Point	160.1	97.23			G-T	0.247

Table 5c.

<i>Euphrasia</i> Trait	Location	Mean	Std. Dev.	Kruskal-Wallis Effect Size	p	Dunn's Test with adj-p	
Height (cm) n=623	Two Harbors	5.82	2.53	0.167	<0.001	A-G	<0.001
	Gooseberry	11.41	5.98			A-T	0.015
	Temperance	10.16	4.34			A-TH	<0.001
	Artist Point	8.89	4.54			G-T	0.417
						G-TH	<0.001
						T-TH	<0.001
Stem Diameter (cm) n=623	Two Harbors	0.63	0.25	0.0634	<0.001	A-G	0.020
	Gooseberry	0.62	0.24			A-T	0.830
	Temperance	0.74	0.28			A-TH	0.020
	Artist Point	0.72	0.29			G-T	0.004
						G-TH	0.858
						T-TH	0.004
Leaves n=623	Two Harbors	20.57	14.17	0.091	0.008	A-G	0.005
	Gooseberry	19.89	12.83			A-T	<0.001
	Temperance	28.42	18.46			A-TH	0.005
	Artist Point	16.65	12.53			G-T	<0.001
						G-TH	0.984
						T-TH	<0.001
Flowered n=623	Two Harbors	0.52	0.50	0.079	<0.001	A-G	0.826
	Gooseberry	0.71	0.45			A-T	0.001
	Temperance	0.91	0.28			A-TH	<0.001
	Artist Point	0.72	0.45			G-T	0.002
						G-TH	0.001
						T-TH	<0.001
Branches n=623	Two Harbors	3.97	2.34	0.048	<0.001	A-G	1
	Gooseberry	2.42	1.32			A-T	<0.001
	Temperance	2.40	1.55			A-TH	1
	Artist Point	2.14	1.66			G-T	<0.001
						G-TH	1
						T-TH	<0.001
Date of First Flower n=93	Two Harbors	206.72	9.00	0.204	<0.001	A-G	<0.001
	Gooseberry	202.18	10.36			A-T	0.006
	Temperance	199.39	8.23			A-TH	<0.001
	Artist Point	195.98	8.45			G-T	0.009
						G-TH	0.007
						T-TH	<0.001
Flower Number n=623	Two Harbors	1.80	1.73	0.098	<0.001	A-G	0.202
	Gooseberry	2.44	1.73			A-T	<0.001
	Temperance	2.86	2.82			A-TH	<0.001
	Artist Point	1.98	1.65			G-T	0.019
						G-TH	<0.001
						T-TH	<0.001

Table 6. Linear selection analysis on populations of *Primula* (6a), *Pinguicula* (6b), and *Euphrasia* (6c) across sites spanning a latitudinal gradient (47°N–48°N). Direct (β_i) and Total Selection (S) on selected traits are shown for each site. Model statistics and sample size are listed for each population. Significant terms are bolded.

Table 6a.

<i>Primula</i>	Trait	$B_i(Direct)$	p	$S(Total)$	p
Gooseberry n = 186	Plant Height	0.19	<0.001	0.77	<0.001
	Rosette Width	-0.05	<0.001	0.11	0.14
	Leaf Number	0.00	0.91	-0.06	0.37
Temperance n = 181	Plant Height	0.22	<0.001	0.91	<0.001
	Rosette Width	-0.04	<0.001	0.28	<0.001
	Leaf Number	0.00	0.79	0.24	0.0001
Artist Point n = 175	Plant Height	0.19	<0.001	0.75	<0.001
	Rosette Width	-0.02	0.07	0.28	0.0001
	Leaf Number	-0.02	0.11	0.22	0.004

Table 6b.

<i>Pinguicula</i>	Trait	$B_i(Direct)$	p	$S(Total)$	p
Gooseberry n = 57	Plant Height	0.13	0.09	0.05	<0.001
	Plant Width	0.00	0.81	0.28	<0.001
	Flowering Date	-0.08	0.08	-0.54	<0.001
	Leaf Number	0.04	0.40	0.31	0.001
Temperance n = 65	Plant Height	0.11	0.01	0.48	<0.001
	Plant Width	0.08	0.03	0.50	<0.001
	Flowering Date	-0.00	0.88	-0.11	0.42
	Leaf Number	0.02	0.61	0.29	<0.001
Artist Point n = 78	Plant Height	0.15	<0.001	0.30	<0.001
	Plant Width	-0.04	0.17	0.13	<0.001
	Flowering Date	-0.02	0.54	0.04	0.04
	Leaf Number	0.00	0.89	0.22	0.003

Table 6c.

<i>Euphrasia</i>	Trait	$B_i(Direct)$	p	$S(Total)$	p
Two Harbors n = 73	Plant Height	-0.03	0.004	0.38	<0.001
	Stem Diameter	0.02	0.09	0.63	<0.001
	Flowering Date	-0.04	0.001	-0.25	0.09
	Leaves	0.07	<0.001	0.91	<0.001
Gooseberry n= 65	Plant Height	0.00	0.75	0.52	<0.001
	Stem Diameter	0.01	0.24	0.48	<0.001
	Flowering Date	0.00	0.64	-0.45	0.12
	Leaves	0.08	<0.001	0.55	<0.001
Temperance n = 101	Plant Height	0.02	0.14	0.52	<0.001
	Stem Diameter	0.04	0.04	0.63	<0.001
	Flowering Date	0.01	0.31	-0.39	<0.001
	Leaves	0.10	<0.001	0.76	<0.001
Artist Point n = 113	Plant Height	0.01	0.20	0.55	<0.001
	Stem Diameter	0.01	0.51	0.56	<0.001
	Flowering Date	0.00	0.84	-0.18	0.27
	Leaves	0.08	<0.001	1.00	<0.001

Table 7. Count-based population viability analysis for *Primula mistassinica* based on two consecutive years (2020 and 2021) of demographic data. Population data was compiled from three permanent 1×1 m plots.

Site	2020 Population	2020 Mortality	2021 Population	λ
Gooseberry	190	0.13	237	1.25
Temperance	192	0.11	239	1.25
Artist Point	177	0.05	230	1.30

Table 8. Count-based population viability analysis for *Pinguicula vulgaris* based on two consecutive years (2020 and 2021) of demographic data. Population data was compiled from three permanent 1×1 m plots.

Site	2020 Population	2020 Mortality	2021 Population	λ
Gooseberry	173	0.01	179	1.03
Temperance	148	0.06	162	1.09
Artist Point	147	0.02	170	1.16



Figure 1. Flowering arctic relicts: *Primula mistassinica* (a), *Pinguicula vulgaris* (b), and *Euphrasia hudsoniana* (c).

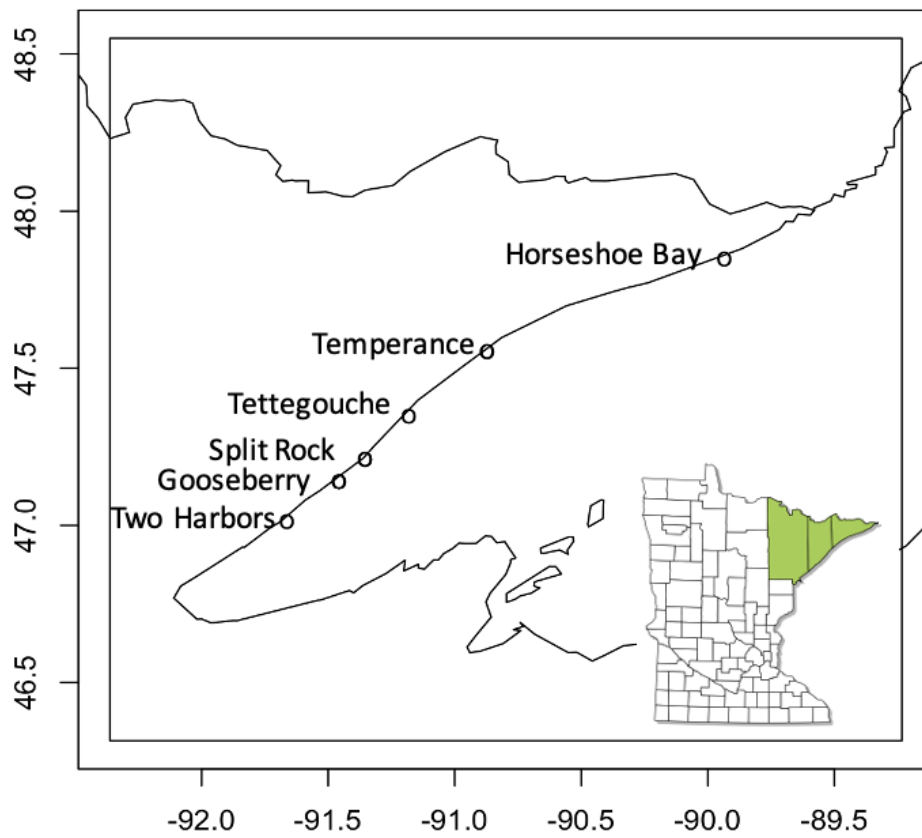


Figure 2. Locations of the nine resurveyed Relevé sites of the arctic relicts studied. Two surveys (and resurveys) were conducted at Gooseberry, Split Rock, and Temperance sites.

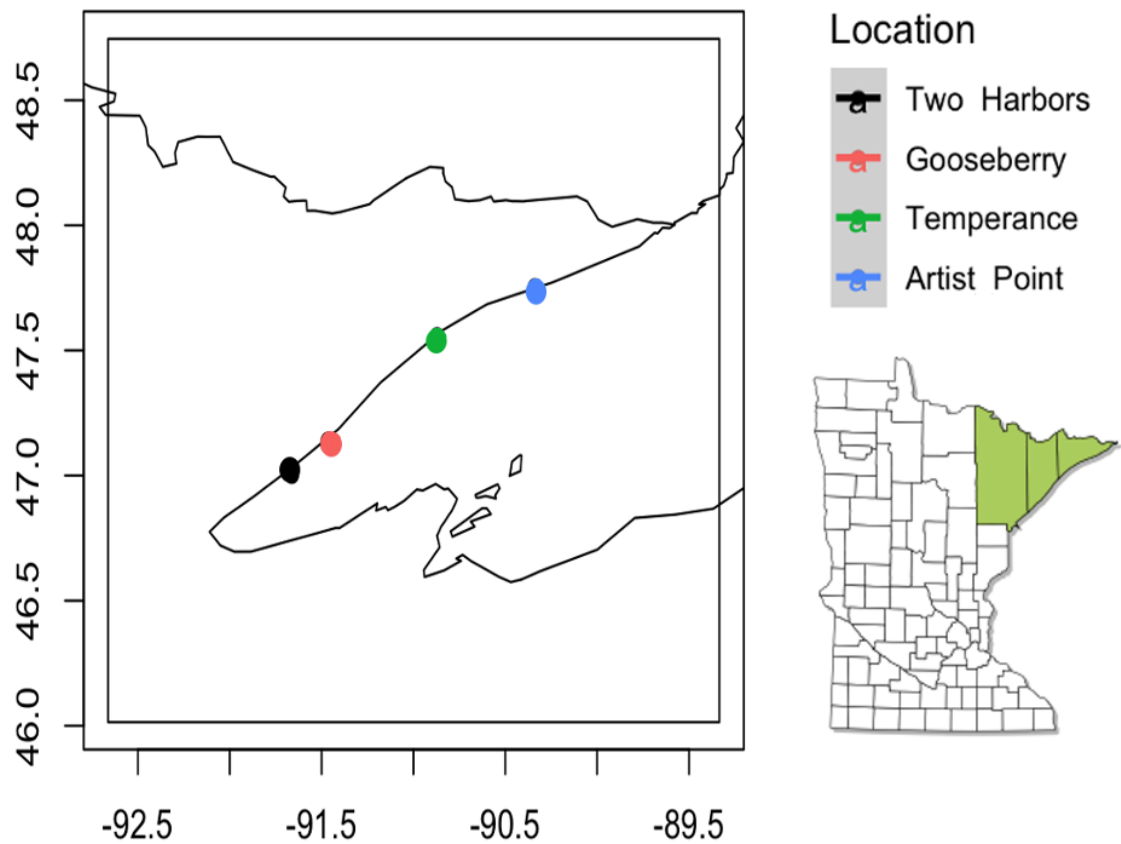


Figure 3. Selected sites for phenotypic selection and population viability analyses. Sites were intentionally selected to span a latitudinal gradient. In 2020, *Pinguicula* and *Primula* populations were measured at three sites: Gooseberry Falls State Park, Temperance River State Park, and Artist Point in Grand Marais. For *Euphrasia hudsoniana*, an additional southern site was located at Two Harbors Lighthouse.

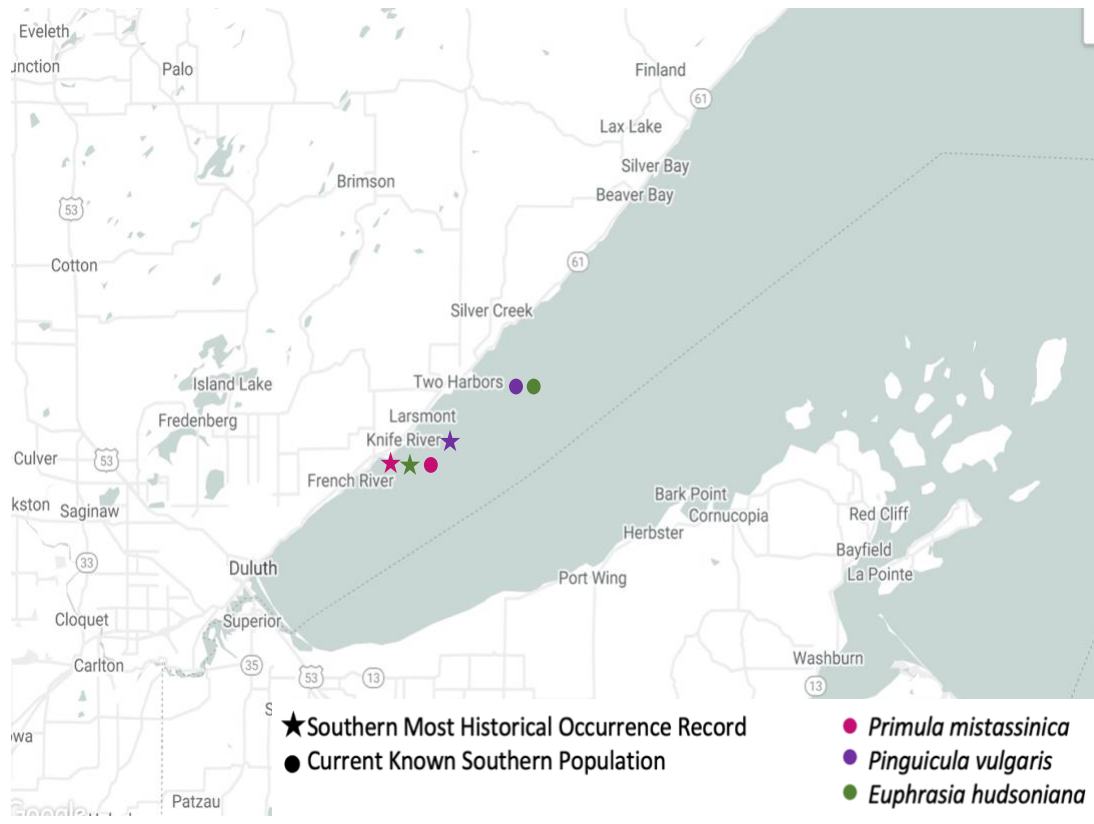


Figure 4. Historical (star) southernmost locations of *Primula mistassinica* (1941), *Pinguicula vulgaris* (1948), and *Euphrasia hudsoniana* (1944) and current (circle) southernmost populations of the same species, as found in 2020.

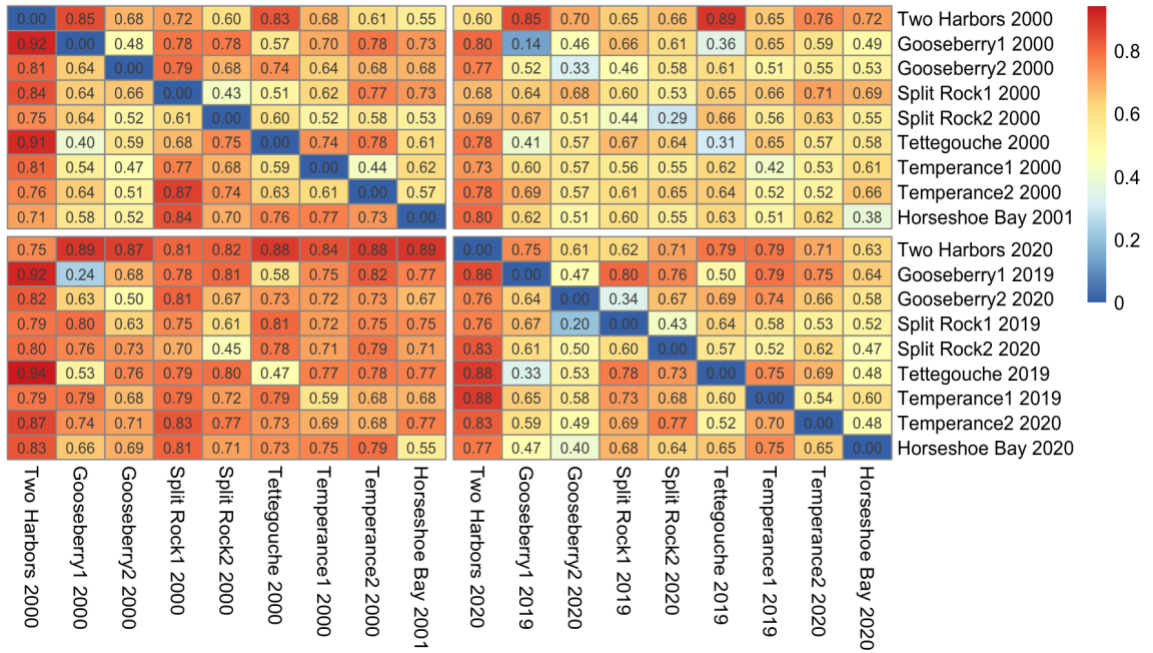


Figure 5. Temporal and spatial dissimilarity matrix with pairwise Bray-Curtis dissimilarity values (above diagonal) and Jaccard dissimilarity values (below diagonal). A value of 0 indicates no difference between community composition between compared sites and a value of 1 indicates complete difference between sites.

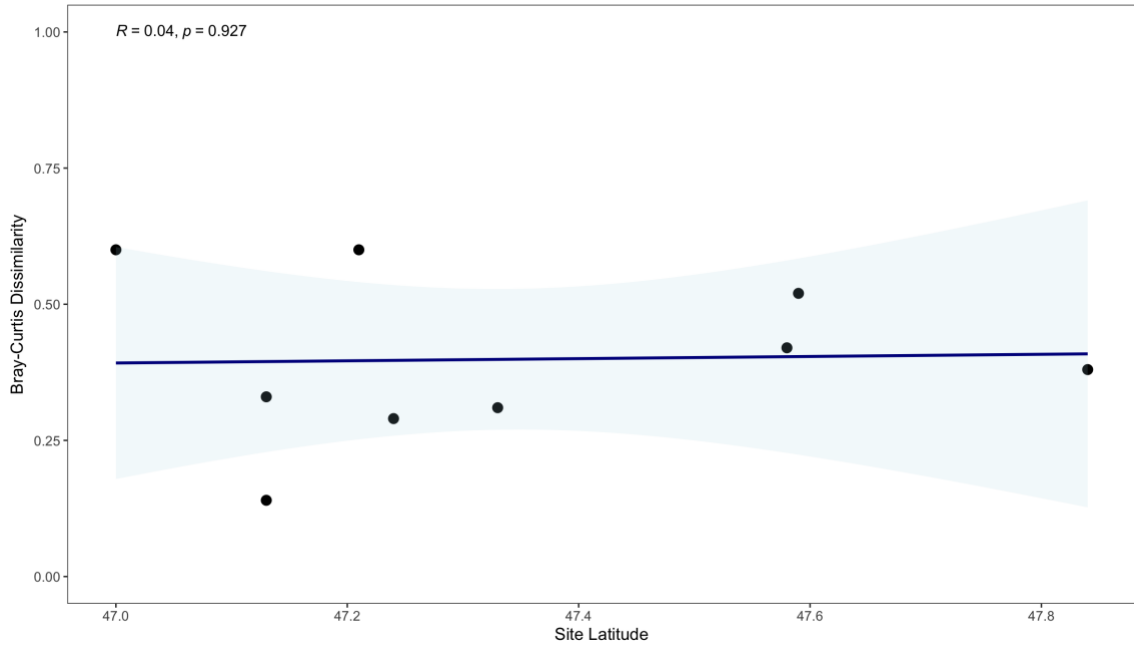


Figure 6. Bray-Curtis dissimilarity values between resurveyed sites (dots) as a function of survey site latitude. Correlation and p-value are shown in upper left corner.

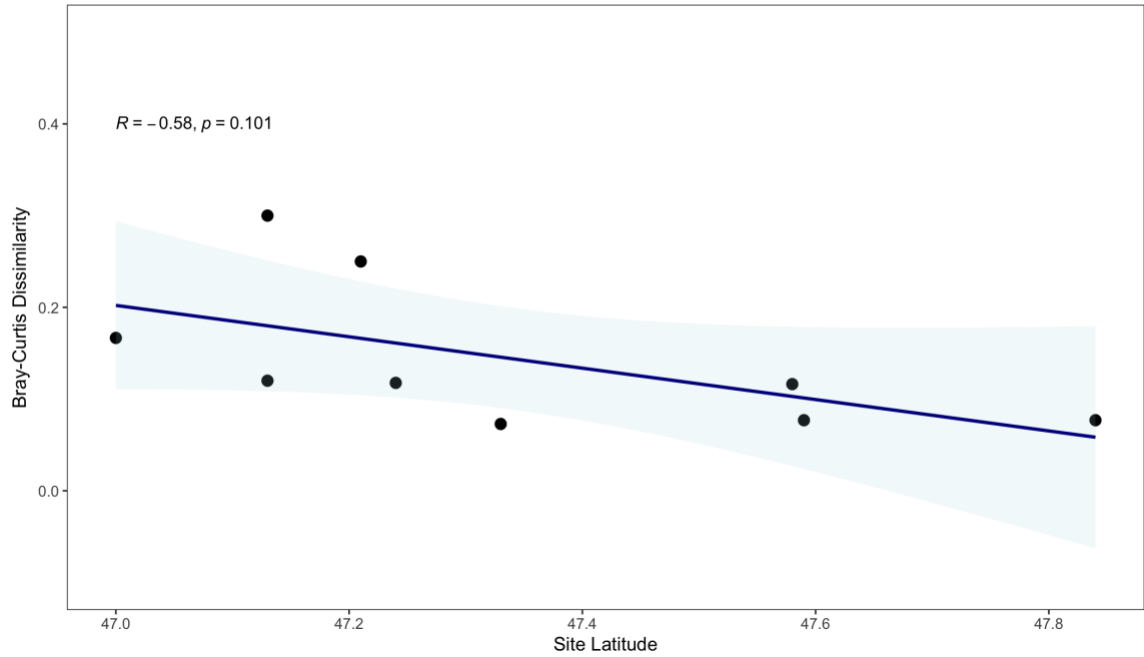


Figure 7. Bray-Curtis dissimilarity values between resurveyed sites (dots), including arctic relicts only, as a function of survey site latitude. Correlation and p-value are shown in upper left corner.

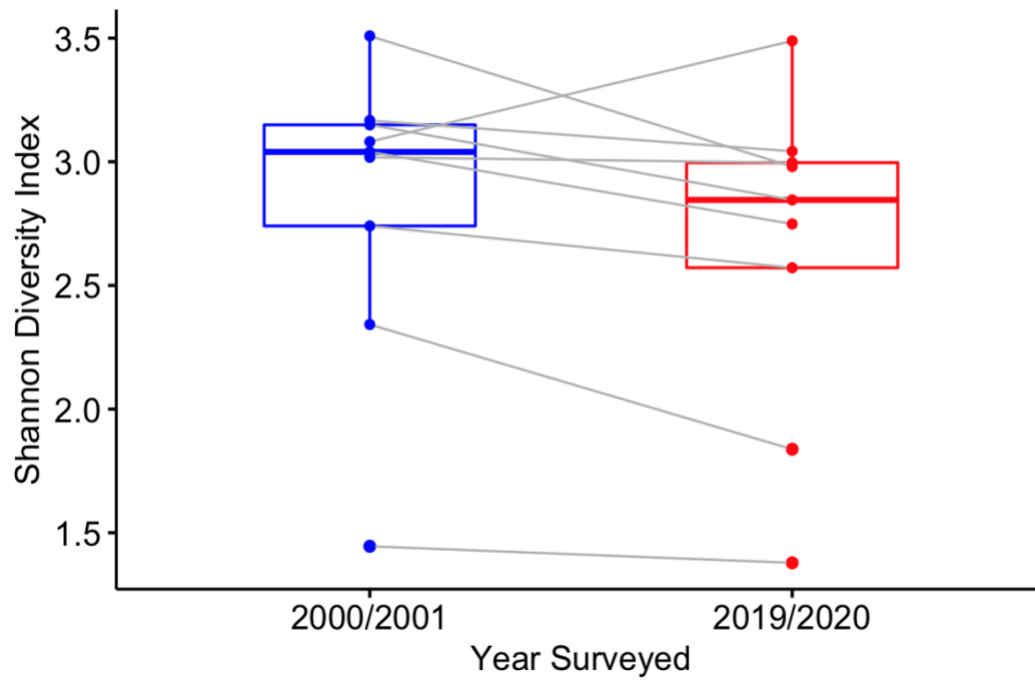


Figure 8. Box plot of temporal change in Shannon Diversity Index of nine surveyed sites. Lines connect historical to modern survey values. The thick horizontal line represents group mean with the box extending to upper and lower quartiles.

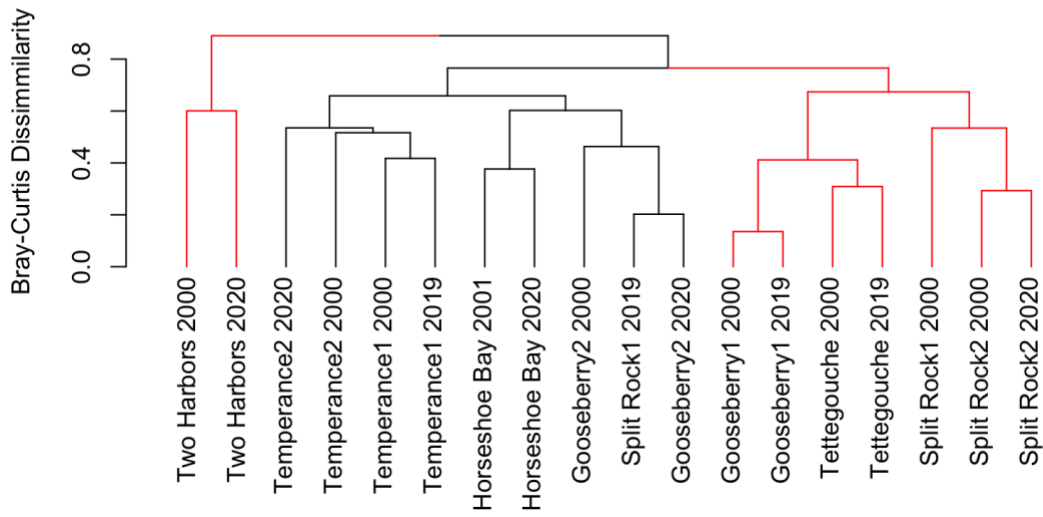


Figure 9. Cluster analysis of all surveyed sites using Bray-Curtis dissimilarity values and complete linkage methods. Color defines broad groupings of northern and southern sites.

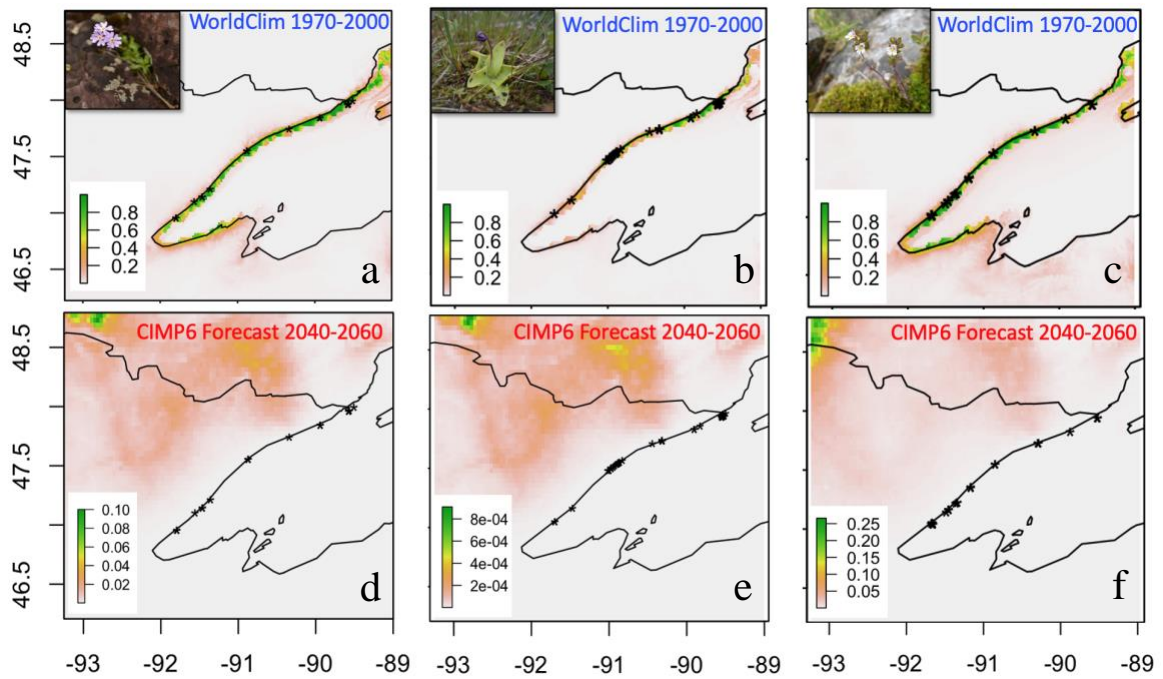


Figure 10. Ecological niche models for *Primula* (a,d), *Pinguicula* (b,e), and *Euphrasia* (c,f). Models (a,b,c) were constructed using species occurrence record locations (asterisks) and selected WorldClim 1970–2000 bioclimatic variables. Future niche models (d,e,f) were made using the same bioclimatic variables from CIMP6 2040–2060 forecast models. Each model includes a habitat suitability legend with model-specific values in the lower left corner.

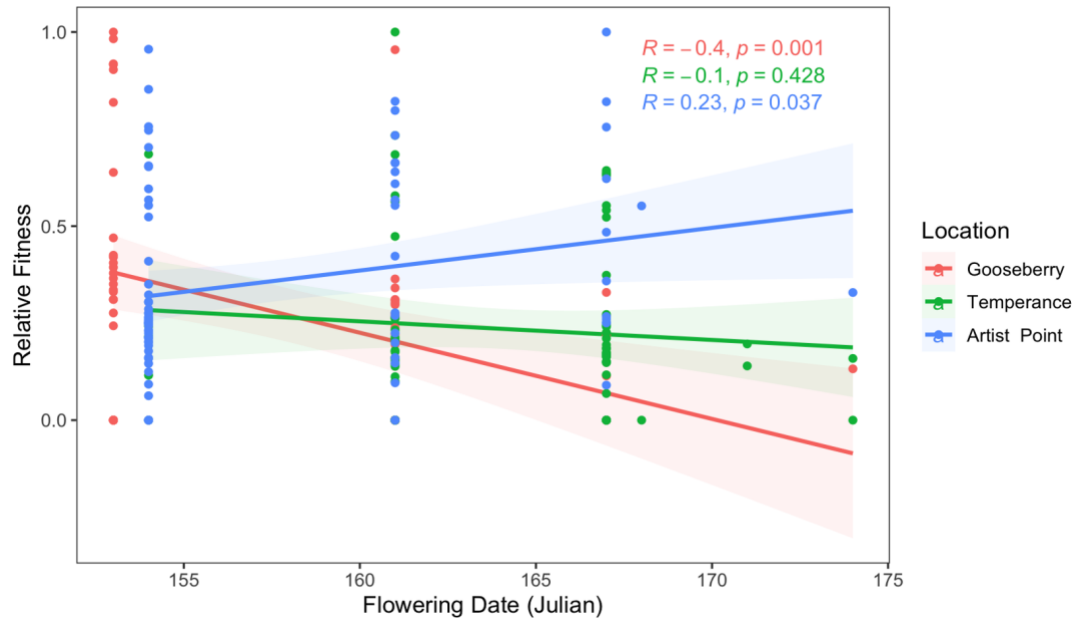


Figure 11. Total linear selection analysis of flowering date in *Pinguicula vulgaris* at three different sites. Dots represent individual values. Correlation and p-values for each site are listed in the upper right corner and 95% confidence levels are shown as the light-colored area surrounding each line.

References

- Baker, R., & Dnr, M. (n.d.). *Minnesota's List of Endangered, Threatened and Special Concern Species*. 18.
- Ballinger, T. J., Overland, J. E., Wang, M., Bhatt, U. S., Hanna, E., Hanssen-Bauer, I., Kim, S.-J., Thoman, R. L., & Walsh, J. E. (2020). *Arctic Report Card 2020: Surface Air Temperature*.
- Becher, H., Brown, M. R., Powell, G., Metherell, C., Riddiford, N. J., & Twyford, A. D. (2020). Maintenance of species differences in closely related tetraploid parasitic Euphrasia (Orobanchaceae) on an isolated island. *BioRxiv*, 2020.04.29.067579.
- Bontrager, M., & Angert, A. L. (2018). Gene flow improves fitness at a range edge under climate change. *BioRxiv*, 399469.
- Bray, J. R., & Curtis, J. T. (1957). An Ordination of the Upland Forest Communities of Southern Wisconsin. *Ecological Monographs*, 27(4), 325–349.
- Bridle, J. R., & Vines, T. H. (2007). Limits to evolution at range margins: When and why does adaptation fail? *Trends in Ecology & Evolution*, 22(3), 140–147.
- Brodie III, E., Moore, A., & Janzen, F. (1995). Visualizing and Quantifying Natural-Selection. *Trends in Ecology & Evolution*, 10, 313–318.
- Cahill, A. E., Aiello-Lammens, M. E., Fisher-Reid, M. C., Hua, X., Karanewsky, C. J., Ryu, H. Y., Sbeglia, G. C., Spagnolo, F., Waldron, J. B., & Wiens, J. J. (2013). Causes of warmedge range limits: Systematic review, proximate factors and implications for climate change. *Journal of Biogeography*, 14.
- Casper, S. J., & Stimper, R. (2009). Chromosome numbers in Pinguicula (Lentibulariaceae): Survey, atlas, and taxonomic conclusions. *Plant Systematics and Evolution*, 277(1), 21–60.
- Caswell, H. (2001). *Matrix Population Models: Construction, Analysis, and Interpretation*. Sinauer Associates. *SERBIULA (Sistema Librum 2.0)*.
- Ceballos, G., Ehrlich, P., Barnosky, A., Garcia, A., Pringle, R., & Palmer, T. (2015). Accelerated Modern Human-Induced Species Losses: Entering the Sixth Mass Extinction. *Science Advances*, 1, e1400253.
- Chadde, S. W. (2019). *Minnesota Flora: An Illustrated Guide to the Vascular Plants of Minnesota* (2nd edition). Orchard Innovations.

- Channon, B., & Heard, L. M. B. (1997). *Guide to a Native Vegetation Survey: Using the Biological Survey of South Australia*. Department of Housing & Urban Development.
- Chapin, F. S., Shaver, G. R., Giblin, A. E., Nadelhoffer, K. J., & Laundre, J. A. (1995). Responses of Arctic Tundra to Experimental and Observed Changes in Climate. *Ecology*, *76*(3), 694–711.
- Chevin, L.-M., Lande, R., & Mace, G. M. (2010). Adaptation, Plasticity, and Extinction in a Changing Environment: Towards a Predictive Theory. *PLOS Biology*, *8*(4), e1000357.
- Cohen, J., Screen, J. A., Furtado, J. C., Barlow, M., Whittleston, D., Coumou, D., Francis, J., Dethloff, K., Entekhabi, D., Overland, J., & Jones, J. (2014). Recent Arctic amplification and extreme mid-latitude weather. *Nature Geoscience*, *7*(9), 627–637.
- Darwin, C. (1862). On the Two Forms, or Dimorphic Condition, in the Species of *Primula*, and on their remarkable Sexual Relations. *Journal of the Proceedings of the Linnean Society of London. Botany*, *6*(22), 77–96.
- Doak, D. F., & Morris, W. F. (2010). Demographic compensation and tipping points in climate-induced range shifts. *Nature*, *467*(7318), 959–962.
- Dyke, A., Moore, A., & Robertson, L. (2003). Deglaciation of North America. In *Geological Survey of Canada*.
- Edwards, C. E. (2015). Looking to the future of conservation genetics: The case for using quantitative genetic experiments to estimate the ability of rare plants to withstand climate change. *American Journal of Botany*, *102*(7), 1011–1013.
- Elith, J., Kearney, M., & Phillips, S. (2010). The art of modelling range-shifting species. *Methods in Ecology and Evolution*, *1*(4), 330–342.
- Feeley, K. J., Bravo-Avila, C., Fadrique, B., Perez, T. M., & Zuleta, D. (2020). Climate-driven changes in the composition of New World plant communities. *Nature Climate Change*, *10*(10), 965–970.
- Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, *37*(12), 4302–4315.
- French, G. C., Ennos, R. A., Silverside, A. J., & Hollingsworth, P. M. (2005). The relationship between flower size, inbreeding coefficient and inferred selfing rate in British *Euphrasia* species. *Heredity*, *94*(1), 44–51.

- Gilmartin, P. M. (2015). On the origins of observations of heterostyly in *Primula*. *New Phytologist*, 208(1), 39–51.
- Given, D. R. (1981). *The arctic-alpine element of the vascular flora at Lake Superior*. Ottawa: National Museums of Canada.
- Global Assessment Report on Biodiversity and Ecosystem Services*. (2019, May 17). IPBES Secretariat.
- Gratani, L. (2014). Plant Phenotypic Plasticity in Response to Environmental Factors. *Advances in Botany*, 2014, e208747.
- Guggisberg, A., Mansion, G., Kelso, S., & Conti, E. (2006). Evolution of biogeographic patterns, ploidy levels, and breeding systems in a diploid–polyploid species complex of *Primula*. *New Phytologist*, 171(3), 617–632.
- Hampe, A., & Jump, A. S. (2011). Climate Relicts: Past, Present, Future. *Annual Review of Ecology, Evolution, and Systematics*, 42(1), 313–333.
- Hampe, A., & Petit, R. J. (2005). Conserving biodiversity under climate change: The rear edge matters. *Ecology Letters*, 8(5), 461–467.
- Harrison, S., Spasojevic, M. J., & Li, D. (2020). Climate and plant community diversity in space and time. *Proceedings of the National Academy of Sciences*, 117(9), 4464–4470.
- Kelso, Sylvia. 2020. *Primula*. Flora of North America North of Mexico, Provisional Publication. Flora of North America Association. November 5, 2020. fna.huh.harvard.edu/files/Pittosporaceae.pdf. Accessed [7/19/2021].
- Kremers, K. S., Hollister, R. D., & Oberbauer, S. F. (2015). Diminished Response of Arctic Plants to Warming over Time. *PLOS ONE*, 10(3), e0116586.
- Lande, R., & Arnold, S. J. (1983). The Measurement of Selection on Correlated Characters. *Evolution*, 37(6), 1210–1226. JSTOR.
- Legendre, L. (2000). The genus *Pinguicula* L. (*Lentibulariaceae*): An overview. *Acta Botanica Gallica*, 147(1), 77–95.
- Leroy, B., Meynard, C. N., Bellard, C., & Courchamp, F. (2016). Virtualspecies, an R package to generate virtual species distributions. *Ecography*, 39(6), 599–607.
- Lesica, P., & McCune, B. (2004). Decline of Arctic-Alpine Plants at the Southern Margin of Their Range Following a Decade of Climatic Warming. *Journal of Vegetation Science*, 15(5), 679–690. JSTOR.

- Levin, D. A., Francisco-Ortega, J., & Jansen, R. K. (1996). Hybridization and the Extinction of Rare Plant Species. *Conservation Biology*, *10*(1), 10–16. JSTOR.
- Liebst, B., & Schneller, J. J. (2005). How selfing and intra- and interspecific crossing influence seed set, morphology and ploidy level in *Euphrasia*: An experimental study of species occurring in the Alps of Switzerland. *Plant Systematics and Evolution*, *255*, 193–214.
- Lotts, K. C., Waite, T. A., & Vucetich, J. A. (2004). Reliability of Absolute and Relative Predictions of Population Persistence Based on Time Series. *Conservation Biology*, *18*(5), 10.
- Mason, S. C., Palmer, G., Fox, R., Gillings, S., Hill, J. K., Thomas, C. D., & Oliver, T. H. (2015). Geographical range margins of many taxonomic groups continue to shift polewards. *Biological Journal of the Linnean Society*, *115*(3), 586–597.
- Menges, E. S. (2000). Applications of Population Viability Analyses in Plant Conservation. *Ecological Bulletins*, *48*, 73–84.
- Milbau, A., Vandeplas, N., Kockelbergh, F., & Nijs, I. (2017). Both seed germination and seedling mortality increase with experimental warming and fertilization in a subarctic tundra. *AoB Plants*, *9*(5), plx040.
- Minnesota Department of Natural Resources. 2013. A handbook for collecting vegetation plot data in Minnesota: The relevé method. 2nd ed. Minnesota Biological Survey, Minnesota Natural Heritage and Nongame Research Program, and Ecological Land Classification Program. Biological Report 92.
- Moore, A. (2000). *North Shore Land Use Issues: The Real Costs of Growth*. 5.
- Mueller-Dombois, D., & Ellenberg, H. (1974). Aims and methods of vegetation ecology. In *John Wiley and Sons*.
- Nadeau, C. P., & Urban, M. C. (2019). Eco-evolution on the edge during climate change. *Ecography*, *42*(7), 1280–1297.
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2020). *vegan: Community Ecology Package (2.5-7)* [Computer software].

- O'Reilly, C. M., Sharma, S., Gray, D. K., Hampton, S. E., Read, J. S., Rowley, R. J., Schneider, P., Lenters, J. D., McIntyre, P. B., Kraemer, B. M., Weyhenmeyer, G. A., Straile, D., Dong, B., Adrian, R., Allan, M. G., Anneville, O., Arvola, L., Austin, J., Bailey, J. L., ... Zhang, G. (2015). Rapid and highly variable warming of lake surface waters around the globe. *Geophysical Research Letters*, *42*(24), 10,773-10,781.
- Parmesan, C., & Hanley, M. E. (2015). Plants and climate change: Complexities and surprises. *Annals of Botany*, *116*(6), 849–864.
- Pearson, G. A., Lago-Leston, A., & Mota, C. (2009). Frayed at the edges: Selective pressure and adaptive response to abiotic stressors are mismatched in low diversity edge populations. *Journal of Ecology*, *97*(3), 450–462.
- Phillips, S., Dudík, M., & Schapire, R. (2004). *A Maximum Entropy Approach to Species Distribution Modeling*. 21.
- Phillips, S. J., Anderson, R. P., & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, *190*(3), 231–259.
- Post, E., Steinman, B. A., & Mann, M. E. (2018). Acceleration of phenological advance and warming with latitude over the past century. *Scientific Reports*, *8*(1), 3927.
- Schlaepfer, D. R., Braschler, B., Rusterholz, H.-P., & Baur, B. (2018). Genetic effects of anthropogenic habitat fragmentation on remnant animal and plant populations: A meta-analysis. *Ecosphere*, *9*(10), e02488.
- Schlichting, C. D. (1986). The Evolution of Phenotypic Plasticity in Plants. *Annual Review of Ecology and Systematics*, *17*, 667–693.
- Sell, P. D., & Yeo, P. F. (1970). A revision of the North American species of *Euphrasia* L. (Scrophulariaceae). *Botanical Journal of the Linnean Society*, *63*(3), 189–234.
- Sheth, S. N., & Angert, A. L. (2018). Demographic compensation does not rescue populations at a trailing range edge. *Proceedings of the National Academy of Sciences*, *115*(10), 2413–2418.
- Stubben, C., & Milligan, B. (2007). Estimating and Analyzing Demographic Models Using the popbio Package in R. *Journal of Statistical Software*, *22*(1), 1–23.
- Swart, N. C., Cole, J. N. S., Kharin, V. V., Lazare, M., Scinocca, J. F., Gillett, N. P., Anstey, J., Arora, V., Christian, J. R., Hanna, S., Jiao, Y., Lee, W. G., Majaess, F., Saenko, O. A., Seiler, C., Seinen, C., Shao, A., Sigmond, M., Solheim, L., ... Winter, B. (2019). The Canadian Earth System Model version 5 (CanESM5.0.3). *Geoscientific Model Development*, *12*(11), 4823–4873.

- Thomas, C. D., Bodsworth, E. J., Wilson, R. J., Simmons, A. D., Davies, Z. G., Musche, M., & Conradt, L. (2001). Ecological and evolutionary processes at expanding range margins. *Nature*, *411*(6837), 577–581.
- van Kleunen, M., Weber, E., & Fischer, M. (2010). A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters*, *13*(2), 235–245.
- Villellas, J., Doak, D. F., García, M. B., & Morris, W. F. (2015). Demographic compensation among populations: What is it, how does it arise and what are its implications? *Ecology Letters*, *18*(11), 1139–1152.
- Vucetich, J. A., & Waite, T. A. (2003). Spatial patterns of demography and genetic processes across the species' range: Null hypotheses for landscape conservation genetics. *Conservation Genetics*, *4*(5), 639–645.
- Wiens, J. J. (2016). Climate-Related Local Extinctions Are Already Widespread among Plant and Animal Species. *PLoS Biology*, *14*(12), e2001104.
- Zefferman, E., Stevens, J. T., Charles, G. K., Dunbar-Irwin, M., Emam, T., Fick, S., Morales, L. V., Wolf, K. M., Young, D. J. N., & Young, T. P. (2015). Plant communities in harsh sites are less invaded: A summary of observations and proposed explanations. *AoB PLANTS*, *7*(plv056).
- Zettlemoyer, M. A., & Peterson, M. L. (2021). Does Phenological Plasticity Help or Hinder Range Shifts Under Climate Change? *Frontiers in Ecology and Evolution*, *0*.
- Zlonis, K. J., & Gross, B. L. (2018). Genetic structure, diversity, and hybridization in populations of the rare arctic relict *Euphrasia hudsoniana* (Orobanchaceae) and its invasive congener *Euphrasia stricta*. *Conservation Genetics*, *19*(1), 43–55.