Population Genetics and Population History of *Moehringia macrophylla*, a Rare and Endangered New England Serpentine Plant

by

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Dedicated to Laura Palumbo, whose warmth, unfailing kindness, and curiosity I will always carry with me.

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TABLE OF CONTENTS

LIST OF FIGURES.......... i
LIST OF TABLES.......... ii
ABSTRACT.......... iii
INTRODUCTION.......... 1
METHODS.......... 9
RESULTS.......... 15
DISCUSSION.......... 22
WORK CITED.......... 30

LIST OF FIGURES

Figure 1. Gel electrophoresis image of *M. macrophylla* and *M. lateriflora* samples....12
Figure 2. Polished billets from *M. macrophylla* sample sites....15
Figure 3. Petrographic microscopy of rock samples from *M. macrophylla* sample sites.... 16
Figure 4. DAPC plot of clustered genetic populations.... 20
Figure 5. sNMF plots for K=2 through K=5....21

LIST OF TABLES

Table 1: Average FIS values per population.. 17
Table 2: Number of private alleles per population...18
Table 3: Average Pi value of nucleotide diversity per population......................... 19

ABSTRACT

As climate change intensifies, habitats around the world are shifting, shrinking and growing. An understanding of species distribution is important as they must adapt and move in response to these changes. The ability to perform genetic research has expanded greatly in recent years, allowing researchers to sequence whole genomes, compare populations, and perform a wide variety of analyses. I worked with the species *Moehringia macrophylla*, a small flowering plant that grows on rocky outcrops and cliff crevices throughout New England. Its habitat is one usually considered to be harsh and uninhabitable for most other plant species, yet *M. macrophylla* has found its home there. *M. macrophylla* also makes for a rich study system because it follows a disjunct distribution pattern across the continent, with large uninhabited areas between habitats and a patchy distribution within the regions it does inhabit. I've used methodology of population genetics to dive into the makeup of *M. macrophylla* and pick apart questions of genetic diversity, population structure, and isolation. I've also investigated the geological habitat of sample locations to determine the edaphic pressures *M. macrophylla* is living in. I'm interested in how these methods can be used in the context of focused conservation efforts and land use decisions, and what these results may indicate for the adaptation of *M. macrophylla*.

INTRODUCTION

As climate change induces range shifts across plant species, loss of both inter-specific and intra-specific diversity is a concern for species persistence (Exposito-Alonso et al. 2022, Abhari et al. 2024). Knowledge of species' adaptive potential, genetic makeup and population size can complement effective conservation efforts. Analyzing genetic diversity patterns, population structure, isolation, and gene flow can provide a basis of understanding the adaptive potential of the species as well as provide a better understanding of environmental pressures on the population's survival.

Study Species

This thesis is an exploration into the genetics and habitat of New England populations of *Moehringia macrophylla* (Hook.) Fenzl*,* a delicate flowering plant that grows on edaphically harsh serpentine outcrops. *M. macrophylla'*s patchy distribution, unique edaphic survival, and disjunct biogeography make this species a rich study system. *M. macrophylla* is a member of the plant family

Caryophyllaeae and is known commonly as largeleaf or bigleaf sandwort. *M. macrophylla* is only found in North America and it is native to multiple disjunct, or disconnected, regions throughout the continent, notably in large regions of western North America, and in smaller areas within the Great Lakes region and eastern North America ("Moehringia macrophylla (Hook.) Fenzl" n.d.). The continent-wide disjunct geographic distribution is in itself an interesting puzzle of historical biogeography, and within New England its distribution is patchy and fragmented. This species is rare and endangered in New England, classified as "Level 2: A Regionally Rare Taxa" and listed as endangered in both Connecticut and Massachusetts though not in Vermont (Brumback et al. 1996, Flora of North America Editorial Committee 2005, "Large-leaved Sandwort | Mass.gov" n.d., "Serpentine Outcrop | Vermont Fish & Wildlife Department" n.d.). Because there are so few sites of *M. macrophylla* in New England, even the more robust populations may be at risk especially due to mining or other human disturbance (Ruesink 2003).

Within the eastern North American region, populations of *M. macrophylla* are patchy. They may have little ability to shift or expand their range if they are restricted to the island-like niches that they occupy, whether that's due to ecotypic differentiation, competition, or restrictions to a broader distribution. The rare and endangered status of this species presents a risk to its future persistence and survival, especially if populations show low genetic diversity and high isolation. The dispersal mechanism for other species of *Moehringia* is thought to be mainly by ants due to eliasome presence on the seeds, which is a common indicator of myrmecochory, and ant presence at plant sites (Casazza et al. 2008). This may lead to even lower gene flow between populations, and thus further impact the adaptive potential of this species.

Because eastern North American populations of *M. macrophylla* are often small and physically isolated, they may be at particular risk to climate change since smaller populations tend to harbor less genetic diversity than large populations (Frankham et al. 2004). This is compounded by how fragmented these populations are, whether that is due to competition on non-serpentine substrates or limits of the species' habitat tolerance (Frankham et al. 2004). Being able to examine the genetic diversity between and within populations can provide a better idea of *M. macrophylla'*s adaptability to a shifting climate. This pattern of physically isolated populations may be due to a physiological need for particular edaphic conditions, or could be due to increased competition on more welcoming substrates. In this thesis I am examining the gene flow and genetic overlap between these seemingly isolated New England populations by comparing to *Moehringia lateriflora,* a species that is sister to *M. macrophylla* but has a widespread and continuous distribution throughout the globe. I am also examining the shared ancestry of *M. macrophylla* populations through population structure analyses.. Past research on species that occupy similar disjunct patterns between western North America, the Great Lakes region and eastern North America has hypothesized that their disjunction could be due to a formerly widespread range undergoing extirpation, with populations persisting in glacial refugia during the Pleistocene (Drummond et al. 2022)

Beyond understanding the impacts that the patchy and fragmented distribution of populations has, I investigated the substrate that *M. macrophylla* grows on in order to contribute to a foundational understanding of why these distribution patterns may exist. Records from different states indicate that the habitats *M. macrophylla* occupies may not be uniform throughout the region. In CT the species is found in cold pockets of algific talus slopes, without mention of particular substrates, while MA inventory records indicate that the species is exclusive to serpentine outcrops (Mehrhoff 1989, Weatherbee and Crow 1990). Vermont records indicate that *M. macrophylla* is consistently found on serpentine outcrops, as well as some records on dunite or more broadly in talus areas (Zika and Dann 1985, “Serpentine Outcrop | Vermont Fish & Wildlife Department” n.d.).

Serpentine

Although it is a more broadly used term, serpentine at its most technical refers to a mineral composition of $Mg_3Si_2O_5(OH)_4$ in a sheet structure, mainly seen as chrysotile, lizardite, and antigorite (Coleman and Jove 1991). It is formed from olivine-rich peridotite as it moves from the mantle to the crust and undergoes the hydrothermal process of hydration when coming in contact with seawater (Coleman and Jove 1991). Rocks that are composed mainly of the mineral form of serpentine are referred to as serpentinite (Moores 2011). In general usage, it is a term used to refer to outcrops and soils where serpentine is present and influencing the ecology and geology of the area or habitat (Rajakaruna et al. 2009). Given its occurrence with peridotite and other rocks serpentine is commonly found in, it is often referred to as mafic or ultramafic. The soils derived from ultramafic rocks are notably high in magnesium and nickel, low in essential plant nutrients such as nitrogen and potassium, and contain low Ca:Mg ratios (Harrison et al. 2004, Brady et al. 2005).

The presence of serpentine environments and their associated endemic species is widespread throughout the globe, with hotspots found in California, Cuba, New Caledonia, Turkey, Japan, and beyond (Brooks 1987, Harrison et al. 2004, Kawase et al. 2009). In North America, much of the noted serpentine outcrops and regions are found throughout the west coast, with ample evidence of many endemic species and local adaptation unique to serpentine conditions (Rajakaruna et al. 2009, Wright and Stanton 2011). As such, the bulk of the North American studies and information on serpentine ecology are conducted in the western regions of North America, and the geoecology of serpentine habitats in eastern North America remains relatively understudied despite documentation of outcrops throughout much of the region (Rajakaruna et al. 2009).

Serpentine environments are uninhabitable to most plants due to the harsh edaphic conditions. The species found in serpentine environments have adapted in a variety of ways to be able to grow there, and many of the species are endemic to serpentine outcrops and soils. The identified stressors for serpentine plant life are both chemical and physical. The main area of adaptation needed by serpentine-tolerant species is the ability to withstand low Ca:Mg ratios in the soil, specifically low calcium content that is amplified by the negative effect of Mg on Ca availability (Brady et al. 2005). Serpentine tolerant species are hypothesized to be adapted to this in a variety of mechanisms, notably an avoidance of Mg toxicity, a Mg requirement, or the ability to hyperaccumulate metals (Brady et al. 2005). Physically harsh conditions include shallow soils and high drought impact, an aspect that is likely compounded by the effects of climate change (Damschen et al. 2011).

In Eastern North America, serpentine outcrops form a belt that stretches from as far south as Alabama along the Appalachians to Newfoundland and Labrador (Brooks 1987). While New England records indicate that *M. macrophylla* grows on serpentine, there is no public record of how this was determined and habitat descriptions are often lacking specifics. It is possible that *M. macrophylla* grows on non-serpentine rocks in a region that has been broadly defined as containing serpentine outcrops or grows on both serpentine and non-serpentine rocks or soils. It is important to determine whether this species does grow on serpentine in New England, as this would inform its conservation and be another step in uncovering its range limits and edaphic tolerances.

Purpose

With further study into *M. macrophylla* population genetics and habitat, I hope to contribute to the information needed for conservation and continuing study of this unique species and habitat. This project was split into two sections. The first and most substantial part was uncovering the population-level genetics of *M. macrophylla* in New England, which may be able to inform conservation and land use decisions. The second was to assess whether this species is growing on serpentine in MA and VT in order to better understand the edaphic conditions it can tolerate, and contribute to future research *M. macrophylla's* adaptations and distributions.

Investigating Population Genetics and Population History

Population genetics can be particularly useful for understanding the distribution and interactions of rare and endangered species, as well as fragmented or isolated populations (Turchetto et al. 2016, Rose and Sytsma 2023). A comparative analysis of genetic structure and diversity among populations of *M. macrophylla* in New England can offer insight to both historical and current relationships between populations and uncover potential limitations to this species' persistence in the future. These analyses can also inform future persistence and viability of these species, particularly in response to climate and land use changes. Due to limited dispersal via ants and the large distances between populations, I hypothesized that there is limited gene flow between populations leading to high genetic differentiation. Given how isolated these populations are, I expected that there is low genetic diversity within populations due to high levels of inbreeding, though this may be complicated by the presence of historical genetic diversity if rates of clonal reproduction are high.

Investigating Serpentine Presence in Massachusetts and Vermont

Due to the current lack of information on *M. macrophylla* habitat and substrate, I contextualized my genetic research with analyses of substrate at *M. macrophylla* locations. This is an important factor to consider given the specific adaptability needed to grow in serpentine environments. Because of this informational gap, the second goal of my thesis was to perform mineral analyses on rock samples from *M. macrophylla* locations to identify whether or not serpentine is present in the outcrops *M. macrophylla* inhabit.

METHODS

Sampling

Leaf samples of *M. macrophylla* were collected by Dr. Chloe Pak Drummond in 2025 and samples were silica dried. Seven samples were collected from one site in CT, 10 samples were collected from two sites in MA, and 44 samples were collected from seven sites in VT. Five samples of *Moehringia lateriflora,* a sister species of *M. macrophylla*, were collected from one site in MA. These samples are labeled as OUT since I am using them as an outgroup in genetic analyses. Due to non-disclosure agreements and the rare status of these species, I am not able to disclose specific site locations. Rock samples were also collected from two sites in MA and three sites in VT near leaf collection sites.

Rock Analysis

I worked with Dr. Claire Pless to visually analyze the rock samples. Using a rock saw, I opened the samples and cut the rocks into standard slide-sized billets that included as much of the non-weathered rock as possible. I then ground and polished the billets up to a 1200 grit and attached the polished surface to slides with epoxy. I cut the billet from the slide and ground the thin section down to ~30 µ thick. Dr. Pless and I then analyzed the thin sections under different angles of polarized light using a petrographic microscope and compared the light refractions to standard color samples using a refraction index.

DNA Extraction and Quality Control

DNA was extracted from tissue samples during the summer and fall of 2025. Extractions were performed with the Qiagen DNeasy Plant Mini Kit according to manufacturer protocol, and eluted with 100µL of nuclease-free water. DNA purity was tested using a NanoDrop spectrophotometer and DNA quantity was tested using a Qubit assay reader.

Sequencing

To ensure that these DNA samples could be digested with restriction enzymes, a process needed for sequencing, I tested the samples for digestion using *EcoRI* to check that there were no inhibitors for enzyme activity (Figure 1). Samples were sent to the Biotechnology Center at the University of Wisconsin-Madison for double-digest RAD sequencing (ddRADseq), which is a reduced-representation genomic sequencing approach (Peterson et al. 2012). Given that *M. macrophylla* is likely a polyploid, and because there is not a reference genome to work with, I chose to specifically have samples sequenced using ddRADseq. This process is a modification of RADseq with two main changes. In ddRADseq a second restriction enzyme digest is added and genomic fragments are selected for sequencing based on specific size. These steps ensure a specific set of DNA fragments are sequenced and that the same loci are sequenced across all samples, which is particularly helpful for allele determination in polyploids (Peterson et al. 2012). My 67 samples were barcoded during library preparation in order to be pooled and multiplexed during sequencing on the NovaSeq Illumina platform. The samples were sequenced paired-end at 2x150, aiming for ~3.7 million reads per sample.

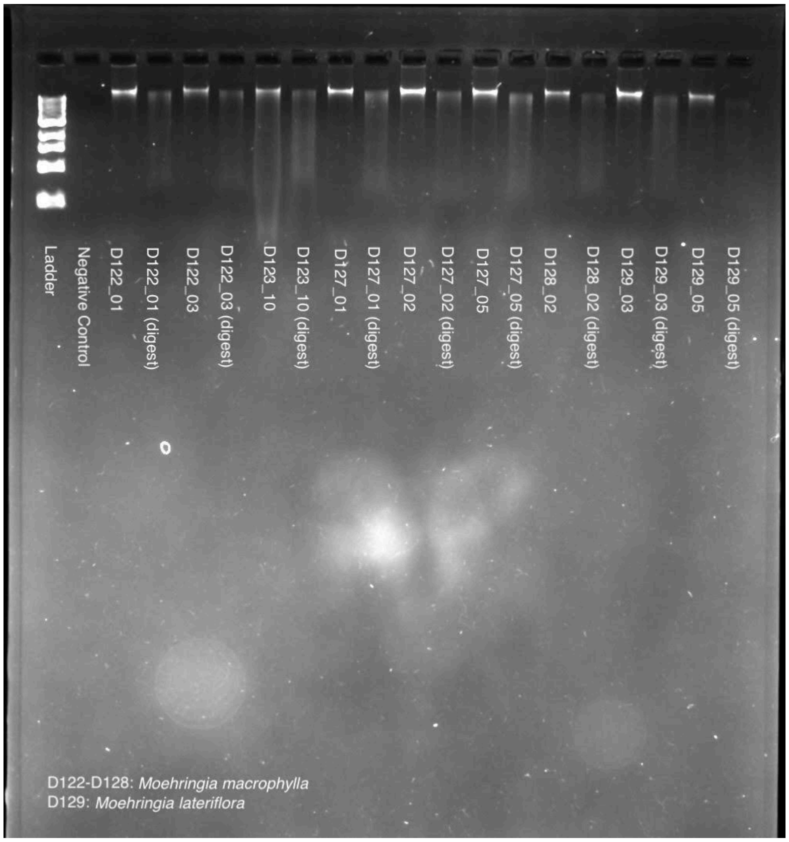


Figure 1. Gel electrophoresis image of *M. macrophylla* and *M. lateriflora* samples both digested and undigested.

Data Assembly

Read data was received from UWBC with Illumina sequencing adapters removed. Using the Unity cluster at University of Massachusetts Amherst, which has parallelizing capability and sufficient memory, I processed my data using Cutadapt and the *de novo* Stacks 2.0 pipeline (Martin 2011, Catchen et al. 2011, 2013). Since I was assembling my sequence data *de novo*, without a reference, I tested different values of the assembly parameters to determine the optimal parameters for my dataset. I followed the assembly optimization process proposed in Paris et al (Paris et al. 2017). I used the pipeline components cutadapt, ustacks, cstacks, sstacks, tsv2bam, gstacks, and populations. I tested the minimum number of reads to seed a stack between 2 and 8 (referred to as *m*), and originally determined 4 to be the most ideal based on plotting. With the minimum number of reads set to 4, I tested the maximum distance in nucleotide differences allowed to cluster putative alleles in a stack (referred to as *M*) between 2 and 8 with a filtering of 75%. Across all M values, there was a substantial drop in the number of loci retained, which diverged from expected procedure. Because of this, I continued with an *m* value of 3 which aligns with suggested protocol when read depth is substantial enough, which is true for this set of data (Paris et al. 2017). I used an *M* value of 1, since there was little increase in the number of polymorphic loci between a value of 1 and 2. I also opted to filter at 50% instead of 75%, which combined with the above alterations resulted in more expected loci retention.

M. macrophylla is understood to be a polyploid species, specifically a tetraploid in which 2n=48 (Löve 1982). Standard genotype calling methods are typically designed to interpret data of diploid organisms. In the case of Stacks, I specified that there were 4 alleles per locus instead of 2 to account for this (Bourke et al. 2018, Salvado et al. 2022).

Data Analyses

I calculated F_{IS}, number of private alleles, and nucleotide diversity in the populations component of the Stacks pipeline. In order to infer admixture within my data I used sNMF (Sparse Nonnegative Matrix Factorization), by running the sNMF function within the “LEA” R package (Frichot et al. 2014, Frichot and François 2015). I chose to run an sNMF because of its ability to work with polypoid data, as well as its shorter run times when computing large datasets compared to the more commonly used program STRUCTURE (Salvado et al. 2022). After running the sNMF function I plotted the cross-entropy criterion against possible K values, or ancestral populations, in order to determine which K value was a good fit for this data. After multiple runs, I found a K value of 3 or 4 to be a consistent elbow point, or a low point before a peak. I plotted K values of 2 through 5 to get a broader look at population structure. I chose the run with the lowest cross-entropy for the chosen K value. For a K value of 3, which was the lowest, the best run had a cross-entropy of 0.2577802.

When deciding on a multivariate analysis approach, I opted to perform a Discriminant Analysis of Principle Components (DAPC). I chose to use DAPC due to its focus on between-group variability, lack of reliance on Hardy-Wienberg equilibrium, and its noted use in previous polypoid studies (Jombart et al. 2010, Dufresne et al. 2014). A DAPC is optimized when the most appropriate number of clusters in the data is represented, and those cluster alignments are maximized. I chose to retain all ~60 PCs from the find.cluster function, and to retain 5 clusters based on the BIC plot. I chose the number of clusters, or genetically similar groupings, based on each value's interaction with Bayesian Information Criterion (BIC). I chose to work with 4 clusters because that was the minimum number preceding a sharp increase in BIC values, as outlined by Jombart et al (2010). After plotting the Eigenvalues of this analysis, I chose to keep the first 4 discriminant functions.

RESULTS

Rock Analysis

Polished billet sections immediately showed similarity to serpentine due to the coloration, physical structure, and softness of the rock (Figure 2). Thin sections under 400x petrographic microscopy showed alignment to reference images of serpentine, and fell under the first order of coloration under cross polarized light (Figure 2). The samples also showed cleavage and the fibrous texture that is indicative of typical serpentine structure (W. A. Deer, R. A. Howie, J. Zussman 1967, Schmidt 2023). Through this imaging I was able to infer serpentine presence in all samples, with varying amounts of weathering.

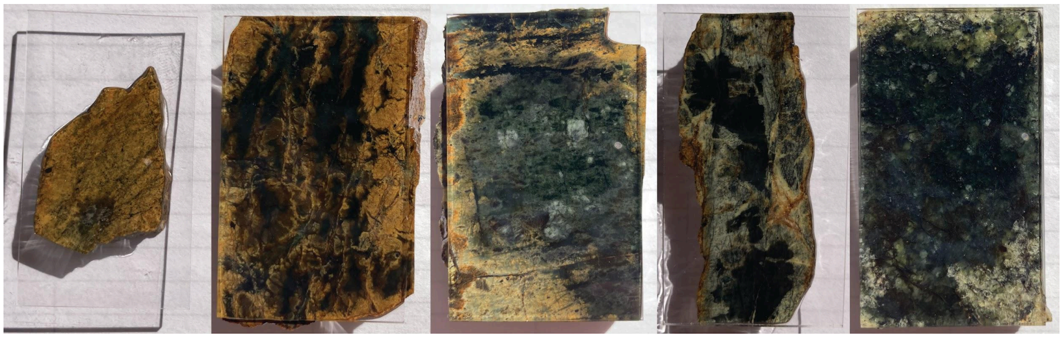


Figure 2: Polished billets from Moehringia macrophylla sample sites (from left to right) MA1, MA2, VT1, VT7, and VT6.

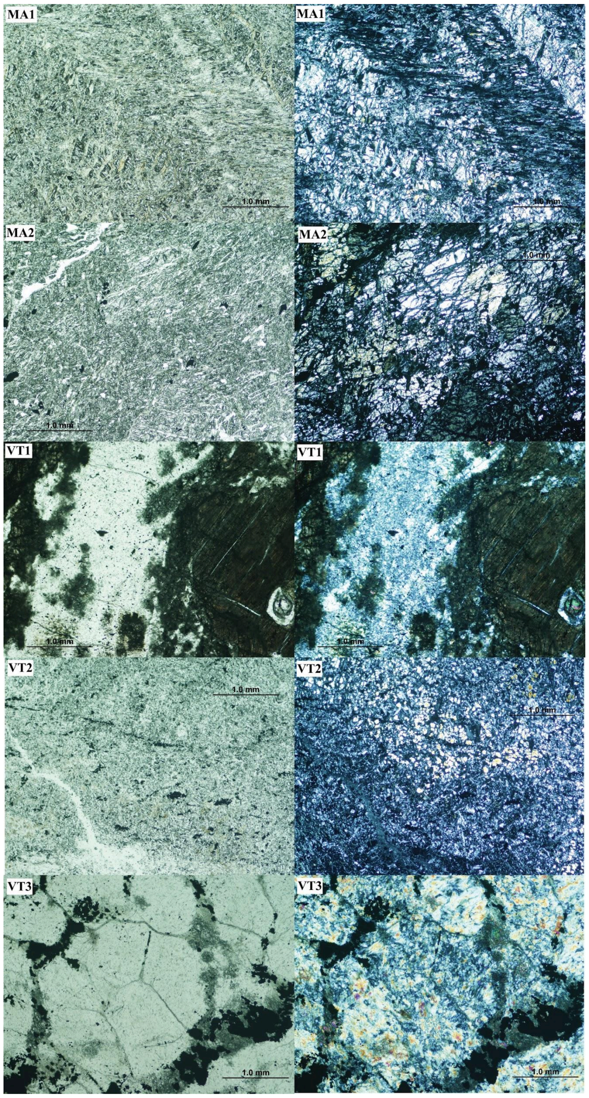
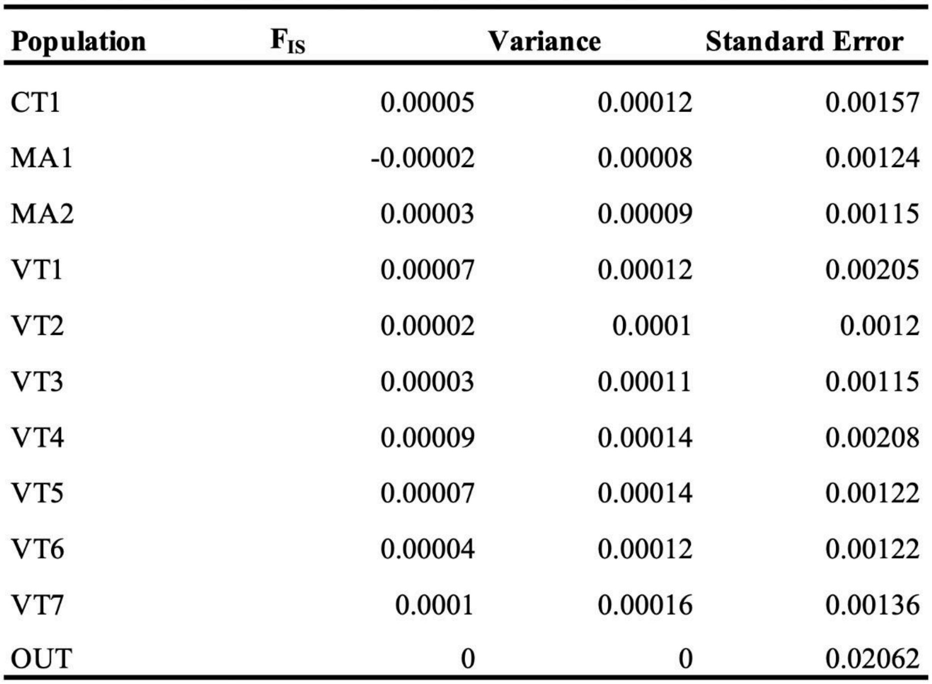


Figure 3: Approximately 30µ thick sections of rock samples from Moehringia macrophylla sample sites (MA1 and MA2 are from 2 different sites in Massachusetts and VT1-3 are from 3 different sites in Vermont) under a petrographic microscope at 400x. Images on the left column are under plane polarized light. Images on the right column are under cross polarized light. Scale bars are present on images.

Genetic Diversity Analyses

All *M. macrophylla* populations except for MA1 exhibit positive average F_{IS} values (Table 1). With one exception, the *M. macrophylla* populations are orders of magnitude greater than the *M. lateriflora* population.

Table 1: Average F_{IS} values per population



Population	F_{IS}	Variance	Standard Error
CT1	0.00005	0.00012	0.00157
MA1	-0.00002	0.00008	0.00124
MA2	0.00003	0.00009	0.00115
VT1	0.00007	0.00012	0.00205
VT2	0.00002	0.0001	0.0012
VT3	0.00003	0.00011	0.00115
VT4	0.00009	0.00014	0.00208
VT5	0.00007	0.00014	0.00122
VT6	0.00004	0.00012	0.00122
VT7	0.0001	0.00016	0.00136
OUT	0	0	0.02062

The number of private alleles in *M. macrophylla* populations were higher than that of the *M. lateriflora* population, particularly VT1 and VT4 (Table 2).

Table 2: Number of private alleles per population

Population	**Private Alleles**
CT1	189
MA1	146
MA2	155
VT1	304
VT2	143
VT3	134
VT4	305
VT5	138
VT6	141
VT7	142
OUT	1

All *M. macrophylla* populations had lower average nucleotide diversity than the *M. lateriflora* population (Table 3).

Table 3: Average Pi value of nucleotide diversity per population.

Population	π	Variance	Standard Error
CT1	0.00028	0.0001	0.00002
MA1	0.00023	0.00009	0.00002
MA2	0.00024	0.0001	0.00002
VT1	0.00026	0.00009	0.00001
VT2	0.00026	0.00011	0.00002
VT3	0.00025	0.0001	0.00002
VT4	0.00029	0.0001	0.00001
VT5	0.0003	0.00012	0.00002
VT6	0.00027	0.00011	0.00002
VT7	0.00031	0.00013	0.00002
OUT	0.00055	0.00018	0.00055

Population Structure Analyses

The DAPC analysis suggested the strongest clustering at K=5, and showed clustering of populations VT1-VT3 as well as populations VT4-VT7. CT1, MA1, and MA2 all showed more distinct grouping separate from other populations (Figure 3). *M. lateriflora* was clustered with the VT4-VT7 grouping.

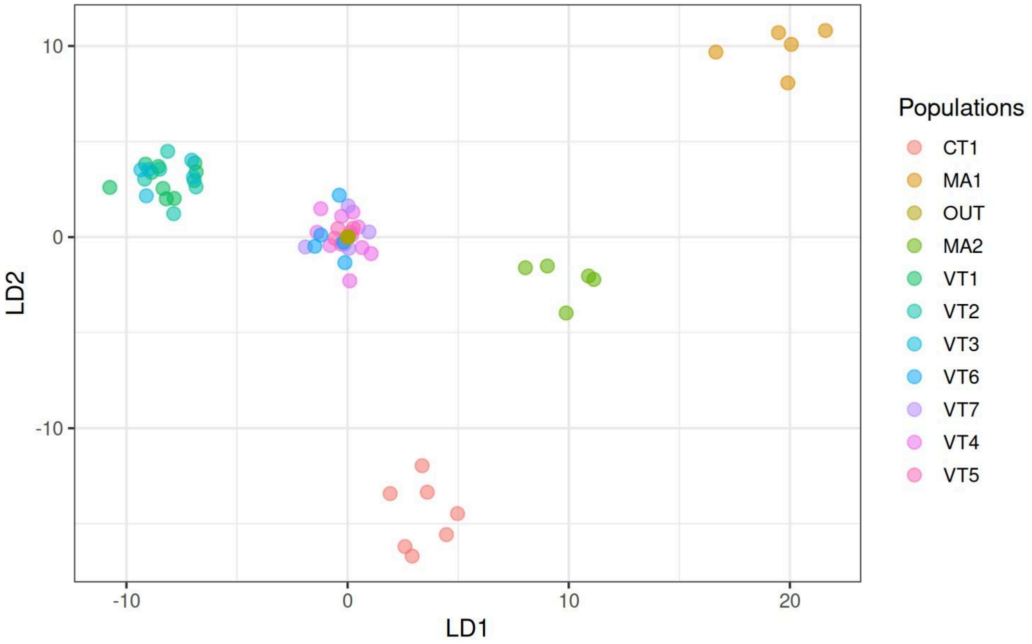


Figure 4: DAPC plot of clustered genetic populations, colored by geographic populations.

The sNMF analysis included K values of 2 through 5, which were all similarly acceptable values across repetitions as inferred by their low cross entropy values, hence why I am reporting on all 4 values. The plotting of sNMF showed consistent coloration throughout individual columns. Geographic populations are not clustered completely separate from each other, but CT1 shared ancestry with MA1 and MA2 shared ancestry with VT5.

Figure 5: sNMF plots for K=2 through K=5. Columns show individuals, grouped by population. Colors represent shared genetic ancestry when grouped in different numbers of clusters, or K values.

DISCUSSION

Population Genetic Analyses

The results from these genetic analyses suggest that New England populations of *M. macrophylla* have lower nucleotide diversity, higher numbers of private alleles, and higher levels of inbreeding when compared to *M. lateriflora.* This supports my hypotheses of genetic isolation between populations of *M. macrophylla*. Low genetic diversity as a result of isolated populations may be a concern for the adaptive potential of these populations, especially considering the rare and endangered status of *M. macrophylla* in New England. In addition to the fragmented and patchy distribution of *M. macrophylla* populations, their small population sizes may also be a contributing factor to their isolation and inbreeding.

An F_{IS} analysis shows the ratio of variance between an individual and the population it is a part of, and can be an indicator of genetic drift within populations (Weir and Cockerham 1984, Kanaka et al. 2023). The positive results of the F_{IS} analysis for all but one of the *M. macrophylla* populations indicate some excess amount of homozygosity that departs from Hardy Weinberg equilibrium. Within *M. macrophylla* populations, VT4 has the highest F_{IS} value followed by VT5 and VT1. MA1 is an outlier within *M. macrophylla* populations due to its negative F_{IS} value. While sometimes referred to as the “inbreeding coefficient”, a positive F_{IS} value on its own does not guarantee inbreeding as the sole reason for this departure from HWE as a whole. Within the context of this thesis, with other diversity measures and population structure analyses also accounted for, we can see that the F_{IS} results align with a common theme of isolated and inbred populations. For further research, a comparison of these values to F_{IS} values of other population groups would create a clearer image of how isolation across populations and regions may differ. I would be particularly interested in how these results compare to F_{IS} results from populations in Western North America where *M. macrophylla* is more abundant or from the Great Lakes region where records do not indicate serpentine as a substrate where *M. macrophylla* is found.

Private alleles refer to alleles that are only present in one population of those sampled. This can indicate levels of gene flow between populations, with a higher number of private alleles indicating less gene flow and more isolation. The high amount of private alleles in *M. macrophylla* populations indicate low amounts of gene flow. VT4 and VT1 have the highest number of private alleles, which is consistent with the patterns of F_{IS} values between populations. There is no clear geographic pattern that indicates one group of populations are more isolated than another geographic grouping. The lack of private alleles in the *M. lateriflora* population is surprising given that it is being compared to the populations of another species.

The two analyses discussed so far mostly infer information about the populations' relationships with each other. While this is useful information to have, I also wanted to look at the diversity within individual populations by measuring nucleotide diversity. In addition to providing insights into within-population diversity, this is also a clearer comparison between species since the analysis does not rely on comparisons between populations when compared to private alleles. All *M. macrophylla* samples show lower diversity compared to the *M. lateriflora* population which is consistent with my hypotheses and the results of the previous analyses.

The DAPC analysis shows differentiation that aligns with geographic populations. Two clusters contain multiple VT populations, suggesting that those populations have gene flow within that smaller geographic region. Both CT and MA populations remain distinct from VT populations. The *M. lateriflora* samples showed complete overlap with the most central cluster of *M. macrophylla.* This is surprising given that they are from separate species, but consistent with the low number of private alleles in the *M. lateriflora* population. This could be due to nuances of evolutionary history such as recent speciation events or human error such as contamination.

To interpret the sNMF analyses, I chose to focus on the K value of 5 given that it shows the most detail while still being an acceptable K value. The sNMF plot shows shared ancestry across all populations, although some differentiation is apparent. While there is some overlap of shared genetic ancestry between populations, the specific columns (representing individuals) are rarely combinations of separate ancestry groupings. The K values of 2-5 indicate admixture, or shared ancestry indicating that these populations are not completely isolated from each other. It's important to note that I am not able to infer if this shared ancestry is a result of ongoing admixture, a snapshot of historical diversity that has stayed consistent due to heightened clonal reproduction, or some combination of the two.

M. macrophylla is a species that reproduces both sexually and asexually through rhizomatic shoots. This may lead to a higher level of genetic diversity even without higher gene flow between populations due to the retention of historical diversity through cloning.

These analyses suggest that gene flow and genetic diversity is limited, confirming the genetic effects of a patchy and fragmented population distribution. Increased isolation and reduced genetic diversity can indicate the risk of reduced adaptive potential. To further understand the risks facing these populations, further methods of diversity and structure analysis should be pursued. Analyses like isolation by distance and phylogenies would provide a better image of the structure and divergence of these populations. Similarly to the diversity analyses, I would be interested in a structural analysis that compares multiple regions of *M. macrophylla* which would provide more context for the results of New England populations. A phylogenetic analysis, especially one that contains multiple regions of *M. macrophylla* populations would also be revealing. As climate change continues, the suitable habitat of species continues to shift. This may be confounded by any restriction to particular outcrops or edaphic conditions.

Distribution

The North American disjunct distribution of *M. macrophylla* follows a pattern present in other species. The locally disjunct populations in New England are hypothesized to be relics of glacial refugia (Drummond et al. 2022). This is supported by *M. macrophylla's* presence in cold microclimates of rock crevices and talus slopes. I suggest that this hypothesis may be complicated by the harsh edaphic conditions that *M. macrophylla* grows in. Serpentine outcrops are uncommon and discontinuous in New England, making it additionally unusual that it would be the habitat of *M. macrophylla* purely as a result of its microclimate (Brooks 1987, Rajakaruna et al. 2009). While I do not have mineral analyses for all New England populations of *M. macrophylla*, the 5 population sites I did sample from have serpentine present even in areas where this has not been previously noted. Harsh or low fertility soils can be a refuge from competition if the species is tolerant of the edaphic conditions in a way competing species may not be (Damschen et al. 2019). This can impact how these populations respond to increasing climate change since shifts in the competitive balance between species can be more impacted in a habitat that is less commonly suitable (Damschen et al. 2019).

Future experimental studies that look at competition dynamics would further our understanding of why *M. macrophylla* is found in this disjunct pattern as well as on serpentine. Additionally, studies that include population sampling from across the species' entire North American range would provide a more complete understanding of the biogeography of this species. For example, a phylogenetic analysis that included an estimate of timing could shed light on when populations diverged from each other and would be especially interesting if it included more regions beyond New England populations. The literature around *M. macrophylla* is still limited in other regions, so further research into its habitat in a broader view would provide great context. I'm particularly interested in whether populations in western North America and the Great Lakes region have similar patterns of occupying cold microclimates, and if they are growing on serpentine specifically. Populations in Minnesota are recorded as growing on rocky outcrops without particular mention of serpentine ("Moehringia macrophylla" n.d.). West coast populations are recorded as present in a broader range of habitats, including mention of ultramafic presence but encompassing wetlands and meadows as well as rocky areas without particular mention to serpentine ("Moehringia macrophylla - Burke Herbarium Image Collection" n.d., "Moehringia macrophylla - Calflora" n.d.). Populations growing clearly off of serpentine may provide a useful comparison, especially when investigating possible serpentine adaptation of *M. macrophylla.* West coast populations are not classified as rare or endangered, which could also provide a useful comparison to New England populations.

Climate Change and Conservation

The status of *M. macrophylla* as a rare and endangered plant in New England presents a need for conservation, especially when faced with the growing impacts of climate change. Understanding the isolation and diversity of these populations, especially if more research is done on adaptive potential, may be a basis for more focused efforts and further conservation research. Serpentine as a confirmed substrate for some populations may also be a piece of conservation planning. Since serpentine outcrops are a unique but not a well studied habitat type in New England, further research into the conservation needs and protection of these ecosystems may contribute to positive conservation efforts.

Limitations

Much of my limitations with this project result from how understudied *M. macrophylla* is within the literature, and as such the lack of information I am able to build on. Particularly relevant is the lack of information around *M. macrophylla's* potential adaptation to serpentine as well as information about the habitat and substrate that *M. macrophylla* is present on throughout its wider distribution throughout North America.

Because this study is only comparing New England populations, I am not able to compare these results to that of populations that have continuous distributions, are confirmed to be found on other substrates, or otherwise provide a contrasting group of populations. I hope that this research can be continued with further sampling and analysis.

Further Research

As mentioned previously, more widespread sampling across the continent would be a key next step in this research, and allow for a much greater context in which to interpret the results I have been able to obtain. Geologic research at more sample sites and in other regions of *M. macrophylla* presence would also add to this context.

Experimental studies like reciprocal transplant and common garden experiments could provide information on the adaptation or plasticity of *M. macrophylla* (Wright and Stanton 2011).

Conclusions

With this thesis, I hope to provide a first step towards further research of *M. macrophylla* in New England. I want to emphasize the unique risk that this species is facing in regards to climate changes and land use changes. I hope this can emphasize the importance of understanding and caring for unique edaphic habitats such as serpentine outcrops, and unique species such as *M. macrophylla.*

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