Ecological Niche Differentiation of the *Adiantum pedatum* complex in the Green Mountains

Morgan W. Southgate

Abstract

How do substrate preference and other ecological factors impact the distribution and interaction of closely related species? I pursued answers to this question through study of the *Adiantum pedatum* complex, a clade of maidenhair ferns that exhibit a complex distribution pattern in northeastern North America directly tied to the geological chemistry of the region. Whereas *A. pedatum* is widely distributed in calcium-rich woodlands, *A. aleuticum* and *A. viridimontanum* are both restricted to serpentine-influenced substrates in northern VT and southern Quebec. *A. viridimontanum*, though a serpentine endemic, is a tetraploid hybrid lineage derived from a sterile hybrid between the maidenhair ferns *A. aleuticum* and *A. pedatum*, which are diploid. This reticulate evolution, uniting ecologically disparate ancestors, leads to the hypothesis that the two serpentine maidenhair ferns are ecologically isolated from one another as a result of inherent hybrid ecological intermediacy, competitive exclusion due to interspecific competition, or a combination of the two. To determine if the ecological niches of *A. aleuticum* and *A. viridimontanum* are differentiated from one another, multiple populations of all three species of maidenhair ferns were located in 10 field sites across Vermont. Their ecological niches were characterized in terms of light availability, topography, soil properties, and natural community type. Soil samples were submitted to the UVM Soil Lab for detailed analysis and flow cytometry was used to verify field identifications. Ecological data were analyzed using principal components analysis and discriminant function analysis, in order to identity the factors most important in distinguishing the ecological niches of the three maidenhairs. Initial results suggest that the ecological niche of the hybrid maidenhair fern is intermediate between those of its progenitors, supporting the hypothesis that the niches of the two serpentine maidenhair ferns are ecologically differentiated from one another.

Introduction

Plant distribution results from a complex interplay between the abiotic and biotic ecological factors that characterize the landscape in which a plant lineage evolves. The presence of a particular plant in a given location reflects the convergence of ecological conditions in which the plant is physiologically able to live. The range of ecological conditions that different plants are able to inhabit varies widely. Some plants are generalists, able to grow in a wide range of habitats, whereas other plants are specialists, restricted to a narrow range of habitats. These habitats vary in abiotic conditions such as temperature, precipitation, topography, light availability, and bedrock and soil type. Biotic variables also define the habitat in which plants grow; these variables include relations with mycorrhizal fungi and other soil microbes, as well as with animals that act as pollinators, seed dispersers, and herbivores. Additionally, plants interact with one another, acting as hosts, competitors, parasites, and facilitators.

Species distribution is also constrained by the different reproductive biologies of the major lineages of land plants. The distribution of flowering plants is influenced to some degree by their relations with pollinators and seed dispersers, because the ranges of the co-evolutionary partners must overlap to some degree for the plant to continue to reproduce successfully. In contrast, fern distribution largely reflects the distribution patterns of climatic and edaphic factors, without the additional complication of co-evolutionary relationships to consider (Barrington 1993). Fern spores are dispersed by the wind, which obviates the necessity of co-evolution for dispersal with insects and other animals (Barrington 1993). As ferns lack associated pollinators and dispersers, the number of ecological factors to consider in analyzing evolutionary history is reduced, making patterns of distribution reduced, making patterns of distribution in terms of abiotic ecological conditions easier to discern.

Study System

The present study focuses on the ecology and distribution of the *Adiantum pedatum* complex, a clade of maidenhair ferns (Paris & Windham 1988, Lu et al. 2011) in northeastern North America. This clade is the northern-most element of the diverse genus *Adiantum,* with a distribution extending across North America to eastern Asia and the Himalayas (Paris & Windham 1988). In North America there are three species of the complex represented; they inhabit remarkably different habitats. The diploid maidenhair fern *Adiantum pedatum* L. (the northern maidenhair fern) is widely distributed in calcium-enriched forests of northeastern North America. *Adiantum aleuticum* (Rupr.) Paris (the western or Aleutian maidenhair fern), also a diploid, is found on serpentine bedrock and serpentine-derived forests in both western and northeastern North America; in the East it is restricted to these substrates, whereas in the West it has a wider substrate tolerance. The tetraploid species *Adiantum viridimontanum* Paris (the Green Mountain maidenhair fern), is endemic to serpentine-derived substrates in northern Vermont and southern Quebec (Paris 1991) with a small outlying population in the central portion of the Maine coast. The systematic relationships of these three species was clarified by Paris & Windham (1988), who established *A. pedatum* and *A. aleuticum* as separate diploid species. Their discovery of a fertile tetraploid hybrid lineage derived from the two widespread diploid species led to the recognition of *A. viridimontanum* by Paris (1991).

Figure 1: The three members of the *Adiantum pedatum* complex in North America. From left to right, *A. aleuticum* (West Farman Hill, VT), *A.viridimontanum* (Belvidere Mines, VT), and *A. pedatum* (Elmore State Park, VT). Growing with *Campanula rotundifolia*, *Populus balsalmifera*, and *Caulophyllum thalictroides*

Biogeography

The biogeographical history of the *Adiantum pedatum* complex in North America is relevant to the origin of the allotetraploid lineage *A. viridimontanum.* The work of Paris & Windham (1988) confirmed that *A. aleuticum* is disjunct between western and eastern North America. The disjunct distribution likely resulted from migration of this taxon from western to eastern North America during glacial retreat in the Holocene, followed by extirpation of the populations in central North America and restriction to serpentine in the far eastern portion of its range (Barrington & Paris 2007). Presumably after the migration of *A. aleuticum* to eastern North America, hybridization with *A. pedatum* yielded one or more sterile diploids which gave rise to the fertile tetraploid species *A. viridimontanum* (Paris 1991; Barrington & Paris 2007). The origin of *A. viridimontanum* was a sympatric speciation event, as speciation occurred within the same geographic area made possible by the reproductive isolation following from the origin of the polyploid. The number of times that the allotetraploid has originated from a sterile hybrid between the two diploid maidenhairs remains unknown.

There is the potential for multiple and even ongoing hybridization events between the two diploid maidenhairs *Adiantum aleuticum* and *A. viridimontanum*, as their distributions overlap in some places where the allopolyploid is also found. The formation of hybrid species between ecologically disparate progenitors is made possible when the different habitat types are contiguous (Barrington 1993). Thus the allotetraploid lineage may be relatively young, as the conditions necessary for formation of the sterile diploid hybrid are still in existence.

More direct evidence for the recent origin of *Adiantum viridimontanum* is seen in three ways: restricted geographic range, the presence of multivalents in meiotic sporocytes, and duplicated gene loci (Paris & Windham 1988). Restricted geographic range of *A. viridimontanum* may indicate recent origin because old polyploids commonly surpass the ranges of their diploid progenitors (Stebbins 1940). Paris & Windham (1988) found that ten of the 13 loci examined in the course of their analysis were still producing multiple isozymes, while two loci were producing the isozyme of the serpentine progenitor. As duplicated gene loci are diagnostic of polyploid species of recent origin (Haufler & Soltis 1986), the many duplicate loci present in the genome of the allotetraploid maidenhair provides evidence that it a relatively young lineage (Paris & Windham 1988). Paris & Windham (1988) also observed the presence of multivalents at meiosis in the allotetraploid, providing evidence that the process of chromosomal diploidization is in an early stage due to recent polyploidization (Paris & Windham 1988).

Morphology

The genus *Adiantum* is characterized by the presence of false indusia, in which the sori are sub-marginal and borne abaxially on the recurved lobes of the frond margin (Paris 1991). Members of the *A. pedatum* complex are easily recognized by their pseudo-pedate leaf architecture; the leaf is pinnate but through continuous elaboration of the basal pinnae the fronds appear to have a pedate leaf design (Paris 1991). The three species of the *A. pedatum* complex in northeastern North America vary in pinnule and leaf morphology. The leaves of *Adiantum pedatum* are usually arching, while those of *A. aleuticum* are held more stiffly erect. The allotetraploid *A. viridimontanum* is intermediate between its progenitors in frond orientation (Paris 1991).

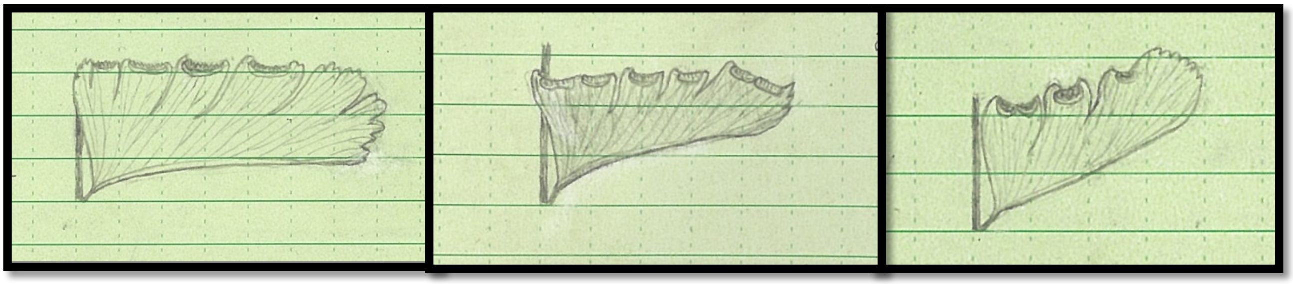

Figure 2: Field sketches of the medial- ultimate segment morphology of the three species. From left to right, 2a) A. pedatum (MS 101), 2b) A. viridimontanum (MS 149), A. aleuticum (MS 156). Ultimate segments drawn at 2x life size, and each grid is 1 x 1 cm.

The position of the pinnule relative to the blade of the leaf varies both ecotypically and by species in the *Adiantum pedatum* complex. Whereas the pinnules of *A. pedatum* lie within the plane of the leaf blade, the pinnule rotation of *A. aleuticum* and *A. viridimontanum* exhibits ecotypic variation. When growing in exposed sites, both serpentine maidenhair ferns tend to have stiffly erect fronds with small ultimate segments twisted on their stalks, but when occupying shadier habitats their fronds tend to be more arching with larger ultimate segments that lie within the plane of the blade (Paris 1991). The ecotypic variation superimposed on these three similar species explains the numerous historical disagreements regarding the classification of maidenhair species in northeastern North America (Fernald 1905, Cronquist et al. 1972, Cody 1983, Lellinger 1985).

Despite the morphological complexity of the species constituting the *Adiantum pedatum* complex, characteristics of the medial ultimate segments can be diagnostic for identification (Paris 1991). The medial ultimate segments of *A. pedatum* are oblong, usually less than 3.2 times as long as broad, with narrow incisions in the acroscopic margin and rounded apices (Figure 2a). The medial ultimate segments of *A. aleuticum* are longer, usually more than 3.2 times as long as broad, with deep incisions on the acroscopic margin, false indusia mostly less than 3.5 mm in length, and petiolules shorter than 0.9 mm (Figure 2c). The pinnules of *A. viridimontanum* are usually more or less triangular, with false indusia mostly longer than 3.5 mm, shallow incisions on the acroscopic margin of the ultimate segments, and with petiolules generally longer than 0.9 mm (Figure 2b). Additionally, the croziers and expanding leaves of the Green Mountain maidenhair fern are often tinged reddish pink.

Ecology

The *Adiantum pedatum* complex is an excellent target for ecological inquiry, as the distribution of the three species in northeastern North America is directly related to patterns of geology that underlie the region. *A. pedatum* is distributed in rich northern hardwood forests from Quebec to the mid-western United States. Rich northern hardwood forests are places where colluvial processes, calcareous bedrock, or some combination of these two conditions provide plants with a steady nutrient supply (Sorenson & Thompson 2000). *Acer saccharum* and *Fraxinus americana* are canopy dominants in rich northern hardwood forests; other abundant tree species include *Tilia americana, Fagus grandifolia,* and *Betula alleghaniensis* (Sorenson & Thompson 2000). The deciduous trees of this natural community usually shelter a rich diversity of herbs on the forest floor, including *A.*

pedatum, Laportea canadensis, Caulophyllum thalictroides, Carex plantaginea, and many others herbaceous calciphiles (Sorenson & Thompson 2000).

In contrast, the serpentine maidenhair ferns *Adiantum aleuticum* and *A. viridimontanum* are restricted in distribution to the northern portion of a serpentine belt approximately 10 miles in width which extends from Newfoundland to Alabama (Paris & Windham 1988). Serpentine, referring both to bedrock and to soils derived therefrom, is a kind of ultramafic rock which originated deep in the mantle of the earth, and therefore is more similar in chemical composition to the mantle of the earth than to its crust (Sorenson & Thompson 2009). Serpentine tends to have low concentrations of important plant nutrients including calcium, nitrogen, potassium, and phosphorus, but high concentrations of magnesium and heavy metals such as chromium, nickel, and cobalt, which are toxic at high concentrations (Paris & Windham 1988). This unique soil chemistry, combined with the shallow soil and high degree of exposure typical of serpentine outcrops, provides adverse growing conditions for the growth of most plants (Brady et al 2005). Because of the stressful growing conditions, serpentine outcrops are sparsely vegetated with a low diversity of plant species. *Picea rubens* and *Betula populifolia* are usually present forming a sparse canopy in some areas, with *Juniperus communis* var. *depressa* common in the understory. Herbaceous plants are few, and commonly include *Campanula rotunifolia, Deschapsia flexuosa, Arenaria stricta, Dryopteris marginalis,* and *Cerastium arvense*. More uncommon herbs of serpentine outcrops include *A. aleuticum* and *A. viridimontanum* as well as *Arenaria marcenses, A. macrophylla*, and *Empetrum nigrum* (Sorenson & Thompson 2000).

Plants adapted to the harsh conditions of serpentine often grow nowhere else, and so serpentine outcrops tend to have many endemic plants relative to other areas (Harris & Rajakaruna 2009). This endemism likely results from the island-like distribution of serpentine outcrops in the surrounding forest, which promotes divergence and speciation (Kruckeberg 1986). The topographic and geochemical discontinuities of the edaphic element can facilitate the formation of endemics in two ways (Stebbins 1940; Kruckeberg 1954). Neoendemic species form as a result of in site evolution resulting from divergence following colonization of a new substrate (Kruckeberg 1954; Baldwin 2005). In contrast, paleoendemic species can form from relict populations of a formerly widespread distribution that contracted due to changing environmental conditions (Kruckeberg 1954). The conditions of relaxed interspecific competition on serpentine may allow disjunct populations of multiple species to persist at the limits of their range (Drury 1969).

Electrophoretic investigation of the *Adiantum pedatum* complex by Paris & Windham (1988) provided evidence that in northeastern North America *A. aleuticum* represents a paleoendemic lineage, as low genetic diversity in the eastern populations of this taxon suggests that many biotypes have been lost. Although *A. aleuticum* has flexible edaphic requirements in western North America and so therefore cannot be considered endemic to serpentine across the entirety of its range, in northeastern North America it is restricted to serpentine with the characteristics of a paleoendemic lineage. The allotetraploid lineage *A. viridimontanum* is truly endemic to serpentine, as it is restricted to serpentine throughout its range (Harris & Rajakaruna 2009). The Green Mountain maidenhair fern is an interesting case of edaphic endemism; although it inherited a serpentine-adapted genome from *A. aleuticum,* and so could be considered a paleoendemic species itself, this lineage is also an allopolyploid containing a rich-woods adapted genome from *A. pedatum.* Speciation through allopolyploidy is often associated with diversity in geo-edaphic phenomena, as hybridity often acts to extend the range of an edaphic specialist (Kruckeberg 1986). Regardless of how the two species came to inhabit serpentine, both lineages are restricted to serpentine except in cultivation (Don Avery, pers. comm.); they frequently grow together on serpentine outcrops.

Research Focus

The co-occurrence of the two closely related maidenhair species leads to the contention that they nevertheless occupy distinct ecological niches, following from the competitive exclusion principle (Grinnell 1904), which predicts that two species competing for the same limited resources cannot stably co-exist with one another. Here I present an array of evidence with the goal of characterizing ecological niche differentiation in the *Adiantum pedatum* complex in northeastern North America. My aim is to test the hypothesis that niche differentiation exists between *A. aleuticum* and *A. viridimontanum*. The niche differentiation may have been present since the origin of the allopolyploid, or disruptive selection between the two serpentine maidenhairs could have secondarily yielded the niche difference. The alternate hypotheses regarding this question are as follows:

- H1: There is ecological niche differentiation between the two serpentine maidenhair ferns because the allotetraploid species is inherently intermediate in habitat preference between its two progenitors, due to inheriting both their genomes and thus their adaptations to different habitats.
- H2: There is ecological niche differentiation between *Adiantum aleuticum* and *A. viridimontanum* because of competitive exclusion only – interspecific competition between the two serpentine maidenhair fern species has resulted in directional selection of one or both lineages away from occupying similar ecological niches.
- H3: There is ecological niche differentiation caused by both the tetraploid's inherent ecological intermediacy and competitive selection with *Adiantum aleuticum* acting on existing difference.
- Null hypothesis: The ecological niches of *A. aleuticum* and *A. viridimontanum* are not differentiated from one another.

The primary focus of this project is the niche differentiation between the two serpentine maidenhairs, but study of the niche characteristics of *Adiantum pedatum* is also crucial to determine if the ecology of the hybrid maidenhair lineage is intermediate in any sense between its progenitors. The reticulate evolutionary event that reunited the ecologically disparate lineages of *Adiantum pedatum* and *A. aleuticum* resulted in the formation of a lineage possessing the genome of both a serpentine and rich-woods ancestor. Although *Adiantum viridimontanum* shares a habitat with its serpentine progenitor, the ecology of the allotetraploid is likely influenced to some degree by the ecological contribution of its rich-woods ancestor.

In order to characterize ecological niche differentiation during the course of this work, I chose to use ploidy difference inferred from flow cytometry data as the basis for distinguishing the two maidenhairs on serpentine outcrops, given the difficulty of accurate identification using only morphological characters. Ploidy level is useful for identification of species within the complex because *Adiantum pedatum* and *A. aleuticum* are diploid, while *A. viridimontanum* is tetraploid. As *A. pedatum* occupies rich northern hardwood forests and usually does not co-occur with the serpentine maidenhairs, diploids growing on serpentine are most likely *A. aleuticum*. An advantage of using flow cytometry to verify field identifications is that triploid plants can also be identified. Sterile triploid ferns result from backcrosses between diploid and tetraploid individuals; in the present case, they have the potential to form between either *A. aleuticum* or *A. pedatum* and *A. aleuticum.*

Methods

Field Research

Data to test the hypothesis that the ecological niches of the two serpentine maidenhair ferns are differentiated from one another were gathered during the summer of 2016. Maidenhair habitat characteristics were studied at ten sites across Vermont, five on serpentine bedrock and five on non-serpentine bedrock (Figure 3, Appendix Table 1). The serpentine sites included Browns Ledges (Lowell VT), West Farman Hill (Westfield VT), Mississquoi Ledges (Lowell, VT), Mineral Springs Knob (Westfield, VT), and Belvidere Mines (Eden, VT). The non-serpentine sites included Farrell Woods (Montpelier, VT), Chapel Woods (Montpelier, VT), Elmore State Park (Elmore, VT), Calvin Coolidge State Forest (Shrewsbury, VT), and Green River Reservoir State Park (Hyde Park, VT). Following from the concept that ecological niche is the combination of factors that leads to maximum species abundance (Grinell 1917), I chose to survey the largest patches of each species of maidenhair fern in the site, usually places

Table 1: Attributes compiled for each study patch

Attribute	Methodology & Comments
Location and elevation	Determined by GPS point
Species Identity	Documented with sketches and photos
Number of genets	By rough count
Spatial Area	By estimate of patch dimensions
Natural Community type	Classification according to Sorenson & Thompson (2000)
Associated species	Within 20 ft. of the patch
Percent canopy cover	Determined by densiometer
Micro and macro scale topography	Measurements of slope and aspect and assessments of hillslope form and position
Depth of leaf litter and soil layers	Multiple measurements taken at various points
Texture & composition of soil	Determined by feel
Disturbance?	Signs noted

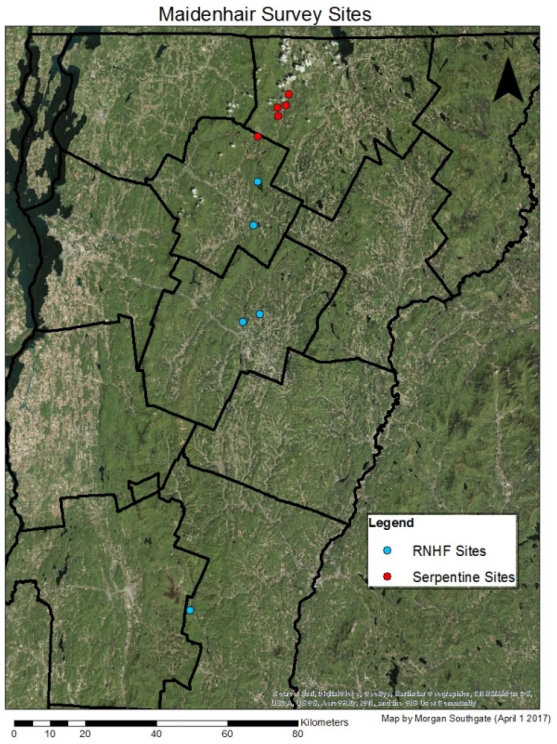

Figure 3: Rich northern hardwood and serpentine sites studied during the summer of 2016

where multiple clumps (ramets) formed one or more patches (genets). Where possible, most subpopulations were selected for survey in order to maximize variation in aspect and elevation in each site.

For each patch chosen for study, I assembled a set of patch attributes including the natural community type, light availability, topography, and substrate of the landscape immediately surrounding the subpopulation (Table 1). At each survey patch, I also collected materials for later analysis, including frond tissue for flow cytometry and morphological documentation, and soil samples for soil chemistry analysis. I made a landscape sketch of the species composition and topography at each site. GPS points of were taken at many un-surveyed patches in order to collect information regarding species distribution within each site.

Flow Cytometry

Flow cytometry of selected *Adiantum* samples was carried out in order to estimate DNA ploidy. Leaf tissue collected over the summer was stored at 2° C without prior drying for use within several weeks to eight months of collection. Tissue preparation for flow cytometry analysis followed the protocol of Bainard et al. (2011), but with either propidium iodide or Sybr green as the fluorophore. The leaf tissue was placed in a petri dish, into which was added 1.2 mL of LBO1 buffer, 100 µL of RNase, and 10 µL of DNA-specific dye. The tissue was finely chopped in the solution and strained through a 30 µL filter. The resulting suspension of released nuclei was then used immediately or stored at 34° for use within a day. Nuclei of *Nephrolepis biserrata*, with a known 1C value of 9.52 pg (Obermeyer et al. 2002), were used as an external standard and prepared separately from the maidenhair tissue following the same protocol. Samples were analyzed using a Coulter Epics XL flow cytometer (Beckman Coulter Genomics/Genewicz, South Plainfield, New Jersey, USA) maintained at the Plant Biology Department, University of Vermont. Histograms of the event numbers were analyzed using FlowJo software (FlowJo 2010). When determining 1C sample values, the median of the sample peak was compared to the median of the standard peak. The range of 1C values for each ploidy was determined by using the halfway point between the mode 1C values of the diploid and tetraploid as the upper and lower limit of the range of 1C values for each ploidy respectively.

Soil Chemistry Analysis

The soil samples from the ten field sites were analyzed by the University of Vermont's Agricultural and Environmental Testing Laboratory. The analysis provided measurements of pH, nutrient content (both macro- and micro-nutrients), base saturation of potassium, magnesium, and calcium, effective cation exchange capacity (ECEC), percent organic matter, and concentrations of heavy metals including nickel, lead, copper, zinc, cadmium, and chromium.

Data Analysis

ArcGIS maps were compiled of the species distribution in each site. The quantitative data from the ecological data-set collected in the field and the soil analysis results were combined and analyzed using multivariate statistics. The computer language R and the interface R Studio(R Core Team 2017) was used for all statistical analysis.

For multivariate analysis, missing values in the data-set (including heavy metal presence in soil from rich northern hardwood forest samples and sites with absent soil layers) were set as 0. Two approaches to multivariate statistical analysis were employed, which differed in the approach to how the species identification of each patch was used for the analysis.

1) Principal components analysis, a multivariate statistical technique that does not use a priori class information for the analysis, was used to assess how well the ecological niche variation observed in each survey patch sorted naturally into species groups. The prcomp function in R (R Core Team 2017) was utilized for this analysis with the internal settings of the function set to center and scale the data prior to analysis. The ggplot2 package (Wickham 2009) was used to visualize the PCA results.
2) Discriminant function analysis, a supervised statistical technique which uses class information to group observations prior to the analysis, was used to find the linear combination of predictor variables which most clearly separated survey patches by species identity. As discriminant function analysis groups the data by species initially, the ecological niche data gathered for patches with more than one species and hybrid patches were omitted from the analysis. Additionally, the correlation of all ecological variables was determined prior to analysis and one member of variable groupings with a correlation of 0.90 and above was omitted from the analysis. Discriminant function analysis requires more observations than variables, and so the ecological data-set was broken into two lots for analysis: the data collected on site, and the data from the soil analysis results. The lda function in the MASS package (Venables & Ripley 2002) was used to perform discriminant function analysis in two different ways. First, half the samples were set as a training group for species discrimination, and the calculated fits for each species group were then applied to the rest of the data. The results of this form of discriminant function analysis were plotted using the ggplot2 package (Wickham 2009). Second, discriminant function analysis was conducted by setting prior probabilities equal to the species proportions within the dataset. This variant of discriminant function analysis was used to calculate ecological factor weightings for each linear discriminant.

The most important ecological variables in distinguishing between the three species of maidenhair fern were identified as the factors with the largest weightings along the first and second axes of both types of multivariate analysis. These ecological variables were tested for significance between all three species of maidenhair fern and between only the serpentine species using ANOVA, and plotted using boxplots.

Results

Principal Components Analysis

The first principal component resulting from the principal components analysis (PC1) explained 30.06% of the variation in the data, and the serpentine and woodland maidenhair fern species are resolved in cohesive separate clusters along this axis (Figure 4). Considering the serpentine maidenhairs, the Aleutian maidenhair fern patches form a well-defined cluster distant from the northern maidenhair fern patches, but they overlap almost completely with those of the green mountain maidenhair fern. However, the Green Mountain maidenhair fern cluster extends well beyond the Aleutian maidenhair fern cluster towards the northern maidenhair fern cluster. Thus, overall the niche space

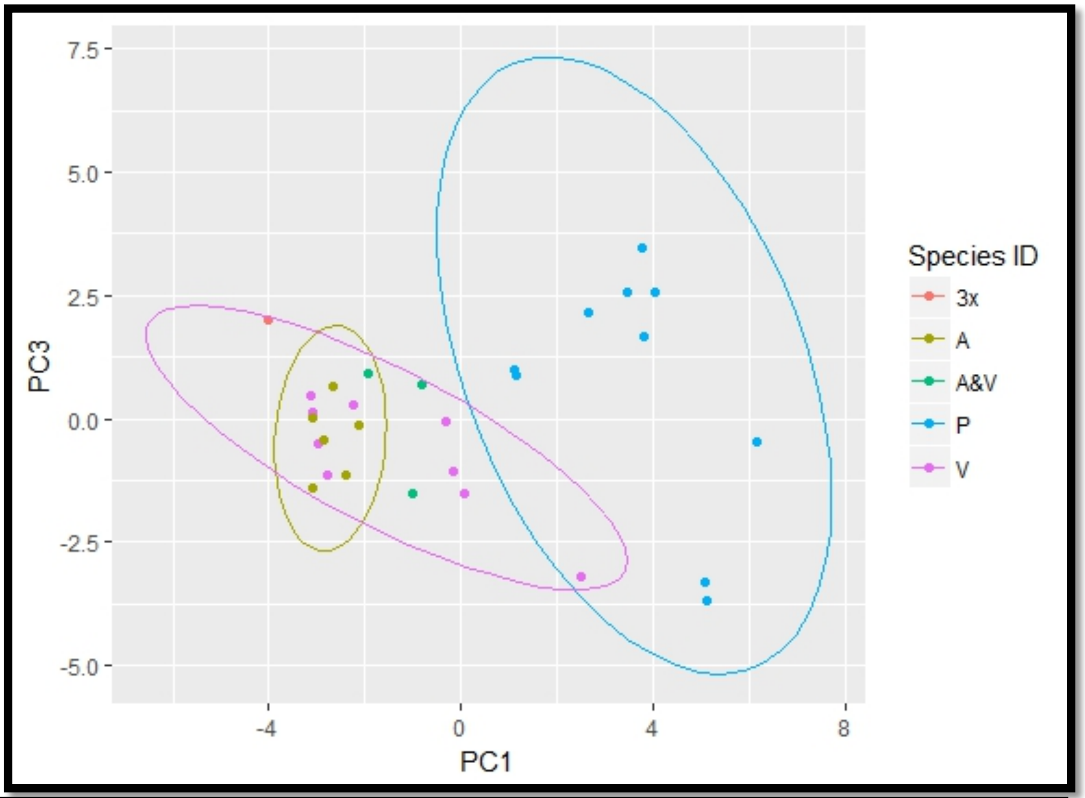

Figure 4: A plot of the first and third principal components derived from a PCA of the quantitative ecological data.

occupied by the hybrid lies between that of its progenitors. The sample patches that include both species of serpentine maidenhairs lie at the center of the cluster of patches from serpentine substrates.

The second principal component explained 16.98% of the variance present between the maidenhair fern species. Although the third principal component explained only 9.63 % of the variance between species, more separation by species group was observed along this axis than the second principal component. Therefore, the first and third principal components were plotted and analyzed. Along the third principal component, most of the points making up the cluster of *Adiantum pedatum* are is slightly above the cluster of the serpentine maidenhair ferns. In the plot of the first and second principal components, no such separation along the vertical axis is observed.

The ecological variables with the greatest factor loadings along the first principal component were the concentration of magnesium ions in the soil, the average depth of the A layer of the soil, the ECEC of the soil, the percent organic matter present in the soil, and the concentration of potassium ions in the soil (Table 2). The ecological variables with the greatest factor weightings along the third principal component were the concentration of calcium and potassium ions in the soil, the calcium base saturation, the average soil depth, and the elevation (Table 2).

Table 2: Factor weightings for the first two principal components from the PCA of maidenhair ecological data, sorted from highest to lowest weightings.

Ecological Variable	PC1	PC3
Avg Depth A	0.269	-0.094
Al	0.212	-0.127
Avg Soil Depth	0.199	-0.28
Ca Base Saturation	0.199	0.384
Avg Depth B	0.189	-0.031
Cu	0.189	-0.202
% Canopy Cover	0.176	0.079
S	0.132	-0.175
Fe	0.127	-0.27
Ca	0.121	0.455
Zn	0.11	0.123
Avg Litter Depth	0.101	0.047
Elevation	0.099	0.312
Mn	0.091	0.023
Na	0.071	-0.22
Avg Depth O	0.07	-0.069
K Base Saturation	0.003	-0.153
Microslope	-0.095	0.052
Exchangeable Acidity	-0.1	0.073
pH	-0.121	-0.004
B	-0.125	-0.156
MacroAspect	-0.143	0.153
MicroAspect	-0.153	0.168
Ni	-0.212	-0.128
Macroslope	-0.216	-0.06
Pb	-0.22	0.052
P	-0.224	0.054
Mg Base Saturation	-0.231	-0.223
K	-0.239	0.006
Organic Matter %	-0.246	0.118
ECEC	-0.264	0.03
Mg	-0.278	-0.157
Proportion of between-species variance explained:	**30.06 %**	**9.63%**

Table 3: Factor weightings for the first two linear discriminants from both discriminant function analyses of maidenhair ecological data, sorted from highest to lowest weightings.

Ecological Variable	LD1	LD2
Avg Depth A	1.406	-1.149
Microslope	1.259	0.146
% Canopy Cover	1.12	-0.722
Avg Depth B	0.521	0.02
Macro Aspect	0.292	-0.911
Elevation	0.027	0.079
Avg Litter Depth	-0.015	0.548
Avg Depth O	-0.479	0.835
Macroslope	-0.702	-0.902
Proportion of between-species variance explained:	**86.49%**	**13.50%**
Cd	6.723	0.762
Ca	6.151	5.006
K Base Saturation	4.397	3.024
B	3.218	-1.599
Al	3.13	1.441
Organic Matter%	1.119	-7.097
Na	0.574	1.527
Cu	-0.349	1.669
S	-0.66	-0.21
Ni	-1.066	-2.935
Mn	-1.225	-1.974
Zn	-1.286	-0.366
Fe	-2.339	-5.479
Cr	-2.644	-0.689
Mg	-2.659	15.353
K	-2.726	-1.432
P	-3.448	1.71
Pb	-5.215	0.011
pH	-7.961	-10.912
Proportion of between-species variance explained:	**97.49%**	**2.51%**

Discriminant Function Analysis

The plot of the first two linear discriminants produced from the training sample method of discriminant function analysis shows the three species of maidenhair fern separating primarily along the first linear discriminant. *Adiantum pedatum* clusters along the right portion of the first linear discriminant, while the serpentine maidenhairs cluster on the left portion. Slight separation between the serpentine maidenhairs is visible along

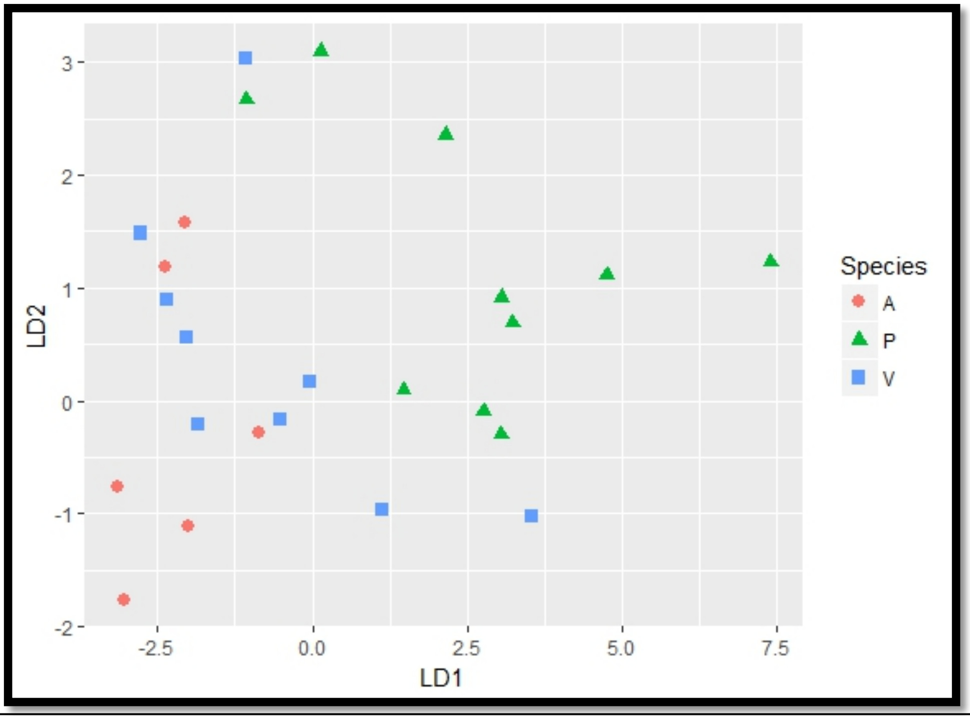

Figure 5: A plot of the first two linear discriminants produced by the training sample method of DFA

the first linear discriminant. Although the *A. viridimontanum* cluster overlaps almost entirely with that of *A. aleuticum,* it also extends towards the *A. pedatum* cluster. Slight separation by species is also visible along the second linear discriminant. The center of the *A. pedatum* cluster appears above the majority of the serpentine maidenhair cluster.

The first linear discriminant produced by the prior probabilities method of discriminant function analysis explained 86.5% of the variance between species (Table 3). The ecological variables with the largest factor weightings along the first linear discriminant were the average depth of the A layer of the soil, the microslope, the percent canopy cover, the macroslope, and the average depth of the B layer of the soil. The second linear discriminant of the ecological data-set gathered in the field explained 13.5 % of the variance between species. The ecological variables with the largest factor weightings contributing to this second linear discriminant were the average depth of the A layer of the soil, the macro-aspect, the macroslope, the average depth of the O layer, and the percent canopy cover.

The first linear discriminant produced by the prior method of discriminant function analysis of the soil chemistry data explained 97.5% of the variation between the three species. The five ecological factors with the largest factor weightings contributing to the first linear discriminant were the pH of the soil, levels of cadmium, calcium, and lead ions in the soil, and potassium base saturation of the soil. The second linear discriminant explained 2.5% of the variance between the maidenhair species. The top five factor weightings were levels of magnesium, iron, and calcium ions in the soil, as well as the pH and percent organic matter of the soil.

Univariate Analysis

The only ecological variable shared between the five largest weightings of the first principal axis of both types of multivariate analysis was the depth of the A layer. An ANOVA test of the variation in the depth of the A layer between species returned a significant result (p = 0 .0048). However, an ANOVA test of the difference in the depth of the A layer between only the serpentine species revealed an insignificant difference (p = 0.113). A boxplot of the depth of the A layer by species shows that the measured depth range of the A soil layer in patches of *Adiantum viridimontanum* is intermediate between the measured depth ranges of its progenitors.

Ecological variables in common between the five largest factor weightings of the first principal component and second linear discriminant were the concentrations of magnesium and iron ions in the soil as well as the percent organic matter in the soil. The difference in magnesium concentrations between species was highly significant as tested by ANOVA (p = 0.000000119), but not significantly different between only the serpentine species (p= 0.951). The boxplot of magnesium levels present in the soil by species shows

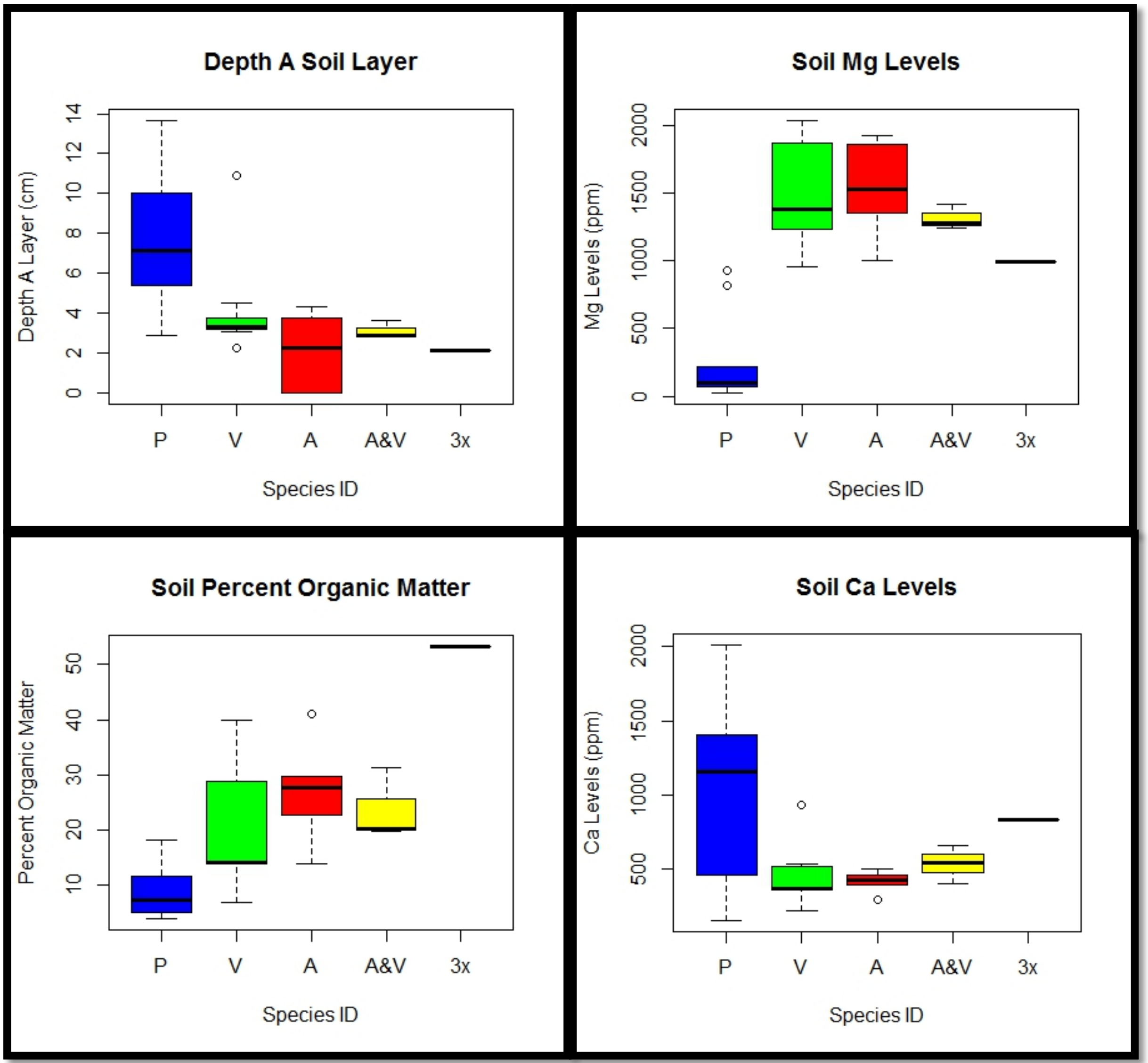

Figure 6: Boxplots of ecological variables identified as important through multivariate analysis. 6a) (top left): Depth of the A layer by species. 6b)(top right) the levels of Mg (ppm) present in the soil by species. 4c) (bottom left): percent organic matter content in the soil by species. 4d) (bottom right) the levels of Ca in the soil by species.

little difference between the two serpentine maidenhairs, but significant difference between *A. pedatum* and the serpentine maidenhairs. The differences in the percent organic matter in soil between all species were highly significant as tested by ANOVA (p = 0.000295).The difference in percent organic matter between the serpentine maidenhair ferns *Adiantum aleuticum* and *A. viridimontanum* was non-significant as tested by ANOVA (p=0.281). However, the boxplot of percent organic matter by species shows a slight pattern of hybrid intermediacy, in which *A. viridimontanum* occupies a percent range of organic matter in the soil that is slightly intermediate between the ranges occupied by its progenitors (Figure 6).

The only factor in common between the five largest factor weightings of the first linear discriminant and the third principal component was the concentration of calcium present in the soil. The difference in calcium levels was highly significant between all species of maidenhair (p=0 .008354) but not between the two species of serpentine maidenhair (p =0 .752).

Flow Cytometry

A histogram of the 1C values from calculated from flow cytometry results (Appendix Table 1) shows two peaks corresponding to the genome sizes of diploid and tetraploid maidenhair fern samples. The peaks were interpreted using symmetry around the mode, so that the halfway point between the two peaks marked the upper and lower limits of the diploid and tetraploid 1C values, respectively. Using this method the range of diploid 1C values was interpreted to extend from 4-6 pg, the range of triploid 1C values from 7-8 pg, and the range of tetraploid 1C values from 8-11 pg. The 1C values that were larger than 11 pg were not taken into account in calculating the 1C value for each ploidy, as these results were likely anomalous due to measurement error. The mean 1C value for *Adiantum aleuticum* was calculated to be 5.53 pg with a standard deviation of 0.65 pg, while the mean

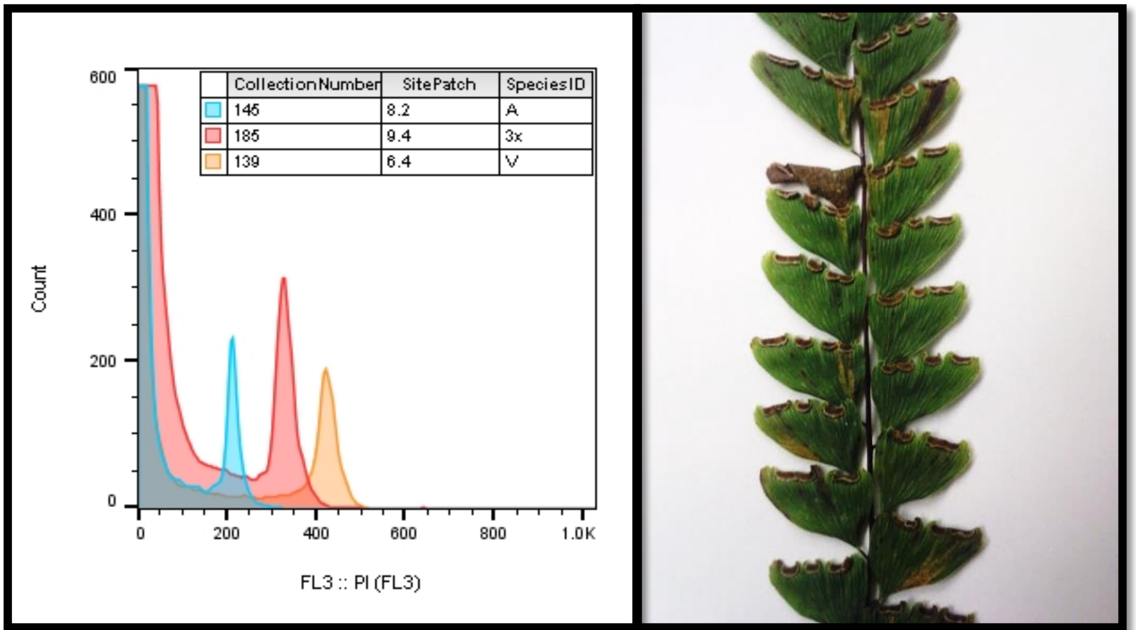

Figure 7: The flow cytometry results indicating the presence of a triploid hybrid (left) and the morphology of mid-ultimate segments of the hybrid (right*).*

1C values of *A. viridimontanum* was calculated to be 10.71 pg with a standard deviation of 1.56 (Appendix Table 3).

A triploid hybrid from West Farman Hill was documented using flow cytometry (Figure 7). The peak of the triploid is intermediate in position between the two diploids. Multiple trials of the same sample resulted in identical results, and the 1C value of the triploid was calculated to be 7.5 pg. (Appendix Table 4).

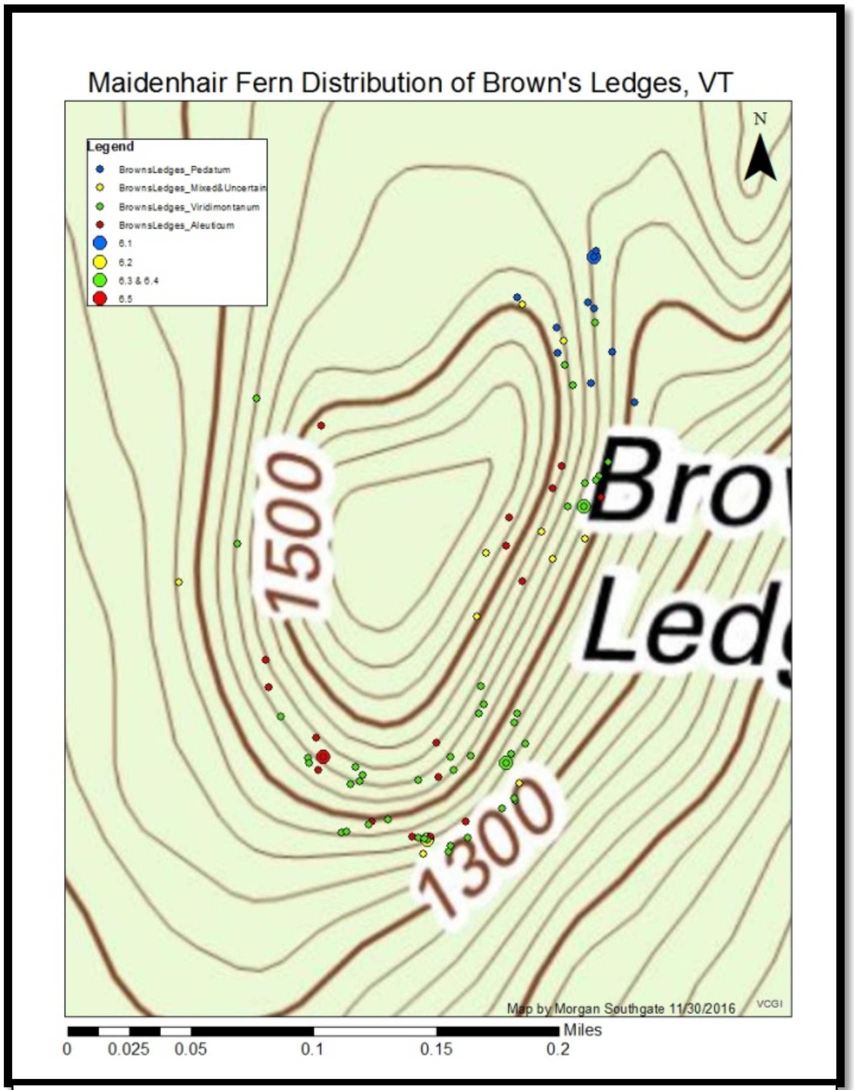

Figure 8: Maidenhair fern species distribution of Brown's Ledges, Lowell, VT

Geospatial Mapping

Large-scale patterns of species distribution in the geospatial data indicate that the two serpentine maidenhairs co-occur (e.g. Brown's Ledges, Figure 8). At the Brown's Ledges serpentine outcrop, patches of the two species are interspersed at close proximity to one another, and there are patches with both species present. The co-occurrence of *Adiantum aleuticum* (shown with red dots in Figure 9) and *A. viridimontanum* (shown with green dots in Figure 9) is seen in how patches of the two serpentine maidenhair ferns intermingle with one another across many elevations and aspects of each site. Furthermore, there are some patches that were identified as mixed in species composition, including site patch 6.2 (shown with yellow dots in Figure 9). On Brown's Ledges patches of *A. pedatum* (shown with blue dots in Figure 9) were also identified growing within the northern distribution limit of the serpentine maidenhairs on the outcrop.

Discussion

Niche differentiation

Results from both the multivariate and univariate statistical analyses provide evidence of ecological niche differentiation between *Adiantum aleuticum* and *A.*

viridimontanum. The ecological factors most important in explaining the variance between species groups, as indicated by the largest factor weightings calculated by the multivariate analyses, are the depth of the A layer of the soil, the concentration of magnesium ions in the soil, the percent organic matter in the soil, and the concentration of iron ions in the soil. ANOVA tests of the variance between these ecological variables by species revealed significant differences between all three species of maidenhair fern, but non-significant differences between only the serpentine maidenhair ferns. Thus ecological niche differentiation between the serpentine maidenhairs is not possible to resolve using only singular ecological variables.

Despite this lack of significance between the serpentine species based on ANOVA, there is a pattern of hybrid ecological intermediacy visible in the boxplots of some ecological factors. In both the boxplot of the depth of the A soil layer and that of the percent organic matter in the soil, the measured range of *A. viridimontanum* is intermediate between the measured ranges of its progenitors. For other important ecological variables, including as the levels of magnesium and calcium ions in the soil, there was no discernible difference between the serpentine maidenhairs but a significant difference between the serpentine and woodland species.

Thus the habitats of the two serpentine maidenhair species appear to be similar in terms of some variables but distinct in terms of others. Although the differences in single ecological variables between the serpentine maidenhair ferns were non-significant, several results were close to significance and some showed a pattern of ecological intermediacy for the allotetraploid hybrid. Thus it seems that the ecological niches of the two serpentine maidenhairs cannot easily be differentiated by taking only a single ecological variable into account, but the consideration of multiple factors simultaneously provides evidence for differentiation.

In the plot of the first two principal components produced from the principal components analysis, the niche space of *Adiantum viridimontanum* appears intermediate between the spaces of its progenitors along the first principal component. The *A. viridimontanum* cluster overlaps almost completely with the cluster of its serpentine progenitor, but also extends into the multivariate space between *A. pedatum* and *A. aleuticum* much past the boundaries of the *A. aleuticum* cluster*.* Although the differentiation between the serpentine maidenhairs and the rich woods species is more distinct, as expected given the ecological disparity of their habitats, the differentiation between *A. aleuticum* and *A. viridimontanum* is in how the niche space of the latter extends towards the niche of *A. pedatum* beyond the niche boundaries of the former. The hybrid origins of the allotetraploid lineage correspond with this inference, as *A. viridimontanum* appears to be able to colonize habitats intermediate between its progenitors. This hybrid ecology, attained through the joint integration of two genomes, one adapted to serpentine and the other to rich woods habitats, likely enables *A. viridimontanum* to occupy a range of conditions intermediate between its progenitors.

The ecological variables that have the largest factor weightings along the first principal component were the concentration of magnesium present in the soil and the depth of the A layer of the soil. The factor weighting of magnesium concentration is negative along the first principal component, which explains the clustering of the serpentine maidenhairs in the left portion of the PCA plot and the rich woods species in the right portion. The weighting of the depth of the A soil layer is positive, meaning that the depth of this layer increases along the first principal component. The placement of the serpentine maidenhairs in the left portion of the plot correspond to shallow depth of the A layer, while the placement of the rich woods species on the right portion of the plot correspond to a deeper A layer of the soil.

The ecological variables which loaded heavily on the third principal component included the concentration of calcium ions in the soil, the calcium base saturation of the soil, the elevation, and the average soil depth. The positive weighting of both the level of calcium ions and the calcium base saturation determines the position of *Adiantum pedatum* slightly above the cluster of the serpentine maidenhairs on the third principal component. Elevation is another factor with a large positive weighting which contributes to the location of the *A. pedatum* cluster appearing along the third principal component. The other variable which loaded heavily along PC3, the average soil depth, had a negative factor weighting. This result is confusing given that observations and boxplots of the soil depth indicate that *A. pedatum* inhabits deeper soil. However, this result may be due in part to how some points of the *A. pedatum* cluster are below that of the serpentine maidenhair clusters.

Ecological niche width

The wider extent of the *Adiantum viridimontanum* cluster compared to that of *A. aleuticum* along the first principal component of the PCA indicates that the allopolyploid species inhabits a wider range of ecological conditions. Based on the factor weightings which load most heavily on the first principal component and in which there is a visible (although non-signficant) difference between the serpentine maidenhair species, *A. viridimontanum* appears to have the capacity to inhabit soils with a deeper A layer and a lower percent organic matter content than *A. aleuticum*, although it also grows in similar soil conditions to its serpentine progenitor.

The observations of serpentine maidenhair habitat made during the past summer of field research support the hypothesis that the ecological niche of *Adiantum viridimontanum* overlaps with that of *A. aleuticum* but also extends to occupy a wider range of soil conditions. *A. viridimontanum* was observed in many different expressions of serpentine-influenced habitats, often serpentine-influenced forests contiguous with serpentine outcrops, where *A. aleuticum* was not present. These locations likely had a deeper A layer of the soil and lower percent organic matter than the survey patches on serpentine outcrops. However, most patches of *A. aleuticum* were located on serpentine outcrops, with *A. viridimontanum* growing nearby. Thus both serpentine maidenhairs seem to grow readily on talus slopes and the thin soil of serpentine outcrops, and *A. aleuticum* seems to thrive within the most extreme rock crevices, but only *A. viridimontanum* is distributed in the serpentine-influenced forest around serpentine outcrops.

Water and Nutrient Flow

The distribution of maidenhair ferns in northeastern North America appears to be strongly influenced by substrate mediated through water flow, in a way that is unique for each species. For *Adiantum viridimontanum* there is an especially interesting distribution pattern related to the flow of water and nutrients. In several serpentine sites, the allotetraploid species was found growing in serpentine-influenced forests along the outwash derived from the serpentine outcrops above. The dedicated restriction to these water run-off routes may be related in part to seeking out high moisture, but given that this species can thrive in low-moisture environments it is more likely seeking out the serpentine-influenced soil. Additionally, the allotetraploid hybrid seems to thrive when growing in disturbed areas. This love of disturbance is seen at Belvidere Mines, where *A. viridimontanum* grows densely over tailing piles and mine outwash, and also on old logging roads on West Farman Hill, where it grows as the dominant herb on the old tracks created by heavy machinery. This proclivity towards colonization of disturbed habitat may be related to release of serpentine nutrients from lower soil layers when the top layers are disturbed.

The influence of water and nutrient flow is also seen in the distribution of the other two members of the *Adiantum pedatum* complex. *A. aleuticum* seems to thrive at the base of serpentine outcrops and overhangs. This tendency indicates that places where water and nutrient flow down the serpentine outcrops are collected, such as crevices, hollows, and the bases of the outcrops, may provide a relatively moisture- and nutrient-rich micro-habitat suitable for colonization by *A. aleuticum.* Similarly, in rich northern hardwood forests, *A. pedatum* often occupies small cove-shaped features in the landscape, where the concavity to the slope promotes the accumulation of nutrients from water flow. Here, the accumulation of calcium is likely especially important in determining the distribution of the northern maidenhair fern.

Inherent hybrid intermediacy or competitive exclusion?

Multiple forms of evidence, including observations as well as the results of multivariate analyses, support the hypothesis that the ecological niches of *Adiantum viridimontanum* and *A. aleuticum* are differentiated from one another. The allotetraploid lineage appears ecologically intermediate between its progenitors in multivariate space, and this pattern is visible for some single ecological variables. Additionally, observations suggest that while both serpentine maidenhairs are distributed on serpentine outcrops, only *A. viridiontanum* inhabits the serpentine-influenced forests adjacent to the outcrops. However, the two possible causes of niche differentiation – 1) intrinsic ecological intermediacy of the hybrid due to the inheritance of two genomes adapted to different environments, or 2) acquired ecological differentiation evolved because of competitive exclusion between the two serpentine species – cannot be clearly distinguished using present data.

The rationale for the first hypothesis is that ecological niche differentiation between the serpentine maidenhairs may have resulted from the very origin of the *Adiantum viridimontanum* lineage, as the combination of genomes adapted to both serpentine and rich woods habitats may have enabled the allotetraploid to occupy an intermediate ecological niche from the start. This explanation seems likely because *A. viridimontanum* has always possessed two ecologically disparate genomes, and so may have occupied an ecological niche intermediate between that of its progenitors since its origin. The results showing ecological intermediacy of the allotetraploid provide possible support for this hypothesis, as the niche space of the hybrid appears between its progenitors *A. pedatum* and *A. aleuticum* in multivariate space. The probable recent origin of the *A. viridimontanum* lineage also provides some support for this hypothesis, as the effects of directional selection through competitive exclusion may not have had sufficient time to cause evolutionary change in either serpentine maidenhair lineage.

However, the pattern of hybrid ecological intermediacy observed may also result from competitive exclusion between the two serpentine maidenhairs following the origin of the allotetraploid lineage. If the ecological niches of *Adiantum aleuticum* and *A. viridimontanum* were not differentiated from one another initially, interspecific competition between one or both species may have acted as a selective force favoring plants with ecological niches distinct from one another. As the hybrid maidenhair fern possesses the genome of a rich-woods ancestor, competition with the diploid serpentine maidenhair could function to cause directional selection of the *A. viridimontanum* lineage towards the niche space of *A. pedatum.* Possible support for this hypothesis is provided by the geospatial mapping and flow cytometry results, which document the co-occurrence of the two serpentine maidenhair fern species on both macro-and micro- scales. On a macro perspective, maps made of species distribution by site, such as in Figure 2, show intermingling of different species patches across the landscape. On a micro scale, flow cytometry results indicated that two of the patches surveyed with a field identification of *A. viridimontanum* also contained *A.aleuticum,* and one of the patches initially identified as *A. aleuticum* also contained *A. viridimontanum.* Therefore the two serpentine maidenhairs do sometimes grow so closely together that the two species seem to be in active contact through the rhizosphere. This co-occurrence on multiple scales suggests that there are conditions in which interspecific competition between the two closely related species could be operative.

The third alternate hypothesis is that the two potential causes of hybrid ecological intermediacy have acted in combination to produce the observed ecological niche differentiation. Perhaps the ecological niche *Adiantum viridimontanum* has indeed been inherently intermediate between that of its progenitors since origin. If selection driven by interspecific competition between the serpentine maidenhairs acted on existing ecological difference between the two, it would likely function to increase the degree of ecological niche differentiation observed by favoring ecological niche genotypes of the serpentine maidenhairs that reduced competitive interaction. This selective force may have favored the observed distribution of *A. viridimontanum* away from the center of serpentine outcrops into the serpentine-influenced woodlands beyond, where competition with *A. aleuticum* is minimal.

Serpentine-Influenced Rich Northern Hardwood Forests

Adiantum pedatum was found growing in the serpentine-influenced forests of Browns Ledges and Belvidere Mines (survey patches 6.1 and 10.3). These patches of *A. pedatum* documented on serpentine sites are very interesting from an ecological perspective, because unlike most sites surveyed the designation between serpentine and rich woods habitat is impossible to make. The two community types essentially overlapped with one another, forming what can best be described as a serpentine-influenced northern hardwood forest. The soil results show a combination of calcium and magnesium enrichment, the indicative elements of rich northern hardwood forests and serpentine influenced habitats respectively. The finding of *A. pedatum* on serpentine-influenced sites corroborates the finding of Hoess et al. (2016) who documented this taxon on the serpentine barrens of Pennsylvania and Maryland.

The morphology of these patches strongly indicates the species identity to be *Adiantum pedatum,* but given the notoriously complex morphology of this clade, these identifications should be verified using gene sequence data. In the plot of the PCA results, the two patches of *A. pedatum* surveyed from serpentine-influenced sites are visible as the points of *A. pedatum* which cluster most closely to the serpentine maidenhairs. Assuming that the species identification is correct, this is fascinating in that it documents all three species co-occurring within close distance of one another, meaning that continued origin of the sterile diploid hybrid is very possible. The tolerance of *A. pedatum* for growth in serpentine-influenced forests may be the situation in which the lineage first hybridized with *A. aleuticum*. Additionally, the co-occurrence of all three species in the same sites makes possible the formation of triploid backcrosses between *A. viridimontanum* and either of the two diploid maidenhair ferns.

Species Identity & Hybridization

The 1C values derived from the flow cytometry results are consistent with the expected genome size for the diploid and tetraploid individuals. The mean 1C value for the diploid samples was calculated to be 5.47 pg, with a standard deviation of 0.66 pg. The error term for this result overlaps with the published result for the 1C value of *Adiantum pedatum* of 5.13 pg (Bainard et al 2011). The estimate of the genome size of *A. aleuticum* is thus consistent with the published value of the 1C value of the other diploid maidenhair fern. The mean 1C value for the tetraploid samples was calculated to be 10.03, which corresponds to roughly twice the size of the diploid 1C value I retrieved.

Most of the flow cytometry results were easy to interpret confidently, with the peak of *Adiantum aleuticum* appearing at roughly half the peak placement of *A. viridimontanum,* and most event numbers were well over 1000. However, later trials produced more confusing results, including a series of peaks with a mean 1C value of 14.05 pg. The ploidy level corresponding to this 1C value is a pentaploid maidenhair fern, which is very unlikely. Most of these large results were produced from the last flow cytometry sessions, suggesting that nuclei degradation over the eight month storage time may be the cause of the erroneously large results.

The 1C value of the triploid hybrid found on West Farman Hill was calculated to be intermediate between the genome sizes of its progenitors at 7.5 pg. Although the progenitors of this triploid hybrid cannot be determined based on current data, it seems likely that this triploid individual resulted from a hybridization event between the diploid and tetraploid serpentine maidenhair ferns. On West Farman Hill, *Adiantum pedatum* was not found, meaning that that the closest documented patch of this taxon is across the valley at the foot of Brown’s Ledges. While this distance does not eliminate the possibility of hybridization between *A. pedatum* and *A. viridimontanum*, it makes it less likely. The co-occurrence of the two serpentine maidenhairs within the same site makes hybridization between them more likely. The morphology of the triploid hybrid maidenhair supports this hypothesis, as it shows traces of the morphology of both of its probable progenitors. The pinnules are roughly triangular in shape and with shallow incisions in the acroscopic margin, in this regard similar of *A. viridimontanum*, but with short, crescent-shaped false indusia reminiscent of *A. aleuticum* (Figure 8).

Conclusion

Initial results of this research showing ecological intermediacy of the allotetraploid hybrid maidenhair fern *A. viridimontanum* support the hypothesis that the ecological niches of *Adiantum aleuticum* and *A. viridimontanum* are differentiated from one another. However, the two possible causes the observed differentiation, namely competitive exclusion or inherent hybrid ecological intermediacy, cannot be distinguished based on present data.

The ecological complexity of this clade is remarkable, and certainly not untangled or even fully comprehended in a single year of study. I believe that the ecological patterns discerned here are meaningful but do not fully capture the range of ecological variation present within each species. The power of the multivariate statistical analyses implemented for the purposes of discerning ecological niche differentiation would be much improved by an increase in sample size, as the current data-set includes more variables than observations, which limits the capacity of discriminant function analysis to assess the data-set as a whole.

In the next field season the sampling depth will be increased through more comprehensive sampling of each site and expansion of the survey to include sites not yet studied. The data collected from both summers of field research, as well as gene sequencing to confirm the species identifications of *Adiantum pedatum* documented in serpentine sites, will form the basis for a Master's thesis on the ecology and distribution of the *A. pedatum* complex, with the goal of clarifying the evolutionary and ecological forces shaping ecological niche differentiation between *A. aleuticum* and *A. viridimontanum*.

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Appendix

Table 1: Survey Sites

Site Patch#	Site Name	Town	Coordinates
1.1	Farrell Woods	Montpelier	44.28627, -72.58031
1.2	Farrell Woods	Montpelier	44.285929, -72.579581
2.1	Chapel Woods	East Montpelier	44.30490, -72.51934
2.2	Chapel Woods	East Montpelier	44.30450, -72.51974
3.1	Elmore SP	Lake Elmore	44.53037, -72.54168
4.1	Coolidge SF	Plymouth	43.55442, -72.76347
4.2	Coolidge SF	Plymouth	43.55691,-72.76359
5.1	Mineral Springs Knob	Westfield	44.86280, -72.41673
5.2	Mineral Springs Knob	Westfield	44.86346, -72.41615
5.3	Mineral Springs Knob	Westfield	44.86296, -72.41655
6.1	Browns Ledges	Lowell	44.83243, -72.45392
6.2	Browns Ledges	Lowell	44.82899, -72.45531
6.3	Browns Ledges	Lowell	44.82944, -72.45465
6.4	Browns Ledges	Lowell	44.83096, -72.45900
6.5	Browns Ledges	Lowell	44.82948, -72.45616
7.1	Green RRSP	Hyde Park	44.64053, -72.52703
8.1	Mississquoi Ledges	Lowell	44.80700,-72.45557
8.2	Mississquoi Ledges	Lowell	44.80702, -72.45556
8.3	Mississquoi Ledges	Lowell	44.80758, -72.45580
8.4	Mississquoi Ledges	Lowell	44.80674, -72.45579
8.5	Mississquoi Ledges	Lowell	44.80676, -72.45587
8.6	Mississquoi Ledges	Lowell	44.807574,-72.454993
9.1	W Farman Hill	Westfield	44.83426, -72.42493
9.2	W Farman Hill	Westfield	44.83400, -72.42625
9.3	W Farman Hill	Westfield	44.83414, -72.42913
9.4	W Farman Hill	Westfield	44.83402, -72. 426306
10.1	Belvidere Mines	Eden	44.75639,-72.52723
10.2	Belvidere Mines	Eden	44.75713, -72.52892
10.3	Belvidere Mines	Eden	44.75656, -72.52737

Appendix Table 2: Flow cytometry results

Site Patch #	Collection #	Event #	1C Value(pg)	Ploidy	Species ID
5.1	215	838	9.67	4x	V
5.1	216	2362	15.65	5x?	?
5.2	210	5103	11.79	4x	V
5.2	211	2361	17.93	5x	?
5.3	175	1252	10.35	4x	V
5.3	214	689	13.63	5x?	?
6.2	130	1673	4.48	2x	A
6.2	131	1150	5.27	2x	A
6.2	199	1422	12.39	4x	?
6.2	200	1143	9.5	4x	V
6.3	134	2748	10.61	4x	V
6.3	135	72	5.96	2x	A
6.3	225	1447	14.05	5x?	?
6.4	139	2602	9.68	4x	V
6.5	164	1581	6.38	2x	A
8.2	144	841	5.37	2x	A
8.2	145	1962	4.84	2x	A
8.5	149	2246	11.96	4x	V
8.5	151	1726	10.17	4x	V
8.6	154	1600	9.05	4x	V
9.2	156	844	4.95	2x	A
9.2	157	2066	6.33	2x	A
9.2	191	922	5.64	2x	A
9.3	160	1232	15.81	5x?	?
9.3	161	1477	17.31	5x?	?
9.4	185	3883	7.5	3x	Triploid
10.1	169	3289	12.28	4x	?
10.1	170	1169	14.01	5x?	?
10.2	171	3219	8.99	4x	V
10.2	172	1501	8.59	4x	V
10.2	173	1477	12.79	4x	?

Appendix Table 3: Mean 1C values calculated from flow cytometry results

Ploidy	Mean 1C Values	SD 1C Values
2x	5.47	.66
4x	10.03	1.09
3x	7.5	NA