

**POPULATION GENETICS OF MICHAUX'S SUMAC,
SMOOTH SUMAC, AND THEIR HYBRIDS**

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ABSTRACT

Michaux's sumac (*Rhus michauxii*) is a federally endangered rhizomatous shrub endemic to the southeastern United States, with two of the largest populations located at Maneuver Training Center--Fort Pickett, VA (Fort Pickett), and a nearby property, Deepwater. Michaux's sumac requires soil disturbance and fire to maintain healthy populations. Before being added to the endangered species list, 47% of populations were extirpated due to habitat loss, fire suppression, and hybridization with smooth sumac (*Rhus glabra*). Concerns with hybridization include hybrid swamping if hybrids are fertile, or outbreeding depression if hybrids display reduced fitness. I used genotyping-by-sequencing to estimate the extent of hybridization at Fort Pickett and Deepwater, and to assess how such hybridization may impact survival of Michaux's sumac as a distinct species at each of these locations. Additionally, population structure was examined using DAPC (discriminant analysis of principal components) and Admixture analyses to determine whether the colonies at Fort Pickett and Deepwater make up separate populations, meta-populations, or one large population. Analysis of 107,344 SNPs (single nucleotide polymorphisms) using *Introgress* and Admixture software suggested widespread hybridization at both Fort Pickett and Deepwater, with hybrids present in most of the sampled colonies. Population structure analyses revealed differentiation between the Fort Pickett and Deepwater populations, but little evidence of separate populations among the colonies sampled at Fort Pickett. These results are important for conservation planning to ensure the long-term survival of Michaux's sumac at Fort Pickett and Deepwater and can be used to help inform future management decisions.

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GENERAL AUDIENCE ABSTRACT

Michaux's sumac (*Rhus michauxii*) is a small, federally endangered shrub endemic to the southeastern United States, with populations remaining in North Carolina, Virginia, and Georgia. To date, the largest known colonies of Michaux's sumac are in Virginia at Maneuver Training Center--Fort Pickett, VA (Fort Pickett), and at a nearby privately owned property called Deepwater. Michaux's sumac requires soil disturbance and fire to reduce competition and maintain healthy populations. It currently faces threats from habitat loss due to agricultural land use and fire suppression, and hybridization with a closely related species, smooth sumac (*Rhus glabra*). Hybridization is a threat to Michaux's sumac at Fort Pickett and Deepwater because it co-occurs with smooth sumac throughout the area. This study determined how much smooth sumac and Michaux's sumac are hybridizing in these locations and assessed whether hybridization is a threat to the long-term survival of the populations at each site. A secondary goal of the study was to gain a better understanding of how genetically similar the colonies within and between locations are to one another. Understanding the level of hybridization and the population structure of Michaux's sumac is important for making management decisions to protect the species. I found widespread hybridization between Michaux's and smooth sumac, with hybrid individuals at nearly all the colonies sampled. Additionally, there is evidence that Fort Pickett and Deepwater comprise two distinct populations, but the colonies inside each area are likely not separate populations. These results will inform future conservation management decisions for the species.

DEDICATION

Dedicated to Jimmy for his unwavering support and encouragement, and to Zooey, Lexi, Clark, and Gus for always making me laugh.

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CHAPTER 1: A REVIEW OF THE LITERATURE

Introduction

Michaux's sumac (*Rhus michauxii* Sargent) is a dioecious, rhizomatous shrub that was first described by André Michaux in 1794 as *Rhus pumila*, due to its short stature. In 1895, Charles Sargent renamed *Rhus pumila* to *Rhus michauxii* after it was discovered that a different species had already been given the name *R. pumila* (Barden and Matthews 2004). Michaux's sumac is endemic to the southeastern coastal plain and lower Piedmont of the eastern United States, with occurrences originally recorded in Georgia, the Carolinas, and Florida. Michaux's sumac's abundance has declined sharply, with 47% of the known populations extirpated by 1989 (USFWS 1989). The main factor contributing to its decline is habitat loss due to human activity, including agricultural land usage, silviculture, industrial and residential development, and fire suppression. An additional threat to the species survival is the isolation of single sex-colonies. Currently, Michaux's sumac can be found only in small populations in Virginia, North Carolina, and Georgia, with the largest known colonies located on land managed by the U.S. Department of Defense. Due to its steep decline and threats to its habitat, as well as inadequate regulations for its protection, Michaux's sumac was added to the Endangered Species list in 1989 (USFWS 1989).

Morphology

Michaux's sumac ranges in height from 0.2 to 1 m tall and is densely pubescent, which sets it apart from other sumac species (Weakley et al. 2012). Leaves consist of 9 to 13 oblong, serrated leaflets that are 4 to 9 cm in length on a smooth wingless rachis. Small yellowish-green flowers form in a terminal cluster and bloom in June. In females, small terminal clusters of bright red fruit develop from August to September. While Michaux's sumac is typically dioecious, there is

evidence that stems may be hermaphroditic or change from male to female or vice versa from year to year depending on resource availability (Savage et al. 1991). Emrick and Jones (2008) also found that high competition reduces the density of all flowering stems in a colony, with female stems affected more strongly than male stems.

Reproduction

Dioecious flowering plants are rare, making up only 6% of angiosperms, and often are at higher risk of extinction due to pollination failure and isolation of single-sex populations (Renner and Ricklefs 1995; Youngsteadt et al. 2019). While Michaux's sumac can also reproduce clonally through rhizomatous growth, to sexually reproduce it relies on insect pollinators (USFWS 1993). Youngsteadt et al. (2019) found that Michaux's sumac attracts diverse pollinators at rates high enough to sustain sexual reproduction in populations with stems of both sexes. Bees in the genus *Megachile* were the most successful pollinators for the species, but a total 55 different arthropod species were recorded visiting Michaux's sumac flowers. The authors concluded that pollination failure is not likely a threat to Michaux's sumac in populations with both male and female stems. Restoration of colonies with stems of both sexes is vital to the continued sexual reproduction of Michaux's sumac.

Germination

Michaux's sumac has a low germination rate, but little is known about its germination requirements (Wilkinson et al. 1996). Its seeds are encased in a hard coating that is impervious both to water and to dry heat that would be associated with fire (Bolin et al. 2011). Scarification with sulfuric acid and boiling water in laboratory studies resulted in successful germination at

low rates (Wilkinson et al. 1996). The natural mechanism for breaking dormancy in Michaux's sumac seeds is currently unknown, but is believed to be related independently or in combination to daily minimum and maximum temperature changes and scarification in the digestive tracts of birds that eat the fruit (Bolin et al 2011).

Range

Michaux's sumac typically grows in a sandy or sandy loam soil and requires a combination of soil disturbance and fire to thrive (USFWS 1993). Soil disturbance is believed to stimulate rhizomatous growth, while fire reduces competition for sunlight and soil nutrients (Emrick and Jones 2008). Michaux's sumac is commonly found on sites with high levels of P, Ca, and K; it is unclear whether these nutrient levels are a requirement for development or an artifact of the frequent fires required for Michaux's sumac survival (Emrick and Hill 1998). Michaux's sumac is shade-intolerant and grows best in sandy or rocky open woods.

In 1993, the largest population at the time was discovered at Army National Guard - Maneuver Training Center-Fort Pickett (Fort Pickett) in southeastern Virginia, near Blackstone (Flemming and Van Alstine 1994). Fort Pickett is located in the lower Piedmont, with mild winters and hot, humid summers. Rainfall is evenly distributed throughout the year and averages 102cm. Soils are generally nutrient-poor sandy loam, which is ideal for Michaux's sumac (Emrick and Hill 1998). Vegetation at Fort Pickett is consistent with a typical eastern deciduous forest, with the most common forest cover types consisting of natural and planted pine, pine-hardwood, upland hardwood, bottomland hardwood, and swamp hardwood. A small portion of training land (5%) consists of maintained mid-early successional grassland and scrubland for military training and wildlife management (Emrick and Hill 1998). Fort Pickett covers

approximately 18,282 ha, with 10,120 ha of training land and 4,251 ha of Controlled Access Area (CAA) for live-fire exercises. Frequent fires related to military training activity create ideal habitat conditions for Michaux's sumac to grow, and consequently, most of the Michaux's sumac colonies at Fort Pickett occur within the CAA (Emrick and Hill 1998, Smith and Van Alstine 1995). While many previously discovered populations were single sex and isolated on roadsides or railroad tracks, the Fort Pickett population is comprised of several self-sustaining colonies with both male and female stems, making it particularly valuable for conservation (Emrick and Hill 1998).

In 2011, the largest population to date was discovered on private land owned by the Ward Burton Wildlife Foundation (WBWF; Teets and Emrick 2012). The 191-ha property, known as Deepwater, was acquired by WBWF as part of the Army Compatible Use Buffer program. Deepwater is approximately 600m from the southeast border of Fort Pickett, and roughly 4km from the closest known colony of Michaux's sumac on Fort Pickett. Because of its proximity to Fort Pickett, Deepwater has comparable climatic and geological conditions. The soil composition at Deepwater consists of a top layer of sandy loam soil over a layer of clay loam. Prior to WBWF acquiring Deepwater, the property was harvested, burned, and planted with loblolly pine. A 2011 survey of Deepwater found 124 total colonies of Michaux's sumac, with a higher stem density of male, female, and total stems compared to Fort Pickett, and over 50% of counted stems being fertile (Teets and Emrick 2012). After the discovery of Michaux's sumac on the property, a prescribed burn has been implemented every 3-5 years to reduce shade and soil resource competition to help support the Michaux's sumac population there (Hammond 2016).

Transplantation efforts

Since its listing, there have been multiple transplantation efforts to maintain Michaux's sumac populations. The first published attempt at transplanting 47 rhizomes from 5 different wild populations was unsuccessful (Boyer 1993). Following this attempt, Emrick (2003) successfully transplanted rhizomes from a small colony of individuals at Fort Pickett to mitigate conflicts with the construction of a live-fire range. While this transplantation was considered successful, only about 25% of rhizomes resulted in above-ground stems the following growing season.

In an effort to increase transplantation success and rescue a clonal colony from destruction during the construction of a North Carolina expressway, Braham et al. (2006) designed and implemented a study testing the success of different transplantation techniques. Two propagule types – roots only and roots plus shoots – were either transplanted immediately or grown in a greenhouse for 10 months before being transplanted. While the propagule type did not appear to impact survival, individuals grown in the greenhouse had a significantly higher survival rate than those transplanted directly to a new site. Both transplanted populations were successful and resulted in healthy, well-established colonies. Using the methods described by Braham et al. (2006), Emrick et al. (2018) successfully transplanted rhizomes from three additional colonies located on a firing range at Fort Pickett to the same site as the 2003 transplantation. These studies showed that the establishment of new colonies through transplantation is possible, but care must be taken when choosing both the colonies to transplant and the transplantation site.

Hybridization

Rhus glabra (smooth sumac) is a closely related sumac species that has been documented to hybridize with Michaux's sumac (Hardin and Philips 1985; Yi et al. 2004). Smooth sumac is one of the most widespread woody species in North America, and co-occurs throughout Michaux's sumac's range, including at both Fort Pickett and Deepwater (Burke and Hamrick 2002; Weakley et al. 2012; Teets and Emrick 2012). Much like Michaux's sumac, it is drought-resistant and shade-intolerant (Weakley et al. 2012). It is found in open fields, roadsides, and burned areas on acidic sandy or gravelly soil. Smooth sumac is a rhizomatous, dioecious shrub, and ranges in height from 0.5-6.0m (Johnson 2000). Leaves are 3-5 cm long and consist of 11-31 lanceolate or oblong-lanceolate leaflets that are 7-9cm long (Weakley et al. 2012). Leaflets are dark green on top and pale on the underside. Small greenish-yellow flowers are produced in erect terminal clusters from June to July, with clusters of 100-700 small red fruit forming from August to September. Smooth sumac and Michaux's sumac share many phenotypic characteristics, but smooth sumac is typically taller, has more leaflets per leaf, and lacks Michaux's sumac's characteristic dense pubescence. The first third of smooth sumac's flowering season overlaps with the last third of Michaux's sumac's flowering season (Hardin and Philips 1985).

Instances of hybridization between Michaux's sumac and smooth sumac have been recorded in the field, and experimental crosses have shown that about 20% of hybrid seeds from those crosses are viable (Hardin and Philips 1985). Few genetic studies have been completed, however, to determine the extent of hybridization and its possible impacts on the parental species. Hybrid swamping and outbreeding depression between an endangered species and a widespread species often reduce long-term fitness of the endangered species (Gompert et al. 2017). Hybrid swamping occurs when hybrid individuals are fertile and cross with individuals of

the rarer species, causing a genetic assimilation, or swamping, over time from the more common species. Outbreeding depression has the opposite effect, with hybrid individuals exhibiting reduced fitness levels, causing the parental species to expend energy reproducing only to have low to no offspring survival.

Burke and Hamrick (2002) completed one of the first genetic studies of the hybridization between Michaux's sumac and smooth sumac. They identified a fixed allelic difference at the *Idh2* locus and were able to confirm the occurrence of hybridization in five of eleven sampled populations at Fort Pickett. Despite this result, the number of hybrids per population was low, and hybridization was considered uncommon, with the authors stating that hybridization is likely not a threat to Michaux's sumac populations at Fort Pickett. Using Nei's genetic distance, hybrid populations were more similar to Michaux's sumac (Nei's $I = 0.97$), than smooth sumac (Nei's $I = 0.89$). While this study sought to genetically confirm hybridization in natural populations, they were unable to determine the frequency of hybridization, or to confidently confirm hybrid fertility or backcrossing. Additionally, hybrid populations sampled in the field were phenotypically *R. michauxii*-like and likely only represented a portion of total hybrids between the species.

Genetic Diversity

Endemic species, like Michaux's sumac, typically have lower levels of genetic diversity than more widespread species, with both fewer polymorphic loci, and fewer alleles per polymorphic locus (Hamrick et al. 1992). The loss of genetic diversity in these species increases their risk for extinction due to limited opportunities for sexual reproduction resulting from their fragmented ranges (Sherman-Broyles et al. 1992). A lack of opportunities for sexual

reproduction is especially concerning for isolated single-sex colonies of Michaux's sumac, as they must rely solely on clonal vegetative growth through the rhizomes, which reduces their ability to adapt to changing environments.

Sherman-Broyles et al. (1992) used allozyme markers to compare levels of genetic diversity among nine populations of Michaux's sumac, six populations of smooth sumac, and six populations of winged sumac (*Rhus copallinum* L.) in North Carolina. Across 17 loci, they found that Michaux's sumac had significantly less genetic diversity than both smooth and winged sumac, with fewer polymorphic loci and fewer alleles per polymorphic locus both at the species level and within populations. These results are typical of endemic species with similar life histories, and the authors noted that it is likely that Michaux's sumac experienced a genetic bottleneck and has always exhibited less genetic diversity than other *Rhus* species. This study also found that Michaux's sumac had higher levels of among-population differentiation, which could be due to clonal reproduction, founder effects, and random genetic drift than did other sumac species. Of the nine Michaux's sumac populations in this study, five were single-sex colonies, and two colonies had unbalanced sex ratios with few female stems, meaning that likely only limited sexual reproduction was occurring.

Burke and Hamrick (2002) used allozyme markers to determine and compare levels of genetic diversity in six populations of Michaux's sumac, one population of smooth sumac, and four populations of putative hybrids at Fort Pickett and one population of Michaux's sumac in North Carolina. A total of 11 polymorphic loci were resolved, with the *Idh2* locus acting as a diagnostic locus for hybrids because of fixed allelic differences between the parental species. All four putative hybrid populations and one putative Michaux's sumac population were genetically identified as hybrids using the fixed *Idh2* marker locus. The five identified hybrid

populations were found to have higher levels of genetic variation than both parental species populations studied. Additionally, Michaux's sumac populations at Fort Pickett showed higher levels of genetic diversity than the North Carolina populations studied by Sherman-Broyles et al. (1992), likely because the colonies at Fort Pickett are larger and contain both sexes, while most of the North Carolina populations sampled were small, single-sex colonies that would be more affected by random genetic drift. Fort Pickett populations also displayed lower levels of among-population genetic differentiation than the North Carolina populations, likely because colonies are much closer and may experience gene flow between colonies.

Genotyping-by-sequencing

Both of the studies described above used allozyme markers to measure genetic diversity, but new DNA sequencing tools have since been developed, such as genotyping-by-sequencing (GBS) that could provide more insight into population structure. Genotyping-by-sequencing (GBS) is a low-cost, high-throughput approach that can be applied to a variety of species and studies. It utilizes restriction enzymes to reduce genome complexity by avoiding highly repetitive sequences (Elshire et al. 2011). GBS is especially useful for studies on wild populations of non-model organisms, particularly for those without an existing reference genome, as it allows for the genomes of hundreds of individuals to be sequenced without prohibitive costs (Narum et al. 2013). GBS does have drawbacks however. Due to the random distribution of enzyme cut sites throughout the genome, specific regions of the genome cannot be targeted; only certain-sized fragments with enzyme cut sites on both sides are sequenced (Holliday et al. 2018). Additionally, missing data is a common problem when using GBS. Despite these limitations, GBS is commonly used in population genomics, both for studying

within- and between-population diversity, and for studies focusing on hybridization (Narum et al. 2013). GBS has been used successfully across a range of species and studies, and remains a popular choice in ecological and conservation genomics studies.

Conclusion

Due to its endemic nature, population genetic research on Michaux's sumac has been relatively limited. Previous studies using allozyme markers were able to confirm hybridization and determine and compare genetic diversity in populations of Michaux's sumac (Sherman-Broyles et al. 1992; Burke and Hamrick 2002), but the extent of hybridization between Michaux's sumac and smooth sumac is a knowledge gap that needs to be filled. In the time since these historical studies were conducted, the largest population of Michaux's sumac to date, Deepwater, was discovered. Additionally, new genomic tools, like GBS, have become available that will allow for a greater understanding of hybridization and an updated assessment of the within and between population genetic diversity in both the Fort Pickett and Deepwater populations. GBS sequencing will allow for a better understanding of population structure and help determine the genetic interconnectedness of the Fort Pickett and Deepwater populations. It can help elucidate whether individual colonies represent single populations, meta-populations, or multiple populations.

In order to be removed from the Endangered Species list, there must be 19 self-sustaining populations in locations where they, and their habitat, are protected from human-related and natural threats that might interfere with their long-term survival (USFWS 1993). A self-sustaining population is defined as a population expanding either clonally or reproducing sexually and which is genetically viable. As of 2014, based on the Natural Heritage Program's

ranking criteria, there are 22 populations throughout Georgia, North Carolina, and Virginia that could be considered self-sustaining, but require further monitoring for confirmation of this status (USFWS 2014).

Identifying populations with the highest levels of genetic diversity could be extremely helpful in designing and implementing transplantation or propagation efforts to establish new populations and increase the number of self-sustaining, protected populations. Additionally, understanding how many distinct, genetically viable, protected, Michaux's sumac populations already exist can also help inform management decisions, and potentially allow for the down-listing or de-listing of Michaux's sumac under the U.S. Endangered Species Act.

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CHAPTER 2: POPULATION GENETICS OF MICHAUX'S SUMAC

Introduction

Michaux's sumac (*Rhus michauxii* Sargent) is a federally Endangered, dioecious, rhizomatous shrub endemic to the southeastern United States (USFWS 1989). It was named by Charles Sargent in 1895, although he initially falsely believed it to be "...one of the most poisonous plants in North America", which earned it the colloquial name false poison sumac (Barden and Matthews 2004). Michaux's sumac is shorter than other *Rhus* species, and ranges in height from 0.2-1m tall, which has earned it another one of its colloquial names, dwarf sumac (Weakley et al. 2012). Its leaves consist of 9 to 13 oblong, serrated leaflets that are 4 to 9 cm in length on a smooth, wingless rachis. The entire plant is densely pubescent, distinguishing it from other closely related species.

Small yellowish-green flowers form in a terminal cluster and bloom in June. Michaux's sumac is insect-pollinated and requires colonies with stems of both sexes in order to sexually reproduce (Youngsteadt et al. 2019). From August to September, bright red fruit are borne in terminal clusters on female plants (USFWS 1989). While Michaux's sumac is typically dioecious, there is evidence that some stems may be hermaphroditic (Savage et al. 1991). Stems have also been observed to change from male to female or vice versa from year to year depending on resource availability.

Michaux's sumac is endemic to the inner coastal plain and Piedmont of the southeastern United States, with occurrences originally recorded in Georgia, the Carolinas, and Florida (USFWS 1993). It grows best in sandy or sandy loam soils and is typically found in sandy or rocky open woods. Michaux's sumac requires soil disturbance and fire to maintain healthy populations, with the soil disturbance believed to stimulate rhizomatous growth, and fire to

reduce competition for sunlight and soil resources (Emrick and Jones 2008). Michaux's sumac's abundance has declined sharply, with 47% of known populations extirpated by 1989 (USFWS 1989). Because of this decline and threats to the survival of the species – including agricultural land usage, silviculture, industrial and residential development, isolation of single-sex colonies, fire suppression, and hybridization with smooth sumac (*Rhus glabra*) – Michaux's sumac was added to the Endangered species list in 1989. Currently, populations of Michaux's sumac can be found only in Virginia, North Carolina, and Georgia, with the largest known colonies occurring on military land.

In 1993, a large population of Michaux's sumac was discovered at Army National Guard - Maneuver Training Center Fort Pickett (Fort Pickett) in southeastern Virginia, near Blackstone Virginia (Flemming and Van Alstine 1994). The Fort Pickett population consisted of 74 colonies, with many self-sustaining colonies containing stems of both sexes (Smith and Van Alstine 1995; Emrick and Hill 1998). This made the Fort Pickett population particularly valuable, as many other known colonies at the time were small single-sex colonies isolated to roadsides, railroads, and along power lines. Frequent fires associated with military training at Fort Pickett are believed to contribute to the health of the colonies found there.

In 2011, the largest population of Michaux's sumac known to date was discovered at a 191-ha property known as Deepwater that was obtained by the Ward Burton Wildlife Foundation (WBWF) through the army compatible use buffer program (Emrick and Teets 2012). Deepwater is about 600m from the southeast border of Fort Pickett, and about 4km from the closest known colony of Michaux's sumac. Shortly before Deepwater was obtained by WBWF, it was harvested and burned, and loblolly pine was planted, which likely contributed to the success of the Michaux's sumac population there. A survey of the colonies at Deepwater by Teets and

Emrick (2012) found a higher density of flowering stems of both sexes compared to other previously discovered populations, with 50% more fertile stems overall than Fort Pickett. After the discovery of Michaux's sumac on the property, a prescribed burn has been implemented every 3-5 years to reduce shade and soil resource competition to help support the Michaux's sumac population there (Hammond 2016).

A major threat to Michaux's sumac throughout its range is hybridization with smooth sumac (*Rhus glabra*; Hardin and Phillips 1985). Smooth sumac is a widespread woody plant species in North America, and is closely related to Michaux's sumac, with one study even suggesting that Michaux's sumac may have been derived from smooth sumac (Sherman-Broyles 1992). Like other *Rhus* species, including Michaux's sumac, smooth sumac is a dioecious, rhizomatous shrub. It is 0.5- 6.0m tall, with leaves that are 3-5 cm long and consist of 11-31 lanceolate or oblong-lanceolate leaflets that are 7-9cm long (Weakley et al. 2012). Small greenish-yellow flowers are produced in erect terminal clusters from June to July, with clusters of small red fruit forming from August to September. The first third of smooth sumac's flowering season overlaps with the last third of Michaux's sumac's flowering season (Hardin and Phillips 1985). Hybridization with such a widespread species is a major threat to Michaux's sumac, with the associated threats of both outbreeding depression and genetic swamping (Gompert et al. 2017). Outbreeding depression occurs when hybrid individuals exhibit reduced fitness, causing parental species to expend energy reproducing with little offspring survival. Hybrid swamping occurs when hybrid individuals are fertile and backcross with individuals of the more rare species, causing a genetic assimilation, or swamping, over time from the more common species.

Instances of hybridization with smooth sumac have been recorded in the field, and tested with controlled crosses in the laboratory, with 20% of seeds from these crosses viable (Hardin and Philips 1985). Using allozymes, Burke and Hamrick (2002) identified a fixed allelic difference at the *Idh2* locus that could be used as a diagnostic locus to genetically confirm hybridization. In a study of eleven colonies from Fort Pickett, hybridization was confirmed in five colonies. They noted that hybridization in those colonies did not appear to be widespread and was likely not a threat to Michaux's sumac. However, while they were able to confirm hybridization, because of limitations of having just one genetic marker, they were unable to identify potential later-generation hybrids.

Another threat to Michaux's sumac is a lack of genetic diversity. Endemic species have fragmented ranges and are therefore at a higher risk for extinction and loss of genetic diversity (Hamrick et al. 1992). Sherman-Broyles et al. (1992) found that Michaux's sumac colonies in North Carolina had much lower levels of genetic diversity than to other *Rhus* species. Additionally, they found that Michaux's sumac had higher levels of among-population differentiation, which could be due to clonal reproduction, founder effects, and random genetic drift. A follow-up study by Burke and Hamrick (2002) looked at genetic diversity in populations at Fort Pickett using allozymes. They found that the larger colonies containing stems of both sexes found at Fort Pickett had higher levels of genetic diversity and more gene flow among colonies than did smaller, more isolated colonies studied in North Carolina. This finding reinforces the threat of isolation of single-sex colonies to the long-term survival of Michaux's sumac.

In the time since the last genetic studies on both hybridization and genetic diversity were conducted, the largest colony-based upon stem number-to date, Deepwater, was discovered.

Additionally, new next-generation DNA sequencing tools have become available that can provide more insight into the extent of hybridization and population structure of these colonies. The goals of this study were to: 1) determine the extent of hybridization in colonies of Michaux's sumac at Fort Pickett and Deepwater; 2) determine whether hybrids are fertile and backcrossing to the parental species; 3) better understand the population structure of the Deepwater and Fort Pickett populations, and determine whether they are one population, a meta-population, or many populations. This information can inform management decisions, and potentially allow the down-listing or de-listing of Michaux's sumac under the U.S. Endangered Species Act.

Materials and Methods

Collection

In June of 2019 and 2020, leaflet samples were collected from Fort Pickett Army Maneuver Training Center ($n=276$) and Deepwater army compatible use buffer zone ($n=187$) near Blackstone, Virginia (Figure 1). Each individual was field-identified as either Michaux's sumac ($n=348$), smooth sumac ($n=42$), or a hybrid ($n=73$). The youngest 3-5 leaflets were taken from each sampled individual using forceps and placed in labeled coin envelopes. Sample envelopes were stored in plastic bags containing silica gel until they could be moved to -80°C freezers. Additional data, including colony number, plant height, flowering status, flower sex, and GPS coordinates, were recorded for each individual. For a subset of individuals, full leaf samples were taken for later morphometric analysis.

Genomic Library Preparation

Between 0.5-1.0g of leaf tissue was weighed into a 2-ml conical tube for each sample depending on leaf quality, with fresh young tissue requiring less tissue to reach the desired DNA

concentration. Tissue samples were ground to a fine powder using the 2000 Geno/Grinder bead mill (SPEX Sample Prep, Metuchen, NJ) at 1100rpm for 4 cycles of 45 seconds each with sample tubes submerged in liquid nitrogen after each grinding cycle to prevent thawing. DNA was initially extracted from all samples using a modified Qiagen DNeasy plant mini kit (Qiagen, Hilden, Germany) extraction protocol with 600 μ l 24:1 phenol:chloroform used in place of the QiaShredder columns to increase yield. All sample concentrations were quantified using a Nanodrop ND-1000 spectrophotometer. Samples with low concentrations ($< 5\text{ng}/\mu\text{l}$), were re-extracted using a CTAB extraction protocol followed by purification using the Monarch PCR and DNA Cleanup Kit (New England Biolabs, Ipswich, MA).

GBS library preparation was carried out for all samples with a concentration greater than $5\text{ng}/\mu\text{l}$. For each sample, 200ng of DNA were digested with 1 μ l *ApeK1* enzyme at 75°C for 2 hours. Illumina-compatible individual barcode adapters were then ligated to digested samples using 1.6 μ l T4 DNA ligase (New England Biolabs, Ipswich, MA). Samples were incubated at 22°C for 1 hour followed by 30 minutes at 65°C . To check samples for successful ligation, 18 cycles of PCR were performed, and samples were then run in a 1% agarose gel for 1 hour at 100V. Samples that successfully amplified ($n=390$) were used for pooling; 6 μ l of each sample were chosen randomly for pooling into each library, with 77-81 samples per library, and a total of 5 libraries. After pooling, samples were purified with the Monarch PCR and DNA cleanup kit (New England Biolabs) following the manufacturer's protocol, with a total final elution of 40 μ l per library.

In ten 50- μ l reactions, 20 μ l of each pooled library (2 μ l per reaction) were amplified through 18 cycles of PCR. All ten PCR reactions for each library were then combined, and purified using the Monarch PCR and DNA cleanup kit following manufacturer's protocol, with a

total elution of 30 μ l per library. Size selection for amplification fragments between 250-600bp was performed using the Blue Pippin instrument (Sage Science, Inc., Beverly, MA). After size selection, DNA concentrations for each library were quantified using the Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, Waltham, MA), and 1 μ l of each library was diluted to 3ng/ μ l for use on a Bioanalyzer instrument (Agilent 2100; BioAnalyzer, Santa Clara, CA). The Bioanalyzer high sensitivity DNA kit (Agilent 2100; BioAnalyzer, Santa Clara, CA) was used to check each library for proper sizing and quality following the manufacturer's protocol. Libraries were then sequenced on an Illumina HiSeq 6000 instrument with an S-Prime flow cell in 150bp paired-end mode at the Duke Center for Genomic and Computational Biology (Duke University, Durham, NC).

Data analysis

Libraries were demultiplexed using the Stacks (Catchen et al. 2013) *process_radtags* function. Quality trimming, paired-end assembly, read mapping, and SNP calling was done using the Ddocent pipeline (Puritz et. al 2014). In the first step of the pipeline, reads were quality trimmed using TRIMMOMATIC (Bolger et al. 2014) to remove any bases below a quality score of 20 and Illumina adapters. In the second step, Rainbow (Chong et al. 2012) was used to create a *de novo* reference sequence, and BWA (Li & Durbin 2009) was used to align the reads to this reference sequence. In the final step, FREEBAYES (Garrison & Marth 2012) was used for SNP calling and VCFtools (Danecek et al. 2011) was used for filtering to keep only SNPs called in >90% of individuals. The default parameters were used across the entire pipeline. Ddocent assembled a total of 129,736 sequences into 14,721 contigs.

Downstream filtering was done using GATK -4.0.1.1 (Van der Auwera and O'Connor 2020) to remove SNPs with a depth-normalized quality score (QD) less than 2.0, a root mean square of the mapping quality (MQ) less than 40.0, a strand bias value (FS) greater than 40.0, a median mapping quality (MQRankSum) less than -12.5, an Allele-specific Rank Sum Test (ReadPosRankSum) score less than -8.0, and a strand bias estimate (SOR) greater than 3.0. QD filters out low quality bases, and is normalized by the sequencing depth in order to put the quality score into perspective based on the amount of coverage available, since reads with deep coverage may have artificially high quality scores. MQ estimates the overall mapping quality of the reads and is averaged across all the samples to filter out bases with low mapping quality. Similarly, MQRankSum uses a Rank sum test to compare the mapping quality of reads supporting the reference allele versus those supporting the alternate allele and filters out alternate alleles with a low mapping quality score compared to the reference allele. FS estimates strand bias using Fisher's Exact Test, and filters out reads with strand bias present. Strand bias is a sequencing bias that favors one DNA strand over the other and can lead to incorrect allele calls if it is present. Similarly, SOR estimates allele-specific strand bias using the Symmetric Odds Ratio Test, which is an updated form of Fisher's Exact Test and takes into account larger amounts of data with higher coverage. ReadPosRankSum is an allele-specific rank sum test that compares the reference and alternate alleles and checks for evidence of bias in the position of alleles within the reads that support them. Alleles near the end of reads are typically caused by sequencing error, so this test can determine if there is any difference in where the alleles are found relative to the ends of reads, and remove reads if alternate alleles are found near the ends of reads more often than reference alleles are. VCFtools (Danecek et al. 2011) was also used for further filtering to remove any SNPs with more than 10% missing data, a minor allele frequency less

than 0.01, and with two or fewer alleles. After filtering, data for 382 individuals and 107,344 loci were kept for further analysis.

Admixture (Alexander 2011) was used to estimate ancestry of individuals to help determine population structure in the sampled colonies. Admixture was run first using all individuals sampled, but subsequently was run with only individuals field-identified as Michaux's sumac, individuals from Fort Pickett only, and individuals from Deepwater only. In all cases, cross validation was used to determine the best K -value between 1 and 10. Log likelihood was plotted for each of the four runs, and the best K -value for each run was determined. Results for each were then plotted in R and mapped to each colony based on each individual's GPS coordinates using the *scatterpie* package.

Population structure was further examined using a DAPC analysis in the Adegenet R package (Jompert 2008) to identify genetic clusters. First, the *find.clusters* function was used to identify the optimal number of clusters using sequential k means clustering, with the max number of clusters set to 60. All PCs were retained, and the number of clusters ($k=4$) was chosen on the basis of the lowest associated Bayesian Information Criterion (BIC), or until increasing K no longer resulted in an improvement of BIC. Next, the *dapc* function was used to describe the clusters by transforming the data using PCA and retaining the minimum number of principle components that maximize the variance explained ($n=150$) based on the graph of number of PCs retained versus cumulative variance.

Hybrid indices between 0 and 1 were calculated in R Version 3.5.3 (R Core Team 2020) for 322 individuals using *est.h* in the Introgress software package (Gompert and Buerkle 2010). Of the samples field-identified as Michaux's sumac, 50 were selected from the DAPC analysis as having the highest likelihood of being Michaux's sumac, with the 30 most phenotypically *R*.

michauxii-like used in parental population 1. All samples field-identified as smooth sumac ($n=30$) were used in parental population 2. Hybrid indices were then plotted against field identifications to determine the rate of misidentification. Additionally, based on their hybrid indices, individuals were *post-hoc* re-identified as Michaux's sumac (0.0-0.20), smooth sumac (0.80-1.0), F₁ hybrids (0.40-0.60), or hybrids backcrossed to Michaux's (0.21-0.39) or smooth (0.61-0.79) sumac. These cutoffs were chosen based on the idea that an F₁ hybrid would receive 50% of their DNA from each parent, resulting in a hybrid index of .50. Following this, an F₁ hybrid backcrossed to Michaux's sumac would have about 75% Michaux's sumac ancestry and 25% smooth sumac ancestry, resulting in a hybrid index of about 0.25, and an F₁ hybrid backcrossed to smooth sumac would have about 75% smooth sumac ancestry and 25% Michaux's sumac ancestry, resulting in a hybrid index of about 0.75. Cutoffs for each classification were then evenly chosen around these values.

Population connectivity (F_{st}) and nucleotide diversity (π) were calculated using VCFtools (Danecek et al. 2011). Smooth sumac samples were removed from the dataset before calculations were done. F_{st} was calculated between all 27 colonies sampled at Fort Pickett, and the Deepwater population as a whole, as well as the Fort Pickett population as a whole compared with Deepwater using the *weir-fst-pop* function, which uses Weir and Cockerham's (1984) F_{st} statistic. Colonies with fewer than 4 samples were removed from the F_{st} analysis, and results were visualized using the *heatmaply* R package (Galili et al. 2021). Heterozygosity was calculated using VCFtools -- *het* function for all colonies and both Fort Pickett and Deepwater as a whole. π was calculated for each of the colonies at Fort Pickett and Fort Pickett as a whole, as well as for the Deepwater population using the *window-pi* function with a window size of 10kb.

For the calculation of pi , filtering was redone on the original dataset to exclude the minor allele frequency filtering step.

Results

Hybridization

Introgress analysis showed a high rate of misidentification using standard field observable phenotypic characteristics of Michaux's sumac, with 173 (59%) individuals field-identified as Michaux's sumac being genetically identified as hybrids, and two individuals (1%) genetically identified as smooth sumac (Table 1; Figure 2.1, Figure 2.2, Figure 6). Additionally, hybrids were identified at Deepwater and in 25 of the 27 colonies sampled at Fort Pickett (93%). Deepwater and 14 of the 27 colonies sampled at Fort Pickett (52%) were found to also have true Michaux's sumac present. Of the 229 hybrid individuals identified, 113 (49%) were F₁ hybrids, 79 (35%) were hybrids backcrossed to Michaux's sumac, and 37 (16%) were hybrids backcrossed to smooth sumac. Additionally, when ordered by species genetic identification, Admixture analysis of all samples and locations at $k=2$ (Figure 3.1) showed a clear separation between Michaux's and smooth sumac, with hybrid individuals showing intermediate assignment values. Admixture analysis with only Fort Pickett samples at $k=2$ (Figure 3.2) also showed a clear separation between Michaux's sumac, smooth sumac, and their hybrids. When these results are mapped to the samples' GPS points (Figure 3.3), geographically widespread hybridization was clear, with admixed individuals present in most colonies. DAPC analysis (Figure 4.1) also showed a clear separation between species, with one cluster containing predominantly Michaux's sumac from Fort Pickett, a second cluster containing predominantly Michaux's sumac from Deepwater, a third cluster containing predominantly smooth sumac, and

a fourth cluster composed of mostly hybrid individuals. The two Michaux's sumac clusters and the one smooth sumac cluster grouped on opposite sides, with the hybrid cluster in the center.

Population structure

Admixture analysis including all samples and locations at $k=3$ sorted by colony and genetic ID (Figure 3.4) showed a separation between individuals sampled at Fort Pickett versus Deepwater, across all species. A secondary analysis including only individuals genetically identified as Michaux's sumac at $k=2$ (figure 3.6) supported this finding, with a distinct separation between Fort Pickett and Deepwater samples. Looking at only samples from Fort Pickett at $k=2$ (Figure 3.2), there was no clear distinction amongst colonies, only species. Analysis including only samples from Deepwater found $k=1$ as the best fit to the data. Admixture results including all samples at $k=3$ mapped to their GPS points (Figure 3.5) shows a geographic separation between Fort Pickett and Deepwater populations, with colonies at Fort Pickett that are closer to Deepwater appearing more closely related to Deepwater than those farther north.

DAPC results identified by colony (Figure 4.2) showed little distinction in clusters between colonies at Fort Pickett, but showed a clear separation between Fort Pickett and Deepwater samples. The same figure colored by Fort Pickett versus Deepwater sampling locations clarified this further, with the two clusters identified as predominantly Michaux's sumac appearing to be separated based on their sampling locations with Deepwater and Fort Pickett samples clustering separately (figure 4.3).

Analysis of heterozygosity across all loci found mean observed heterozygosity ranging from 0.085 to 0.146 (Table 3). Analysis of nucleotide diversity across all colonies and Deepwater found mean π values ranging from 1.00×10^{-5} – 1.13×10^{-4} (Table 4). The average F_{st} estimate

between colonies at Fort Pickett was 0.13, and the F_{ST} estimate between Fort Pickett as a whole and Deepwater was 0.026. F_{ST} estimates between all the colonies and Fort Pickett (Table 2; Figure 5) ranged from 0 - 0.368, with the highest F_{ST} between colonies 076 and 203, likely because of the small sample sizes within those colonies (Table 1). Most F_{ST} values were between 0.10-0.19, followed by F_{ST} values less than 0.010, and the fewest F_{ST} values above 0.20.

Discussion

Hybridization

Our results indicate that hybridization is widespread throughout both Fort Pickett and Deepwater populations of *Rhus michauxii* and *glabra*. While previous genetic studies (Sherman-Broyles 1992; Burke and Hamrick 2002) found that hybridization appeared to not be a threat to the long-term survival of Michaux's sumac at these locations, this study finds the opposite. Hybrids are fertile and readily backcross to both Michaux's sumac and smooth sumac in nearly all colonies studied. Even colonies without smooth sumac directly located within or near the colony appear to be affected, with 93% of the sampled colonies at Fort Pickett containing at least one hybrid individual. Because hybrids are widespread, and have been confirmed to be fertile, hybrid swamping is a threat to Michaux's sumac populations, particularly at Fort Pickett where hybridization appears to be more geographically widespread. Hybrid swamping occurs when there is a genetic assimilation over time from the more widespread species. With 69% (263/382) of all samples analyzed containing alleles from smooth sumac, this is a very real concern for Michaux's sumac.

The high rate of misidentification of hybrid individuals in this study using standard field characteristics is also a concern, as distinguishing between Michaux's sumac and hybrids in the field is vital to the management of the species. In total, 59% of samples that were field-identified

as Michaux's sumac using standard field characteristics were genetically identified as hybrids, including F₁ hybrids and hybrids backcrossed to both Michaux's and smooth sumac. Field identification guidelines for sample collection focused mainly on height, degree of pubescence, and rachis coloration. Our findings indicate that many hybrid individuals, even those backcrossed to smooth sumac, may be phenotypically similar to Michaux's sumac in these characters. However, of the individuals field-identified as hybrids, only three (5%) were genetically identified as either Michaux's or smooth sumac; the remaining 56 were either F₁ hybrids ($n=37$) or hybrids backcrossed to smooth sumac ($n=19$). The individuals field-identified as hybrids displayed an intermediate phenotype, indicating that some hybrid individuals, including those with a higher percentage of smooth sumac ancestry, can exhibit more intermediate characteristics. A clearer understanding of the range of hybrid phenotypes is vital for accurately differentiating between true Michaux's sumac and hybrids in the field.

These findings have direct management implications, so it is important to note that our *Introgress* results are subject to some error since the analysis relies on the selection of non-admixed individuals for the parental populations, and this may not be the case for all individuals selected for our analysis. Additionally, since Michaux's sumac and smooth sumac, are closely related, they likely have some standing variation from the ancestor as well as a history of introgression and shared alleles independent of hybridization, which can make the classification of hybrid indices less straight-forward. Finally, the classification of hybrid individuals as F₁ or backcrossed hybrids is imperfect as the cutoff selection for these classes was somewhat subjective reflecting the natural distribution of the dataset. Despite this potential for error, our *Admixture* and DAPC analyses supported our findings that hybridization is widespread, with F₁

and backcrossed individuals present, and that there was a high rate of misidentification of hybrid individuals using standard field characteristics.

Population structure

Based on Admixture and DAPC results, the Deepwater and Fort Pickett populations are genetically distinct populations, despite their close geographic proximity. In both analyses, there was a clear distinction in clustering between the two sampling locations (Figure 3.6; Figure 4.3). While the sampling locations as a whole may represent separate populations, it appears that there is still gene flow occurring between Deepwater and some of the colonies at Fort Pickett that are geographically closer to Deepwater. From a management perspective, these results indicate that Fort Pickett and Deepwater make up two of the 19 self-sustaining populations required for the recovery of Michaux's sumac (USFWS 1989).

Within Fort Pickett, the colonies appear to make up a single population. While F_{st} estimates between some colonies are biologically meaningful ($>.20$), this appears to be due to small sample sizes and higher levels of hybridization in some colonies compared to others rather than due to population structure (Table 1). Colonies 60B, 203, 087, and 076 all have a sample size of less than 4, and appear to account for many of the higher F_{st} estimates. Overall, pairwise F_{st} estimates between most colonies were relatively low and indicated continued gene flow between nearby colonies. This finding is in agreement with Burke and Hamrick's (2002) findings that colonies at Fort Pickett experience high levels of gene flow due to their close proximity.

As found in previous studies (Sherman-Broyles 1992; Burke and Hamrick 2002), the genetic diversity of Michaux's sumac at both Fort Pickett and Deepwater is low. This is to be expected because of its limited geographic distribution and small population sizes, and is in line with those of other species with similar life history traits such as *Paeonia decomposita*, *Frangula*

alnus, and *Alnus maritima* (Wang 2020; Finlay et al. 2017; Gibson 2008). As noted by Hamrick et al. (1992), woody species with an endemic distribution typically have lower levels of genetic diversity than more widespread species, and Michaux's sumac has previously been found to have lower levels of genetic diversity than both smooth and winged sumac. All of the colonies at Fort Pickett and Deepwater had similar levels of genetic diversity.

Conclusion

This study found widespread hybridization between Michaux's sumac and smooth sumac throughout both Fort Pickett and Deepwater. Hybrids were shown to be fertile and able to backcross to both parental species, which is a major threat to the continuing survival of the Endangered Michaux's sumac. Advancements in molecular genetics have allowed for the detection of hybridization in a way that was not possible when the Endangered Species Act was created, leading to difficulties in determining how to handle cases of hybridization in endangered species (O'Brien et al. 1991). Our results are important for the future management of Michaux's sumac at Fort Pickett and Deepwater, and should be taken into account when making decisions related to its recovery. Determining an acceptable level of hybridization and the value of hybrid individuals to the continued survival of Michaux's sumac are important management decisions, and our results will likely be an important factor in these decisions. While both Fort Pickett and Deepwater are individual self-sustaining populations, both populations have relatively low levels of genetic diversity and face threats from hybridization. This study provides important data for future management of Michaux's sumac and should be considered when evaluating the species recovery in relation to the recovery goals set by USFWS. Further studies into the phenotypic variation of hybrid individuals are necessary to better identify hybrids in the field.

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TABLES

Table 1 Number of samples from each colony and their phenotypic field identification compared to the genetic identification from *Introgress* analysis. The Fort Pickett sampling location is made up of the 28 preceding colonies. Location is the colony or larger sampling location (Fort Pickett or Deepwater), *n* is the total number of samples analyzed for each location, phenotypic field ID is the initial species identification, Genetic ID is the species ID from *Introgress* analysis, and Avg. hyb. index is the average hybrid index for each colony of sampling location with the standard error calculated for each (excluding *glabra* since all samples were used as the parental population).

Location	<i>n</i>	Phenotypic field ID			Genetic ID			Avg. hyb. index
		<i>Michauxii</i>	Hybrid	<i>Glabra</i>	<i>Michauxii</i>	Hybrid	<i>Glabra</i>	
000	11	8	3	0	0	10	1	0.57 +/- .04
011	5	5	0	0	0	5	0	0.38 +/- .02
014	11	10	1	0	0	11	0	0.51 +/- .03
020	9	9	0	0	2	7	0	0.40 +/- .05
044	10	9	0	1	7	2	1	0.19 +/- .06
046	12	8	0	4	1	8	3	0.42 +/- .03
052	14	12	2	0	8	6	0	0.28 +/- .05
060	9	8	1	0	1	8	0	0.48 +/- .05
068	8	8	0	0	7	1	0	0.21 +/- .04
071	12	11	1	0	2	10	0	0.40 +/- .05
076	2	0	2	0	0	2	0	0.51 +/- .05
077	5	5	0	0	1	4	0	0.23 +/- .01
081	8	8	0	0	0	8	0	0.50 +/- .03
084	7	0	7	0	0	7	0	0.53 +/- .03
087	3	2	1	0	0	3	0	0.45 +/- .12
100	14	10	4	0	4	10	0	0.42 +/- .05
101	7	7	0	0	6	1	0	0.19 +/- .05
202	10	9	1	0	9	1	0	0.10 +/- .05
203	2	2	0	0	2	0	0	0.15 +/- .01
204	5	5	0	0	0	5	0	0.56 +/- .03
205	5	5	0	0	5	0	0	0.12 +/- .01
208	9	9	0	0	0	9	0	0.44 +/- .03
302	4	4	0	0	3	1	0	0.17 +/- .02
60A	10	3	6	1	0	9	1	0.53 +/- .02
60B	3	3	0	0	0	3	0	0.52 +/- .001
DF6	9	9	0	0	0	9	0	0.50 +/- .04
RT615	5	3	2	0	0	5	0	0.45 +/- .09
<i>Glabra</i>	23	0	0	23	0	0	23	NA
Fort Pickett Total	232	172	31	29	58	145	29	0.40 +/- .01
Deepwater Total	150	120	28	2	61	84	5	0.35 +/- .02
All Total	382	292	59	31	119	229	34	0.38 +/- .01

Table 2 Pairwise *Fst* results for each individual *Rhus* colony at Fort Pickett and the Deepwater (DW) sampling location as a whole.

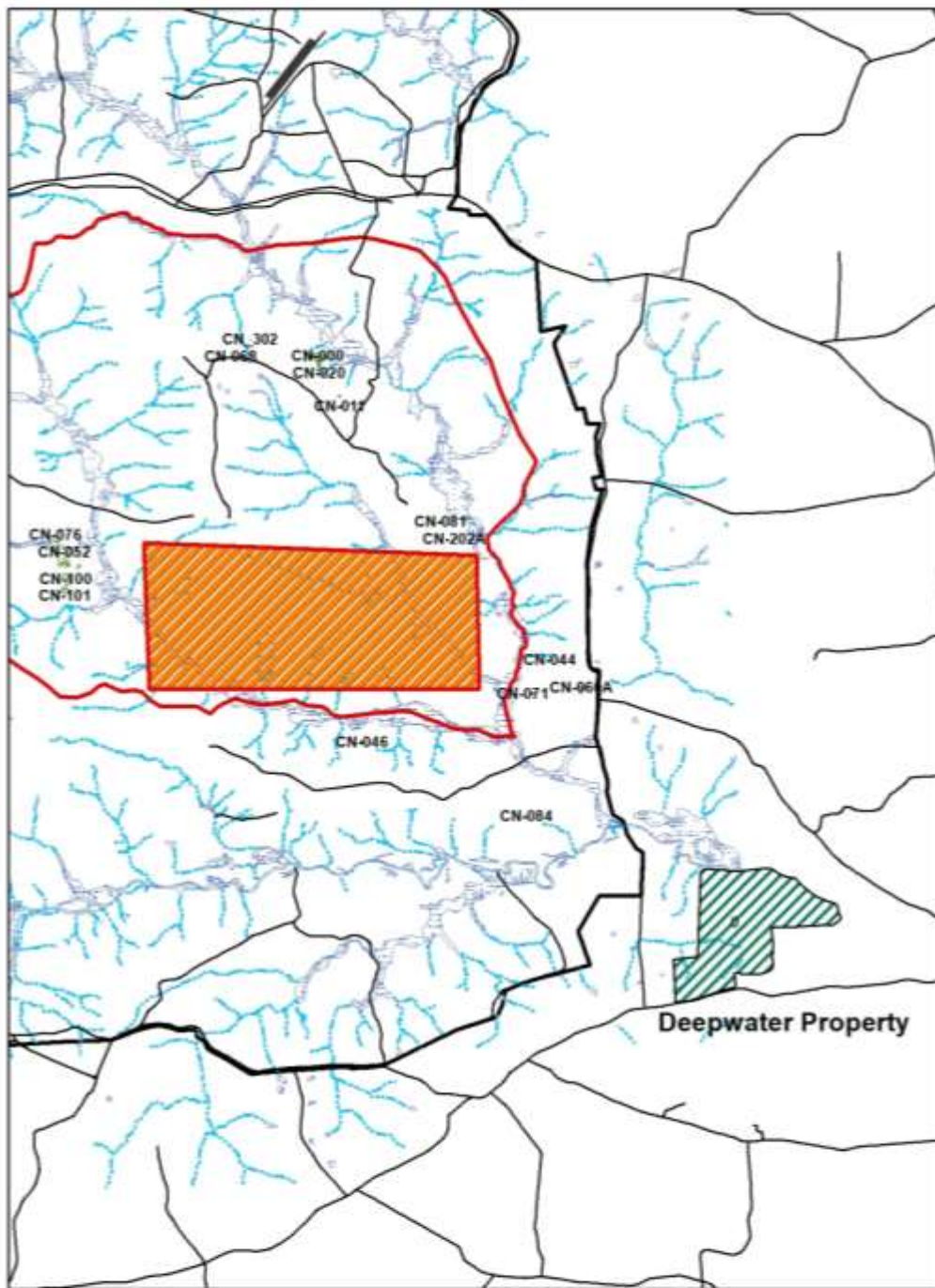
	000	011	014	020	044	046	052	060	068	071	076	077	081
000	X												
011	0.115	X											
014	0.046	0.113	X										
020	0.037	0.129	0.067	X									
044	0.112	0.168	0.135	0.098	X								
046	0.064	0.112	0.068	0.060	0.100	X							
052	0.051	0.143	0.084	0.044	0.080	0.069	X						
060	0.097	0.097	0.088	0.108	0.112	0.086	0.128	X					
068	0.114	0.195	0.137	0.071	0.137	0.118	0.094	0.163	X				
071	0.045	0.127	0.058	0.029	0.091	0.050	0.040	0.101	0.086	X			
076	-0.015	0.157	-0.018	0.036	0.180	0.059	0.063	0.001	0.196	0.043	X		
077	0.034	0.226	0.149	0.095	0.176	0.135	0.118	0.171	0.117	0.114	0.209	X	
081	0.109	0.186	0.107	0.101	0.180	0.115	0.129	0.153	0.163	0.097	0.163	0.172	X
084	0.135	0.172	0.121	0.143	0.190	0.124	0.168	0.108	0.210	0.135	0.140	0.222	0.189
087	0.130	0.179	0.124	0.139	0.189	0.110	0.150	0.055	0.216	0.132	0.168	0.241	0.207
100	0.036	0.112	0.054	0.033	0.084	0.037	0.032	0.097	0.083	0.031	0.000	0.096	0.097
101	0.066	0.166	0.091	0.034	0.100	0.082	0.026	0.143	0.076	0.047	0.109	0.108	0.130
202	0.094	0.132	0.111	0.083	0.090	0.081	0.071	0.098	0.118	0.080	0.122	0.151	0.142
203	0.079	0.222	0.104	0.058	0.137	0.092	0.025	0.113	0.107	0.068	0.368	0.144	0.184
204	0.108	0.202	0.116	0.113	0.200	0.120	0.141	0.139	0.188	0.108	0.155	0.186	0.173
205	0.121	0.209	0.143	0.104	0.138	0.114	0.067	0.162	0.153	0.109	0.230	0.196	0.198
208	0.050	0.108	0.065	0.065	0.100	0.059	0.054	0.086	0.135	0.058	0.024	0.152	0.122
302	0.089	0.193	0.103	0.061	0.141	0.114	0.074	0.149	0.101	0.077	0.157	0.166	0.167
60A	0.092	0.127	0.103	0.110	0.126	0.098	0.127	-0.014	0.176	0.098	0.056	0.178	0.156
60B	0.143	0.252	0.150	0.145	0.243	0.173	0.160	0.045	0.239	0.126	0.260	0.263	0.253
DF6	0.137	0.135	0.130	0.140	0.165	0.118	0.158	0.073	0.196	0.138	0.092	0.204	0.170
rt615	0.117	0.133	0.103	0.143	0.164	0.112	0.162	0.019	0.215	0.132	0.039	0.216	0.180
DW	0.062	0.075	0.078	0.059	0.050	0.043	0.054	0.048	0.082	0.050	0.004	0.103	0.106

Table 3 Proportion of observed (Observed Het.) and expected heterozygosity (Expected Het.) for all sites averaged over each colony or sampling location.




<u>Population</u>	<u>Observed Het.</u>	<u>Expected Het.</u>
CN-000	0.121	0.144
CN-011	0.146	0.142
CN-014	0.108	0.149
CN-020	0.118	0.140
CN-044	0.085	0.148
CN-046	0.111	0.144
CN-052	0.091	0.144
CN-060	0.114	0.141
CN-068	0.091	0.143
CN-071	0.102	0.148
CN-076	0.081	0.157
CN-077	0.109	0.140
CN-081	0.145	0.143
CN-084	0.131	0.144
CN-087	0.114	0.145
CN-100	0.098	0.147
CN-101	0.079	0.149
CN-202	0.114	0.143
CN-203	0.094	0.138
CN-204	0.144	0.140
CN-205	0.100	0.140
CN-208	0.123	0.144
CN-302	0.113	0.144
CN-60A	0.110	0.143
CN-60B	0.123	0.140
CN-DF6	0.137	0.142
CN-rt615	0.112	0.145
Pickett	0.111	0.144
Deepwater	0.106	0.144

Table 4 Table of minimum, maximum, and mean π values for each colony and Fort Pickett and Deepwater sampling locations as a whole.

<u>Population</u>	<u>Min π</u>	<u>Max π</u>	<u>Mean π</u>
CN-000	5.26E-07	1.04E-03	5.48E-05
CN-011	2.22E-06	1.16E-03	9.44E-05
CN-014	4.33E-07	6.13E-04	5.35E-05
CN-020	6.54E-07	1.32E-03	6.68E-05
CN-044	6.54E-07	6.46E-04	4.40E-05
CN-046	6.54E-07	1.01E-03	6.45E-05
CN-052	2.65E-07	6.72E-04	4.29E-05
CN-060	6.54E-07	8.26E-04	5.19E-05
CN-068	8.33E-07	9.32E-04	4.77E-05
CN-071	3.62E-07	8.62E-04	4.20E-05
CN-076	1.67E-05	5.33E-04	3.17E-05
CN-077	2.22E-06	1.26E-03	6.97E-05
CN-081	8.33E-07	1.09E-03	7.09E-05
CN-084	1.10E-06	9.00E-04	5.81E-05
CN-087	6.67E-06	1.33E-03	8.03E-05
CN-100	2.65E-07	8.07E-04	4.48E-05
CN-101	1.10E-06	7.28E-04	4.80E-05
CN-202	5.26E-07	9.55E-04	6.44E-05
CN-203	1.67E-05	1.23E-03	1.13E-04
CN-204	2.22E-06	1.25E-03	7.45E-05
CN-205	2.22E-06	1.23E-03	7.17E-05
CN-208	6.54E-07	7.96E-04	5.89E-05
CN-302	3.57E-06	9.79E-04	8.62E-05
CN-60A	6.54E-07	7.26E-04	1.00E-05
CN-60B	6.67E-06	1.16E-03	9.80E-05
CN-DF6	6.54E-07	1.21E-03	5.91E-05
CN-rt615	2.22E-06	7.72E-04	5.90E-05
Pickett	6.93E-08	9.05E-04	4.86E-05
Deepwater	6.27E-08	8.81E-04	4.42E-05



Legend

-  Michaux's Sumac Colonies sampled
-  duded_impact_area
-  surface_water_body

0 0.375 0.75 1.5 2.25 3 Miles



Figure 1 Sampling locations, including the Deepwater property and 27 colonies sampled at Fort Pickett.

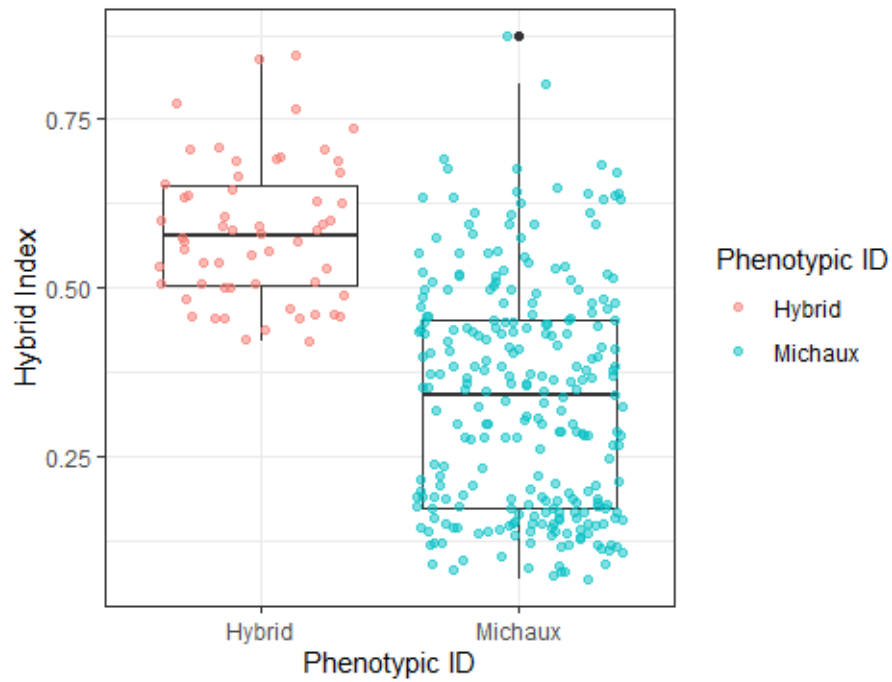


Figure 2.1 Boxplot of phenotypic identification (Field ID) versus hybrid index calculated using *Introgress* analysis. Red dots represent hybrid indices of individuals field-identified as hybrids, while blue dots represent hybrid indices of individuals field-identified as true Michaux’s sumac. All individuals field identified as smooth sumac were used in the parental population of the analysis, so no hybrid index was calculated for those samples. An individual with a hybrid index of 0-0.20 is regarded as Michaux’s sumac, 0.20-0.80 as a hybrid, and 0.80-1.0 as smooth sumac.

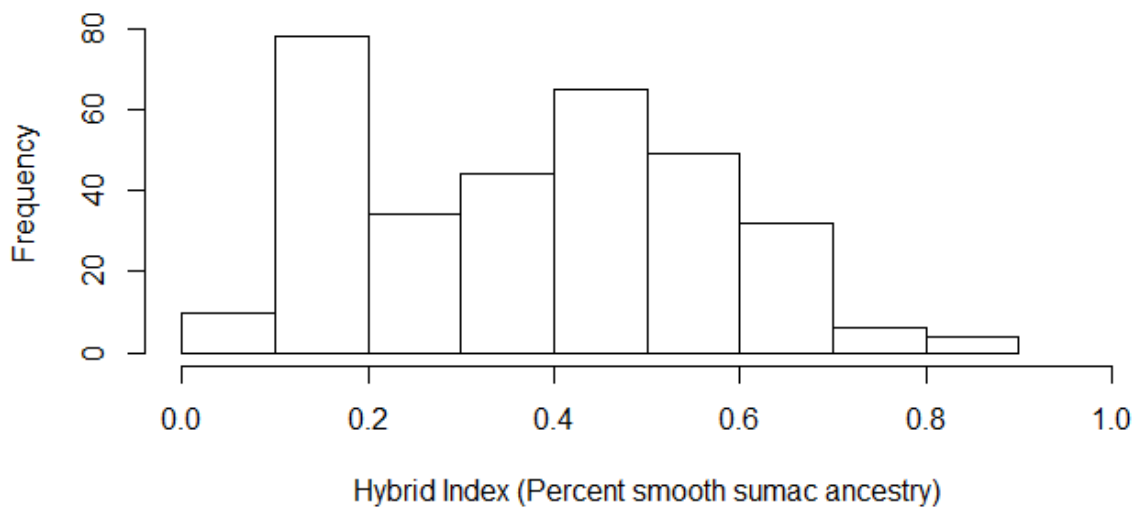


Figure 2.2 Histogram of hybrid indices versus frequency for all samples in the *Introgress analysis*, excluding the 60 individuals selected as the parental populations. A hybrid index of 0 represents Michaux’s sumac, and a hybrid index of 1 represents smooth sumac, so the hybrid index represents the percent smooth sumac ancestry in an individual. An individual with a hybrid index of 0-0.20 is Michaux’s sumac, 0.20-0.40 is a hybrid backcrossed to Michaux’s sumac, 0.40-0.60 is an F₁ hybrid, 0.60-0.80 is a hybrid backcrossed to smooth sumac, and 0.80-1.0 is smooth sumac.

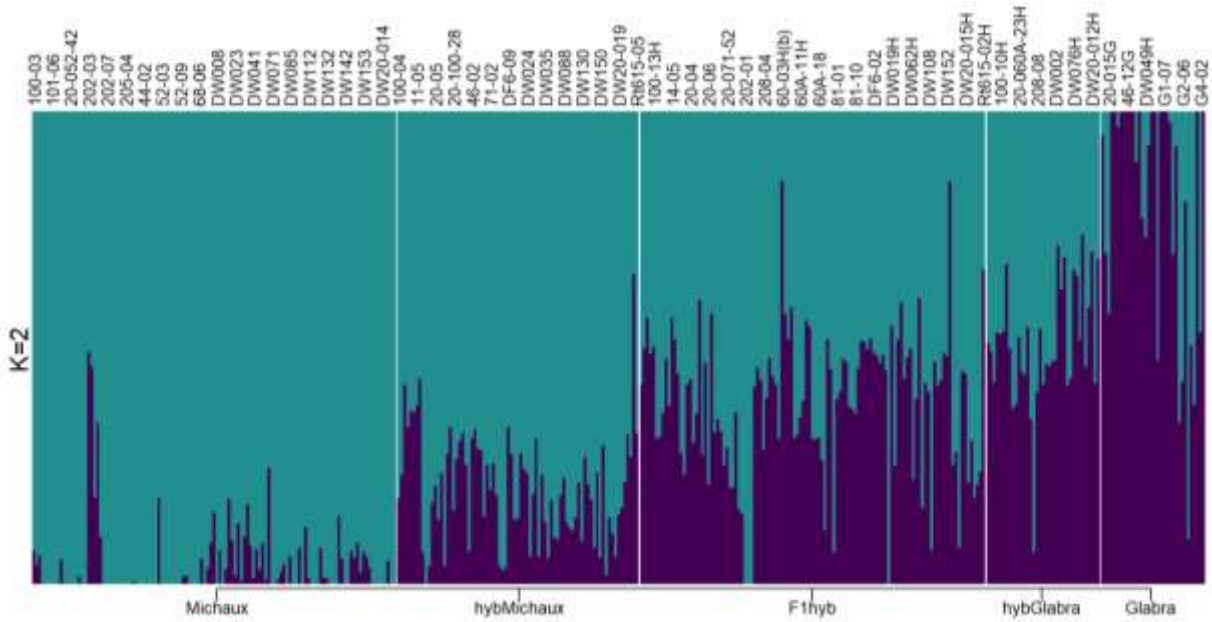


Figure 3.1 Admixture analysis results for all 382 *Rhus* individuals at $k=2$. Samples are ordered by genetic id based on *Introgress* analysis, with a white space dividing each group as identified in the field. Individuals are labeled across the top and species groupings are labeled along the bottom. Michaux’s sumac is on the left, labeled as “Michaux”, hybrids backcrossed to Michaux’s sumac are second from the left labeled as “hybMichaux”, F_1 hybrids are in the middle labeled as “F1hyb”, hybrids backcrossed to smooth sumac are second from the right labeled as “hybGlabra”, and smooth sumac samples are on the far right labeled as “Glabra”.

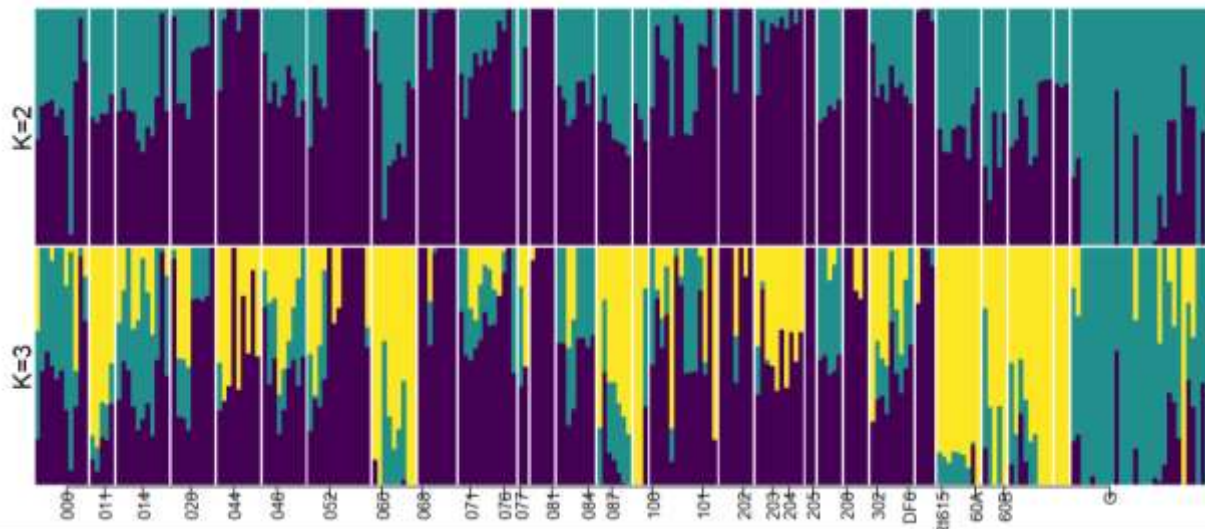


Figure 3.2 Admixture results from only Fort Pickett samples at $k=2$ and $k=3$. Individuals are ordered by colony number with white spaces dividing each. All colonies contain individuals field-identified as Michaux’s sumac or hybrids except colony “G”, which is a colony of only smooth sumac samples collected as a parental reference group.

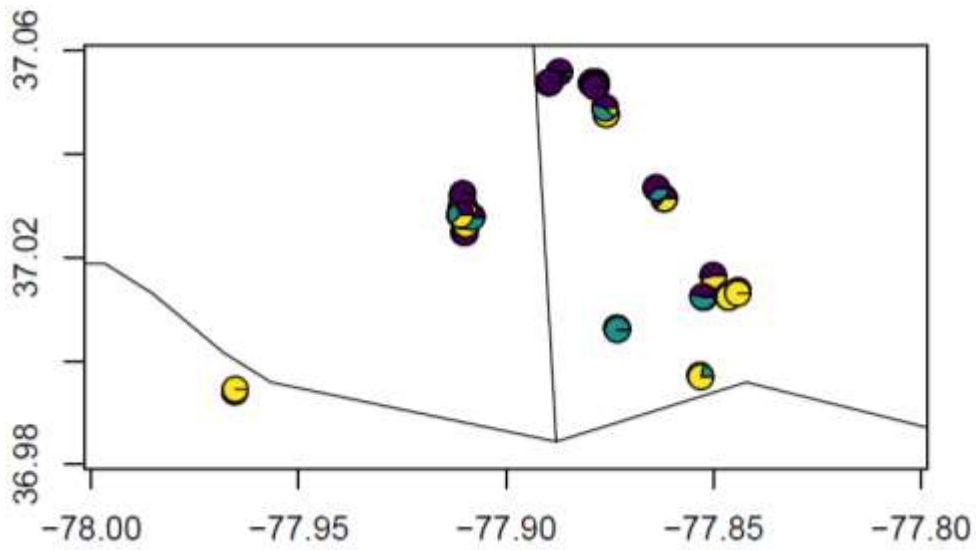


Figure 3.3a

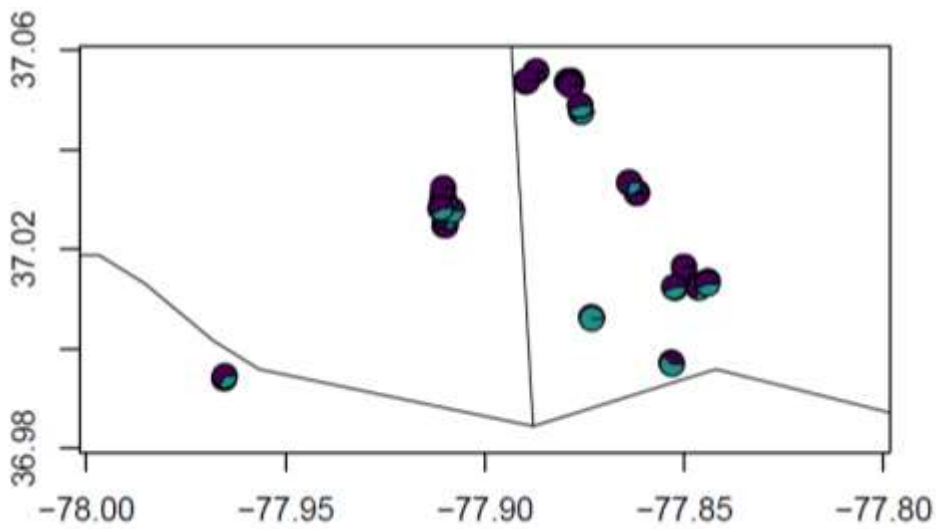


Figure 3.3b

Figure 3.3 Admixture results from analysis including only Fort Pickett samples mapped to individual's GPS coordinates for $k=2$ (a) and $k=3$ (b). Map lines represent county lines, and figure axes indicate GPS coordinates.

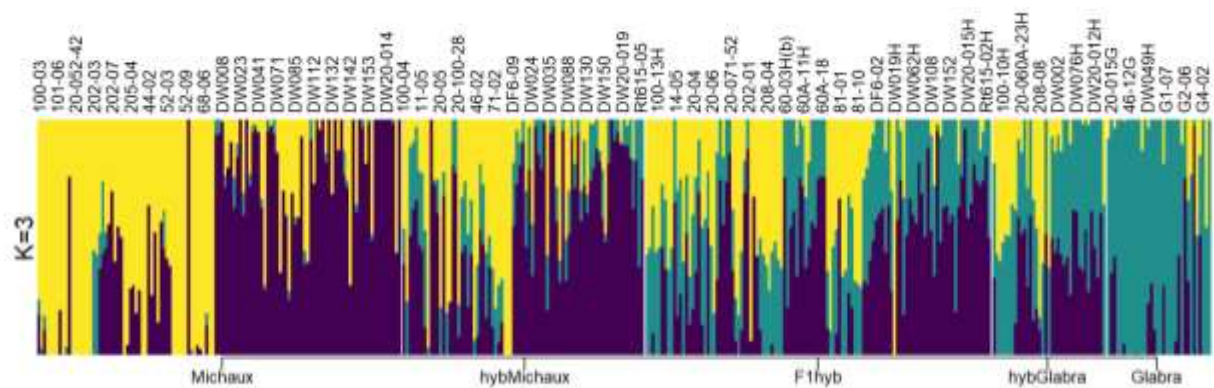


Figure 3.4 Admixture analysis results for all 382 individuals at $k=3$. Samples are ordered by genetic id based on *Introgres* analysis, with a white space dividing each group. Individuals are labeled across the top and species groupings are labeled along the bottom. Michaux’s sumac is on the left, labeled as “Michaux”, hybrids backcrossed to Michaux’s sumac are second from the left labeled as “hybMichaux”, *F1* hybrids are in the middle labeled as “F1hyb”, hybrids backcrossed to smooth sumac are second from the right labeled as “hybGlabra”, and smooth sumac samples are on the far right labeled as “Glabra”.

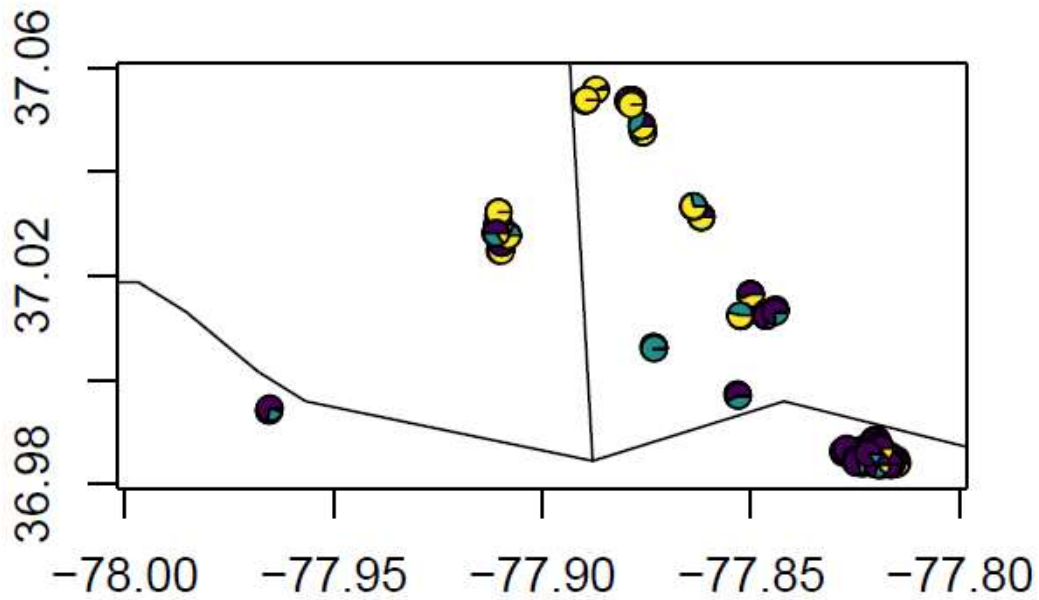


Figure 3.5 Admixture results for all 382 samples at $k=3$ mapped to individual sample GPS coordinates. Map lines represent county lines, and figure axes indicate GPS coordinates. The cluster in the bottom right corner represents the Deepwater sampling location.

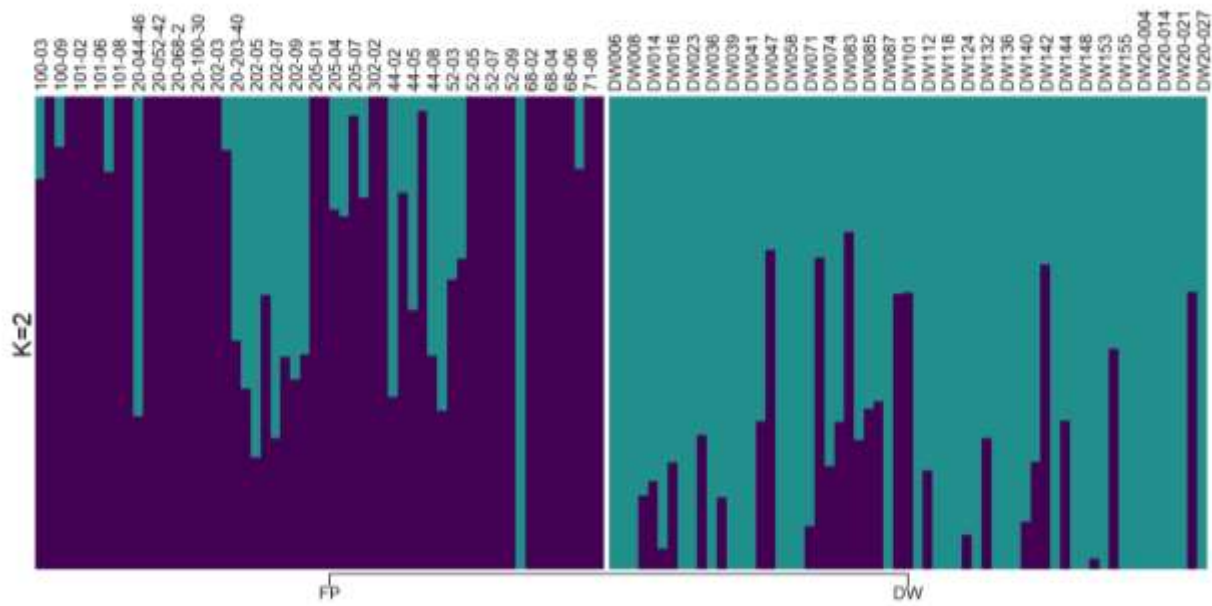


Figure 3.6 Admixture results including only individuals genetically identified as Michaux’s sumac through *Introgress* analysis at $k=2$. Samples are ordered by sampling location, with FP representing Fort Pickett, and DW representing Deepwater. Individual samples are labeled along the top.

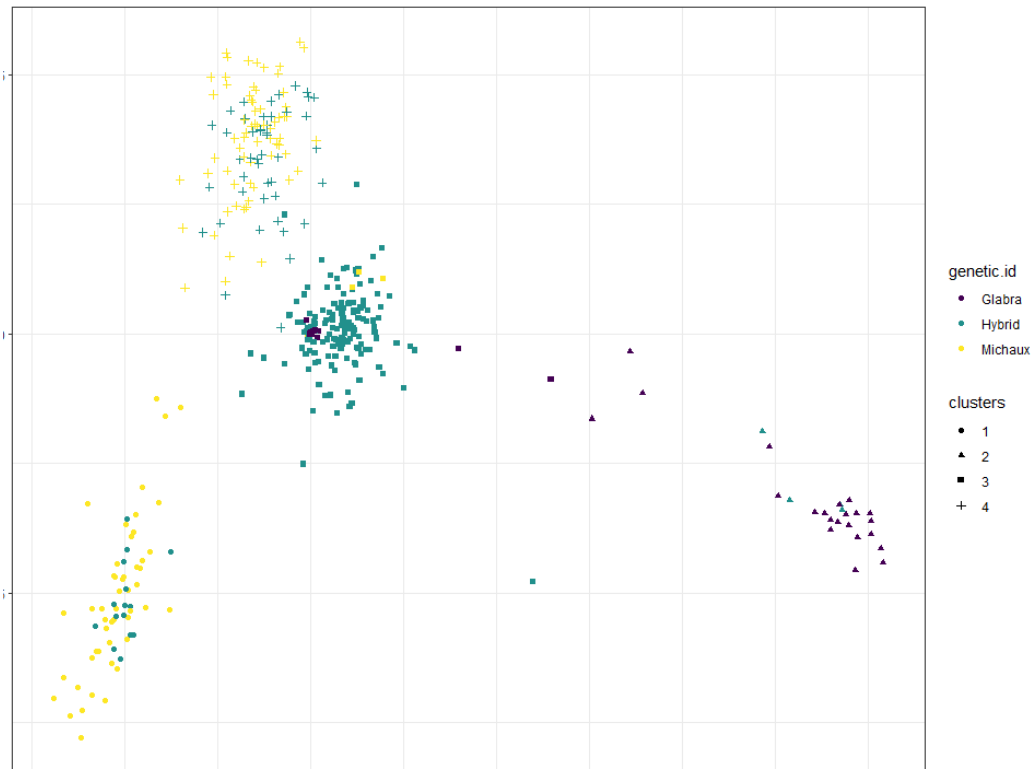


Figure 4.1 DAPC analysis results for $k=4$ clusters. Clusters are represented by different shapes, with cluster 1 labeled with circles in the bottom left, cluster 2 labeled with triangles on the right, cluster 3 labeled with squares in the middle, and cluster 4 labeled with pluses at the top left. Individual sample's genetic ids are labeled with different colors across the clusters, with individuals identified as smooth sumac colored purple, individuals identified as hybrids colored blue, and individuals identified as Michaux's sumac colored yellow.

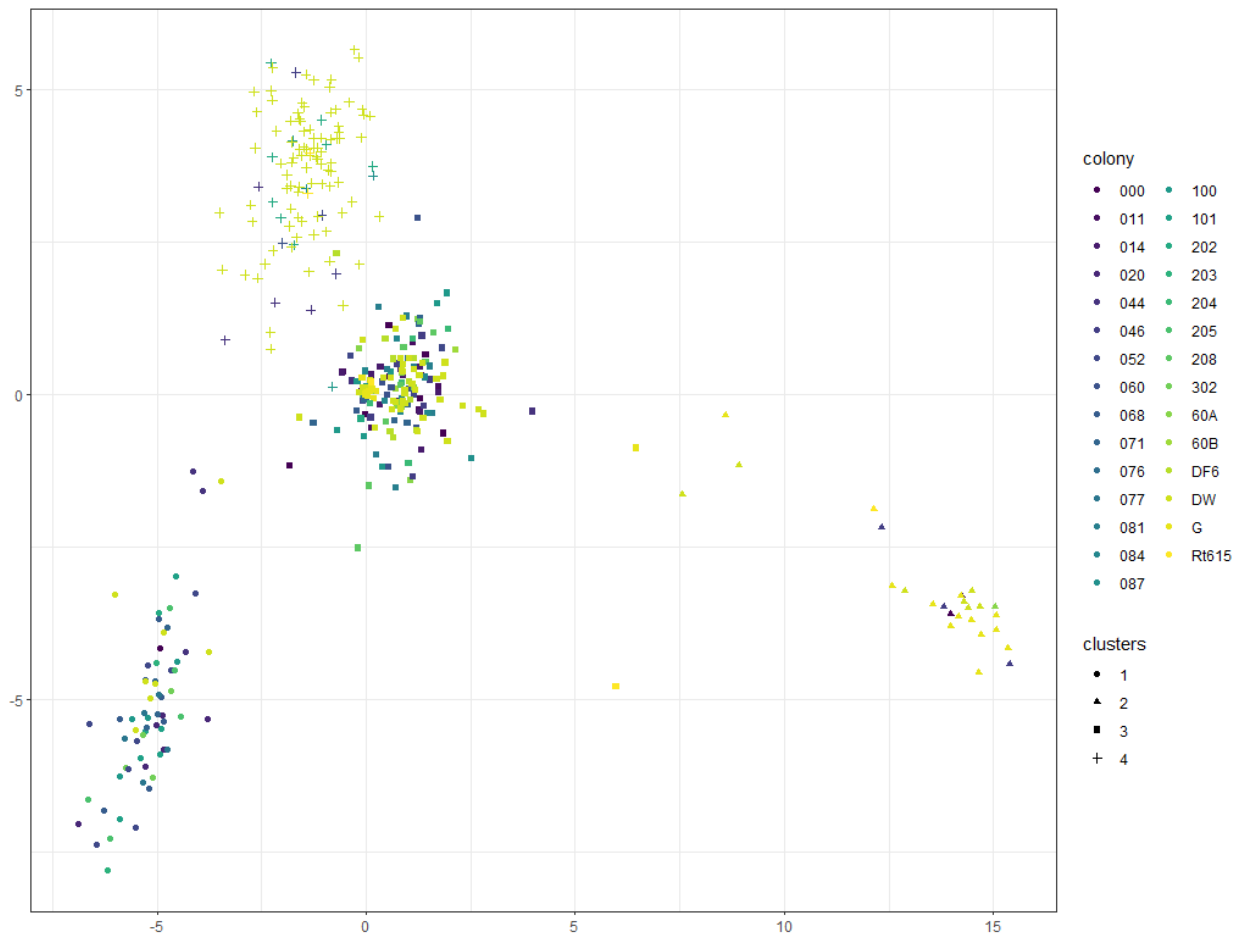


Figure 4.2 DAPC analysis results for $k=4$ clusters. Clusters are represented by different shapes, with cluster 1 labeled with circles in the bottom left, cluster 2 labeled with triangles on the right, cluster 3 labeled with squares in the middle, and cluster 4 labeled with pluses at the top left. The colony each individual was sampled from is labeled by color. Numbers in the legend represent colony numbers, with DW representing Deepwater, and G representing the smooth sumac colony used as a parental reference group.

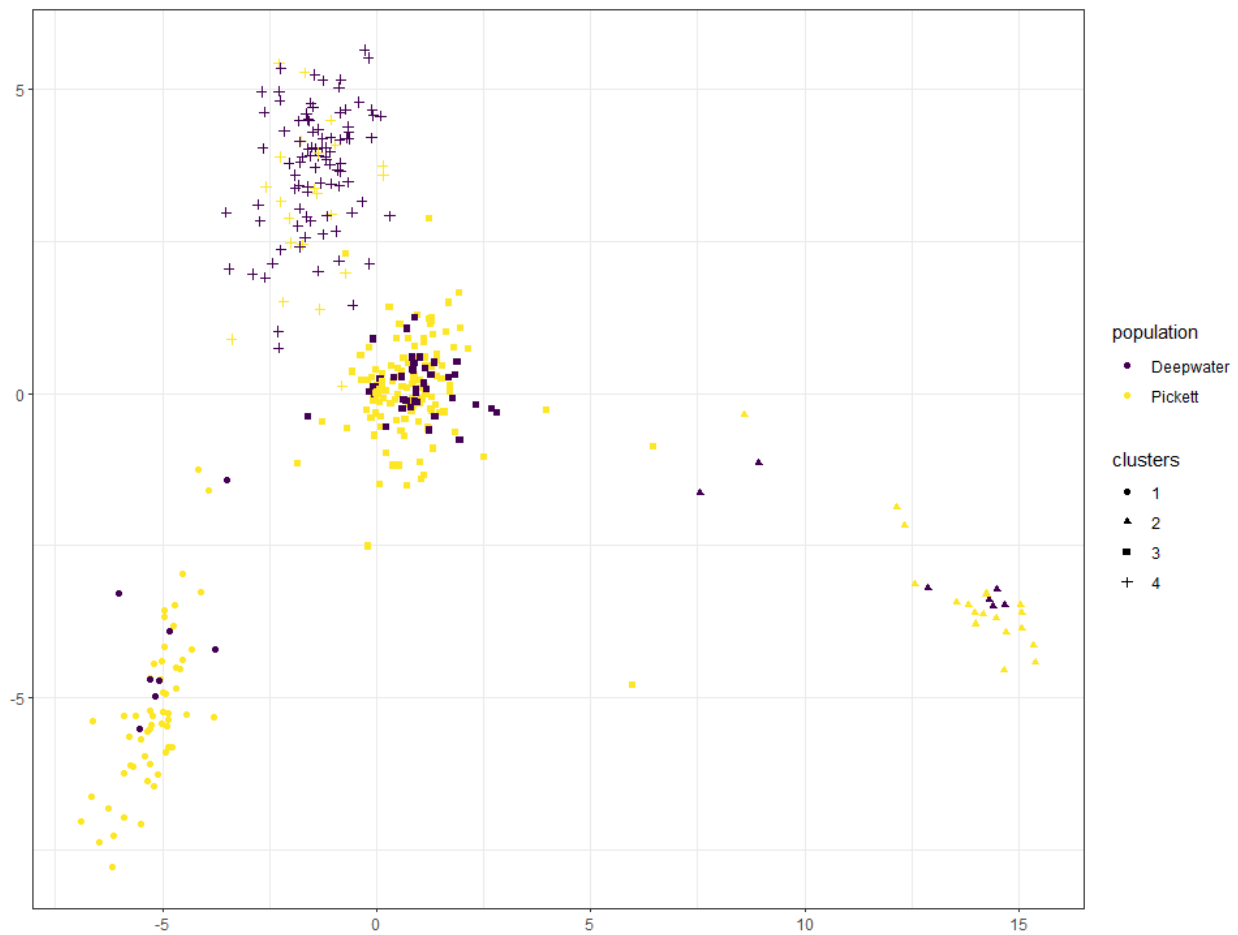


Figure 4.3 DAPC analysis results for $k=4$ clusters. Clusters are represented by different shapes, with cluster 1 labeled with circles in the bottom left, cluster 2 labeled with triangles on the right, cluster 3 labeled with squares in the middle, and cluster 4 labeled with pluses at the top left. The sampling location each individual was sampled from is labeled by color, with purple representing individuals from Deepwater and yellow representing individuals from Fort Pickett.

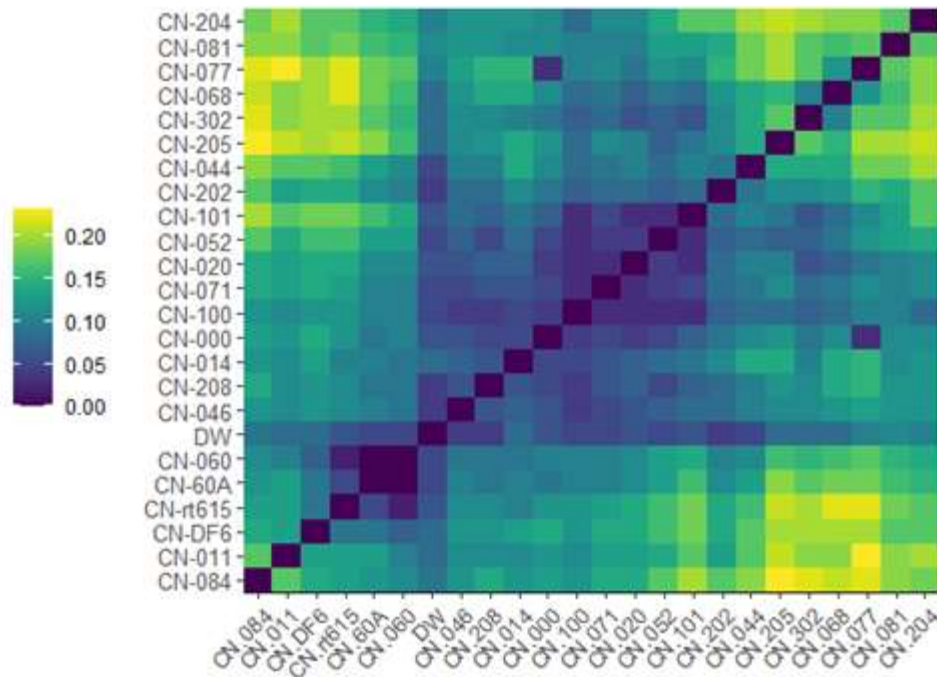


Figure 5 Heat map of F_{st} estimates between the 25 colonies at Fort Pickett with a sample size larger than 3 (excluding colonies 60B, 203, 087, and 076) and the Deepwater population. Darker purples and blues represent values closer to zero, while greens and yellows represent higher values closer to 0.20. Values closer to zero indicate that populations are more similar to each other, while higher values indicate less gene flow between colonies. Colonies are labeled along both axes, with CN for colonies at Fort Pickett, and DW for Deepwater. Heat map is symmetrical across the diagonal.

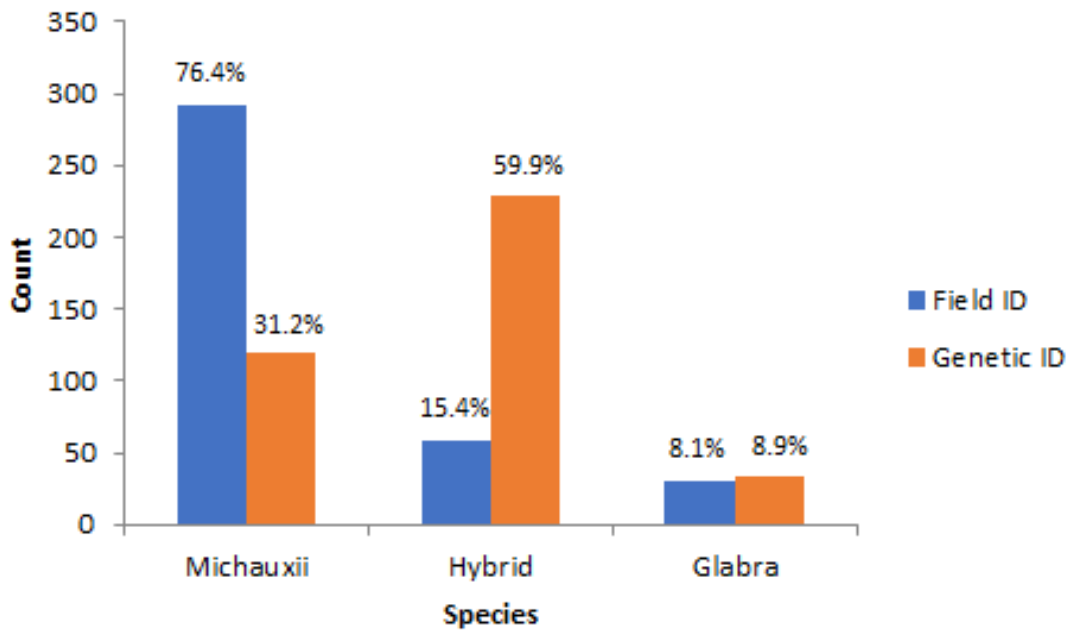


Figure 6 Bar chart displaying the count of each species based on field identification (Field ID in blue) or genetic identification (Genetic ID in orange). Percentages of total samples collected are displayed above each chart for each type of identification.

CHAPTER 3: FUTURE PERSPECTIVES

My research highlights the threat that interspecific hybridization poses to Michaux's sumac colonies in Virginia, but the level of hybridization in populations in North Carolina and Georgia is still unknown. Understanding the degree of hybridization across Michaux's sumac's entire range is vital to managing the species. Additionally, characterizing the known populations of Michaux's sumac over the landscape, particularly the 22 populations throughout Georgia, North Carolina, and Virginia that may be considered self-sustaining based on the Natural Heritage Program's ranking criteria, would help managers to better understand the current recovery status of the species (USFWS 2014). Using the methods from our study of the Fort Pickett and Deepwater populations, researchers could further understand the levels of hybridization and genetic diversity across all the known populations across the range of Michaux's sumac, and conservationists could better manage the species as a whole by prioritizing populations with higher levels of genetic diversity and lower levels of hybridization for management actions such as transplantation and propagation efforts.

Wider sampling across Michaux's sumac range could also allow researcher to gain insight into the life history of the species. Many species, including both plant and animal species, survived in refugia during the Wisconsin glaciation, before recolonizing their modern, interglacial ranges (Hewitt 2000; Fedorov et al. 2002; Loehr et al. 2006; Aldenholven et al. 2010; Roberts and Hamann 2015). Additionally, the separation of different populations of the same species into different glacial refugia is known to have played an important role in speciation events for many species (Lang et al. 2002). Based on Sherman-Broyles et al.'s (1992) hypothesis that Michaux's sumac and smooth sumac share a recent common ancestor, it may be the case that the ancestral sumac inhabited more than one glacial refuge before recolonizing its extant range. This geographic isolation may have resulted in different evolutionary trajectories for the

populations, with Michaux's sumac adapting to its environment with different phenotypic characteristics, such as its short stature and dense pubescence, as compared to smooth sumac's taller stature and lack of pubescence. With the loss of geographic isolation in their modern ranges, the closely related species are again able to interbreed. Using genetic studies, such as a phylogeographic study, researchers could gain a better understanding of the life history of Michaux's sumac, and understand whether and how the ice age and isolation in distinct glacial refugia impacted the speciation of Michaux's sumac from its putative progenitor.

Phylogeographic studies can help to provide an understanding of the life history of a species, and can elucidate the relationships between closely related species. Previous phylogenetic studies that included Michaux's sumac found differing relationships between Michaux's sumac and smooth sumac using chloroplast DNA as compared to nuclear DNA (Yi et al. 2004; Yi et al. 2007). Using chloroplast DNA, smooth sumac and Michaux's sumac formed a clade, with staghorn sumac (*Rhus typhina*) found to be a sister species to that clade. However, using nuclear DNA, Michaux's sumac was found to be a sister species to the clade containing smooth and staghorn sumac. Due to this discordance between methods, further phylogeographic studies may help to further elucidate the relationship between Michaux's and smooth sumac, and help to confirm or dismiss the hypothesis put forth by Sherman-Broyles et al. (1992) that smooth sumac is the progenitor of Michaux's sumac.

In addition to wider sampling across known populations, a broad search to find and characterize previously undiscovered populations, including those on private land, would be beneficial to the recovery of the species. The largest known population to date, Deepwater, was discovered by chance in 2011 on private land (Teets and Emrick 2012). A broader search and the wider dissemination of information about the species could lead to similar discoveries. The

discovery of new populations, particularly self-sustaining populations, could be an important part of the recovery of the species, and would allow for the management and protection of those populations from any potential threats they may face in the future.

While genetic studies may provide more insight into Michaux's sumac's recovery status and life history, further studies of different management tools and their usefulness in promoting the recovery of Michaux's sumac may prove important to the future of the species. Prescribed burns have been found to help support Michaux's sumac populations by reducing competition for sun and soil resources, with a prescribed burn implemented every three to five years in the Deepwater population (Hammond 2016). Additionally, Emrick and Jones (2008) found that high competition reduces the density of all flowering stems in a colony, with female stems affected more strongly than male stems. With prescribed burns able to reduce competition, this leads to the question, would an increased fire frequency through prescribed burns be a practical solution to help support sexual reproduction in currently non-flowering or single sex colonies? Determining a feasible way to implement prescribed burns in other populations, and determining whether they would be an effective population growth-promoting tool for those populations could help to support recovery of Michaux's sumac throughout its range.

There have been observations of Michaux's sumac flowers changing sex from male to female or vice versa from year to year based on resource availability, but little research has been done into this phenomenon (Savage et al. 1991). Determining if an increase in soil nutrients through fertilizer applications could help to increase the percentage of flowering stems, or promote a more balanced ratio of male to female stems could provide an important tool for the management of Michaux's sumac populations. A better understanding of the resources needed for the production of flowers of both sexes could help to prevent the isolation of single-sex

colonies, thereby helping to increase sexual reproduction to increase the genetic diversity of many small colonies.

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