GENETIC DIFFERENCES WITHIN AND AMONG AMERICAN GINSENG (*PANAX QUINQUEFOLIUS* L.) LOCATIONS IN WESTERN NORTH CAROLINA AND NORTHERN VIRGINIA

A Thesis
by
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Abstract

GENETIC DIFFERENCES WITHIN AND AMONG AMERICAN GINSENG (*PANAX QUNIQUEFOLIUS* L.) LOCATIONS IN WESTERN NORTH CAROLINA AND NORTHERN VIRGINIA

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American ginseng (*Panax quinquefolius* L.) is widely viewed as a medicinal plant and has been harvested in the mountains of North America since the late 1600s. American ginseng has a range that stretches from southern portions of east Canada, south to the north Georgia mountains and as far west as South Dakota. Populations appear to have diminished in size, according to historical records; the plants self-pollinate more than they outcross and there are no known seed dispersers. Additionally, harvesters of American ginseng are thought to occasionally mix seeds from different populations, although the extent of seed mixing is not known. It is my hypothesis that this life history would seem to isolate populations, allowing them to become genetically distinct from one another, and keep genetic diversity low within populations. To explore these ideas, amplified fragment length polymorphisms (AFLPs) were used to examine the genetic diversity within and among locations of American ginseng in North Carolina and northern Virginia. Locations sampled in this study include: Great Smoky Mountains National Park, The Balsam Mountain Preserve, The Blue Ridge Parkway, Shenandoah National Park, Caldwell County, N.C.,

Wilkes County, N.C. and Oconee State Park. Clear genetic differences emerged when comparing geographically separated locations of American ginseng. Sample sites within a geographical location, however, showed no clear genetic differences. Overall, and contradictory to my original hypothesis, there was a high level of genetic diversity within sites but not as much diversity among locations. The levels of variation and the genetic differences that were found in this study suggest that there is still a large amount of genetic diversity. That diversity may be maintained by the self-pollinating nature of American ginseng. Conservation efforts should be enacted that encourage population growth and the maintenance of current levels of genetic diversity.

Dedication

I would like to dedicate this work to my family and, especially, my Mother. They always encouraged me to ask questions and gave me confidence to pursue my goals no matter what they were. It is because of them that I grew to love nature and the outdoors, so it is also because of them that I was able to study something that I love. I would also like to dedicate this work to the friends that provided an escape from school and research, but were also interested enough to ask questions. There is no such thing as a “self-made man.” I am evidence of that because it is due to these people that I am who and what I am.

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Introduction

Research Goals

American ginseng, *Panax quinquefolius* L., is one of 12 species in the *Panax* genus, which is in the family Araliaceae. Ten of the 12 *Panax* species are natives to east Asia and 2, *P. quinquefolius and P. trifolius,* or dwarf ginseng, are native to eastern North America (Wen & Zimmer 1995). *Panax* is one of about 120 genera to show this east Asian and east North American disjunct distribution. American ginseng and dwarf ginseng are not closely related within the *Panax* genus, however. American ginseng is more closely related to its Asian sister species *P. ginseng* and *P. japonicas*. This would suggest at least two separate divergence events with dwarf ginseng diverging much earlier than American ginseng (Wen & Zimmer 1995).

American ginseng is a perennial, understory plant of eastern temperate forests in North America that has been harvested for medicinal use since the 1700s. The roots of the plant are dug up and dried; most are shipped from North America to China and Japan where they serve as the main component of many herbal tonics (Kauffman 2006). Traditionally, roots collected from wild populations are thought to be more potent for medicinal use than are roots from plants that have been cultivated. For this reason, wild American ginseng plants are in high demand and are still heavily harvested. In addition to these anthropogenic pressures on wild populations, pressures from whitetail deer *(Odocoileus virginianus)* herbivory threaten to drive down American ginseng populations to unsustainable sizes (Van Der Voort & McGraw 2006; Furedi & McGraw 2004).

Although American ginseng exhibits a mixed mating system, it has been shown to self-pollinate more than it outcrosses (Lewis & Zenger 1983). Studies by Furedi and McGraw (2004) have also shown that seeds do not germinate after they have traveled through the digestive tracts of deer. Turkey and grouse have also been shown to macerate seeds to the point that they are not able to germinate (McGraw personal communication) so, to date, there are no known seed dispersers of American ginseng other than gravity. To complicate the genetic picture further, harvesters of American ginseng are thought to mix seeds from different populations, but the extent of this mixing is not known. The reproductive strategy of American ginseng would seem to confine populations to a single location for long periods of time which may cause them to become genetically distinct from one another by making them more homogeneous within, and more heterogeneous among, populations.

The questions I want to address in this thesis are the following (1) Are geographically separate locations of American ginseng genetically different from one another and (2) are there measurable and significant genetic differences within geographical locations? Due to the high selfing rates, the lack of seed dispersers, the pressure of overharvesting since the 1700s, and the unknown extent to which seeds between populations are mixed by harvesters, I hypothesize that (1) geographically separate locations will be genetically different from one another and (2) that genetic diversity will be low within, but high among, geographic locations.

Because of its slow decline since the late 1700s and small population size now, the conservation of American ginseng has become an important research topic (Gagnon 1999; Van Der Voort & McGraw 2006). When conserving species, one of the first topics

researchers should address is the genetic diversity that is available in populations. Populations with high levels of genetic variation are thought to stand a better chance at survival than populations with low genetic diversity (Dudash & Fenster 2000). This is because those with higher levels of genetic diversity, theoretically, have a better chance of adapting to a changing environment. American ginseng has been harvested, and over harvested, for several hundred years. It also has a reproductive strategy that would seem to cause populations to become more homogenous within and more heterogeneous among populations. If this is the case, conservation efforts should focus on preserving genetic diversity among populations before it declines dramatically.

Although studies have been completed that look into the genetic variability of American ginseng (Bai et al. 1997; Boehm et al. 1999; Schluter & Punja 2002; Grubbs & Case 2004; Cruse-Sanders & Hamrick 2004a; Cruse-Sanders & Hamrick 2004b; Zhuravlev et al. 2010), many compared cultivated to non-cultivated sites, and used unreliable markers or markers that may not have captured as much of the genetic picture as can be captured with amplified fragment length polymorphism (AFLP) markers. Previous studies also compared *P. quinquefolius* to *P. ginseng* or they looked to see if selective breeding was possible with the genetic diversity that is available in American ginseng farms. This study differs from previous ones in that it compares only samples found in the wild and it uses the AFLP technique, which is reliably repeatable, needs no knowledge of the species' DNA and has a high resolving power (Vos et al. 1995).

Habitat Characteristics

American ginseng, *Panax quinquefolius* L., is a perennial understory herb that is found in eastern North American from Ontario and Quebec, west to Kansas and south to the mountains of northern Georgia (Anderson et al. 1993). American ginseng is restricted to cove hardwood communities where it grows in moist, well-drained soils and slopes of up to 60% (Anderson et al. 2002). It has been found to tolerate a wide range of soil pH, soil texture and soil fertility (Anderson et al. 2002). The ability of American ginseng to survive a variety of soil conditions may be due to its arbuscular mycorrhizal associations (Seo and Anderson 1990). Shade requirements for American ginseng range from 70% to 90% (Anderson et al. 1993). Higher levels of light have been found to reduce total leaf area as well as increase leaf senescence of *P. ginseng*, a close relative of *P. quinquefolius* (Parmenter & Littlejohn 2000). These light requirements are the reason American ginseng is thought to be restricted to north facing slopes, but it is often found on other aspects as well. In deep coves, it may be found on slopes facing any direction, as long as those slopes are protected from direct sunlight. McGraw et al. (2003), however, found that east and west facing slopes tended to have the highest concentrations of plants, especially when aspect was coupled with elevation. Populations found at middle and high elevations in their study tended to be more prevalent on east facing slopes while populations at lower elevations were primarily located on west facing slopes (McGraw et al. 2003). McGraw et al. (2003) suggest that if north facing slopes are optimal habitat, or at least thought to be optimal by ginseng harvesters, then American ginseng may be being pushed to east and west facing slopes by harvest pressures.

Reproductive Biology

American ginseng is a member of the Araliaceae family and, as is common of this family, has palmately compound leaves (Weakly 2011). Leaves have 3-5 leaflets and are arranged in a whorl around a central stalk (Proctor et al. 2003). The small, whitish-green, flowers are arranged on a peduncle that extends 2-25 cm out of the middle of the whorl of leaves. The petals range from 0.5-1.0 mm in length with sepals absent in most cases (Schlessman 1985). Flowers usually do not appear until plants are at least 5 years of age and each flower produces a 10 mm drupe that is red upon maturation. Each drupe produces 1-3 seeds (Anderson et al. 2002).

Pollinators are not well known or documented for the species, but some generalist pollinators, such as halictid bees and syrphid flies, have been reported (Duke 1980; Carpenter & Cottam 1982; Catling & Spicer 1995). American ginseng is self-compatible and self-pollination is thought to be the main mode of fertilization (Lewis & Zenger 1983). Flowers that were covered to prevent pollinators from visiting them produced just as many or more seeds than those flowers that were not covered (Lewis & Zenger 1983). Seeds are thought to be dispersed by gravity or water during periods of high rainfall. Once fruits fall to the forest floor, the seeds must pass through two winters before they will germinate (McGraw et al. 2005). Germination rates of seeds once they fall from the parent plant to the forest floor depend on the maturity of the berry at that time. When fruits are still green, 20% of seeds tend to germinate, but when fruits are mature and red, just over 50% germinate (McGraw et al. 2005).

Harvest History

American ginseng was first discovered and used medicinally by Native Americans (Pritts 1995). It was used because the shape of the root oftentimes resembles that of the human body so Native Americans thought the root would be good medicine for the whole body as well (Veninga 1973). There is some evidence that Native Americans even followed a conservation regimen when harvesting plants, such as taking only the fourth plant in a group, and they may have replanted seeds after they harvested plants (Foster 1991; Kauffman 2006). In China, long before American ginseng was being traded for profit, *P. ginseng* was being harvested for medicinal use as well. When extensive harvest pressure was placed on *P. ginseng* in China in the early 1700s, Father Jartoux, a French Jesuit missionary, wrote a letter to other missionaries indicating the extreme demand for the herb (Kauffman 2006). He speculated that there may have been some ginseng growing in present day Canada because of the similar climate (Kauffman 2006). Upon reading the letter, Father Francois Lafiteau, who was stationed in Canada at the time, sought out and, 3 months later with the help of Native Americans, found American ginseng (Kauffman 2006). Soon after, in the 1710s, harvest of American ginseng moved from Canada down through the Great Lakes region and, by the 1720s, American ginseng was second in trade only to fur (Goldstein 1975; Gagnon 1999; Kauffman 2006).

In the early to mid-1700s heavy harvest of the plant included "gold rush" style camps in which harvesters would dig 40-60 pounds of ginseng root each for days at a time (Pritts 1995). These types of camps could, and did, generate tons of ginseng root for export (Kauffman 2006). At first most exports went through England, Holland and France, but soon entrepreneurs realized the huge profits that could be made by trading directly with China

(Kauffman 2006). In 1784 the Empress of China left from New York carrying almost 30 tons of ginseng (Kauffman 2006). John Jacob Astor, founder of the American Fur Company, was an active trader in ginseng after realizing a $55,000 profit from a single trip in 1782 (Williams 1957) and it has been suggested that ginseng gathering was more profitable than the fur trade in some parts of the Americas in the late 1700s (Williams 1957; Veninga 1973). Even one of the most well-known fur traders of the time, Daniel Boone, was a ginseng dealer. In 1787 he harvested and purchased, for trade, 12-14 tons of roots only to lose it all in an accident while in transport to Philadelphia (Hammond 1999). Even after such a large loss, he collected almost as much the very next year (Kauffman 2006). The plentiful harvest of American ginseng continued through the mid-1800s. By the late 1800s and early 1900s reports of ginseng becoming more and more scarce began to surface, and by 1896, trade with China had fallen off to less than one-third of what was traded in the late 1850s to early 1860s (Nash 1898; Kauffman 2006). The intense harvest pressure coupled with the extensive logging in the Appalachians from the 1870s to the 1940s certainly reduced ginseng numbers drastically (Martin 1992; Kauffman 2006).

Due to the decline in natural populations, there was an increased interest in cultivating American ginseng. George Stanton, a former tin smith who turned to farming in central New York, began successfully cultivating the plant with some commercial success and others soon began to follow his practices (Butz 1897; Nash 1898). A period from 1880-1903 became known as the “Ginseng Boom” due to the success of cultivated ginseng (Persons 1994). In 1904, however, a blight, *Alternaria panax*, ravaged cultivated populations, forcing many growers out of the business such that only 23 acres of ginseng was being cultivated in the U.S. (Persons 1994). To the relief of many, in 1906 Dr. I. C. Curtis

developed a recipe of copper sulfate and lime, known as Bordeaux mixture, which was successful in killing the blight (Persons 1994). Soon after the development of this antifungal treatment, cultivating ginseng became profitable again, and by 1929 there were 434 acres under cultivation in the US (Persons 1994). Just as the ginseng trade was recovering from blight and over-collection, the one-two punch of the second Sino-Japanese War and World War II put a halt on all American ginseng exports to China (Persons 1994). However, since 1960 a steady increase in both price and demand has occurred. In 1992, 800 tons of ginseng root, which included 70 tons of wild root, was certified for export (Persons 1994). Cultivated plants, however, do not sell for as much as wild plants. In 2007, wild roots sold for a price that was seven times higher than that of cultivated root (Cheng & Mitchell 2009). This price difference is driven by the notion that wild roots are, somehow, "better" medicinally (Lim et al. 2005; Schlag & Mcintosh 2006).

Since wild plants are worth more on the export market, harvest pressure remains high and this is the cause of much of the poaching that occurs on private land and in state and national parks up to the present day. The increase in poaching over the last 100 years has placed so much stress on wild populations that there is evidence that some current populations are no longer of a sustainable size (Gagnon 1999; Van Der Voort & McGraw 2006). There is also evidence, based upon herbaria records, that since large roots are most desirable harvesters have focused on large plants and large roots, leading to reduction in root size in wild populations over the past three centuries (McGraw 2001). Smaller roots mean harvesters will need to harvest, or poach, more plants to make the payoff worth the effort. This reduces the size of populations that are already small and, if root size is genetically

determined, could put selective pressure on populations that will continue the trend toward smaller root size.

Due to the reduced number of plants in the wild and international trade, American ginseng was placed on the treaty drawn up by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Under CITES, states have to monitor the impact of harvest and regulate the harvest of American ginseng. These regulations mean that in North Carolina plants of a certain age, mostly five years and older, can be harvested and harvesting can only occur after seeds have matured and the fruit is red. Plant age can be determined by counting bud scars on the neck of the root as one scar is left for each year of growth (USFWS 2009). Seeds are also often required to be planted in close vicinity to the parent plants (USFWS 2009; McGraw et al. 2010). These laws are often very hard to enforce as poachers have to be caught in the act in order to be prosecuted. It is also up to each state as to how the state would like to regulate the harvest of American ginseng.

Along with the implementation of harvesting laws by many states, some national parks have monitoring and marking programs to try to slow and deter the illegal harvest of roots in the park. Jim Corbin, a plant protection specialist with the North Carolina Department of Agriculture, has led the way in Great Smoky Mountains National Park by marking roots with a bright orange powder (Nickens 2001; Corbin 2002). The application of the dye is a labor intensive process that requires one to remove the soil from each individual root then sprinkle the powdered dye over the root surface (Corbin 2002). The dye remains on the root for some time, but after a few years it may need to be reapplied. Even though the dye makes roots illegally harvested from the park easy to identify, over 80 poachers have been caught with roots covered in orange (Corbin 2002). Other attempts have been made to

stop poachers in the park such as using motion-detecting cameras and even a dog that can smell marked roots (Nickens 2001; Kauffman 2006). No matter what method is used, poaching will probably remain high as long as wild roots have such a high market value.

Genetic Diversity

The genetic diversity of American ginseng has been a topic of interest for population and conservation biologists for many years. As noted earlier, American ginseng has no known agents of seed dispersal other than gravity and water, and tends more toward self-pollination than it does toward outcrossing. These factors alone would allow for American ginseng populations to diverge and become genetically distinct from one another. However, harvesters and poachers have reduced populations down to much smaller, isolated patches, dispersed both plants and seeds from cultivated to non-cultivated sites, established new populations and moved wild plants and/or seeds from one extant population to another. These factors may cause populations to appear more closely related to one another than they would be without the impact of human intervention for at least the past three centuries. Given the history of harvesting from wild populations, establishment of cultivar populations, and purposeful transplanting of wild-grown individuals within and between known and new populations, any analysis of the current genetic structure of this quasi-cultivar should be viewed through this lens of complexity.

One of the first studies to look at the genetic diversity of American ginseng was carried out on plants and seeds acquired from ginseng farmers in Ontario, Canada (Bai et al. 1997). This study focused mainly on determining if the genetic diversity of cultivated ginseng was high enough to carry out crossing experiments in order to establish superior

lines of ginseng for cultivation. High levels of genetic diversity were found when the 36 individual plants were compared to each other using randomly amplified polymorphic DNA, or RAPD analysis (Bai et al. 1997). From this high level of genetic diversity, it was suggested that a superior line of ginseng could be selected for in order to develop those qualities that are desired in the plant (Bai et al. 1997). However, this study suffered from a number of drawbacks that make it difficult to compare with other studies. First, it was limited in scope because it only focused on cultivated plants. Secondly, this study used the RAPD technique, which has been found to be unrepeatable due to its high sensitivity to the conditions of the Polymerase Chain Reaction (PCR). In fact, the RAPD technique is so sensitive that the use of different brands of thermocyclers was found to be sufficient to cause changes in results (Weising et al. 2005).

Boehm et al. (1999) looked into species differences between *P. ginseng* and *P. quinquefolius*, as well as differences between cultivated and wild type populations of *P. quinquefolius* using the RAPD technique. As expected, they found substantial differences between the two species. They also found that wild populations of American ginseng were genetically different when compared to cultivated populations. Wild populations in Wisconsin were also found to be different from those in Great Smoky Mountains National Park whereas wild populations found in Pennsylvania more closely resembled populations of cultivated plants (Boehm et al. 1999). This last finding could either be because cultivated populations were started from wild Pennsylvania populations or because wild populations in Pennsylvania had, in the past, been adulterated with cultivars.

Since Boehm et al. (1999) had established that there was indeed a genetic difference between cultivated and natural populations, the question remained as to how much genetic

variation there was in commercial ginseng farms and whether genetic variation was being lost in commercial farms because of selection. Schluter and Punja (2002) explored these questions as well as the question of whether or not the selfing nature of American ginseng would affect genetic variation. Using RAPDs, they discovered that most of the variation was found within cultivated and natural populations and not between them (Schluter & Punja 2002). They also found that cultivated populations had more genetic diversity than did natural populations, which suggests that cultivated populations have not gone through extensive selection pressures and that seed mixing has probably occurred, which would increase genetic diversity of these populations (Schluter & Punja 2002). Seeds harvested from the same mother plant showed less genetic diversity than those from cultivated populations as a whole, which is not surprising considering the level of genetic material that must be shared by siblings. Less genetic diversity among siblings indicates that continued selfing could result in genetically different populations, or cultivars (Schluter & Punja 2002). That said, they did find some genetic diversity between siblings in both plants that had been bagged to prevent cross pollination as well as in those in which cross pollination had not been prevented. Between these two samples, interestingly, the degree of genetic variation was not different. This suggests that heterozygosity in the parent plant, and not cross pollination, would be the likely explanation for the differences (Schluter & Punja 2002). Like the studies before it, this study focused mainly on the genetic diversity of cultivated populations in relation to natural populations. Of the 641 plants studied, only 58 were from three natural populations and these populations were 50+ km from one another. Also, different tissue types were sampled; leaves were sampled from some populations while seeds were sampled from others. Questions have been raised regarding the ability to use different

source tissues and still generate consistent and comparable results when using PCR-based experiments. Weising et al. (2005) reviewed several studies where PCR product taken from different tissues of the same plant yileded different results. The difference is thought to occur because of the way DNA in different tissues is methylated (Weising et al. 2005).

Because previous studies focused mostly on cultivated plants with little emphasis on wild populations, Grubbs and Case (2004) focused more heavily on wild populations. The goals of their investigation were to determine how allozyme variation was partitioned within and among populations, to see if wild and cultivated populations had genetic differences, and to see if they could find evidence of a predominant breeding strategy. They then compared their results to previous results that used RAPD markers instead of allozymes. The results of their study, using allozyme markers, showed that wild populations had more variation partitioned among themselves than they did within. This could be explained by a lack of gene flow among wild populations. Less variation in wild populations was found when compared to cultivated ones and this finding was similar to that found in RAPD studies. This can be explained by the higher rates of gene flow in a cultivated population than would occur in the wild. They also found a high estimate of inbreeding that is consistent with the self pollinating breeding system of American ginseng. Even though wild and cultivated populations were different from one another, only 3% of the total variation could be explained by this factor alone. Gene diversity was also not different between wild and cultivated populations and only one unique allele found in wild populations could not be found in cultivated ones.

A prevailing assumption in the American ginseng community is that no truly wild American ginseng populations exist in the 21st century. However, Grubbs and Case (2004)

point out that, based upon differences between wild and cultivated populations, it is more plausible that wild populations are still truly wild. They also concluded that wild populations should be considered wild unless other information confirming human interaction can be established. This is an important conclusion because if the idea that "no wild populations exist" is assumed to be correct, conservation strategies would, no doubt, be impacted. Because of the high estimates of inbreeding, Grubbs and Case (2004) warn aginst mixing wild populations because they may be locally adapted. Selfing populations are thought to purge deleterious recessive genes even though the population itself tends toward homozygosity. Introducing new genes to the population may interfere with locally-adapted gene complexes and cause populations to become less fit.

The first study to tackle the issue of genetic diversity within natural populations was that of Cruse-Sanders and Hamrick in 2004. Their aim was to measure genetic diversity across populations in the southeast USA using allozyme variation and to determine if the diversity of those populations was affected by harvesting pressures (Cruse-Sanders & Hamrick 2004a). Along with this information, demographic data were also collected and compared to see how harvest pressure may affect the average number of plants, ages of plants, and number of "prongs," or leaves, per plant (Cruse-Sanders & Hamrick 2004a). Heterozygosity was found to be less than expected, which seems to be in contradiction to Schluter and Punja (2002). Another difference Cruse-Sanders and Hamrick (2004a) found was that about half of the genetic diversity was among populations. Positive correlations between genetic distance as well as geographic distance were found, suggesting that populations showed isolation by distance, or IBD. Unprotected populations were found to have much less genetic diversity than protected populations, which could be explained by

constant harvest pressure (Cruse-Sanders & Hamrick 2004a). The use of allozymes in this study avoided the complications that are seen using RAPDs. The use of allozymes, however, has some of its own problems. For example, as proteins, they tend to underestimate diversity because different DNA base sequences can lead to the same protein structure. This may explain the high homozygosity that was found by Cruse-Sanders and Hamrick (2004a).

In a subsequent study Cruse-Sanders and Hamrick (2004b) looked into the spatial and genetic structure of American ginseng in wild populations and how it may differ in protected and unprotected populations. Because of microhabitat requirements and restricted seed movement, the authors hypothesized there would be significant fine scale genetic structure. They also thought that unprotected populations would have less fine scale genetic structure than would protected populations, because plants, particularly older ones, are being removed from unprotected populations. As expected, spatially, American ginseng was clumped with juvenile plants being close to adults. Fine scale genetic structure was also found in most of the populations studied (Cruse-Sanders & Hamrick 2004b). This was expected because of low distance seed dispersal and the selfing nature of American ginseng. In populations where juvenile and adult plants were looked at separately, juvenile plants showed fine scale genetic structure while adult plants did not. This would also be consistent with plants that self pollinate and have limited seed dispersal. Because there would be little to no outcrossing, adult plants would not be genetically as similar as the juveniles that came from those adult plants. The patterns seen in this study suggest there are family-structured groups within populations of American ginseng (Cruse-Sanders & Hamrick 2004b).

One of the most recent papers to be published related to the topic of genetic diversity within *Panax* was that of Zhuravlev et al. (2010). *Panax ginseng* is a close relative to *P.*

quinquefolius and both species share similar selection pressures and harvest histories. In their study, estimates of genetic diversity, the distribution of that diversity, and the relatedness of populations to one another were made using AFLP techniques. Ten populations were sampled for a total of 167 plants. Of the 282 fragments that were scored from two AFLP primers, 281, or 99.6%, were polymorphic across all individuals. Mean levels of polymorphisms within a population were found to be 55.6%. Analysis of molecular varience (AMOVA) partitioned the majority of the variance, 64.5%, as being within populations, leaving 35.5% among populations. Even with the high level of within-population variation, all pairwise comparisons of populations were significantly different. The authors suggested that the pattern of high within population variation coupled with significant pairwise population differentiation may be explained by human activities as they relate to moving plants and overexploitation of *P. ginseng* populations (Zhuravlev et al. 2010).

Materials and Methods

Sample Site Data

American ginseng plots were surveyed across the following locations: Shenandoah National Park (SHEN), Great Smoky Mountains National Park (GSMNP), The Blue Ridge Parkway (BRP), the Balsam Preserve (BAL), Caldwell County, N.C. (CALD), Wilkes County N.C. (WILKES) and Oconee State Park (SC) (Figures 1, 2 and 3). Plots were located by GPS coordinates previously established by park rangers or other researchers or plants were found by searching in habitat suitable for their growth. Once GPS coordinates were found, a 30 m x 30 m grid was established by laying two 30 m tapes perpendicular to each other on the ground so that they crossed at the 15 m mark. One tape was placed in a north-south orientation while the other tape was placed in an east-west orientation. This created four quadrants that were searched thoroughly for American ginseng. Once plants were found they were marked with a flag and measured for height. The number of leaves, or prongs, were also counted and recorded. Using a Garmin 60CSX handheld GPS unit (Garmin, Kansas City, Kansas), coordinates were taken for the site. Ownership and protection status of the land being surveyed was noted as being either public or private and as either protected or unprotected. Sites were also noted as being either known cultivated, presumed cultivated, presumed wild, or unknown harvest history. The elevation of the site was taken using the GPS unit. An inclinometer was used to measure the slope (in degrees) where the site was located. The aspect of the slope was taken with a handheld compass and the slope was classified as a ridge, sideslope, toe slope, terrace, or in a flood plain. A tree wedge prism was

used to measure the density of trees above the sample site. Canopy composition was categorized as either evergreen (>75% cover), deciduous (>75% cover) or mixed (both present and <75% each), and canopy closure estimates were made as open (0% closure), sparse cover (1-25% closure), somewhat closed (25-50% closure) or mostly closed (>50% closure). Any disturbances that may have been noticeable, such as logging, blowdowns, defoliation, deer browse, poaching, old homesites, or exotics, were also noted. For each site the number of American ginseng plants in each size or prong class was recorded. If any notes or photos of the site were taken, then they were also recorded on the data sheet. Along with these demographic data, the 30 m plot was surveyed for a list of potential indicator species which included the following: *Asplenium platyneuron L.* (ebony spleenwort), *Hepatica nobilis* Schreb. var. *acuta* (Pursh) Steyerm (sharp-lobed hepatica), *Trillium spp*., *Actaea racemosa* L. (black cohosh), *Sanguinaria canadensis* L. (bloodroot), *Podophyllum peltatum* L. (mayapple), *Caulophyllum thalictroides* (L.) Michx. (blue cohosh), *Lindera benzoin* (L.) Blume (spice bush), *Arisaema triphyllum* (L.) Schott (Jack-in-the-pulpit), *Hydrastis canadensis* L. (goldenseal), *Botrychium virginianum* (L.) Sw. (rattlesnake fern), *Adiantum pedatum.* L. (maidenhair fern), *Asimina triloba* (L.) Dunal (pawpaw), *Asarum spp.* L. (wild ginger) and *Trillium spp. L.* This list of potential indicator species was generated by the USGS. All of these data were kept on standardized data sheets that each researcher used in order to maintain continuity (Appendix A).

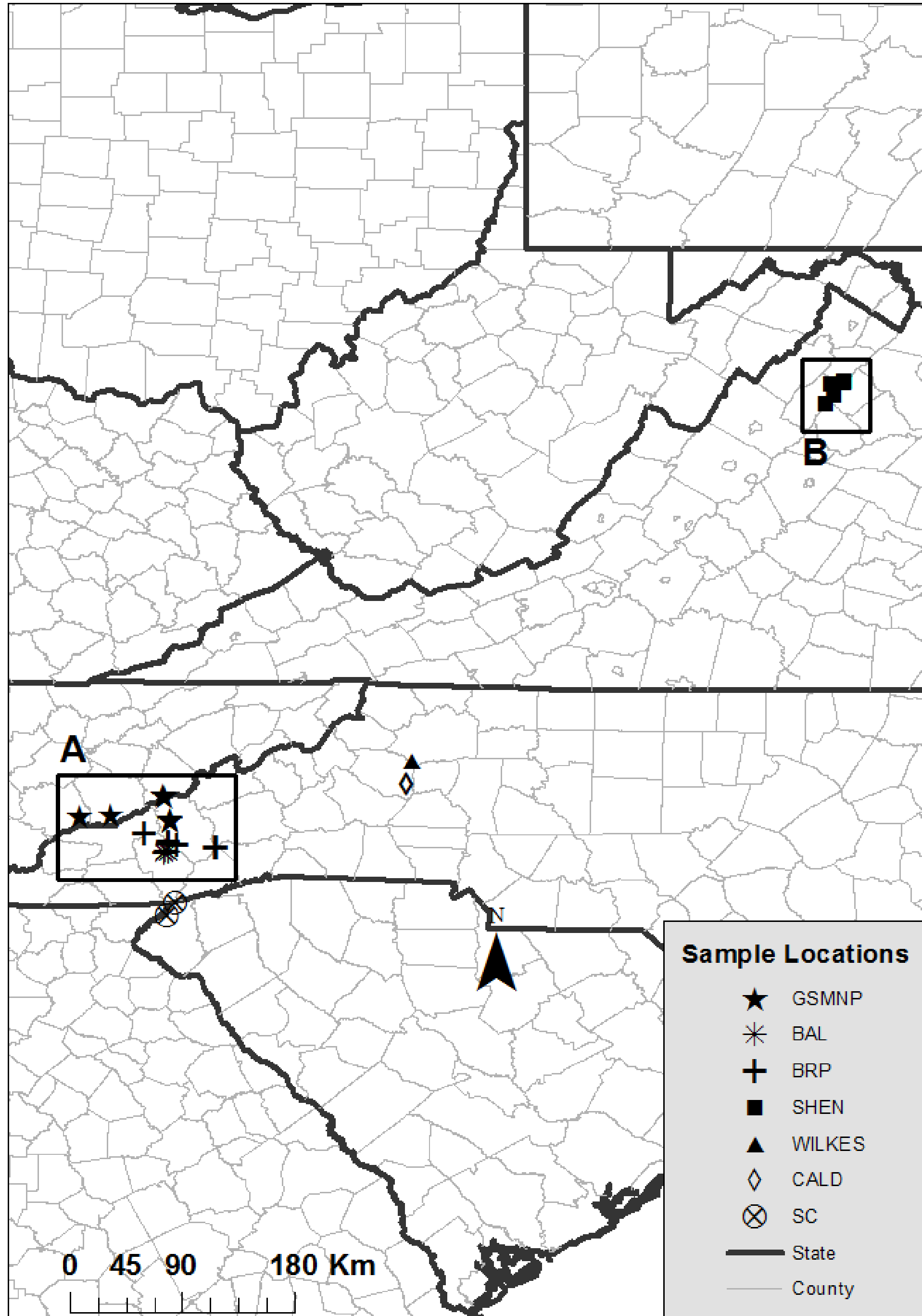

Figure 1. American ginseng sample sites in Great Smoky Mountains National Park (GSMNP), the Balsam Preserve (BAL), the Blue Ridge Parkway (BRP), Shenandoah National Park (SHEN), Wilkes County (WILKES), Caldwell County (CALD), and Oconee State Park (SC). Boxes A and B indicate areas that are enlarged in figures that follow.

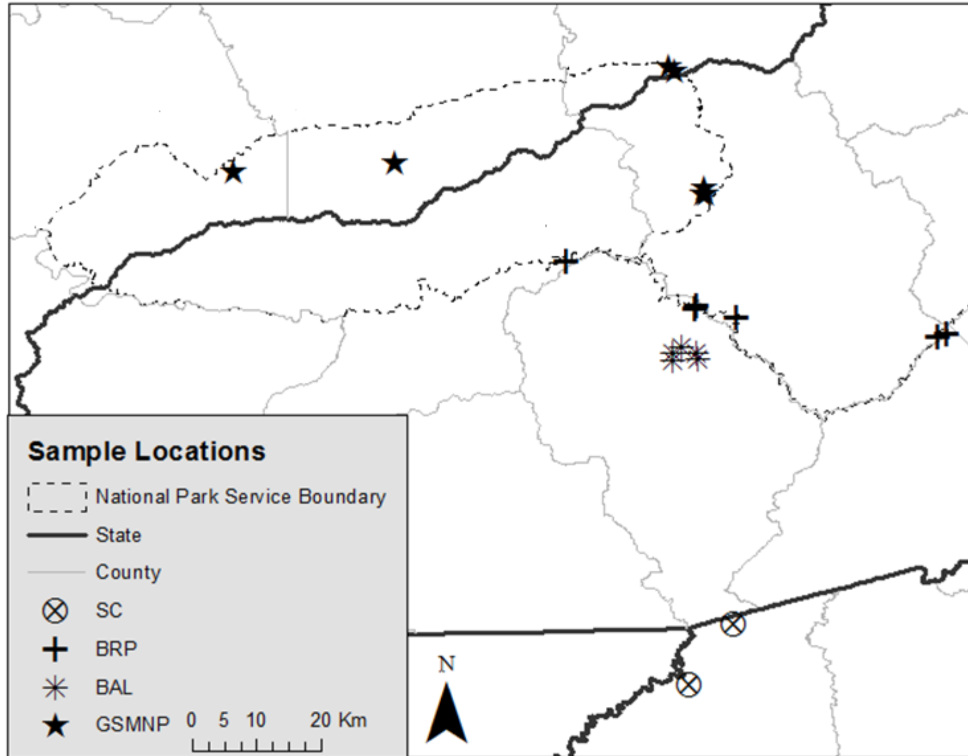

Figure 2. American ginseng sample sites from Box A in figure 1. The dotted line represents the boundary for Great Smoky Mountains National Park (GSMNP) and the Blue ridge Parkway (BRP).

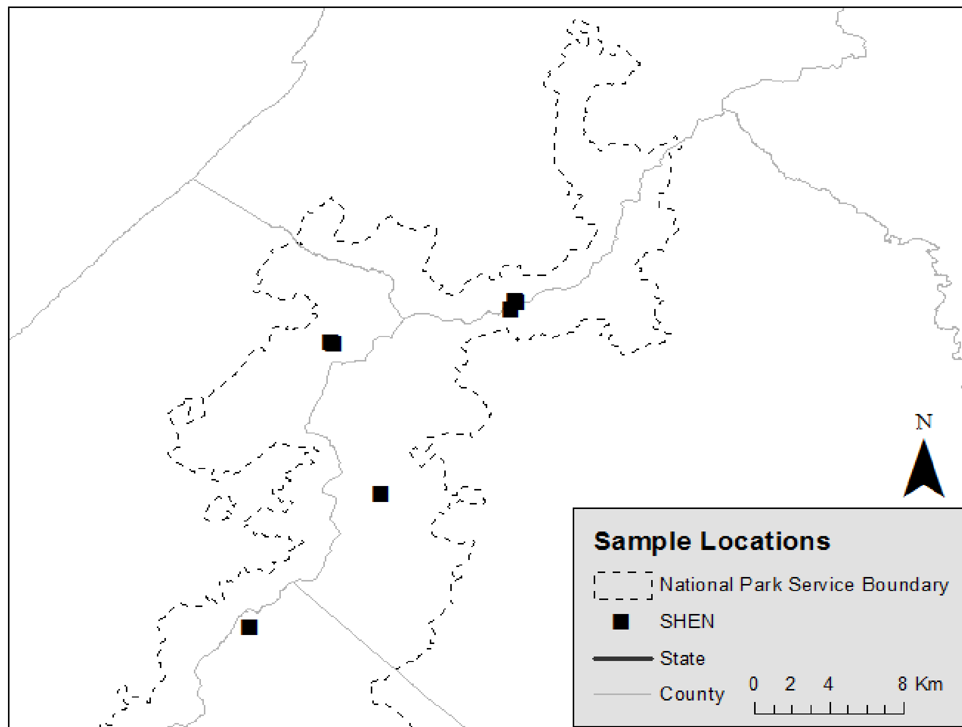

Figure 3. American ginseng sample sites from Box B in figure 1. The dotted line represents the boundary for Shenandoah National Park (SHEN).

Lab Analysis

All of the lab work in this study, both the DNA extraction and AFLP protocol, was completed in the lab of Dr. Tim King of the United States Geological Survey (USGS) Leetown Science Center in Leetown, West Virginia. Genetic samples were collected using Whatman FTA® cards. This method was minimally destructive in that a small portion of a leaf was firmly pressed into the card so that fluids and tissue from the leaf penetrated the cellulose fibers of the FTA card. Four separate samples, one from each plant sampled, were placed on each card and cards were stored in sandwich bags along with a desiccant of silica gel to keep samples dry. Genetic samples were marked with a two letter state abbreviation, a number that indicated the site and a letter that indicated the individual within that site. Samples were taken from up to eight individuals, when possible, from each site. The maximum of eight plants were chosen to make the most use of time and resources and based upon a previous study conducted by the USGS that found an average of eight plants per site (Thatcher et al. 2006). When possible, individuals were chosen so that they were spaced evenly in each of the four quadrants of the 30 m plot.

Whatman FTA cards containing genetic samples were taken back to the lab where DNA extractions were performed. A single hole paper punch was used to remove two samples from each Whatman FTA card and those punches were placed into a 1.5 ml microcentrifuge tube. To each tube, 600 µl of a cell lysis solution was added along with three µl of ProK. These tubes were inverted 25 times to mix and were then incubated at 55°C overnight. Following overnight incubation, 3 µl of RNAse was added. Tubes were again mixed by inverting 25 times and incubated for 60 min at 37°C. Samples were then

cooled to room temperature, and 200 µl of a protein precipitation solution was added followed by a 20 second vortex mixing. After vortexing, samples were placed in an ice bath for 5 mins followed by centrifugation at 13,000 x g for three minutes. A tight white pellet formed at the bottom of the microcentrifuge tubes and the supernatant above the pellet was poured off into a new 1.5 ml microcentrifuge tube. To the supernatant, 600 µl of 100% isopropyl alcohol along with one µl of a 20 mg/ml glycogen solution was added. Tubes were then gently mixed by inverting 50 times and incubated for five minutes at room temperature. After incubation, tubes were again centrifuged at 13,000 x g for five minutes in order to pelletize the DNA. The supernatant was poured off and 600 µl of 70% EtOH was added, followed by inverting tubes several times to wash the DNA. Tubes were again centrifuged at 13,000 x g for one minute and then the EtOH was carefully poured off. Tubes were left open and allowed to air dry for 10-15 minutes or until the smell of EtOH was no longer detected. Twenty µl of a DNA hydration solution was added to the dry DNA pellet and it was allowed to rehydrate overnight at room temperature. DNA samples were stored in a -80°C freezer for later use with care taken not to put samples though too many freeze-thaw cycles in order to avoid mechanical shearing of DNA. Concentrations of DNA were recorded using a Nanodrop ND-1000 spectrophotometer (ThermoScientific, Wilmington, DE).

In order to ensure that both EcoRI and MseI would cut *P. quinquefolius* DNA, 6 µl of two different DNA samples from Ohio were cut with each restriction enzyme individually and with both restriction enzymes together. The restriction was allowed to run overnight at room temperature. Following restriction, 5 µl of product was run through a 1.5% agarose gel and a smear from the 100-1500 bp range was observed in order to confirm complete restriction.

AFLP Procedure

Once it was confirmed that both EcoRI and MseI restriction enzymes would cut ginseng DNA, the AFLP protocol from Applied Biosystems was started. Using a selective amplification kit purchased from Applied Biosystems containing 8 EcoRI and 8 MseI selective primers that could give 64 primer combinations, primer pairs were run together until three sets of primers were found that yielded consistent peaks over 100 relative florescence units (RFU) across the entire fragment range in a capillary array sequencer. Once those pairs were found, a gel was run using product from the pre-selective amplification step to ensure fragment sizes were in the appropriate range, from 100 to 1500 base pairs. If the correct size range was found, those primers were then used to selectively amplify DNA from all research sites. Polymerase chain reaction was completed in a PTC-200 Thermal Cycler (CR-MJ Research, Waltham, Massachusetts) and sequencing was carried out in a 3100 XL Genetic Analyzer (Applied Biosystems, Carlsbad, California).

After sequencing, a presence-absence table was generated such that peaks, or bands, present in one sample received a “1” while those absent in a sample received a “0”. This process was automated in Genemapper 3.7 (Applied Biosystems, Carlsbad, California) where all peaks above 100 RFU were counted as “present” and those below 100 RFU were treated as noise and, therefore, not counted.

Statistical Analysis

The bulk of the statistical analysis was completed in GenAlEx 6.41, a macro that runs in Excel (Peakall & Smouse 2006). GenAlEx produced a distance matrix that was used in

AMOVAs, spatial autocorrelation and principal component analyses. The false discovery rate correction (Benjamini & Hochberg 1995) was used when multiple pairwise comparisons were made between sites or locations. The AMOVA partitioned the variation "within" and "among" each site or geographic location, depending on which was being tested.

In addition to GenAlEx 6.41, STRUCTURE 2.3.3 was also used to analyze the data matrix. STRUCTURE is model-based clustering method for inferring population structure using genotype data (Pritchard et al. 2000). It assigns individuals from a matrix to a population, or multiple populations, based on probability and user input, such as the number of populations (K), assumed to be present. The burn-in for each run was set at 10000 iterations and the number of Markov Chain Monte Carlo (MCMC) iterations after the burn-in was also 10000. After K was determined, the appropriate STRUCTURE output was loaded into CLUMPP 1.1.2 in order to modify the bar graph by adding labels and altering the color to make a more presentable figure (Jakobsson & Rosenberg 2007).

Using the above process, all samples within a geographic location were grouped together so they could be compared to all other locations. For instance, all plants from the GSMNP were grouped together and compared to all samples taken in SHEN, and so on. After locations were compared, smaller scale genetic differentiation was tested. To do this finer scale test, sample sites within a location were compared to one another. For instance, all 7 sample sites within GSMNP were compared to one another to see if differences existed among sites within the park. When analyzing at this finer scale, sample sites that had less than two plants were removed from the analysis. This removal was done because one individual cannot be used in an AMOVA when comparing it to other sites.

Results

Sample Site Data

Thirty-one sites were surveyed for American ginseng at 7 different locations. Twenty-five, or 81%, of sites surveyed had American ginseng within the plot (Table 1). The 7 sample locations along with sites surveyed at each location are shown in Table 1. In the 25 sites where American ginseng was found, the average number of plants per site was 8 with a range of 1 to 21 plants. The number of American ginseng plants surveyed was 189 with most being 3 pronged and the least being 4 pronged (Figure 4). For genetic samples, the maximum sample size per site was set to 8 before the study began, so a total of 119 genetic samples were taken (Table 1). In some instances, insufficient DNA was extracted from the FTA cards, so those samples were removed from the study.

Table 1. Location and sample site information

Location	Sites	# of plants found	# of genetic samples
Great Smoky Mountains National Park			
	GSMNP3	5	5
	GSMNP12	6	5
	GSMNP13	7	7
	GSMNP14	1	1
	GSMNP15	2	2
	GSMNP17	1	1
	GSMNP20	7	7
Balsam Mountain Preserve			
	BAL1	10	8
	BAL2	3	3
	BAL3	2	2
	BAL4	5	2
	BAL5	4	3
Wilkes County			
	WILKES	6	6
Blue Ridge Parkway			
	BRP2	7	7
	BRP3	12	7
	BRP4	21	8
	BRP7	0	0
	BRP8	0	0
	BRP9	0	0
	BRP10	0	0
Caldwell County			
	CALD80	18	7
	CALD81	7	4
Oconee State Park			
	SC2	0	0
	SC19	0	0
Shenandoah National Park			
	SHEN1	6	4
	SHEN2	11	8
	SHEN3	2	2
	SHEN4	2	2
	SHEN5	18	8
	SHEN6	7	6
	SHEN7	19	4
Total	31	189	119

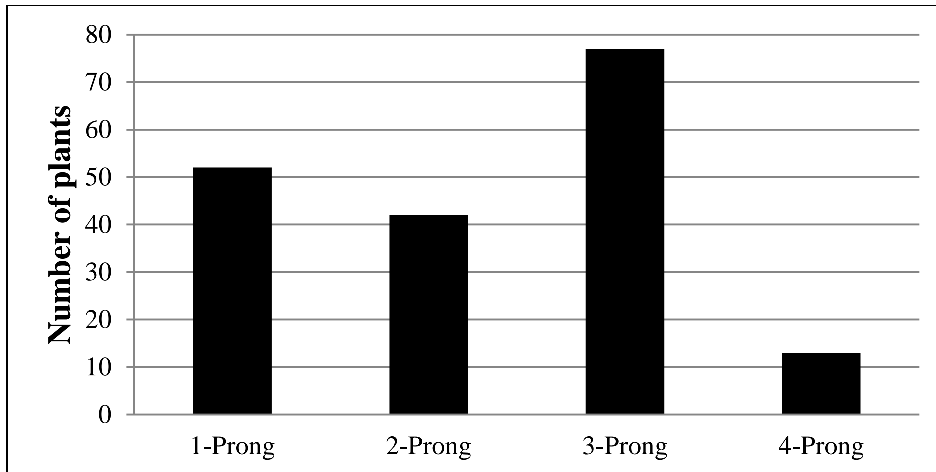

Figure 4. Number of plants with 1, 2, 3 and 4 prongs.

In the survey of co-occurring plants, I found that trillium, bloodroot, black cohosh and maidenhair fern were the most common co-occurring plants. They were found in 23, 20, 17, and 17 of the 25 sites where ginseng was located, respectively. The average elevation in which plants were found was 903 m with the minimum being 362 m, which was located on private land in CALD, and the maximum elevation was 1534 m, which was on the BRP. The average slope in which American ginseng was found was 19% and ranged from 5% to 30%. The aspect of most slopes fell to the north, between 45° and 315°, showing that, in this survey, plants do tend to grow predominantly on north facing slopes, but there were some slopes that faced as far south as 204°. Using the tree wedge prism a range of 12 to 39 m^2/ha basal area was found, with the average being 25 m^2/ha. In sites where American ginseng was found, the dominant overstory cover was comprised of deciduous tree species. The entire set of these community data can be found in Appendix B.

AFLP Procedure

After extraction, DNA concentrations ranged from 0.03 ng/µl to 81.01 ng/µl. Regardless of the variation, Vos et al. (1995) did not find significant fall out of amplification

product when using the AFLP technique until DNA concentrations fell below 2.5 pg/µl. The lowest concentration in this study was around 30 pg/µl, which is 12 times higher than what Vos et al. (1995) found to be insufficient. Restriction of DNA with both EcoRI and MseI gave a smear on a 1.5% agarose gel (Figure 5). A subsequent 1.5% agarose gel, run with pre-selectively amplified product, gave a smear in the 100 to 1500 bp size range (Figure 6).

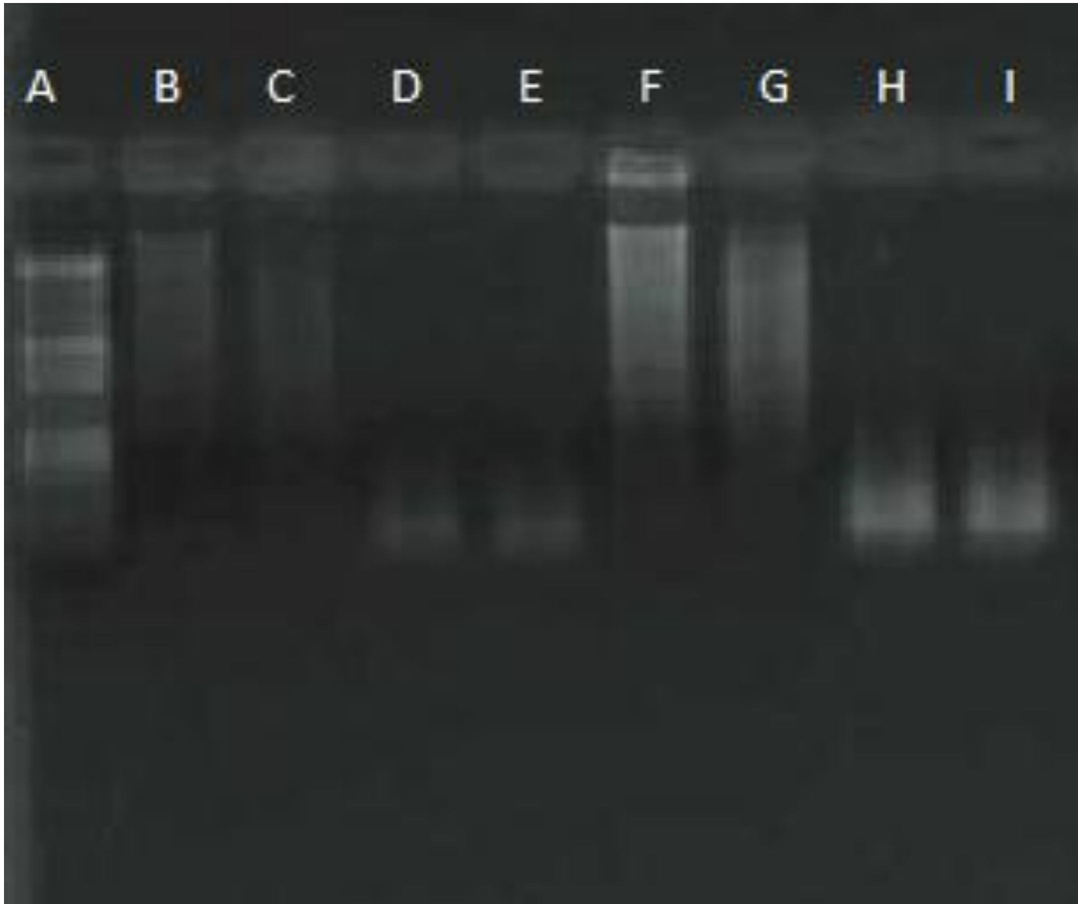

Figure 5. *P. quinquefolius* samples cut with MseI and EcoRI. Lane A is a one KB ladder while lanes B-E is sample one and F-I is sample two. Lanes B and F are uncut DNA, lanes C and G are cut with EcoRI, lanes D and H are cut with MseI and lanes E and I are cut with both EcoRI and MseI together.

Figure 6. Agarose gel (1.5%) run with 16 samples after pre-selective amplification. The first lane is a one KB ladder and the last lane is a HindIII size standard. The white box indicates the 1500-100 base pair range that the product should be found in.

The comparison of all 64 AFLP primer pair combinations yielded 2 primer pairs that gave consistent readable peaks higher than 100 RFU. These primer pairs were EcoRI-ACT/MseI-CAA (1A), and EcoRI-ACC/MseI-CTT (4H). Using these two primer pairs, 240 peaks were generated. All of these peaks were polymorphic between individuals since no peak was found in every single individual. Frequency information, such as total number of bands, the percentage of polymorphic bands, number of private bands and mean heterozygosity are found in Table 2.

Table 2: AFLP frequency information for each sample site in Great Smoky Mountains National Park (GSMNP), the Balsam Preserve (BAL), the Blue Ridge Parkway (BRP), Shenandoah National Park (SHEN), Wilkes County (WILKES), and Caldwell County (CALD). Percentage of polymorphic loci within the site (%P) and the mean heterozygosity (Mean He) are shown.

Population	**# samples**	**# Bands**	**% P**	**No. Private Bands**	**Mean He**
GSMNP3	5	98	20.83%	0	0.080
GSMNP12	5	111	35.83%	1	0.134
GSMNP13	7	120	44.58%	0	0.156
GSMNP15	2	85	15.42%	0	0.064
GSMNP20	7	121	49.17%	0	0.173
BAL1	8	106	38.75%	0	0.143
BAL2	3	95	38.33%	0	0.150
BAL3	2	65	24.58%	0	0.102
BAL4	2	87	28.33%	1	0.117
BAL5	3	103	40.42%	0	0.154
WILKES	6	117	38.75%	1	0.133
BRP2	7	136	47.08%	1	0.144
BRP3	7	126	41.25%	0	0.130
BRP4	8	135	49.58%	1	0.155
CALD	11	123	37.08%	2	0.105
SHEN1	4	93	17.92%	0	0.064
SHEN2	8	152	62.50%	1	0.192
SHEN3	2	96	36.67%	0	0.152
SHEN4	2	87	7.08%	0	0.029
SHEN5	8	130	42.50%	0	0.138
SHEN6	6	115	29.17%	0	0.111
SHEN7	4	113	37.08%	0	0.126

Statistical Analysis

Using an AMOVA, most of the locations were found to be different from one another (p=0.007), after doing a B-H False Discovery Rate correction, with only one exception (Benjamini & Hochberg 1995; Table 3). That one exception was that GSMNP was not significantly different from BMP. Most of the variation (75%) was found to be partitioned within sites leaving 11% among locations and 13% among sites (Table 4). Principal coordinates one, two, and three accounted for 79% of the genetic variation and also showed

clustering of sites at the location level (Figures 8 and 9). STRUCTURE analysis supported the AMOVA and PCA findings by showing negligible differences between GSMNP and BAL, but clear differences between all other geographic locations (Figure 10). Spatial autocorrelation found that sites that are close to each other (≤ 25 km), are more genetically similar to one another than sites that are separated by distances greater than 25 km (Figure 7).

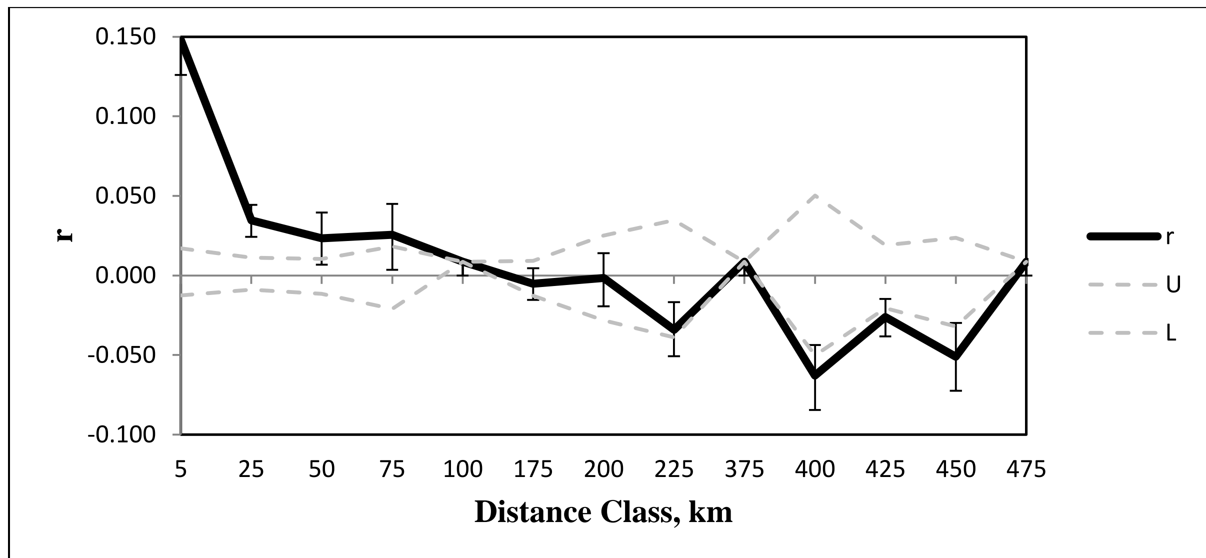

Figure 7: Spatial autocorrelation of samples sites. Dashed lines U and L are the 95% confidence limits and r is the correlation coefficient.

Table 3. Pairwise comparison of Great Smoky Mountains National Park (GSMNP), the Balsam Preserve (BAL), the Blue Ridge Parkway (BRP), Shenandoah National Park (SHEN), Wilkes County (WILKES), and Caldwell County (CALD) locations using PhiPT values generated from an AMOVA in GenAlEx. PhiPT values are below the diagonal while significance values are above the diagonal. Red indicates significant differences after doing a False Discovery Rate correction (p=0.007).

	GSMNP	**BAL**	**WILKES**	**BRP**	**CALD**	**SHEN**	
GSMNP		0.0206	0.0001	0.0001	0.0004	0.0001	**GSMNP**
BAL	0.0559		0.0004	0.0002	0.0025	0.0001	**BAL**
WILKES	0.2698	0.2643		0.0001	0.0003	0.0005	**WILKES**
BRP	0.1209	0.1258	0.144		0.0004	0.0001	**BRP**
CALD	0.1095	0.1443	0.268	0.0598		0.0005	**CALD**
SHEN	0.1291	0.1297	0.2135	0.1029	0.1199		**SHEN**
	GSMNP	**BAL**	**WILKES**	**BRP**	**CALD**	**SHEN**	

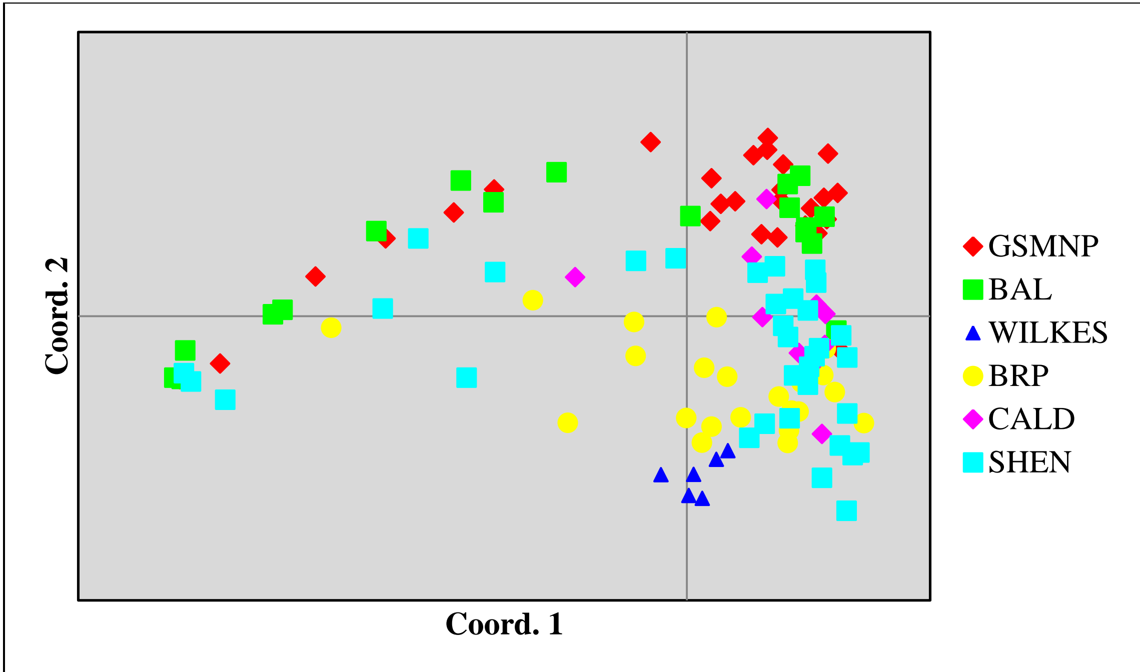

Figure 8. Coordinates 1 and 2 from a PCA generated in GenAlEx. Locations include Great Smoky Mountains National Park (GSMNP), the Balsam Preserve (BAL), the Blue Ridge Parkway (BRP), Shenandoah National Park (SHEN), Wilkes County (WILKES), and Caldwell County (CALD)

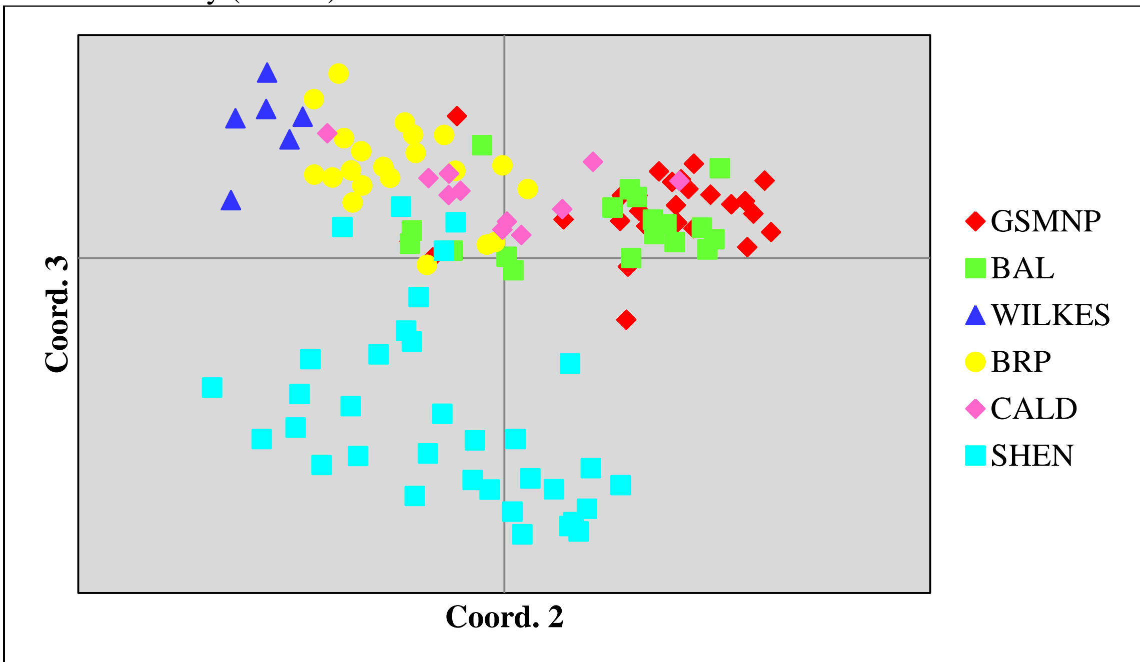

Figure 9. Coordinates 2 and 3 from a PCA of locations done in GenAlEx. Locations include Great Smoky Mountains National Park (GSMNP), the Balsam Preserve (BAL), the Blue Ridge Parkway (BRP), Shenandoah National Park (SHEN), Wilkes County (WILKES), and Caldwell County (CALD).

Table 4: Partition of variation based upon AMOVA.

Source	df	SS	MS	Est. Var.	%
Among Locations	5	513.18	102.636	3.079	11%
Among sites	16	599.118	37.445	3.578	13%
Within sites	95	1947.12	20.496	20.496	75%
Total	116	3059.42		27.153	100%

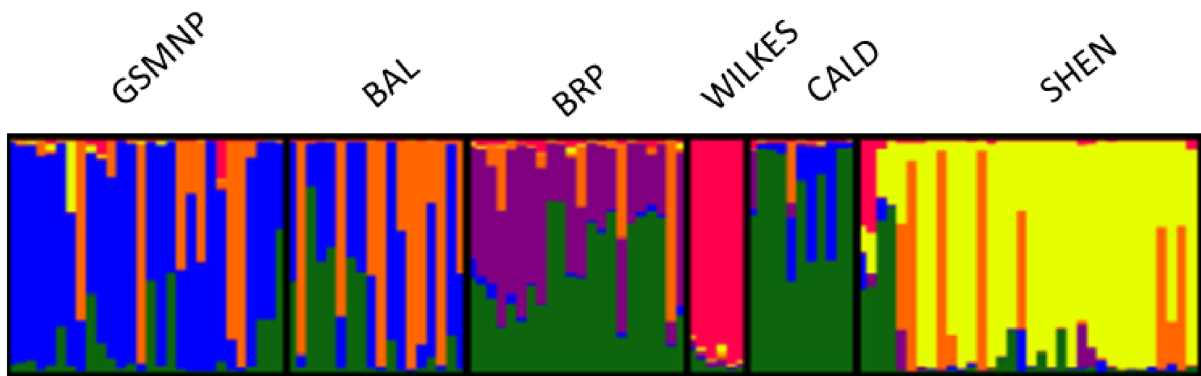

Figure 10. STRUCTURE output showing differences among Great Smoky Mountains National Park (GSMNP), the Balsam Preserve (BAL), the Blue Ridge Parkway (BRP), Shenandoah National Park (SHEN), Wilkes County (WILKES), and Caldwell County (CALD). Colors represent different populations (K) found by the STRUCTURE software. Each small bar represents a single plant within a location and colors within that bar represent the likelihood that that particular plant belongs to a given location. Dark black lines separate each location.

Most sites within GSMNP were not significantly different from one another. The only significant difference found was that GSMNP12, GSMNP13, and GSMNP15 were different from GSMNP3 (Table 5). Ninety percent of the genetic variation was found within sites, leaving only 10% among sites. Principal coordinates one, two and three, which account for 72% of the variation combined, showed some grouping within sample sites, but also a large amount of overlap between sample sites (Figure 11 and 12). STRUCTURE analysis did not show clear differences between sites GSMNP3 and GSMNP12, GSMNP13, or GSMNP15 as was suggested by AMOVA and PCA analyses (Figure 13).

Table 5. Pairwise comparison of sites in Great Smoky Mountains National Park using PhiPT values generated from an AMOVA in GenAlEx. PhiPT values are below the diagonal while significance values are above the diagonal. Red indicates significant differences after false discovery rate correction (p=.04).

	GSMNP3	GSMNP12	GSMNP13	GSMNP15	GSMNP20	
GSMNP3		0.0077	0.0079	0.0001	0.0813	**GSMNP3**
GSMNP12	0.1391		0.1819	0.0944	0.1005	**GSMNP12**
GSMNP13	0.1041	0.0494		0.0828	0.2478	**GSMNP13**
GSMNP15	0.3699	0.2229	0.1942		0.1129	**GSMNP15**
GSMNP20	0.1014	0.091	0	0.1099		**GSMNP20**
	GSMNP3	**GSMNP12**	**GSMNP13**	**GSMNP15**	**GSMNP20**	

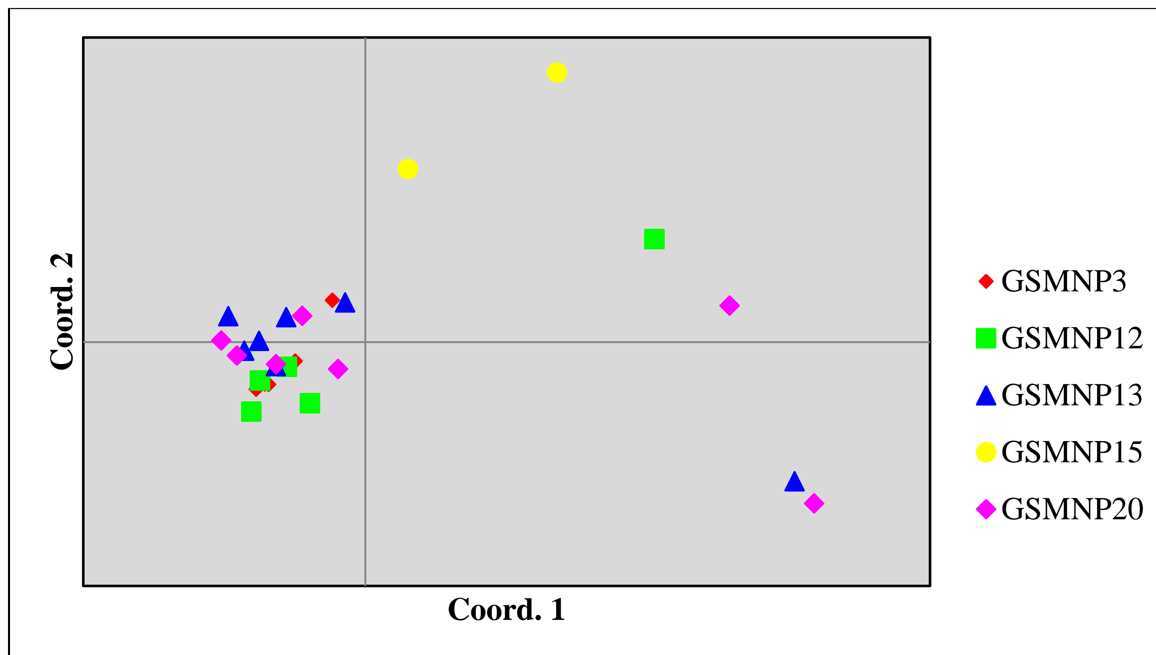

Figure 11. Coordinates 1 and 2 from PCA of Great Smoky Mountains National Park sample sites.

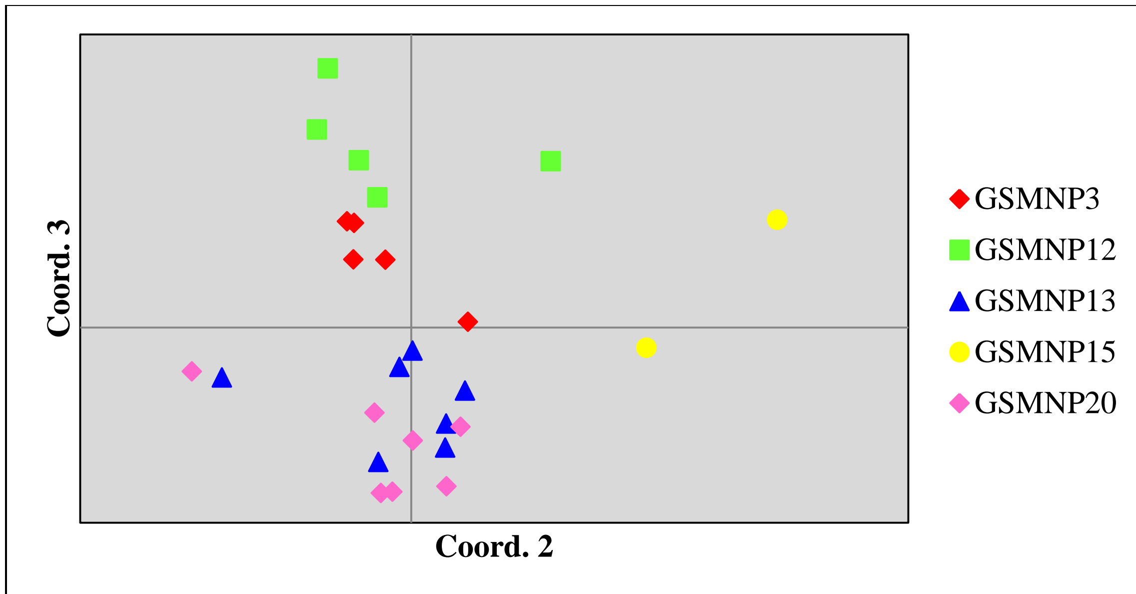

Figure 12. Coordinates 2 and 3 from PCA of Great Smoky Mountains National Park sample sites.

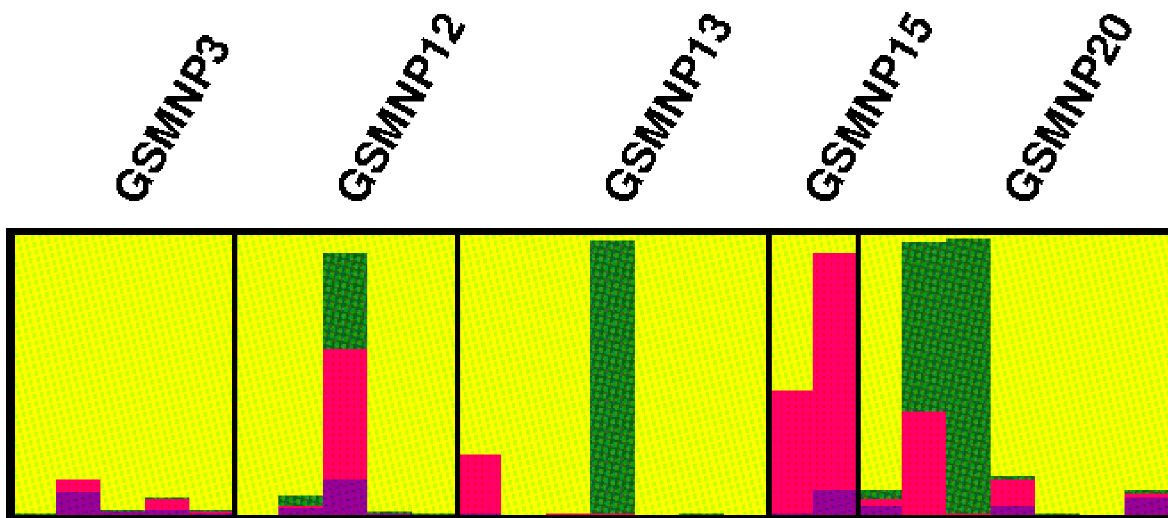

Figure 13. STRUCTURE analysis showing no clear differences among Great Smoky Mountains National Park sample sites. Colors represent populations (K) found using STRUCTURE analysis. Each bar represents a single plant within the sample site. Black lines separate sample sites.

Genetic differences among sample sites within the BAL were not found. AMOVA indicated that there were no significant differences among sites within the preserve (Table 6). Ninety-nine percent of the variation was found to be within sites while only 1% was among sites. Principle coordinates analysis did show some grouping within BAL1, but also showed

a large amount of variation within all other sample sites (Figures 14 and 15). STRUCTURE supports the PCA by showing BAL1 form a tight group with the exception of two individuals (Figure 16).

Table 6. Pairwise comparison of sample sites in the Balsam Mountain Preserve. PhiPT values are below the diagonal while significance values are above the diagonal. No significant differences were found between BAL sample sites.

	BAL1	BAL2	BAL3	BAL4	BAL5	
BAL1		0.3861	0.0886	0.0899	0.0796	**BAL1**
BAL2	0.0398		0.4006	0.3019	0.4016	**BAL2**
BAL3	0.2508	0		0.3333	0.3839	**BAL3**
BAL4	0.1399	0	0		0.4239	**BAL4**
BAL5	0.1291	0	0	0		**BAL5**
	BAL1	**BAL2**	**BAL3**	**BAL4**	**BAL5**	

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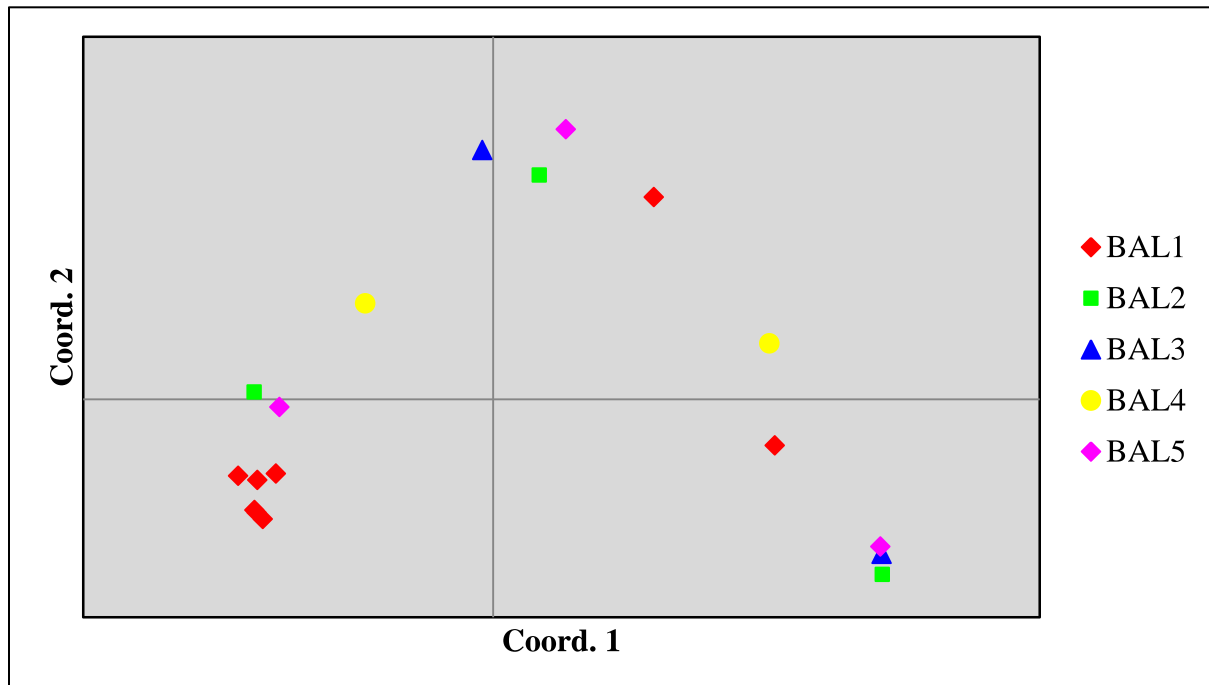

Figure 14. Coordinates 1 and 2 from a PCA of Balsam Mountain Preserve sample sites.

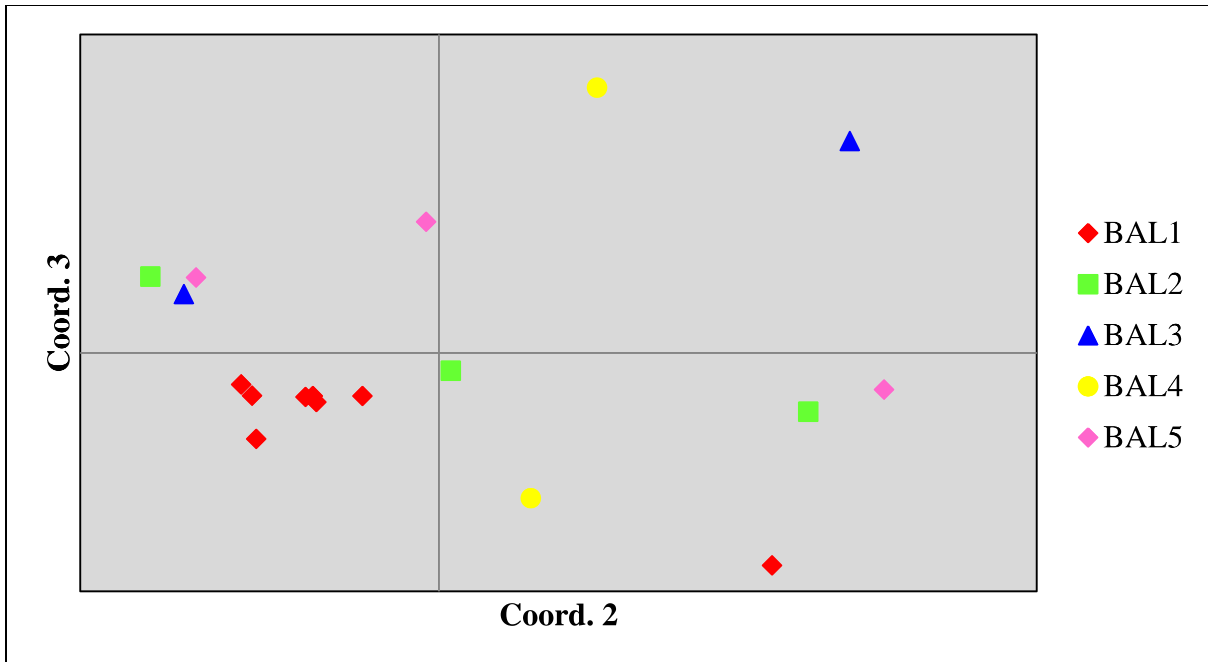

Figure 15. Coordinates 2 and 3 of a PCA for Balsam Mountain Preserve sample sites.

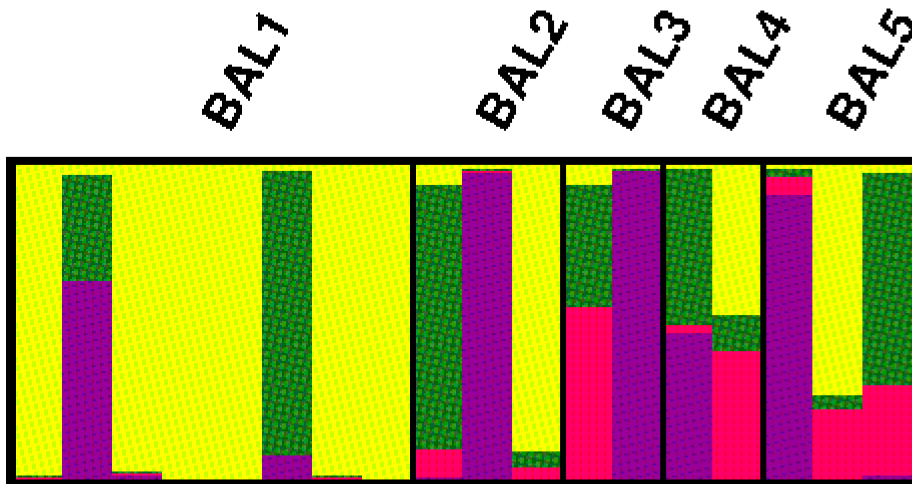

Figure 16. STRUCTURE analysis of Balsam Mountain Preserve location.

BRP3 and BRP4 are significantly different from BRP2 (p=0.033), but BRP3 and BRP4 are not significantly different from one another (Table 7). AMOVA indicate a high amount of within site variation, at 96%, with only 4% being among sites. Principle coordinates 1, 2 and 3, which explain 62.7% of the variation, also indicates differences may

exist between BRP2 and BRP3 (Figures 17 and 18). STRUCTURE analysis also indicates that there are differences between BRP2 and the other two sample sites (Figure 19).

Table 7. Pairwise comparison of sample sites in the Balsam Mountain Preserve. PhiPT values are below the diagonal while significance values are above the diagonal. Red indicates significant differences between sites after B-H False Discovery Rate correction (p=0.033).

	BRP2	**BRP3**	**BRP4**	
BRP2		0.011	0.024	**BRP2**
BRP3	0.047		0.293	**BRP3**
BRP4	0.055	0.006		**BRP4**
	BRP2	**BRP3**	**BRP4**	

.

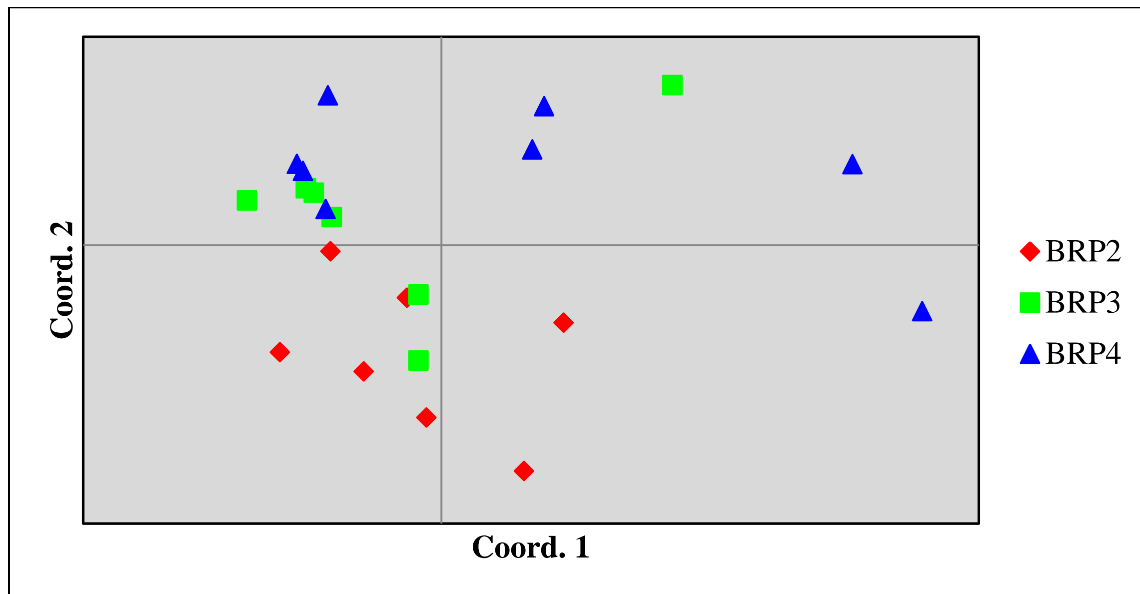

Figure 17. Coordinates 1 and 2 of PCA for Balsam Mountain Preserve sample sites.

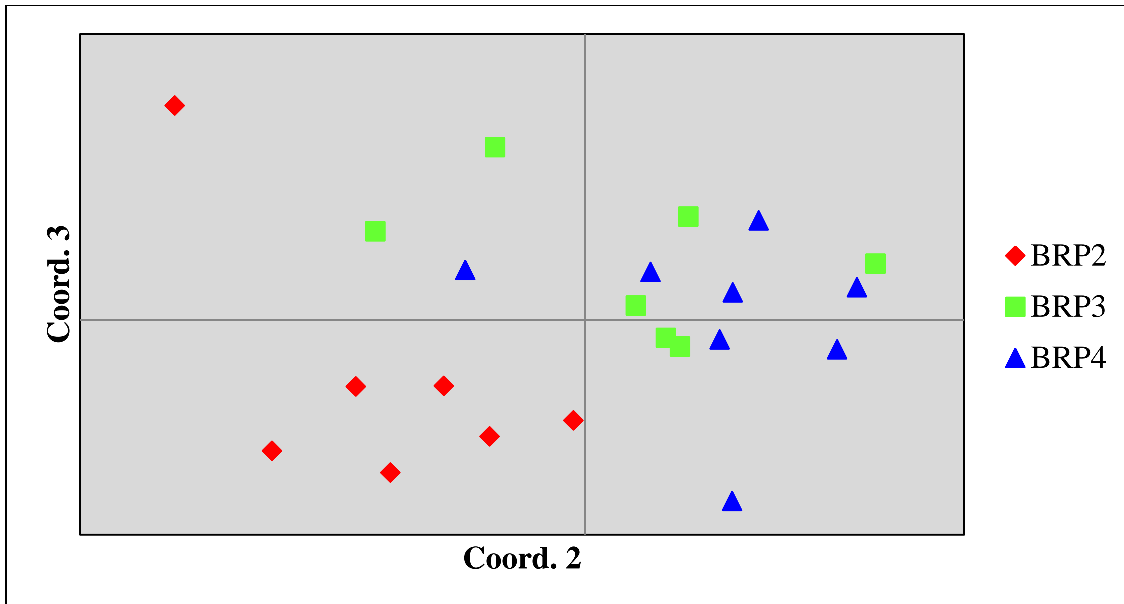

Figure 18. Coordinates 2 and 3 of PCA for Balsam Mountain Preserve sample sites.

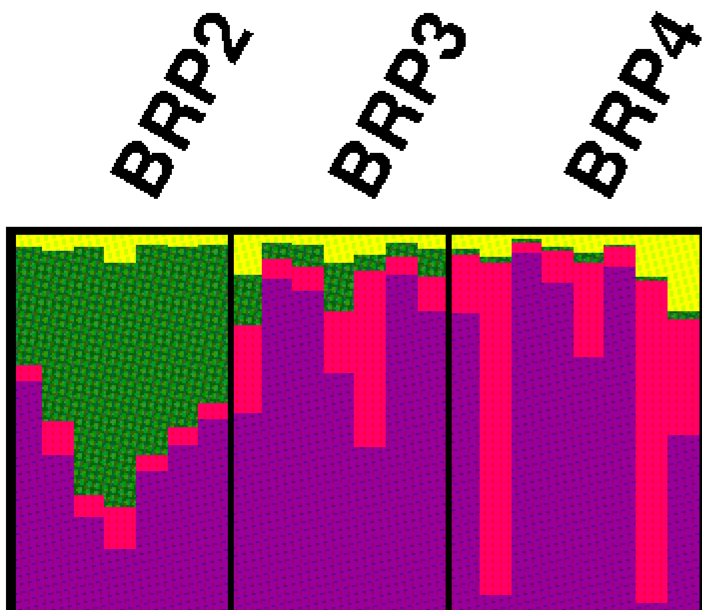

Figure 19. STRUCTURE analysis of Balsam Mountain Preserve sample sites.

There were pairwise differences between sample sites within SHEN. A pairwise distance matrix generated from an AMOVA found that SHEN3 and SHEN4 were not

different from any other VA site and SHEN2 was not different from SHEN7 (Table 8). All of the other pairwise comparisons were different from one another after using the B-H False Discovery Rate correction (p=0.031; Table 8). The partition of the genetic variation in AMOVA showed that 85% was within sites while 15% was among sites. Principle components 1, 2 and 3, which explained 73% of the variation, did not show clear clumping of samples within a site (Figure 20 and 21). STRUCTURE showed a clear difference between VA1 and all other sites, but VA2-VA7 did not appear different from one another (Figure 22).

Table 8. Pairwise distance matrix of Shenandoah National Park sample sites. Numbers below the diagonal are PhiPT values while those above are significance values. Red indicates significance after false discovery rate correction at p=0.031.

	SHEN1	**SHEN2**	**SHEN3**	**SHEN4**	**SHEN5**	**SHEN6**	**SHEN7**	
SHEN1		0.022	0.066	0.066	0.002	0.005	0.028	**SHEN1**
SHEN2	0.173		0.398	0.111	0.014	0.012	0.267	**SHEN2**
SHEN3	0.248	0		0.335	0.045	0.067	0.331	**SHEN3**
SHEN4	0.26	0.07	0.066		0.331	0.081	0.137	**SHEN4**
SHEN5	0.167	0.13	0.204	0		0.004	0.01	**SHEN5**
SHEN6	0.273	0.149	0.23	0.115	0.125		0.004	**SHEN6**
SHEN7	0.311	0.022	0	0.205	0.23	0.243		**SHEN7**
	SHEN1	**SHEN2**	**SHEN3**	**SHEN4**	**SHEN5**	**SHEN6**	**SHEN7**	

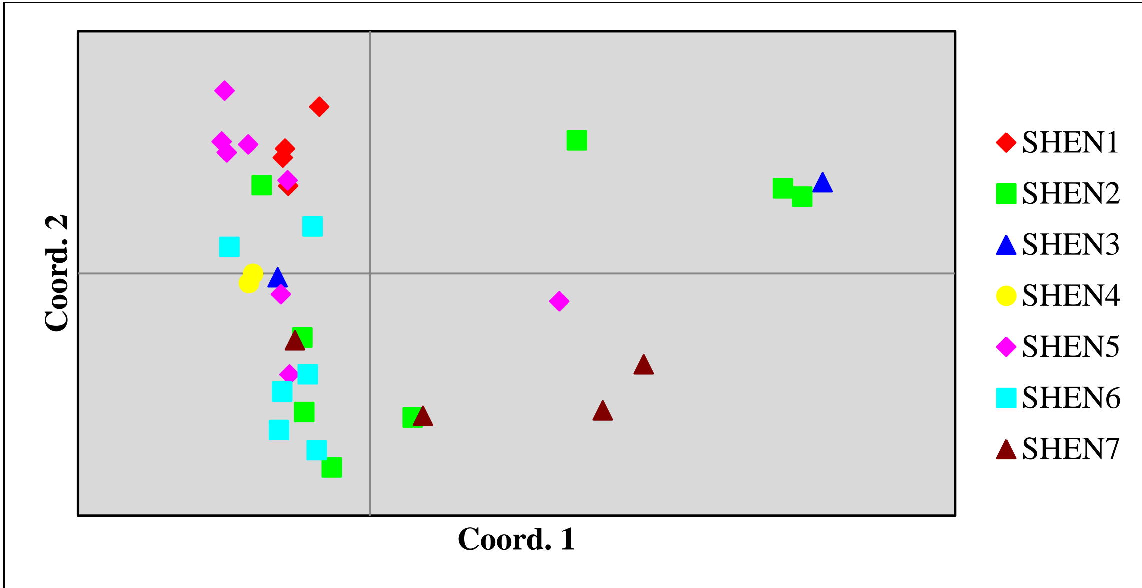

Figure 20. Coordinates 1 and 2 showing the overlap among Shenandoah National Park sample sites.

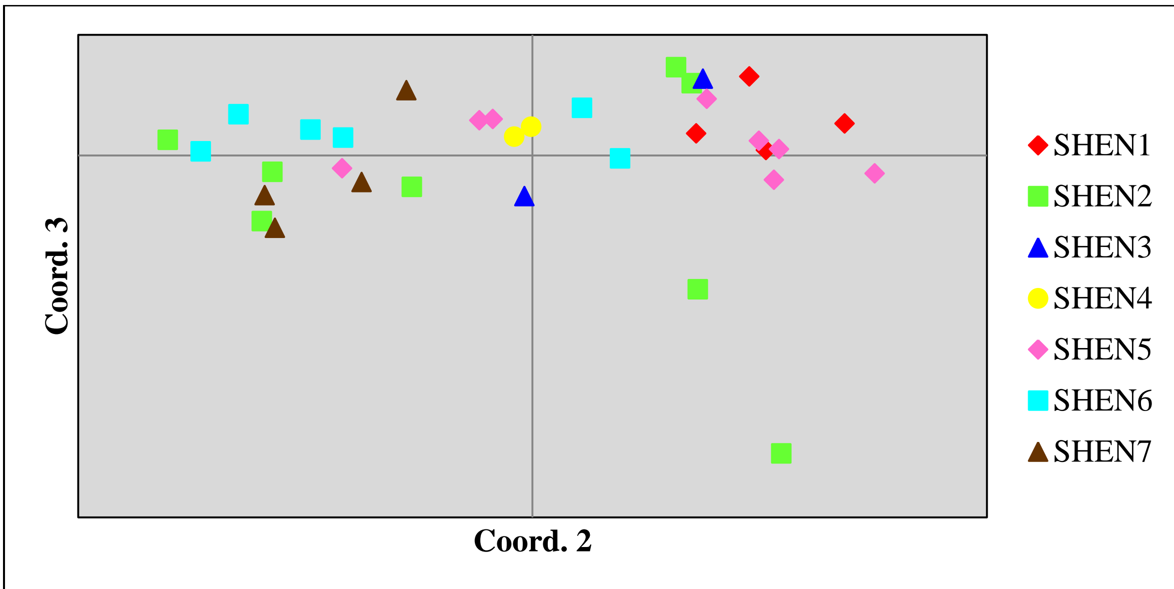

Figure 21. Coordinate 2 and 3 of Shenandoah National Park sample sites.

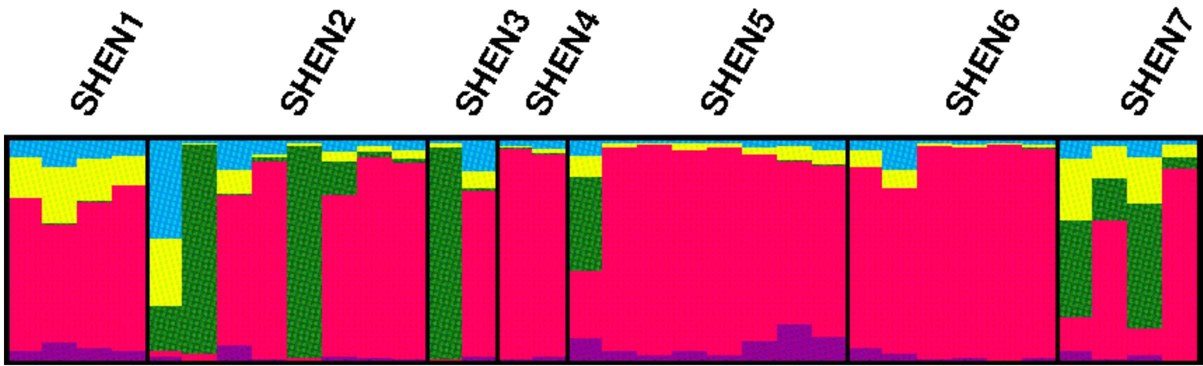

Figure 22. STRUCTURE output showing Shenandoah National Park sample sites.

Discussion

Genetic differences among geographic locations were clear and distinguishable. For instance, GSMNP is genetically different from SHEN, BRP, CALD, and WILKES. The finding that most of the genetic variation was located within sites and some of those sites are still different from one another indicates that American ginseng has not suffered a major loss of genetic variation overall. My hypothesis of low within site variation and high among site variation was rejected. It was thought that most of the variation would occur among sample sites and locations, not within, because of American ginseng's lack of seed dispersal and high level of self-pollination, but this clearly was not the case. This finding closely matches what Honnay and Jacquemyn (2006) found about self-pollinating plant species. They found that, regardless of population size, selfing plants were able to maintain higher diversity levels than plants that were obligate out-crossers. As habitats are fragmented, obligate out-crossers lose potential mates, while self-pollinators do not suffer the same loss of mate. The pattern found here was also similar to that found by Zhuravlev et al. (2010) when they looked at *P. ginseng* in Russia. The similarity between the two studies is not surprising given that the species are closely related, share very similar life history strategies and are under similar harvest pressures. In both studies, within population variation was higher than among population variation. Populations in Russia have seen harvest pressure from humans for a much longer time period than American ginseng populations have experienced. This longer period of harvest pressure exposure may have reduced variation within *P. ginseng* populations and that

observed reduction could be a prediction for the future of *P. quinquefolus* populations in North America unless drastic conservation efforts are taken.

Genetic differences are harder to discriminate at finer spatial scales in this study, like those within locations. For example, there are minimal differences between sample sites within GSMNP or within SHEN. Even though some of the sample sites are different from others, no clear pattern emerges from these few differences. The lack of difference at this smaller spatial scale may be due to human-initiated disturbances. Plants within GSMNP or SHEN, for instance, have been moved from one site to another by poachers and by rangers replanting poached plants. In GSMNP specifically, most of the sites that were surveyed were close to trails and roads which means they were easily accessible to potential harvesters. In fact, during this study a poacher was encountered while digging plants at one sample site. In addition to transplanting roots, mixing of seeds also occurs, and most probably has since the onset of American ginseng cultivation in the late 1800s, if not earlier. Harvesters pool seeds from collection sites and plant them among other ginseng sites and they buy seeds from cultivation farms to plant in wild populations. Pooling seeds in this fashion would increase the variation within a site and obscure any fine-scale patterns that may have naturally occurred. Lastly, it could be that sites in this study were actually part of a once very large population. This would mean that sites within any location are actually subpopulations of what were much larger, continuous and extensive populations. Based upon the historic literature, there is little doubt that American ginseng was much more abundant in the forest understory and, therefore, populations were much larger than present day. Larger populations also tend to have higher levels of genetic diversity than smaller populations, so the high levels of genetic diversity found in this study support that assumption.

It is interesting that the WILKES location appears to be so different in both the principal components analysis and the STRUCTURE analysis, and that it appears to have less variation than other locations. WILKES was located in a privately owned section of woods that is part of a hunting club, just off a logging road many of the club members used for access. As previous studies have found, populations that are not on protected land tend to have less variation than those that are found on protected land (Cruse-Sanders & Hamrick 2004a). This is thought to be because the constant removal of adult plants from the population imposes a constant bottleneck on those plants that are left behind. While this study was not designed to tease apart genetic differences in protected and unprotected land, this finding appears consistent with those of Cruse-Sanders and Hamrick (2004a). John Young of the USGS, through personal communication about a currently unpublished work, reported that his research has shown that when using microsatellite markers, WILKES samples cluster with samples taken from Ohio and the Tennessee Valley. If these samples were taken from another site or are from seed of Ohio or the Tennessee Valley populations, then that may explain why they appear so different in this study as well.

Studies of species with similar self-pollinating reproductive strategies to American ginseng reveal differences and similarities as they pertain to the allocations of genetic differentiation. Schonswetter et al. (2004) found that *Comastoma tenellum*, which is a self-pollinating Alpine species in the family Gentianaceae, held most of its variation at the regional level, with the least amount of variation being found within populations. Schonswetter et al. (2004) conclude that the patterns they found are consistent with selfing annual populations that do not immigrate or cross pollinate. A couple of factors could cause the differences between this study and Schonswetter et al. (2004). There is no report that *C.*

tenellum is harvested or traded to the extent that American ginseng is. This would limit the impact humans would have on the genetic picture. High within population genetic diversity in American ginseng populations could be a result of harvester seed mixing. American ginseng is also a perennial species so the same individuals contribute to the genetic picture year after year. Because there is little to no outcrossing, and because of the family type structuring that was found by Cruse-Sanders and Hamrick (2004b) within American ginseng populations, differences that arise between plants within a population would persist for longer periods of time. A study conducted by Gale et al. (2010) on the endangered orchid *Nervilla nipponica* found higher diversity within populations than among populations, which is similar to this thesis. This diversity pattern was partially attributed to the fact that orchids have very fine powder-like seeds that can be wind dispersed over very long distances. This long distance seed dispersal could give a similar genetic picture as the potential seed mixing that occurs in American ginseng populations.

Conservation

Even though there still appears to be a lot of genetic variation within American ginseng populations, the small size of those populations may threaten that diversity. Small populations are usually not able to adapt to change as easily as larger populations because, as mentioned above, small populations tend to have less genetic variation. Currently, harvesters and whitetail deer are driving down the sizes of American ginseng populations, which could ultimately lead to a loss of overall genetic diversity. One way the current level of genetic diversity can be preserved is to encourage the use of cultivated plants for medicinal use rather than the use of wild plants. There is a pervasive idea that "wild" American ginseng

roots are more potent than harvested roots. The price that cultivated roots bring is, therefore, much lower than that of roots harvested in the wild. Due to the fluctuation from day to day, it is hard to put a consistent number on the price of cultivated or wild ginseng; however in 2011 a local dealer in North Carolina estimated that cultivated roots sold for $40 a dried pound while wild roots sold for $500-550 per dry pound. So, even though ginseng can be easily cultivated in mass quantities, hunters continue to harvest in the wild because of the stark price difference. A study by Assinewe et al. (2003) that compared the phytochemistry of wild and cultivated roots showed no significant difference in ginsenoside content between the two groups of plants. Given that cultivated roots yield the same concentration of ginsenosides as "wild" ones, much of the harvest pressure could be lifted from wild plants if cultivated plants were used more extensively. The increased use of cultivated roots has not happened yet because hundreds of years of history and tradition need to be overcome as it pertains to American ginseng use as a medicine. The only way to overcome this history is through the continual education of those that consume American ginseng products to convince those people that cultivated roots are just as effective.

Harvest pressure may also be lifted if the legal age for collecting American ginseng plants was raised from 5 years old to 10 years old. This change was attempted by the USFWS in 2005 but was reversed in 2006 and, thus, there was not enough time to see if increasing the harvest age would have any effect. It is unclear if science or politics drove the decision for the USFWS to change the legal age of harvested plants back to 5 years old in 2006. During this time, there was some resistance from harvesters, especially individuals that practice woods-grown cultivation. Harvesters expressed concern about being able to decide if plants were 10 years old or not without digging roots. Woods-grown cultivators

feared that because their cultivation technique often yields roots that appear to be wild they would have been subject to the law as well.

Given that much time and money is put into starting an American ginseng crop, woods-grown cultivators may have a valid fear. To circumvent this, individuals that practice woods-grown cultivation techniques could be allowed to show documents of seeds or plants purchased to prove they are cultivating, or there could be a permitting system put into place allowing cultivators to set themselves apart from wild harvesters. Regardless of how woods-grown cultivators fit into the picture, harvesting wild plants at 5 years of age is detrimental to population size and likely to genetic diversity as well. Anderson et al. (2002) indicate that wild plants have not even reached reproductive age within 5 years, so they have not had time to contribute to the seed bank or the genetic diversity of the population before they are removed. To protect the genetic diversity as it currently stands, the age of legal harvesting should be raised from 5 to 10 years old.

Whitetail deer also need to be controlled more effectively, not only for the sake of American ginseng, but also for the sake of other imperiled plants in general. Furedi and McGraw (2004) found that in the majority of populations they studied, deer ate 50% or more of the reproductive plants and also consumed a substantial portion of the fruits in those populations. In the same study it was found that American ginseng seeds the deer consume do not germinate after passing through the digestive system (Furedi & McGraw (2004). In a subsequent study, it was found that annual mean deer browse rates varied from 19% to 42%, but harvest rates by humans across the same time span ranged from 0.45% to 3.04% (McGraw & Furedi 2005). This would mean that deer may pose a more serious threat to American ginseng populations than harvesters do. At current browse rate levels, McGraw

and Furedi (2005) conclude that 800 plants would be needed per population to remain sustainable or that deer browse rates would have to be decreased by at least 50%. It is not thought that deer are preferentially choosing to eat American ginseng, so current browse rates may be having similar effects on other imperiled plants. Increasing the hunting seasons or bag limits for deer hunters may help American ginseng populations to grow larger and, at the same time, help preserve current diversity levels.

As this and other studies have shown, American ginseng populations have retained a significant amount of genetic diversity, in spite of the harvesting by humans and the foraging by deer. However, this does not mean that this diversity could not and will not be reduced in the future if conservation actions are not taken. As mentioned above, increased age requirements and the reduction of whitetail deer populations may go a long way in restoring American ginseng to pre-trade levels. In preserving the size of populations we would also preserve the genetic diversity within those populations. Doing so will make populations more resilient to potential environmental changes, and potential extirpation in the future.

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Appendix A: Example of data sheet used during American ginseng survey.

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USGS 2007-2009 American Ginseng Study If found, please call 304-724-4469

[X] New Survey [ ] Re-survey

Date: 8-13-08 (MM/DD/YY) Start Time: ______ End Time: ______ Surveyors: ______

Land Unit/Area Description: Balsam Preserve

Ownership/Harvest Protection Status:

[ ] Public [X] Private Protection status unknown [ ]

[X] Protected [ ] Unprotected

Population Source:

[ ] Known cultivated [ ] Presumed Wild

[ ] Presumed cultivated [ ] Unknown

Coordinates: N____° ____' W____° ____' +-____m

* Degrees.Decimal minutes (WGS84), Mark as averaged waypoint in GPS

Plot number: NC 089 BAL 5 (Example: WV-001)

* Plot number consists of state and plot sequence number by state

Elevation (m): 949 **Aspect (degrees):** 100 **Slope (degrees):** 30

Slope Position: Ridge Sideslope Toe Slope Terrace Flood Plain **Wedge Prism:** # of trees: 17

Canopy Composition: Evergreen (>75% of cover) Deciduous (>75% of cover) Mixed

Canopy Closure: [ ] Completely Open (0% closure) [ ] Sparse cover (1-25% closure) [ ] Somewhat closed (25-50% closure) [X] Mostly Closed (> 50% closure)

Disturbance: [ ] none [ ] logging [ ] blowdown [ ] defoliation [ ] homesite [ ] exotics [ ] poaching [ ] deer browse ______ other

Ginseng Present: [X] **Ginseng Absent:** [ ] **Total Found:** 34

Ginseng Stem count:

Plant Size	1- prong	2-prong	3-prong	4+ prong
Number of plants		|	|||	

Ginseng genetics sample: (max of 8 per plot: divide total # plants by 8 to get skip value: ex. If 24 plants found, sample every 3rd plant: spread sampling equally among plot quadrants if possible: write plot number and sample in each square of FTA card)

Sample	Prongs:	Height:	Bud scars:	NS/EW dist	Sample	Prongs:	Height:	Bud scars:	NS/EW dist
a	3	8		/	e				/
b	3	10		/	f				/
c	2	10		/	g				/
d	3	10		/	h				/

Co-occurring herbaceous species:

____ Ebony Spleenwort	____ Mayapple	____ Rattlesnake fern
____ Sharp-lobed hepatica	____ Blue cohosh	X Maidenhair fern
X Trillium spp.	X Spice bush	____ Paw paw
X Black cohosh	X Jack-in-the-pulpit	____ Wild ginger
X Bloodroot	____ Goldenseal	____ Tulip Poplar

Site Photos:
1) 2) 3) 4)

Notes: ______

Appendix B: Demographic data of sites surveyed for American ginseng.

IDENT	Search Type [1]	Search Method [2]	Ownership [3]	Protection Status [4]	Population Source [5]	EPE (m)	Elevation (m)	Aspect (degrees)	Slope (degrees)	Slope Position	Wedge Prism (. of trees)	Canopy Composition [6]	Canopy Closure [7]	Disturbance[8]	Logging[8]	Blowdown[8]	Defoliation[8]	Homesite [8]	Exotics[8]	Poaching[8]	Deer Browse [8]
GSMP003	NS	PS	Pub	Pro	PW	6.3	590	30	18	Side	9	D (>75% of cover)	MC	N							
GSMP012	NS	PS	Pub	Pro	PW	6.2	650	340	21	Side	10	D (>75% of cover)	MC	N							
GSMP13	NS	PS	Pub	Pro	PW	4.3	1034	20	15	Toe	9	D (>75% of cover)	MC	N							
GSMP14	Re-S	PS	Pub	Pro	PW	6.3	1111	340	22	Side	10		MC	N							
GSMP15	NS	PS	Pub	Pro	PW	4.7	764	325	10	Side	8	D (>75% of cover)	MC	N							
GSMP17	Re-S	PS	Pub	Pro	PW	6.4	1117	300	24		11	D (>75% of cover)	MC	N							
NC-001	NS	PS	Pri	UnPro	PW	6.2	437	284	25	Side	11.5	D (>75% of cover)	MC	N	N	N	N	N	N	N	N
NC-002	NS	PS	Pub	Pro	PW	8.6	865	320	15	Side	12	D (>75% of cover)	MC	Y	N	N	N	N	N	N	N
NC-003	NS	PS	Pub	Pro	PW	6.7	1246	290	25	Side	10	D (>75% of cover)	MC	Y	N	N	N	N	N	N	N
NC-004	NS	PS	Pub	Pro	PW	5.4	1195	226	18	Side	11	D (>75% of cover)	MC	Y	N	N	N	N	N	N	N
NC-007	NS	TS	Pub	Pro	PW	8.7	1486	280	12	Side	15	E (>75% of cover)	MC	Y	N	N	N	N	N	N	N
NC-008	NS	TS	Pub	Pro	PW	7.7	1424	124	20	Side	14	D (>75% of cover)	MC	Y	N	N	N	N	N	N	N
NC-009	NS	TS	Pub	Pro	PW	9.1	1534	20	5	Side	7	M	S/w C	Y	N	N	N	N	N	N	N
NC-010	NS	TS	Pub	Pro		8.7	1519	60	15	Side		M	S/w C	N	N	N	N	N	N	Y	N
NC-080	NS	PS	Pri	UnPro	PW	3.2	362	290	15	Side	11	D (>75% of cover)	MC	Y							
NC-081	NS	PS	Pri	UnPro	PW	4.7	371	90	20	Side	8	D (>75% of cover)	MC	N							
NC-085	NS	PS	Pri	Pro		6.9	1405	300	23	Side	10	D (>75% of cover)	MC	Y							
NC-086	NS	PS	Pri	Pro	PW	4.8	1405	60	20	Side	5	D (>75% of cover)	MC	Y							
NC-087	NS	PS	Pri	Pro		6.2	1143	230	19	Side	9	D (>75% of cover)	MC	N							
NC-088	NS	PS	Pri	Pro		5.5	1086	330	19	Side	8	D (>75% of cover)	MC	N							
NC-089	NS	PS	Pri	Pro		0	949	100	30	Side	17	D (>75% of cover)	MC	N							
SC-002	NS	TS	Pub	UnPro	PW	5.3	805	52	25	Side	11	D (>75% of cover)	MC	N							
SC-019	NS	TS	Pub	UnPro		4.6	659	322	18	Side	12	D (>75% of cover)	MC	N							
VA-001	Re-S	PS	Pub	Pro	PW	4.8	841	204	6	Side	11	D (>75% of cover)	MC	Y	N	N	N	N	N	N	N
VA-002	Re-S	PS	Pub	Pro	PW	3.7	862	204	14	Side	10	D (>75% of cover)	MC	N	N	N	Y	N	N	N	N
VA-003	Re-S	GO	Pub	Pro	PW	4.2	761	0	0		0			N	N	N	N	N	N	N	N
VA-004	Re-S	GO	Pub	Pro	PW	4.2	606	0	0		0			N	N	N	N	N	N	Y	N
VA-005	Re-S	PS	Pub	Pro	PW	4.9	603	30	24	Side	8	D (>75% of cover)	MC	N	N	Y	N	N	N	N	N
VA-006	Re-S	PS	Pub	Pro	PW	6.2	523	42	24	Side	9	D (>75% of cover)	MC	N	N	N	N	N	Y	N	N
VA-007	Re-S	PS	Pub	Pro	PW	4.4	777	100	18	Side	15	D (>75% of cover)	MC	Y	N	N	N	N	N	N	N

Notes: [1]New survey or resurvey, [2]Plot survey or times search, [3]Public or Private, [4]Protected or unprotected, [5]Presumed Wild, [6]Deciduous or evergreen, [7]Mostly closed or somewhat closed, [8] yes or no

Appendix B: continued

IDENT	Disturbance(Other)	Ginseng Present/Absent [9]	Total Found	1-prong	2-prong	3-prong	4-prong	Ebony Spleenwort [10]	Sharp-lobed hepatica [10]	Trillium spp [10]	Black Cohosh[10]	Bloodroot[10]	Mayapple [10]	Blue Cohosh [10]	Spicebush [10]	Jack-in-the-pulpit [10]	Goldenseal [10]	Rattlesnake Fern [10]	Maidenhair Fern [10]	Paw Paw [10]	Wild Ginger[10]
GSMP003		P	5	1	1	3	0	F	F	T	T	T	F	T	F	F	F	F	T	F	T
GSMP012		P	6	3	3	0	0	F	F	T	T	T	F	F	F	F	F	F	T	F	T
GSMP13		P	6	0	2	4	0	F	F	T	F	T	F	F	F	F	F	F	T	F	F
GSMP14		P	1	0	0	1	0	F	F	T	F	T	F	F	F	F	F	F	T	F	F
GSMP15		P	2	2	0	0	0	F	T	T	T	T	F	T	F	F	F	F	F	F	T
GSMP17		P	1	0	0	1	0	F	F	T	F	T	F	F	F	F	F	F	F	F	T
NC-001	hunting access road in plot	P	6	3	3	0	0	F	F	F	T	T	F	F	F	F	F	T	T	F	F
NC-002		P	7	5	0	2	0	F	F	T	F	F	F	T	F	T	F	T	T	F	F
NC-003		P	12	1	2	8	1	F	F	T	F	T	F	T	F	T	F	F	T	F	F
NC-004		P	21	16	1	4	0	F	F	T	F	T	F	T	F	T	F	T	T	F	F
NC-007		A	0	0	0	0	0	F	F	T	T	F	F	F	F	F	F	F	F	F	F
NC-008		A	0	0	0	0	0	F	F	T	T	T	F	T	F	T	F	F	F	F	F
NC-009		A	0	0	0	0	0	F	F	T	F	F	F	F	F	F	F	F	F	F	F
NC-010	Large areas of freshly overturned soil	A	0	0	0	0	0	F	F	T	F	F	F	F	F	T	F	T	F	F	F
NC-080	old road bed in plot	P	18	3	6	9	0	F	F	T	T	F	F	F	F	F	F	F	F	F	F
NC-081		P	7	1	2	4	0	F	F	T	T	T	F	F	F	F	F	F	T	F	F
NC-085	within last hundred years	P	10	0	2	5	3	F	F	F	T	T	F	T	F	F	F	F	F	F	F
NC-086		P	3	0	1	1	1	F	F	F	T	T	F	T	F	F	F	F	F	F	F
NC-087		P	2	0	0	2	0	F	F	T	F	T	F	F	F	F	F	F	F	F	F
NC-088		P	5	1	2	1	1	F	F	T	T	T	F	T	F	F	F	F	T	F	F
NC-089		P	4	0	1	3	0	F	F	T	T	T	F	F	T	T	F	F	T	F	F
SC-002		A	0	0	0	0	0	F	F	F	F	F	F	F	F	F	F	F	F	F	F
SC-019		A	0	0	0	0	0	F	F	F	F	F	F	F	F	F	F	F	F	F	F
VA-001		P	6	2	1	3	0	F	F	F	T	T	F	T	T	F	F	T	T	F	T
VA-002	10	P	11	0	3	8	0	F	F	F	T	T	F	T	T	F	F	T	T	F	T
VA-003		P	0	0	0	0	0	F	F	F	F	F	F	F	F	F	F	F	F	F	F
VA-004	?	P	0	0	0	0	0	F	F	T	F	F	F	F	F	F	F	T	T	F	F
VA-005		P	18	5	4	5	4	T	T	T	T	F	F	F	T	F	F	T	T	F	T
VA-006	Garlic mustard, ailanthus	P	7	1	4	2	0	T	F	T	T	T	F	F	T	F	F	T	T	F	T
VA-007		P	19	6	2	8	3	F	F	T	F	F	F	F	F	F	F	T	F	F	T

Notes: [9]Present or absent, [10]True or false

Vita

David DeViney was born on July 11, 1978 in Hickory, North Carolina. He is the son of Gwen and Rex Teague and Liz and Marshall DeViney. He attended elementary and middle school in Granite Falls, NC and high school in Hudson, NC. Upon high school graduation in 1996, David went to North Carolina State University for a single semester. He then returned home to Dudley Shoals, North Carolina where he worked for Corning Cable Systems for almost 4 years. David left Corning Cable Systems to pursue his education, enrolled at Caldwell Community College and Technical Institute and completed his A.S. two years later. David transferred to Appalachian State University in Boone, NC during Spring 2004 and in the Fall of 2005 completed his B.S. in Ecology and Environmental Biology with a Basic Science concentration. After a semester away from school, he was accepted into graduate school at Appalachian State University under the advisement of Dr. Gary Walker. He completed his course work and research and then took a job at Caldwell Community College and Technical Institute while simultaneously writing his thesis. David completed his thesis in May 2013 and was awarded his M.S. in Biology. He continues to work at CCC&TI.