

Genetic Diversity of *Panax quinquefolius* L. Across Geographic Distance

Tiana Miller
Biology Department
The University of North Carolina Asheville
One University Heights
Asheville, North Carolina 28804 USA

Faculty Advisor: Jennifer Rhode Ward

Abstract

Panax quinquefolius L., commonly known as American ginseng, is a perennial herb imperiled in parts of its range and listed under Appendix II of the Convention on International Trade in Endangered Species due to threats from overharvesting. American ginseng roots are commonly used in Traditional Chinese Medicine to treat a range of ailments. The preference for human-shaped roots has caused an increase in demand for wild grown plants, which has caused a drastic decrease in range-wide abundance of American ginseng populations. In this study, seven microsatellite loci were used to examine patterns of genetic diversity in American ginseng populations. Allelic and genotypic diversity varied by population but not by population protection status. In addition, populations showed significant genotypic overlap. Reasons for genetic similarities among populations are unclear but could be due to contemporary gene flow amongst populations or historic fragmentation of a larger population. Results could be used to repatriate poached plants or prioritize site protection.

1. Introduction

Panax quinquefolius L., commonly known as American ginseng, is a long-lived herbaceous perennial commonly used in Traditional Chinese Medicine (TCM). This member of the Araliaceae family is found throughout eastern North American deciduous forests, particularly in the Appalachians, Ozarks, and eastern Canada. American ginseng is a slow growing plant, usually taking 4-10 years to reach reproductive maturity. Once mature, the plant produces greenish-white perfect or near perfect flowers, and later bears bright red drupe fruits³. American ginseng is classified under Appendix II of Convention on International Trade in Endangered Species (CITES) as a species threatened with extinction from excessive overharvesting⁴.

American ginseng is commonly used in TCM as a cooling agent⁴. It can treat a variety of ailments such as diabetes, cancer, cold and flu, sexual impotence, stress, dyspepsia, and vomiting, which has made American ginseng highly sought after. The main bioactive components in American ginseng are ginsenosides. There are over 40 different species of ginsenosides naturally found in the *Panax* genus¹⁶. Ginsenosides are chemicals found most concentrated in the roots of ginseng, as well as other parts such as the leaves and berries. In TCM, there is a preference for body, or human-like, shaped roots, which are naturally found in wild populations⁵, which typically occur in small clumps². This has caused the demand for wild American ginseng to increase since the 1700s⁴.

Over harvest is a threat to American ginseng. Its high market value has made it a cultural and economic staple of the Appalachian market⁶, and this species is the United States' most abundant CITES-listed export. In 2014, a dried pound of American ginseng sold for an average \$800⁹, but rose to \$1000 by 2016⁸. Export to China from the US has tripled or quadrupled since the 1800s⁹, and the increase in market demand has led to decreased abundance of American ginseng range-wide. Since roots are the focal product, harvest is lethal.

Climate change is also a concern for the future of American ginseng. Temperatures, which are predicted to rise by 2.3-5.6 °C by the end of the century⁹, pose a serious threat. Climate change could increase spring frosts, leaving

populations vulnerable to freezing for a larger portion of the year⁹. This could also lead to lower seed production, and photosynthetic rates⁹. Climate change could cause extreme periods of drought and heavy rainfall. Previous studies have also shown that increased precipitation can help American ginseng growth to an extent before it causes harm to the American ginseng plant⁹. It is unknown exactly how future variable temperatures and precipitation patterns will affect American ginseng.

Deer grazing is another major concern for American ginseng. White-tailed deer are not dispersers of seeds, despite previous beliefs⁶, since the deer crush seeds before ingestion¹⁰. White-tailed deer are a keystone species whose range has expanded in North America, largely due to low predator densities. Deer-grazing events and the prospect of increased deer-grazing events as population sizes continue to increase cause an increased extinction risk for American ginseng.

Songbirds, specifically wood thrushes, are the main dispersers of American ginseng seeds³. Wood thrushes have a 16-minute flight pattern, so their ability to disperse seeds across geographic distance relies on their short flight pattern⁸. Seed dispersal is vital for a population as it can reduce competition amongst plants, inbreeding with relative plants, and isolation from potential mates. It can also increase the expansion of new populations. Future seed dispersal patterns could potentially be vital for American ginseng's future. Dispersing further away from deer trails, known human harvesting sites, and to other environmental locations as the climate continues to change could influence whether a population persists into the future.

This research has three main objectives. First, I examined genetic diversity across North Carolina populations of American ginseng, where I expect to see much interpopulation differentiation. Second, I queried relationships between genetic diversity and a population's protection status. I expect protected populations to have more genetic diversity since they are better protected against threats to American ginseng. Finally, I examined relationships between geographic and genetic diversity. Since populations are geographically isolated, I expect to see that they are also genetically distinct.

2. Methods

To examine the genetic diversity of American ginseng, seven different microsatellite markers¹ were used to determine allelic presence and variability amongst populations. Microsatellite DNA markers are repeating segments of DNA that can be used to characterize genetic population structure, degrees of selfing, and potential level of introgression of cultivated seeds into previously wild populations¹.

Tissue samples were collected non-destructively from seven wild American ginseng populations found within western North Carolina and one wild outgroup population from Virginia. Five of the populations occur in protected sites, while the other three occur in unprotected sites. Most populations collected for this work have had at least one confirmed instance of poaching. A modified CTAB method²¹ was used to extract DNA from leaflets, and DNA was resuspended by adding 100 μ L of 1x TE buffer. DNA quantity and quality was measured with a Nanodrop ND-1000TM spectrophotometer. Ethanol precipitation and dilution was used to achieve final DNA concentrations of 10 - 100 ng/ μ L.

Five μ L of template DNA and 8 μ L of PCR cocktail (Table 1) was added to each PCR tube, and PCR tubes were placed in the BIO-RAD T100TM Thermal Cycler. The PCR program was run at 2 min at 94 °C, 35 cycles of 40 s at 94 °C, 40 s at 56 °C and 60 s at 72 °C, followed by a final extension for 10 min at 72°C. To determine the DNA quality, the PCR products were run at 100 V on a 2% agarose gel using TBE buffer. Gels were photographed on a UVP BioDoc-It^{2R} Imager, and successful amplicons with complementary fluorophores were multiplexed. Two to four microliters of multiplexed PCR product was added to 8 μ L of a 1:50 mix of Hi-Di Formamide (Applied Biosystems) and GeneScanTM 500 ladder (containing LIZ dye).

Allele presence and frequency were determined in Geneious 2021.1¹⁹. Data analyses were conducted using the *polysat*²² package in R¹². The *alleleDiversity* function in *polysat*²² was used to examine the amount of unique alleles (A) across all 7 microsatellite loci in each population. Genotypic diversity was estimated with a Shannon Index. Genetic distance between individuals and across populations was measured using a Principle Coordinate Analysis. Differences in A and the Shannon Index were compared across population protection status using ANOVA in R¹².

Table 1. PCR Cocktail Recipe (N = 1)

Ingredient	Quantity
Master Mix	6.5 μ L
Fluorescently tagged Forward Primer	0.4 μ L
Reverse Primer	0.4 μ L
PCR Water	0.7 μ L

3. Results

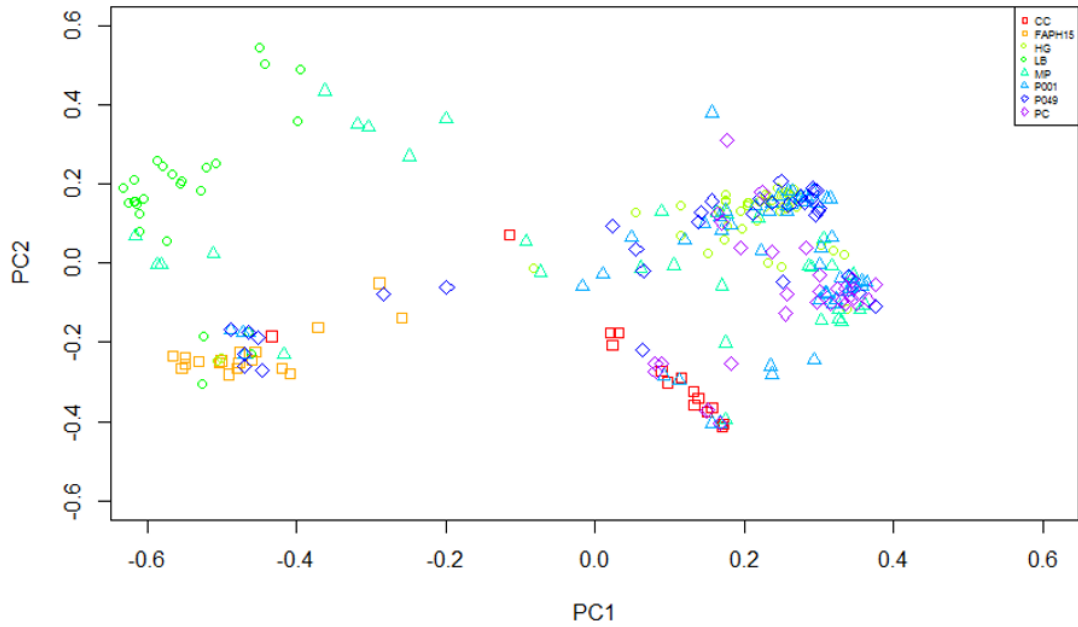


Figure 1. Principle Coordinate Analysis showing genetic clustering patterns denoting genotypic similarities across eight American ginseng populations. Each axis represents a composite combination of genetic variation across all 7 microsatellite loci.

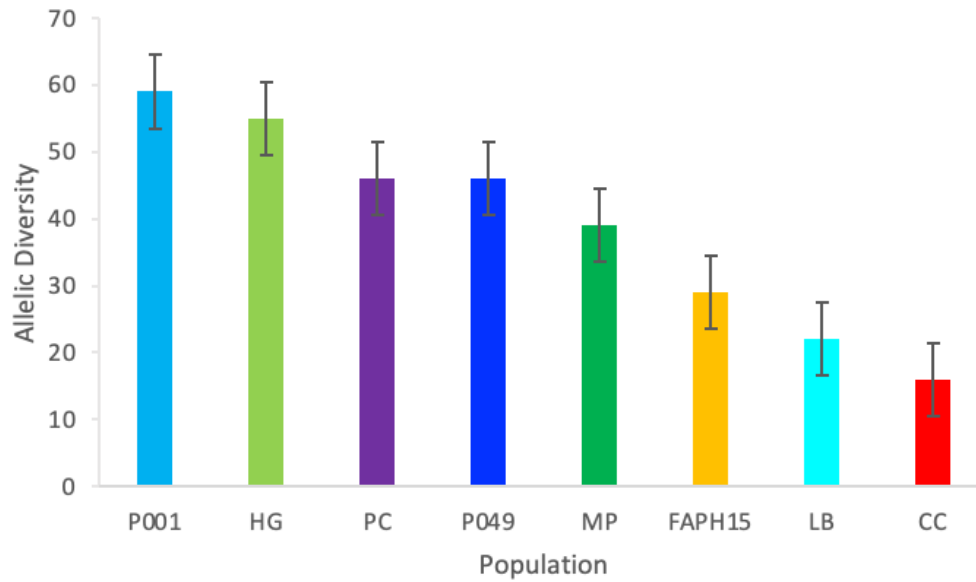


Figure 2. Mean (+/- 1 SE) allelic diversity across all seven loci in all eight populations of American ginseng. The MP, P001, and P049 populations occur in unprotected sites, while HG, PC, FAPH15, LB, and CC populations occur in protected sites.

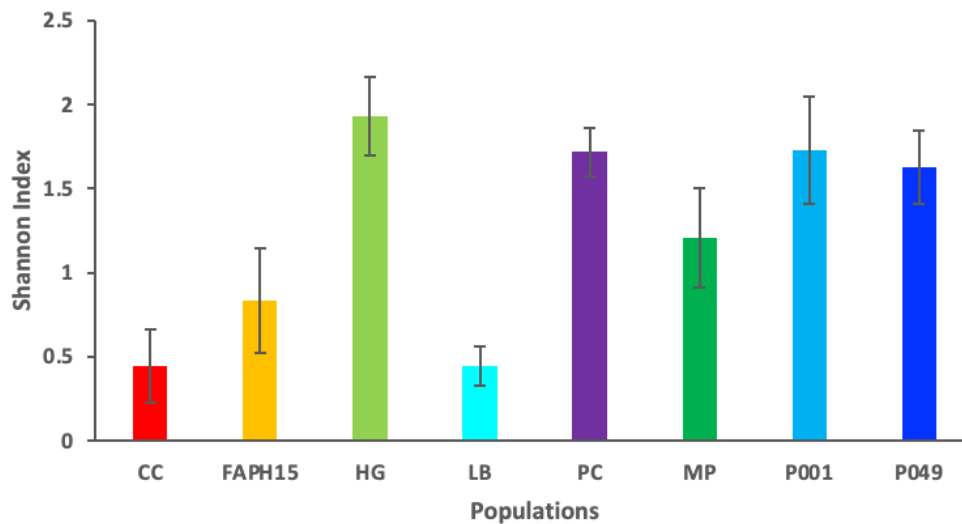


Figure 3. Mean (+/- 1 SE) Shannon Index in eight populations of American ginseng. The MP, P001, and P049 populations occur in unprotected sites while HG, PC, FAPH15, LB, and CC populations occur in protected sites.

Populations are geographically but not genetically distinct (Figure 1). There is a significant difference in allelic diversity across populations (ANOVA $F(7,48) = 2.74, P = 0.018$), but not across protection status of populations (Figure 2). There is a significant difference in genotypic diversity across populations (ANOVA $F(7,48) = 2.48, P = 0.00005$) but not by protection status of populations.

4. Discussion

The eight American ginseng populations examined in this study are geographically but not genetically distinct. Populations varied by allele and genotypic diversity, but population protection status did not influence allelic or

genotypic diversity. This is particularly interesting in the FAPH15 population, an outgroup population from Virginia that is over 650 km from the nearest population in North Carolina. There could be a few explanations for the overlay in genotypic diversity. Current gene flow may explain the overlapping genotypes amongst populations². Another proposed theory could be that these small, spatially dispersed populations used to be a part of one giant population. There is a theory posed by the results of a 2012 paper¹ that there are only three true genetically distinct populations of American ginseng throughout its entire natural distribution. There is a Wisconsin population, a Canadian population, and an Appalachian population. This could explain the genetic similarities and closeness found across all populations, even seen in the FAPH15 population, which is an outgroup from Virginia. Perhaps the Appalachian region populations were originally just one population that has since been fragmented, potentially due to overharvesting and increased land development. The overlay in genotypic diversity could support this theory of there only being three genetically distinct populations of American ginseng¹.

Current gene flow could also be an explanation to genotypic overlay (Figure 1). If genetic material, such as pollen or seeds, are being moved from one population to the next, we could see an overlay in genetic diversity within populations (Figure 1). This could be due to the movement of seeds from one population to the next via wood thrushes, falling down mountainsides into another nearby population, and flooding¹⁷. Insect pollination could also potentially explain the movement of genes from one population to the next. Another aspect could be the movement of seeds via humans, in particular poachers.

Both allelic (Figure 2) and genotypic diversity varied by population, but not by the protection status of populations. However, there was a trend towards non-protected land being more allelically and genotypically diverse than protected land. There could be a demographic explanation behind these results such as populations with bigger sizes having more allelic and genotypic diversity. The reproductive statuses of a population, such as those with more reproducing adults, may also influence the allelic and genotypic diversity of a population. There is a known correlation between size and reproductive ability in American ginseng¹², where larger plants are more sexually reproductive than smaller plants. This could be explored more on a genetic level as demographic data was not examined in this research and could be a next step for further research. Demography has also been related to harvesting indices, as populations with higher harvesting instances yield smaller leaves¹⁴. Examining the effect this could have on a population's genetics by comparing populations that have not been harvested/poached with ones that have could be a future research opportunity as well.

In order to determine whether or not geographic distance truly influences genetic diversity in American ginseng, further landscape genetic analyses must be conducted such as a Mantel test. This would correlate genetic diversity and geographic distance to analyze the exact role geographic distance has on genetic diversity. A STRUCTURE genetic heat map could also be beneficial in visualizing potential gene flow amongst populations. Other areas for future research include continuing to genotype samples collected from previous and future dates, expanding population data, correlating genetic data with demographic data, and expanding microsatellite repertoire. A further understanding of this species on a genetic level can aid in conservation efforts by providing genotypic insight for conservation policies.

5. Conclusion

Two current theories to help explain the data: current gene flow or the populations sampled in this study having historically been a single population that has since fragmented. Gene flow between populations is beneficial to species. If a bottleneck event were to occur from any of American ginseng's many threats, there could still be a possibility that new genetic material could be introduced to another population, further reducing the chance of inbreeding. The results of this paper and the future research that comes from it will be used in a much wider American ginseng conservation project sponsored by the U.S. Fish & Wildlife Service through genotyping efforts. The goal of this conservation project is to genotype American ginseng populations using microsatellite markers. By using microsatellite markers to characterize individual populations, it could allow researchers to determine which plant came from which site and attempt to repatriate a plant by returning it to the site from which it came from. The characterization of American ginseng from a poached plant could also alert the U.S. Fish & Wildlife Service as to which populations should be more heavily protected. Genotypic information has been seen to aid in conservation decisions in other species facing similar harvesting pressures such as commercial timber, *Dimorphandra exaltata*¹⁸, and *Prunus africana*²⁰, whose genotyping efforts have provided essential information for conservation policies.

6. Acknowledgements

I am grateful for my committee members: Dr. Jennifer Rhode Ward, Dr. Jonathan Horton, Dr. Graham Reynolds, Dr. Melinda Grosser. I also thank collaborators from Warren Wilson College: Dr. Alisa Hove, Bassam Shawamreh, Emmanuella Afrane, and Kiersten Keith-Pederson, and from the University of North Carolina Asheville: Claudia Prieto, Lindsey Spence, Yasmin Dajani, Gradise Nishimwe, and Shravya Sarengapalli. Funding for this project was granted by the North Carolina Friends of Plant Conservation.

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