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Genetic diversity in two populations of American Ginseng (*Panax quinquefolius* L.) before and after poaching

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Abstract

Panax quinquefolius L. (American ginseng) is an herbaceous perennial plant native to North America. It is a congener of about 16 species of Asian ginseng, and the roots of most species produce ginsenosides used in both western and traditional Chinese medicine practices. Because ginseng species are in high demand and command lofty prices due to their cultural and medical use, these plants are susceptible to poaching. Poaching is a harmful activity that can cause a reduction in genetic diversity over time. Many plants' genetic diversity is positively correlated with population size; therefore, a decrease in the number of individuals due to poaching results in the reduction of genetic diversity. Decreasing genetic diversity can negatively impact a species' survival and fitness, and cause an increase in the rate of extinction. This study aimed to examine changes in the genetic diversity of two small, poached populations of ginseng before and after poaching. DNA was extracted and amplified at microsatellite loci, then fragment sizes were quantified and compared for genetic diversity over time. Results indicated genetic diversity did not differ between populations or change after poaching. In addition, diversity was not related to plant age. This study shows that poaching did not immediately reduce genetic diversity, perhaps because of population interconnectedness. Results might be useful in conservation efforts to preserve the genetic and demographic integrity of wild American ginseng.

1. Introduction

The relationship between genetic diversity and population size determines a species' fitness. Evolutionary factors such as genetic drift explain genetic variation among populations. Negative effects such as reduced genetic variation increase the probability of inbreeding due to genetic drift within small populations[1,2]. Many plant species' genetic diversity is directly aligned with population size, therefore a decrease in the number of individuals results in the reduction of genetic diversity[3]. A decline in genetic diversity can have profound evolutionary consequences as it can reduce the fitness of a species. Numerous studies demonstrate inbreeding as a result of a small population size, which ultimately lowers the genetic variation. For example, within the last 50 years, detrimental effects of anthropogenic activity and competition among plant species have caused a rapid decline in the population size of the endangered European eastern pasqueflower (*Pulsatilla patens*), and researchers found overall very high levels of inbreeding depression and low levels of heterozygosity within the 29 populations sampled[4]. However, other small populations of plant species grown under similar conditions have shown opposing effects. Other research suggests that long-lived perennial plants may have less severe consequences of genetic drift. A study of genetic diversity in a diminishing primrose (*Primula vulgaris*) population across a fragmented agricultural landscape showed that the population maintained high levels of genetic diversity with a low occurrence of inbreeding. The high heterozygosity and low inbreeding could be due to the connection of the fragmented population to others via pollen-mediated gene flow, which could counteract genetic erosion[5].

Harmful human activity such as overexploitation may cause a decrease or cessation of gene flow due to population fragmentation and lower population densities[1]. Poaching and overharvesting are a threat to the genetic and phenotypic

diversity of a species, and plants with high socio-economic value are at greater risk of poaching[6]. Profitable wild plant species such as American ginseng (*Panax quinquefolius*), bamboo (*Bambusa vulgaris*), and eyebright (*Euphrasia officinalis*) are plagued with the likelihood of overexploitation due to the demand of consumer use and commercial trade[7]. In addition to threats posed by overharvesting, forest herbaceous perennials require years to reach full maturity, and wild plants' ability to take root and propagate is diminished through premature harvesting[8].

Analyzing DNA variation is one way to track the effects of poaching on wild plant populations. One approach uses microsatellite DNA markers to examine the genetic relationships within individuals among and between populations. These highly polymorphic repetitive DNA sequences are typically intergenic and consist of blocks of 1 to 6 nucleotides. Detecting microsatellite polymorphisms is accomplished through polymerase chain reaction amplification with fluorescently labeled primers, followed by gel electrophoresis and fragment analysis[9,10]. Microsatellites do not usually detect selective pressures since they are examined at multiple loci and are principally neutral markers, and they can be used to investigate allelic diversity within and among species[11].

This study focused on American ginseng (*Panax quinquefolius* L.), a long-lived harvested perennial herb indigenous to North America. A mature ginseng plant is characterized by thick taproots and a whorl of compound leaflets. The maturity of ginseng is strongly correlated with size, therefore young juvenile plants tend to be smaller, while adult plants possess greater leaf area and stalk height[12]. American ginseng is different from Asian ginseng in its secondary compounds, called ginsenosides. Ginsenosides are triterpenoid saponins found within the root of ginseng, which contain carbon rings in a steroid-like configuration. Modulation between each form of ginsenoside is reliant on the type of sugar and the placement of hydroxyl groups. Ginsenosides, exist in higher concentrations in American ginseng in comparison to Asian ginseng, therefore making it more valuable in the medicinal market. American ginseng has been shown to mitigate symptoms of Alzheimer's disease, reduce hypertrophy, and exhibit anti-microbial properties[13,14,15,16,17].

Due to ginseng's medicinal properties and values, it has become susceptible to overharvesting. This plant has been identified as a risk for becoming endangered over the past four decades by organizations such as the Convention on International Trade in Endangered Species (CITES)[12]. China is American ginseng's number one consumer and export recipient, and this consumption pattern has persisted for decades. In the 1800s, more than 290,000 kg of dry ginseng roots were shipped from North America to the Asian continent each year[18]. Additionally, in the nineteenth century, 95% of American ginseng was shipped to China; however, this volume has been declining due to the restrictions put on ginseng because of overharvesting. The value of wild ginseng in 2021 ranges from $700 - $850 per pound[19].

The objective of this study was to investigate the genetic diversity of two small wild ginseng populations over time. In addition, the relationship between plant size and genetic diversity was examined. I expected that genetic diversity would decline after poaching and that the largest plants would be more genetically diverse than smaller ones.

2. Methods

All ginseng plants were marked with metal tags, and poaching was suspected because plants were gone but tags were left. Each population was monitored before and after a known poaching event. American ginseng (*Panax quinquefolius* L.) leaflets were harvested summer of 2021 from two small populations in Buncombe County, North Carolina with known poaching; tissue was collected by Warren Wilson College and UNC Asheville personnel and frozen at -20 °C until extraction. DNA was extracted from thirty-six new plants using a modified CTAB method, which began by manually grinding tissue with a mortar and pestle. Cell lysis was performed by adding a 2% CTAB buffer to the ground leaf tissue. Samples were incubated for an hour with occasional mixing every fifteen minutes. Protein was removed from the aqueous solution and placed into new microcentrifuge tubes to be stored in the refrigerator until the next day. Refrigerated phenol was added to deaminate the protein, then the sample was spun with the microcentrifuge at 14,000 rpm for fifteen minutes at 4°C. The aqueous layer was removed, and an equal volume of chloroform: isoamyl alcohol was added to the solution. After the samples were spun again in the microcentrifuge the phenol was removed. Isopropanol was added and mixed through the microcentrifuge. The supernatant was poured out, and 70% ethanol was added. To remove salts it was spun in the microcentrifuge and the supernatant was poured out afterward. Samples were air-dried under the fume hood for 24 hours and left overnight in the refrigerator to resuspend in 100 µL of TE buffer.

The quality of DNA concentration was determined using a Nanodrop ND-1000 TM spectrophotometer. PCR was performed in a Bio-Rad T100 ™ thermal cycler. PCR tubes each contained 6.5 µL of GoTaq© green master mix, 0.4 uL of dyed forward primer, 0.4 uL of reverse primer with both primers at 10 uM concentration, 0.7 µL of PCR water, and 5 µL of DNA sample for a total reaction volume of 13 µL. The thermocycler program consisted of initial

denaturation for 2 min at 94°C, 35 cycles of 40 s at 94°C, 40 s at 56°C, 60 s at 72°C for extension, and ended with 10 min at 72°C.

Electrophoresis on 2% agarose gels was performed to assess DNA amplification success. Fluorescently tagged PCR products were multiplexed and sent to North Carolina State University's Genomic Science Laboratory for fragment analysis. Geneious™ was used to determine fragment sizes. Unanalyzed fragments from *Panax quinquefolius L.* leaves previously harvested by J. Rhode Ward were examined as well. Data from Geneious™ was used to calculate allelic diversity and polymorphism, and these values were analyzed in RStudio (RStudio Team 2021). Allelic diversity is used to quantify different allelic types caused by mutations separating in a population. Polymorphism serves as a genetic marker because one or more alleles occupies the gene's locus within a population. Two-way ANOVA was used to compare diversity pre- and post-poaching as well as between sites. ANOVA was also used to examine the relationship between the number of leaves and diversity measures.

3. Results

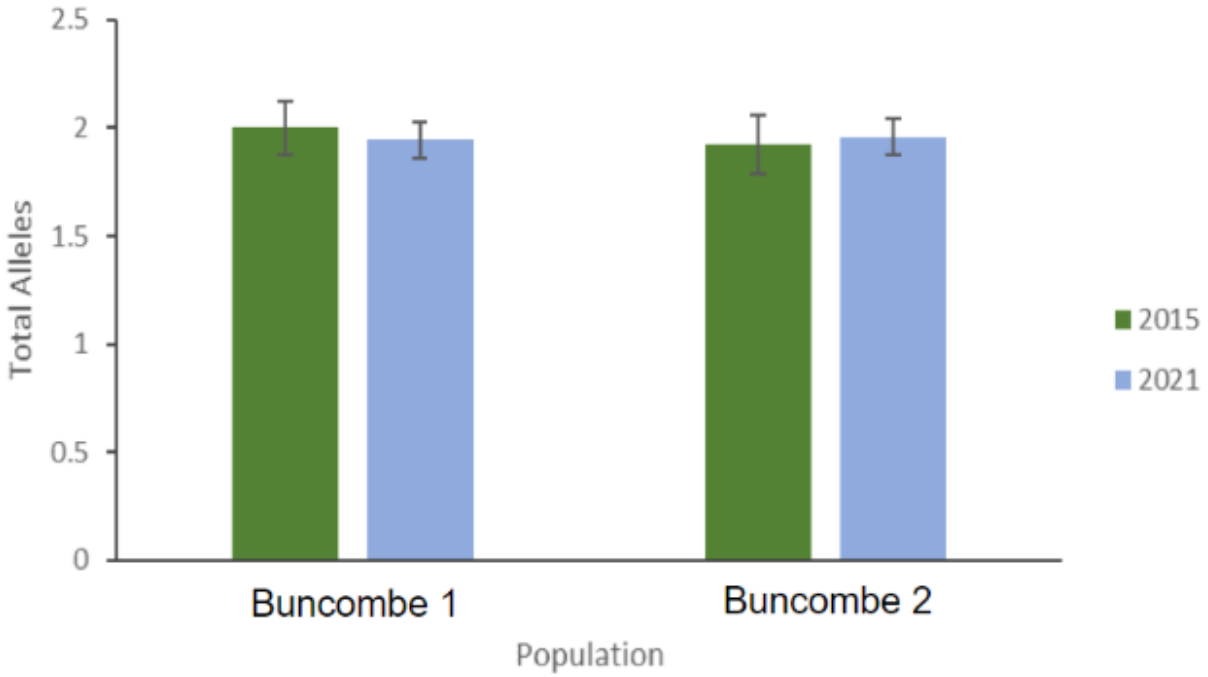

Figure 1. Average of allelic diversity in two ginseng populations in 2015 and 2021 (ANOVA; $F_{3,77} = 0.113$, $p = 0.738$).

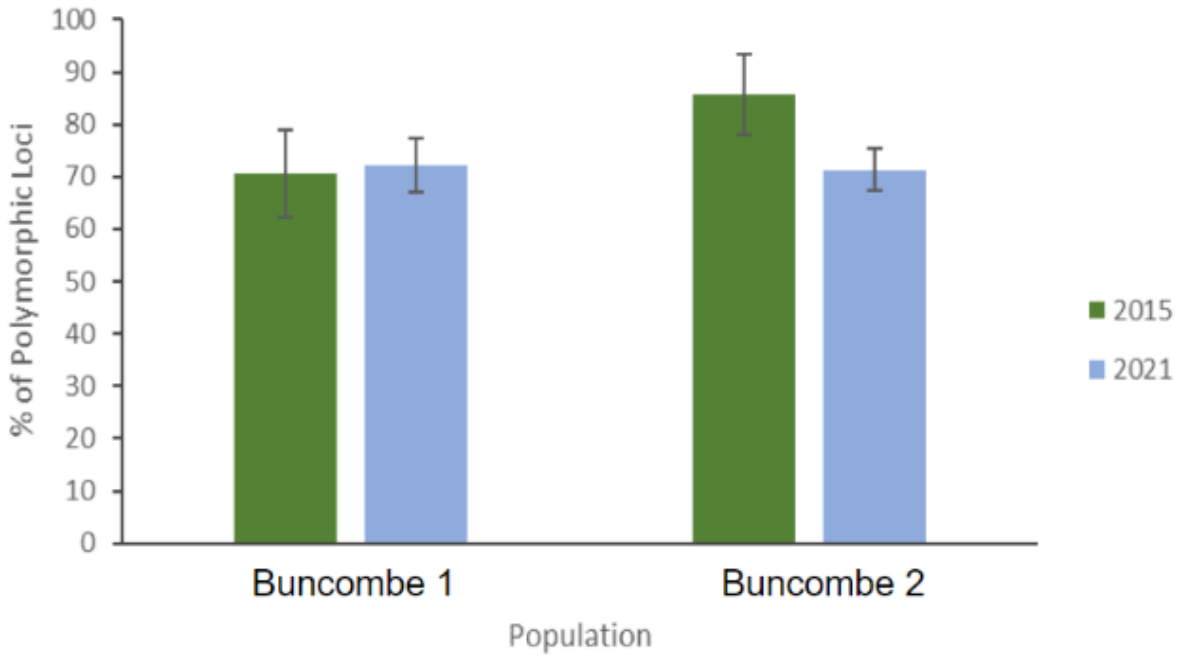

Figure 2. Average of percent polymorphic loci in two ginseng populations in 2015 and 2021 (ANOVA; $F_{3,77}$ = 1.148, p = 0.287).

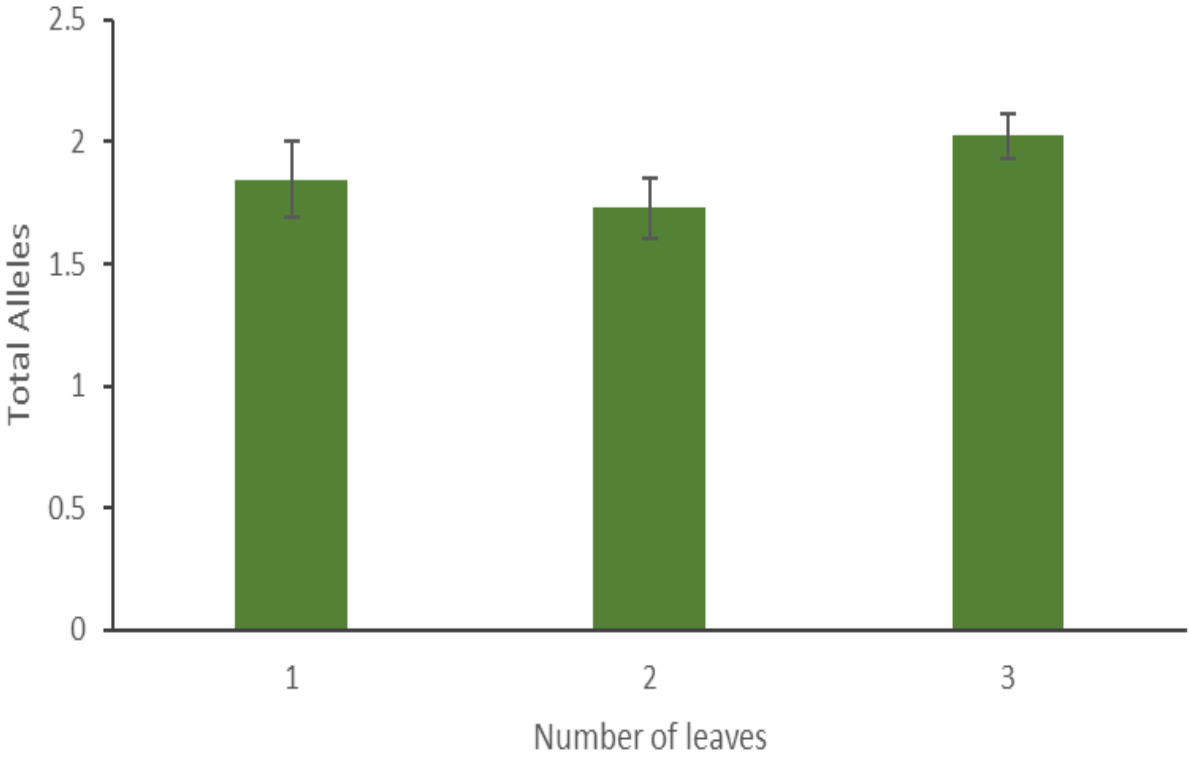

Figure 3. Average of allelic diversity by plant size in a ginseng population (ANOVA; $F_{1,46}$ = 1.985, p = 0.166).

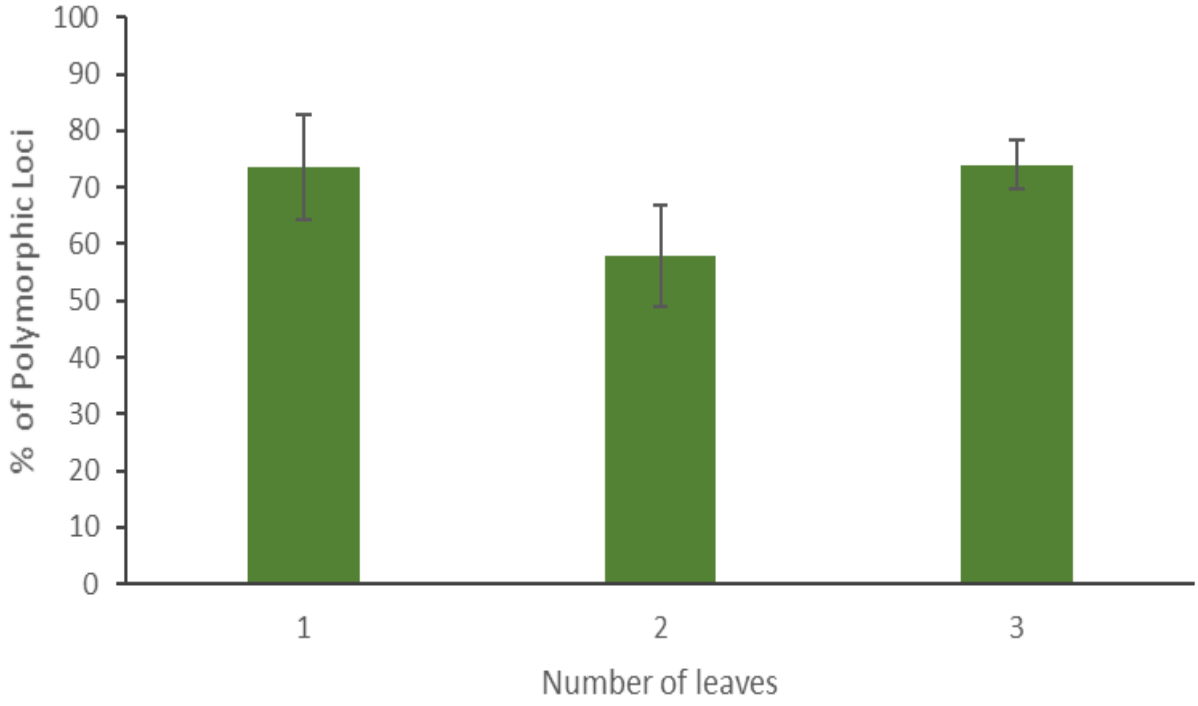

Figure 4. Average of percent of polymorphic loci of plant size in a ginseng population (ANOVA; $F_{1,46} = 0.605$, $p = 0.441$).

4. Discussion

There was not a significant difference in genetic diversity between populations or change after poaching. A lack of difference in genetic diversity could be due to the ability of ginseng to self-pollinate[22]. Most of the time self-pollination leads to the consequence of having lower genetic diversity, whereas cross-pollination allows for more genetic diversity[23]. However, one study revealed that in the case of habitat fragmentation, self-pollinating plants were able to maintain high genetic diversity rather than cross-pollinators. A bigger loss was suffered by cross-pollinators because of the reduction of potential breeding partners, whereas self-pollinators did not suffer the loss of a potential breeding partner[24]. This coincides with previous research done on ginseng populations. For example, one study tested 21 populations of wild ginseng to examine the genetic diversity in harvested and protected populations. Researchers found both populations had a significant amount of homozygosity. They suspected this was the result of harvesting leading to non-random mating and increased self-pollination[25].

There was not a significant correlation between genetic diversity and plant size class. This can be attributed to the fact that ginseng has the ability to regress in size class. One study explored the relationship between age and size in ginseng populations under high harvest pressures and low harvest pressures. In ginseng plants of the same age, data revealed under high harvest pressures plants modified themselves to have smaller leaf areas and overall height than those in the low-pressure harvest. Therefore, smaller leaf areas and reduction of overall height prompted ginseng plants to have greater fitness than those who had bigger ones when experiencing general harvest pressures[26]. Another investigation on ginseng revealed a 9.8% decline in leaf number since the 1900s. Additionally, this study revealed an overall significant decline in size properties (root length, sympodium height, peduncle length) of ginseng in the midwest and south have declined compared to ginseng grown in the north[27].

Future work could include expanding this research to other demographic locations. Currently, 19 states in the United States allow for the harvest and export of wild ginseng[28], it would be important to understand if poaching or overharvesting affects the genetic diversity of other ginseng populations. Furthermore, allocating more resources to examine the relationship between harvest pressures and size class of ginseng. Understanding the implications of such

relationships will determine conservation efforts to maintain demographic integrity and reproductive potential in this species.

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