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GENETIC VARIABILITY IN *HYDRASTIS CANADENSIS L.* USING RAPD ANALYSIS

A Thesis Presented

by

KERRY J. KELLEY

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

February 2009

Plant & Soil Sciences

Genetic Variability in *Hydrastis canadensis* L.

Using RAPD Analysis

A Thesis Presented

by

Kerry J. Kelley

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Finally, thank you to all my family and friends for your love and support.

ABSTRACT

GENETIC VARIABILITY IN *HYDRASTIS canadensis L.*
USING RAPD ANALYSIS

FEBRUARY 2009

KERRY J. KELLEY, B.A. MOUNT HOLYOKE COLLEGE

M.A. UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Lyle Craker

Hydrastis canadensis L. (goldenseal) is an endangered perennial wildflower species native to eastern North America. In this study, several populations of goldenseal, (both cultivated and wild type) were analyzed for genetic variability. The samples were collected from plant populations in North Carolina, Ohio, Pennsylvania and West Virginia and preserved using silica gel during collection. Random amplified polymorphic DNA (RAPD) analysis technique was used to generate DNA profiles from individual plants and to estimate genetic variability between groups (cultivated and wild type), among populations within groups and within populations using analysis of molecular variance (AMOVA) and a UPGMA clustering phenogram. Our results demonstrate that the bulk of genetic diversity may be within and among populations, but not between groups. This indicates the need for preservation and conservation efforts at the population level. The next step would be to study goldenseal populations more in depth for underlying causes of the genetic variability observed in this study. Further study of genetic variability with different molecular markers may be needed to clarify the level of diversity for the species at the group level. Increased knowledge of genetic variability and the identification of accessions of goldenseal would prove useful for reintroduction and cultivation strategies.

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CHAPTER I

INTRODUCTION

Goldenseal *(Hydrastis canadensis* L.) is a perennial medicinal plant of economic importance. Native to Eastern North America, the plant is harvested primarily for the root that contains the bioactive alkaloids berberine, hydrastine, and canadine. These root constituents have been shown to have effective anti-inflammatory, antibiotic and anti-pyretic effects (Murray and Pizzorono 1991; Villinski *et al.* 2003). The goldenseal plant, which has long been used in Native American and folk remedies, has been of commercial interest since the 1800s (Foster 1995). The latest figures available from The American Herbal Products Association on harvest tonnage of combined wild and commercially grown goldenseal root and rhizome are 42 tons in 2004 and 41 tons in 2005, an increase from 21 tons in 2003. The survey indicates a larger increase for cultivated goldenseal, as compared with wild-harvested goldenseal, than for any other herb surveyed with the exception of *Echinacea purpurea* L. Wild-harvested goldenseal, however, still has the greater market share at 59% in 2005 compared to that of the cultivated (AHPA 2007).

Although goldenseal has been protected by the Convention for International Trade on Endangered Species (CITES) since 1997 (Robbins 1998), the plant is currently listed as endangered, imperiled, threatened or vulnerable in most states within the natural range (USDA 2008). Unfortunately, wild populations continue to dwindle due to loss of habitat and over-harvesting, contributing to a decline in the numbers of wild populations (Davis 1999; McGraw *et al.* 2003; Mulligan and Gorchov 2004).

While some of losses in the wild might be solved though increased commercial cultivation, large-scale goldenseal cultivation remains problematic. The primary problems with goldenseal cultivation are the lack of seed viability, long-term seed storage issues, and increased disease susceptibility that make root cuttings the

preferred method of propagation (Davis and McCoy 2000). Although recent advances have been made in *in-vitro* propagation, clonal type propagation in relation to genetic diversity of the plants produced remains an issue (Hall and Camper 2002; Liu *et al* 2004; He *et al.* 2007).

In the wild, the reproductive system of goldenseal is capable of producing seed-set sexually, which requires pollination and may occur by self-pollination or out-crossing. Known pollinators are small polylectic bees, and may also include syrphid flies and some larger bees. Seed dispersal is mainly by birds. Breeding system does not seem likely to contribute to goldenseal scarcity or abundance, however, seed-set rates may be a contributing factor (Sinclair *et al*. 2000; Sanders 2004.). Growth from vegetative offshoots is the most prominent and effective reproductive method, especially after disturbances in the wild, which some studies have shown may actually benefit goldenseal and other woodland herbs. These plants have evolved in an environment in which large, frequent disturbances were the norm, but are no longer occurring at that level (Sinclair and Cartling 2003; Van der Voort *et al*. 2003; Sanders and McGraw 2005a; Albrecht and McCarthy 2006).

Disturbances may be classified under a broad range of activities, from natural to man-made, and would include-fire, flooding, herbivore browsing, harvest, logging, land development and recreational land use (Liebmann *et al*. 1998; Sanders and McGraw 2005b). Problematically, any of these conditions, however, decrease genetic variability within surviving populations of goldenseal as certain plants and populations are destroyed. To fully understand the potential problem, estimates on the genetic diversity of native populations of goldenseal are necessary to discern any problems and stimulate measures, such as cultivation, agro-forestry practices, conservation efforts, and population restoration in the wild. Identification of accessions with higher concentration

of the bioactive constituents could reduce collection pressure (Kapteyn and Simon 2002; Segarra-Moragues *et al.* 2005; Sinclair *et al.* 2005; Yasmin *et al.* 2006).

Studies on genetic diversity within populations have been simplified by the introduction of molecular analysis techniques, such as randomly amplified polymorphic DNA (RAPD) analysis (Welsh and McClelland 1990; Williams *et al.* 1990). RAPD analysis is a multilocus arbitrary fingerprinting technique that can be used for determining genetic relationships of various species (Ramshini *et al*. 2005; Hoque *et al.* 2005; Sadder 2006), as well as determining the components of herbal medicinal mixtures (Shinde *et al.* 2007). In addition, RAPD analyses are efficient, economical and tend to produce genetic markers suited to the assessment of population, race and species-specific genetic variation (Aagaard *et al.* 1998).

In this study, samples of goldenseal collected from 17 wild and cultivated populations though-out North Carolina, Ohio, Pennsylvania, and West Virginia were analyzed for genetic variation using RAPD markers to determine the level of genetic diversity between the groups (cultivated and wild type), among populations within groups and among individual plants within each population.

CHAPTER II

MATERIALS AND METHODS

Plant material

Goldenseal (*Hydrastis candensis* L.) plants from 17 populations located in North Carolina, Ohio, Pennsylvania, and West Virginia were used in this study (Figure 1). Populations were classified as either cultivated or wild type growth (Table 1). At each location a minimum of 10 randomly selected plants were sampled by collection of leaf tissue (approximately 4 cm^2) from individual plants. At locations where plants were extensively populated or distributed over a broad area, additional plant samples were collected. The collected leaf tissue was placed in plastic bags with fine mesh, silica gel (28-200 mesh size, Fisher Scientific) containing moisture level indicator beads to dry and preserve the tissue. After drying, the leaf tissue samples were separated from the silica gel mixture and placed in microfuge tubes for storage at ambient temperature.

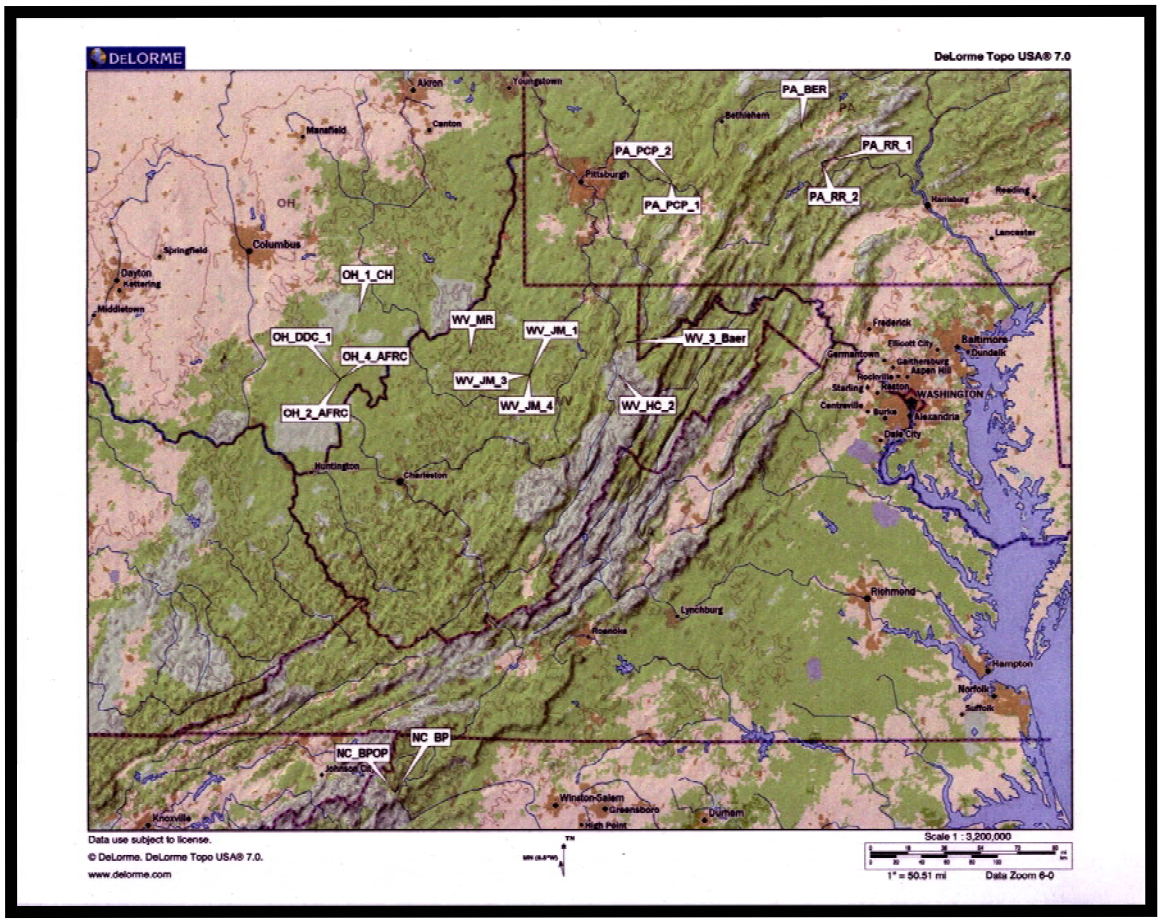

Figure 1. Map of goldenseal collection sites created from GPS (Global Positioning System) data taken at time of sample collection (GPS data not included for protection of locations).

Table 1. Sampled populations. [1] The population names and abbreviations were assigned to distinguish the samples and have no other relationship to the sample.

Cultivated Populations	Location	Date Sampled	Abbreviation
B_NC (Pines)	North Carolina	8/03	NC_BP
B_(Poplars)	“	“	NC_BPOP
Ohio 1_CH	Ohio	“	OH_1
Ohio 2_AFRC Appalachian Forest Resource Center	“	“	OH_2
Ohio 4_AFRC Appalachian Forest Resource Center	“	“	OH_4
DDC_1	“	“	OH_DDC_1
Wild Type Populations			
BER	Pennsylvania	“	PA_BER
PCP_1	“	“	PA_PCP_1
PCP_2	“	“	PA_PCP_2
RR_1	“	“	PA_RR_1
RR_2	“	“	PA_RR_2
CB_3	West Virginia	“	WV_3_Baer
HC_2	“	“	WV_HC_2
JM_1	“	“	WV_JM_1
JM_3	“	“	WV_JM_3
JM_4	“	“	WV_JM_4
MR	“	“	WV_MR

DNA isolation

For DNA extraction, the leaf tissue were removed from the microfuge storage tubes, carefully rinsed with distilled water, and placed in fresh, sterile 1.5 mL microfuge tubes. The DNA was extracted from the collected leaf tissue using a modified hot CTAB procedure (Xie, *et al.* 1999) combined with the DNA extaction microprep method (Fulton 1995).

The CTAB extraction buffer consisted of 2% (W/V) hexadecyltrimethylammonium bromide (CTAB, Sigma), 100 mmol/L Tris-HCL (pH 8.0), 1.4 mol/L NaCl, 20 nmol/L EDTA, 1.5% polyvinyl-pyrrolidone (PVP, Sigma), and 0.5% 2-mercaptoethanol (Sigma).

The buffer mixture was heated at 65 °C in a water bath to bring the PVP into suspension and the buffer constituents were thoroughly mixed.

Plant samples were ground in hot CTAB extraction buffer, incubated in a 65 °C water bath for 45 min. and mixed by inversion at 10 min. intervals. The mixture was extracted twice with chloroform and the DNA was precipitated, using cold (-20 °C) isopropenol, for the first extraction, and cold (-20 °C) ethanol with cold (-20 °C) sodium acetate (pH 5.2) for the second extraction. The DNA pellets were air dried for 30 min, and resuspended in 100µL TE buffer for storage at 4 °C. All DNA pellets obtained from the same leaf tissue were combined into one sterile 1.5 mL microfuge tube. DNA was checked for quality by electrophoresis on a 1% agarose gel prepared in 1x TAE (Tris-base, glacial acetic acid, 0.5 M EDTA) buffer, visualized with UV light and digitally photographed (Fujifilm Luminescent Image Analyzer LAS-3000). Undigested and *Eco*RI digested aliquots (3 µL) of each DNA sample were electrophoresed alongside uncut λ-phage DNA standards (50 µg and 100 µg) to assess DNA quality and quantity.

Amplification

After PCR optimization, all DNA samples were amplified using a 1/20 dilution of DNA (1 µL DNA to 19 µL ddH_2O) and six arbitrary 10-mer RAPD primers (average GC content of 63%, average annealing temperature of 37 °C) (Integrated DNA Technologies, Coralville, IA) (Table 2). All reactions were done using a single thermocycler (RoboCycler® Gradient 96, Stratagene) with a cycle profile of one cycle at 95 °C for 5 min. followed by 45 cycles at 95 °C for 1 min., 36 °C for 1 min. and 72 °C for 2 min.

Amplified PCR products were separated by electrophoresis along with 100 bp standard (100bp DNA Step Ladder, Promega) on 2% agarose gels, prepared in 1x TAE buffer. DNA banding profiles were visualized with UV light using a Fujifilm Luminescent Image Analyzer LAS-3000 and digitally photographed for later analysis with Science Lab

2005 Multi Gauge software (Version 3.0). The DNA amplification and separation for each sample/primer combination was done twice for reproducibility of band scoring.

Table 2. Characteristics of RAPD primers used in this study.

Primer	Nucleotide sequence	GC%	Number of fragments	Fragment size range (bp)
1	GGTGCGGGAA	70	26	350-2300
2	GTTTCGCTCC	60	23	300-1400
3	GTAGACCCGT	60	11	300-850
4	AAGAGCCCGT	60	18	300-1200
5	AACGCGCAAC	60	19	300-1400
6	CCCGTCAGCA	70	21	300-1700

Electophoresis analysis

Base pair sizes of amplified DNA fragments from the RAPD primers were estimated by reference to a known 100 base pair ladder. Bands above 2800 bp or below 270 bp were not included since, these size fragments are generally accepted to be unreliable for assessment. The amplified fragments were characterized by size and intensity for all scorable bands using Science Lab 2005 Multi Gauge software (Version 3.0). The presence or absence of fragments was recorded as either 0 (absent), 1 (present) or ? (unknown or missing data). Those samples for which the DNA quality was insufficient for amplification were not included in the study.

Statistical analysis

Fragments from the two PCR runs from each sample and primer were compared and only those fragments shared over the two runs were used in the statistical analysis. The finalized fragment data from all six primers were pooled to define a single binomial haplotype for each of the 134 samples. The binomial data set was processed using, ARLEQUIN (Population Genetics Analysis Program 3.0 PC, Excoffier *et al*. 2005) and PHYLIP (Phylogenetic Inference Package 3.68 Mac, Felsenstein 1989, 2008). To determine genetic differentiation, all data sets were analyzed as samples within

populations (134 individual samples within 17 populations), among populations within groups (17 populations distributed within two groups), and between groups (two groups, defined by cultivated and wild type location) for genetic differentiation.

The haplotype binomial data set was analyzed via several methods within ARLEQUIN to obtain standard indices for molecular diversity, Analysis of Molecular Variance (AMOVA), population pairwise Fst values, and a pairwise molecular distance matrix using Euclidian square method with Bootstrap analysis (over 20,000 bootstraps) of the average F-statistics over all loci.

A distance matrix, produced from the binomial data set input into the RESTDIST program of PHYLIP, was processed via the program NEIGHBOR for output as a phenogram using DRAWGRAM (PHYLIP 3.68).

CHAPTER III

RESULTS

PCR, using the 6 RAPD primers, yielded different polymorphic banding patterns that were unique to each primer and distinguishable over all samples. Examples of these recognizable patterns and their reproducibility were clearly observable in the PCR results for a population of West Virginia samples, using primers 4 and 5 (Figures 2 and 3).

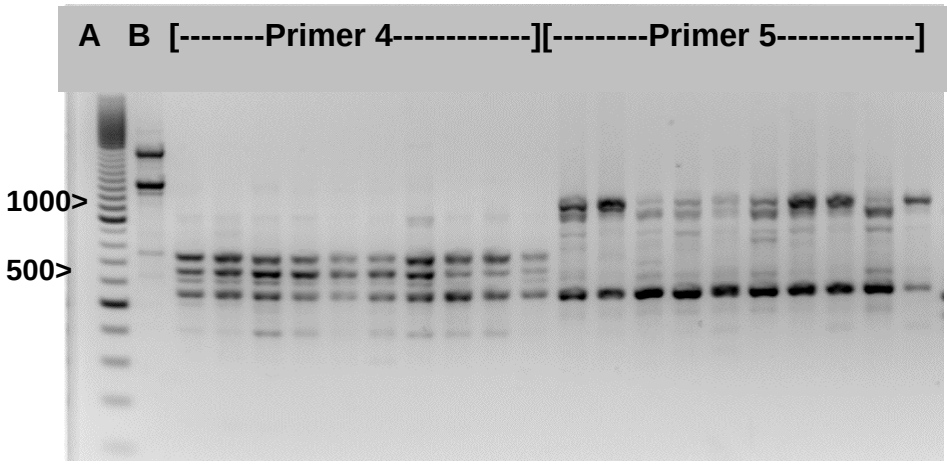

Figure 2. Band patterns produced by RAPD Primers 4 and 5 in samples 1-10 from population WV_JM-1 during the 1st run of PCR. “A” and “B” are the 100 bp standard and the positive control, respectively.

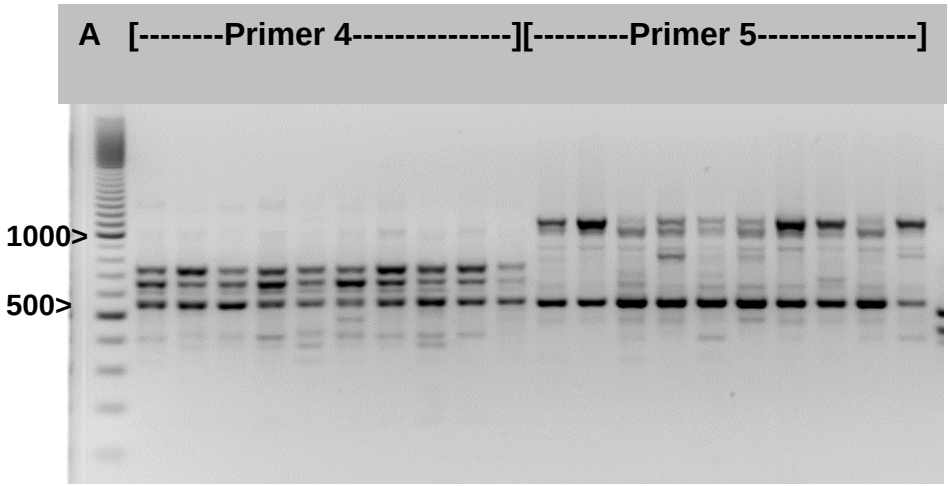

Figure 3. Band patterns produced by RAPD Primers 4 and 5 in samples 1-10 from population WV_JM-1 during the 2nd run of PCR. “A” is the 100 bp standard.

The RAPD profiles of North Carolina_BP (NC_BP) sample 6, and Ohio_4 (OH_4) sample 10 (Figure 4), are based on the primers 1-6, and are an example of variability. While there are recognizable patterns for each primer for both samples, there are bands, in primers 1, 3, 5 and 6, that differ from sample to sample.

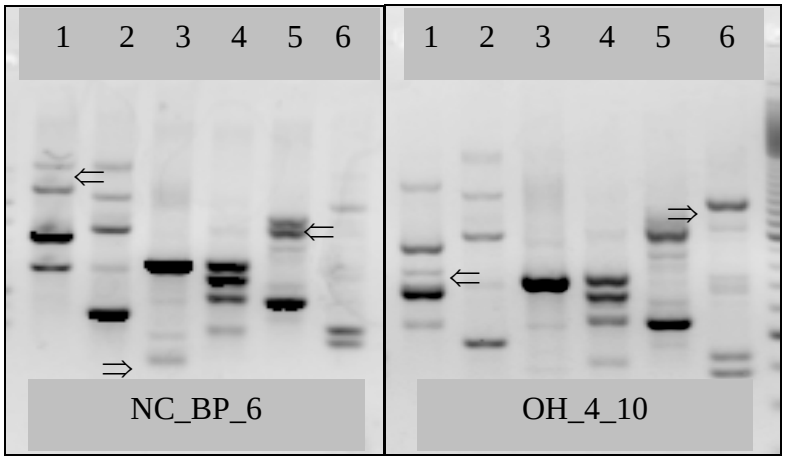

Figure 4. Band patterns of North Carolina_BP, sample 6, and Ohio_4, sample 10, using RAPD primers 1-6. [1]The arrows indicate bands amplified in one sample and not the other.

The AMOVA analysis based on the pairwise distance method (Table 3) shows that there is large genetic variance (66.67%) among the populations within the groups (Vb). Genetic variance of the samples within populations (Vc) is 23.58% and 9.75% for among groups (Va). A Euclidean square distance matrix was used for the pairwise distance matrix AMOVA computations (Jukes & Cantor 1969; Jin & Nei 1990; Tamura 1992; Kimura 1980; Tajima & Nei 1984; Tamura & Nei 1993).

Table 3. Analysis of Molecular Variance (AMOVA) for pairwise distances, calculated from 2 groups, made up of 17 populations, containing 134 individual samples based on 118 RAPD markers.

Source of variation	Variance components	Variation (%)	p	Fixation indices
Between groups	0.393 Va	9.75	0.045+ -0.006	FCT: 0.098
Among populations within groups	2.631 Vb	66.67	0.000	FST: 0.764
Within populations	0.968 Vc	23.58	0.000	FSC: 0.738

A global AMOVA with weighted averages over all (118) loci was also performed. A comparison of the AMOVA and Global AMOVA percentage of variation, shows agreement of their results (Figure 5).

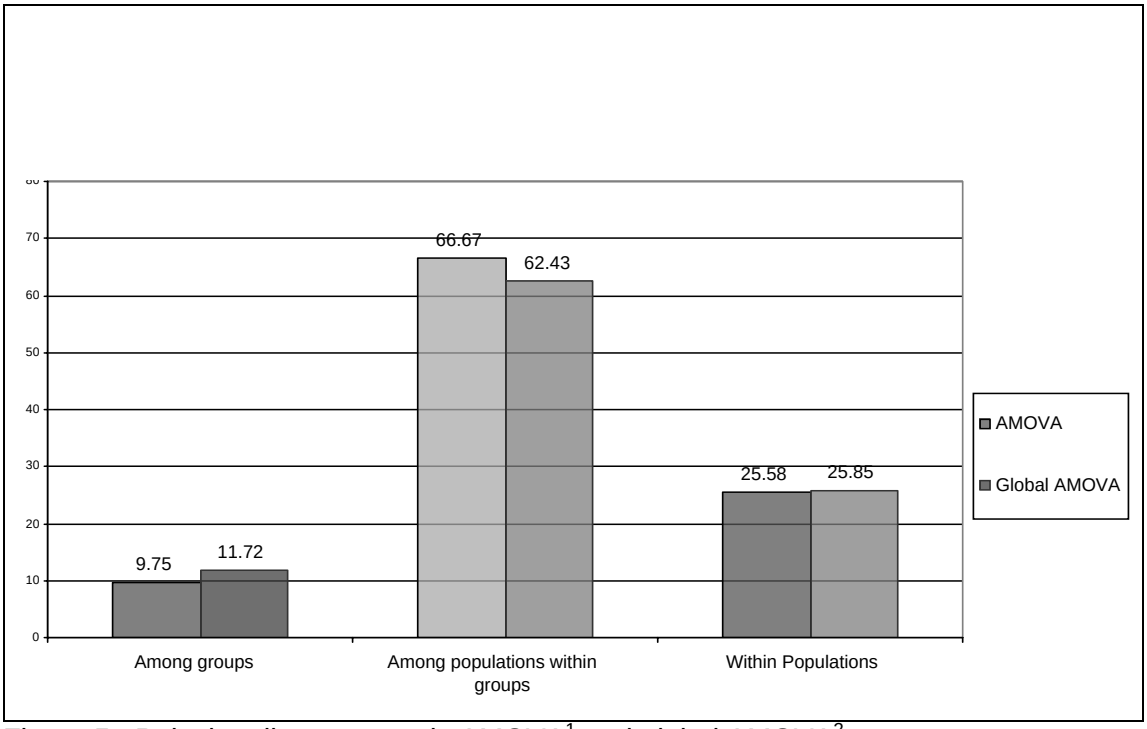

Figure 5. Pairwise distance matrix AMOVA[1] and global AMOVA[2].
[1] Percent variation among groups, among populations within groups, and within populations. [2] Results as a weighted average over 118 loci, percent variation among groups, among populations within groups, and within populations.

Traditional F-Statistics, or fixation indices, which describe the level of heterozygosity in a population, show large genetic differentiation among populations within groups (FSC = 0.738) and also among populations relative to the total variance (FST = 0.764).

Variance among groups relative to the total variance is moderate (FCT = 0.098) (Table 3).

Global AMOVA average F-statistics for genetic differentiation over all loci for among populations within groups (FSC = 0.707), among groups relative to the total variance (FST = 0.741), and variance among groups relative to the total variance (FCT = 0.117), are highly similar to the pairwise distances F-statistics values.

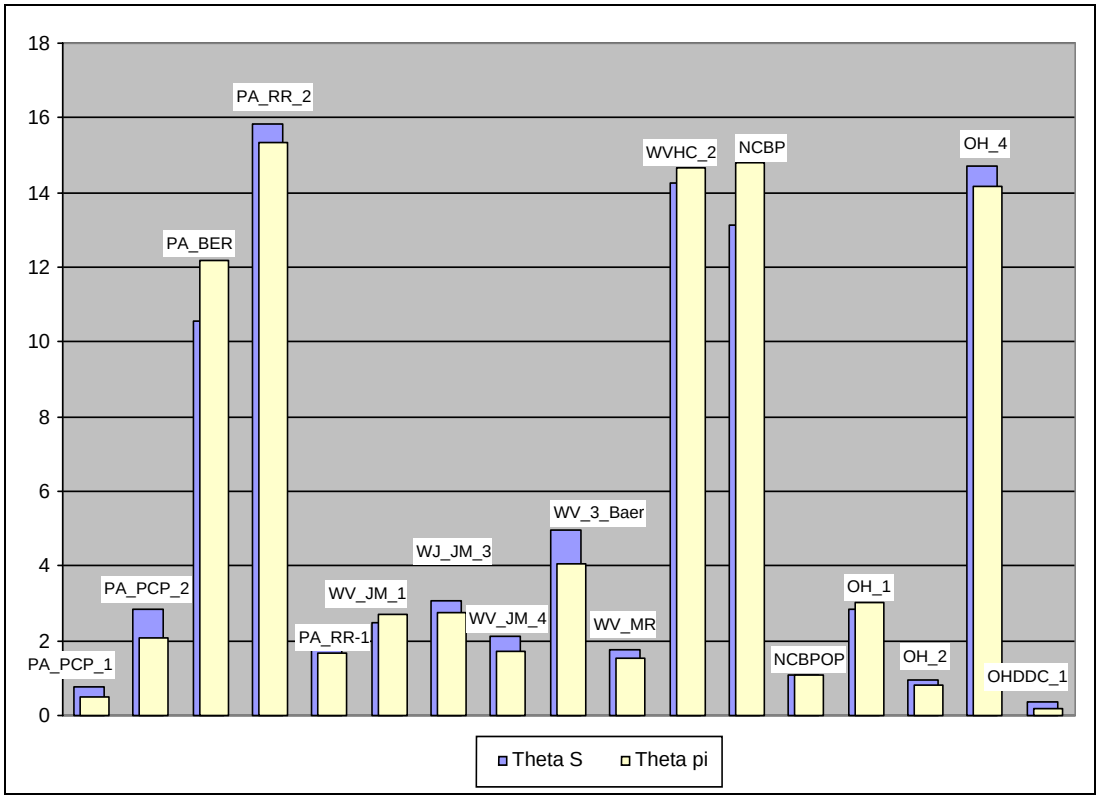

Figure 6. Molecular diversity indices, Theta S and Theta pi for all populations.

The molecular diversity indices for Theta S and Theta pi graphically illustrate the diversity of populations within groups (Figure 6). These indices are unbiased estimators of population genetic structure, where Theta (θ), statistically summarizes the distribution of variation within and among populations when samples are assumed to represent characteristics of the larger group from which they are sampled, as if a larger number of samples were actually drawn from that population—random-effect sampling (Weir and Cockerham 1984; Weir and Hill 2002). Theta S defines the infinite site equilibrium

relationship between polymorphic sites, sample size and θ, for a sample of non-recombining DNA (Tajima 1989a). Theta pi describes the infinite site equilibrium relationship between the mean number of pairwise differences and θ (Tajima 1983b). Note that these indices show higher levels of diversity for five of the 17 populations studied, PA_BER , PA_RR_2, WV_HC_2, NC_Baker's Preserve (NC_BP), and OH_4, and show that the two indices are in concordance (data not shown).

The data output from SEQBOOT was used to process our binomial data set using bootstrap analysis (1000 bootstraps). The resulting data set was then input into RESTDIST to create a distance matrix, which was used as an input file for NEIGHBOR. A phenogram (Figure 7) was plotted by DRAWGRAM using output from the NEIGHBOR program and utilizing the option for Unweighted Pair Group Method with Arithmetic mean (UPGMA), linkage clustering method. (PHYLIP, Ver. 3.68 Mac, Felsenstein 1989, 2008).

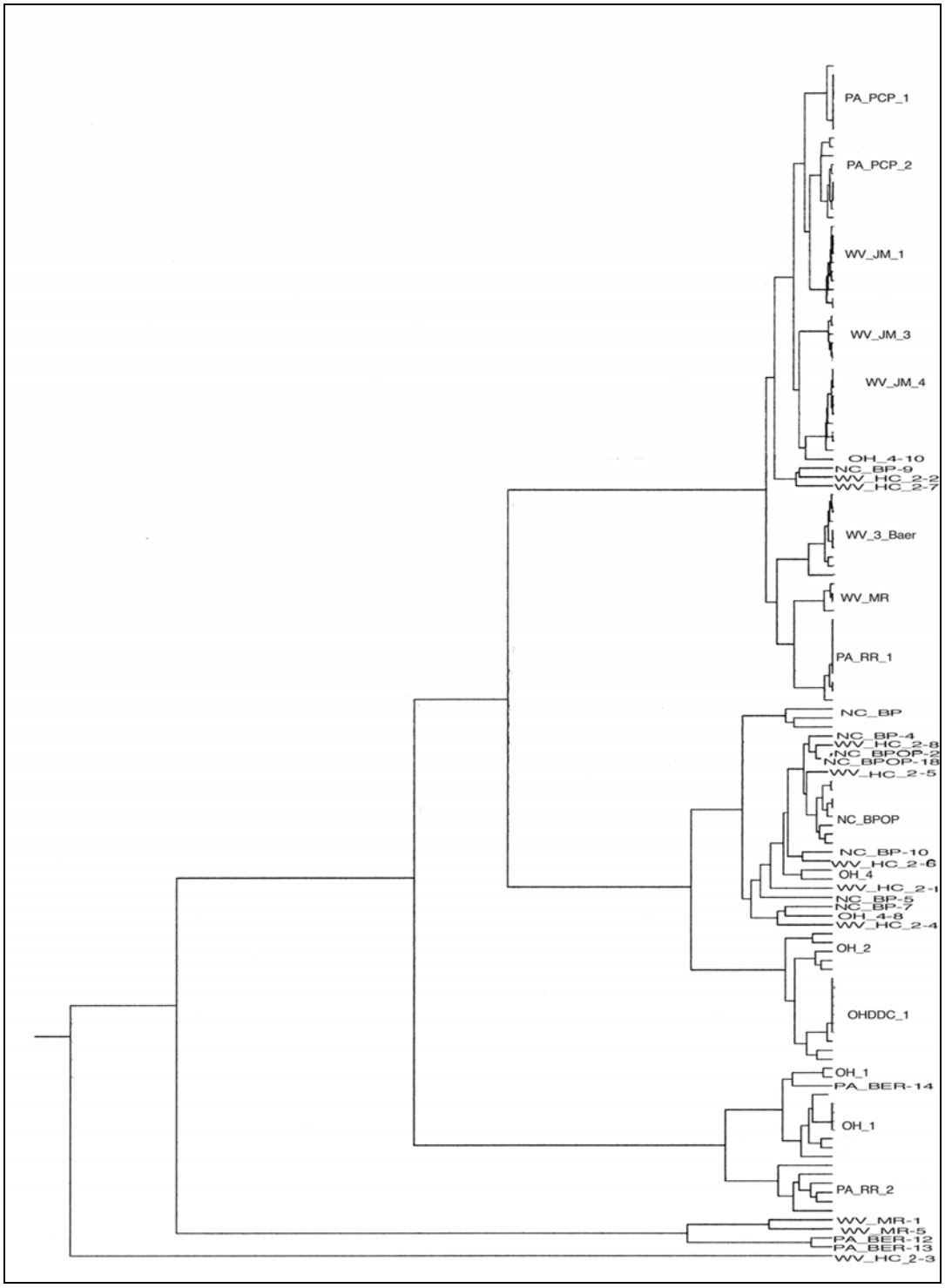

Figure 7. Phenogram of 134 samples within 17 populations, using Unweighted Pair Group Method with Arithmetic mean (UPGMA), and bootstrapping, drawn with DRAWGRAM (PHYLIP, Ver.3.68).

CHAPTER IV

DISCUSSION

Information about genetic variation within an endangered medicinal plant species, such as goldenseal, can assist in conservation efforts for that species and provide future breeders and herbal-based medicine researchers with additional data to support their work. A genetic variation study of goldenseal is necessary and timely as the species is endangered in the wild, cultivation is somewhat problematic and the plant is of economic and medicinal interest.

When initiated, this study assumed no great genetic variation among the individual samples, within populations or within groups would be observed. This hypothesis has been rejected as not supported by the evidence (FST: p = 0.000+-0.000, FSC: p = 0.000+-0.000), although the results for among groups are not conclusive (FCT: p = 0.046+-0.0069) (Rice 1989).

The samples collected in this study were preserved in silica gel for later RAPD analysis. Drying plant material with silica gel is a proven method for preservation of plant material. Despite the extended amount of time between collection and DNA extraction of the samples (4 years), the DNA yield was more than sufficient for effective and reproducible RAPD analysis (Chase & Hills 1991; Thompson & Henry 1993; Schierenbeck 1994; Xie *et al.* 1999).

Reproducibility of RAPDs and artifactual variation of banding patterns have been noted as potential issues (Ellsworth *et al.* 1993; Perez *et al.* 1998). Differing stringency conditions for annealing temperatures, the use of different thermocyclers for PCR reactions and primer optimization may have an effect on PCR outcome. These issues, however, have been addressed by carefully adjusting temperature profiles while

optimizing PCR reactions for each primer and proper calibration of thermocyclers (Penner *et al.* 1993; Bahy *et al.* 2006).

One hundred eighteen RAPD markers used in this study were produced with consistent reliability. Two multi-locus AMOVA analyses were performed using the Pairwise difference method for distances (pairwise differences between haplotypes) (Table 3), and the global AMOVA-results as a weighted average over polymorphic loci only (results not shown). The strong correlation between these two AMOVA analyses (Figures 4 and 5) indicates that there are only small amounts of missing data (Schneider *et al.* 2000). The cut off parameter of the AMOVAs for missing data was 0.05.

The AMOVA results did indicate large genetic variation among populations of goldenseal within groups and among samples within populations, but not between the groups (cultivated and wild type). The groups—cultivated (North Carolina, Ohio) and wild type (Pennsylvania, West Virginia) characterize the overall type of growth conditions at the locations where samples were collected for this study. Little genetic variation in goldenseal was noted between the groups (9.84). The lack of genetic variation between the cultivated and wild type goldenseal might be further investigated to clarify this issue. Perhaps using allozyme markers in tandem with RAPDs would be beneficial, as these have been shown to be a better predictor of total species genetic diversity, over RAPD based marker data. RAPD based diversity values tend to increase with increasing distributional range, whereas with allozymes this in not the case. Comparing results from RAPD marker and allozymes for the same samples may help to see the overall and within population diversity with greater clairity (Nybom and Bartish 2000).

The percent variation among populations within groups, however, had the greatest variation relative to the total variance (66.67%) in the present study. This may be attributed to the genetic diversity of specific populations within the two groups. Although both the cultivated and wild type groups contained populations with high

molecular diversity indices, overall there were a greater number of populations with moderate to high molecular diversity within the wild type group. The percentage of variation within populations was also large (23.58%). Fixation indices (F-statistics) confirmed the population variation with FSC = 0.738 (within populations), FST: 0.764 (among populations within groups) and FCT: 0.098 (between groups). F-statistics greater than 0.25 are consistent with large genetic differentiation, while values of 0.0 – 0.05 suggest little genetic differentiation (Wright 1978).

The molecular diversity indices (Figure 6), Theta S (Tajima 1989a) and Theta pi (Tajima 1983), further illustrated the relatively high level of molecular diversity existing in several populations (Theta S = 10.56 - 15.818, Theta pi = 12.2 - 15.333), although the molecular diversity was moderate (Theta S = 1.751 - 4.948, Theta pi = 1.533 - 4.044) for most samples and some samples exhibited quite low levels of diversity (Theta S = 0.353 - 1.060, Theta pi = 0.2 - 1.088). Each group had at least one population with very high genetic diversity levels, populations labeled NC_BP, OH_4, PA_BER and PA_PCP_2 and WV_HC_2. While the moderate levels of molecular diversity overall are suggestive of a combination of vegetative and seed based population expansion for goldenseal, the very low molecular diversity indices for some populations may indicate either increased vegetative expansion for the wild type populations and clonal propagation methods for the cultivated populations. The very high molecular diversity indices for the five populations above may be attributed to population age and geographic isolation for the wild type populations, or seed based propagative methods for the cultivated.

Overall, the phenogram of the 134 samples (Figure 7), constructed using bootstrapping of distance matrices and UPGMA cluster analysis, is supported by the AMOVA analyses and graphically illustrates the closeness of samples within populations and the within population variation percentages, as well as the Theta molecular diversity

indices. With the exception of 22 outlying samples, all samples could be divided into their within population clusters.

Some of these outlying samples were placed within other populations or on branches with samples from other populations. According to the RAPD profiles, these outlying samples are more closely related in genetic phenotype to the population or samples with which they were paired, than to their population of origin. For example, PA_BER-12 and 13 were closely paired with WV_MR-1 and 5, PA_BER-14 was grouped with OH_1 samples, OH_4-10 was closely placed with WV_JM_4, and NC_BP-9 was paired with WV_HC_2-2 and 7. This type of pairing could be attributed to importation or exchange of plants among the populations, as the sites were close enough geographically for introduction of plants from other locations to occur. Some of the studied populations, although they were thought to be more wild and undisturbed by human intervention than others, may have had more plant introduction than suspected. Because no records are available, repopulation of an area with plants from another location cannot be eliminated and such repopulation could reasonably be suspected to have occurred over the years.

There are two clusters of samples from various populations that are arranged within the NC_BPOP population region of the phenogram. NCBP-4, WV_HC_2-8, NCBPOP-2 and 18, were closely grouped, as were NCBP_7, WV_HC-4 and OH_4-8. WV_HC_2-5 was also grouped with the NC_BPOP population set and NC_BP-10 was paired with WV_HC_2-6. These samples, while they may be paired with other samples outside their population group, show though their branch locations, a close genetic similarity to the NC_BPOP population.

Other samples (PA_BER-12, PA_BER-13, WV_MR-1, WV_MR-5, WV_HC_2-1, WV_HC_2-3, and WV_HC_2-4) in the phenogram were assigned to separate branches, indicating no close relationships. For example, the two samples from WV_MR were

paired with two PA_BER samples, apart from all other populations and samples, and this type of pairing may imply a greater degree of molecular variation for those samples. Theta S (1.751) and Theta pi (1.533) values for WV_MR indicate a limited molecular diversity for the population as a whole although this molecular diversity may be due to these highly variable samples increasing the diversity for the population overall, indicating a need for further investigation of this population (Figure 6).

A previous study (Segarra-Moragues *et al.* 2005) with RAPD markers indicated that perennials with mixed breeding systems show moderate levels of variation, compared with obligatory out-crossing species. Most of our results were in the moderate range. However, there are five populations (PA_BER, PA_RR_2, WV_HC_2, NC_BP and OH_4) that, according to the molecular diversity indices, have the greatest levels of diversity, and are clearly more genetically variable then other populations in the current study. This diversity may imply that other populations in the study have been more affected by human interaction, harvest disturbance or vegetative propagation within those populations. Because both NC_BP and OH_4 are cultivated populations, the plants tested may come from varied root stock sources or cross-bred seed originally. The PA_BER and PA_RR_2 populations are more isolated populations. These appear to be more variable, possibly due to the increased longevity of the plants (due to lack of harvesting) that may lead to greater sexual reproduction relative to clonal expansion via vegetative propagation.

Due to the issues surrounding goldenseal propagation and breeding methods, any definitive conclusions regarding overall species genetic variability for this plant at this time may be premature. This study, however, demonstrated that the bulk of the species genetic variability may be with-in and among the populations. The next logical research step would be to study more populations and more samples for underlying causes of the observed genetic differences noted in this study.

The current study on genetic diversity of goldenseal does indicate a need for preservation and conservation of the species at the population level, as drastic changes in population sizes can have a negative effect on the amount of DNA polymorphism within a population (Tajima 1989b). Restoration efforts may also be assisted by this information, as single source plantings may perform better than those with multiple sources (Sanders and McGraw 2005b). Thus, knowledge of the accessions used for plantings would prove beneficial in those efforts.

Future goldenseal measures of genetic diversity should perhaps explore different markers, such as amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) and restriction fragment length polymorphism (RFLP), to determine the effectiveness and suitability of the each method for evaluating goldenseal. Such diversity measures as have proven successful for other species (Fisher *et al.* 2000; Garcia *et al.* 2000; Tel-Zur *et al.* 2004). AFLPs have already been successfully used for identification of goldenseal accessions from Florida, Georgia and Tennessee (Zhou & Sauvé 2006).

During studies on the potency of individual plants for medicinal purposes, molecular markers should be included with the HPLC and/or anti microbial screening reports for correct identification of the plants (Kharma and Hassawi 2006; Villinski *et al.* 2003). Then as cultivation practices improve, the most productive plants would be identifiable for large-scale production and the information could lead to improvements through breeding or engineering metabolic pathways in medicinal plants to increase yield (Canter *et al.* 2005).

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