PHYLOGEOGRAPHY OF AMERICAN GINSENG (*PANAX QUINQUEFOLIUS* L., ARALIACEAE): IMPLICATIONS FOR CONSERVATION

A Thesis
by
CIARA MARINA LOCKSTADT

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APPROVED BY:

Eva Gonzales
Chairperson, Thesis Committee

Gary Walker
Member, Thesis Committee

Annkatrin Rose
Member, Thesis Committee

Sue Edwards
Chairperson, Department of Biology

Edelma Huntley
Dean of Research and Graduate Studies

Abstract

PHYLOGEOGRAPHY OF AMERICAN GINSENG (*PANAX QUINQUEFOLIUS* L., ARALIACEAE): IMPLICATIONS FOR CONSERVATION

Ciara M Lockstadt

M.S. Appalachian State University

Chairperson: Eva B Gonzales

Historical climate change has caused shifts in species' distributions in eastern North America. Pleistocene glaciations shrunk ranges into refugia, from where they later spread northward following recession of the ice. I analyzed the post-glacial history of the declining native medicinal forest understory plant American ginseng (*Panax quinquefolius* L., Araliaceae). My research objectives were to 1) test the hypothesis that *P. quinquefolius* shows a phylogeographical break east and west of the Appalachian Mountains, 2) infer the locations of glacial refugia in the species' range during the Last Glacial Maximum 3) identify regions of high genetic diversity and 4) develop conservation recommendations, including Evolutionarily Significant Units (ESU). I sequenced fourteen regions of chloroplast DNA from 158 populations across the eastern North American range of *P. quinquefolius* to reveal six mutations and seven haplotypes. I found weak phylogeographical structure due to an overlap of lineages within the Appalachian Mountains. The center of diversity was in the southern Appalachian Mountains, indicating possible southern refugia for *P. quinquefolius*. In addition, I found two unique lineages, signifying potential refugia, in the Blue Ridge Mountains and the Ozark Mountains. Since lineages overlap, future research is necessary to define Evolutionarily Significant Units. Conservation efforts should focus on preserving each unique lineage in germplasm banks and in the wild if possible.

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Dedication

I would like to dedicate this work to my parents, who have been the best friends a daughter could ever ask for over these past three years.

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Foreword

This thesis is formatted with the journal "Conservation Genetics". Through my quest for this delicate medicinal herb in the forests of eastern North America, I have developed a deep understanding on how difficult it is for this plant to survive in today's modern world. Though I saw the effects of deer herbivory and met ginseng harvesters that contribute to the extinction risk of this plant, I also met numerous independent landowners and government and non-government agencies who strictly guard their "sang" populations to prevent them from harvest. I hope this research contributes valuable insights into the conservation of one of the most heavily overharvested species in North America, American ginseng (*Panax quinquefolius* L., Araliaceae).

Introduction

Climate oscillations, species' life history, and landscape can affect the geographic distribution of genetic patterns observed in the vegetation of eastern North America. Pleistocene glaciations forced plants into refugia that were traditionally presumed to be in the southeastern portion of the continent (Deevey 1949; Stewart et al. 2010). The most recent Wisconsin glaciation, which resulted in fluctuations in temperature as the Laurentide ice sheet expanded to lower latitudes from 110,000 years before present (BP) until it's complete recession 8,000 BP (Davis 1983), had the greatest effect on the contemporary geographic structure of lineages. The ice began receding as climates warmed following the last glacial maximum (LGM) 18,000 BP (Davis 1983), and post-glacial northward expansion from isolated refugia, was mitigated by landforms that either hindered or allowed gene flow. Thus, intraspecific lineages that accumulated unique mutations tend to be geographically distributed based on dispersal characteristics and landscape features of eastern North America (Soltis et al. 2006; Taberlet et al. 1998). Inquiries into the factors that determine historical lineage distributions and those that drive postglacial expansion have sparked considerable interest as phylogeographical tools continue to reveal the history of eastern North America's forests.

Though fossilized pollen and macrofossils are a reliable method for dating the locations and migration patterns of high-pollen producing species and species that preserve well in the macrofossil record, mapping molecular phylogenies is a complementary technique for plants occurring in low densities or that do not preserve well as macrofossils. Jackson et al. (2000) used pollen and macrofossils to determine that the unglaciated area of eastern North America was comprised of a coexistence of coniferous and deciduous forest in the Lower Mississippi Valley (LMV) during the LGM. However, they point out that they cannot determine from their palynological data if small densities of deciduous species occurred east of the LMV because these species may not have

produced enough pollen to be detected in pollen cores. In addition, small herbaceous species do not produce enough pollen to be detected in palynological records nor do they preserve well in the macrofossil record. We also cannot assume that herbaceous forest understory plants responded to climate change congruently with their well-preserved associated canopies because most tree species uniquely expanded their ranges as the ice receded to form the contemporary forest assemblages seen today (Davis 1983; Delcourt & Delcourt 1987; Jackson et al. 2000). Mapping the distribution of genetic diversity across the eastern North American landscape is now the predominant method for reconstructing post-glacial histories for these small herbaceous species that do not preserve well in the fossil record. Additionally, fossils cannot reveal evolutionarily unique lineages and their migration patterns, so molecular techniques have been used in intraspecific post-glacial reconstructions for both herbaceous species and trees.

Avise et al. (1979) were the first to popularize reconstructing postglacial histories using mitochondrial and nuclear genetic markers. They found that haplotypes of gophers were distributed regionally east or west of the Apalachicola River, indicating that this river represented a phylogeographic break in lineage distribution. Since then, slowly evolving non-recombining maternally inherited markers, such as in mitochondrial and chloroplast DNA (cpDNA), have demonstrated their usefulness in phylogeographic studies. They show intraspecific polymorphisms that can be used to trace maternal migration patterns and can reveal when seed migration is inhibited by landscape barriers (Avise et al. 1987). Specifically, the non-coding regions of the chloroplast genome are commonly implemented in plant phylogeographic studies because they have a high enough mutation rate to identify lineage-specific changes (Wolfe et al. 1987, Schaal et al. 1998; McLachlan et al. 2005;) and can be used to infer seed migration patterns from geographically mapping haplotypes (Comes and Kadereit 1998).

Much like the aforementioned Apalachicola River phylogeographic break (Avise et al. 1979), several eastern North American species show an east - west division of lineages within the Appalachian Mountains (Soltis et al. 2006). This division is usually attributed to species' ranges

contracting during Pleistocene glaciations causing the formation of isolated refugia east or west of the southern Appalachian Mountains (Soltis et al. 2006). These refugia contain populations that would have persisted through many generations during glaciations allowing them time to accumulate unique mutations (Comes and Kadereit 1998; Taberlet et al. 1998; Provan & Bennett 2008). These refugia serve as the source populations for seeds founding previously glaciated northern regions. In many species, migration from isolated refugia on either side of the southern Appalachians was hindered by the topographic relief features of the mountains that could be difficult for seeds to disperse across, which would explain why an east - west division of lineages is often seen within the mountains (Soltis et al. 2006). Indeed migration from refugia located east and west of the southern Appalachian Mountains resulting in an east - west division of lineages was documented in the eastern tiger salamander *Ambystoma tigrinum tigrinum* (Church et al. 2003), in the dicot *Apios Americana* (Joly and Bruneau 2004) and in the monocot *Trillium grandiflorum* (Griffin and Barrett 2004).

Patterns of genetic diversity align with more recent fossil predictions that Pleistocene glacial refugia for deciduous species existed not only in regions east and west of the southern Appalachians, but also in the Atlantic and Gulf Coastal Plains of southeastern North America (Davis 1983; Delcourt and Delcourt 1987; Swenson & Howard 2005; Soltis et al. 2006). Fossil and chloroplast DNA evidence is concordant in signifying Pleistocene refugia throughout this region in contemporary common widespread deciduous trees, such as *Acer* spp. (Davis 1983; McLachlan et al. 2005), *Fagus* spp. (Whitehead 1973; Williams 2002; McLachlan et al. 2005; Morris et al. 2010), *Liquidambar* spp. (Williams et al. 2004; Morris et al. 2008), and *Quercus* spp. (Delcourt & Delcourt 1984, 1987; Magni et al. 2005). Gonzales et al. (2008) determined that the southernmost populations of geographically restricted cpDNA haplotypes in the understory herb *Trillium cuneatum* inferred multiple refugia in the Gulf Coastal Plain. In the herbaceous aquatic plant *Sagittaria latifolia* the center of cpDNA diversity was located in this region, leading Dorken and Barrett (2004) to conclude the Gulf and Atlantic Coastal Plains as refugial regions.

Since some refugia are predicted to be in the southern portion of a species' range, these areas are expected to accumulate mutations making them rich in haplotype diversity, while the formerly glaciated area exhibits a lack of genetic diversity (Hewitt 2000; Martin & McKay 2004). This pattern is typical of species that co-occur today, and it is likely that they were distributed together throughout the Pleistocene, as is the case for numerous eastern North American animals (Martin and McKay 2004) and some plants, such as the aquatic herb *Sagittaria latifolia* (Dorken & Barrett 2004) and red maple, *Acer rubrum* (McLachlan et al. 2005). The pattern of "southern richness and northern purity" (Hewitt 1996) is attributed to two factors. First, multiple refugia are presumed to have persisted in the south, while northern populations were repetitively extirpated during successive glaciations (Hewitt 2000). Second, lineages that were on the "leading edge" of post-glacial northward expansion would have colonized new habitats first, thereby preventing later arrivals from entering already established populations (Nichols & Hewitt 1994).

In addition to the southeastern refugia, northern refugial locations have been proposed in the Blue Ridge Mountains close to the Laurentide ice sheet (Church et al. 2005). This area harbors rare lineages or disjunct species (Buhlmann & Mitchell 1999; Fleming & Van Alstine 1999; Mitchell & Buhlmann 1999; Roble 1999) presumed to be relicts of once widespread species whose surrounding populations were extirpated during the Wisconsin glaciation (Carr 1938; Braun 1947). This area is predicted to be relatively warm (18°C) during Pleistocene glacial summers (Jackson et al. 2000) and has many sheltered incised valleys that could have created refugial microclimates within the harsh glacial surroundings (Stewart & Lister 2001). Species occurring in refugia in the Blue Ridge Mountains probably existed in populations not large enough to be detected in the pollen record (McLachlan & Clark 2004), but genetic evidence found rare haplotypes in *Quercus rubra* (Magni et al. 2005) and there is a 'relict' population of the salamander *Ambystoma tigrinum tigrinum* in this region (Church et al. 2003).

Few studies have looked at contiguous genetic patterns across all of eastern North America because of the small geographic range of the study organism or limited geographic scope of interest

of the study. Studying the geographic distribution of genetic lineages in widespread organisms can provide inferences into species' large-scale responses to climate change. Thus, there is a need for research on species with distributions that encompass eastern North America. Here, I present a study using cpDNA variation to reconstruct the postglacial history of a widespread yet rare deciduous forest understory herb, American ginseng (*Panax quinquefolius* L., Araliaceae), which has a range that extends from Quebec to Georgia and from the east coast of North America to Nebraska. It is most common along the Appalachian Mountains (Anderson et al. 1993; Robbins 1998). Today *P. quinquefolius* occurs on north-facing slopes in the understory of other widespread species whose glacial and post-glacial history has been studied using fossil and molecular data, such as oaks (*Quercus spp*.), maples (*Acer spp*.) and beech (*Fagus spp*.) (Charron & Gagnon 1991). *Panax quinquefolius* may have occurred with a similar assemblage of temperate species during the Pleistocene. Though human dispersal (Boehm et al. 1999) has been recorded in *P. quinquefolius*, seeds are mainly dispersed by gravity (Lewis & Zenger 1982), so it may show similar distributions of genetic diversity as other species with short-distance dispersal, such as *Trillium cuneatum* (Gonzales et al. 2008) and *Trillium grandiflorum* (Griffin & Barrett 2004).

Panax quinquefolius is a valuable medicinal herb that is rapidly declining throughout its eastern North American range. It is threatened across its entire distribution by illegal harvest (Nantel et al. 1996; Van der Voort and McGraw 2006; McGraw et al. 2010), white-tailed deer herbivory (Furedi & McGraw 2004; McGraw & Furedi 2005), invasive species (Wixted & McGraw 2010), and habitat loss (Charron & Gagnon 1991). *Panax quinquefolius* is listed in Appendix II of Convention on International Trade in Endangered Flora and Fauna (CITES) and is classified under different levels of protection status under the Endangered Species Act (ESA) in 10 U.S. states (U.S. Division of Agriculture, Plants Database). The U.S. Fish and Wildlife Service (FWS) is responsible for determining if harvest is non-detrimental to the survival of the species for CITES and for determining conservation strategies for the species under the ESA.

In some species, the FWS and CITES rely on Evolutionarily Significant Units (ESU; Ryder 1986) as a method for dividing species into units for individual management based on their conservation priority. Ryder (1986) originally introduced the concept of ESUs for the purpose of conserving intraspecific diversity for present and future generations and defined that ESUs be concordant across datasets. Varying opinions on the ESU criteria discuss the importance of adaptive traits, which signify that an ESU has alleles that are important in selection (Dizon et al. 1992; Crandall et al. 2000), and neutral variation in plastid and nuclear markers, which indicate long-term and short-term unique evolutionary histories of lineages and reproductive isolation (Avise et al. 1987; Avise 1998). Since nuclear, biparentally-inherited markers show divergence between portions of *P. quinquefolius'* range (Cruse-Sanders & Hamrick 2004), it is possible that there will be geographic structuring of cpDNA lineages as required by the ESU definition. The FWS and US National Marine Fisheries Service jointly define an ESU as being reproductively isolated and representing an important component of the evolutionary legacy of the species (61 FR 4722 February 7, 1996; 56 FR 58612-58618 November 20, 1991), while CITES does not have a clear definition and appears to define ESUs on a case-by-case basis (Fraser & Bernatchez 2001). Though the FWS's application of ESUs in defining Distinct Population Segments (DPS) in high-profile vertebrate conservation cases has proven valuable in deciding which populations deserve protection (Pennock & Dimmick 1997; Waples 1998), the benefits of its application to endangered and rare plant management have yet to be realized because FWS does not define DPS's for plants (Pennock & Dimmick 1997). Nonetheless, a few studies have justified defining ESUs for plants and have successfully done so by delineating them based on both neutral and adaptive markers (Cavers et al. 2003; Kang et al. 2007; Ge et al. 2011). Thus, there needs to be more research done to support that ESUs warrant use for management and conservation of plant species.

The primary objective of this study is to reconstruct the phylogeographic history of American ginseng (*Panax quinquefolius*) and apply it to conservation. Specifically, I ask the following questions: 1) Does the distribution of *P. quinquefolius* lineages support the hypothesis of an east -

west phylogeographical break similar to that documented in other species? 2) Where are potential locations of *P*. *quinquefolius* refugia, specifically in the southern portion of the species' range or in the Blue Ridge Mountains? 3) Are there any regions of high genetic diversity? 4) What are the implications of phylogeographic history to the conservation of this rapidly declining species?

Methods

Sampling

In total I sampled 245 individuals across 158 populations in 24 states to represent most of the geographic distribution of *P*. *quinquefolius* (Table 1; Figure 1). I recorded GPS coordinates of the locations not disclosed here in the interest of protecting wild populations from potential illegal harvest. In addition, I sampled plants from three growers in Wisconsin, Maryland, and North Carolina. Sample sizes varied from one to eight individuals per population depending on whether the population was large enough to collect eight plants.

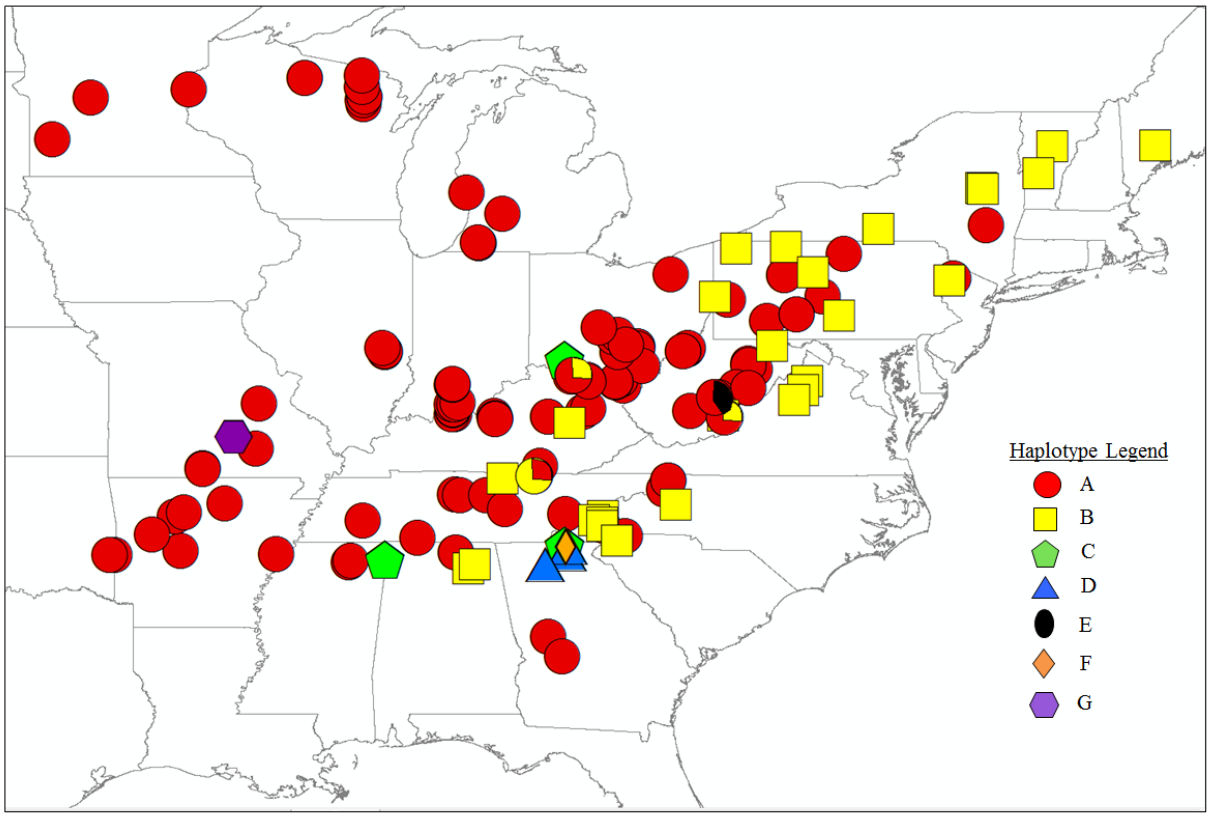

Figure 1. Geographic distribution of *Panax quinquefolius* sampling locations and cpDNA haplotypes in the eastern United States. Colors and shapes correspond to different haplotypes.

Table 1. Geographic location, population code, sample size, and haplotype distribution of wild and cultivated *Panax quinquefolius* populations sampled.

Population name	State	County	*N*	Haplotype
AL-001	AL	Marshall	4	B
AL-002	AL	DeKalb	4	B
AL-003	AL	Madison	4	A
AL-004	AL	Madison	4	A
AR-002	AR	Lee	4	A
AR-004	AR	Stone	1	A
AR-005	AR	Saline	1	A
AR-006	AR	Pope	1	A
AR-007	AR	Pope	1	A
AR-008	AR	Logan	1	A
GA-B	GA	Union	3	D
GA-BC	GA	Union	2	D
GA-H	GA	Macon	3	A
GA-J	GA	Pickens	3	D
GA-P	GA	Union	1	C
GA-U	GA	Upson	4	A
GA-Un	GA	Union	1	F
IL-001	IL	Coles	1	A
IL-002	IL	Coles	1	A
IL-003	IL	Coles	1	A
IN-001	IN	Perry	1	A
IN-002	IN	Perry	1	A
IN-003	IN	Perry	1	A
IN-004	IN	Perry	1	A
IN-005	IN	Perry	1	A
IN-006	IN	Perry	1	A
IN-007	IN	Perry	1	A
IN-008	IN	Crawford	1	A
IN-009	IN	Orange	1	A
IN-010	IN	Orange	1	A
IN-011	IN	Orange	1	A
KY-001	KY	Bullitt	1	A
KY-002	KY	Nelson	1	A
KY-003	KY	Nelson	1	A
KY-004	KY	Nelson	1	A
KY-006	KY	Menifee	1	A
KY-007	KY	Rowan	1	A
KY-011	KY	McCreary	4	A

KY-201	KY	Fayette	1	A
KY-202	KY	Fayette	1	A
KY-203	KY	Powell	1	B
Mdw[a]	MD	Garrett	3	A
MD-001	MD	Garrett	1	B
ME-001	ME	Androscoggin	1	B
MI-001	MI	Barry	1	A
MI-002	MI	Muskegon	1	A
MI-004	MI	Cass	1	A
MI-005	MI	Cass	1	A
MN-001	MN	Lyon	1	A
MN-002	MN	Chisago	1	A
MN-003	MN	Kandiyohi	1	A
MO-004	MO	Dent	1	G
MO-006	MO	Shannon	1	A
MO-008	MO	Ozark	1	A
MO-009	MO	Ozark	1	A
MO-011	MO	Franklin	1	A
MS-001	MS	Tishomingo	4	C
MS-002	MS	Union	4	A
MS-003	MS	Union	1	A
GSMP-018	NC	Haywood	1	B
NCOnt[a]	NC	Madison	4	A (3) B(1)
NC-ASU	NC	Watauga	1	A
NC-BM1	NC	Ashe	1	A
NC-BM2	NC	Ashe	1	A
NC-002	NC	Swain	1	B
NC-003	NC	Jackson	1	B
NC-081	NC	Caldwell	1	B
NC-089	NC	Jackson	1	B
NC-090	NC	Jackson	1	A
NJ-004	NJ	Sussex	1	A
NY-001	NY	Tompkins	1	B
NY-002	NY	Shcuyler	1	B
NY-004	NY	Greene	1	A
NY-006	NY	Hamilton	1	B
NY-007	NY	Hamilton	4	B
NY-100	NY	Fulton	1	B
NY-102	NY	Fulton	1	B
NY-103	NY	Fulton	1	A
OH-001	OH	Lawrence	1	A
OH-002	OH	Scioto	1	A
OH-003	OH	Hocking	1	A

OH-004	OH	Hocking	1	A
OH-005	OH	Hocking	1	A
OH-006	OH	Washington	1	A
OH-007	OH	Washington	1	A
OH-008	OH	Washington	1	A
OH-009	OH	Washington	1	A
OH-010	OH	Scioto	1	A
OH-011	OH	Scioto	1	A
OH-012	OH	Scioto	1	A
OH-013	OH	Brown	4	C
OH-015	OH	Brown	1	A
OH-016	OH	Brown	4	A
OH-017	OH	Brown	4	A
OH-018	OH	Brown	4	A(3) B(1)
OH-019	OH	Adams	4	A
OH-020	OH	Adams	4	A
OH-024	OH	Summit	2	A
OH-025	OH	Ross	1	A
OH-026	OH	Ross	1	A
OH-027	OH	Pickaway	1	A
OH-029	OH	Fairfield	1	A
OH-030	OH	Hocking	1	A
OH-032	OH	Franklin	1	A
OK-001	OK	LeFlore	1	A
OK-002	OK	LeFlore	1	A
PA-001	PA	Centre	1	A
PA-003	PA	McKean	1	B
PA-005	PA	Monroe	1	B
PA-006	PA	Cameron	4	B
PA-007	PA	Perry	1	B
PA-008	PA	Westmoreland	1	A
PA-009	PA	Beaver	4	B
PA-011	PA	Crawford	1	B
PA-012	PA	Beaver	1	A
PA-013	PA	Jefferson	3	A
PA-014	PA	Jefferson	2	A
PA-018	PA	Bedford	1	A
PA-019	PA	Bedford	1	A
PA-021	PA	Tioga	1	A
SC-001	SC	Greenville	1	A
SC-002	SC	Pickens	1	B
SC-003	SC	Pickens	1	B
TN-001	TN	Blount	1	A

TN-002	TN	Lawrence	1	A
TN-003	TN	Davidson	1	A
TN-004	TN	Wilson	1	A
TN-005	TN	DeKalb	1	A
TN-006	TN	Overton	4	B
TN-007	TN	Van Buren	1	A
TN-008	TN	Madision	1	A
TN-010	TN	Scott	4	A (1) B(3)
TN-011	TN	Scott	1	A
VA-004	VA	Page	1	B
VA-005z	VA	Page	1	B
VA-009	VA	Madison	1	B
VA-018	VA	Greene	1	B
VT-001	VT	Washington	1	B
VT-002	VT	Rutland	1	B
HSU[a]	WI	Marathon	4	A
WI-001	WI	Langlade	1	A
WI-003	WI	Langlade	1	A
WI-005	WI	Oconto	1	A
WI-006	WI	Oconto	1	A
WI-008	WI	Forest	1	A
WI-011	WI	Forest	1	A
WI-014	WI	Price	1	A
WI-015	WI	Price	1	A
WV-001	WV	Tucker	1	A
WV-002	WV	Tucker	1	A
WV-003	WV	Tucker	1	A
WV-004	WV	Randolph	1	A
WV-005	WV	Fayette	1	A
WV-006	WV	Webster	3	A(2) E(1)
WV-007	WV	Pocahontas	1	A
WV-105	WV	Webster	1	A
WV-108	WV	Greenbriar	4	B
WV-109	WV	Greenbriar	4	A(3) B(1)

[a] cultivated populations

N number sampled

Lab analyses

I extracted DNA in the field using FTA PlantSaver Cards (Whatman, Piscataway, NJ) or collected leaf tissue and transported it to the lab on ice and stored it at -80 degrees Celsius. For DNA extraction from frozen leaflets I ground samples using liquid nitrogen and then used the DNeasy Plant Mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. I extracted DNA from the FTA Cards as part of the FTA PCR protocol using the FTA Card Extraction Kit according to the manufacturer's instructions.

I screened for polymorphisms by amplifying 14 non-coding regions of the chloroplast genome using published universal primers (Taberlet et al. 1991; Hamilton 1999; Ebert and Peakall 2009). Initially, I tested one individual in populations in four geographically distant regions of ginseng's range, and later, upon finding variation, I sequenced one individual in the remaining populations for those primers. However, in two primers I assumed that most populations did not contain variations because preliminary results indicated that it was likely that only one population contained the mutation. I also extracted and amplified an additional one to three individuals in 30 populations to test for within-population haplotype polymorphism in a region that showed extensive haplotype overlap (Table 2; Supplementary Figure 1) and in three grower's populations to see which haplotypes they distribute. The PCR mixture consisted of 9 µL nuclease-free water (Promega, Madison, WI), 12.5 µL Go*Taq* Hot Start Green Master Mix (Promega, Madison, WI), 1 µL each of forward and reverse primer diluted to 0.033 µM/µL (Integrated DNA Technologies, Coralville, IA) and 1 µL of template DNA diluted to 15 to 70 ng/ µL per sample. I used the following PCR protocol: 1 cycle of 5 minutes of denaturation at 94 °C, 40 cycles (30 s at 94 °C; 30 s for annealing at the variable primer annealing temperature of the primer used (Table 2); 1 minute for elongation at 72 °C), and 1 cycle for 7 minutes at 72 °C). Amplified DNA was run on a one percent agarose gel and visualized with staining by ethydium bromide or GelRed (Phenix Research Products, Candler, NC). PCR products were cleaned and sequenced by Retrogen Inc. (San Diego, CA). I initially sequenced PCR products with the

forward primer and subsequently confirmed polymorphisms in a new PCR reaction and sequencing with the reverse primer. Sequences were manually aligned, edited and formed into contigs using Sequencher 4.10.1 (Gene Codes Corporation, Ann Arbor, MI, USA). All sequences have been deposited in GenBank (Supplementary Table 1).

Table 2. Summary of primers, including the region of the chloroplast genome amplified, average product sizes, annealing temperatures, presence or absence of intraspecific variability, number of individuals sequenced, and the source of the primer.

Variable regions				Nonvariable regions			
Chloroplast region sequenced	Product size (bp)	Annealing temperature (°C)	*N*	Chloroplast region sequenced	Product size (bp)	Annealing temperature (°C)	*N*
psb K - *trn* S (GCU)[t]	694	58.6	249	*rpl* 20 - 5' *rps*12[*]	810	47.8	4
trn G (UCC) ex2 - *trn* G (UCC) ex1[t]	708	58.6	249	5'exon *trn* L *(UAA)* - 3' exon *trn L (UAA)*[§]	540	54.3	2
rps 2 - *rpo* C2[t]	390	46.0	14	*mat* K - *trn* K (UUU) ex2[t]	936	54.3	5
rpo C1 ex2 - *rpo* C1 ex1[t]	890	47.3	9	*rps* 16 ex1- *rps* 16 ex2[t]	912	54.3	3
				atp F ex2 - *atp* H[t]	460	50.8	6
				trnC (GCA) - *pet*N[t]	474	56.4	9
				*trn*T (GGU) - *psb*D[t]	900	49.6	4
				psbC - *psb*Z[t]	850	47.3	9
				*trn*G (GCC) - *rsp*14[t]	575	49.6	9
				*psa*A - *ycf* 3 ex3[t]	850	51.9	9

[*] Hamilton 1999

[§] Taberlet et al. 1991

[t] Ebert and Peakall 2009

N number individuals sequenced

Phylogenetic relationships

I used the most parsimonious, maximum likelihood, and neighbor-joining tree-building methods to look for congruence between phylograms. I gathered sequence data from GenBank for a congener, *P. ginseng* (Accession number AY582139; Kim and Lee 2004), and another close relative, *Eleutherococcus senticosus* (Accession number JN637765; Yi et al. unpublished) to use as outgroups. I generated the most parsimonious haplotype network using a 95% connection limit from concatenated cpDNA sequences using the approach of Templeton et al. (1992) built into the TCS 1.21 software (http://darwin.uvigo.es/ software/ tcs.html). Since the confidence level to connect the outgroups was less than 95%, I determined the minimum connection limit for linking these species to the *P. quinquefolius* haplotype network. I employed the statistical parsimony, neighbor-joining and maximum likelihood methods of Phylip (Felsenstein 1989; http://evolution.genetics.washington.edu/ phylip.html) with 10,000 bootstraps to compare the haplotype relationships derived using TCS 2.1.

Analysis of genetic structure and diversity

I visualized geographic patterns of genetic diversity by mapping haplotypes using ArcMap 10 (ESRI, Redlands, CA) and by determining barriers to seed flow using Barriers 2.2 (Manni et al. 2004; http://www.mnhn.fr/ mnhn/ecoanthropologie/software/ barrier.html). Barriers 2.2 associates a genetic distance along Delaunay triangulation connections between three sampling locations to determine the closest geographic distance and furthest genetic distance. It then uses Monmonier's algorithm to trace a route between sampling locations to determine where genetic distance is the greatest to indicate a barrier to seed-mediated gene flow. Barriers 2.2 constructs first-order barriers by identifying the greatest genetic distance between two locations and working onward from this point until it closes in upon itself or reaches the edge of the sampling range. A second-order barrier would correspond to the barrier that began construction

using the second highest genetic distance and so on for up to the 10th order barrier. I bootstrapped the barriers 100 times using the SeqBoot and Dnadist programs from the Phylip package.

I used the aforementioned maps to group wild populations across *P. quinquefolius'* range at the species, regional and population levels (Supplementary Figure 2). I investigated genetic diversity and structure at the species level by analyzing all 155 wild populations only. Since preliminary data showed one lineage to be restricted to within and east of the Appalachian Plateau province in the Appalachian Mountain physiographic region, I tested for statistical significance supporting a phylogeographic break in this province by dividing all 155 populations into two regions, east or west of the plateau, to see if the distribution of the genetic diversity between the two regions was significantly divided by the plateau. The Barriers 2.2 analysis (Manni et al. 2004) identified a statistically generated area of genetic discontinuity southeast of the Appalachian Plateau, so I further subdivided this region into the southeastern and northeastern subregions and then compared them to the region west of the Appalachian Plateau in order to determine how this barrier may influence genetic patterns between the two regions (Supplementary Figure 2). We tested for the significance of the greatest contiguous genetic discontinuity to gene flow identified by Barriers 2.2 (Manni et al. 2004) by grouping all 155 populations into two regions, north or south of the geographic and genetic boundary. Since previous studies have proposed a pattern of "southern richness and northern purity" (Hewitt 1996) of lineage distribution between the unglaciated and glaciated areas, respectively, I also compared genetic parameters for all 155 wild populations on previously glaciated versus unglaciated regions. Finally, in order to gain insights into potential seed-mediated gene flow among populations, I estimated genetic parameters for a subset of 30 populations for which I analyzed multiple individuals per site in an area that showed extensive haplotype overlap.

I estimated diversity at the species, regional and population levels using two methods. I first calculated the Shannon Diversity Index (I) for haplotype diversity using the formula $I = \sum p_i \ln_e p_i$ (Sherwin et al. 2006), and second I determined diversity (h) or the probability that two individuals will be different when selected from the species range based on haplotype frequencies using the formula $h = 1 - \sum p_i^2$, where p_i is the frequency of the i-th haplotype in the species (Peakall et al. 1995), using the GenAlEx 6.5 Add-in for Microsoft Excel (Peakall & Smouse 2005; http://www.anu.edu.au/ BoZo/GenAlEx/). I used these two methods to calculate diversity for the species using all 155 populations (h_T; I_T) and at the regional level for the two regions east (h_E; I_E) and west (h_W; I_W) of the Appalachian Plateau, the northeastern (h_{NE}; I_{NE}) and southeastern (h_{SE}; I_{SE}) subregions in the region east of the Appalachian Plateau, the regions north (h_{NB}; I_{NB}) and south (h_{SB}; I_{SB}) of the Barriers 2.2 boundary (Manni et al. 2004), and the glaciated (h_G; I_G) and unglaciated (h_{UG}; I_{UG}) regions, and for the subset of 30 populations tested for within population haplotype polymorphism (h_{30}; I_{30}).

I investigated partitioning of genetic diversity for the species and among regions and populations by conducting an Analysis of Molecular Variance (AMOVA) using GenAlex 6.5. I conducted a hierarchical AMOVA to determine the genetic differentiation among the region to the west of the Appalachian Plateau and the two subregions to the east of the plateau (G_{ST}; Pons & Petit 1995). I also found the genetic differentiation between the western region and the two eastern subregions when taking into account the genetic distances between haplotypes (N_{ST}; Pons & Petit 1996) by implementing 1,000 permutations in the Permut software (Pons & Petit 1995; http://www.pierroton/inra.fr/genetics/labo/Software). I determined G_{ST} and N_{ST} among the 30 populations in the region of haplotype overlap. If N_{ST} is greater than G_{ST}, then there is more phylogeographical structure between groups compared because closely related haplotypes are found more often in the same geographical area than would be expected by chance (Pons & Petit 1996).

Results

Chloroplast DNA sequence polymorphism

I sequenced 8,152 bp combined across the 14 primer pairs sampled. While ten chloroplast genome fragments were not variable, four primer pairs showed a total of six polymorphisms (Table 3). This variation resulted in seven haplotypes. There was one indel and one substitution in the *psbK* - *trnS* (GCU) region, two substitutions in the *trnG* (UCC)ex2 - *trnG* (UCC)ex1 region, one 23 bp indel event in the *rps2* - *rpoC2* region, and one substitution in the *rpoC1*ex2 - *rpoC1*ex1 region.

Table 3. Genomic regions of cpDNA polymorphisms in *Panax quinquefolius* haplotypes and outgroups.

Haplotype	*psbK* - *trnS* (GCU)	*trnG* (UCC)ex2 - *trnG* (UCC)ex1	*rps2* - *rpoC2*	*rpoC1*ex2 - *rpoC1*ex1
A	…C…TT…	…G…A…	...GTTATTATCTTTATTACTAAATC…	…C…
B	…C…T-…	…G…T…	...GTTATTATCTTTATTACTAAATC…	…C…
C	…A…TT…	…G…A…	...GTTATTATCTTTATTACTAAATC…	…C…
D	…C…TT…	…T…A…	...GTTATTATCTTTATTACTAAATC…	…C…
E	…C…T-…	…G…A…	...GTTATTATCTTTATTACTAAATC…	…C…
F	…C…T-…	…G…A…	…----------…	…C…
G	…C…TT…	…G…A…	...GTTATTATCTTTATTACTAAATC…	…T…
Panax ginseng	…C…TT…	…G…A…	…----------…	…C…
Eleutherococcus senticosus	…C…--…	…G…A…	…----------…	…C…

Phylogenetic relationships

Phylogenetic analyses using different approaches were partially congruent (Figures 2-5). All analyses show haplotype F to be ancestral with 100 percent bootstrap support. All analyses identified haplotype E to be second-most ancestral with bootstrap values above 74 percent in the neighbor-joining and most parsimonious trees generated and above 95 percent in the TCS most parsimonious haplotype network. The most parsimonious and maximum likelihood tree-building methods found haplotype B to be a sister lineage to haplotype E, while the neighbor-joining tree found haplotype B to be more recently derived. All methods group haplotypes A, C, D and G as of more recent origin. The relationships of haplotypes B through G vary among tree construction methods with low bootstrap values.

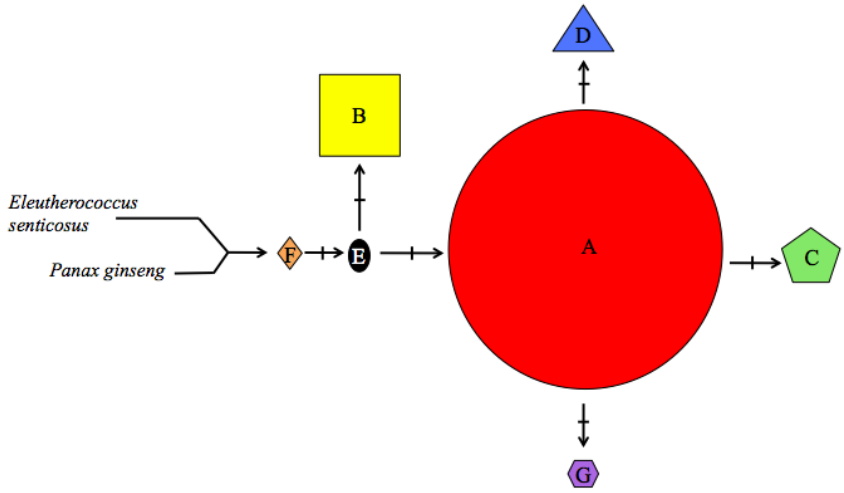

Figure 2. Minimum spanning network of *Panax quinquefolius* cpDNA haplotypes and the outgroups analyzed, *Panax ginseng* and *Eleutherococcus senticosus*. Haplotype shape and color correspond to Figure 1 and perpendicular lines on the arrows represent the number of changes between haplotypes. Size of the shape of each haplotype corresponds to the frequency of that haplotype within the species.

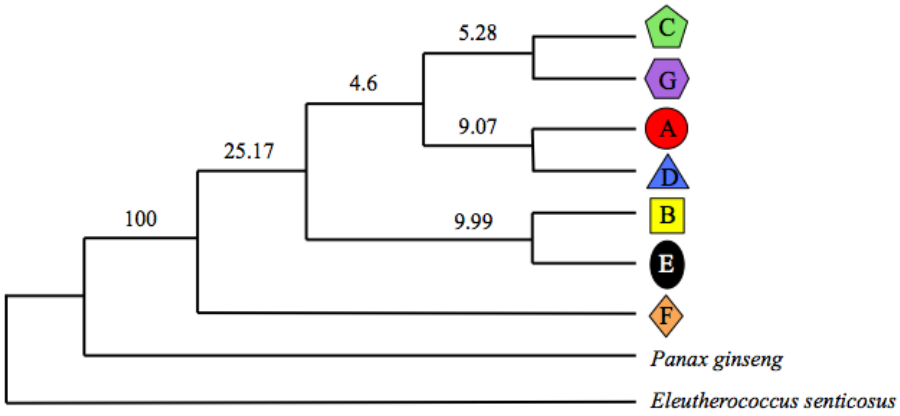

Figure 3. Consensus tree generated from the maximum likelihood method in the Phylip package of concatenated cpDNA sequences for *Panax quinquefolius* haplotypes and the two outgroups. Numbers above branches are the percent of times that branch was present out of 10,000 bootstraps.

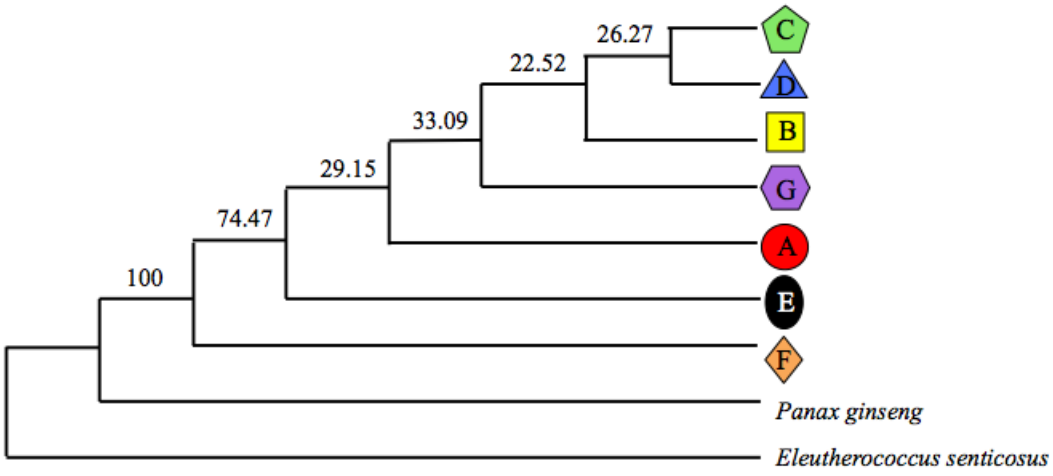

Figure 4. Consensus tree generated from the neighbor-joining method in the Phylip package of concatenated cpDNA sequences for *Panax quinquefolius* haplotypes and the two outgroups. Numbers above branches are the percent of times that branch was present out of 10,000 bootstraps.

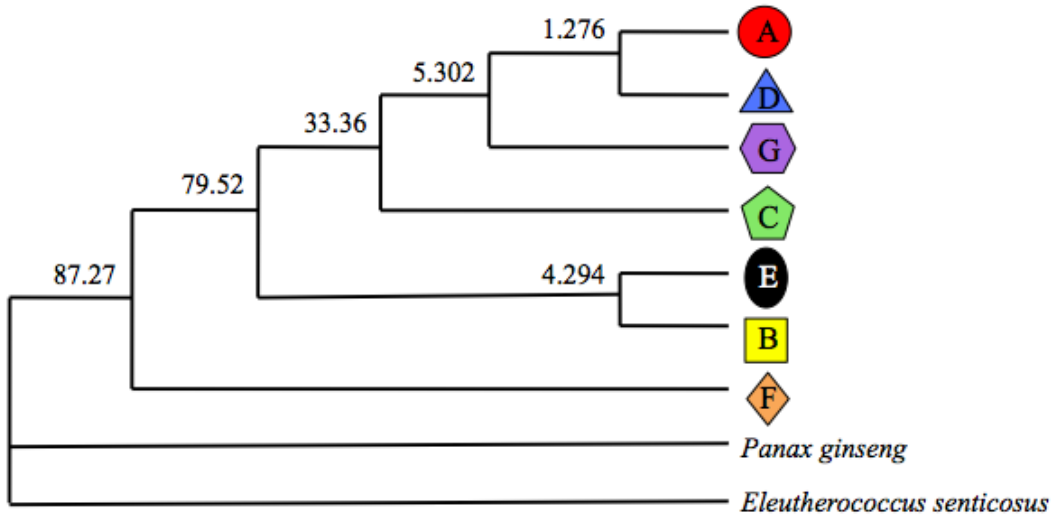

Figure 5. Consensus tree generated from the parsimony method in the Phylip package of concatenated cpDNA sequences for *Panax quinquefolius* haplotypes and the two outgroups. Numbers above branches are the percent of times that branch was present out of 10,000 bootstraps.

Genetic structure and diversity

Panax quinquefolius has two common and widespread haplotypes with partially overlapping ranges, while the distribution of the other five rare haplotypes is restricted. The most frequent and widespread haplotype, haplotype A, occurs in 80 % of the populations sampled (Figure 6) and is distributed over the entire sampling range except for the far eastern edge. The second most common haplotype, which occurs in 22.8 % of populations, is distributed within and east of the Appalachian Plateau. Haplotype C is located in two populations within the southern part of the species' range and one population in southern Ohio. Haplotype D is distributed in three populations in northern Georgia. Haplotypes E, F and G occur in one population in the Blue Ridge Mountains of West Virginia, the southern Appalachian Mountains of Georgia, and the Ozark Mountains of Missouri, respectively. Haplotypes C, D, E, F and G were distributed in less than four percent of the populations sampled. Four out of 30 native populations and one out of three grower's populations were polymorphic. The growers' plants were mainly haplotype A, but one individual from one cultivated population from North Carolina was of haplotype B.

I identified five overlapping barriers that form a main contiguous genetic discontinuity in the southeastern portion of *P*. *quinquefolius*' range and five smaller unconnected discontinuities throughout the species distribution using Barriers 2.2 (Manni et al. 2004; Supplementary Figure 3). The contiguous boundary was created by the overlap of the second, fifth, sixth, seventh and eighth orders barriers and stretched from northern Alabama to South Carolina. Most of this contiguous barrier was south of 36 degrees N. The other five barriers encircled areas of haplotype overlap or the locations of rare lineages.

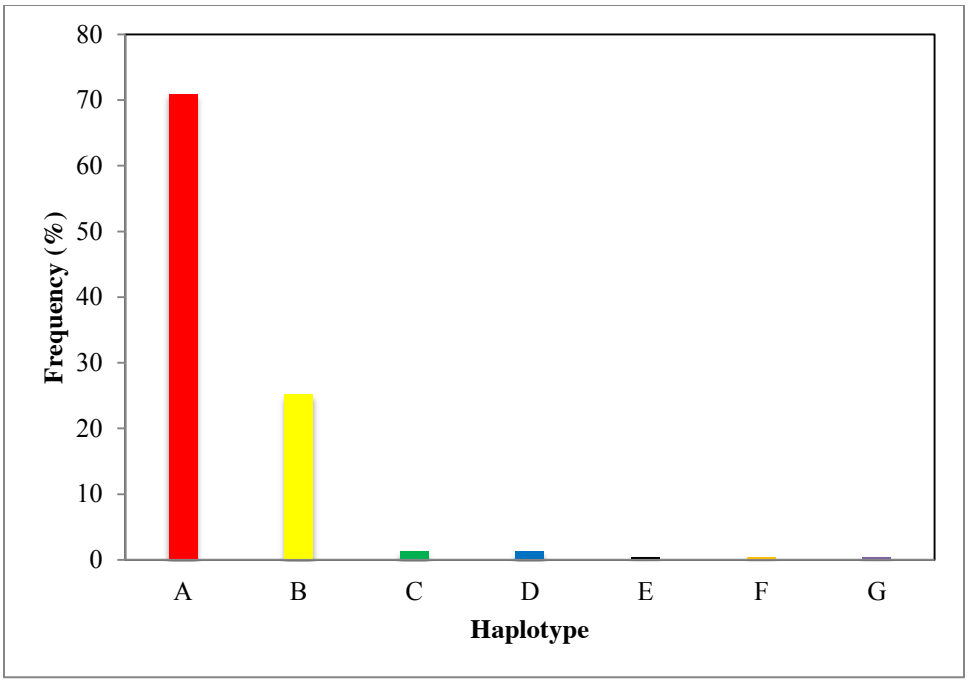

Figure 6. Frequency of haplotypes in *P*. *quinquefolius* individuals sampled

Total species diversity and the Shannon Index of Diversity for all 155 wild populations was low (h_T=0.010 ± 0.005, I_T = 0.015 ± 0.007; Table 5). In both calculations the western region (h_W = 0.246; I_W = 0.519) was less diverse than the eastern region (h_E = 0.578; I_E = 1.007; $p<4.28e^{-13}$), and the northeastern subregion was less diverse (h_{NE} = 0.492; I_{NE} = 0.726) than the southeastern subregion (h_{SE} = 0.659; I_{SE} = 1.242; $p<1.7e^{-7}$) within the eastern region. I found similar levels of diversity in the glaciated and unglaciated regions (h_G = 0.455; h_{UG} = 0.496) and the amounts of diversity using the Shannon Index (I_G = 0.647; I_{UG} = 0.960) were not significant ($p>0.154$). The region to the south of the barrier was more diverse (h_{SB} = 0.674; I_{SB} = 1.257) than the region north of the barrier (h_{NB} = 0.410; I_{NB} = 0.736; $p<5.3e^{-10}$) in both analyses used. Diversity for the subset of 30 populations was low (h_{30} = 0.052; I_{30} = 0.077); 26 populations were fixed for the same haplotype and only 4 were polymorphic.

Table 5. Genetic diversity and Shannon Index of Diversity and their standard deviations calculated using haplotype frequencies for species, regional, and population level analyses.

Method of partitioning populations	h	I
Species	0.010 ± 0.005	0.015 ± 0.007
Regional - West/ East		
West	0.264	0.519*
East	0.578	1.007*
Regional - West/ Northeast/ Southeast		
Western region	0.246	0.519*
Northeastern subregion	0.492	0.726*
Southeastern subregion	0.246	1.242*
Regional - Glaciated/ Unglaciated		
Glaciated region	0.455	0.647^{ns}
Unglaciated region	0.496	0.960^{ns}
Regional - North/ South		
North	0.410	0.736*
South	0.674	1.257*
30 Populations	0.052 ± 0.025	0.077 ± 0.037

* $P < 0.000$

^{ns} Not significant

h Genetic diversity

I Shannon's Diversity Index

The AMOVA analysis showed that 24 % of diversity is partitioned between the regions west and east of the Appalachian Plateau, while 76 % of diversity is found within the two regions ($p<0.01$; Table 6). The hierarchical AMOVA revealed that only 7 % of diversity was partitioned between the eastern and western regions and that 22 % of diversity was partitioned among the western region and two eastern subregions, while 72 % of diversity was partitioned within the two regions ($p<0.01$). When I compared the regions north and south of the former Laurentide ice sheet margin only 0.5 % of diversity was non-significantly ($p<0.27$) partitioned between the glaciated and unglaciated regions while most of the diversity was partitioned within regions (99.5 %). However, when I divided the populations latitudinally north or south of the boundary identified using Barriers 2.2, I found diversity to be partitioned between regions (28 %) and within regions (72 %; $p<0.01$). Finally, the 30-population level analysis in the region of overlap revealed that 88 % of the diversity was partitioned among populations and that only 12 % of the diversity was partitioned within populations ($p<0.01$).

The genetic differentiation among the western region and the two eastern subregions was greater ($G_{ST}=0.339$) when I did not consider genetic distances between haplotypes ($N_{ST}=0.214$). Using the subset of 30 populations, I found that N_{ST} (0.838) was slightly greater than G_{ST} (0.834).

Table 6. Partitioning of genetic diversity at the regional and population levels.

Source	df	SS	MS	Est. Var.	%
Regional - West/ East					
Between regions	1	8.155	8.155	0.068	24% *
Within regions	232	49.341	0.213	0.213	76% *
Regional - West/ Northeast/ Southeast					
Between regions	1	8.155	8.155	0.019	7% *
Among regions and subregions	1	2.881	2.881	0.060	22% *
Within regions and subregions	231	46.459	0.201	0.201	72% *
Regional - Glaciated/ Unglaciated					
Between regions	1	0.324	0.324	0.001	0.5% ^{ns}
Within regions	232	57.172	0.246	0.246	99.5% ^{ns}
Regional - North/ South					
Between regions	1	5.126	5.126	0.086	28% *
Within regions	232	52.370	0.226	0.226	72% *
Population					
Among Populations	29	30.028	1.035	0.275	88% *
Within Populations	79	2.917	0.037	0.037	12% *

* $P < 0.01$

^{ns} Not significant

Discussion

The post-glacial evolutionary history of American ginseng is more complex than originally anticipated. The weak phylogeographical structure caused by haplotype overlap created a partial east - west phylogeographical break in the Appalachian Plateau, with no clear division of lineages. The cluster of overlapping haplotype diversity in the southeastern portion of the species' range suggests that this was a region of Pleistocene refugia for multiple lineages. Unique haplotypes in the Blue Ridge and Ozark Mountains suggests existence of refugia further north than is apparent from fossil records of species occupying the same niche. Unexpectedly, the most widespread haplotype, which covers the entire species' range, is more recently derived, while the most ancestral lineages are unique and appear to be going extinct. The overlap of the two dominant lineages makes it difficult to define geographically delineated ESU's because populations in the region of overlap may not be reproductively isolated. The southeastern portion of ginseng's range is the center of genetic diversity, meaning that this region may deserve high conservation priority.

Phylogeographic patterns and location of refugia

There is weak phylogeographical structuring of *P. quinquefolius* cpDNA haplotypes because five rare lineages are interspersed within the ranges of two main widespread and partially overlapping lineages. Haplotype B creates a partial east - west geographic break because it is restricted to the area within and east of the Appalachian Plateau physiographic region. However, this break is not considered "phylogeographic" because it did not divide *P. quinquefolius* lineages. Despite the lack of phylogeographic structure, statistical analyses support a significant partitioning of genetic diversity (24 %, $P < 0.01$) between the regions east and west of the

Appalachian Plateau. Soltis et al. (2006) predicted and documented this east – west break, or "Appalachian discontinuity", occurring within the Appalachian Mountains in numerous eastern North American species, such as fish, algae, plants and mammals. In contrast, *P. quinquefolius* follows a less common pattern because this discontinuity is shifted west to create an Appalachian Plateau discontinuity. Nonetheless, this region has restricted haplotypes to within and east of the plateau in species that are presently co-distributed with *P. quinquefolius*, such as the American beech tree, *Fagus grandifolia* (McLachlan et al. 2005; Morris 2010) and the spring peeper frog, *Pseudocris crucifer* (Austin et al. 2004). This region also appears to restrict lineages to the west as haplotypes have also been distributed solely within and west of the plateau in white trillium, *Trillium grandiflorum* (Griffin & Barrett 2004) and the tiger salamander, *Ambystoma tigrinum tigrinum* (Church et al. 2003).

The Appalachian Plateau discontinuity in *P. quinquefolius* can be attributed to range contraction during the Wisconsin glaciation followed by range expansion when climates warmed. The present-day southernmost distribution of haplotype B is within the southern Appalachian Mountains in Alabama, indicating that this area harbored a refugium for this lineage. When climates warmed, haplotype B dispersed from this refugium within the valleys of the Appalachian Mountains in a southwestern to northeastern direction because these valleys provided restricted migration corridors. They formed in a southwest to northeast pattern and are bound by ridges on either side that would have been difficult for *P. quinquefolius'* gravity-dispersed seeds to migrate over. Furthermore, in the event of seeds dispersing westward over the ridges of the mountains, they may have been unable to establish new populations in the Interior Plains if they did disperse past the plateau. Since the Appalachian Plateau physiographic region represents a shift in environmental conditions, such as temperature or rainfall, between the Appalachian Mountains and the Interior Plains of eastern North America, it may have prevented maladapted mountain lineages from surviving dry, warm conditions in the plains. Though Souther et al. (2012) did not find adaptive differences in populations in two areas of West Virginia within the Appalachian

Mountains that are exposed to different temperature regimes, Souther and McGraw (2011) conducted a similar study where they expanded their sampling to cover six states and did find evidence suggesting temperature adaptations between populations between the Interior Plains and Appalachian Mountains. Therefore, it is possible that there is an "environmental barrier" preventing lineage B from growing in the Interior Plains.

The center of genetic diversity is in the southeastern part of *P. quinquefolius'* range in the southern Appalachians of Georgia and South Carolina, which is consistent with the hypothesis of southern refugia (Deevy 1949). Barriers 2.2 separated this area into a southeastern subregion within the region east of the Appalachian Plateau, which was found to be more diverse than the northeastern subregion and the region to the west of the plateau. Further statistical analyses supported a pattern of "southern richness and northern purity" in respect to the southeastern Barriers 2.2 boundary. Interestingly, this "southern richness and northern purity" pattern is traditionally thought to be found either side of the former ice margin (Hewitt 1999), yet the AMOVA comparison and Shannon Index were not significant between the unglaciated and glaciated regions, indicating that the former ice margin did not affect the distribution of genetic diversity in the species. This could have been an effect of there being equal frequencies of haplotypes in the glaciated and unglaciated regions, despite there being less total number of haplotypes in the glaciated region. The southern Appalachian Mountains also harbor unique and ancestral haplotypes, which are characteristics of a region that protected refugia populations through glacial maxima allowing them time to accumulate mutations (Comes & Kadereit 1998; Taberlet et al. 1998; Provan & Bennet 2008). Additionally, southeastern refugia were supported in other phylogeographic studies (Soltis et al. 2006) and paleoecological Pleistocene reconstructions of forest assemblages (Davis 1983; Delcourt & Delcourt 1984; Jackson 2000).

Unfortunately, it is not possible to tell if the center of diversity in northern Georgia was an actual refugia region for the haplotypes that currently occur there or if they existed further south during the Pleistocene. For example, since the southernmost haplotype occurrences are

often considered to be the seed-source for populations of the same lineage that extend north from it (Hewitt 1996) and the southernmost population in Georgia containing haplotype A is of natural origin, it appears to be a refugium for this lineage. Therefore, populations of haplotype A in southern Georgia could have been the source population for migrants into the center of diversity in northern Georgia where haplotype A also occurs. Gonzales et al. (2008) pointed out a similar situation in the forest understory herb sessile trillium, *Trillium cuneatum*, where it was difficult to tell if the southernmost extent of a haplotype's range was an actual refugium or if populations existed further south during the Wisconsin glaciation. Here it is possible that populations of haplotypes B, C, D and F occur or did occur in southern Georgia along with haplotype A, but were missed in sampling or have been extirpated due to habitat destruction, harvesting or climate change. This emphasizes the difficulty in discerning refugia for haplotypes that are widespread because it is not possible to determine the locations or number of refugia or to see overlapping intrahaplotype migration paths (McLachlan 2005).

The unique and ancestral haplotype E located in the Blue Ridge Mountain physiographic region of West Virginia supports my hypothesis of a northern glacial refugium. Intriguingly, *P. quinquefolius'* haplotype network shows that haplotype E evolved from haplotype F, which is located in the southern Appalachian Mountains. Since it has been proposed that disjunct populations in the Blue Ridge Mountains of West Virginia represent relicts of once-widespread haplotypes that were mainly extirpated during the Pleistocene glaciations (Carr 1938; Braun 1947), it is possible that haplotype E evolved from haplotype F in the southern Appalachians then expanded to the West Virginia Blue Ridge Mountains before the Wisconsin glaciation. Then when climates cooled populations of haplotype E between the southern Appalachians and northern Blue Ridge region were extirpated, while this specific West Virginia area remained temperate and moist enough to be hospitable to deciduous species, such as *P. quinquefolius*, during the Wisconsin glaciation (Church et al. 2003). Indeed this area has been reported to harbor disjunct or relict populations in almost 100 species that are both closely and distantly

related to *P*. *quinquefolius* (Buhlmann et al. 1999; Fleming and Alstine 1999; Roble 1999 ; Church et al. 2003).

There are two possible explanations regarding the odd northern location of haplotype G in the Ozark Plateau. First, this could represent a post-glacial mutation, thereby rejecting the idea of this area as a refugium during the Wisconsin glaciation or it could be a possible refugium. Considering that chloroplast DNA mutates at a rate of one to three changes every million years (Wolfe et al. 1987), it is likely that the mutation that created haplotype G preceded the last glacial period. This population could be the result of a once widespread haplotype that was extirpated in surrounding regions that were not sheltered by the topographic relief of the Ozark Mountains. Then as climates warmed, this haplotype was restricted from expanding out of the mountains, which would explain why Barriers 2.2 identified a statistically significant boundary to seed dispersal surrounding this specific population. Genetic evidence has supported an Ozark refugium in royal catchfly, *Silene regia*, which has similar distribution and reproductive and seed dispersal characteristics as *P*. *quinquefolius* (Dolan 1994), and in numerous aquatic vertebrates that exist in deciduous forests alongside *P*. *quinquefolius* including the spring peeper frog, *Pseudocris crucifer* (Austin et al. 2004), the hellbender salamander, *Cryptobranchus alleganiensis* (Sabatino & Routman 2009), and the minnow, *Notropis nubilus* (Berendzen et al. 2010) that span the range of *P*. *quinquefolius*.

Since coalescent theory predicts that the most widespread haplotypes are ancestral because they have had more time to disperse and recently evolved haplotypes had less time, resulting in restricted ranges (Hudson 1990; Neigel et al. 1991), it is interesting that *P*. *quinquefolius* displays the opposite of this trend. While the restricted distributions of haplotypes D and G and their recent evolution from the widespread haplotype A are consistent with this theory, the more ancestral lineages, E and F, do not support this hypothesis. Haplotype F has an ancestral 23bp indel that is present in one *P*. *ginseng* sequence on GenBank (Accession number AY582139.1) but absent in another (JN700447.1), leading to the conclusion that this might be the

result of incomplete lineage sorting. The divergence time between *P. quinquefolius* and *P. ginseng* dates to a recent Tertiary vicariance event (Wen 1999; Xiang et al. 2000), which can account for the sharing of haplotypes between the sister species (Wen & Zimmer 1996). This ancestral haplotype and the other ancestral haplotype, E, are each unique to one individual in one population, highlighting that a cautionary approach must be taken when assuming that the most widespread haplotype is ancestral or that a lack of migration of a haplotype indicates recent origins. A similar pattern was documented in American beech, *Fagus grandifolia* (Morris et al. 2010), broadleaf arrowhead, *Sagittaria latifolia* (Dorken & Barrett 2004) and yellow poplar *Liriodendron tulipifera* (Sewell et al. 1996). It is possible that these species and *P. quinquefolius* all contain evolutionary relict haplotypes that were once widespread but are now reduced to single locals because of population extinctions during climate cooling without subsequent recolonization when climates warmed in the late Pleistocene (MacDonald 2001).

Conservation recommendations

Since haplotypes overlap extensively in *P. quinquefolius*' range, ESUs cannot be defined based on cpDNA data for practical purposes as originally proposed. Dizon et al. (1991) and Moritz and Faith (1998) recommended that ESUs be geographically isolated because regional structuring of lineages allows for land managers to estimate which ESU that populations of concern are in, without having to spend the resources to genetically analyze it. Though each lineage was not isolated, much research has been done to find regional differences in allozyme and RAPD markers, phenology, morphology and disease susceptibility between plants (Bai et al. 1997; Schluter & Punja 2002; Cruse-Sanders & Hamrick 2004a, 2004b; Grubbs & Case 2004) and evidence that natural selection has led to certain genotypes possibly being adapted to local conditions (Lim et al. 2007; Souther & McGraw 2010). So, it is possible that future research covering the entire range of the species will provide the information necessary to define

Management Units, which are a conservation unit similar to the ESU but divide units based on significant divergence in allele frequencies (Moritz 1994a).

Since ESUs cannot be defined based on my data and the ESA does not protect privately owned populations, it is practical to conserve publicly owned populations of each *P*. *quinquefolius* lineage with a focus on the rare haplotypes because there is a greater chance of these haplotypes going extinct. Those populations containing the rare haplotype D in northern Georgia, haplotype E in West Virginia, and haplotype C in Ohio are on public property and harvest from them should be limited. Preventing harvest in these populations is critical especially in those haplotypes found only in one plant within one population, as is the case for haplotypes E and F, because harvest of this single plant could cause the extinction of these evolutionarily distinct lineages. Conserving haplotype F is especially important because this entire population is comprised of a single plant that contains the lineage that is ancestral to all of the *P*. *quinquefolius* in North America. Despite the fact that harvest is unregulated on the private property where this single plant of haplotype F grows, many private property owners, including the owner of this population, heavily guard their plants from harvest and only disclose their locations to researchers because they think the study would contribute to conserving the species. Therefore, private property owners may be willing to participate in conservation efforts and should be made aware that they harbor an important component of the evolutionary history of the *P*. *quinquefolius* species. Additionally, publicly owned populations near unique lineages growing on private property should be genetically analyzed for cpDNA diversity in case seeds from the unique private populations were able to disperse onto public property. Moreover, in recognizing that the social and economic pressure on land managers to allow harvest may result in harvest of populations containing rare lineages, it is recommended that seeds from each lineage be collected and stored in germplasm banks.

Despite previous reports of human-mediated seed dispersal (Boehm et al. 1999), I cannot determine if human transplants between natural populations played a role in the contemporary

distribution of *P*. *quinquefolius* using cpDNA sequences. If cpDNA haplotypes had been more geographically structured, then finding a population containing a haplotype out of its typical range would indicate possible human transplants. In addition, it has been proposed that harvesters supplement wild populations with cultivated seeds (Boehm et al. 1999), yet the biggest seed supplier in Wisconsin sells seeds of the most common wild haplotype (A). Therefore, if a seed of haplotype A were planted in the wild throughout the range of *P*. *quinquefolius*, it would be impossible to tell if that seed were of non-native origins because haplotype A is prevalent throughout *P. quinquefolius'* range. Future work on the bi-parentally inherited rapidly evolving nuclear genome may be necessary to resolve if wild populations are of cultivated origins. Some harvesters' claims that all wild populations originated from cultivated seeds are unfounded, because growers only sell seeds of two haplotypes. If all wild populations were from cultivated seeds, then all seven lineages would have been found in sampling growers' seeds. Despite being unable to determine if human-mediated seed dispersal occurs using cpDNA markers, transplantation of seeds between populations of different lineages should be prevented because mixing of lineages could result in the degradation of possible coadapted gene complexes thereby reducing the fitness of that lineage via outbreeding depression (Templeton et al. 1986).

CONCLUSION

My study used phylogeographical tools to reconstruct the post-glacial history of a widespread forest understory species, *P. quinquefolius*. Genetic diversity was clustered in the southern Appalachian Mountains, indicating that this area harbored refugia for multiple lineages. I found less geographic structuring of haplotypes than anticipated, making it difficult to define conservation units. Nonetheless, I was able to identify populations containing rare lineages that may be critical to conserving the species and genetically unique lineages that should be preserved in germplasm banks. Future work should focus on defining Management Units for conservation purposes.

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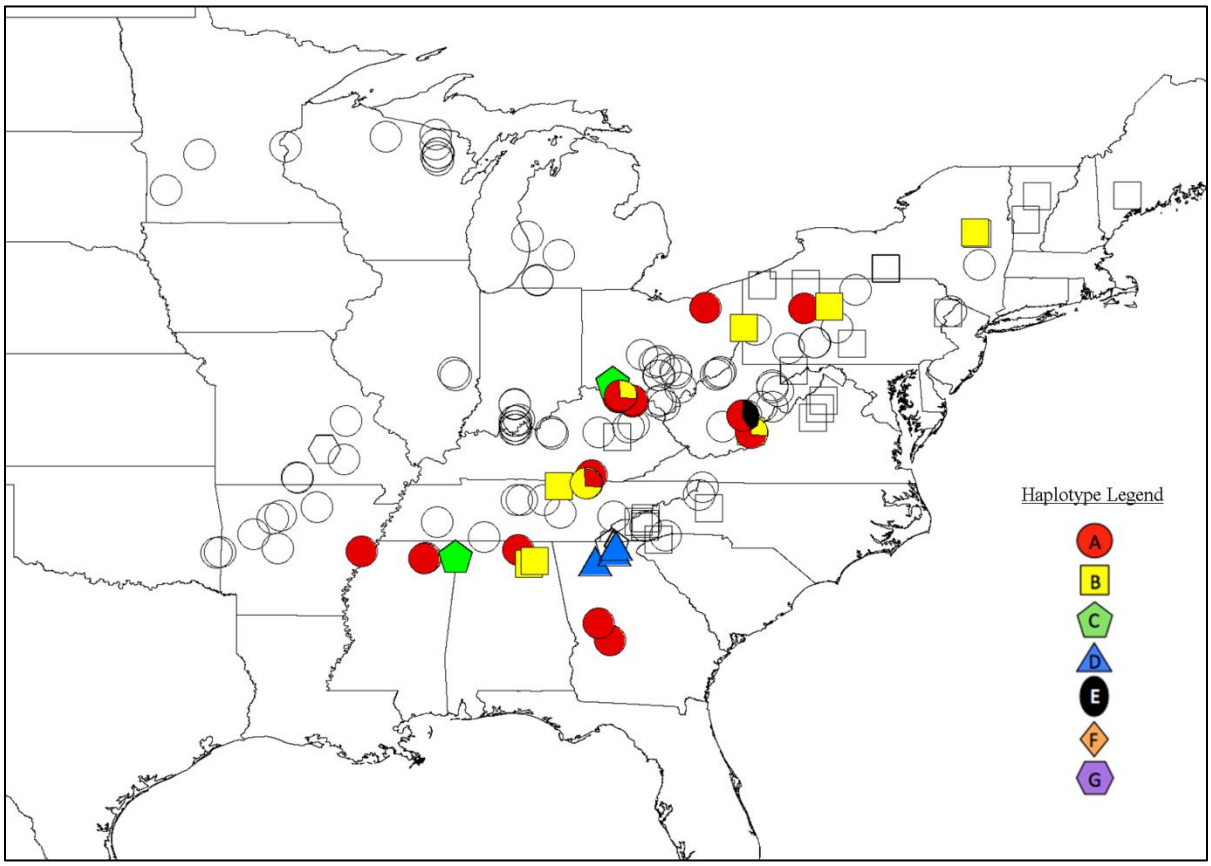
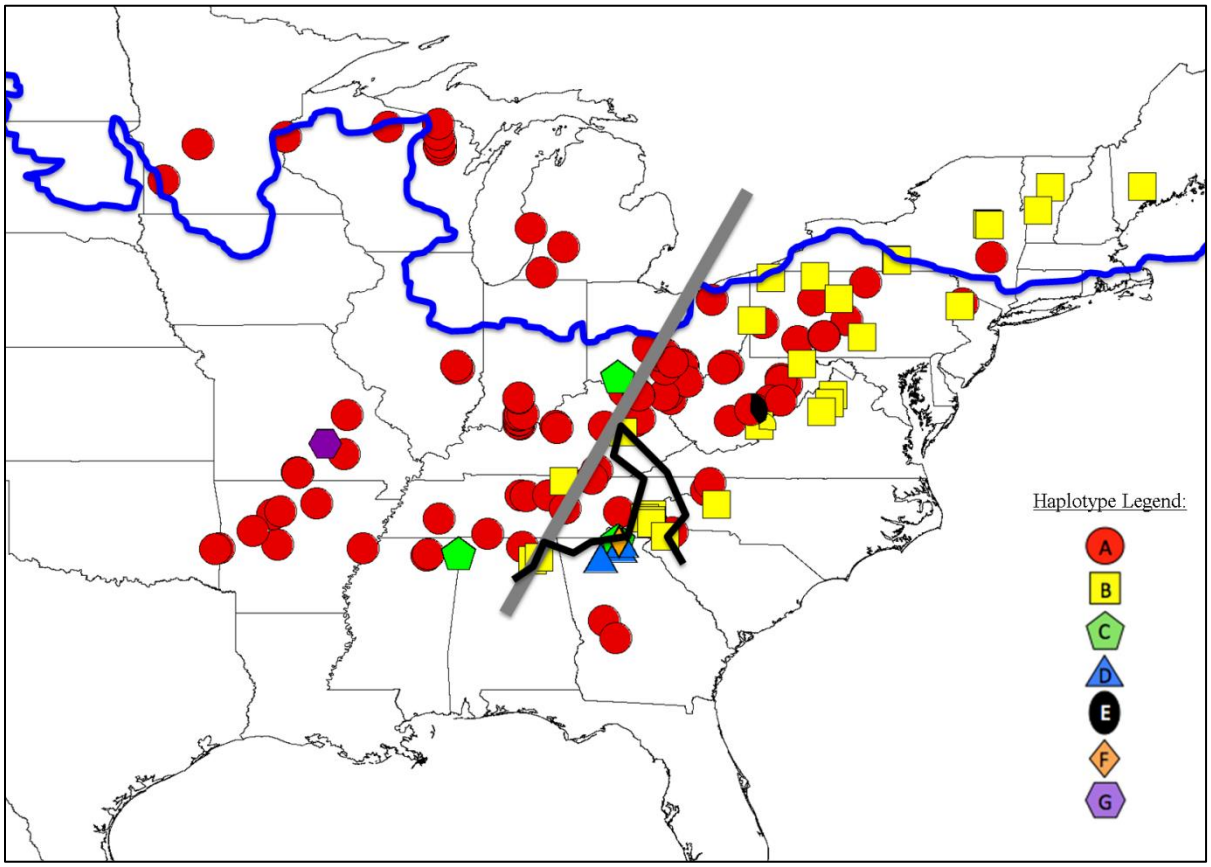
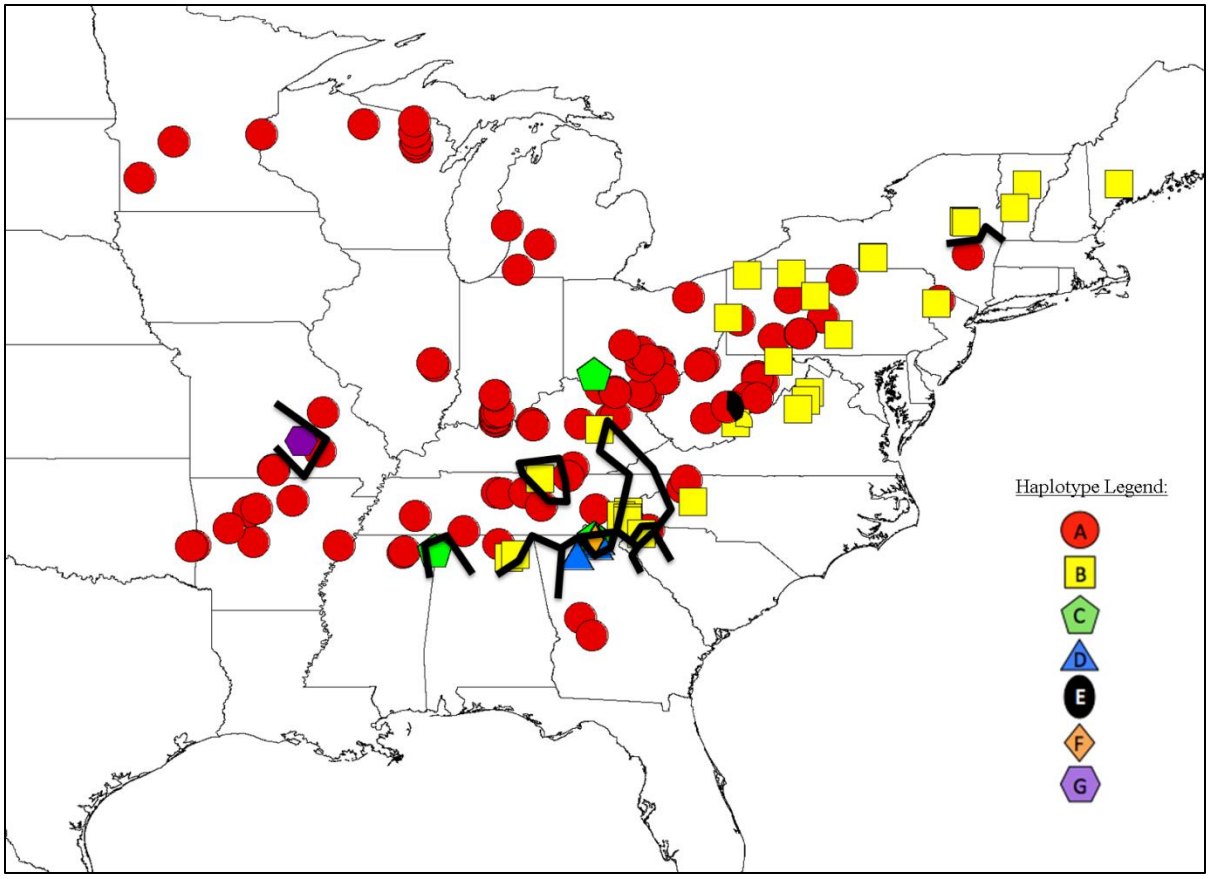
Appendix 1: Supplementary Figures and Tables



Supplementary Figure 1. Locations of the 30 populations (colored symbols) sampled for within-population haplotype diversity in the region of extensive haplotype overlap. Empty symbols indicate the haplotype of sampling locations that were not tested for within-population haplotype polymorphism.



Supplementary Figure 2. Map of population groups used to generate genetic diversity parameters. In my first regional analysis, populations to the west of the Appalachian Plateau (grey line) were considered to be in the western region, while populations to the east of the plateau were considered to be in the eastern region. In my second regional analysis, the barrier (black line) divided the region east of the Appalachian Plateau into the northeastern and southeastern subregions. In my third analysis, I compared the regions north and south of the barrier. In my fourth regional analysis, I compared populations north and south of the former ice margin (blue line).



Supplementary Figure 3. Geographic location of genetic discontinuities identified using Barriers 2.2 (Manni et al 2004).

Supplementary Table 1. Accession numbers for sequences obtained in this study, including their genomic region, primer names, primer sequences,the sample submitted to GenBank, the haplotype, and the species

Genomic region	Forward primer name	Forward primer sequence	Reverse primer name	Reverse primer sequence	Sample	Haplotype (if variable)	Species	Accession number
chloroplast matK gene for maturase, located within the intron of trnK	ANU_cp003-L	aaatatttctgttgatacattcg	ANU_cp005-R	gggttgctaactcaatggta	GABC	nv	*Panax quinquefolius*	KC217535
ribosomal protein S16 (rps16)	ANU_cp007-L	cttcgagatcgaacatcaat	ANU_cp008-R	aaaacgatgtggtagaaagc	NY4c	nv	*Panax quinquefolius*	JX896431
psbK-trnS intergenic spacer	ANU_cp013-L	tgtttggcaagctgctgtaa	ANU_cp014-R	gggttcgaatccctctcttt	AL1a	B	*Panax quinquefolius*	JX896432
psbK-trnS intergenic spacer	ANU_cp013-L	tgtttggcaagctgctgtaa	ANU_cp014-R	gggttcgaatccctctcttt	AL3a	A	*Panax quinquefolius*	JX896433
psbK-trnS intergenic spacer	ANU_cp013-L	tgtttggcaagctgctgtaa	ANU_cp014-R	gggttcgaatccctctcttt	GAP	C	*Panax quinquefolius*	JX896434
tRNA-Gly (UCC) intron and tRNA-Gly (UCC) exon	ANU_cp016-L	gcgggtctcgtttagtggtaaag	ANU_cp017-R	cgttagcttggaaggctagg	AL1a	B	*Panax quinquefolius*	JX908829
tRNA-Gly (UCC) intron	ANU_cp016-L	gcgggtctcgtttagtggtaaag	ANU_cp017-R	cgttagcttggaaggctagg	AL3b	A	*Panax quinquefolius*	JX908830
tRNA-Gly (UCC) intron and tRNA-Gly (UCC) exon	ANU_cp016-L	gcgggtctcgtttagtggtaaag	ANU_cp017-R	cgttagcttggaaggctagg	GAB1	D	*Panax quinquefolius*	JX908831
atpF gene, atpF-atpH intergenic spacer and atpH gene	ANU_cp025-L	tcggtattaagcccgaaact	ANU_cp026-R	gcttttatttgcgaacccttt	AR2e	nv	*Panax quinquefolius*	KC217536
rps2 gene, rps2-rpoC2 intergenic spacer, and rpo C2 gene	ANU_cp031-L	ccatgaccaaaatgaactcc	ANU_cp032-R	gcgtcggaaatgagagatatt	GAU1	A	*Panax quinquefolius*	KC297163
rps2 gene, rps2-rpoC2 intergenic spacer, and rpo C2 gene	ANU_cp031-L	ccatgaccaaaatgaactcc	ANU_cp032-R	gcgtcggaaatgagagatatt	PGa	na	*Panax ginseng*	KC297164

rps2 gene, rps2-rpoC2 intergenic spacer, and rpo C2 gene	ANU_cp031-L	ccatgaccaaaatgaactcc	ANU_cp032-R	gcgtcggaaatgagagatatt	GAUn	F	*Panax quinquefolius*	KC297165
rpo C1 intron	ANU_cp033-L	ttcgaattaaacctcgtaatcg	ANU_cp034-R	catgtgtggtatttgaaacgtc	MO4f	G	*Panax quinquefolius*	KC354696
rpo C1 exon and rpo C1 intron	ANU_cp033-L	ttcgaattaaacctcgtaatcg	ANU_cp034-R	catgtgtggtatttgaaacgtc	NY4c	A	*Panax quinquefolius*	KC354697
tRNA-Cys-petN intergenic spacer and PetN (petN) gene	ANU_cp037-L	caggggactgcaaatcctt	ANU_cp038-R	taccattaaagcagcccaag	GAUn	nv	*Panax quinquefolius*	KC354698
psbD-TrnK intergenic spacer and psbD gene	ANU_cp045-L	aaggcataagtcatcggttc	ANU_cp046-R	agcgaaataagcagcaagga	MO4f	nv	*Panax quinquefolius*	KC354695
psbC gene and psbC-tRNA-Ser intergenic spacer	ANU_cp047-L	ggcgtagctaccgagatcaa	ANU_cp048-R	tgcaaaaacagctaattggaaa	NCBM1	nv	*Panax quinquefolius*	KC354699
trnG-rps14 intergenic spacer	ANU_cp049-L	ttgccaaggagaagatacgg	ANU_cp050-R	tagtgcacctacacgccttc	NCBM1	nv	*Panax quinquefolius*	KC217531
psaA-ycf3 intergenic spacer and ycf3 gene	ANU_cp051-L	gttccggcgaacgaataat	ANU_cp052-R	gtcggatcaagctgctgag	NY4c	nv	*Panax quinquefolius*	KC217534
tRNA-Leu (trnL) intron and tRNA-Leu exon	C	cgaaatcggtagacgctacg	D	ggggatagagggacttgaac	NC1c	nv	*Panax quinquefolius*	KC217532
rpl20-rps12 intergenic spacer	rpl 20	tttgttctacgtctccgagc	5'-rps 12	gtcgaggaacatgtactagg	AR4a	nv	*Panax quinquefolius*	KC217533

na Not applicable

nv Not variable

Vita

This research is part of my Master of Science degree from Appalachian State University in Boone, NC, USA. I completed my Bachelor of Science with a major in Natural Resources Conservation and Management and a minor in Geography from the University of Kentucky in 2009. The concentration area of my undergraduate degree was Plant Conservation. During this portion of my education I took courses that emphasized economic botany and the lasting effects of climatic oscillations. I fused these two interests thereby continuing my education as represented by the present thesis. I completed my Master of Science degree in 2013 and I hope to continue working in the field of plant conservation.