Genet Resour Crop Evol (2012) 59:1553–1568
DOI 10.1007/s10722-011-9784-4

RESEARCH ARTICLE

RAPD-based assessment of genetic relationships among and within American ginseng (*Panax quinquefolius* L.) populations and their implications for a future conservation strategy

Erin M. Schlag · Marla S. McIntosh

Received: 24 May 2011 / Accepted: 12 December 2011 / Published online: 1 February 2012

Abstract American ginseng (*Panax quinquefolius*) is a native North American medicinal plant that is becoming increasingly vulnerable despite government harvest restrictions. To better understand the genetic diversity and gene flow of American ginseng, we studied RAPD variation in cultivated and wild populations. Classical and Bayesian analogues of genetic diversity statistics were estimated in seven wild and two cultivated populations. The wild populations were more highly structured ($G_{st\beta} = 0.41$) than the cultivated populations ($G_{st\beta} = 0.24$). The genetic diversity within populations ranged from $H_{e\beta} = 0.05$ to 0.38. Based on genetic pairwise distances, six of the wild populations clustered with the locally-derived cultivated population, while one wild population was more similar to the non-local cultivated population than the local populations. This wild population was highly diverse ($P = 1.0$; $U = 1.0$) suggesting that it was supplemented from exotic seed. A set of eight RAPD markers was identified that differentiated plants of local and non-local origin. As a conservation strategy, we recommend that regional gene banks be established based on molecular and geographic diversity to preserve the locally adapted germplasm. These regional gene banks would serve as a conservation tool and also provide a source of genes for genetic improvement of cultivated ginseng.

Keywords *Panax quinquefolius* · Plant conservation · Genetic diversity · Medicinal plants

E. M. Schlag · M. S. McIntosh (✉)
Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20742, USA
e-mail: mmcintos@umd.edu

E. M. Schlag
e-mail: eschlag1@swarthmore.edu

Present Address:
E. M. Schlag
Department of Biology, Swarthmore College, Swarthmore, PA 19081, USA

History

Economically important wild species are frequently endangered due to overharvest. This has certainly been the case for ginseng, a valued medicinal herb commonly used in Traditional Chinese Medicine. The survival of the most widely used ginseng species, Asian ginseng (*Panax ginseng* C. A. Mey.) and American ginseng (*Panax quinquefolius*), have been threatened by the overharvest of wild populations. As early as 1000 BC, the demand for Asian ginseng vastly exceeded the supply, creating an economic incentive to find new sources of this costly herb (Duke 1989; Persons and Davis 2005). In 1713, the Royal Society published a letter from Father Jartoux, a Jesuit missionary in China, containing a description of ginseng's botany, habitat, medicinal uses, and its exorbitant market value in China. Most noteworthy

was his observation that if ginseng is "to be found in any other Country in the World, it may be particularly in Canada, where the Forests and Mountains, according to the relation of those that have lived there, very much resemble these here." (Jartoux et al. 1713). This letter inspired Joseph Lafitau, also a Jesuit missionary, to search for ginseng in eastern Canada, where in 1716, he discovered American ginseng, a congener of Asian ginseng (Appleby 1983). American ginseng rapidly became a lucrative export and overharvest soon depleted Canadian ginseng populations. This fueled the search for additional populations, which were soon found across the eastern US (Duke 1989; Persons and Davis 2005; Pritts 1995; Taylor 2006). In response to the strong export demand and declining wild populations, growers began to cultivate ginseng in shade-covered lath houses in the 1800s (Nash 1898). Theoretically, cultivated ginseng could alleviate the harvest pressure on wild ginseng populations except that wild and cultivated ginseng have separate markets (Burkhart and Jacobson 2009) and the market value for wild roots is typically 10 times higher than for cultivated roots (Cheng and Mitchell 2009). This two-tiered price and demand structure encourages wild harvest at levels that continue to threaten the survival of wild ginseng populations.

Study species

American ginseng (*P. quinquefolius*) is a mixed-mating, perennial herb that is native to the understory of mature deciduous forests throughout eastern USA and Canada. Ginseng grows slowly in the wild and requires at least three growing seasons to produce berries. Ginseng berries ripen in early September and each berry contains 2–3 seeds that are primarily gravity dispersed and germinate within 2 m of the maternal plant (Anderson et al. 2002). Populations of wild ginseng exhibit low recruitment and establishment rates but plants are long-lived. A wild ginseng plant was recently discovered growing in Maryland that was estimated to be a century old (Moses 2007), but plants older than 10 years are uncommon in wild populations (Anderson et al. 2002), most likely due to extensive wild harvesting. Ginseng, when cultivated in fields under shade cloth, grows more rapidly and can be marketable after 4 years of growth (Persons and Davis 2005). Fast-growing cultivated ginseng roots are easily distinguished from roots that are grown in an undisturbed natural forest habitat and lack the gnarly shape, dark color, and density that are desirable characteristics of wild ginseng roots.

Declining populations

American ginseng roots are sold throughout the world in a variety of forms. Initially, ginseng was used as an herbal medicine for its multiple therapeutic benefits but has recently also become a popular dietary supplement marketed to boost energy. American ginseng has been listed in Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) since 1975 (UNEP-WCMC 2011). Species listed in Appendix II are potentially threatened with extinction from excessive harvest. The US Fish and Wildlife Service (USFWS) and Canada Environment are authorized by their governments to closely regulate the export of wild ginseng to ensure that export will not pose a risk to species survival. These governmental agencies are responsible for issuing "non-detriment findings" based on the best currently available scientific research (US Fish and Wildlife Service 2009, 2011; Environment Canada 2011; US Fish and Wildlife Service 1994, 2006). In Canada, the harvest and export of wild ginseng has been considered unsustainable and has been illegal since 1989. This decision is largely based on "the biological characteristics of the species (slow-growing, high seed mortality, low seedling establishment, mature woodland habitat), in addition to the low abundance of the species, declining population trends, as well as difficulties associated with protection and conservation" (Environment Canada 2011). Cultivated ginseng roots, however, continue to be an important Canadian export (Agriculture and Agri-Food Canada 2011). In the US, the status of wild ginseng populations is more secure than in Canada and is exported more than any other wild-harvested native plant species listed under CITES (US Fish and Wildlife Service 2009). Since 2006, in order to ensure the survival of the species, the USFWS has banned the export of wild and wild-simulated ginseng roots that are younger than 5 years of age. In addition, the nineteen US states that export wild American ginseng have implemented laws and certification programs that ban the harvest of wild ginseng before

the berries are mature and require "diggers" (harvesters of wild ginseng roots) to plant the berries to replace the harvested plants.

Despite harvest and trade restrictions, wild American ginseng populations in the US continue to decline (NatureServe 2011). The decline in status has been attributed to a combination of noncompliance and insufficient enforcement of the laws, habitat loss, and excess deer browse (Cruse-Sanders and Hamrick 2004; McGraw and Furedi 2005; Van Der Voort et al. 2003). In an effort to reduce harvest pressure on wild populations, growers and diggers are encouraged to plant wild-simulated ginseng as an economically viable harvest alternative to wild ginseng (US Fish and Wildlife Service 2009). Unlike wild ginseng, wild-simulated ginseng is intentionally seeded or transplanted into a native habitat then left to grow without further cultivation. Wild and wild-simulated roots have similar morphology phenotype, market value, and share the same export and trade regulations (US Fish and Wildlife Service 2011). State ginseng root certification programs that distinguish between wild and wild-simulated roots depend on digger information, which can be unreliable and inaccurate. The establishment of wild-simulated populations and supplementation of wild populations is alleged to be common and a recent survey of ginseng dealers suggests that government statistics collected on "wild" roots harvested are most likely a mixture of wild and wild-simulated roots (McGuffin 2009). The USFWS considers the inability to quantify the amount of wild-simulated ginseng reported as "wild" to be a major obstacle to assessing the impact of harvest on wild American ginseng (US Fish and Wildlife Service 2011). Also, if "wild" populations are supplemented or solely derived from intentional planting of non-local seed, there are concerns related to potential genetic erosion and its effect on the long-term viability of the species (Mooney and McGraw 2007). Thus, for effective genetic conservation of ginseng, there is a critical need to quantify, assess, and monitor the extent and the genetic origin of wild-simulated and supplemented wild populations.

Genetic diversity

Research on the genetic diversity within and among wild and cultivated ginseng populations has provided essential information used to develop effective policies, regulations, and recommended practices for conservation. Table 1 provides a summary of published estimates of genetic statistics estimating the genetic diversity among and within American ginseng populations (Boehm et al. 1999; Cruse-Sanders and Hamrick 2004; Grubbs and Case 2004; Lim et al. 2007; Schluter and Punja 2002). These analogous genetic statistics were based on the analysis of allozyme or RAPD markers from studies conducted on numerous wild and cultivated populations found throughout different geographic regions in the US and Canada. In general, the total genetic variation of American ginseng was high at the species level and across both wild and cultivated populations. Studies comparing wild and cultivated populations have concluded that these different types of ginseng populations have remained genetically distinct (Boehm et al. 1999; Grubbs and Case 2004; Schluter and Punja 2002). The proportion of total genetic variation among wild populations was found to be higher than among cultivated populations, but wild populations that have been protected from harvest were less highly structured than unprotected populations. The G_{st} levels of protected wild populations were characteristic of mixed-mating species (Boehm et al. 1999; Schluter and Punja 2002), while the G_{st} levels of unprotected wild populations were higher and characteristic of inbreeding populations. Unprotected populations have been declining in size and population size has been found to be significantly correlated with allozyme diversity (Cruse-Sanders and Hamrick 2004; Grubbs and Case 2004). Thus, high levels of genetic structure have been attributed to limited gene exchange and extensive episodes of genetic drift associated with small population size (Grubbs and Case 2004). Geographic diversity has also been found to be associated with genetic differences between wild populations. Based on RAPD marker frequencies, wild populations from geographically different regions have been found to be genetically distinct (Boehm et al. 1999; Lim et al. 2007).

Cultivated populations have also been found to be genetically diverse, but in contrast to wild populations, cultivated populations are less structured and harbor proportionately less genetic diversity among populations and more among populations (Bai et al. 1997; Boehm et al. 1999; Grubbs and Case 2004; Schluter and Punja 2002). However, the cultivated populations studied reflect the eclectic nature of cultivated

Table 1 Summary of genetic diversity estimates from published studies of wild and cultivated American ginseng (*P. quinquefolius*) populations

References	*N*	Type	Geographic area	Marker	GD within populations		GD among populations		GD within species	
					SM	H_e	*SM*	G_{st}	*SM*	H_e
Boehm et al. (1999)	14	Wild, protected, unprotected	US: PA, TN, WI	RAPD	0.17[b]	–	–	–	0.24	–
	9	Cultivated	US: WI, KY, MO, NC; CA: BC, PEI, NVSC	RAPD	0.15[b]	–	–	–	0.15	–
Schluter and Punja (2002)	3	Wild, protected	CA: QU	RAPD	0.21[b]	–	–	0.28	0.27	–
	8	Cultivated	US: WI; CA: BC, ON, QU, NVSC	RAPD	0.30[b]	–	–	0.18	0.31	–
Lim et al. (2007)	15	Wild, unprotected	US: NY, KY, NC, PA, TN, VA, WI	RAPD	0.26	–	0.27[c]	–	–	–
Cruse-Sanders and Hamrick (2004)	21	Wild, protected, unprotected	US: GA, NC, WV, MD	Allozyme	–	0.07*	–	–	–	0.16
	8	Wild, protected	US: NC	Allozyme	–	0.08*	–	0.17	–	–
	13	Wild, unprotected	US: GA, NC, WV, MD	Allozyme	–	0.07*	–	0.49	–	–
Grubbs and Case (2004)	32	Wild, protected, unprotected	Eastern US, CA	Allozyme	–	0.11	–	0.63	–	0.47
	12	Cultivated	Eastern US	Allozyme	–	0.20	–	0.24	–	0.31
Schlag and McIntosh (This paper)	7	Wild, unprotected	MD	RAPD	0.20	0.19	–	0.41	0.33	0.33
	2	Cultivated	MD, TN/WI[a]	RAPD	0.32	0.31	–	0.24	0.37	0.36

N number of populations, *SM* mean pair-wise simple-matching genetic distance, H_e mean expected heterozygosity based on all markers (denoted by *) or polymorphic markers only

[a] Grown in MD from TN and WI seed sources

[b] Averaged across regions

[c] NY versus Non-NY

populations, which should be recognized for valid comparisons between studies. On one end of the spectrum, cultivated populations are unrelated assemblages of germplasm from multiple sources and geographic regions; thereby facilitating gene flow across geographic regions. At the other end, cultivated populations are grown as landraces from seed of selected plants from local wild populations or their progeny selected by the grower from their production fields.

Molecular markers

Molecular markers have become an essential tool for the studying genetic diversity. Molecular and statistical techniques have been rapidly evolving to advance genetic research and choosing the most suitable technique from this wide array can be complicated and controversial. What constitutes the optimum molecular marker depends on a variety of factors, including the specific research objectives as well as practical considerations (Sunnucks 2000; van Tienderen et al. 2002; Nybom 2004; Bonin et al. 2007). The earliest molecular markers, allozymes, RAPDs, and AFLPs have the advantages of ease, speed, and low cost as compared to the newer, more sophisticated molecular marker techniques based on microsatellites that offer greater sensitivity, resolution, and genome coverage (Agarwal et al. 2008). Microsatellite-based markers are often preferred because they are codominant, hypervariable, highly reproducible between laboratories, and produce data suited to classical genetic statistics. However, microsatellite markers are locus-specific, which is a drawback for studying plant species that lack extensive genetic research. For

American ginseng, the disadvantages of the time and cost associated with the development of micro-satellites markers can outweigh their advantages. Furthermore, a comprehensive analysis of published studies using RAPDs, ISSR's, AFLPs, and microsatellite markers in plants found that estimates of intraspecific genetic diversity were similar, regardless of marker type, and could be compared across studies (Nybom 2004).

For our study, a marker technique was sought that would be sufficiently informative to clarify the genetic relationships among the study populations and had the potential to become a practical means for genetic fingerprinting of ginseng plants. Due to limitations on time, expense, and laboratory equipment and because American ginseng does not have locus-specific microsatellite markers, RAPD primers were deemed the most suitable genetic markers for our research objectives.

Both allozyme and RAPD markers have been used to estimate genetic diversity and gene frequencies in American ginseng populations. Allozyme markers have the advantage of being co-dominant but RAPDs have the advantages of being DNA-based and more polymorphic. Although early research utilizing RAPD markers was widely criticized for its poor reproducibility, subsequent studies have demonstrated that RAPD banding patterns are reliable and reproducible for intraspecific genetic diversity studies when careful laboratory practices and sample replication are implemented (Boehm et al. 1999; Skroch and Nienhuis 1995). Another early criticism of RAPD and other dominant markers is that classical statistical methods were developed for co-dominant markers and require knowledge of inbreeding and Hardy–Weinberg equilibrium. To overcome this statistical insufficiency, Bayesian approaches have been developed to estimate genetic diversity parameters that are appropriate for dominant markers (Holsinger et al. 2002). Because the properties and robustness of the different diversity measures remain controversial, Bonin et al. (2007) have suggested that genetic diversity based on dominant markers should be estimated using both classical and Bayesian statistical and compared to ensure consistent results.

Study objectives

In our study, genetic distances and genetic diversity within and among seven wild local populations, a cultivated local population, and a cultivated non-local population from Maryland were estimated using RAPD markers. The main objectives of the study were to: (1) assess and compare the genetic diversities among and within wild and cultivated populations; (2) infer the geographic origin, whether local or non-local, of plants based on their genetic distances; (3) evaluate the potential for using RAPD fingerprinting as a tool for ginseng conservation; (4) use findings to propose a conservation strategy for supplementation of wild ginseng populations.

Materials and methods

Study populations

A total of 197 plants from seven wild and two cultivated American ginseng populations growing in western Maryland were investigated. The seven wild populations (P1–P7) were located in forests typical of American ginseng's natural habitat. One of the cultivated populations (MD) was assembled of plants representative of a cultivated landrace population derived and selected from seeds of local wild plants. The other cultivated population (TN/WI) was grown from seed purchased from Tennessee and Wisconsin commercial sources, representing a heterogeneous assemblage of seed of exotic origin. Descriptions of the study populations including estimated population size, plant sample size, collection location, and source of origin are given in Table 2. Plant samples from the wild populations located in two counties in western Maryland, Garrett County (GC) and Washington County (WC), were obtained from local collectors (Table 2; Fig. 1). Distances between the GC populations ranged from 6 to 40 km. The WC populations were closer, ranging from <0.1 to 2.5 km apart. The average distance between the GC and the WC populations was 136 km. Plant samples from cultivated fields in Maryland were obtained from Maryland growers and grouped into populations based on grower information on their seed source.

DNA extraction and RAPD PCR

RAPD analysis was conducted on leaf tissue samples for the majority of plants. Root tissue was analyzed for 18 cultivated plants, twelve MD and six TN/WI plants,

Table 2 Description of American ginseng (*P. quinquefolius*) samples from populations in Maryland

Population	Type	Site (county)	Seed source	*n*	*N*
MD	Cultivated	A, F, G, W	Local	50	–
TN/WI[a]	Cultivated	A, W	Non-local	17	–
P1	Wild	G	Local	21	30
P2	Wild	G	Local	16	20
P3	Wild	G	Local	20	30
P4	Wild	G	Local	21	30
P5	Wild	W	Local	23	60
P6	Wild	W	Local	10	20
P7	Wild	W	Local	19	30
Total				197	

n sample size, *N* estimated population size, *A* Allegany Co., *F* Fredrick Co., *G* Garrett Co., *W* Washington Co

[a] Tennessee (TN) and Wisconsin (WI) commercial seed

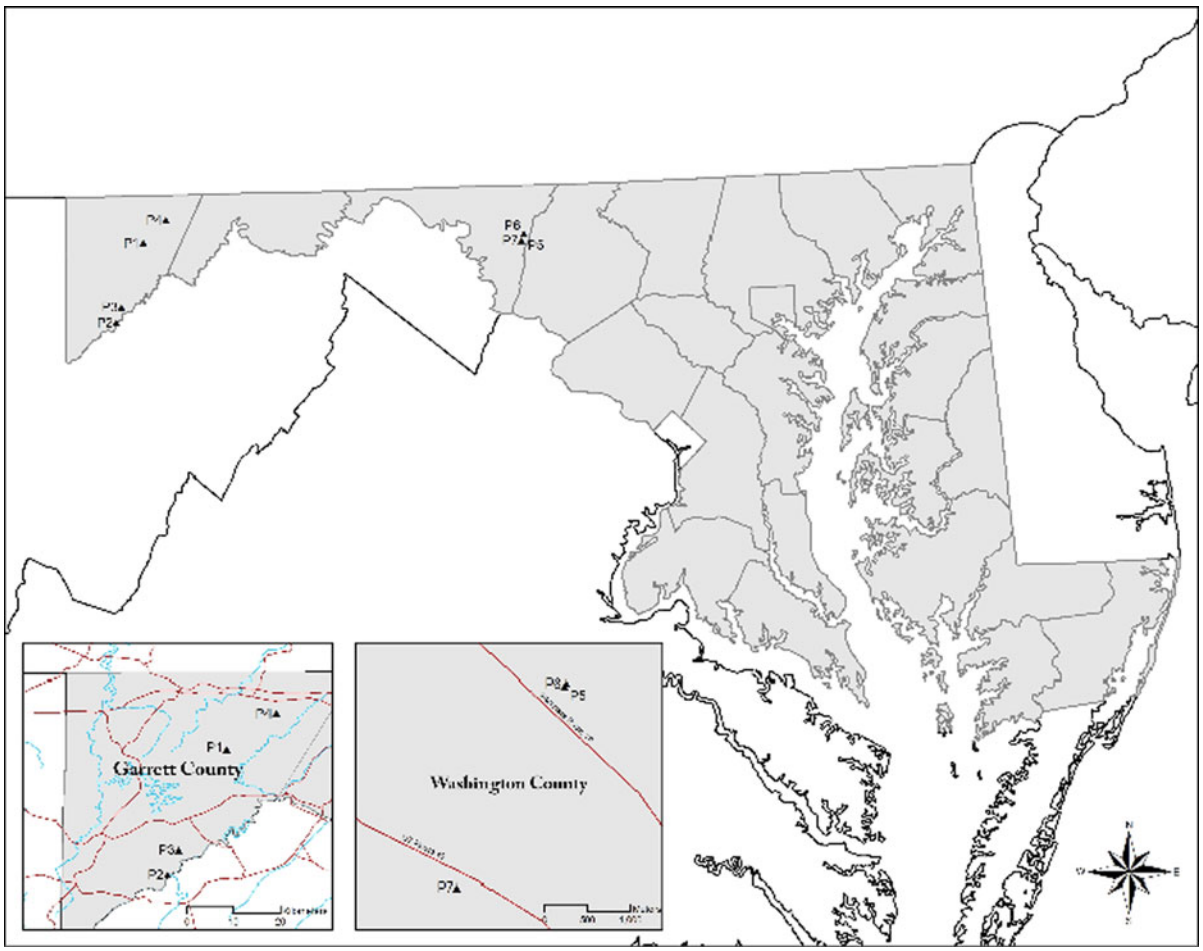

Fig. 1 Locations of wild American ginseng (*Panax quinquefolius*) populations sampled in Maryland

where only roots had been collected. The RAPD profiles of the root samples were consistent with the leaf samples from the same population.

Leaves were freeze-dried for 24 h and immediately ground to a fine powder in a 1.5-ml microcentrifuge tube with a micropestle. Roots were freeze-dried for

72 h and ground in a Wiley Mill (20 mesh) (Foss Tecator AB, Höganäs). DNA was isolated from approximately 20 mg of leaf or root powder following the standard DNeasy® Plant Mini Kit protocol (Qiagen, Valencia). DNA was eluted with 150 µl DNeasy AE buffer twice and the DNA concentration was estimated spectrophotometrically (A260). DNA concentrations did not differ greatly and an aliquot of all samples was diluted 1:4 (5–10 ng μl^{-1}) for use in RAPD PCR. Samples that did not amplify well were diluted until amplification improved.

A total of 180 decamer primers were initially screened for polymorphisms on a subset of eight leaf samples representative of the wild and cultivated populations sampled. Primers were obtained either from Qiagen (Valencia) (based on the sequences provided by Operon Technologies, Alameda) or from the Nucleic Acid Protein Service Unit at the University of British Columbia (Vancouver). Twenty decamers that produced highly reproducible polymorphic markers were selected for RAPD analysis of all 197 samples. Polymorphic markers were scored twice for twenty representative samples and percent error (number of mismatches/total number of marker comparisons × 100) was calculated to estimate marker reproducibility in this study. Less than 5% scoring error was calculated for the twenty replicated samples, indicating that at least 95% of the RAPD markers scored were reproducible. Polymorphic markers were named according to decamer code and the size of the amplified fragment.

Mixtures for RAPD PCR (25 µl) reactions contained 20 ng DNA, 10 mM KCl, 10 mM $(NH_4)SO_4$, 20 mM Tris–HCl, 3 mM $MgSO_4$, 0.1% Triton X-100, 0.1 mM dNTPs, 35 ng of a single decamer and 1 unit taq polymerase (NEB, Beverly). Amplification was performed on a GeneAmp® PCR system (Perkin-Elmer, Boston) or an Eppendorf Mastercycler Gradient (Brinkmann Instruments, Inc., Westbury) DNA thermocycler for 45 cycles according to the procedure of Williams et al. (1990). Approximately 15 µl of the reaction was loaded onto a 2% agarose gel containing ethidium bromide (0.5 µg ml^{-1}) and PCR fragments were separated by electrophoresis (2 h, 120 V). RAPD fragments were illuminated under UV light and images were captured with an Eagle Eye II instrument using EagleSight software (Stratagene, La Jolla).

Genetic statistics

Polymorphic RAPD bands were assumed to represent neutral, independent genetic loci for genetic analyses and were scored as present (1) or absent (0) for each plant ($n = 197$) and used to calculate the percent polymorphic markers using a 95% criterion (P) and the percent of plants displaying unique multilocus RAPD fingerprints (U). Plant samples missing data for >10% of markers were excluded from statistical analyses.

Genetic diversity was estimated and partitioned between and among populations using three different approaches in order to facilitate comparisons with previous studies and to verify the validity of estimates regardless of requisite assumptions. The statistics were: (1) mean pair-wise simple-matching genetic distance (SM), (2) Bayesian analogs of diversity statistics and (3) Shannon's Diversity Index. Correlation between diversity estimates was tested as a measure of reliability and robustness.

We estimated genetic variation within and among populations using SM to facilitate direct comparisons with previous studies. Genetic distance was calculated as the complement to the simple-matching similarity coefficient (Sneath and Sokal 1973) using PROC Distance, method = match (SAS V. 9.1.2). The multilocus RAPD fingerprint was treated as a haplotype and a distance index was calculated based on marker sharing among individuals. Monomorphic markers (99% criterion) were excluded from analyses to allow for valid comparisons with genetic diversity estimates from previous ginseng studies.

Because RAPD markers are dominant and the level of inbreeding in the ginseng populations was unknown, a Bayesian approach that incorporates uncertainty about inbreeding levels within the sampled populations was also used to estimate genetic diversity (Holsinger 1999; Holsinger et al. 2002). This approach was implemented in the program Hickory V1.0 (Holsinger and Lewis 2003) with the default parameters as described by Holsinger et al. (2002). The full, $f = 0$, and free Bayesian models provided good fits compared to the $\theta = 0$ model. We report estimates based on the full model, which is considered the most robust (Holsinger and Lewis 2003).

Bayesian analogs of Nei's expected heterozygosity ($H_{e\beta}$) were used to estimate diversity within wild and

cultivated populations (Nei 1973). Bayesian analogs of Weir and Cockerham's θ (θ_β), (Wier and Cockerham 1984); and Nei's G_{st} ($G_{st\beta}$) (Nei 1973) provided estimates of diversity among wild and cultivated populations. Credible intervals (CI), analogous to confidence intervals but based on the posterior distributions of Bayesian estimates provided a measure of reliability (Holsinger 1999).

Shannon's Diversity Index was also used to partition RAPD diversity. Although it is a measure of phenotypic diversity, it does not require knowledge of heterozygosity and is equivalent to genetic diversity estimates if heterozygotes are infrequent. Shannon's Diversity Index was calculated for each for each marker in each population as $H'_j = -\sum p_i \log_2 p_i$, where p_i was the relative frequency of the presence or absence of a RAPD marker in that population. Markers that were monomorphic within a population but polymorphic within the species were included in the analysis as zeroes (Bussell 1999). Shannon's Diversity Index within populations was standardized by dividing by 2 (diversity ≤ 0.5) to facilitate comparison with heterozygosity estimates. The average genetic diversity across populations was calculated as $H'_{pop} = 1/n\sum H'_j$, where n is the number of populations. The total genetic diversity of the populations analyzed was calculated as $H'_{sp} = -\sum p_i \log p_i$, where p_i was the frequency of the presence or the absence of a RAPD band in all samples pooled across populations. The genetic variation among populations was calculated as $G_{st} = 1 - (H'_{pop}/H'_{sp})$. Overall estimates of diversity were calculated from the average per-marker values of H'_j, H'_{pop}, H'_{sp} and G_{st}.

Nonmetric multidimensional scaling (NMDS) was used to visualize relationships among individuals and populations in two dimensions. Pairwise genetic distances between individuals ($n = 197$) and mean pairwise genetic distances between populations ($n = 9$) were reduced to two dimensions using PROC NMDS (SAS v.9.1) and plotted.

Results

RAPD profile

A total of 180 primers were screened and twenty primers that produced highly reproducible polymorphic markers were selected for RAPD analyses. These primers amplified 38 polymorphic bands (66% of total markers) and yielded from one to four high intensity and highly reproducible polymorphic markers. Total marker frequencies over all ginseng populations ranged from 2% to 100%. All markers were present in at least one ginseng population but not in all populations. These 38 RAPD markers differentiated between populations based on differences in their presence and frequency within different populations. For example, out of 38 total markers, 20 markers were present and two were polymorphic in P4; whereas, all 38 markers were present and polymorphic in P7.

RAPD markers proved to effectively discriminate between populations and plants of local versus non-local origin. A small core subset of eight markers was identified that could be used to consistently differentiate populations (Table 3). These markers were

Table 3 Relative frequencies of a set of RAPD loci differing between local and non-local American ginseng (*P. quinquefolius*) populations

Locus	Wild								Cultivated		
	P1	P2	P3	P4	P5	P6	P7	Total	MD	TN/WI	Total
n	21	16	20	21	23	10	19	130	50	17	67
$A07_{100}$	0.00	0.00	0.05	0.00	0.00	0.00	0.11	0.02	0.02	0.59	0.16
$A12_{485}$	0.00	0.00	0.00	0.00	0.26	0.00	0.32	0.09	0.04	0.76	0.22
$F20_{750}$	0.00	0.00	0.00	0.00	0.13	0.00	0.50	0.09	0.10	0.71	0.25
$F20_{925}$	0.00	0.00	0.00	0.00	0.09	0.00	0.61	0.10	0.04	0.65	0.19
$U01_{600}$	0.00	0.00	0.00	0.00	0.00	0.00	0.47	0.07	0.00	0.65	0.17
$Z04_{1800}$	0.00	0.00	0.00	0.00	0.13	0.00	0.74	0.13	0.12	0.82	0.30
226_{700}	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.02	0.02	0.35	0.10
227_{1300}	0.00	0.00	0.00	0.00	0.00	0.00	0.39	0.05	0.00	0.59	0.15

frequent in the non-local cultivated TN/WI population and the wild P7 population but rare or absent in the local cultivated MD population and the wild P1–P4 and P6 populations. Four of these markers were found in the P5 population but at frequencies lower than the P7 or TN/WI populations.

Genetic diversity

Genetic diversity was measured as percent polymorphic markers (P_{95}) and percent of unique multilocus RAPD fingerprints (U) within a population (Table 4). Percent polymorphism was high across both the wild (90%) and cultivated (97%) populations. The total percent of plants with unique RAPD fingerprints was very high (99%) across the cultivated populations but only moderate (58%) across the wild populations. These statistics were markedly higher than allozyme-based estimates (Grubbs and Case 2004) and suggest that RAPD markers are more polymorphic and informative than allozyme markers. In their allozyme study, Grubbs and Case (2004) attributed the differences between cultivated and wild populations to different allele frequencies rather than uniqueness, whereas Schluter and Punja (2002) using RAPDs found a single marker that was polymorphic and present only in wild populations.

Within the wild populations, the percent polymorphism and percent of plants with unique fingerprints was highly variable and differed by population and county of origin but was independent of population size. The wild GC populations were less polymorphic and had fewer plants with a unique fingerprint compared to the wild WC populations (Table 4). Within the P4 population, only 5% of the loci were polymorphic and only 14% of the plants had unique fingerprints. The other GC populations had relatively low percent polymorphism and low to moderate percent unique fingerprints. These results indicate that P4 has a high level of gene fixation and contains plants are that are mostly genetically identical. Thus, P4 is at high risk of extinction and the other GC populations are also genetically vulnerable. In contrast, each of the WC populations had high percentages of polymorphic loci and over 95% of plants in each population had unique fingerprints, suggesting that the WC populations are less vulnerable.

Both cultivated populations had high percentages of polymorphic loci and unique fingerprints. As expected, the TN/WI population was highly diverse

Table 4 Estimates of genetic diversity within American ginseng (*P. quinquefolius*) populations

Population	n	P_{95}	U	H'_j	SM	$H_{e\beta}$
Cultivated						
MD	50	74	98	0.28 (0.17)	0.27 (0.11)	0.27 (0.25–0.30)
TN/WI	17	90	100	0.36 (0.16)	0.37 (0.11)	0.35 (0.33–0.38)
Mean	34	82	99	0.32	0.32	0.31 (0.29–0.33)
Wild						
P1	21	32	33	0.07 (0.11)	0.06 (0.06)	0.08 (0.06–0.13)
P2	16	29	63	0.11 (0.18)	0.10 (0.07)	0.12 (0.10–0.15)
P3	20	55	75	0.20 (0.20)	0.21 (0.09)	0.19 (0.17–0.21)
P4	21	5	14	0.02 (0.07)	0.03 (0.04)	0.05 (0.03–0.08)
P5	23	82	96	0.32 (0.17)	0.32 (0.11)	0.30 (0.28–0.33)
P6	10	61	100	0.25 (0.22)	0.26 (0.08)	0.24 (0.21–0.27)
P7	19	100	100	0.41 (0.10)	0.42 (0.17)	0.38 (0.36–0.40)
Mean	19	52	69	0.20	0.20	0.19 (0.18–0.21)
Total						
Wild	130	90	58	0.35 (0.15)	0.33 (0.15)	0.33 (0.32–0.34)
Cultivated	67	97	99	0.39 (0.10)	0.37 (0.16)	0.36 (0.35–0.38)
Mean	99	94	76	0.37	0.35	0.35 (0.34–0.36)

n number of plants sampled, P_{95} percent polymorphism (95% criterion), U percent of plants with unique RAPD fingerprints, H'_j mean Shannon's diversity index standardized for a maximum diversity of 0.5 (± SD), SM mean pair-wise simple-matching genetic distance (±SD), $H_{e\beta}$ Bayesian analog of expected heterozygosity (95% credible interval)

and 90% of the loci were polymorphic and 100% of the plants had unique fingerprints. Within the MD population, the percent polymorphism was 74%, which was lower that the TN/WI population and within the same range as the WC wild populations. As found in the WC wild populations and the TN/WI cultivated population, nearly 100% of the MD plants had unique fingerprints. Notably, despite one or more generations of phenotypic selection by the grower, these results show that the MD population maintained levels of genetic diversity similar or higher than local wild populations and no indication of gene fixation.

Genetic diversity statistics

The within population genetic diversity was estimated based on three analogous statistics: (1) average simple matching genetic distances (SM); mean Shannon's Diversity Index (H'_j); and Bayesian analog of Nei's expected heterozygosity ($H_{e\beta}$). These statistics differ in approach and requisite assumptions but their estimates were highly correlated ($r > 0.99$), which provides evidence as to the robust nature of these RAPD-based estimates. For simplicity, our discussion will focus on the Bayesian estimates, because they do not rely on statistical priors and also provide 95% credible intervals for statistical comparisons.

The total genetic diversity across wild populations ($H_{e\beta} = 0.33$) was high (Table 4). The mean within-population diversity of the wild populations was also high ($H_{e\beta} = 0.19$) but differed significantly (95% credible intervals) between populations. The genetic diversity within the GC populations (P1–P4) was low, especially for P1 and P4 ($H_{e\beta} = 0.05$ and 0.08, respectively). The genetic diversity within the WC populations (P5–P7) was relatively high compared to the other unprotected wild populations. Based on pairwise comparisons ($\alpha = 0.05$), the genetic diversity within each WC population was higher than within each GC wild population. Most notably, the genetic diversity within the P7 population was unusually high and similar to the species-level diversity estimated using allozyme markers (Tables 1, 4) (Grubbs and Case 2004).

The populations in our study were small, but in contrast to findings of Grubbs and Case (2004), the substantial differences among the within-population genetic diversities were not associated with population size (Tables 1, 4). The wild populations in our study were comprised of only 20–60 plants, but the average within-population diversity of wild Maryland populations was similar to or higher than the average within-population diversity estimates in previous studies (Boehm et al. 1999; Cruse-Sanders and Hamrick 2004; Grubbs and Case 2004; Schluter and Punja 2002) (Table 1). However, the average within population diversity differed between Maryland counties and was considerably higher within WC populations ($H_{e\beta} = 0.31$) than within the GC populations ($H_{e\beta} = 0.18$). Thus, assessing genetic diversity of individual populations is important.

Similar to previous studies, the total genetic diversity across the cultivated populations ($H_{e\beta}$ [*total cultivated*] = 0.36) was higher than the total genetic diversity across the wild populations. In our study, the genetic diversity within the more geographically limited MD cultivated population was high ($H_{e\beta} = 0.27$) but less than the WI/TN cultivated population ($H_{e\beta} = 0.35$).

Genetic structure

Genetic variation among wild populations ($G_{st\beta}$) accounted for 41% of the total wild genetic variation (Table 5). This was higher than the average for a mixed-mating dicot, such as ginseng, ($G_{st} = 0.24$,

Table 5 Estimates of genetic structure among wild American ginseng (*P. quinquefolius*) populations

Populations	*N*	G_{st}	$G_{st\beta}$	θ_β
Wild (P1–7)	7	0.45 (0.12)	0.41 (0.36–0.44)	0.46 (0.39–0.52)
Garrett Co. (P1–4)	4	0.52 (0.27)	0.52 (0.45–0.57)	0.56 (0.46–0.65)
Washington Co. (P5–7)	3	0.20 (0.16)	0.11 (0.08–0.15)	0.16 (0.11–0.22)
Cultivated	2	0.16 (0.18)	0.24 (0.22–0.28)	0.31 (0.23–0.39)
Wild versus cultivated	2	0.03 (0.08)	0.04 (0.03–0.05)	0.07 (0.05–0.11)

N number of populations, G_{st} partitioning of Shannon's diversity index (SD), $G_{st\beta}$ Bayesian analog of Nei's G_{st} (95% credible interval), θ_β Bayesian analog of Weir and Cockerham's θ (95% credible interval)

0.19) and more comparable to a self-pollinating species where $G_{st} = 0.45$, 0.59 for allozyme and RAPD markers, respectively (Hamrick and Godt 1996; Nybom and Bartish 2000). The full Bayesian model (DIC = 737) was strongly preferred to the $\theta = 0$ model (DIC = 2,627) further indicating the differentiation among populations. The GC populations were more highly structured than the WC populations. The genetic variation between wild populations accounted for 52% of the total genetic diversity for GC populations compared to 11% of the total genetic diversity for the WC populations. As expected, the level of genetic structure for the cultivated populations was less than for the wild populations. The genetic variation between the two cultivated populations accounted for 24% of the total genetic variation. Although the genetic differentiation between the wild and the cultivated populations was very low and accounted for only 4% of the overall total genetic diversity, this comparison is based on distances averaged over populations and does not reflect the significant differences among populations within each population type.

Genetic distance

Genetic relationships among wild and cultivated populations and individual plants are displayed in two-dimensions following NMDS of pairwise distances (Fig. 2). Figure 2a displays the genetic

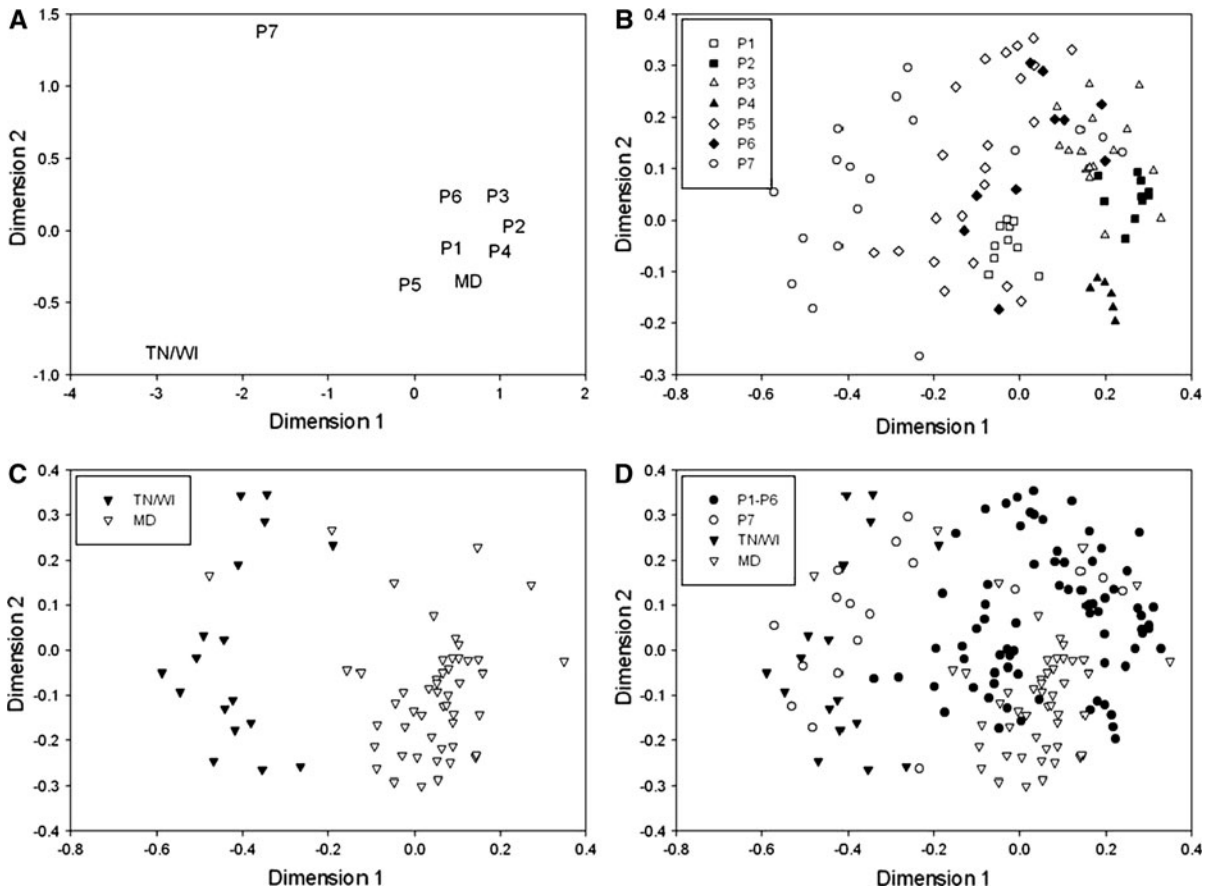

Fig. 2 Nonmetric multidimensional scaling showing genetic patterns among and within wild (P1–P7), local cultivated (MD) and non-local cultivated (TN/WI) populations of American ginseng. Simple-matching distances were calculated based on the RAPD fingerprint (38 loci) of each plant. **a** Distribution of cultivated and wild populations. **b** Distribution of plants within wild populations (P1–P7). **c** Distribution of plants within local (MD) and non-local (TN/WI) cultivated populations. **d** Distribution of plants ($n = 197$) within wild and cultivated populations. Individual plants may be hidden if RAPD fingerprints are not unique

relationships among the studied populations. The wild populations P1–6 and the MD cultivated population were clearly separated from the P7 wild and the TN/WI cultivated populations. The P1 and TN/WI populations were also distant from each other. The plot of the individual wild plants (Fig. 2b) shows that plants collected in GC (P1–P4) formed a cluster that was separated in Dimension 1 from plants growing in WC. The plants from GC were separated into populations in Dimension 2. Plants from the WC P7 population were the most distant from the GC plants. Plants from P5 and P6 were intermediate between P7 and P1–P4 in Dimension 1 and spatially diffuse in Dimension 2. The individual plants from the cultivated populations (Fig. 2c) formed two clusters separated in Dimension 1 with minimal overlap between populations. The MD plants were more tightly grouped than the TN/WI plants indicating a greater genetic relatedness in this group. The genetic relationships among the wild and cultivated populations visualized in Fig. 2a are further explained on an individual plant level in Fig. 2d. Of particular interest is that most plants in the P7 and TN/WI plants are not only distinct from plants in the other populations but also show extensive overlap in both dimensions (Fig. 2d). Within the P7 population, only 20% of the plants grouped with wild populations P1–P6 and 80% grouped with the TN/WI population. As expected from Fig. 2a, most of the MD cultivated plants were central on Dimension 1 among plants from P1 to P6 although approximately 50% were at the lower end of Dimension 2. The cultivated MD population was similar to all but one wild population and clearly distinguishable from the TN/WI cultivated population (Fig. 2), which supports the assumption that the MD cultivated population is a local landrace.

Discussion

Genetic diversity of ginseng in North America

Understanding of the genetic diversity of American ginseng is complicated due to the confluence of divergent natural and artificial forces that shape the structure and integrity of wild populations. Beginning with the discovery and subsequent commercialization of American ginseng, the fate of wild ginseng populations and its surrounding human populations have been intertwined. While ginseng has influenced the culture, heritage, and economy of eastern rural American populations, especially in the Appalachian region, the human actions of harvesting, supplementation, and cultivation have impacted the survival and genetic diversity of ginseng populations (Persons and Davis 2005; Pritts 1995). However, the extent that humans have impacted the genetic diversity and gene flow of American ginseng remains unresolved. Studies of genetic markers frequencies in wild and cultivated populations of American ginseng have contributed to the understanding the impacts of human activity and associated threats to the survival American ginseng. But the very fact that genetic diversity estimates vary within and between studies (Table 1) illustrates the complex nature of American ginseng's gene flow.

Our study of ginseng populations growing in Maryland, similar to other studies, found that genetic diversity was high across both wild and cultivated ginseng populations but maintained differently. The wild populations appeared to be more strongly influenced by mating strategy and harvest pressure than the cultivated populations. The wild populations exhibited high levels of structure and a lack of diversity within some, but not all, of the wild populations. This is probably caused by harvest pressure resulting in small population sizes and population isolation. For the cultivated populations, our results were also consistent with other studies, where the genetic diversity within cultivated populations was equal to or larger than between the populations. The diversity within the locally-derived MD cultivated population plants was less than the diversity within the non-local TN/WI population grown from different commercial sources. This suggests that the genetic diversity between and within cultivated populations was largely dependent on human decisions related to the seed source and suggest that the average diversity of cultivated populations between studies should only be compared with caution.

Previous studies have provided estimates of genetic diversity of wild populations but have not considered the probability that wild populations may contain wild-simulated plants grown from local or exotic seed. Our analysis of RAPD markers provides the first DNA-based evidence that a purportedly wild population of American ginseng had been supplemented with exotic seed. Based on genetic distances of plants collected from seven wild American ginseng populations with no known history of planting or harvesting

activity, we found that one of the wild populations (P7) was more genetically similar to the non-local cultivated ginseng population than to any of the other local populations. We conclude that P7 contained a significant proportion of exotic supplemented plants because: (1) the within population RAPD variation was higher than typical for a mixed-mating species (Hamrick and Godt 1996; Nybom and Bartish 2000) and far exceeded the range previously reported for wild ginseng populations (Table 1); (2) genetic distances between plants within the population were discontinuous. Eighty percent of the plants grouped with the TN/WI population while 20% grouped with the local MD plants; (3) the P7 population contained eight markers that were displayed at high frequency in the TN/WI population but were largely absent from the other studied populations.

In an effort to reduce harvest pressures on existing wild ginseng populations without reducing their genetic integrity and fitness, the USFWS advocates supplementing wild populations and planting wild-simulated populations but opposes supplementing and/or restoring native wild populations "that are refugia for the species" with commercially grown or non-local seeds and propagules. Supplementation and restoration of wild ginseng populations is encouraged because most wild populations are below the minimum viable population size and vulnerable to the negative effects of genetic drift and inbreeding depression. Conversely, supplementation with commercial or non-local sources is discouraged due to the potential associated risks of genetic pollution, outbreeding depression, and/or maladaptation to the local environment. Wild ginseng populations have been found to experience low gene flow among populations and high rates of self-pollination within populations (Cruse-Sanders and Hamrick 2004; Grubbs and Case 2004), which are characteristics of species most prone to outbreeding depression from the introduction of exotic germplasm (Allendorf et al. 2001; Ellstrand and Elam 1993; Parker 1992). Although results of a short-term study of outcrossing on population fitness (Mooney and McGraw 2007) indicated that outbreeding depression may be less of a problem than inbreeding depression, long-term research is needed before extrapolating from these results. Since ginseng is an economically important medicinal herb, there is additional concern that supplementation will disrupt gene linkages or introduce new genes associated with the expression of secondary metabolic traits. The bioactivity of ginseng is attributed to ginsenosides and differences in ginsenoside composition have been found to be associated with the region of origin (Schlag and McIntosh 2006). Thus, introducing non-local genes could alter the phytochemical as well as the genetic integrity of local populations.

The conservation guidelines, policies, and regulations for American ginseng are based on the best available biological and trade information on the status of the species, which may be leading to false conclusions about harvest pressure and stability of wild populations due to the inability to quantify the amount of wild-simulated ginseng reported to be "wild". Since the distinction between wild and wild-simulated roots relies on the sellers claim, ginseng buyers generally believe that a significant portion of roots being sold as wild are actually wild-simulated (Persons and Davis 2005). Also, without a reliable way to distinguish between plants of local and non-local origin, USFWS cannot investigate the risks of the wild-simulated planting of nonlocal commercially produced seeds. Our findings demonstrate that RAPD fingerprints could be used as a practical and reliable tool for investigating the extent and impacts of planting non-local seed in native habitats. For example, using a core subset of eight RAPD markers, we identified plants within a "wild" population that we deduced to be wild-simulated from a non-local source. However, we caution that genetic markers cannot be used to identify or track the extent of wild-simulated plants grown from local seed. In fact, although wild and cultivated roots can be separated based on root phenotype, our study found that a locally-derived cultivated population was genetically similar to the local wild populations.

RAPD markers

As discussed previously, there is a significant need to develop a molecular marker system to address the many unresolved questions related to the genetic history, status, and trends for American ginseng. For major crop plants, the many advantages of microsatellites make them the preferred marker for genetic studies (Agarwal et al. 2008; Kalia et al. 2011). However, a PCR-derived marker, such as RAPDs that uses non-specific primers, is most suitable for most wild plant species, such as American ginseng, due to

the lack of locus-specific genetic sequence information (Nybom and Bartish 2000). Thus, RAPD markers were utilized because they are relatively inexpensive, have modest equipment requirements, and are reliable when good scientific practices are rigorously applied (Skroch and Nienhuis 1995). Also, RAPD markers have been previously used for genetic studies of American ginseng and are known to be relatively polymorphic.

Based on the results of our study, we believe that a RAPD marker system could be used extensively as a practical and reliable genetic tool for the strategic genetic conservation of American ginseng. The estimates of genetic diversity based on dominant RAPD markers were confirmed to be robust since estimates of genetic diversity using three different statistical approaches were highly correlated ($r = 0.99$). Our study also adds to the mounting evidence that RAPD-based estimates of population diversity and structure within a species can be compared conservatively across studies (Nybom and Bartish 2000; Nybom 2004). One advantage of Bayesian analogs over the other diversity indices is that they allow calculation of a 95% credible interval that is similar to a confidence interval and can be used for statistical comparisons between populations. The narrow range of the 95% credible intervals for the estimated diversities within populations (Table 4) and among populations (Table 5) indicates a high level of confidence in the RAPD-based estimates.

Results of our study indicate that RAPDs could also be used as a rapid and economical method for genetic fingerprinting of ginseng. This would be particularly useful for determining the genetic identity and origin of roots for export and trade certification and identifying wild populations that are actually wild-simulated and supplemented with seed from non-local plants. RAPDs are well-suited for fingerprinting ginseng because they are minimally destructive and can be conducted using small samples of leaf tissue of a growing plant or of harvested roots at the point of certification, export, or sale. As in our study, a small core set of RAPD markers could be used to differentiate between local and non-local plants.

Regional seed banks

Previous studies have addressed issues related to protecting and regulating the harvest of wild ginseng to best conserve the integrity and genetic diversity of ginseng. Here, we propose a framework for supplementing and establishing ginseng populations for population conservation. Our intent is to stimulate discussion, debate, development, and ultimately implementation of a system of regional gene banks. These gene banks would contain catalogued collections of accessions to provide support for research, genomic conservation, and genetic improvement of American ginseng. For each regional bank, a genetically diverse plant and seed collection representing native and locally-adapted genotypes would be assembled and maintained. Seed and plants in each bank would capture the regional genetic diversity and also become a source of breeding stock for regionally certified seed. Regionally certified seed would be produced to facilitate supplementation and restoration of native ginseng populations with seed known to be genetically similar to native populations and adapted to a specific geographical growing region. We also recommend the establishment of a bi-national (US and Canada) committee to advise, coordinate, and advocate for a system of regional gene banks. This committee would include representatives of major stakeholder groups.

To assemble each gene bank, seed and leaf tissue would be collected from wild ginseng populations throughout its geographical range. A core differential set of RAPD markers could be used to screen plants for geographical origin, level of polymorphism, and percent unique loci that would be used to select accessions to form a regional core seed collection. The core collections would be intended to maximize diversity and minimize redundancy of accessions. Because wild ginseng populations tend to be highly structured, this should be accomplished by maximizing the number of populations in the collection. The regional core collections could be grown either *in situ* or *ex situ* but should be maintained within the region of collection.

Ideally, a large reserve seed collection should also be established for long-term storage of genetic diversity. Alternatively, cultivated production fields already provide an extensive, easily obtainable, and diverse genetic reserve. Ginseng, cultivated since the mid 1800s, remains virtually undomesticated. Cultivated populations are highly diverse and offer a broad foundation for genetic improvement through traditional plant breeding strategies (Boehm et al. 1999;

Schluter and Punja 2002; Bai et al. 1997; Grubbs and Case 2004). The genetic composition of cultivated ginseng populations varies by grower. Common among older established fields, cultivated populations were established from local wild populations and newer fields are planted from seed of plants selected from these fields. In our study, the MD population represented this landrace population type. Most of the MD plants shared a genetic fingerprint common among local wild populations even though this population was derived from plants that were selected over generations by the grower. Our results suggest that cultivated populations could be identified as locally-derived based on DNA analysis and serve as *in situ* gene banks and repositories for locally co-adapted gene complexes.

Acknowledgments The authors thank R. Trumbule for making this study possible by recruiting the support of Maryland ginseng growers and collectors, locating ginseng populations, and collecting the plants analyzed for this study. We thank L. Harding for generously providing plants and knowledge of ginseng. T. Keen is acknowledged for creating the map of the ginseng populations. This work was supported by the Maryland Department of Agriculture.

References

Agarwal M, Shrivastava N, Padh H (2008) Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Rep 27(4):617–631

Agriculture and Agri-Food Canada (2011) Canada's North American ginseng. http://www.marquecanadabrand.agr.gc.ca/tools-utils/5270-eng.htm

Allendorf FW, Leary RF, Spruell P, Wenburg JK (2001) The problems with hybrids: setting conservation guidelines. Trends Ecol Evol 16(11):613–622

Anderson RC, Anderson MR, Houseman G (2002) Wild American ginseng. Nativ Plants J 3(2):93–97, 100–105

Appleby JH (1983) Ginseng and the royal society. Notes Rec R Soc Lond 37(2):121–145

Bai D, Brandle J, Reeleder R (1997) Genetic diversity in north American ginseng (*Panax quinquefolius* L.) grown in Ontario detected by RAPD analysis. Genome 40:111–115

Boehm CL, Harrison HC, Jung G, Nienhuis J (1999) Organization of American and Asian ginseng germplasm using randomly amplified polymorphic DNA (RAPD) markers. J Am Soc Hortic Sci 124(3):252–256

Bonin A, Ehrich D, Manel S (2007) Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. Mol Ecol 16(18):3737–3758. doi:10.1111/j.1365-294X.2007.03435.x

Burkhart EP, Jacobson MG (2009) Transitioning from wild collection to forest cultivation of indigenous medicinal forest plants in eastern North America is constrained by lack of profitability. Agrofor Syst 76(2):437–453. doi:10.1007/s10457-008-9173-y

Bussell JD (1999) The distribution of random amplified polymorphic DNA (RAPD) diversity amongst populations of *Isotoma petraea* (lobeliaceae). Mol Ecol 8(5):775

Cheng L, Mitchell P (2009) Status of the Wisconsin ginseng industry, p 44. http://www.aae.wisc.edu/pubs/misc/docs/Mitchell.WI.Ginseng.Industry.2009.pdf. Accessed 23 Nov 2011

Cruse-Sanders JM, Hamrick JL (2004) Genetic diversity in harvested and protected populations of wild American ginseng, *Panax quinquefolius* L. (Araliaceae). Am J Bot 91(4):540–548. doi:10.3732/ajb.91.4.540

Duke JA (1989) Ginseng: a concise handbook. Reference Publications, Inc., Algonac

Ellstrand NC, Elam DR (1993) Population genetic consequences of small population size: implication for plant conservation. Ann Rev Ecol Syst 24:217–242

Environment Canada (2011) Non-detriment findings—American ginseng for Canada. http://www.ec.gc.ca/cites/default.asp?lang=En&n=8A26C423-1. Accessed 27 Sept 2011

Grubbs HJ, Case MA (2004) Allozyme variation in American ginseng (*Panax quinquefolius* L.): variation, breeding system, and implications for current conservation practice. Conserv Genet 5:13–23

Hamrick JL, Godt MJW (1996) Effects of life history traits on genetic diversity in plant species. Philos Trans R Soc Lond B 351:1291–1298

Holsinger KE (1999) Analysis of genetic diversity in geographically structured populations: a Bayesian perspective. Hereditas 130:245–255

Holsinger KE, Lewis PO (2003) Hickory: a package for analysis of population genetic data, v1.0. http://darwin.eeb.uconn.edu/hickory/hickory.html

Holsinger KE, Lewis PO, Dey DK (2002) A Bayesian approach to inferring population structure from dominant markers. Mol Ecol 11(7):1157–1164. doi:10.1046/j.1365-294X.2002.01512.x

Jartoux F (1713) The description of a tartarian plant, call'd ginseng; with an account of its virtues. In a letter from father Jartoux, to the procurator general of the missions of India and China. Taken from the tenth volume of letters of the missionary jesuits, printed at Paris in octavo, 1713. Philosophical Transactions (1683–1775), vol 28. The Royal Society of London, Paris

Kalia RK, Rai MK, Kalia S, Singh R, Dhawan AK (2011) Microsatellite markers: an overview of the recent progress in plants. Euphytica 177(3):309–334. doi:10.1007/s10681-010-0286-9

Lim W, Mudge KW, Weston LA (2007) Utilization of RAPD markers to assess genetic diversity of wild populations of north American ginseng (*Panax quinquefolium*). Planta Med 73(1):71–76

McGraw JB, Furedi MA (2005) Deer browsing and population viability of a forest understory plant. Science 307(5711):920–922

McGuffin M (2009) The "wild" American ginseng industry: collectors and cultivators. www.fws.gov/international/DMA_DSA/CITES/plants/ginseng.html. Accessed 9, 2011

Mooney EH, McGraw JB (2007) Effects of self-pollination and outcrossing with cultivated plants in small natural

populations of American ginseng, *Panax quinquefolius* (Araliaceae). Am J Bot 94(10):1677–1687
Moses S (2007) Kitzmiller man digs up enormous ginseng root. Cumberland Times-News, September 9, 2007
Nash GV (1898) American ginseng: commercial history, protection, and cultivation. Revised By Maurice G. Kains edn. Government Printing Office, Washington
NatureServe (2011) Natureserve explorer: an online encyclopedia of life [web application]. Version 7.1. NatureServe. http://www.natureserve.org/explorer. Accessed 11 Jan 2011
Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321–3323
Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecol 13(5):1143–1155
Nybom H, Bartish IV (2000) Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspect Plant Ecol Evol Syst 3(2):93–114
Parker MA (1992) Outbreeding depression in a selfing annual. Evolution 46(3):837–841
Persons WS, Davis JM (2005) Ginseng, goldenseal and other woodland medicinals. Bright Mountain Books, Inc., Fairview
Pritts KD (1995) Ginseng: how to find, grow, and use America's forest gold. Stackpole Books, Mechanicsburg
Schlag EM, McIntosh MS (2006) Ginsenoside content and variation among and within American ginseng (*Panax quinquefolius* L.) populations. Phytochemistry 67(14): 1510–1519
Schluter C, Punja ZK (2002) Genetic diversity among natural and cultivated field populations and seed lots of American ginseng (*Panax quinquefolius* L.) in Canada. Int J Plant Sci 163(3):427–439
Skroch P, Nienhuis J (1995) Impact of scoring error and reproducibility of RAPD data on RAPD based estimates of genetic-distance. Theor Appl Genet 91(6–7):1086–1091
Sneath PHA, Sokal RR (1973) Numerical taxonomy. W. H. Freeman & Co., San Francisco
Sunnucks P (2000) Efficient genetic markers for population biology. Trends Ecol Evol 15(5):199–203
Taylor DA (2006) Ginseng, the divine root. Algonquin Books, Chapel Hill
UNEP-WCMC (2011) UNEP-WCMC species database: CITES-listed species. On the world wide web. http://www.cites.org/eng/resources/species.html. Accessed 25 Sept 2011
US Fish and Wildlife Service (1994) Proposed rule for the export of American ginseng harvested in 1994–1996 seasons. Fed Regist 59(185):49046–49052
US Fish and Wildlife Service (2006) Non-detriment finding on CITES export permit applications for wild-simulated American ginseng 2006–2008 (trans: Department of the Interior FWS). Washington, D.C.
US Fish and Wildlife Service (2009) General advice for the export of wild and wild-simulated American ginseng (*Panax quinquefolius*) harvested in 2009 and 2010 from states with approved cites export programs. (trans: US-FWS). Scientific findings, Washington, D.C.
US Fish and Wildlife Service (2011) Advice for the export of roots of wild and wild-simulated American ginseng (*Panax quinquefolius*) lawfully harvested during the 2011 harvest season in 19 states. Washington, D.C.
Van Der Voort ME, Bailey B, Samuel DE (2003) Recovery of populations of goldenseal (*Hydrastis canadensis* L.) and American ginseng (*Panax quinquefolius* L.) following harvest. Am Midl Nat 149(2):282–292
van Tienderen PH, de Haan AA, van der Linden CG, Vosman B (2002) Biodiversity assessment using markers for ecologically important traits. Trends Ecol Evol 17(12):577–582
Wier BS, Cockerham CC (1984) Estimating *f*-statistics for the analysis of population structure. Evolution 38:1358–1370
Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535