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Genetic Diversity of Wild and Cultivated Populations of American Ginseng (*Panax Quinquefolium*) from Eastern North America Analyzed by RAPD Markers

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ABSTRACT : The objective of this study was to assess genetic diversity among 6 different wild ginseng populations from New York, Kentucky, North Carolina, Pennsylvania, Tennessee and Virginia, and to compare these wild populations to one cultivated population. RAPD markers were used to estimate the genetic difference among samples from the 7 populations. The 64 random primers were screened, and 15 primers were selected which exhibited the 124 highly reproducible polymorphic markers. The ratio of discordant bands to total bands scored was used to estimate the genetic distance within and among populations. Multidimensional scaling (MDS) of the relation matrix showed distinctive separation between wild and cultivated populations. The MDS result was confirmed using pooled chi-square tests for fragment homogeneity. This study suggests that RAPD markers can be used as population-specific markers for American ginseng.

Key words : RAPD, wild ginseng, Panax quinquefolium, genetic diversity, population, American Ginseng

INTRODUCTION

Asian ginseng (*Panax ginseng* L.) has been used as traditional medicine in China and Korea for thousands of years (Proctor *et al.*, 1996; Ren & Chen, 1999). For over 200 years, the roots of American ginseng, *Panax quinquefolium* L., have been exported to east Asia to be used as a complement to *Panax ginseng* in traditional Asian medicinal practice (Court *et al.*, 1996a; Court *et al.*, 1996b; Sokhansanj *et al.*, 1999). Since most of the more than 60 metric tons of wild ginseng that is legally harvested in the U.S. is exported to Asian countries, *Panax quinquefolium* has been listed in Appendix II of the Convention on International Trade in Endangered Species (CITES) since 1973. Only reproductively mature plants may be harvested. Seeds from collected plants must be planted at the collection site.

There is an ongoing debate about the long-term sustainability of current harvest levels and conservation practices regulations between "shang hunters" (collectors of wild ginseng for profit) and other conservationists (Harris, 1999). These two groups are by no means mutually exclusive. Many shang hunters have replanted cultivated seeds in addition to seeds from harvested plants to offset the effects of harvesting wild plants (Grubbs & Case, 2004; Harris, 1999; Persons, 1994).

In contrast to the views of the proponents of reseeding "wild" ginseng, some in the professional conservation research have expressed reservations about the introduction of exotic germplasm (Gabel, 2002). Grubbs & Case (2004) found that genetic diversity within wild populations is lower than within cultivated populations and that genetic diversity among wild populations is greater than among cultivated populations with allozyme analysis. They suggested that the practice might disrupt fitness-conferring, co-adapted gene complexes, consequently reduce fitness and ultimately threaten the long-term survival of wild populations. Another recent population genetic study based on allozyme analysis exhibited that within-population genetic diversity was greater for protected populations than for unprotected ones, due to inbreeding of the latter related to lower population size associated with harvest (Cruse-Sanders *et al.* 2004). Conservation recommendations suggested by the authors included prohibiting harvest of certain wild populations containing the greatest genetic diversity.

In addition to studies based on allozyme analysis, several recent studies using molecular markers have confirmed confirm that there is genetic variation between wild and cultivated populations, and among wild populations. Boehm *et al.* (1999), using RAPD markers, reported that there were genetic differences among wild populations from Tennessee, Pennsylvania, and Wisconsin, but that the wild Pennsylvania population resembled a cultivated Wisconsin population, suggesting that "genetic pollution" of the Pennsylvania "wild" ginseng has occurred as a result of the shang hunter mediated reseeding of cultivated (Wisconsin) seeds. Shulter & Punja (2002),

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also working with RAPD markers, reported that wild populations from Quebec differed among Quebec wild populations and differed from cultivated populations from the same region. Bai *et al.* (1997) used RAPD markers to demonstrate that there were genetic differences among cultivated populations from Ontario. Population-specific markers for Asian ginseng (*Panax ginseng*) have been reported by several authors (Kim *et al.*, 2003; Lim *et al.*, 1993; Um *et al.*, 2001).

Molecular markers have also been used to estimate genetic variation among *Panax species* for taxonomic purposes and for the commerce-related authentication of *Panax species* to verify "truth in labeling". Amplified fragment length polymorphism (AFLP), directed amplification of minisatellite region of DNA, and randomly amplified polymorphic DNA (RAPD) were capable of differentiating P. *ginseng* from P. *quinquefolius* (Ha *et al.*, 2001; Ha *et al.*, 2002; Ngan *et al.*, 1999; Um *et al.*, 2001; Wang *et al.*, 2001). Differentiation between *Panax ginseng* and *Panax quinquefoliumhas* also have been demonstrated with RAPD markers (Shaw & But, 1995) and DNA sequences (Ha *et al.*, 2001; Ha *et al.*, 2002; Ngan *et al.*, 1999; Wang *et al.*, 2001).

The pharmacological effects of ginseng are thought to be mediated by a group of triterpene saponins known as ginsenosides. Differential effects of specific ginsenosides on mammalian and human physiology have been documented (Inhee *et al.*, 2001; Rudakewich *et al.*, 2001; Toda *et al.*, 2001) and show promise in the treatment of cancer (Murphy, 2000) and diabetes (Attele *et al.*, 2002). Ginsenoside content has been used to characterize wild populations of *Panax quinquefolium* (Mudge *et al.*, 2000; Mudge *et al.*, 2004; Smith *et al.*, 1996). However, the characterization of wild populations of *Panax quinquefolium* with using both molecular markers and ginsenoside analysis has not previously been attempted.

Because of the relatively short history of domestication, *Panax quinquefolium* has no distinct cultivars or selections and cultivated material consists of unimproved land races (Boehm *et al.*, 1999; Proctor & Bailey, 1987). Hence, information about the genetic diversity of American ginseng derived from molecular markers and other population genetics experimental approaches could be useful for genetic improvement of cultivated ginseng through breeding and/or clonal selection (Grubbs & Case, 2004; Boehm *et al.*, 1999).

RAPD analysis has several benefits compared with other molecular marker-based technologies such as AFLP, SSR, and RFLP. It is cost effective and relatively simple. Also, it needs only small amounts of DNA (Karp *et al.*, 1997). The objectives of this experiments were to identify RAPD markers for population differentiation among *Panax quinquefolium* populations and use them to assess genetic diversity among wild ginseng populations from the Catskill region of New York State as well as populations from other states.

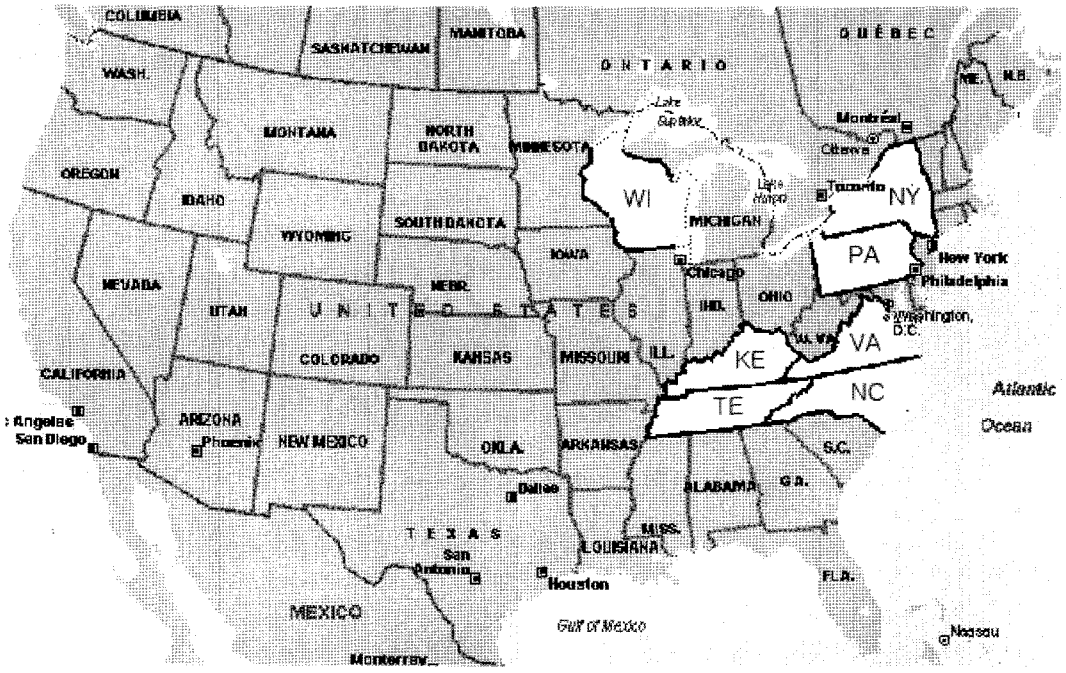

Fig. 1. A map showing 7 states in U.S.A where *Panax quinquefolium* was collected. KE = Kentucky, NC = North Carolina, PA = Pennsylvania, TE = Tennessee, VA = Virginia, WI = Wisconsin, NY = New York.

Table 1. County of origin of wild and cultivated populations of American ginseng (*Panax quinquefolium*) and number of root samples for DNA extraction and RAPD analysis. NY5 = New York populations, KY = Kentucky, NC = North Carolina , PA = Pennsylvania, TN = Tennessee , WI = Wisconsin (cultivated), VA = Virginia

Population	NY County or State	No. of samples
NY5	New York	11
KY	Kentucky	10
NC	North Carolina	9
PA	Pennsylvania	10
TN	Tennessee	10
VA	Virginia	11
WI	Wisconsin cultivated	10

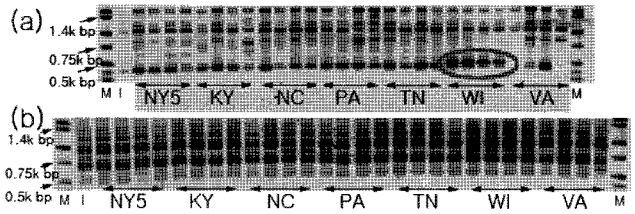

Fig. 2. (a) RAPD profiles from six wild and one cultivated populations of American ginseng amplified by primer OPD05, (b) RAPD profiles amplified by primer OPD03. M = DNA marker, I = Internal Standard, NY5 = New York populations, KY = Kentucky, NC = North Carolina, PA = Pennsylvania, TN = Tennessee, WI = Wisconsin (cultivated), VA = Virginia.

MATERIALS AND METHODS

Sample Collection

Ginseng plants were collected from 6 reproductively isolated wild populations from 6 states (Table 1, Fig. 2) including New York, Kentucky, Tennessee, North Carolina, Pennsylvania, and Virginia, and one cultivated population from Wisconsin (Table 1, Fig. 1). Table 1 indicates the number of individual plant samples from each population used for DNA analysis. To maintain genotypes, collected plants were transplanted to each of two different forest gardens, including Cornell University's Arnot Teaching and Research Forest, near Van Etten, New York. Whole plant samples for transplant from the wild consisted of a rhizome with apical bud and the attached storage root, after removal of the above ground shoot. Samples for DNA analysis were collected during August 2002 from the Arnot Forest site. For DNA extraction from each sampled plant, one entire palmately compound leaf was removed from shoot, put immediately into plastic Whirl Pak bags (NASCO-Modesto, Modesto, California) and placed in an insulated cooler with dry ice. Within several hours the samples were transferred to an ultra-low freezer at −70℃.

DNA isolation

The procedure for extraction of genomic DNA was modified from the published CTAB method (Bernatzky & Tanksley, 1986). A leaf sample of 100 ㎎ was weighed and ground in liquid nitrogen. The ground tissue was incubated at 65 ℃ for 30-60 min in 700 ul CTAB (1.8 ㎖, hexacethltrimethylammonium bromide) 2×extraction buffer (100 ㎖ 1 M Tris, pH 8.0; 280 ㎖ 5 M NaCl; 40 ㎖ 0.5 M EDTA; 580 ㎖ double distilled H_20; 20 g CTAB) with 2 ul beta-mercaptoethanol. DNA was extracted again if the UV absorption ratio, A260/280, was greater or less than the range 1.7-1.9.

The remaing precedures were well explained in Bernatzky & Tanksley (1986).

Primer Selection

A total of 64 primers were screened for polymorphic bands with a combined sample of extracted DNA bulked from all of the populations. Selection of some of these primers was based on successful results reported in previously published studies involving American ginseng, while others were selected randomly. Twenty two primers were selected based on the result by Bai *et al.* (1997), Boehm *et al.* (1999) and Schulter & Punja (2002). Two sets of 20 random primers, Kit-N and Kit-AD, were purchased from Operon Technologies (Alameda, Calif.). Two Primers were selected randomly. After screening these 64 random decamer primers, 15 were selected based on repeatability and maximum polymorphism. The 15 primers selected for further screening were AD01, AD02, AD11, AD15, N2, N19, OPD03, OPD05, OPO15, OPU10, OPU15, UBC81, UBC98, UBC164, and UBC203.

Conditions for DNA Amplification by PCR and Electrophoresis

A Taq PCR Core Kit from Quiagen (Valencia, CA) was used for the amplification of DNA by the polymerase chain reaction. The PCR reaction mixture (20 μL) was mixed by manufacture's recommendation. DNA amplification was performed in an Eppendorf thermo cycler (Master Cycler, Westbury, NY). The initial cycle was 2 min at 94 ℃, 10 min at 35 ℃, and 2 min at 72 ℃. Subsequent cycles were 45 s at 94 ℃, 45 s at 35 ℃ and 2 min at 72 ℃, followed by 10 min at 72 ℃ for the last cycle (Bai *et al.*, 1997). Approximately 17 uL of amplified DNA was loaded onto a 1.5% agarose gel in a TBE buffer (89 mM Tris Base, 89 mM Boric Acid, 2 mM EDTA) at 150 V for 2 hours. Amplification products were stained with ethidium bromide for visualization on a UV transilluminator

(Alpha Innotech Corporation, Alpha Imager TM 2200, San Leandro, California).

Statistical analysis

Stained gels were scored for the presence (1) or absence (0) of co-migrating polymorphic bands ranging from 0.3 to 2 kb. A standard DNA ladder was used to measure the molecular weight of co-migrating bands.

Simple matching coefficients were determined from a table of all samples indicating the presence or absence of individual bands. GD (genetic distance) was defined as 1 -simple matching coefficient. The resulting 111×111 distance matrix was fitted in two dimensions using monotonic multidimensional scaling (MDS) with the SAS System for Windows v.8 (Nienhuis *et al.*, 1995). Genetic structure was calculated by PopGene32.

All pairwise comparisons were calculated for differences in individual marker frequencies. The χ^2 analysis was used for pair wise comparisons. For χ^2 calculations the genetic differences were tested by comparing fragment frequencies on a band-by-band basis. The mean fragment frequency was used, under the null hypothesis, as the expected fragment frequency for χ^2 analysis. Non-polymorphic bands were excluded (Tivang *et al.*, 1996). All such observations were grouped into a single class with one degree of freedom (Snedecor & Cochran, 1967).

Dendrograms were created with UPGMA (unweighted pair group method with arithmetic average) cluster analysis and graphically displayed with the SAS System for Windows v.8 (Schluter & Punja, 2002).

Results and Discussion

The 15 primers selected from the original 64 primers produced a total of 124 highly reproducible polymorphic bands. Selected primers produced an average of 8.3 (ranging from 5 to 12) polymorphic bands (Table 2).

Fig. 2 shows examples of typical gels from DNA amplified from all 15 different populations for 2 of the 15 selected primers. Primer OPD05 (Fig. 2(a)) shows a polymorphism unique to 4 samples from cultivated Wisconsin population. Other 6 samples of cultivated Wisconsin population had this unique band. Fig. 2(b) shows a gel from DNA amplified using primer OPD03, where a polymorphic band is evident.

The genetic distance (GD) presented in Table 3 is a measure of the genetic dissimilarity among individual plants within a population or among populations on a scale of 0.0 to 1.0, with the minimum value of 0.0 indicating maximum genetic similarity (minimum diversity) and the maximum value of 1.0 indicating no similarity (maximum diversity). The GD for all 71 *Panax quinquefolium* samples analyzed in this study was 0.256. This is very similar to the GD value of 0.24 that Boehm (1999) reported for all wild American ginseng samples (combined from populations in Pennsylvania, Tennessee, and Wisconsin), and similar to the combined value of 0.27 for 3 wild populations from Quebec reported by Schulter & Punja (2002). Hence, each of these independent estimates of the level of genetic diversity among 3 different sets of wild popu-

Table 2. Number of polymorphic bands generated by selected primers.

Primer	Number of bands
AD01	10
AD02	8
AD11	12
AD15	5
N19	7
N2	6
OPD03	7
OPD05	7
OPO15	8
OPU10	6
OPU15	5
UBC164	12
UBC203	12
UBC81	12
UBC98	7
Average	8.3

Table 3. The mean genetic distance (GD) values based on analysis of RAPF bands and the mean genetic distance values within 7 populations. NY5 = New York, KY = Kentucky, NC = North Carolina, PA = Pennsylvania, TN = Tennessee, WI = Wisconsin (cultivated), VA = Virginia

Population	GD within population
All	0.256
NY5	0.224
KY	0.165
NC	0.172
PA	0.158
TN	0.151
VA	0.176
WI	0.238

lations as determined by Boehm (1999), Schulter (2002), and our own results (Table 3) are very similar. Boehm (1999) reported that the combined GD of all cultivated populations (0.15) was lower than the combined GD of three wild populations (0.24) whereas Schulter & Punja (2002) reported the opposite trend-the combined GD for 4 cultivated populations (British Columbia, Ontario, Nova Scotia, and Wisconsin) was slightly higher (0.31) than the combined GD for 3 wild Quebec populations (0.27).

From the results of our study, Wisconsin cultivated population and had highest GD (Table 3) compared to wild populations. This result is consistent with Grubbs & Case (2004). Usually, ginseng growers in America buy so called "elite" population and plant mixed seed lots. New York state population, NY5 had second highest GD. The NY5 population was collected from Catskill region in central New York state. The seven individual plants of NY5 were differed from other populations in MDS plot (Fig. 3) and cluster analysis (Fig. 4) and four of them were overlapped with other populations. The Catskill region was reputed for high quality wild ginseng (Lim *et al.*, 2005). Also, ginseng collectors replanted the seeds of cultivated populations after collecting wild ginseng to recover population size. It might cause some plant in NY5 to be overlapped with other populations.

We compared the level of genetic diversity between Wisconsin cultivated population and 6 other wild populations with χ^2 analysis. The p value of comparison between Wisconsin cultivated and North Carolina population is 0.048. The genetic diversity between Wisconsin cultivated and North Carolina population (GD = 0.269) is greater than the within-population GD values for each of the 7 individual wild populations, which range from 0.148 (Pennsylvania population) to 0.238 (Wisconsin cultivated population). This is similar to the range of 0.11-0.31 reported by Schulter & Punja (2002) and 0.14 to 0.24 reported by Boehm *et al.* (1999). This apparent difference between Wisconsin cultivated and North Carolina population is supported by geographical distance between two collection states (Fig. 1), which indicated relatively large distance between two collection states. This difference is also apparent from the tendency of individuals from Wisconsin to cluster separately from North Carolina in the MDS plot shown in Fig 3. Also, we found significant difference with χ^2 analysis between New York population and North Carolina population.

Although both χ^2 analysis and MDS plots indicate differences between Wisconsin cultivated and North Carolina population and between New York and North Carolina population, χ^2 analysis of all possible pair wise comparisons were not statistically significant. This is also apparent from the overlap of individuals and close to each other in the MDS plot shown in

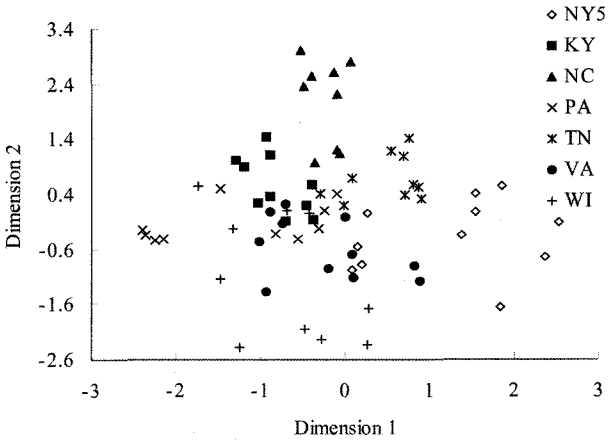

Fig. 3. Multidimensional scaling plot of the genetic distance values for individual Panax quinquefolium plants. NY5 = New York populations, KY = Kentucky, NC = North Carolina, PA = Pennsylvania, TN = Tennessee, WI = Wisconsin (cultivated), VA = Virginia.

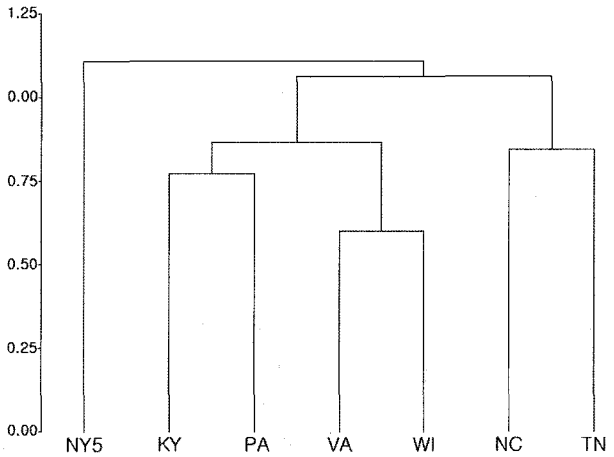

Fig. 4. Cluster analysis of 7 populations from New York (NY5), Kentucky (KY), Pennsylvania (PA), Virginia (VA), North Carolina (NC), Tennessee (TN), Wisconsin (WI, cultivated). X axis is average distance between clusters.

Fig 3. Similarly, Schulter & Punja (2002) could not distinguish among 3 wild populations from Quebec which, like the adjacent states involved in this study, were from a limited geographic region. On the other hand, Boehm *et al* (1999) reported significant differences among 3 wild populations from a considerably wider geographic region (Wisconsin, Tennessee, and Pennsylvania). The lack of significant differences except two compurisons between Wisconsin and North Curolina and between New York and North Curolina in this study could be an artifact of insufficient sample size (9 to 11 individuals per population, Table 1), but this seems unlikely since Schulter & Punja (2002) reported that within-population genetic diversity was comparable based on analysis to either

Table 4. Partitioning of genetic diversity generated by 15 RAPD primers into within and among population components for 6 natural populations and 1 cultivated Wisconsin population of *Panax quinquelolium*

Primer	Hpop	Hsp	Hpop/Hsp	1-Hpop/Hsp
AD01	0.249	0.445	0.560	0.440
AD02	0.199	0.372	0.535	0.465
AD11	0.166	0.352	0.472	0.528
AD15	0.169	0.284	0.595	0.405
N19	0.157	0.376	0.418	0.582
N2	0.338	0.416	0.813	0.188
OPD03	0.196	0.251	0.781	0.219
OPD05	0.168	0.292	0.575	0.425
OPO15	0.156	0.358	0.436	0.564
OPU10	0.162	0.36	0.450	0.550
OPU15	0.19	0.428	0.444	0.556
UBC164	0.161	0.271	0.594	0.406
UBC203	0.182	0.342	0.532	0.468

10 or 20 individual plants per population.

The genetic structure was 0.472 (Table 4). Cruse-Sanders & Hamrick (2004) reported that genetic structure is 0.493. Both genetic structure is close to 0.5. The diversities among and between populations are similar. It implies that distinct genetic differences could develop not only within populations, but also between populatios.

Discussion

Collection pressure on wild populations of American ginseng due to the high price paid for wild-collected roots, and loss or fragmentation of forest habit, may affect the prospects for long-term survival of this species in the wild (Carpenter & Cottam, 1982; Charron & Gagnon, 1991; Lewis, 1984; Lewis & Zenger, 1982; McGraw, 2001). The genetic structure of wild populations based on allozyme analysis by Cruse-Sanders & Hamrick (2004) have shown that genetic diversity, as reflected by expected heterozygosity, was greater in protected populations (e.g. Great Smoky Mountain National Park) than in unprotected, routinely collected populations, suggesting that harvesting from the wild may have significant evolutionary implications for the species. Similar findings based on RAPD marker analysis by Boehm *et al.* (1999), of significant genetic differences among widely geographically separated wild populations, suggest that consideration should be given to modifying conservation strategies to include protection of the genetic integrity of distinct local populations. On the other hand, the comparison of 3 wild populations from a more geographically limited area (Quebec, Canada) by Schulter & Punja failed to detect significant interpopulation differences. The results of our study could be interpreted as consistent with both of these previous RAPD-based evaluations since population of adjacent states were not significantly different among themselves, but were significantly different from widely separated (multi-state). Hence our results, as well as those from the other molecular marker-based studies, suggest that the most appropriate conservation strategy might be protection of the genetic integrity by states wide levels. This might involve policies or regulations that discourage introducing exotic germplasm into a given region by banning or limiting replanting of extra-regional cultivated seed among wild intra-regional populations. On the other hand, our results are not inconsistent with genetic similarity among regional populations due to natural selection of a genetically uniform limited subset of genotypes well adapted to local conditions from an originally more genetically diverse population of exotic seed. These two different scenarios suggest different conservation management strategies and further research will be necessary to determine which is most appropriate for the long-term conservation of the species.

In addition to the conservation-related implications of this study, our results, along with other investigations into genetic diversity among wild American ginseng populations (Bai *et al.* 1997; Boehm, *et al.* 1999; Schulter & Punja 2002), suggest that sufficient genetic diversity exists, both within and among wild populations, to be useful for genetic improvement of cultivated ginseng through breeding or clonal selection.

LITERATURE CITED

Attele AS, ZhouYP, Xie JT ,Wu JA, Zhang L, Dey L , Pugh W, Rue PA, Polonsky KS, Yuan CS (2002) Antidiabetic effects of *Panax ginseng* berry extract and the identification of an effective component. DIABETES 51:1851-1858.

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.

Bernatzky R, Tanksley SD (1986) Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887-898.

Boehm CL, Harrison HC, Jung G, Nienhuis J (1999) Organization of american and asian ginseng germplasm using randomly amplified polymorphic DNA (RAPD) markers. J. Amer. Soc. Hort. Sci. 124:252-256.

Carpenter SG, Cottam G (1982) Growth and reproduction of American ginseng (*Panax quinquefolius*) in Wisconsin, U.S.A. Can. J. Bot. 60:2692-2696.

Charron D, Gagnon D (1991) The demography of northern populations of *Panax Quinquefolium* (American Ginseng). J. Ecol. 79:431- 445.

Court WA, Hendel JG, Elmi J (1996a) Reversed-phase high-performance liquid chromatographic determination of ginsenosides of *Panax quinquefolium*. J. Chromatogr. A. 755:11-17.

Court, WA, LB Reynolds, and Hendel JG (1996b) Influence of root age on the concentration of ginsenosides of American ginseng (*Panax quinquefolium*). Can. J. Plant. Sci. 76:853-855.

Cruse-Sanders JM, Hamrick JL (2004) Spatial and genetic structure within populations of wild American ginseng (*Panax quinquefolius* L., Araliaceae). Journal of Heredity 95: 309-321.

Gabel R (2002) Permit applications for ginseng harvested in 2002, US fish and wildlife service, international affairs. http://international.fws.gov/animals/ginfin02.html.

Grubbs HJ, Case MA (2004) Allozyme variation in American ginseng (*Panax quinquefolius* L.):Variation, breeding system, and implications for current conservation practice. Conserv. Genet. 5:13-14.

Ha WY, Yau FC, But PPH, Wang J, Shaw PC (2001) Direct amplification of length polymorphism analysis differentiates *Panax ginseng* from *P. quinquefolius*. Planta Med. 67:587-589.

Ha WY, Shaw PC, Liu J, Yau FC, Wang J (2002) Authentication of *Panax ginseng* and *Panax quinquefolius* using amplified fragment length polymorphism (AFLP) and directed amplification of minisatellite region DNA (DAMD). J. Agri. Food Chem. 50:1871-1875.

Harris S (1999) Empire state ginseng growers association. http://www.catskillginseng.com/SB.html.

Inhee MJ, Hong HS, Boo JH, Lee KH, Yun SH, Cheong MY, Joo I, Huh K, Jung MW (2001) Ginsenoside Rb1 and Rg1 improve spatial learning and increase hippocampal synaptophysin level in mice. J. Neurosci. Res. 63:509-515.

Karp A, Edwards K, Bruford M, Funk S, Vosman B, Morgante M, Seberg O, Kremer A, Boursot P (1997) Molecular technologies for biodiversity evaluation: opportunitties and challenges. Nat. Biotech. 15:625-628.

Kim JH, Yuk JA, Cha SK, Kim HH, Seong BJ, Kim SI, Choi JE (2003) Genetic variation in pure lines of *Panax ginseng* based on by RAPD analysis. Korean J. Medicinal Crop. Sci. 11:102-108.

Lewis WH (1984) Population structure and environmental corollaries of *Panax quinquefolium* (Araliaceae) in Delaware count, New York. Rhodosa 86:431-437.

Lewis WH, Zenger VE (1982) Population dynamics of the American ginseng *Panax Quinquefolius* (Aralicaceae). Amer. J. Bot. 69:1483-1490.

Lim W, Vermeylen F, Mudge KW (2005) Effects of population, age, and cultivation methods on ginsenoside content of American ginseng (*Panax quinquefolium*). J. Agri. Food Chem. 53:8498-8505.

Lim YP, Shin CS, Lee SJ, Youn YN, Jo JS (1993) Survey of proper primers and genetic analysis of Korean ginseng (*Panax ginseng* C.A. Meyer) variants using the RAPD technique. Korean J. Ginseng Sci. 17:153-158.

McGraw JB (2001) Evidence for decline in stature of American ginseng plants from herbarium specimens. Biol Conserv 98: 25-32.

Mudge KW, Lim W, Lardner J, Jousselin V (2000) Ginsenosides as Quality Indicators in woods grown American ginseng. American ginseng production in the 21st century. Cornell Cooperative Extension of Green County. p:90-96.

Mudge KW, Lim W, Lardner J, Beyfuss RL (2004) Effects of population and age on ginsenoside content of American ginseng (*Panax quinquefolium L.*). Acta. Hort. 629:161-166.

Murphy L (2000) Effects of American ginseng on breast cancer and prostate cancer cells. American ginseng production in the 21st century Cornell cooperative extension of green county. p. 39-45.

Ngan F, Shaw P, But P, Wang J (1999) Molecular authentication of *Panax* species. Phytochemistry 50:787-791.

Nienhuis J, Tivang J, Skroch P (1995) Genetic relationships among cultivars and landraces of Lima bean (*Phaseolus lunatus* L.) as measured by RAPD markers. J. Amer. Soc. Hort. Sci. 120:300-306.

Persons WS (1994) American ginseng farming in its native woodland habitat. in The challenges of the 21st century: proceedings of the International Ginseng Conference-Vancouver 1994. Vancouver Burnaby, B.C. Simon Fraser University. p. 78-83.

Proctor JTA, Bailey WG (1987) Ginseng: Industry, Botany, and Culture, in Horticultural Reviews, J. Janick, Van Nostrand Reinhold Company, New York. p. 187-236.

Proctor JTA, Slimmon T, Saxena PK (1996) Root growth and organogenesis in thidiazuron-treated ginseng (*Panax quinquefolium* L.). HortScience 31:628.

Ren G, Chen F (1999) Simultaneous quantification of ginsenosides in American ginseng (*Panax quinquefolium*) root powder by visible/near-Infrared reflectance spectroscopy. J. Agri.

Food. Chem. 47:2771-2775.

Rudakewich M, Ba F, Benishin C (2001) Neurotrophic and neuroprotective actions of ginsenosides Rb1 and Rg1. Planta Med. 67:533-537.

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. 163:427-439.

Shaw PC, But PPH (1995) Authentication of *Panax species* and their adulterants by random primed polymerase chain reaction. Planta Med. 61:466-469.

Smith RG, Caswell D, Carriere A, Zielke B (1996) Variation in the ginsenoside content of American ginseng, *Panax quinquefolius* L. roots. Can. J. Botany 74:1616-1620.

Snedecor GW, Cochran WG (1967) Statistical methods. Ames, Iowa Univ. Press. p. 132.

Sokhansanj S, Bailey WG, Guo YP, Oliver A (1999) Physical characteristics of north American ginseng (*Panax quinquefolius* L.) root. Can. Agric. Engin. 41:239-246.

Tivang J, Skroch PW, Nienhuis J (1996) Randomly amplified polymorphic DNA (RAPD) variation among and within artichoke (*Cynara Scolymus* L.) cultivars and breeding populations. J. Amer. Soc. Hort. Sci. 121:783-788.

Toda N, Ayajiki K, Fujioka H, Okamura T (2001) Ginsenoside potentiates NO-mediated neurogenic vasodilatation of monkey cerebral arteries. J. Ethnopharmacol. 76:109-113.

Um JY, Chung HS, Kim MS, Na HJ, Kwon HJ, Kim JJ, Lee KM, Lee SJ, Lim JP, Do KR, Hwang WJ, Lyu YS, An NH, HM Kim (2001) Molecular authentication of *Panax ginseng* species by RAPD analysis and PCR-RFLP. Biol. Pharm. Bull. 24:872-875.

Wang J, Ha WY, Ngan FN, But PP, Shaw PC (2001) Application of sequence characterized amplified region (SCAR) analysis to authenticate *Panax* species and their adulterants. Planta Med. 67:781-783.