Wansang Lim[1]
Kenneth W. Mudge[2]
Leslie A. Weston[2]

Utilization of RAPD Markers to Assess Genetic Diversity of Wild Populations of North American Ginseng (*Panax quinquefolium*)

Abstract

The Catskill Mountains of New York State are an important source of wild-collected American ginseng (*Panax quinquefolium*) and, increasingly, of woods-cultivated ginseng. The objective of this study was to assess genetic diversity among 9 different wild ginseng populations in and adjacent to the Catskill Mountain region of New York State and to compare these to wild populations from other states including Kentucky, Tennessee, North Carolina, Pennsylvania, and Virginia, and one cultivated population from Wisconsin. Randomly amplified polymorphic DNA (RAPD) markers were used to estimate the genetic distance among samples from the 15 populations. Pooled DNA from 10 plants of each of 8 New York populations was initially screened with 64 random primers; subsequently, the 15 primers that exhibited the greatest number of reproducible polymorphic markers were selected for further experimentation. Gel electrophoresis with the selected 15 primers produced 124 highly reproducible polymorphic bands. 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 distinctly separate clusters between New York and non-New York populations, indicating separation between these two groupings. The MDS analysis was confirmed using pooled chi-square tests for fragment homogeneity. This study shows that RAPD markers can be used as population-specific markers for *Panax quinquefolium*, and may eventually be utilized as markers for ginsenoside assessment.

Key words
North America · population · *Panax quinquefolium* · Araliaceae · genetic diversity · Catskill Mountains · ginsenoside · RAPD · HPLC

Supporting information available online at
http://www.thieme-connect.de/ejournals/toc/plantamedica

Introduction

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. Since most of the more than 60 metric tons of wild ginseng that are 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.

Several studies of American ginseng using molecular markers have confirmed genetic variation, on the one hand between wild and cultivated populations, and on the other hand among wild populations [1], [2], [3]. Population-specific markers for Asian ginseng (*Panax ginseng*) have also been identified by several authors [4], [5], [6]. 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" [5], [7], [8], [9], [10].

Affiliation
[1] Department of Plant Breeding and Genetics, Cornell University, Ithaca, New York, USA
[2] Department of Horticulture, Cornell University, Ithaca, New York, USA

Correspondence
Dr. Kenneth W. Mudge · Department of Horticulture · Cornell University · Ithaca · New York 14853 · USA ·
Phone: +1-607-255-1794 · Fax: +1-607-255-9998 · E-mail: kwm2@cornell.edu

Received February 14, 2006 · **Accepted** October 23, 2006

Bibliography
Planta Med 2007; 73: 71–76 © Georg Thieme Verlag KG Stuttgart · New York
DOI 10.1055/s-2006-951768 · Published online December 18, 2006
ISSN 0032-0943

There is an ongoing debate about the long-term sustainability of current harvest levels of wild American ginseng between conservationists on the one hand and ginseng or "shang" hunters, on the other, who collect ginseng for profit [11]. Over decades, many ginseng hunters have deliberately replanted wild populations with seeds of cultivated origin to offset the effects of continually harvesting wild plants [11], [12]. Additional information about genetic variation within and among wild populations could be useful in crafting effective conservation strategies.

Because of the relatively short history of domestication, *Panax quinquefolium* currently has no commercially available cultivars or selections, and cultivated material consists mainly of unimproved land races [2], [13]. Although ginseng is very difficult to propagate clonally, several authors have reported successful somatic embryo culture. Hence, information about the genetic diversity of American ginseng derived from molecular markers and other population-based experimental approaches could be useful for future genetic improvement of cultivated ginseng through breeding or clonal selection [2], [12].

RAPD analysis has several benefits compared with other molecular marker-based technologies such as AFLP, DALP, SSR, and RFLP. Among marker-based systems, RAPD is comparatively cost effective, relatively simple, and requires only small amounts of DNA [5]. Although the method is sometimes considered a difficult means by which to standardize reproducible bands, under carefully controlled and restricted reaction conditions reproducible and interpretable RAPD banding patterns can be obtained [14], [15]. Therefore, the objectives of this experiment were to identify a series of RAPD markers to differentiate *Panax quinquefolium* populations in New York State and also to assess the genetic diversity among wild ginseng populations from New York State and populations from other states.

Materials and Methods

Sample collection and DNA isolation

Ginseng plants were collected from 9 reproductively isolated wild populations occurring within 5 New York counties in or adjacent to the Catskill Mountains (see Supporting Information Fig. **1S**) and from 6 other states including 1 wild population each from Kentucky, Tennessee, North Carolina, Pennsylvania, and Virginia, and 1 cultivated population from Wisconsin. Representative voucher specimens from New York populations 2 and 7 (KWM1-pop.2; KWM2-pop.7. respectively) are on file in the Bailey Hortorium at Cornell University. The number of replicate individual plant samples from each population used for DNA analysis is presented in the Supporting Information, Table **1S**. Plants from each of the 9 collected New York populations and out of state populations were transplanted to Cornell University's Arnot Teaching and Research Forest in Newfield, New York, in October 2000 and April 1995, respectively. Whole plant samples for transplant from the wild consisted of a rhizome with the belowground apical bud and the attached storage root, after removal of the above ground shoot. DNA samples for RAPD analysis were obtained in August, 2002 from approximately 7-year-old plants collected from the Arnot Forest site only. For DNA extraction, one entire palmately compound leaf was removed from the shoot of each harvested plant and put immediately into plastic Whirl Pak bags (NASCO; Modesto, CA, USA) and placed in an insulated cooler containing dry ice. Samples were then transferred to an ultra-low freezer at –80 °C within several hours following collection.

Genomic DNA was extracted from each sample using the CTAB hexadecyltrimethylammonium bromide) method as described by Bernatzky and Tanksley [16]. A 100-mg leaf sample was frozen in liquid nitrogen and ground in a 2.5-mL microcentrifuge tube with a plastic pestle. DNA was extracted again if the UV absorption ratio, A260/280, was greater or less than the range 1.7 – 1.9.

Primer selection

A total of 64 primers were initially screened for polymorphic bands using a combined sample of extracted DNA from all of the New York populations. Selection of some of these primers was based on successful results reported in previously published studies involving American ginseng [1], [2], [3], while others were selected by random evaluation. Primers UBC98 and UBC203 were selected based on Bai et al. [1]. Primers POD03, OPD05, OPH04, OPH05, OPO15, OPU10, and OPU15 were selected based on Boehm et al. [2]. Primers UBC6, UBC18, UBC81, UBC164, UBC177, UBC210, UBC227, UBC262, UBC326, UBC398, UBC419, UBC464, and UBC 497 were selected based on Schulter [3]. Two sets of 20 random primers each, Kit-N and Kit-AD, were purchased from Operon Technologies (Kit-N and Kit-AD; Alameda, CA, USA). Primers OPH03 and OPO10 were selected randomly. UBC primers were purchased from the Nucleic Acid-Protein Service Unit at the University of British Columbia (Vancouver, Canada) and all other primers were purchased from Operon Technologies (Huntsville, AL, USA). After screening these 64 random decamer primers, 15 were selected for further experimentation based on maximum reproducibility and 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 Qiagen (Valencia, CA, USA) was used for the amplification of DNA via the polymerase chain reaction. The PCR reaction mixture (20 μL) consisted of 2 μL of 10X PCR buffer, 0.4 μL dNTP mix, 0.1 μL Taq DNA polymerase, 13.95 μL water, 0.8 μL $MgCl_2$ solution (2.5 mM), 2 μL DNA template (5 ng/μL) and 0.75 μL of the appropriate primer (0.2 μM). Primers were dissolved in a 250 μL TE buffer. DNA amplification was performed in an Eppendorf thermocycler (Master Cycler; Westbury, NY, USA). The initial cycle was 2 min at 94 °C, 10 min at 35 °C, and 2 min at 72 °C. The subsequent 46 cycles were 45 s at 94 °C, 45 s at 35 °C and 2 min at 72 °C, followed by 10 min at 72 °C for the last cycle [1]. For electrophoretic separation of the DNA products of the PCR reaction, approximately 17 μL of amplified DNA were loaded onto a 1.5 % agarose gel submerged 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 ImagerTM 2200; San Leandro, CA, USA).

DNA extraction, amplification, and electrophoresis were performed separately upon 8 to 10 individual plants from each of the New York populations and 3 to 4 individual plants of the non-New York populations.

RAPD reproducibility

Before the main experiment was conducted, several preliminary experiments were performed to optimize PCR reactions for reproducibility. These experiments evaluated template concentration, $MgCl_2$ concentration and primer concentration using two separate extractions and gel runs. In addition, each sample was re-extracted when necessary to maximize intensity and reproducibility among bands.

We also utilized a positive control with a previously characterized sample to evaluate reproducibility. Additional negative controls were utilized to identify any potential contaminants. Gel bands were visualized on gel using an AlphaImager 2002 (Alpha Innotech Corporation, Alpha ImagerTM 2200). Primers were selected for further experiments based on their ability to produce clear and reproducible bands. All samples were replicated at least three times and experimental runs were repeated over time.

Statistical analysis

Stained gels were scored for the presence (1) or absence (0) of co-migrating polymorphic bands ranging in size from 0.3 to 2 kb. A standard DNA ladder (Bionexus Inc, All-purpose Hi-Lo DNA maker 50 – 10,000 bp; Oakland, CA, USA) 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. Genetic distance (GD) 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 v8 [17].

All pairwise comparisons, among New York State populations and between New York State and non-New York State populations, were calculated for differences in individual marker frequencies. Chi-square analysis was used for pairwise comparisons. For χ^2 calculations, 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. All such observations were grouped into a single class with one degree of freedom [18].

Dendrograms were created with UPGMA (unweighted pair group method with arithmetic average) cluster analysis and graphically displayed with the SAS System for Windows v8 [3].

Supporting information

The number of samples, the sample collection counties and the cluster analysis of New York populations are available as Supporting Information.

Results and Discussion

Average yield of total genomic DNA from leaves of *Panax quinquefolium* was 61 ng/mg fresh leaf mass. 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 each (Table **1**). The primers, UBC203 and UBC98, produced bands that were similar to those reported by Boehm [2] and Shulter [3], respectively (Table **1**).

Table **1** List of polymorphic band characterized by molecular mass (bp) and frequency of band in parenthesis

Primer	*Polymorphic band*											
AD01	1990(99)	1760(109)	1550(98)	1210(86)	1070(108)	980(88)	770(89)	560(103)	490(67)	480(105)		
AD02	1780(77)	1600(99)	1230(54)	1120(104)	720(106)	580(107)	490(73)	410(64)				
AD11	1930(109)	1820(81)	1690(78)	1320(83)	1100(107)	1020(73)	910(106)	830(108)	770(101)	700(99)	640(110)	590(29)
AD15	1290(90)	950(67)	710(101)	620(84)	490(24)							
N19	1560(100)	890(104)	780(108)	670(73)	570(104)	540(105)	450(106)					
N2	1320(92)	850(108)	650(52)	560(73)	450(110)	410(59)						
OPD03	1430(110)	1302(105)	890(104)	790(63)	590(53)	540(64)	490(92)					
OPD05	1330(93)	1080(93)	900(110)	740(108)	700(52)	560(55)	480(109)					
OPO15	1980(104)	1380(105)	1240(72)	1000(106)	780(91)	610(106)	540(102)	380(110)				
OPU10	1630(87)	1410(17)	1210(99)	1020(22)	840(94)	580(3)						
OPU15	1240(93)	1160(66)	860(109)	610(104)	520(109)							
UBC164	1450(32)	1310(97)	1200(84)	1120(104)	1010(70)	980(98)	810(13)	730(102)	680(66)	630(66)	550(108)	490(53)
UBC203	1990(92)	1900(96)	1710(108)	1490[B] (102)	1380[B] (74)	1300(93)	1170[B] (88)	1040(95)	880[B] (99)	780[B] (79)	710[B] (89)	5420[B] (86)
UBC81	1800(81)	1460(92)	1270(106)	1190(61)	1060(74)	940(104)	830(96)	720(108)	590(44)	520(107)	460(53)	420(67)
UBC98	1560[S](18)	1310(5)	1170[S] (81)	780(22)	730(108)	630[S] (99)	520(85)					

[B] Same band with Boehm [2].

[S] Same band with Shluter [3].

Table **2** Mean genetic distance (GD) values within and between New York and non-New York populations

Population	*GD within population*	*GD between populations*
All	0.259	–
1	0.242	–
2	0.266	–
3	0.246	–
4	0.196	–
5	0.226	–
6	0.197	–
7	0.236	–
8	0.251	–
9	0.086	–
KY	0.195	–
NC	0.161	–
PA	0.159	–
TN	0.153	–
VA	0.180	–
WI	0.231	–
NY vs. Non NY	–	0.272

The genetic distance (GD) presented in Table **2** is a measure of the estimated genetic dissimilarity among individual plants within a population or among populations on a scale of 0.0 to 1.0, with 0.0 indicating maximum genetic similarity (minimum diversity) and 1.0 indicating no similarity (maximum diversity). The average GD for all 111 *Panax quinquefolium* samples analyzed in this study was 0.259. This is very similar to the GD value of 0.24 that Boehm [2] 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 and Punja [3]. Hence, these independent estimates of the level of genetic diversity among 3 different sets of wild populations as determined by Boehm [2], Schulter [3], and this study (Table **2**) are very similar. The average GD of 0.259 in Table **2** shows that the within-population genetic heterogeneity was relatively high, especially for populations 2 and 3 (GD = 0.266 and 0.246 respectively) (Fig. **2**). The fact that banding patterns are not identical within some populations such as populations 4 and 7 in both Fig. **2** and Fig. **5** also indicates within-population heterogeneity. Our results also allow us to compare the level of genetic diversity between 9 wild populations from New York (collectively) with that of the 5 wild populations from other states. The genetic diversity between New York and non-New York populations (GD = 0.272, Table **2**) is greater than the within-population GD values for each of the 14 individual *Panax quinquefolium* populations, which range from 0.086 (New York Population 9) to 0.266 (New York Population 2). Interestingly, this range is similar to that reported by Schulter and Punja (0.11 – 0.31) [3] and that reported by Boehm et al. (0.14 – 0.24) [2].

Using gel electrophoresis, we observed differences in band frequency among populations; for example, Fig. **1** and Table **2** illustrate differences in polymorphic band frequency between non-New York and New York populations. The band at a molecular weight of approximately 800 is frequently present in non New York populations, but is infrequent in New York populations. In Fig. **2**, the band at molecular weight 1406 is present in non-New York populations 12, 13, 14, and 15, but entirely absent in all New York populations. These differences are also apparent from the tendency of individuals from New York to cluster separately from non-New York individuals in the MDS plot shown in Fig. **3**- Although some overlap is observed, χ^2 analysis indicates a statistically significant difference ($p = 0.046$) between the New York and non-New York populations. Cluster analysis was also performed to compare New York and non-New York populations and clearly shows a grouping of the 8 New York populations, distinct from that of the non-New York populations (see Supporting Information Fig. **2S**). Although both χ^2 analysis and MDS plots (Fig. **3**) indicate significant differences between New York and non-New York populations of wild American ginseng, χ^2 analyses of all possible pairwise comparisons among the 9 New York populations were not statistically significant, indicating less genetic diversity among New York populations than between New York and non-New York populations. This is also apparent from the overlap of individuals from the 9 New York populations in the MDS plot shown in Fig. **4**, although individuals from New York populations 2 and 3 tend to cluster separately. Similarly, Schulter and Punja [3] could not distinguish among 3 wild popu-

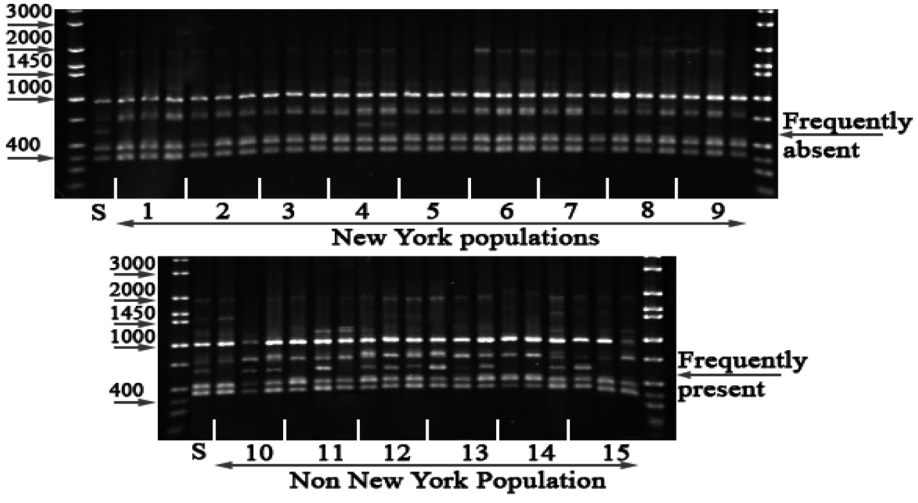

Fig. **1** Agarose gel electrophoresis of ginseng genomic DNA amplified with primer AD 07. A band between 0.7 and 1.4 kb exists more frequently in non-New York populations than in New York populations (the 3 lanes per population are randomly selected to show the specific band between NY and non NY populations).

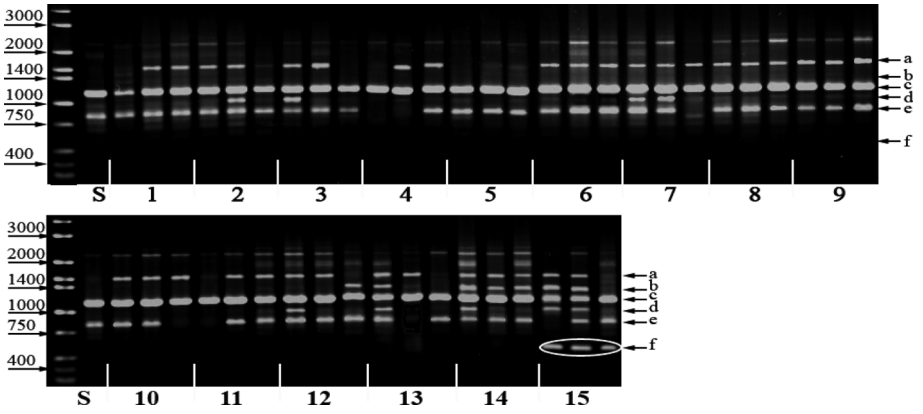

Fig. **2** Agarose gel electrophoresis of ginseng genomic DNA amplified with primer OPU 10. Note the differences in band appearance in Wisconsin cultivated ginseng versus all other wild populations. M = DNA marker, S = Standard; 1 – 9 = New York populations; 10 = Kentucky; 11 = North Carolina; 12 = Pennsylvania; 13 = Tennessee; 14 = Virginia; 15 = Wisconsin cultivated [molecular mass (bp); a = 1625, b = 1446, c = 1206, d = 1019, e = 839, f = 584].

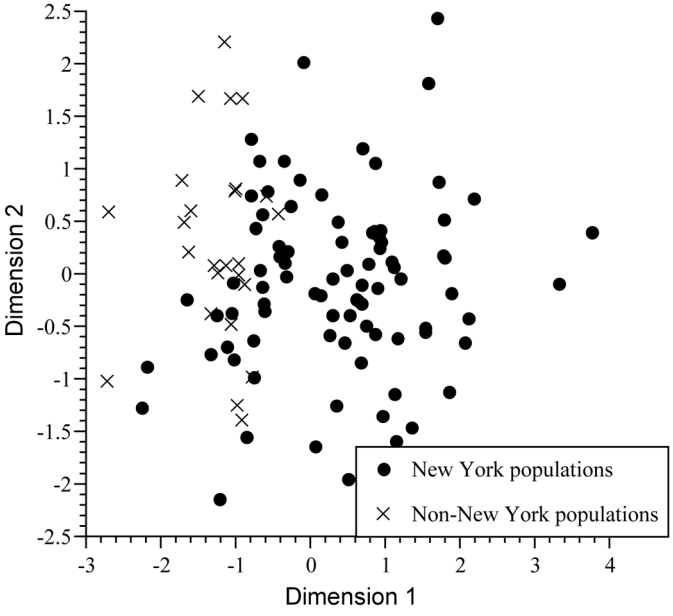

Fig. **3** Multidimensional scaling plot of the genetic distance values for individual *Panax quinquefolium* plants showing separation between New York populations and non-New York populations.

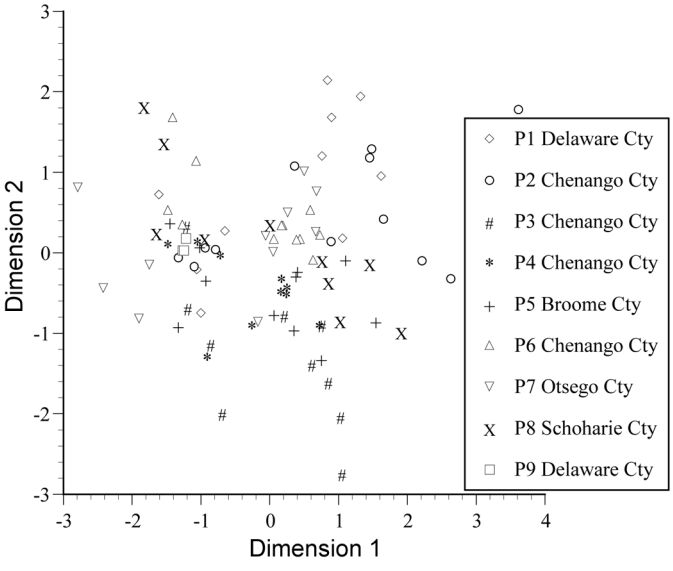

Fig. **4** Multidimensional scaling plot of genetic distance values for individual *Panax quinquefolium* plants from 9 wild New York populations collected in 5 counties, Cty = county.

lations collected from the Canadian province of Quebec which, like the 5 adjacent counties that encompassed the 9 New York populations involved in this study, were from a limited geographic region. In contrast, Boehm et al. [2] reported significant genetic differences among 3 wild populations encompassing a considerably broader geographic region (Wisconsin, Tennessee, and Pennsylvania).

As another dimension of the same study reported here, we found among the same 8 New York *Panax quinquefolium* populations that quantitative and qualitative differences existed among the 6 major ginsenosides as measured by HPLC analysis. Individual ginsenosides varied among ginseng populations [19]. Interestingly, Catskill population 3, which tended to cluster separately from other populations in the MSD plot assessing genetic distance (Fig. **4**), also differed chemically from other populations in that it had the highest content of the bioactive ginsenoside Rd [19]. Also, the RAPD pattern of Catskill Population 3 is different from other New York populations (Fig. **5**).

This suggests that further experimentation with diverse *Panax quinquefolium* populations may result in discovery of unique molecular marker(s) for ginsenocide content.

Perhaps not surprisingly, the cultivated Wisconsin population also differed significantly from other non-cultivated ginseng populations from across the United States, when compared by marker-based analysis. Fig. **2** shows clear differences in polymorphic bands appearing in Wisconsin samples subjected to gel electrophoresis, whereas similar responses or less polymorphism was observed among other non-cultivated ginsengs. This may indicate that the process of cultivation is associated with changes in genotypic diversity over time, in comparison to non-cultivated or wild collected ginseng populations.

Collection pressure on wild populations of American ginseng are a consequence of the high price paid for wild-collected roots (approximately $300 per kg dry weight), and further loss or fragmentation of forest habit may affect the prospects for long-term survival of *Panax quinquefolium* in the wild [20], [21], [22]. Based on allozyme analysis by Cruse-Sanders and Hamrick [23], genetic diversity as reflected by expected heterozygosity was greater in protected populations (i. e., Great Smoky Mountain National Park) than in unprotected, routinely harvested populations, suggesting that harvesting from the wild may have significant long-

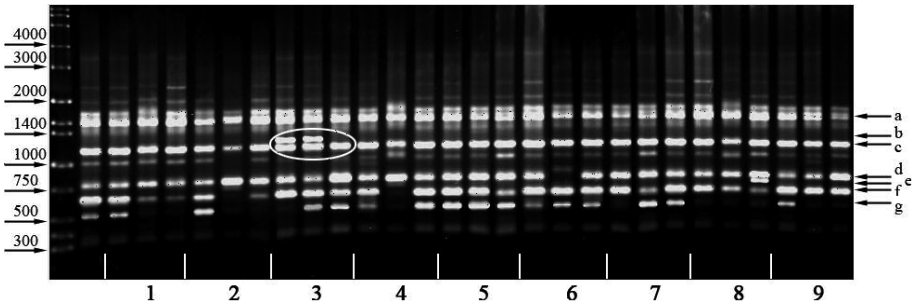

Fig. **5** Agarose gel electrophoresis of ginseng genomic DNA amplified with primer UBC 98. Note the differences in band appearance in New York population 3 versus New York populations 1, 2 and 4 – 9 [molecular mass (bp); a = 1560, b = 1312, c = 1173, d = 784, e = 728, f = 629, g = 524].

term implications for the species. Similar findings by Boehm et al. [2] using RAPD marker analysis to detect significant genetic differences among geographically distant wild populations suggest that consideration should be given to modifying conservation strategies to include protection of the genetic integrity of distinct local populations. The comparison of 3 wild populations from a more geographically limited area failed, however, to detect significant interpopulational differences [8]. Similarly, populations from the Catskill region of New York were not significantly different from each other when compared using RAPD or cluster analyses, but were collectively different from 6 geographically disparate non-New York populations. RAPD analysis also indicated that cultivated Wisconsin populations exhibited marker based differences from all non-cultivated populations. Many marker-based studies performed to date strongly suggest that preservationists consider the future protection of the genetic integrity of regional population centers rather than individual populations. Our results also suggest that discouraging introduction of exotic germplasm into a given region by banning replanting of introduced seed among wild populations could aid in the preservation of genetic integrity. Our findings are also not inconsistent with the observation that a lack of genetic diversity often exists among regional populations, perhaps due to natural selection over time for a genetically uniform and limited subset of genotypes that are well adapted to local conditions.

Experimental assumptions such as these may lead to the development of various approaches for ginseng conservation management in North America. However, further research will be necessary to determine which strategies will be most appropriate for the long-term conservation of *Panax quinquefolium*. In addition to the conservation-related implications of this study, our results and others [1], [2], [3] suggest that sufficient genetic diversity may exist both within and among wild populations for genetic improvement of cultivated ginseng through future advancements in breeding or clonal selection.

References

[1] Bai D, Brandle J, Reeleder R. Genetic diversity in North American ginseng (*Panax quinquefolius* L.) grown in Ontario detected by RAPD analysis. Genome 1997; 40: 111 – 5

[2] Boehm CL, Harrison HC, Jung G, Nienhuis J. Organization of American and Asian ginseng germplasm using randomly amplified polymorphic DNA (RAPD) markers. J Am Soc Hortic Sci 1999; 124: 252 – 6

[3] Schluter C, Punja ZK. Genetic diversity among natural and cultivated field populations and seed lots of American ginseng (*Panax quinquefolius* L.) in Canada. Int J Plant Sci 2002; 163: 427 – 39

[4] Lim YP, Shin CS, Lee SJ, Youn YN, Jo JS. Survey of proper primers and genetic analysis of Korean ginseng (*Panax ginseng* C.A. Meyer) variants using the RAPD technique. Korean J Ginseng Sci 1993; 17: 153 – 8

[5] Um JY, Chung HS, Kim MS, Na HJ, Kwon HJ, Kim JJ et al. Molecular authentication of *Panax ginseng* species by RAPD analysis and PCR-RFLP. Biol Pharm Bull 2001; 24: 872 – 5

[6] Kim JH, Yuk JA, Cha SK, Kim HH, Seong BJ, Kim SI et al. Genetic variation in pure lines of *Panax ginseng* based on by RAPD analysis. Korean J Med Crop Sci 2003; 11: 102 – 8

[7] Ha WY, Yau FCF, But PPH, Wang J, Shaw PC. Direct amplification of length polymorphism analysis differentiates *Panax ginseng* from *P. quinquefolius*. Planta Med 2001; 67: 587 – 9

[8] Ha WY, Shaw PC, Liu J, Yau FCF, Wang J. Authentication of *Panax ginseng* and *Panax quinquefolius* using amplified fragment length polymorphism(AFLP) and directed amplification of minisatellite region DNA(DAMD). J Agric Food Chem 2002; 50: 1871 – 5

[9] Ngan F, Shaw P, But P, Wang J. Molecular authentication of *Panax* species. Phytochemistry 1999; 50: 787 – 91

[10] Wang J, Ha WY, Ngan FN, But PPH, Shaw PC. Application of sequence characterized amplified region (SCAR) analysis to authenticate *Panax* Species and their adulterants. Planta Med 2001; 67: 781 – 3

[11] Persons WS. American ginseng farming in its native woodland habitat. Proceedings of the International Ginseng Conference, Vancouver: 1994

[12] Grubbs HJ, Case MAC. Allozyme variation in American ginseng (*Panax quinquefolius* L.): Variation, breeding system, and implications for current conservation practice. Conserv Genet 2004; 5: 13 – 24

[13] Proctor JTA, Bailey WG. Ginseng: industry, botany, and culture. In: Janick J, editor. Horticultural reviews. New York: Van Nostrand Reinhold Company; 1987: 187 – 236

[14] Sun M, Wong KC. Genetic structure of three orchid species with contrasting breeding systems using rapd and allozyme markers. J Am Soc Hortic Sci 2001; 88: 2180 – 8

[15] Morgan DJW, Reitz SR, Atkinson PW, Trumble JT. The resolution of Californian populations of *Liriomyza huidobrensis* and *Liriomyza trifolii* (Diptera: Agromyzidae) using PCR. Heredity 2000; 85: 53 – 61

[16] Bernatzky R, Tanksley SD. Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 1986; 112: 887 – 98

[17] Nienhuis J, Tivang J, Skroch P. Genetic relationships among cultivars and landraces of Lima bean (*Phaseolus lunatus* L.) as measured by RAPD markers. J Am Soc Hortic Sci 1995; 120: 300 – 6

[18] Tivang J, Skroch PW, Nienhuis J. Randomly amplified polymorphic DNA (RAPD) variation among and within Artichoke (*Cynara Scolymus* L.) cultivars and breeding populations. J Am Soc Hortic Sci 1996; 121: 783 – 8

[19] Lim W, Mudge KW, Vermeylen F. Effects of population, age, and cultivation methods on ginsenoside content of American ginseng (*Panax quinquefolium*). J Agric Food Chem 2005; 53: 8498 – 505

[20] Charron D, Gagnon D. The demography of northern populations of *Panax quinquefolium* (American ginseng). J Ecol 1991; 79: 431 – 45

[21] Gagnon D, Nantel P. Prediction of population extinction of ginseng and wild leek under harvesting pressure. Bull Ecol Soc America 1992; 73: 182

[22] McGraw JB. Evidence for decline in stature of American ginseng plants from herbarium specimens. Biol Conserv 2001; 98: 25 – 32

[23] Cruse-Sanders JM, Hamrick JL. Genetic diversity in harvested and protected populations of wild American ginseng, *Panax quinquefolius*. (Araliaceae). Am J Bot 2004; 91: 540 – 8