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GENETIC DIVERSITY AMONG NATURAL AND CULTIVATED FIELD POPULATIONS AND SEED LOTS OF AMERICAN GINSENG (*PANAX QUINQUEFOLIUS* L.) IN CANADA

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Genetic diversity within Canadian-grown North American ginseng (*Panax quinquefolius* L.) was evaluated using random amplified polymorphic DNA (RAPD) markers. Fifteen primers that produced 35 highly repeatable polymorphic markers were used to screen over 600 plant samples from various Canadian ginseng farms and seed lots. Ten samples from a Wisconsin seed lot and 58 samples from three natural ginseng populations in Quebec were also included for comparison. Genetic distance values, estimated as the complement to the simple matching coefficient, within cultivated populations ranged from 0.21 for a population in Nova Scotia to 0.34 for a British Columbia population, with an overall mean of 0.3. Distance values within three natural populations were either similar (0.33) or lower (0.12, 0.19) when compared with cultivated populations, indicating that populations under cultivation have not undergone a reduction in overall genetic diversity. However, one RAPD marker was polymorphic only in natural populations. Monotonic multidimensional scaling and χ^2 analyses indicated that natural populations were genetically distinct from cultivated ones. Individual plants originating as seeds from the same mother plant had much lower genetic diversity (mean of 0.18) compared with individual field-grown plants chosen at random from the same farm. Segregation of some RAPD markers was observed among the progeny, indicating that parental plants have some degree of heterozygosity and that a level of outcrossing may be present. Estimates of the component for genetic diversity between populations (G'_{ST}) were 18.0% and 28.0% for cultivated and natural populations, respectively; much of the variation was detected within and not between populations. These results imply that North American ginseng is a heterogeneous mix of genetic material and that the observed genetic diversity in cultivated populations in Canada results largely from the mixing of different seed lots. In addition, heterozygosity within the parent plants and cross-pollination appear also to contribute to genetic variation in this species.

Keywords: ginseng, molecular markers, genetic polymorphisms, random amplified polymorphic DNA.

Introduction

One of the most widely used medicinal herbs in the world is ginseng (*Panax* spp.) (Li and Mazza 1999). The two most important species grown commercially are *Panax quinquefolius* L. (North American ginseng), which is native to eastern North America, and *Panax ginseng* C. A. Meyer (Korean ginseng), which is native to northeastern China and the Korean peninsula (Proctor and Bailey 1987). Both species are reported to be allotetraploids with a chromosome number of 2n = 4x = 48 (Hu et al. 1980; Proctor and Bailey 1987). Cultivation of *P. quinquefolius* in Canada began over 100 yr ago when it became apparent that harvesting of wild populations was leading to their extinction (Proctor 1986; Court et al. 1996; Bai et al. 1997). About 3000 ha of *P. quinquefolius* were cultivated in Canada in 1997, primarily in the regions of British Columbia and Ontario (Li and Mazza 1999).

Ginseng is propagated from stratified seeds, which are planted in the fall (September–October) to give rise to first-year seedlings the following spring (April–May). Mature berries containing one to three seeds each are collected when the plants are 3–4 yr old and undergo a stratification period of ca. 12 mo, during which cool/warm periods are provided to enhance embryo maturation (Proctor and Louttit 1995). Seeds are sold or exchanged by growers within and between the major ginseng production areas (Proctor et al. 1999). There are no identified cultivars of ginseng, and no attempts at selection for improved horticultural characteristics have been made yet because of the long reproductive cycle and difficulties associated with seed stratification and germination (Proctor 1986; Proctor and Bailey 1987; Boehm et al. 1999). As a result, ginseng in a commercial field may exhibit considerable phenotypic variability, such as in leaf size and shape, number of flowers, plant height, and susceptibility to disease (Hu et al. 1980; Proctor and Bailey 1987; Bai et al. 1997; Schluter and Punja 2000). Other reported differences among plants in a field include variable inflorescence structure and variation in levels of saponins (or ginsenosides) present in roots and leaves (Proctor 1986; Smith et al. 1996). It has been suggested that North American ginseng is comprised of unimproved land races (Bai et al. 1997; Boehm et al. 1999).

Several reports indicate that *P. quinquefolius* is highly self-fertile because bagging of the inflorescence to exclude pollen from neighboring plants and potential pollinators does not reduce seed set and, in some instances, enhances it (Carpenter 1980; Carpenter and Cottam 1982; Lewis and Zenger 1983; Schluter and Punja 2000). These observations may indicate

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Table 1

Ginseng Samples, Geographic Origins, Collection Dates, and Number of Individuals Used for RAPD Analysis

Code	Geographic origin	Population type	Material collected	Collection date	No. tested
Panax quinquefolius:					
AS	Vernon, B.C.	Cultivated	Leaves	June 1995	7
M697	Merritt, B.C.	Cultivated	Leaves	June 1997	10
Adults	Merrit, B.C.	Cultivated	Leaves	June 1997	10
M897	Merritt, B.C.	Cultivated	Leaves	July 1997	10
CC	Cherry Creek, B.C.	Cultivated	Seeds	August 1997	10
L	Lillooet, B.C.	Cultivated	Seeds	August 1997	10
Wal	Walachin, B.C.	Cultivated	Seeds	August 1997	10
L4	Lillooet, B.C.	Cultivated	Seeds	September 1997	10
L5	Lillooet, B.C.	Cultivated	Seeds	September 1997	10
M898	Merritt, B.C.	Cultivated	Leaves	August 1998	10
#5	Kamloops/Vernon, B.C.	Cultivated	Seeds	September 1998	10
1i	Merritt, B.C.	Maternal family	Seeds	August 1999	9
2i	Merritt, B.C.	Maternal family	Seeds	August 1999	9
3i	Merritt, B.C.	Maternal family	Seeds	August 1999	9
4i	Merritt, B.C.	Maternal family	Seeds	August 1999	9
5i	Merritt, B.C.	Maternal family	Seeds	August 1999	9
6i	Merritt, B.C.	Maternal family	Seeds	August 1999	9
7i	Merritt, B.C.	Maternal family	Seeds	August 1999	6
8i	Merritt, B.C.	Maternal family	Seeds	August 1999	9
9i	Merritt, B.C.	Maternal family	Seeds	August 1999	19
10i	Merritt, B.C.	Maternal family	Seeds	August 1999	9
11i	Merritt, B.C.	Maternal family	Seeds	August 1999	9
12i	Summerland, B.C.	Maternal family	Seeds	July 1999	19
13i	Summerland, B.C.	Maternal family	Seeds	July 1999	19
14i	Summerland, B.C.	Maternal family	Seeds	July 1999	19
15i-B	Summerland, B.C.	Maternal family	Seeds	July 1999	19
16i-B	Summerland, B.C.	Maternal family	Seeds	July 1999	19
17i-B	Summerland, B.C.	Maternal family	Seeds	July 1999	19
18i-B	Peachland, B.C.	Maternal family	Seeds	July 1999	19
19i-B	Peachland, B.C.	Maternal family	Seeds	July 1999	19
20i	Peachland, B.C.	Maternal family	Seeds	July 1999	19
21i	Peachland, B.C.	Maternal family	Seeds	July 1999	19
RD	Kamloops, B.C.	Cultivated	Leaves	July 2000	20
RD-S	Kamloops, B.C.	Cultivated	Leaves	July 2000	40
RD-S2	Kamloops, B.C.	Cultivated	Leaves	July 2000	20
Knox	Vanessa, Ont.	Cultivated	Seeds	August 1997	10
Ont D	Vanessa, Ont.	Cultivated	Seeds	August 1997	10
Ont 3	LaSalette, Ont.	Cultivated	Seeds	September 1997	10
Ont 4	LaSalette, Ont.	Cultivated	Seeds	September 1997	10
#1	Waterford, Ont.	Cultivated	Seeds	September 1998	10
#2	Waterford, Ont.	Cultivated	Seeds	September 1998	10
#3	Waterford, Ont.	Cultivated	Seeds	September 1998	10
#4	Waterford, Ont.	Cultivated	Seeds	September 1998	10
W1	Deux-Montagnes, P.Q.	Natural	Leaves	June 1999	20
W2	L'Assomption, P.Q.	Natural	Leaves	June 1999	20
W3	Chambly, P.Q.	Natural	Leaves	June 1999	18
#6	Pugwash, N.S.	Cultivated	Seeds	September 1998	10
Wis	Wisconsin, U.S.A.	Cultivated	Seeds	April 1999	10
Panax ginseng	Wisconsin, U.S.A.	Cultivated	Seeds	March 1999	5
Panax notoginseng	China	Cultivated	Seeds	May 1999	5

that some of the seeds obtained from ginseng in cultivated fields may be the result of self-pollination. The impact of this reproductive strategy on genetic variation has not been determined. A low level of cross-pollination may occur, however, since generalist insect pollinators, such as bees and ants, have been observed on ginseng inflorescences in the field (Carpenter 1980; Carpenter and Cottam 1982; Lewis and Zenger 1983; Schluter and Punja 2000).

Randomly amplified polymorphic DNA (RAPD) markers have been used in previous studies to estimate genetic diversity and relatedness among populations of many plant species. The results have shown that RAPDs can be used to detect genetic variation among species (Divaret et al. 1999; Rodriguez et al. 1999; Ruas et al. 1999), cultivars (Nicese et al. 1998; Obara-Okeyo and Kako 1998; Moeller and Schaal 1999), populations (Bai et al. 1997; Boehm et al. 1999; Bussell 1999; Fahima et

al. 1999; Gugerli et al. 1999; Loo et al. 1999; Nebauer et al. 1999; Thomas et al. 1999), and even clones (Goto et al. 1998; Watanabe et al. 1998; Al-Zahim et al. 1999; Binsfeld et al. 1999).

Two previous studies have examined the level of genetic diversity in North American ginseng using RAPD markers (Bai et al. 1997; Boehm et al. 1999). A high degree of genetic variation was reported in one cultivated population of *P. quinquefolius* from Ontario, which was initiated from a mixture of seeds collected from different ginseng farms over several years (Bai et al. 1997). Variation within and among populations of cultivated and wildtype *P. quinquefolius* in various regions of North America was characterized by Boehm et al. (1999). The results indicated that there were no distinct genetic populations within cultivated *P. quinquefolius* but that two wildtype populations included in the study were genetically distinct from the cultivated populations and from each other. Therefore, cultivated ginseng appears to be comprised of mixed collections of genetically different plants that vary greatly within a population but much less so among populations.

The extent of genetic variation within and among individual commercial farms and among seed lots of *P. quinquefolius* has not been studied. It is not known whether commercial cultivation may be leading to a reduction in genetic diversity. At present, natural populations of *P. quinquefolius* are protected in Canada in an attempt to preserve genetic diversity in the species, on the assumption that intensive cultivation will erode diversity. If advanced generations of ginseng result primarily from selfing, or if a breeding program is implemented, the impact these factors would have on the current level of genetic diversity in cultivated populations is unknown. Information that quantifies genetic diversity is necessary to determine the impact of selection in future ginseng breeding programs. The objectives of this study were to (i) establish whether RAPDs could provide genotype-specific identification of ginseng, (ii) determine the level of genetic diversity within and among different commercial farms and seed lots of *P. quinquefolius* in Canada, (iii) establish if genetic diversity in three protected natural populations was distinct from that in cultivated populations, and (iv) utilize information about genetic diversity to form hypotheses regarding mechanisms of gene flow.

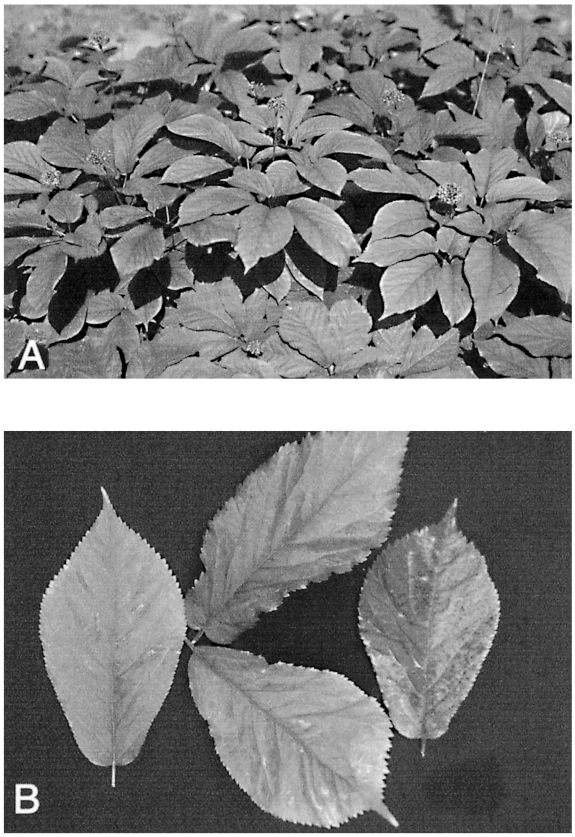

Fig. 1 *A*, Commercial *Panax quinquefolius* garden in Kamloops, British Columbia. Note phenotypic differences among plants with regard to canopy and inflorescence development. *B*, Differences in leaf shape and size observed in four individual plants within a commercial field.

Material and Methods

A total of 641 plant samples was collected from June 1995 to July 2000. Plant samples originated either as leaves from field-grown plants, from cultivated areas (eight fields, 127 samples) or from three reproductively isolated natural populations located near Montreal, Quebec (58 samples), or as seed (stratified and unstratified). One of the cultivated fields (40 samples, labeled "RD-S") contained 5-yr-old individuals sown from the seeds collected from 100 selected plants displaying large root size. Progeny seeds from this population were also collected (20 samples, labeled "RD-S2"). Leaves were collected from 3–5-yr-old plants in the cultivated populations (the ages of plants in natural populations were unknown) and wrapped in moist paper towels after collection. They were frozen in liquid nitrogen within 24 h and stored at −80°C until needed. The seeds were provided either as a sample from a "seed lot," which represented seeds collected from different plants in one cultivated field and bulked (16 seed lots; 160 samples) or as "maternal families," representing seeds collected from mature inflorescences of 21 plants (three locations, 296 samples) and kept separate from all other samples. Early in the season, before flowering began, inflorescences of five of these 21 plants were bagged in fine-meshed, white cloth polyester to exclude pollinators and ensure self-pollination. The seeds had either been stratified before their arrival or were stratified in the laboratory. For stratification, depulped seeds were mixed with about two volumes of moist, autoclaved sand and placed in the dark for 3 mo in an incubator at 4°C, followed by 4–5 mo at 15°C, and then at 4°C until the radicles had emerged. Seeds were planted in sterilized potting medium and seedlings were grown at 20°–23°C with a 16-h photoperiod. For each seed lot, a random sample of 10 seedlings was chosen for RAPD analysis. In some experiments where seed numbers were limited because of inviability or low germination rates, DNA was extracted from the developing embryo within the seed, which was aseptically dissected. The geographic origins of the samples representing *Panax quinquefolius* were British Columbia (BC) in the regions of Cherry Creek, Lillooet, Merrit, Kamloops, Kamloops, and Vernon; Ontario (ON) in the regions of LaSalette, Vanessa, and Waterford; Quebec (PQ) in the regions of Chambly, Deux-Montagnes, and L'Assomption; Nova Sco-

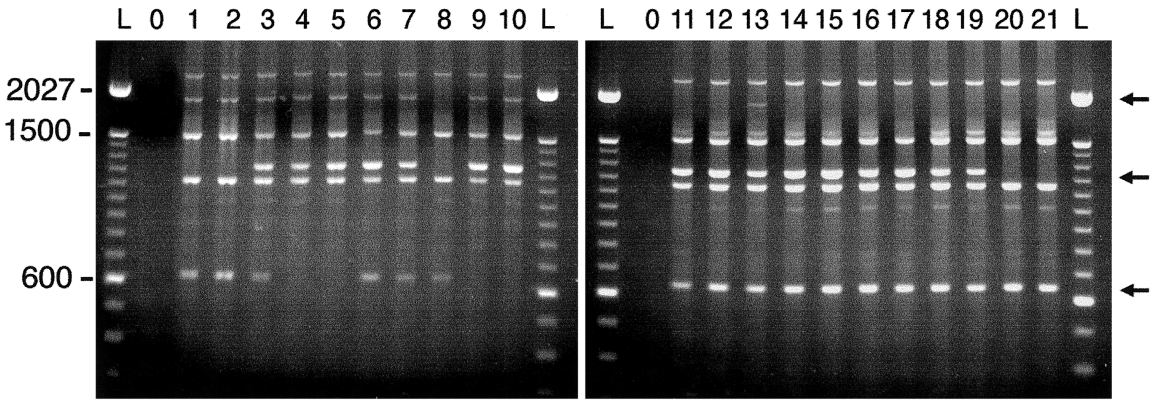

Fig. 2 RAPD amplification of *Panax quinquefolius* samples generated with UBC primer 98. *L*, 100-bp ladder (Gibco BRL). 0, negative control; lanes 1–10, natural ginseng samples; lanes 11–21, cultivated ginseng samples. Arrows show polymorphic bands.

tia (NS) in the region of Pugwash; and Wisconsin (Wis) (table 1). In addition, samples of *Panax ginseng* and *Panax notoginseng* were obtained as seed grown in Wisconsin and China, respectively.

DNA Extraction

About 100 mg of leaf, seed, or embryo tissue was ground with a small quantity of sterile silica sand using a disposable plastic pestle in a 1.5-mL microcentrifuge tube containing 300 μL extraction buffer (200 mM Tris, pH 8; 1.5 M NaCl; 100 mM EDTA, pH 8; 2% SDS). An additional 300-μL extraction buffer was added, and the mixture was incubated at room temperature for 1 h. After centrifugation at 21,000 *g* for 1 min, the supernatant was transferred to a new tube, to which 500 μL phenol : chloroform : isoamyl alcohol (25 : 24 : 1) was added, vortexed to form an emulsion, and centrifuged at 21,000 *g* for 5 min. The supernatant was transferred and reextracted with phenol : chloroform : isoamyl alcohol. Two and one-half volumes of 95% ethanol was added to the supernatant, and the mixture was left overnight at −20°C. The sample was centrifuged at 21,000 *g* for 5 min, and the pellet was washed twice with 70% ethanol and air dried. The resultant pellet was resuspended in DNeasy AP1 buffer (Qiagen DNeasy plant mini kit, Mississauga), and the rest of the extraction procedure was conducted following kit instructions. Extracted DNA was resuspended in DNeasy AE buffer, and the concentration was estimated spectrophotometrically. Ratios of A_{260}/A_{280} and of A_{260}/A_{230} were measured to determine that protein, polyphenolic, and polysaccharide contamination was minimal. If DNA quality was poor, the extraction was repeated. Quantified DNA samples were diluted to 5 ng/μL with DNeasy AE buffer.

RAPD Amplification Conditions

A total of 126 decamer primers was initially screened for polymorphisms among 12 ginseng samples originating from Lillooet, Merritt, Walachin, and Vernon, British Columbia, as well as from several Ontario farms. Primers were obtained either from Operon Technologies (Alameda, Calif.) or from the Nucleic Acid–Protein Service Unit at the University of British Columbia (Vancouver). To identify polymorphic primers, reactions were conducted at least twice. Subsequently, a set of 15 primers was used to screen all 642 samples. The primers were UBC-6, 18, 81, 98, 164, 177, 203, 210, 227, 262, 326, 398, 419, 464, and 497.

A series of optimization experiments was conducted in which concentrations of template, DNA, primers, dNTPs, and Taq polymerase were varied to establish the conditions that produced intense and reproducible banding patterns. In addition, for the first 90 samples, each sample was reextracted and reassayed at different times to establish reproducibility of RAPD patterns. Only intensely stained and consistent markers were considered. Once the conditions that produced identical patterns in the assays were determined, the remainder of the samples were extracted and amplified once. Negative controls were routinely used to check for possible contamination, as were positive controls to ensure reproducibility among reactions.

Mixtures for PCR (25 μL) contained 25 ng DNA, 50 mM KCl, 10 mM Tris, 0.1 mM each of dATP, dCTP, dTTP, and dGTP, 0.2 μM of a single primer, 1.75 mM $MgCl_2$, and 1 unit of Taq polymerase (Gibco BRL). Amplification was performed in a DNA Thermal Cycler 9700 (Perkin-Elmer-Cetus, Norwalk, Conn.) for 46 cycles. Each cycle consisted of denaturation at 94°C for 20 s, annealing at 35°C for 1 min, followed by a 3-min rise to 72°C and primer elongation at 72°C for 1 min. The final primer-elongation segment was extended to 10 min. Approximately 15 μL of the amplified products was loaded onto a 1.5% agarose gel and separated by electrophoresis in TAE buffer (1.6 M Tris, 0.8 M acetic acid, 40 mM EDTA) at 5 V/cm. Amplification products were visualized on a UV transilluminator after staining with ethidium bromide and photographed with Kodak PLUS-X pan film (Eastman Kodak, Rochester, N.Y.).

Genetic Relationships

In this study, populations were defined as groups of plants that originated from the same farm, seed lot, or geographic area (for the cultivated and natural populations). For the seeds, derived from 21 mature inflorescences, a maternal family was defined as all of the progeny of seed embryos derived from a

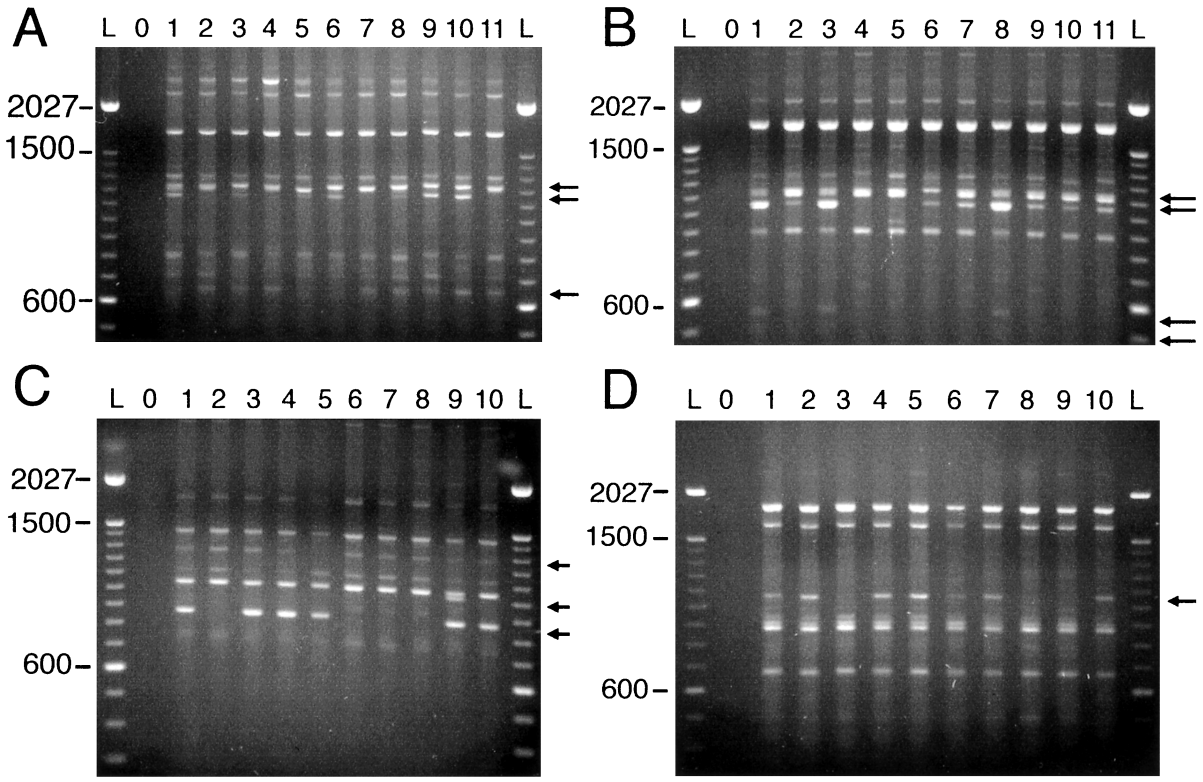

Fig. 3 RAPD amplification of *Panax quinquefolius* samples. *A*, Cultivated ginseng from Ontario (#2) with UBC primer 18. *B*, Cultivated ginseng from Ontario (#2) with UBC primer 262. *C*, Segregation of markers in unbagged ginseng maternal families, UBC primer 177. Lane 1, parent plant; lanes 2–10, progeny. *D*, Segregation of markers in bagged ginseng maternal families, UBC primer 6. Lane 1, parent plant; lanes 2–10, progeny. *L*, 100-bp ladder (Gibco BRL). 0, negative control. Arrows show polymorphic bands.

single plant. Polymorphic bands were scored as present (1) or absent (0), and each fragment was named by the primer number and its approximate size in base pairs. Genetic distance (GD) was estimated based on both the simple matching coefficient (Grower 1972) and Jaccard's similarity coefficient (Jaccard 1908) such that GD = 1 − similarity, for two subsets of the data: (1) among 227 individual plants from cultivated fields and seed lots (excluding selected populations RD-S and RD-S2) and (2) among 296 individuals collected from mature inflorescences of 21 plants (maternal families). The resulting distance matrices were fitted in two dimensions using the monotonic multidimensional scaling (MDS) procedure in S-PLUS 2000 Professional Release 2 (MathSoft, Cambridge, Mass.). Dendrograms were created with UPGMA (unweighted pair group method with arithmetic averaging) cluster analysis using the program Neighbor from PHYLIP version 3.57c (Felsenstein 1995) and visualized with TREEVIEW version 1.5 (Page 1996).

Partitioning of Genetic Variability

Estimates of diversity and partitioning of genetic variability within and among populations were calculated as described by Bussell (1999). Selected populations (RD-S and RD-S2) were excluded from this analysis, and natural and cultivated populations were examined separately. Shannon's Index for each RAPD locus was calculated for each population as $H'_i = -\Sigma p_i \log_2 p_i$, where p_i is the frequency of the presence or absence of a RAPD band in that population. The average diversity over all (cultivated or natural) populations was calculated for each locus as $H'_{pop} = 1/n\Sigma H'_j$, where n is the number of populations (i.e., 3 for natural populations, 25 for cultivated populations). The species diversity was calculated for each locus as $H'_s p = -\Sigma p_s \log_2 p_s$, where p_s is the frequency of presence or absence of the RAPD in the whole sample (i.e., 58 individuals for natural populations, 227 individuals for cultivated populations). The component for diversity within populations is H'_{pop}/H'_{sp}, and the component between populations (G'_{ST}) is $(H'_{sp} - H'_{pop})/H'_{sp}$. The overall G'_{ST} was calculated from the average per-primer values for H'_i, H'_{pop}, and H'_{sp}. G'_{st}, the average of the $(H'_{sp} - H'_{pop})/H'_{sp}$ values for each polymorphic locus, was also calculated.

Estimates of diversity and partitioning of genetic variability within and among maternal families were also conducted, as described above, in order to examine the level of genetic diversity found within individual families. For this analysis, families from bagged inflorescences were examined separately from families originating from nonbagged inflorescences. The entire sample size consisted of 95 individuals originating from bagged inflorescences and 201 individuals originating from nonbagged inflorescences. The number of populations (n) was 5 and 16 for families originating from bagged and nonbagged inflorescences, respectively.

Table 2

Mean Genetic Distance (GD) Values within and among Different Populations of *Panax quinquefolius*

Origin	Region	GD within populations	GD among populations
Cultivated	North America	0.31	…
Cultivated	British Columbia	0.30	…
Cultivated	Ontario	0.32	…
Cultivated	Nova Scotia	0.21	…
Cultivated	Wisconsin	0.29	…
Natural (population W1)	Quebec	0.33	…
Natural (population W2)	Quebec	0.12	…
Natural (population W3)	Quebec	0.19	…
Natural	Quebec	0.27	…
Cultivated (selected for large root)	British Columbia	0.27	…
Cultivated (progeny from selected plants)	British Columbia	0.25	…
Cultivated (nonbagged maternal families)	British Columbia	0.18[a]	…
Cultivated (bagged maternal families)	British Columbia	0.11[a]	…
Cultivated	Ontario vs. British Columbia	…	0.32
Cultivated	Ontario vs. Nova Scotia	…	0.28
Cultivated	Ontario vs. Wisconsin	…	0.35
Cultivated	British Columbia vs. Nova Scotia	…	0.27
Cultivated	British Columbia vs. Wisconsin	…	0.34
Cultivated	Nova Scotia vs. Wisconsin	…	0.30
Cultivated vs. natural	North America vs. Quebec	…	0.43

Note. Values are estimated as the complement to the simple matching coefficient.
[a] Calculated as the mean population genetic distance.

Population Integrity

To complement the analysis, populations comprising cultivated, selected, natural, sibling (maternal families), and regional samples were compared in a pairwise fashion for differences in individual marker frequencies. Following the method proposed by Boehm et al. (1999), the genetic differences calculated with the simple matching coefficient, Jaccard's similarity coefficient, and Shannon's Index were verified by comparing marker frequencies on a marker-by-marker basis. The significance of these observed frequency differences was tested with a χ^2 test of goodness-of-fit (Snedecor and Cochran 1967). Populations were evaluated in a pairwise fashion for each polymorphic marker. Under the null hypothesis, the mean marker frequency of the two populations in each pairwise test became the expected marker frequency. χ^2 values and degrees of freedom were pooled over all polymorphic markers. Markers were defined as polymorphic if their frequencies were not fixed (i.e., 1 or 0).

Results

DNA Quality

The average yield of total DNA was determined to be ca. 42 ng/mg fresh leaf mass or seed embryo mass. For most samples, the A_{260}/A_{230} ratio was near or above 1.0, indicating little polysaccharide or polyphenol contamination. The A_{260}/A_{280} ratio for most samples was between 1.5 and 1.8, indicating an acceptable level of protein contamination. Samples with lower absorbance ratios ($A_{260}/A_{280} < 1.3$) usually amplified well once the DNA had been diluted to 5 ng/μL.

Primer Selection

A total of 126 primers was initially used in this study. Fifty-eight gave weak or no amplification, 28 gave scorable monomorphic amplification patterns, and 40 yielded 1–4 scorable polymorphic RAPD bands ranging in size from 500 to >2027 bp. Of the 40 primers, 15 that generated 35 highly reproducible polymorphic RAPD markers were selected for further analysis of the samples.

Diversity Analysis

Considerable phenotypic variation was observed among ginseng sampled from a commercial field (fig. 1*A*). Individual plants within a field showed differences in leaf morphology (fig. 1*B*). RAPD profiles (figs. 2, 3) and genetic distance values within and among different populations (table 2) reflected the apparent phenotypic variation and indicated that there was a high level of genetic diversity within and among natural, cultivated and regional populations of *Panax quinquefolius*. RAPD profiles among the three different species of *Panax* were very different and clearly indicated that genetic distance among species was much higher than within (data not shown). Distance values within cultivated populations ranged from 0.21 for a population originating from Nova Scotia (#6) to 0.34 for an Ontario population (Knox) with a mean of 0.30 (data not shown). An MDS of the 227 × 227 matrix of genetic distance values for all cultivated samples (minus those selected for large roots and maternal families) did not reveal any clusters associated with a particular population or geographic region (fig. 4*A*). A χ^2 analysis for population band frequency comparison indicated that some populations were significantly different from each other (table 3); however, there were no

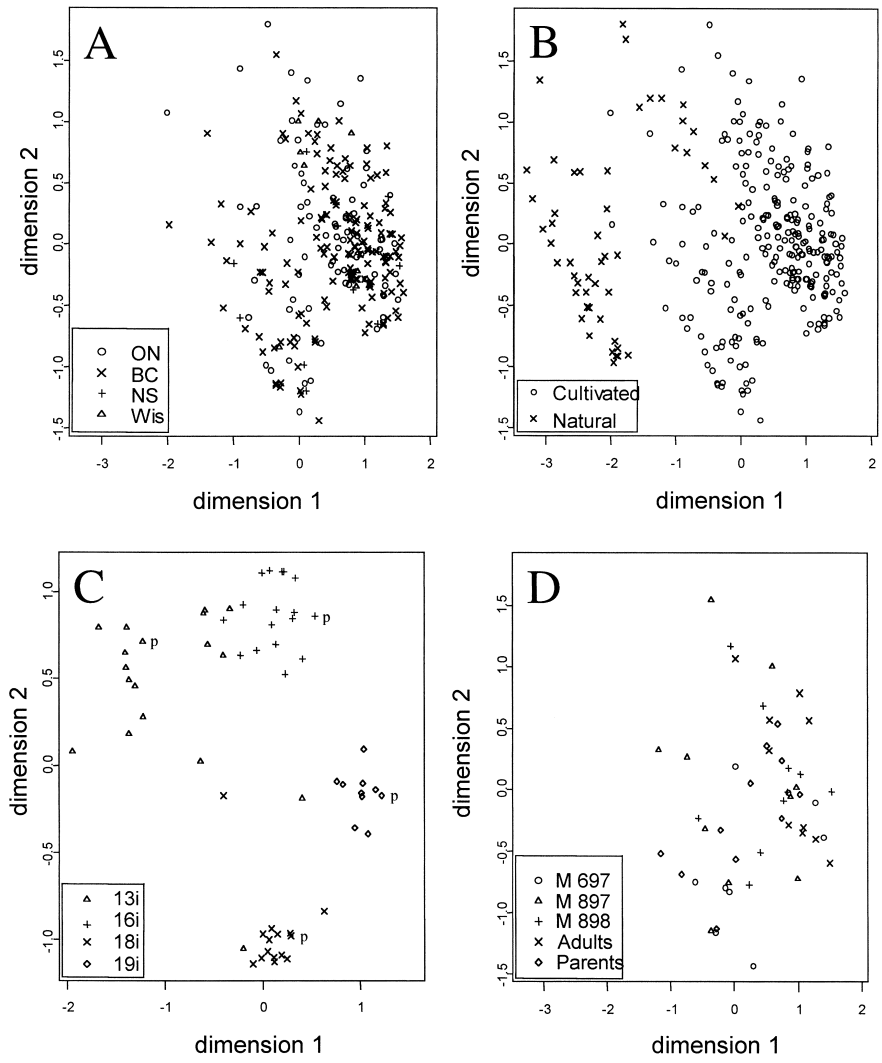

Fig. 4 Multidimensional scaling plot of the genetic distance values for individual *Panax quinquefolius* plants showing distance within and between populations. *A*, Samples from four geographic regions. *B*, Cultivated and natural populations. *C*, Four maternal populations. Parent plants are indicated with "p." *D*, Five Merritt, British Columbia, populations collected at various times.

significant differences ($P \geq 0.05$) between cultivated samples from different Canadian regions (table 6). There was a significant difference between the Wisconsin population and all Canadian regional populations (table 6); however, average genetic distance among different cultivated growing regions (fig. 5) were similar to mean genetic distances within populations (table 2).

To determine the possible effects of selection for improved horticultural characteristics on genetic diversity, a population of 40 individuals (RD-S) sown from the seeds collected from 100 selected plants displaying large root size was analyzed, as well as 20 of their progeny (RD-S2). The genetic distances within the RD-S and RD-S2 populations were 0.28 and 0.25, respectively. These values were very similar to distance values within other cultivated populations, including a population (RD) originating from the same farm where no selection had been made (table 2). A χ^2 analysis showed no significant difference between the selected population (RD-S) and the non-selected population (RD); however, there was a significant difference between populations RD and RD-S2.

Two of the natural populations from Quebec had less genetic variation than cultivated populations in British Columbia, Ontario, Nova Scotia, and Wisconsin, while one was similar (table 2). Distance values ranged from 0.12 to 0.33 with a mean of 0.21, and the overall natural population genetic distance (calculated using all 58 individuals) was 0.27 (table 2). Interestingly, one RAPD marker (UBC 98-650) was found to be polymorphic within the natural populations but monomorphic within all cultivated populations (fig. 2). MDS analysis of the genetic distance matrix for all samples showed that plants from natural populations were genetically distinct from cultivated plants (fig. 4*B*). This was also confirmed by χ^2 analysis that indicated significant differences were present between cultivated and natural populations (table 6). The MDS plot did not separate the three natural populations from one another;

Table 3

χ^2 (Below Diagonal) and *P* Values (Above Diagonal) for Cultivated Ginseng Population Band Frequency Comparisons

	AS	#5	M697	M897	M898	Adults	CC	L	L4	L5	Wal	Knox	Ont D	Ont 3	Ont 4	#1	#2	#3	#4	#6	Wis	RD
AS		0.0416	0.999	0.6607	0.3019	0.7811	0.995	0.3913	0.9524	0.9231	0.987	0.5008	0.6253	0.9171	0.9953	0.7998	0.9939	0.8181	0.9734	0.9996	0.9424	0.7099
#5	50.73		0.0006	0.202	0.2588	0.6625	0.1117	0.0055	0.1185	0.3027	0.1476	0.0029	0.0009	0.0721	0.1386	0.1046	0.1737	0.2635	0.0013	0.0044	0.0132	0.0002
M697	14.73	68.57		0.222	0.011	0.0948	0.3556	0.0004	0.2578	0.1774	0.0849	0.0004	0.0008	0.5723	0.8547	0.1282	0.1185	0.0078	0.2799	0.7328	0.5186	0.0919
M897	31.02	41.71	53.76		0.1556	0.3189	0.3209	0.0002	0.026	0.563	0.5298	0.0073	0.0392	0.5367	0.2221	0.171	0.4545	0.2649	0.1642	0.3693	0.0159	0.0032
M898	38.82	39.97	56.92	43.41		0.687	0.0492	0.0082	0.1894	0.699	0.1017	0.0047	0.3221	0.3633	0.8442	0.1958	0.0828	0.3702	0.0522	0.3556	0.0148	0.0347
Adults	28.31	30.98	46.36	38.38	30.46		0.5133	0.19	0.4405	0.8669	0.3561	0.004	0.1683	0.6166	0.8397	0.2422	0.266	0.7472	0.0042	0.4611	0.1425	0.0518
CC	17.20	56.66	37.49	38.33	49.88	34.06		0.2155	0.2345	0.7562	0.9059	0.0135	0.1886	0.9277	0.9953	0.5728	0.7709	0.3384	0.2254	0.8237	0.6508	0.6782
L	36.67	59.87	70.32	73.16	58.22	42.12	41.27		0.8133	0.0744	0.1875	0.0825	0.343	0.1452	0.1042	0.3137	0.0501	0.5625	0.0025	0.1068	0.0094	0.3089
L4	22.32	45.07	40.00	53.02	42.14	35.59	40.68	27.49		0.7746	0.5474	0.2934	0.6171	0.8033	0.748	0.9342	0.2202	0.7638	0.1534	0.6721	0.15	0.472
L5	23.85	38.79	42.57	33.04	30.2	25.94	28.91	47.71	28.47		0.7506	0.0615	0.3676	0.969	0.985	0.8666	0.6127	0.6721	0.1096	0.9970	0.2973	0.5518
Wal	19.05	43.74	46.98	33.72	45.96	37.48	24.57	42.21	33.36	29.04		0.0122	0.2957	0.9407	0.6181	0.9492	0.9925	0.5386	0.4489	0.6089	0.5537	0.0495
Knox	34.32	62.53	69.90	58.72	60.50	61.15	56.02	47.14	39.03	48.73	56.46		0.0024	0.0153	0.0166	0.3035	0.0029	0.042	0.0042	0.0974	0.0002	0.0000
Ont D	31.76	66.89	67.26	51.03	38.30	42.91	42.17	37.79	31.93	37.21	38.97	63.29		0.4602	0.3877	0.6664	0.482	0.9268	0.3241	0.5206	0.002	0.4725
Ont 3	24.11	47.88	32.85	33.58	37.31	31.94	23.64	43.84	27.75	21.12	22.99	55.45	35.17		0.9174	0.9386	0.9411	0.6292	0.2638	0.9893	0.1665	0.4692
Ont 4	17.07	44.13	26.32	41.06	26.63	26.76	20.02	45.82	29.10	19.37	31.91	55.1	36.75	24.10		0.7742	0.9375	0.7104	0.3522	0.984	0.9684	0.7243
#1	27.84	45.8	44.60	42.81	41.92	40.45	32.84	38.51	39.03	25.95	22.9	38.77	30.9	23.1	28.48		0.8181	0.9707	0.8923	0.9373	0.0652	0.0837
#2	17.56	42.71	45.07	35.29	47.12	39.77	28.56	49.79	23.30	32.02	17.93	62.46	34.71	22.92	23.16	27.36		0.8685	0.5391	0.6224	0.4156	0.0392
#3	27.36	39.84	58.40	39.80	37.15	29.12	37.90	33.05	41.12	30.78	33.54	50.69	23.68	31.68	29.95	20.97	25.84		0.1846	0.5567	0.0503	0.0128
#4	20.73	65.70	39.39	43.07	49.58	61.01	40.96	63.04	28.73	45.53	35.41	61.00	38.25	39.83	37.52	25.08	33.53	42.31		0.6493	0.0495	0.0077
#6	13.44	60.83	29.45	37.17	3.49	35.15	27.21	45.68	30.78	16.32	32.1	46.21	33.91	18.65	19.52	23.17	31.82	33.17	31.26		0.3629	0.4512
Wis	22.90	56.14	32.72	55.28	55.62	43.96	31.23	57.59	43.64	38.93	33.23	73.39	63.94	42.98	21.17	48.42	36.13	49.77	49.85	37.32		0.4353
RD	29.96	73.50	46.54	62.07	51.63	49.62	30.65	38.63	34.92	33.27	44.85	93.47	34.91	34.98	29.64	47.06	51.03	56.27	58.47	35.36	35.7	

Note. Underlined values are significant at $P < 0.05$; df = 35.

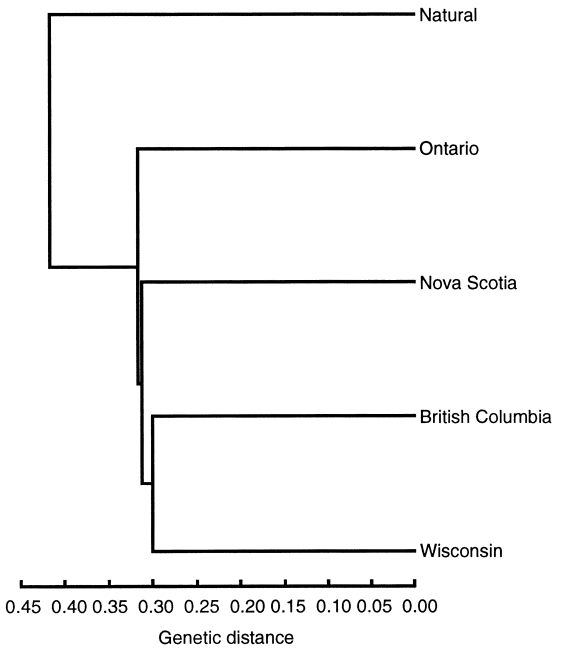

Fig. 5 Dendrogram showing genetic distance (estimated as the complement to the simple matching coefficient) between cultivated populations from four growing regions (Ontario, Nova Scotia, British Columbia, Wisconsin) and natural populations from one growing region (Quebec).

however, χ^2 analysis revealed that the three populations differed significantly from one another (table 6).

Maternal families had less genetic diversity compared to all other cultivated populations (table 2). These families were characterized by being monomorphic for as many as 13 (population 3i) of the 15 primers evaluated (fig. 6). This was in contrast to natural and cultivated populations, which were monomorphic for only 0–5 of the 15 primers evaluated (fig. 6). MDS analysis indicated that siblings tended to cluster (fig. 4*C*), while individual field-gown plants from the same farm chosen at random did not (fig. 4*D*). A χ^2 analysis supported this finding, with significant differences among nearly all maternal families (tables 4, 5). However, some genetic diversity was still observed within these families. Distance values ranged from 0.04 (population 3i) to 0.21 (population 13i) with a mean of 0.17 for families in which the inflorescences were nonbagged, and from 0.06 (population 19i-B) to 0.17 (population 15i-B) with a mean of 0.11 for families from bagged inflorescences (data not shown).

Partitioning of Genetic Variability

Shannon's Index was used to partition the diversity of the various ginseng populations (or families) into within- and among-population (or family) components. For cultivated populations, maternal families and plants selected for large root size were excluded from the calculations. In both cultivated and natural populations, most of the RAPD variation occurred within rather than among populations. G'_{ST} ($[H'_{sp} - H'_{pop}]/H'_{sp}$) was 18.0% and 28.0% for cultivated and natural populations, respectively, when calculated from totaled H'_{pop} and H'_{sp} scores (tables 7, 8), as well as when calculated as G'_{st} (data not shown). For maternal families, most of the variation occurred between families. G'_{ST} was 56.1% when the inflorescence was not bagged and 57.0% when the inflorescence was bagged (data not shown).

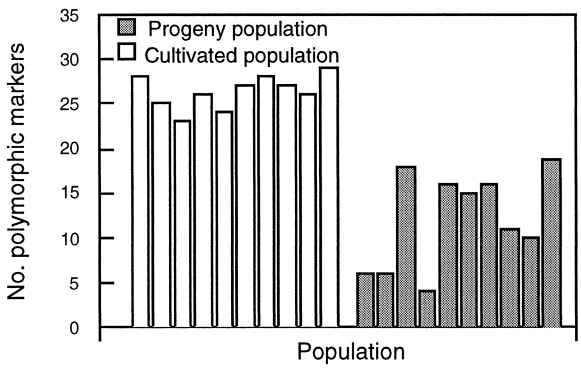

Fig. 6 Number of polymorphic RAPD markers in 10 cultivated populations (M697, adults, M897, CC, L, Wal, L4, L5, M898, 1i–11i parent plants) and 10 maternal families (1i–6i, 8i, 10i–12i). Sample size n = 9 and 10 for maternal families and cultivated populations, respectively.

Sample Size

To determine the effects of sample size on diversity estimates, eight populations (L, CC, 9i, #1, #2, W1, W2, W3) selected at random were analyzed based on either 10 or 20 individuals each. For all cultivated populations, within-population genetic diversity was comparable for both data sets (differences were in the range of 0.01–0.05). For example, within-population genetic diversity values for population L were estimated to be 0.30 using 10 plants and 0.31 using 20 plants. In natural populations, sample size affected the diversity measures in one of the populations (W2). Within-population genetic diversity values for populations W1, W2, and W3 were 0.31, 0.06, and 0.19 for 10 plants and 0.33, 0.12, and 0.19 for 20 plants, respectively.

Discussion

Our study reports the extent of genetic variation within and among cultivated and natural North American ginseng populations and among progeny from individual plants (maternal families). We confirm previous reports of the presence of a high level of genetic diversity among North American ginseng plants, both cultivated and noncultivated (Bai et al. 1997; Boehm et al. 1999). This study has provided additional insight into the reproductive biology of this medicinal plant and of the potential impact of ginseng breeding programs on genetic diversity.

Genetic distance was measured with both the simple matching coefficient and Jaccard's coefficient of similarity. These coefficients consider RAPD bands as phenotypic rather than genetic characters and consider individuals that possess a band in common (Jaccard's) and/or lack a common band (simple matching) to be genetically similar. Nei's genetic distance index was not appropriate for this analysis because it is based on

Table 4

χ^2 (Below Diagonal) and *P* Values (Above Diagonal) for Ginseng Nonbagged Maternal Family Band Frequency Comparisons

	1i	2i	3i	4i	5i	6i	7i	8i	9i	10i	11i
1i		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2i	129		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3i	174	125		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4i	141	126	154		0.000	0.064	0.000	0.000	0.000	0.000	0.000
5i	170	136	150	110		0.000	0.000	0.000	0.000	0.000	0.000
6i	172	141	151	48.5	98.3		0.007	0.000	0.000	0.000	0.000
7i	160	116	82.8	93.3	82	67.8		0.006	0.000	0.000	0.000
8i	146	140	117	117	97.9	126	59.6		0.000	0.000	0.000
9i	86.4	124	123	104	117	132	77.8	93.4		0.000	0.000
10i	98.2	134	159	111	138	162	102	98.4	103		0.000
11i	71.7	135	181	137	115	172	139	132	98.4	114	

Note. Underlined values are significant at $P < 0.05$; df = 35.

allelic frequencies (genetic data) that cannot be accurately determined from RAPD markers. With most RAPD markers, heterozygous individuals cannot be distinguished from homozygous dominant individuals (Bartolozzi et al. 1998; Bussell 1999). The simple matching coefficient provides more information regarding phenotypic similarity among pairs of individuals while Jaccard's coefficient (by considering only the proportion of shared positive bands) reduces the risk of overestimating genetic similarity because, on average, shared bands are more likely than nulls to represent homologous alleles (Grosenberg et al. 1996). Relative genetic distances and overall spatial relationships within MDS plots were very similar regardless of the coefficient used. Therefore, results obtained from the simple matching coefficient were presented. The simple matching coefficient appears to be appropriate when very closely related organisms are the subject of study, and shared absence of bands can be assumed to be as informative as shared presence (Apostol et al. 1993; Bussell 1999).

The dominant nature of RAPDs makes them unsuitable for estimation of population genetic parameters such as *F*-statistics and G'_{ST} unless assumptions are made regarding levels of selfing and null homozygote frequencies and their relationships to heterozygosity (Clark and Lanigan 1993; Lynch and Milligan 1994; Bussell 1999). Another method for partitioning variation, analysis of molecular variance (AMOVA; Excoffier et al. 1992) requires the same assumptions (Bussell 1999). To circumvent this problem, we used Shannon's Index for partitioning genetic diversity into within- and among-population components because it is relatively insensitive to skewing effects caused by undetected heterozygous loci (Dawson et al. 1995).

Genetic distances within cultivated ginseng populations (range of 0.29–0.34) in this study were higher than values of 0.14–0.16 reported by Boehm et al. (1999), but values within natural populations (range of 0.12–0.33) were similar to values of 0.13–0.24 reported previously (Boehm et al. 1999). Overall genetic similarity (the opposite of genetic distance) based on Jaccard's coefficient (data not shown) in cultivated 3–5-yr-old populations (mean = 0.58) was higher than the value of 0.40 reported by Bai et al. (1997) for 24 randomly selected 3-yr-old cultivated plants but is similar to the value of 0.53 reported for selected large-sized 3-yr-old plants (Bai et al. 1997). In these previous studies (Bai et al. 1997; Boehm et al. 1999), the number of polymorphic bands detected was larger than the 35 used in this study. Since our genetic distance values were within the range reported in previous studies, the number of markers used appears to have been sufficient to detect genetic variation in the populations sampled.

Although there was no significant difference in marker frequency for cultivated populations in different Canadian geographical regions (British Columbia, Ontario, Nova Scotia), there were significant differences between some farms and seed lot populations (tables 3 and 6). A χ^2 analysis also showed that there were significant differences between the three natural

Table 5

χ^2 (Below Diagonal) and *P* Values (Above Diagonal) for Ginseng Bagged Maternal Family Band Frequency Comparisons

	12i	13i	14i	15i	16i	17i	18i	19i	20i	21i
12i		0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000
13i	231		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
14i	217	277		0.000	0.000	0.000	0.000	0.000	0.000	0.000
15i	56.1	246	280		0.000	0.000	0.000	0.000	0.000	0.000
16i	117	234	285	212		0.000	0.000	0.000	0.000	0.000
17i	190	258	223	225	185		0.000	0.000	0.000	0.000
18i	295	299	177	328	369	276		0.000	0.000	0.000
19i	160	394	289	172	314	211	295		0.000	0.000
20i	204	257	184	282	270	272	374	369		0.000
21i	244	242	258	322	335	213	291	271	237	

Note. Underlined values in bold significant at $P < 0.05$; df = 35.

populations (table 6). All RAPD markers recorded in this study were shared by all populations. Thus, the variation among populations was detected as differences in marker frequencies rather than the presence of unique markers. The genetic distance values and marker frequencies of a population of progeny from 100 plants selected for large roots (RDS) were very similar to those of a population of cultivated plants from the same farm that had not undergone selection (RD). Without further selection, genetic diversity and marker frequency in the next generation (RD-S2) also remained very similar. These findings indicate that one cycle of selection followed by bulking of seeds did not significantly reduce genetic variation.

Two of the three natural populations had a lower level of genetic diversity than cultivated populations, indicating that *Panax quinquefolius* populations under cultivation have not undergone a reduction in genetic diversity, perhaps because of a bulking and mixing of seed from different geographic locations. However, there was one marker (98-650) that was polymorphic only in natural populations, indicating that natural populations have distinct genetic differences from cultivated populations. Therefore, current efforts to preserve natural populations have some merit.

Genetic variation and its partitioning among and within populations of a plant species is determined by several factors, such as breeding system (outcrossing vs. selfing), seed dispersal mechanisms, life form (annual vs. perennial), and geographic range (Hamrick and Godt 1996). Life form and breeding systems in particular can have significant influences on genetic diversity and its distribution (Hamrick and Godt 1996). Data summarized from allozymes and RAPDs have provided an average $G'_{ST} < 19\%$ for outbreeding species, 21.2%–24.0% for species with a mixed mating system, 58.7%–59.6% for inbreeding dicots, and 41.2% for inbreeding monocots (Hamrick and Godt 1996; Bussell 1999). For cultivated populations of ginseng, most of the RAPD variation was detected within and not among populations ($G'_{ST} = 18.0\%$). This pattern could be explained by the movement of seeds between growers within and among the major production areas, which effectively translates to a high degree of gene flow among populations and minimizes their genetic differences.

Table 6

χ^2 Values for Ginseng Natural, Regional, and Natural versus Cultivated Population Band Frequency Comparisons

Comparison	χ^2	P
W1 vs. W2	245.49	0.000
W2 vs. W3	118.87	0.000
W1 vs. W3	184.95	0.000
ON vs. BC	46.10	0.099
ON vs. NS	48.73	0.062
ON vs. Wis	119.74	0.000
BC vs. NS	48.99	0.059
BC vs. Wis	129.94	0.000
NS vs. Wis	70.94	0.000
Canada vs. Wis[a]	112.59	0.000
Cultivated[b] vs. natural[c]	1234.22	0.000

Note. df = 35.

[a] All Canadian cultivated populations (excluding maternal families and populations selected for large roots).

[b] All cultivated populations (excluding maternal families and populations selected for large roots).

[c] All natural populations (W1, W2, and W3).

Table 7

Partitioning of Genetic Diversity Generated by 15 RAPD Primers into Within- and Among-Population Components for Three Natural Populations of *Panax quinquefolius*

Primer (UBC)	H'_{pop}	H'_{sp}	H'_{pop}/H'_{sp}	$1 - H'_{pop}/H'_{sp}$
81	1.355	1.842	0.736	0.264
98	1.369	2.401	0.570	0.430
177	2.116	2.513	0.842	0.158
227	1.768	1.818	0.972	0.028
262	2.101	3.805	0.552	0.448
419	0.402	0.420	0.957	0.043
464	0.095	0.122	0.781	0.219
497	0.582	0.722	0.806	0.194
6	0.241	0.353	0.683	0.317
18	1.123	1.759	0.638	0.362
164	0.909	1.268	0.717	0.283
203	1.517	1.784	0.850	0.150
210	0.921	1.307	0.705	0.295
326	0.380	0.444	0.856	0.144
398	0.311	0.518	0.599	0.401
Mean	1.057	1.468	0.720	0.280

Maternal families had less genetic diversity compared with other cultivated populations (table 2), and G'_{ST} values (56.1% and 57.0%) indicated that siblings are more genetically similar to each other than they are to unrelated individuals. This indicates that distinct genetic differences could begin to develop within ginseng populations if breeders implemented controlled self-fertilization and developed cultivars for improved agronomic traits in specific regions. However, G'_{ST} values for maternal families also indicated that there is some degree of genetic diversity among siblings. Segregation of RAPD markers among the progeny of both bagged and nonbagged maternal families was observed, indicating that parental plants were likely heterozygous and that some level of outcrossing may occur. The G'_{ST} in natural populations was estimated at 28.0%, a value common to species with a mixed mating system (Hamrick and Godt 1996). In a previous study, indirect estimates of the mating system in *P. quinquefolius* were made by calculating the outcrossing indices (Schlessman 1985). It indicated that flowers of *P. quinquefolius* are morphologically adapted for a mixed mating system of autogamy (pollination within flowers on the same umbel) and xenogamy (pollination between flowers on different umbels) (Schlessman 1985). In the same study, inflorescences that were emasculated to enforce xenogamy produced almost the same proportion of seeds as those that were bagged and not emasculated (Schlessman 1985).

In *Medicago truncatula*, a self-pollinated legume, high intrapopulation variability for RAPD markers was attributed to rare outcrossing events (Bonnin et al. 1996), and a similar situation may be present in *P. quinquefolius*. Theoretical models on the relative frequencies of self-and cross-fertilization have postulated that inbreeding depression in adults diminishes some of the advantages associated with selfing, especially in

Table 8

Partitioning of Genetic Diversity Generated by 15 RAPD Primers into Within- and Among-Population Components for 25 Cultivated Populations of *Panax quinquefolius*

Primer (UBC)	H'_{pop}	H'_{sp}	H'_{pop}/H'_{sp}	$1-H'_{pop}/H'_{sp}$
81	0.743	0.984	0.755	0.245
98	0.847	1.304	0.650	0.350
177	2.285	3.094	0.739	0.261
227	1.851	2.264	0.818	0.182
262	3.150	4.072	0.774	0.226
419	0.840	0.855	0.982	0.018
464	0.662	0.919	0.720	0.280
497	0.377	0.581	0.649	0.351
6	0.873	0.960	0.909	0.091
18	2.873	3.443	0.834	0.166
164	1.343	1.473	0.912	0.088
203	0.594	0.941	0.631	0.369
210	1.529	1.684	0.908	0.092
326	0.984	0.989	0.995	0.005
398	0.843	0.924	0.912	0.088
Mean	1.346	1.641	0.820	0.180

long-lived perennials that experience inbreeding depression over many seasons (Morgan et al. 1997). The models indicate that a perennial life history should maintain a certain level of outcrossing (Morgan et al. 1997; Zhang 2000). In this study, genetic variation values among progeny from bagged versus nonbagged plants were not markedly different. Segregation of RAPD markers was also observed in the first generation selfed progeny of bagged plants, indicating that heterozygosity in the selfed parent plant and not cross-pollination from another plant was the probable explanation. Experiments with controlled crosses between plants with distinct RAPD markers should clarify the contributions of heterozygosity and cross-pollination to genetic diversity in this species.

The level of genetic diversity currently present in cultivated ginseng populations indicates that selection for desirable traits could be conducted based on the existing germplasm base. Several cycles of self-fertilization would be required to stabilize the traits, and it is not known if inbreeding depression would result. Crossing of homozygous lines could produce desirable hybrids with an appropriate complement of traits while producing heterozygosity.

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