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Allozyme variation in American ginseng (*Panax quinquefolius* L.): Variation, breeding system, and implications for current conservation practice

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Abstract

Sustained harvest of wild North American ginseng (*Panax quinquefolius* L.) for over two centuries has led to heightened conservation concern and a recent interest in the population genetics of this species. This study examined allozyme variation from 32 wild and 12 cultivated populations of American ginseng to: (1) document the amount and distribution of genetic variation over a wide portion of the species' natural range, (2) examine genetic differences between wild and cultivated populations, and (3) provide indirect estimates of its breeding system. Strong genetic differences between wild and cultivated populations were found in the amount of variation within populations and the distribution of variation among populations. Wild populations were significantly lower in all within-population diversity measures, but contained significantly higher levels of variation partitioned among populations. Similarities between wild and cultivated populations were also found. As a whole, cultivated and wild groups shared nearly all alleles, and populations of both groups showed strong homozygote excess compared to expectations under random mating. The homozygote excess is best explained by high levels of selfing. In wild populations, significant correlations were found between genetic diversity and estimated population size, and between interpopulation genetic distance and geographic distance. Overall, the results for wild populations suggest that they are influenced by high levels of genetic drift and low migration among populations. Conservation implications for American ginseng are discussed with particular emphases on: (1) the current debate surrounding the existence of wild populations, (2) the ongoing practice of introducing cultivated seed into wild populations, and (3) the collection of genetic material for the establishment of breeding programs.

Introduction

North American ginseng, *Panax quinquefolius* L. (Araliaceae), is a widely used medicinal herb whose wild roots have been harvested and exported to international markets since 1720 (Carlson 1986). Concern over regional depletion of wild populations due to the impact of harvest was indicated as early as 1770 (Kalm 1987) and has continued to be a common theme in the literature (Michaux 1805; Stanton 1892; McGraw 2001). By the last quarter of the nineteenth century, laws limiting wild ginseng harvest were in place in Ontario, West Virginia, and Virginia (Nash 1898). State laws regulating harvest measures are now in place throughout the natural range of American ginseng (Robbins 2000), which extends from southern Quebec and Minnesota, south to Louisiana and Georgia (Anderson et al. 1993; Gleason and Cronquist 1991; Figure 1). International export has been federally regulated since 1973 when American ginseng was listed in Appendix II of the Convention on International Trade in Endangered Species (Robbins 2000). In Canada, all export of American ginseng has been banned since 1990 (Robbins

1998). Although American ginseng is considered uncommon, threatened, rare, or endangered in over 75% of its range (Schlessman 1985), 19 of the 34 US states reporting wild ginseng populations are federally approved for the international export of wild-collected roots (Robbins 2000). In addition, poaching on private lands and in national parks has become a widespread problem of growing concern (P. Ford, US Division of Scientific Authority, personal communication).

Successful cultivation of American ginseng began in the late 1880s in New York (Carlson 1986), and has since extended throughout and beyond the native range of the species (Robbins 1998; Persons 2000). There are no identified cultivars (Schluter and Punja 2000) although previous studies have characterized cultivated American ginseng as consisting of unimproved landraces (Bai et al. 1997; Boehm et al. 1999). Propagule sources for cultivation vary, including the collection of seed or transplants from the wild, other commercial farms, or previous crops. Despite a strong market for cultivated ginseng, wild roots (which are morphologically distinctive from cultivated roots) are in greater demand in the Chinese medicine market due to the belief that wild roots are more medicinally efficacious. Thus, wild roots can command prices over 40 times higher than cultivated roots (Hankins 2000).

Several papers have focused on American ginseng's population and reproductive biology, demography, ecology, and floral biology. These studies characterize American ginseng as a slow-growing herbaceous perennial (Schluter and Punja 2000) of tetraploid origin (4x = 2n = 48; Hu et al. 1980) found on moist, well-drained slopes in eastern hardwood forests (Anderson et al. 1993). It has a long pre-reproductive period of at least 3–8 years and may live longer than 20 years (Carpenter and Cottam 1982; Lewis and Zenger 1982; Schlessman 1987; Charron and Gagnon 1991; Anderson et al. 1993). When mature, it produces a solitary umbel with hermaphroditic flowers that are visited by common generalist pollinators including syrphids and halictids (Carpenter and Cottam 1982; Lewis and Zenger 1983; Schlessman 1985). In addition to outcrossing, several studies have examined whether American ginseng is capable of self-fertilization. These studies frequently report comparable or greater amounts of fruit or seed production in bagged vs. unbagged inflorescences (Carpenter and Cottam 1982; Lewis and Zenger 1983; Schluter and Punja 2000; Case and Bunn, unpublished data). Therefore, it is assumed that selfing occurs in this species (Schluter and Punja 2000), but the relative contributions of selfing vs. outcrossing in natural and cultivated populations is not known. After fertilization, the resulting fruit consists of a 1 to 3 seeded red berry, which is usually passively disseminated (Lewis and Zenger 1982). Low fecundity as well as high seed or seedling mortality is frequently found in wild populations (Hu et al. 1980; Carpenter and Cottam 1982; Lewis and Zenger 1982; Schlessman 1985; Schluter and Punja 2000).

Relatively few studies have examined the population genetics of American ginseng, and these studies have either focused entirely on cultivated populations (Bai et al. 1997) or included a small number of wild populations in studies that emphasize cultivated material (Boehm et al. 1999; Schluter and Punja 2002). All of these studies report high levels of variation at RAPD markers within cultivated material and an absence of unique genetic characteristics associated with any particular cultivated population or region of cultivation. In contrast, studies including wild populations indicate that most wild populations are genetically distinct from cultivated material (Boehm et al. 1999; Schluter and Punja 2002) with a geographical component to the genetic structure of wild populations (Boehm et al. 1999). In the present paper, allozyme analysis is applied to 32 wild and 12 cultivated populations sampled from a large portion of the natural geographic range of American ginseng. The following questions are addressed: (1) What level of allozyme variation is present and how is that variation partitioned within and among populations? (2) Are there genetic differences between wild and cultivated populations? and (3) Do inbreeding coefficients derived from a random sample of individuals in populations suggest a predominant breeding strategy? Finally, the conclusions derived from allozyme data are compared to the conclusions derived from previous studies of RAPD markers in American ginseng, and conservation recommendations based on these data are discussed.

Methods

Sampling

A total of 1177 individuals from 44 populations were examined for this study, representing 35 counties within American ginseng's native range (Figure 1). Thirty-one populations are believed to be natural

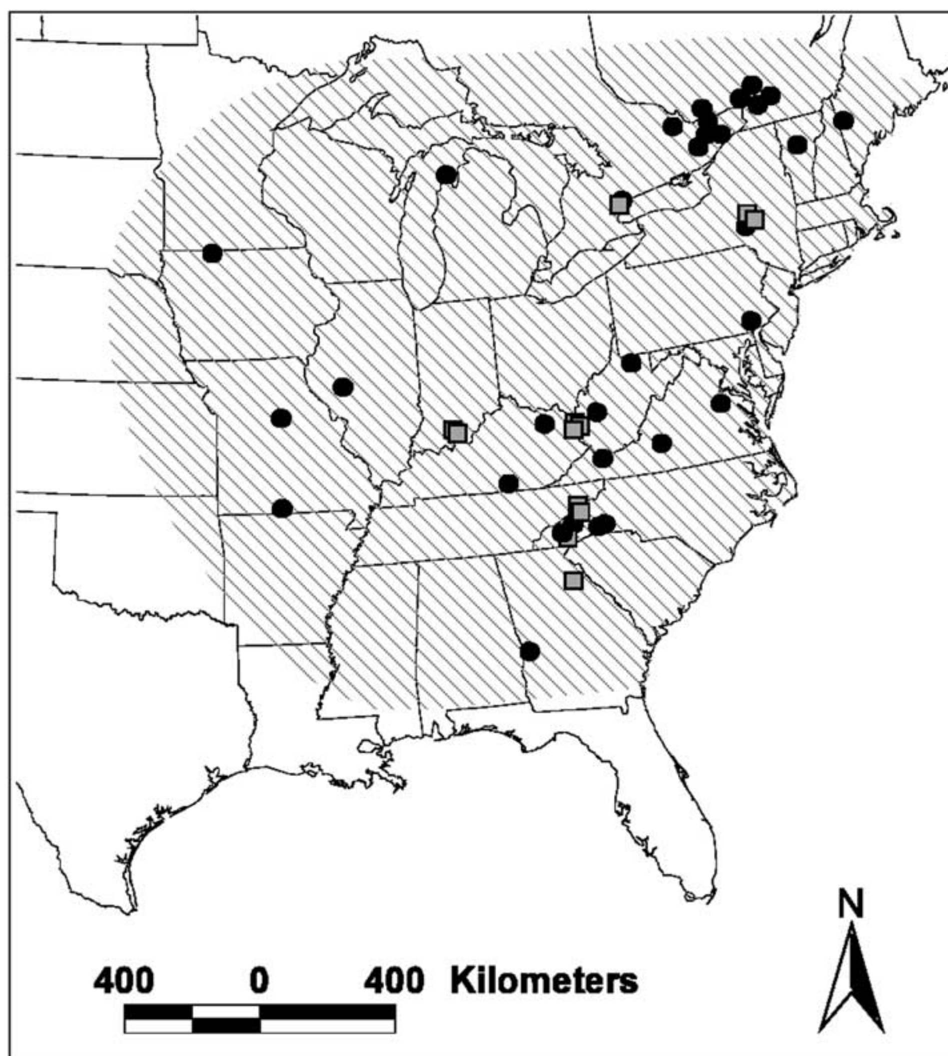

Figure 1. Map of Eastern North America showing approximate natural range of American ginseng (in cross hatching) and the locations of populations sampled for this study (circles and squares indicate wild and cultivated populations, respectively).

occurrences and one population was collected from a research plot in Ontario that was established from wild populations throughout the province. The remaining 12 populations were established through various degrees of human manipulation with diverse origin of propagules including cultivated and wild sources. All these populations were established by growers to harvest for the sale of roots and are hereafter referred to as cultivated. All populations were located with the assistance of state Heritage Programs, herbaria, state and national parks, independent botanists, and those involved directly or indirectly in the ginseng trade. For most populations, volunteers and contracting botanists were identified to collect leaf samples as well as provide estimates of population sizes by counting all plants observed at the field sites.

From each population, all individuals with two or more leaves were sampled except where populations exceeded 100 individuals. In these populations, a maximum of 100 individuals was sampled. Estimated population sizes for sampled wild populations ranged from 4 to 1,000 with a population size of 100 individuals or more in 41% of the populations. Cultivated populations tended to be much larger with 40 to >5,000 individuals per population. For each individual sampled, one to three leaflets were removed and

kept chilled on blue ice during transport to the College of William & Mary. Upon arriving at the college, samples were frozen at $-80\,^{\circ}C$ without chemical processing.

Electrophoresis

Tissue samples were ground with chilled ($4\,^{\circ}C$) mortar and pestles using Gottlieb's (1981) extraction buffer and sand to produce a slurry. The resulting slurry was absorbed onto filter paper wicks and electrophoresed on horizontal 12% starch gels at $4\,^{\circ}C$ according to the methods of Case (1993). Mean population electrophoretic sample size was 27.3 individuals (Table 1). The high degree of fixation and low levels of variation within populations suggested that further runs were unnecessary to establish reliable population allele frequency estimates. Duplicate samples of proteins with similar mobilities across different gels were electrophoresed on a single gel to determine relative mobility differences of those proteins.

Three buffer systems were used to resolve nine enzyme systems which revealed the protein products of nine polymorphic and ten monomorphic putative loci (Table 2). In each enzyme system, the fastest migrating isozymes were designated the products of locus 1 and slower loci were sequentially numbered. Thus, loci encoding the same enzyme but potentially derived from two different ancestral genomes were given separate locus designations. Data for alcohol dehydrogenase, diaphorase, glutamate dehydrogenase, malic enzyme, and phosphoglucomutase were also obtained but were not used in the statistical calculations due to inconsistent resolution or genetically ambiguous electrophoretic patterns.

Genetic interpretation and statistical analyses

Banding patterns were scored as soon as they were visible on gels. Genotypes were inferred from enzyme phenotypes based upon knowledge of the tetraploid ancestry of American ginseng and published information on the quaternary structure expected for each enzyme (Weeden and Wendel 1989; Murphy et al. 1990). Proteins from two loci, *Idh2* and *Skdh*, clearly exhibited segregating asymmetrical staining patterns in heterozygous individuals (i.e., unbalanced heterozygosity). Consequently, allele frequencies at these loci were calculated under the assumption that individuals could possess one to four copies of a specific allele at a locus. The remaining enzyme systems displayed patterns that were consistent with disomic segregation patterns at polymorphic loci and fully differentiated alleles at the putative homoeologous chromosome sets. Interlocus heterodimers in multimeric enzymes were common. Three loci, *Got2*, *Mdh1*, and *Tpi3* each expressed a single active protein that formed interlocus heterodimers with *Got3*, *Mdh2*, and *Tpi2*, respectively. While interlocus heterodimers between these three sets of loci were always present, enzyme activity of the homodimers at *Got2*, *Mdh1*, and *Tpi3* was variable; some populations were fixed for the active or inactive forms, and others were polymorphic for activity. These enzyme phenotypes are identical to those discovered in *Pellaea rufa* (Gastony 1991), which were determined through genetic analysis of segregation patterns to represent the existence of a gene product that was polymorphic for active and silenced forms. Therefore, allele frequencies at *Got2*, *Mdh1*, and *Tpi3* were calculated under the hypothesis that active and inactive alleles constituted the two allelic forms of these loci. However, it is also possible that post-transcriptional processes could produce the inactive product and not necessarily a mutation in the gene itself.

Because genotypic assumptions for *Idh2* and *Skdh* could not be made, and heterozygotes for the active and inactive allelic forms at *Got2*, *Mdh1*, and *Tpi3* could not be identified, these loci were excluded from all statistics that used observed and expected heterozygosities. These statistics included population and species-level estimates of average observed (Ho) and average expected heterozygosity (He), the average population fixation index (Fp) where $Fp = 1 - (Ho/He)$ averaged across loci, and Weir and Cockerham's (1984) F-statistics. The latter generates f (an estimator of inbreeding within populations), θ (an estimator of genetic drift between populations), and F (an estimator of the combined effects of genetic drift and inbreeding). Weir and Cockerham's F-statistics were tested for significant deviation from zero by bootstrapping over all loci using 1,000 replicates. These were calculated with the computer program Genetic Data Analysis (GDA; Lewis and Zaykin 2001) and used the polymorphic loci *Got3*, *Mdh3*, *Pgi1*, and *Pgi2*. For comparison to the F-statistics derived from genotypic data, population differentiation was also estimated from allele frequency data at all polymorphic loci using both hierarchical and nonhierarchical F-statistics of Wright expressed in the form of Nei (1973; Swofford and Selander 1989). In these estimates, the amount of subdivision (Gst)

Table 1. Mean and pooled population diversity statistics. *n* = sample size of individuals in populations; *P* = percentage of polymorphic loci (a locus is considered polymorphic if any variation is found at that locus); *A* = average number of alleles per locus; *Ho* = average observed heterozygosity; *He* = average expected heterozygosity; *Fp* = average population fixation index where $Fp = 1 - (Ho/He)$ averaged across loci. Heterozygosity values were calculated from a subset of the polymorphic loci (see methods section); *P* and *A* were calculated over all loci analyzed. Mean population values with different superscripts indicate significantly different sets of values for wild vs. cultivated populations at the significance levels indicated (Mann-Whitney *U*-tests). Means with the same superscript (a) do not have significantly different sets of values

Group	*n*	*P*	*A*	*Ho*	*He*	*Fp*
	Mean population values					
Wild (32 populations)	27.7^{a}	18.8^{a}	1.20^{a}	0.04^{a}	0.11^{a}	0.62^{a}
Cultivated (12 populations)	26.3^{a}	33.3^{b}	1.40^{b}	0.07^{b}	0.20^{b}	0.65^{a}
All populations (44 populations)	27.3	22.7	1.25	0.05	0.13	0.63
Significance	$P > 0.50$	$P < 0.001$	$P < 0.001$	$P < 0.05$	$P < 0.01$	$P > 0.90$
	Pooled population values					
Wild	886	47.4	1.63	0.04	0.47	0.91
Cultivated	291	47.4	1.57	0.08	0.31	0.74
All populations	1177	47.4	1.63	0.05	0.45	0.89

is reported as a proportion of the total variation (*Ht*) divided among populations. In the hierarchical estimate, population differentiation is subdivided into the proportion of variation within population type (i.e., cultivated vs. wild populations), and the proportion of variation between types. To visualize the genetic variance associated with wild and cultivated populations, a Principal Components Analysis (PCA) of populations using allele frequency data (Ammerman and Cavalli-Sforza 1984; James and McCulloch 1990) was performed.

For the remaining diversity and distance calculations, allele frequency data from all 19 loci were used (Table 2). These statistics included the percentage of polymorphic loci (*P*), the number of alleles per locus (*A*), and Nei's (1978) unbiased genetic distance measure calculated for all pairs of wild populations (excluding the Ontario population that originated from multiple wild populations). To examine a possible association between genetic and geographic distance, the resulting genetic distance matrix was compared to a matrix of linear geographic distances using a Mantel test (Sokal and Rohlf 1995). A total of 10,000 random permutations of one matrix against the other was performed to test the significance of the correlation. For a subset of wild populations for which population size estimates were provided (26 out of 32 wild populations), Spearman's rank correlations (r_s) were calculated for each of *P*, *A*, and *He* with estimated population size (*N*), and with sample size (*n*). The six correlation values were tested for significant ($P < 0.05$) deviations from zero (Sokal and Rohlf 1995).

The Mantel test and the PCA were conducted on the program NTSYS-pc (Rohlf 1988) whereas Wright's *F*-statistics, *P*, *A*, and *He* were calculated using the computer program BIOSYS-1 (Swofford and Selander 1989). Statistical differences between wild and cultivated populations for *P*, *A*, *Ho*, *He*, and *Fp* were tested using Mann-Whitney *U*-tests (Sokal and Rohlf 1995). The statistical significance of differences in *Ht* and *Gst* values for wild and cultivated populations were tested using Wilcoxon's Signed-Ranks test with paired observations (Sokal and Rohlf 1995).

Results

Of the 19 loci used for this analysis (Table 2) 47.4% were polymorphic at the species level (Table 1), with population level values ranging from 5.3% to 47.4% (data not shown). Alleles per locus ranged from 1.05 to 1.47 within populations, and its value was 1.63 at the species level. Average expected heterozygosity estimates of the polymorphic loci *Got3*, *Mdh3*, *Pgi1*, and *Pgi2* ranged from 0 (in 23% of the populations which were all wild) to 0.47 (in one cultivated population). Significant ($P < 0.05$) non-zero correlations were found in wild populations for population size (*N*)

Table 2. Buffer systems, resolved enzymes, and the putative controlling loci used for statistical analyses. Staining protocols followed Soltis et al. (1983) with the exception of aconitase which was modified from Murphy et al. (1990)

Buffer system and reference	Enzyme resolved on buffer system	Text abbreviation for loci used
Histidine (Gottlieb 1981)	6-Phosphogluconate dehydrogenase	*6-Pgd1*[a]; *6-Pgd2*[a]
	Isocitrate dehydrogenase	*Idh1*[a]; *Idh2*[a,c]
	Malate dehydrogenase	*Mdh1*[a,c]; *Mdh2*[a]; *Mdh3*[a,b,c]
	Shikimate dehydrogenase	*Skdh*[a,c]
Lithium-borate (Crawford 1982)	Aconitase	*Aco1*[a]; *Aco2*[a]
	Superoxide dismutase	*Sod1*[a]; *Sod2*[a]
	Triosephosphate isomerase	*Tpi1*[a]; *Tpi2*[a]; *Tpi3*[a,c]
Sodium-borate (Crawford 1982)	Glutamate oxaloacetate transaminase	*Got2*[a,c]; *Got3*[a,b,c]
	Phosphoglucose isomerase	*Pgi1*[a,b,c]; *Pgi2*[a,b,c]

[a]Loci used in statistics not requiring deviations from expected heterozygosities.
[b]Loci used in all statistics requiring deviations from expected heterozygosities (see methods section for details).
[c]Polymorphic loci.

and P ($r_s = 0.38$), and for N and He ($r_s = 0.48$). Border line significance ($P < 0.07$) was found for N and A ($r_s = 0.35$). No significant correlations were found for any of these diversity statistics and sample size ($r_s = -0.02, -0.06, 0.01$ for n with P, n with A, and n with He, respectively; $P >> 0.05$).

With the exception of one population (from Morgan Co., IL) average observed heterozygosities were consistently lower than expected values, resulting in positive and high mean population fixation indices (*Fp*). The overall mean *Fp* for wild populations (0.62) was close to the mean value for cultivated populations (0.65), and the values in the two groups did not differ significantly in rank ($P > 0.90$; Table 1). Likewise, multilocus indirect estimates of inbreeding using Weir and Cockerham's f showed comparable levels of inbreeding between wild ($f = 0.63$) and cultivated ($f = 0.64$) populations. Each value was significantly different from zero ($P < 0.01$; Table 3).

Substantial differences were found in the levels of genetic variation between wild and cultivated populations. Wild populations were significantly lower than cultivated populations for all diversity measures examined ($P = 18.8$, $A = 1.20$, average $Ho = 0.04$ and average $He = 0.11$ for wild populations vs. $P = 33.3$, $A = 1.40$, average $Ho = 0.07$, average $He = 0.20$ for cultivated populations; Table 1). Conversely, total gene diversity was not significantly different between the two groups (*Ht* for wild ginseng = 0.356; *Ht* for cultivated ginseng = 0.275; $P > 0.10$; Table 4). Weighted allele frequencies between cultivated and wild populations differed significantly ($P < 0.05$; *G*-test) at all polymorphic loci (data not shown). Only one unique allele was found between the two groups (i.e., a rare allele at *Idh2* was found exclusively in wild populations).

Wild populations also showed a higher level of variation partitioned among populations in average estimates of *Gst* (wild populations = 63%; cultivated = 24%; Table 4) as well as θ (wild populations = 78%; cultivated = 31%; Table 3). This trend was also consistent among all single locus estimates of *Gst* (data not shown) and θ (Table 3). Consequently, there were significant differences between cultivated and wild populations for *Gst* ($P < 0.01$) and significant deviations from zero for average θ ($P < 0.01$). Furthermore, pair-wise genetic distances of wild populations were significantly ($P < 0.0001$) correlated with geographic distances ($r = 0.41$).

Although wild and cultivated populations differ in their amount and distribution of genetic variation, only 3% of the total genetic variation was attributed to differences between cultivated and wild ginseng (Table 4). This can also be visualized with the PCA (Figure 2) where cultivated populations cluster within wild populations and show less variance in spatial position within the three dimensional PCA plot. The cultivated populations predominantly show variance along axis two which had character loading scores above 0.5 for *Got2*, *Pgi1*, *Pgi2*, and *Tpi3* (data not shown). Only 67% of the total variation was explained by these three axes.

Table 3. Weir and Cockerham's (1984) F-statistics for wild, cultivated (Cult.) and wild + cultivated (All) populations using polymorphic loci for which genotypic assumptions could be made (see methods section); f = an estimate of inbreeding attributable to non-random mating within populations; F = an estimate of the combined effects of genetic drift and inbreeding within populations; θ = an estimate of genetic drift between populations. Values significantly different from zero are indicated in the last row for $P < 0.05$ (*) and $P < 0.01$ (**)

	f			F			θ		
Locus	Wild	Cult.	All	Wild	Cult.	All	Wild	Cult.	All
Got3	0.92	0.69	0.79	0.99	0.78	0.95	0.85	0.30	0.74
Mdh3	0.91	0.97	0.92	0.98	0.98	0.98	0.76	0.25	0.73
Pgi1	0.31	0.32	0.31	0.75	0.32	0.71	0.63	0.00	0.58
Pgi2	0.93	0.95	0.94	1.0	0.99	0.99	0.93	0.69	0.87
All loci	0.63**	0.64**	0.64**	0.92**	0.75**	0.90**	0.78**	0.31*	0.71**

Table 4. Gene diversity statistics (Nei 1973; Swofford and Selander 1989) averaged over all polymorphic loci for different types of American ginseng populations. Total gene diversity (Ht) is shown with the percentage of total variation partitioned within populations, among populations (or within types for All), and among types. Significant differences between cultivated and wild populations are indicated by different superscript letters (Wilcoxon's Signed-Ranks Test)

Populations	Ht	% Within populations	% Among populations/ within types	% Among types
Wild	0.356^a	37	63^a	–
Cultivated	0.275^a	76	24^b	–
All	0.353	43	54	3
Significance	$P > 0.10$	–	$P < 0.01$	–

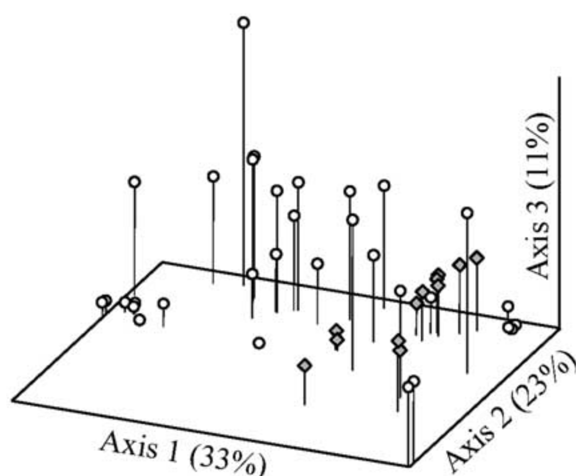

Figure 2. Plot of all populations resulting from Principal Components Analysis using allele frequency data from all loci. The first three axes are given with the percentage of variation explained by each axis. Circles and diamonds represent wild and cultivated populations, respectively.

Discussion

The genetic profile of American ginseng

Genetic variation in American ginseng as measured by species-level estimates of the percentage of polymorphic loci (P) and expected heterozygosity at polymorphic loci (He) are slightly higher than the mean values from allozyme data calculated for dicots as a whole (Hamrick and Godt 1989). Conversely, species-level alleles per locus (A) as well as all population-level estimates of these statistics are slightly lower in American ginseng than the averages for dicots. Conceptually, these results seem somewhat contradictory to the interpretation of the amount of variation in American ginseng using RAPD markers. In those studies, American ginseng was characterized as having a high level of genetic diversity in cultivated and non-cultivated populations (Schluter and Punja 2002). However, RAPD markers and allozyme loci are not necessarily expected to yield directly comparable values of genetic variation (Lynch and Milligan 1994; Krutovskii et al. 1999; Nybom and Bartish 2000). A higher level of intrinsic genetic variation may be present in RAPD markers compared to allozyme loci, and allozymes are limited to protein-encoding loci, which may not be representative of the diversity in the remaining genome.

When genetic variation present in American ginseng is analyzed separately for wild and cultivated populations, significant and substantial differences were found; wild populations showed less genetic variation than cultivated populations for P, A, Ho, and He (Table 1). These differences cannot be attributed to sampling error (Table 1) or to the presence of unique alleles in either cultivated or wild popula-

tions. Instead, this result suggests a fundamental difference in the maintenance of genetic variation in these two groups. Cultivated populations are maintained by regular gene flow as growers supplement their collections with new variation from the wild and from other growers. In contrast, wild populations have undoubtedly experienced far less gene exchange and undergone more extensive episodes of genetic drift. This would especially be true for American ginseng, where wild populations may have experienced over 200 years of repeated harvest, creating successive and severe genetic bottlenecks. The relatively high levels of genetic variation in cultivated populations as well as the apparent gene flow among regions of cultivation parallel the patterns seen in other landraces of crop species (Frankel et al. 1995). Historically, the high levels of genetic variation present in landraces have been important sources of resistance genes and novel adaptations for the production and improvement of cultivars.

Potential differences in gene flow among populations within cultivated or wild material is also evident from the statistics measuring partitioning. As measured by either *Gst* (Table 4) or θ (Table 3), wild material has over 2.5 times more variation distributed among its populations than cultivated material. The direction of this difference is consistent with an analog of the *Gst* estimate provided by RAPD data for American ginseng in Schluter and Punja (2002) although the absolute values of their estimates were considerably lower than estimates from allozymes. Other general conclusions concerning differences between wild and cultivated populations are also consistent between allozyme and RAPD data. For example, multidimensional scaling (MDS) of genetic distance values among individuals showed clear separation of cultivated and wild-collected populations of American ginseng in Schluter and Punja (2002) as well as Boehm et al. (1999). These results led the authors of both RAPD studies to conclude that wild populations were genetically distinct from cultivated populations. It should be emphasized that the differences between wild and cultivated populations apparently reflect a greater contribution from allele frequency differences rather than the existence of unique single-locus genetic variation. This is true for the allozyme data as well as the study by Schluter and Punja (2002).

The most surprising result from the allozyme data is the very high inbreeding estimates as measured by Weir and Cockerham's (1984) f (Table 3) as well as the population fixation index, *Fp* (Table 1). Excess homozygosity can be an artifact due to the inadvertent pooling of genetically distinct sub-populations (i.e., Wahlund's Principle; Hedrick 1985). Although it is possible that a portion of the excess found in American ginseng could be attributed to the Wahlund Principle, the magnitude and consistency of the single-locus deviations strongly suggest that inbreeding is the prevailing cause of the deviation (see Williamson and Werth (1999) for an example of deviations attributed to the Wahlund Principle). With the exception of one locus in one population, every significant deviation was in the direction of increased homozygosity with fixation indices frequently approaching 1.0 (Grubbs 2001). The overall genetic profile of American ginseng is also consistent with a predominant life-history strategy of self-pollination. In Hamrick and Godt (1989), breeding system was found to be the best predictor of the amount and distribution of genetic variation in plant populations. In American ginseng, population levels of *P*, *A*, *He*, and *Gst* most closely match the genetic profile for self pollinating species and are on the extreme ends of the ranges reported in Hamrick and Godt (1989; Table 5).

Table 5. Comparison of mean genetic diversity values in wild American Ginseng populations with averages reported in Hamrick and Godt (1989) for self-pollinating species, species with mixed mating systems (self-pollination + animal pollination), and animal-outcrossed species. *P* = the percentage of polymorphic loci; *A* = alleles per locus; *He* = expected heterozygosity at polymorphic loci (the same as *Hs* in Hamrick and Godt 1989); *Gst* = the proportion of variation distributed among populations

	P	*A*	*He*	*Gst*
Wild ginseng	18.8	1.20	0.11	0.63
Self-pollinating	20.0	1.31	0.15	0.51
Mixed mating	29.2	1.43	0.22	0.22
Outcrossing	35.9	1.54	0.24	0.20

Concern over the detrimental effects of small population sizes is a common theme in conservation biology. Inbreeding depression and loss of alleles due to genetic drift are key genetic consequences of small population size (Ellstrand and Elam 1993). Moreover, the loss of genetic variation is expected to interact with a variety of other ecological and demographic consequences of small population size which can further contribute to population decline (Gilpin and Soulé 1986). The theoretical prediction of the positive association between population size and genetic variation (Montgomery et al. 2000) is consistent

with allozyme data in American ginseng. Significant correlations of wild population sizes with both *He* and *P* were found in addition to a near significant correlation with *A*. These correlations, together with the correlation of genetic distance and geographic distance, suggest that low migration and high genetic drift from prolonged small population sizes have been very important in shaping the population genetic structure of this species. These patterns should become intensified with increased habitat fragmentation and recurrent harvest. Other genetic consequences of small population sizes such as inbreeding depression may not be as pronounced in American ginseng compared to obligately outcrossing taxa. First, populations with a long evolutionary history of self-pollination are expected to express less inbreeding depression than populations with a history of outcrossing. This is apparently due to an increase in homozygosity from selfing and the "purging" of deleterious recessive alleles (Carr and Dudash 1996). In addition, the increased heterozygosity resulting from polyploidy can theoretically act to mask deleterious recessive alleles, although it is clear from the literature that polyploids do not always follow this prediction (Husband and Schemske 1997).

Conclusions and management recommendations

There is a belief, among those directly or indirectly involved in the ginseng trade, that there are no remaining wild populations of American ginseng. Instead, many would argue that most wild extant populations are remnants of deliberate plantings (Harris 1999; Robert Beyfuss, personal communication). This is an important concept because it can influence the motivation and strategies employed for conservation of wild populations. The results from allozyme as well as RAPD marker studies show clear genetic differences between those populations identified as wild or cultivated. In order to explain the genetic patterns of wild populations by human cultivation, it is necessary to assume historical patterns of propagation and seed exchange that are consistent among diverse geographic regions and significantly different from what is known about current practices. Instead, a more plausible explanation is that the patterns identified in the wild populations were largely caused by historical and natural processes of colonization and migration. Therefore, it is important for conservation efforts to acknowledge the genetic differences found between wild and cultivated populations, and also to consider populations wild in the absence of information indicating their establishment by humans.

Current conservation practices may pose serious threats to the genetic integrity of wild populations. One such controversial practice is to use cultivated seed to replenish and supplement existing wild populations (Persons 1994; Harris 1999; Steve Best, personal communication). The genetic consequences of introducing cultivated genotypes into wild populations is uncertain at the present time and needs further investigation. However, theoretical predictions indicate that plants with American ginseng's genetic profile are least likely to respond favorably to the introduction of novel genotypes. The presence of limited gene flow among wild populations and the existence of a high degree of fixation within populations can enhance the formation of both locally adapted genotypes as well as intrinsic co-adapted gene complexes (Templeton 1986). The introduction of novel genotypes could then disrupt these gene combinations and reduce fitness within natural populations (Parker 1992; Waser and Price 1994; Fischer and Matthies 1997). This potential problem in conjunction with the increased possibility of disease transmission and spread through the importation of seeds (Hankins 2000), suggest that the deliberate introduction of cultivated seed into existing populations may pose serious risks to wild populations.

Lastly, it has been suggested that specific wild populations might serve as sources of unique genetic material for the establishment of a core collection of American ginseng (e.g., Boehm et al. 1999). Although unique quantitative trait variation is likely to be present in any given wild population, a sampling scheme focusing on only a few wild populations would likely miss a significant portion of genetic variation in this species. Cultivated populations of American ginseng already contain substantial amounts of genetic variation with only 3% of the species-level variation partitioned between cultivated and wild populations. Cultivated populations are also more similar in their composition to each other than are wild populations. Therefore, to maximize the sampling of genetic variation with minimal effort, it is recommended that breeding efforts focus initially on cultivated material.

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