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GENETIC DIVERSITY IN HARVESTED AND PROTECTED POPULATIONS OF WILD AMERICAN GINSENG, *PANAX QUINQUEFOLIUS* L. (ARALIACEAE)[1]

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Genetic diversity was examined at 16 allozyme loci in 21 wild populations of the medicinal plant American ginseng, *Panax quinquefolius* L. (Araliaceae). This species has been harvested from forests in North America for more than 250 years. Average expected heterozygosity was significantly greater within protected populations ($H_e = 0.076$) than within populations in which harvesting was permitted ($H_e = 0.070$). More notably, genetic structure was greater among unprotected populations ($G_{ST} = 0.491$) than among protected populations ($G_{ST} = 0.167$). These differences in the level and distribution of genetic diversity in American ginseng populations indicate that harvesting may have significant evolutionary implications for this species. Age class structure also shifted toward smaller, nonreproductive plants in unprotected populations. Juvenile plants had lower genetic diversity ($H_e = 0.067$) than reproductive plants ($H_e = 0.076$) suggesting that conserving a proportion of the largest (oldest) plants in each population is important to protect reproductive fitness and the evolutionary potential of the species. Due to its high genetic structure, conservation recommendations include protecting populations throughout the range of *P. quinquefolius*.

Key words: allozymes; Araliaceae; conservation; genetic diversity; ginseng; harvest; medicinal plant; *Panax quinquefolius*.

Globally, plant species are affected by human activities, either directly or indirectly. Ubiquitous impacts from human activities range in scale from global climate change to population fragmentation to direct management of plant resources. These factors, independent of the biology of the species, can have profound evolutionary implications for plants. On a population level, habitat fragmentation, environmental degradation, and overharvesting place pressures on native plant populations, resulting in declines in density and abundance, decreased fitness, and increased isolation (Soule, 1991; Laurance, 1999; Pimm et al., 2001; Vance, 2002), potentially leading to extinction (Frankel and Soule, 1981; Shaffer, 1981; Menges, 1991; Ellstrand and Elam, 1993; Mills and Smouse, 1994; Frankham, 1995; Lande, 1995). Human activities can also change the level and pattern of genetic diversity in native plant species, leading to loss of genetic diversity that may ultimately reduce the evolutionary potential of a species to respond to environmental changes (Ellstrand and Elam, 1993). Population fragmentation, isolation, and lowered population densities may also modify patterns of gene exchange, reducing pollen and seed movement between sites and possibly increasing inbreeding within sites. For many species, genetic diversity is directly related to population size (Godt et al., 1996; Godt and Hamrick, 2001), and levels of genetic diversity may affect individual fitness and potential population persistence (Newman and Pilson, 1997; Fischer and Matteis, 1998).

American ginseng roots are widely collected in the United States for sale in herbal medicines as a cure-all or panacea. Although ginseng can be cultivated, wild roots are considered more potent and are therefore more valuable. Wild roots may sell for U.S. $1105 per kilogram (approximately $500 per lb) (Robbins, 1998). The market for American ginseng has fluctuated throughout the 20th century, but has steadily increased since the 1960s. Currently, population status data are not incorporated into national management programs for *Panax quinquefolius* or into federal decisions regarding export of wild roots (Robbins, 2000). Collection of wild roots less than 5 yr of age, however, is prohibited by the federal government, a measure designed to allow plants an opportunity to reproduce before harvest.

Recent trends suggest that harvest pressure on wild sources of medicinal plants is increasing (Nantel et al., 1996; Sheldon et al., 1996; Robbins, 1998; Laurance, 1999). Research suggests that populations of wild-collected herbs are falling below minimum viable sizes and are going extinct due to collection pressures (Lewis, 1988; Nantel et al., 1996; Sheldon et al., 1996). Because the majority of medicinal plant species are not cultivated, most material for herbal remedies originates in native forests (Pearce, 1997; Sheldon et al., 1997). The current pressure on populations of wild-harvested plants forces the question of how nontimber forest products persist within a landscape of forest fragmentation and habitat loss. Foremost is the question of how evolutionary and demographic processes of wild-collected species are altered by harvesting.

We conducted a genetic survey of *Panax quinquefolius* to estimate the level and distribution of genetic diversity among populations in southeastern Appalachian forests. American ginseng is representative of the economic and cultural value of wild medicinal plants. Our sampling was designed to determine whether the genetic composition of ginseng is affected by harvest pressure by including populations protected from

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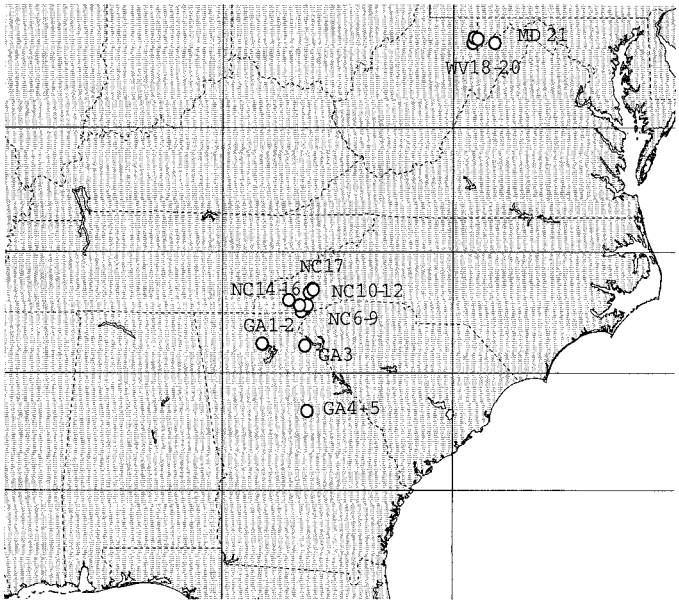

Fig. 1. Location of sample sites for *Panax quinquefolius* in the southeastern United States. Names and description of sample sites are given in Table 1.

harvest and those where collecting is permitted. We also obtained demographic information from protected and unprotected populations in an effort to determine the ecological impact of harvest on American ginseng. Also, information is lacking on population densities, levels of genetic variation, and the distribution of this economically valuable herb (Robbins, 2000). Appalachian hardwood forests, where most wild ginseng populations occur, are also threatened with continued habitat alteration as a result of suburbanization and logging.

MATERIALS AND METHODS

Study organism—*Panax quinquefolius* (Araliaceae) ranges from Quebec and Manitoba south to Georgia, and west to Louisiana, Arkansas, and Oklahoma. It is a long-lived, palmately compound-leaved, forest herb, with aerial shoots that develop in late April and persist until November. American ginseng is pollinated by generalist insects, such as small Halictid bees (Anderson et al., 1993), and is reported to have a mixed-mating breeding system (Carpenter and Cottam, 1982; Lewis and Zenger, 1982; Schlessman, 1985). American ginseng reproduces exclusively by seed after a pre-reproductive period of approximately 3 yr and does not grow from roots clonally (Lewis and Zenger, 1982; Nantel et al., 1996). Green fruits first appear in July and August and mature and redden on the plant from August to November. In an effort to maintain natural population sizes, U.S. Fish and Wildlife regulations require ginseng collectors to harvest only ginseng large enough to reproduce (three- and four-leaf plants) and to plant seeds from harvested individuals within the same population.

Plant material—To determine the genetic structure of *P. quinquefolius* populations, allozyme variation was analyzed via starch gel electrophoresis. During 1999 and 2000, leaf samples were collected from 21 wild populations that occurred in national parks, national forests, and on private land from Georgia to West Virginia, for a total of 1307 plants (Fig. 1). Eight populations were protected, meaning that harvesting has not been permitted since 1940. Thirteen unprotected populations occurred in areas where permits are issued for limited ginseng harvest (Table 1). Poaching likely occurs in both protected and unprotected populations. We collected a few leaflets from a compound leaf of each plant. All plants within a contiguous patch, usually considered a population, were sampled.

At the time of collection, the number of leaves, as well as the presence of flowers or fruits, was noted. In previous demographic research on *P. quinquefolius* using the number of leaves as the size class variable, the number of leaves were determined to reflect age class and to be a good indicator of underground biomass (Lewis and Zenger, 1982; Charron and Gagnon, 1991; Anderson et al., 1993). Small plants are also often considered juveniles, because reproduction is usually delayed until plants have at least three leaves (Lewis and Zenger, 1982; Charron and Gagnon, 1991; Anderson et al., 1993).

TABLE 1. Sampling locations for *Panax quinquefolius*. Results are summarized by region (southern Appalachian, mid-Appalachian) and state (Georgia, North Carolina, West Virginia/Maryland). Protection status: unprotected (U) or protected (P); *N* = number of individuals sampled per population.

State	Population	Protection status	*N*	Location[a]
Southern Appalachian				
Georgia	GA1	U	61	Chattahoochee NF
	GA2	U	48	Chattahoochee NF
	GA3	U	66	Chattahoochee NF
	GA4	U	104	Oconee NF
	GA5	U	25	Oconee NF
North Carolina	NC6	P	43	Coweeta LTER
	NC7	P	52	Coweeta LTER
	NC8	P	47	Coweeta LTER
	NC9	P	32	Coweeta LTER
	NC10	U	27	Pisgah NF
	NC11	U	15	Pisgah NF
	NC12	U	34	Pisgah NF
	NC13	U	39	Pisgah NF
	NC14	P	87	Great Smoky Mountain NP
	NC15	P	106	Great Smoky Mountain NP
	NC16	P	120	Great Smoky Mountain NP
	NC17	P	96	Joyce Kilmer/Slickrock Wilderness Area
Mid-Appalachian				
West Virginia	WV18	U	64	Morgantown
	WV19	U	100	Morgantown
	WV20	U	47	Morgantown
Maryland	MD21	U	94	Savage River State Forest

[a] NF, National Forest; LTER, Long Term Ecological Research site; NP, National Park.

Significant differences in the proportion of plants in each size class and the proportion of reproductive individuals (with fruits or flowers) in each size class for protected and unprotected populations was determined with chi-square tests for marginal homogeneity (JMP, SAS, 1996).

For a more detailed approach to understanding genetic diversity and demography, we compared average within-population genetic diversity among plants in the different size classes. Categories were grouped to include enough individuals for meaningful comparisons of genetic diversity; hence, the number of leaves was used to assign plants to two size classes: small (one- and two-leaf plants) and large plants (plants with three, four, or more leaves).

Allozyme analysis—Sampled leaf material was kept on ice and returned to the University of Georgia within 48 h. There it was crushed with mortar and pestle and a bit of sea sand in a phosphate-polyvinylpyrrolidone extraction (''camellia'') buffer to stabilize the enzymes (Wendel and Parks, 1982). Enzyme extracts were absorbed onto chromatography paper wicks and stored at −70°C until analysis. We consistently resolved seven enzyme systems (abbreviation, number of loci): fluorescent esterase (FE, 1), isocitrate dehydrogenase (IDH, 2), malate dehydrogenase (MDH, 2), menedione reductase (MNR, 2), phosphoglucoisomerase (PGI, 4), triose phosphate isomerase (TPI, 3), and UTP-glucose-1-phosphate (UGPP, 2).

Genetic diversity analysis—Genetic diversity parameters were estimated using a computer program developed by M. D. Loveless and A. F. Schnabel and by POPGENE (Yeh et al., 1997). Gene and genotype frequencies were estimated for each population, as well as genetic diversity statistics at the population, between protected and unprotected populations, and species (pooled) levels (Hedrick, 1985; Hamrick and Godt, 1989). Standard population genetic parameters, reported for making comparisons with published allozyme literature, include percent polymorphic loci (P), mean number of alleles per locus (*A*) and per polymorphic locus (AP), effective number of alleles per locus (A_e), observed heterozygosity (H_o), and expected heterozygosity (H_e). Deviations from Hardy-Weinberg expectations within each population were tested with Wright's (1922) *F* statistic for each polymorphic locus. The level of significance for comparison between observed and expected heterozygosity was determined by a chi-square test (Li and Horvitz, 1953).

Genetic structure—Interpopulation genetic structure was estimated with Nei's (1973, 1977) genetic diversity statistic, G_{ST}, which estimates the proportion of genetic variation among populations at polymorphic loci. Genetic diversity statistics were calculated for each locus and averaged over all loci. Significant differences in allele frequencies among populations were determined with chi-square tests of polymorphic loci, where $\chi^2 = 2N\ G_{ST}\ (a - 1)$, with $df = (a - 1)(n - 1)$, *a* is the number of alleles at the locus, and *n* is the number of populations (Workman and Niswander, 1970). Each pair of populations was used to calculate genetic distance measures (Nei, 1972), and the hypothesis of isolation by distance was tested using the program IBD (Isolation by Distance; Bohonak, 2002). We assessed the level of significance by a Mantel test for matrix correlation between the pairwise value for $F_{ST}/(1 - F_{ST})$ and log geographic distance for each population pair (Rousset, 1997).

Indirect estimates of gene flow were obtained with Wright's (1951) method, using G_{ST} as equivalent to F_{ST}. The estimated number of migrants per generation, Nm, was calculated as $Nm = (1 - G_{ST})/4G_{ST}$. A second measure of Nm was determined from the mean frequency of alleles found in single populations, i.e., ''private alleles'' (Slatkin, 1985; Barton and Slatkin, 1986).

Effects of harvest pressure—The effect of harvest pressure on populations was estimated by comparing levels of genetic diversity between populations for which collection permits were issued (unprotected populations) and populations that have been protected from harvest for more than 60 yr (protected). Genetic diversity statistics were calculated as described earlier. Significant differences in diversity statistics between protected and unprotected populations were determined with an unpaired two-tailed Student *t* test. The test for significant differences in expected heterozygosity (H_e) was performed on jackknifed values (Weir and Cockerham, 1984). Finally, evidence for a recent bottleneck as a result of harvest pressure was determined with Cornuet and Luikart's (1997) BOTTLENECK analysis program. A sign test and Wilcoxon test were used to determine whether there was a significant excess in expected heterozygosity (H_e) with regard to expected equilibrium gene diversity (H_{eq}) computed from the observed number of alleles for each population. The power of each test depended on the number of polymorphic loci and sample size (Cornuet and Luikart, 1997; Luikart et al., 1997).

RESULTS

Across the 21 populations included in the analyses, census size ranged from 15 to 120 plants (Table 1). A chi-square test for marginal homogeneity indicated that the distribution of plants in one-, two-, three- and four-leaf size classes was significantly different between protected and unprotected populations ($P < 0.001$). Generally, unprotected populations had more plants in the one- and two-leaf size class, whereas protected populations had the highest proportion of the three-leaf size class (Fig. 2). There was a steep decline in abundance of the largest size class in all populations, with the four-leaf size class making up a small proportion of all the populations sampled.

A chi-square test indicated a significant difference in overall proportion of reproductive individuals between protected and unprotected populations ($P < 0.05$), with more reproductive individuals in protected populations. Comparisons of reproductive plants per size class (Fig. 3) indicate that the proportion of plants with flowers or fruits was higher in the small size classes (one- and two-leaf plants) in unprotected populations. Although, among large size classes (three- and four-leaf plants) this was not the pattern. The chi-square tests indicated significant differences in the distribution of reproductive individuals among protected and unprotected populations for one-, three- and four-leaf plants ($P < 0.05$), but not for two-leaf plants ($P = 0.07$).

Genetic diversity—Allozyme analyses resolved 16 loci and 32 alleles. At the species level, 10 loci (62.5%) were polymorphic. Loci FE, MNR1, PGI1, PGI2, TPI2, and TPI3 were monomorphic in all populations. An average of 2.70 alleles per polymorphic locus (AP) was found at the species level (Table 2), with an average effective number of alleles (A_e) per locus of 1.26. Expected heterozygosity (H_e) was 0.159 for the species.

Individual populations had a mean percentage of polymorphic loci of 27.3% (P), with a mean number of alleles per polymorphic locus of 2.23 (AP). Percent polymorphic loci ranged from 6.3% in population WV20 to 43.8% in populations GA3 and NC14 (Tables 2 and 3). The highest AP was 2.67 in populations GA4 and NC9. Mean A_e was 1.12; the highest value was 1.28 in population GA3. Within populations, H_e was 0.072 (Tables 2 and 3). Average allelic richness within each population was 20.9.

Generally, observed heterozygosity values were less than expected heterozygosity in all populations, as was the overall mean observed heterozygosity ($H_o = 0.040$). Chi-square tests for deviations from Hardy-Weinberg expectations ($F = 0$) resulted in 52 significant deficiencies and two excesses in 81 comparisons. Mean F_{IS} across all loci was 0.416. Both analyses indicate that there was an excess of homozygotes in the populations relative to that expected with random mating.

Genetic diversity within size classes—When genetic diversity was compared between small (one- and two-leaved plants)

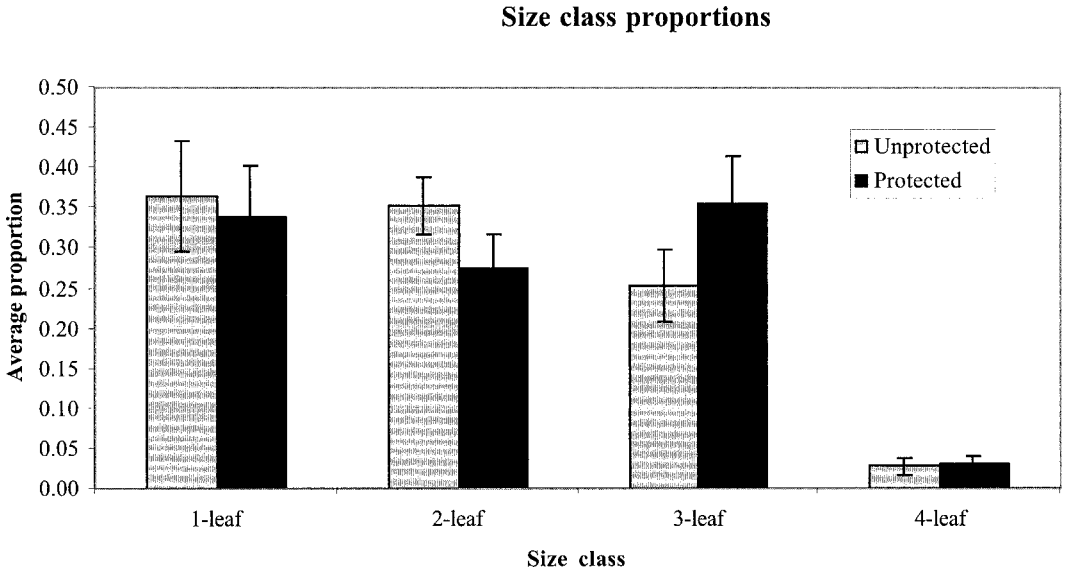

Fig. 2. Proportion of plants of *Panax quinquefolius* in each size class for the unprotected versus protected sites. Error bars are standard errors. For statistical comparisons, plants were grouped into two size classes: small (one- and two-leaf plants) and large (three- and four-leaf) plants.

and large plants (three- and four-leaved plants) within populations, small plants within populations had significantly greater average allelic richness ($P < 0.01$; Table 4). Larger plants also maintained significantly higher average genetic diversity (H_e) than small ones.

Genetic structure—Values of G_{ST} ranged from 0.046 at TPI1 to 0.932 for PGI4, with a mean of 0.493 across the 10 polymorphic loci. Thus, approximately half of the total genetic diversity occurs among populations. All of the polymorphic loci had significant differences in allele frequencies among populations.

Genetic distance among populations ranged from 0.444 between populations GA5 and WV19 and 0.002 between NC15 and NC14 and between GA5 and GA4. We found a significant correlation in pairwise $F_{ST}/(1 - F_{ST})$ and log geographic distance ($P < 0.0001$ from 10 000 randomizations, reduced major axis regression $r^2 = 0.285$), indicating significant isolation by distance. Historical levels of gene flow (Nm) among populations were estimated to be 1.15 from six private alleles with an estimated mean frequency of 0.057. In contrast, Wright's method of estimating gene flow produced a mean Nm of 0.26.

Effect of harvest pressure—Genetic diversity varied between protected and unprotected populations (Table 3). Average H_e for protected populations was 0.076, significantly higher ($P < 0.005$) than the 0.070 in unprotected populations. There was no significant difference in mean P, AP, A_e, or allelic richness, indicating that differences among protected and unprotected populations were due to loss of rare alleles resulting

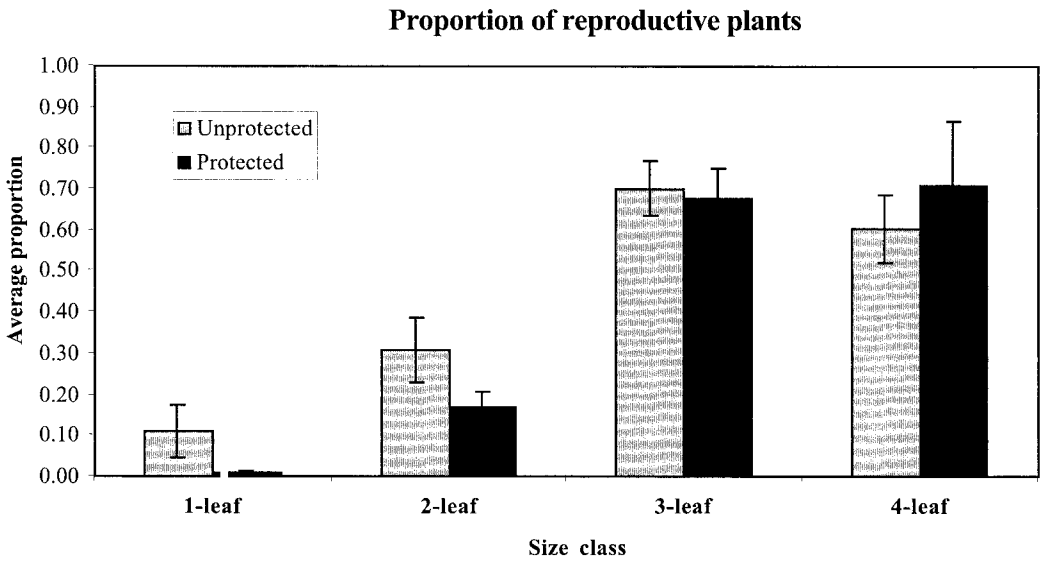

Fig. 3. Proportion of plants of *Panax quinquefolius* with fruits or flowers in each size class, in protected and unprotected populations. Error bars are standard errors.

TABLE 2. Summary of within-population genetic diversity. Genetic diversity statistics are percent polymorphic loci (P); average number of alleles per polymorphic loci (AP); effective number of alleles ($A_e = 1/\Sigma p_i^2$), where p_i is the frequency of the ith allele; allelic richness (AR); observed proportion of heterozygotes (H_o); expected proportion of heterozygotes (H_e). See Table 1 for population location descriptions.

Population ID	P	AP	A_e	AR	H_o (SD)	H_e (SD)
GA1	31.3	2.20	1.20	22	0.047 (0.025)	0.106 (0.050)
GA2	37.5	2.33	1.17	24	0.040 (0.027)	0.083 (0.049)
GA3	43.8	2.14	1.28	24	0.069 (0.027)	0.159 (0.051)
GA4	18.8	2.67	1.08	21	0.075 (0.010)	0.044 (0.033)
GA5	14.3	2.50	1.11	17	0.097 (0.026)	0.059 (0.041)
NC6	25.0	2.25	1.23	21	0.066 (0.033)	0.117 (0.051)
NC7	18.8	2.00	1.09	19	0.039 (0.020)	0.049 (0.033)
NC8	20.0	2.00	1.07	18	0.027 (0.022)	0.044 (0.029)
NC9	20.0	2.67	1.19	20	0.045 (0.032)	0.097 (0.052)
NC10	18.8	2.33	1.08	20	0.032 (0.027)	0.047 (0.038)
NC11	25.0	2.25	1.16	21	0.029 (0.039)	0.089 (0.045)
NC12	31.3	2.20	1.29	22	0.090 (0.043)	0.145 (0.058)
NC13	31.3	2.20	1.07	22	0.027 (0.025)	0.050 (0.023)
NC14	43.8	2.14	1.12	24	0.038 (0.020)	0.084 (0.031)
NC15	18.8	2.33	1.09	20	0.031 (0.016)	0.057 (0.032)
NC16	38.5	2.60	1.15	22	0.049 (0.019)	0.101 (0.043)
NC17	31.3	2.00	1.10	21	0.029 (0.017)	0.056 (0.042)
WV18	31.3	2.00	1.12	21	0.031 (0.020)	0.071 (0.039)
WV19	37.5	2.00	1.03	22	0.009 (0.009)	0.026 (0.012)
WV20	6.3	2.00	1.01	17	0.003 (0.008)	0.006 (0.005)
MD21	31.3	2.00	1.04	21	0.012 (0.012)	0.030 (0.015)
Average	27.3	2.23	1.13	20.9	0.042	0.072
SD	2.4	0.22	0.08	1.95	0.005	0.009

from bottlenecks and genetic drift. There was also a significant difference in H_e between populations of different sizes, but in this case, smaller populations ($n < 80$) had significantly higher H_e, 0.080 ($P < 0.0001$), than large populations ($H_e = 0.057$; Table 3). Small populations (14) had a mean ± SD of 43 ± 15 individuals and large populations (7) had 101 ± 11 individuals. Populations of both sizes were found in protected and unprotected areas.

Genetic structure in protected and unprotected populations—Genetic structure was greater among unprotected populations ($G_{ST} = 0.491$) than among protected populations ($G_{ST} = 0.167$; Table 3), suggesting that unprotected populations may have experienced genetic bottlenecks as a result of consistent harvest pressure. All 10 polymorphic loci had significant heterogeneity in allele frequency among unprotected populations, whereas among protected populations eight of 10 polymorphic loci had significant differences in allele frequencies.

The striking difference in genetic structure might be due to our sampling scheme in which protected populations were sampled across a smaller geographic range than were unprotected populations. However, when the unprotected West Virginia populations were deleted from the analysis, G_{ST} among unprotected populations remained close to 50% (0.478). Therefore, even when corrected for spatial scale, variation in allele frequencies among unprotected populations produced a high level of genetic structure.

Three of 13 unprotected populations had indications of a recent bottleneck (Table 5) based on both sign and Wilcoxon tests, whereas none of the eight protected populations had significant evidence for a recent bottleneck with these tests. A sign test for the number of significant results determined that there were significantly fewer occurrences of a recent bottleneck among protected populations than would be expected at random ($P = 0.0078$). Therefore, there was no evidence of recent bottleneck among protected populations.

DISCUSSION

At the species level, genetic diversity within *P. quinquefolius* is similar to that reported for species with similar life history and ecological traits (Hamrick and Godt, 1996). Although populations are infrequent and patchy, *P. quinquefolius* has a wide geographic range throughout eastern North America and would be expected to maintain moderate to high levels of genetic diversity. The breeding system of *P. quinquefolius* is reportedly mixed selfing and outcrossing (Lewis and Zenger, 1982; Schlessman, 1985; Schluter and Punja, 2002), and seed dispersal mechanism is unknown. Indeed, genetic diversity in American ginseng ($H_e = 0.159$) is between the H_e values for short-lived perennial species with mixed mating systems (0.172) and predominately selfing species (0.135) (Hamrick and Godt, 1996).

Comparisons of observed (H_o) and expected (H_e) heterozygosity within populations of *P. quinquefolius* had a significant excess of homozygosity. This result may be due to nonrandom mating among related individuals or to the mixed mating system reported for this species. It is possible that reductions in population size due to habitat fragmentation or harvest increased selfing or mating among closely related plants resulting in a higher proportion of homozygous individuals within populations. It is also possible that deviations from expected heterozygosity were from sampling over several small subpopulations and treating them as one population (i.e., a Wahlund effect; Hartl and Clark, 1998). Plants within a few wild American ginseng populations were scattered throughout mountain coves and drainages, so we may have collected from more than one subpopulation when collecting leaves. We find this scenario unlikely considering an excess in homozygosity

TABLE 3. Summary of mean genetic diversity at the species and population levels for *Panax quinquefolius*. Genetic diversity statistics are described in the text and in Table 2. Genetic structure, G_{ST}, is the proportion of total diversity that is due to differences in allele frequencies among populations. In this case, 49% of the total diversity (all populations combined) is attributable to differences in allele frequencies in the different populations. The G_{ST} among unprotected populations is 0.478 when geographically distant West Virginia populations are not included in the calculation. Statistical comparisons of genetic diversity within populations of different sizes were made between small ($n < 80$ individuals) and large ($n > 100$ individuals) populations.

	P	AP	A	A_e	AR	H_e	G_{ST}
Species level	62.5	2.70	2.06	1.26	25.0	0.159	0.493
Population level (mean)	27.3	2.23	1.33	1.13	20.9	0.072	—
Protected populations (mean)	28.0	2.23	1.34	1.13	20.6	0.076***	0.167
Unprotected populations (mean)	27.5	2.22	1.33	1.13	21.1	0.070	0.491
Small populations (mean)	25.3	2.22	1.31	1.15	20.6	0.080***	—
Large populations (mean)	32.5	2.23	1.39	1.09	21.6	0.057	—

*** $P < 0.0005$.

was observed at loci in all of the populations, regardless of the area sampled. Further analysis of the fine-scale genetic structure within populations of *P. quinquefolius* may resolve this question (Cruse-Sanders and Hamrick, unpublished manuscript).

Genetic structure—Genetic diversity within American ginseng was highly structured among populations, with approximately 50% of the total genetic variation found among populations. The genetic structure estimated for populations of *P. quinquefolius* was greater than that reported for other plant species with similar life history strategies and was most similar to the mean calculated for annual plants with predominantly selfing mating systems (0.553) (Hamrick and Godt, 1996).

Genetic structure can be used to infer historical rates of gene movement among populations, although the results must be interpreted with caution (Brossart and Prowell, 1998; Sork et al., 1999; Whitlock and McCauley, 1999). Gene flow (Nm) among populations was estimated to be 1.15 based on Slatkin's (1985) private allele method. Alternatively, levels of gene flow based on Wright's (1951) method averaged 0.26. These estimates suggest that historical rates of gene flow have been too low to counteract the effects of genetic drift (Wright, 1931). We found significant evidence for isolation by distance within this species, which indicates population division and divergent evolution throughout the range of American ginseng.

Genetic structure in this study (Table 3) was greater than that reported based on RAPD data among cultivated (G_{ST} = 0.18) and natural (G_{ST} = 0.28) populations of American ginseng in Canada (Schluter and Punja, 2002). There are at least two possible explanations. First, genetic structure in this study was estimated from populations sampled over a larger geographic range. Furthermore, harvesting from wild populations was banned in Canada in 1989 (Nantel et al., 1996). If high genetic structure among populations in the United States is, at least in part, a result of harvesting, the lower genetic structure among populations in Quebec may reflect a release from harvest pressure. This hypothesis is supported by the relatively low genetic structure among protected populations from this study (G_{ST} = 0.167).

Comparison with genetic diversity of more northern populations also suggests an effect of post-glacial dispersal resulting in loss of alleles and lower H_e in the more northern populations. This pattern has been noted for other Appalachian species such as *Helonias bullata* (Godt et al., 1995). However, this pattern is only suggestive for ginseng since our sampling design is not adequate to fully describe geographic variation throughout the entire range of the species.

Demographic impacts of harvest—The size class structure of ginseng plants is different between protected and unprotected populations. Protected populations maintain a higher proportion of older, potentially reproductive plants than unprotected populations. Furthermore, we found a significant dif-

TABLE 4. Summary of mean genetic diversity within size classes of sampled populations. Small size class includes plants with one and two leaves, and the large size class includes plants with three and four leaves. Genetic diversity statistics are described in Table 2 and in the text. Significantly greater H_e in larger plants is due to more even allele frequencies.

Size class	P	AP	A_e	AR	H_o	H_e
Small plants	25.9	2.19	1.11	20.5*	0.039	0.067
Large plants	22.8	2.17	1.14	19.9	0.051	0.076**

* $P < 0.05$; ** $P < 0.01$.

TABLE 5. Results of statistical tests for evidence of a recent bottleneck. Numbers reported are P values. IAM is the infinite allele model, and SMM is the stepwise mutation model.

	Sign test		Wilcoxon test	
ID	IAM	SMM	IAM	SMM
Protected				
NC15	0.27	0.58	0.47	0.58
NC6	0.20	0.32	0.06	0.06
NC7	0.37	0.45	0.38	0.37
NC8	0.63	0.56	1.00	1.00
NC9	0.10	0.59	0.13	0.25
NC17	0.62	0.60	0.81	0.81
NC16	0.37	0.50	0.25	1.00
NC14	0.12	0.13	0.63	0.06
Unprotected				
GA5	0.74	0.72	0.50	0.50
NC11	0.45	0.55	0.37	1.00
WV19	0.05	0.03	0.02	0.02
GA3	0.47	0.34	1.00	1.00
GA4	0.58	0.42	0.81	0.25
NC12	0.25	0.64	0.31	0.87
NC13	0.02	0.03	0.02	0.03
GA3	0.02	0.17	0.02	0.12
NC10	0.44	0.34	0.12	0.13
MD21	0.35	0.27	0.06	0.06
WV20	0.59	0.55	0.50	0.50
GA1	0.36	0.47	0.15	0.21
WV18	0.64	0.58	0.81	0.81

ference in proportions of reproductive individuals in protected and unprotected populations. In unprotected populations, small plants (one- or two-leaf plants) were more likely to have flowers or fruits compared to protected populations. Our results suggest that reproductive parameters can change with a demographic shift in a harvested population, which can lead to a significant drop in population growth rate. Hackney and Mcgraw (2001) found that small, less dense populations of American ginseng produced few seeds per flower and per plant, possibly due to reduced pollinator efficiency. Our findings suggest that this effect could be amplified due to a shift in age class structure with harvest. If unprotected populations have fewer flowering plants than expected based on census size, a drop in reproductive output and lower population growth rate could result.

Within populations, we found that large (three- and four-leaved) plants maintain significantly higher expected heterozygosity than small (one- and two-leaved) plants. This observation could be due to higher survival of more heterozygous individuals through time. Alternatively, this may represent a generational change in overall genetic diversity. Based on previous demographic work with *P. quinquefolius*, large plants are the oldest and reproductively mature individuals within populations (Schlessman, 1985; Anderson et al., 1993; Nantel et al., 1996). Larger (reproductive) plants might not be randomly mating or have low fecundities as a result of low post-harvest population densities. Furthermore, if larger, older individuals reproduce prior to harvest, it is possible that a proportion of their seeds does not germinate or is taken from the population to be planted elsewhere, resulting in low recruitment. This is likely, considering anecdotal information that ginseng diggers routinely move seeds and roots among locations to ensure that ginseng plants are found in locations they regularly harvest (V. Nazarea, University of Georgia, personal communication).

A compounding effect results when the age class distribution in harvested populations is altered, leading to long-term evolutionary effects. Based on studies of herbarium specimens, McGraw (2001) found a significant decrease in ginseng plant stature over 150 years and concluded that the decrease resulted from the sustained harvest of large plants. Our results suggest that there is also a genetic effect of harvest on populations that compounds the harvest pressure imposed on the largest, and presumably most reproductively viable plants within populations of American ginseng.

Documented changes in plant size and genetic diversity in American ginseng populations point to possible negative evolutionary consequences of harvest. Ratner and Lande (2001) provided a theoretical analysis of the evolutionary and demographic implications of size-selective harvesting in populations and found that harvest could lead to significant changes in the average size of individuals within populations. Although, in the short term, demographic effects may be of immediate conservation importance (Schemske et al., 1994), the evolutionary effects of selective harvesting can exacerbate the demographic consequences of collecting.

Genetic diversity in protected and unprotected populations—Trends in genetic diversity from this study indicate that protected populations harbor significantly higher levels of genetic diversity (H_e) than unprotected populations. Although other measures of genetic diversity, P, AP, A_e, did not differ significantly between the two types of populations, the greater H_e indicates that allele frequencies at loci are more even in protected populations. These differences could be due to the current harvesting status of these populations, but there could also have been an association between protected populations and the history of the forested site where the population was found. If protected populations were more likely to be found in pristine habitats and unprotected populations in more disturbed, fragmented habitats, the pattern in genetic diversity might be due to historical bottleneck or drift in low-quality habitats as well as harvest practices (J. M. Cruse-Sanders, unpublished data).

The most noteworthy difference between protected and unprotected populations was the level of genetic structure among populations within each group. Significant variation in allele frequencies and high genetic structure, especially among unprotected populations, is consistent considering the effect harvesting has on populations. As mature plants are collected from the population, allele frequencies should change among populations and genetic structure should increase. This suggests that many populations throughout the range of the species may have experienced reduced effective population sizes due to habitat disturbance and/or harvesting. When populations become small, genetic drift causes different alleles to become fixed in different populations leading to increased genetic structure.

We found no evidence for recent population bottlenecks among any of the protected populations. The lack of evidence for a bottleneck in some populations may be because a bottleneck did not occur or that it is older than this method can detect. Additionally, as has been noted, our populations are not in Hardy-Weinburg equilibrium, which violates the assumptions of the BOTTLENECK program (Cornuet and Luikart, 1997). Therefore, results of this analysis should be interpreted with caution, although they do support our findings of possible negative evolutionary impacts of harvesting (i.e., reduced genetic diversity and increased genetic structure).

Conservation implications—An effective conservation strategy would protect populations throughout the range of ginseng to maximize the maintenance of genetic diversity, because almost 50% of the total genetic diversity occurs among unprotected populations. Schoen and Brown (1991) pointed out that species with higher G_{ST} values also have more variation in genetic diversity parameters (P, H_e, etc.) than species with less genetic structure. This makes it difficult to predict levels of genetic diversity for specific populations (Schoen and Brown, 1991) and to develop effective conservation strategies (Ceska et al., 1997). For *P. quinquefolius*, our results highlight the need for empirical studies to identify populations with higher levels of genetic diversity and unique alleles.

Conservation recommendations resulting from this study include prohibiting certain populations of American ginseng from being harvested because protected populations generally maintained more genetic diversity than unprotected populations. Demographic research on ginseng in Quebec and in Great Smoky Mountain National Park (GSMNP) set the minimum viable population size at 172 plants (Nantel et al., 1996; Gagnon, 1999), yet when populations in GSMNP were censused, only two populations met that criterion (Rock et al., 1999). Nonetheless, protected populations within GSMNP generally harbor more genetic diversity than populations in adjacent harvested areas. Continued harvesting could have long-term negative conservation implications and evolutionary

consequences. Based on our results indicating that older plants maintained more genetic diversity, we recommend that conserving a proportion of the largest (oldest) plants in each population would best protect the reproductive fitness and evolutionary potential of the species.

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