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Allozyme Variation and Population Genetic Structure of an Invasive Plant, *Ageratina altissima* (White Snakeroot), in Seoul

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Allozyme studies have been widely used to estimate genetic variation and to describe genetic structure in natural populations. In many cases, the genetic diversity of recently established populations is generally lower than that of central populations. In addition, the genetic composition of an invasive species is influenced by its history of introduction as well as its ecological characters. *Ageratina altissima* (L.) R. King & H. Robinson (white snakeroot) is a perennial herb native to the eastern United States and Canada, and is currently receiving much attention for its rapid invasion of the Korean forests. Starch gel electrophoresis was used to assess the genetic variability at 11 putative loci in seven introduced populations of *A. altissima* in Seoul. Populations of *A. altissima* maintained lower levels of allozyme diversity (expected heterozygosity = 0.063) than those reported for other taxa with similar ecological traits. The degree of differentiation observed among *A. altissima* populations was considerably low. It is suggested that the populations were recently established from only a few founders via dispersal by human activities, resulting in the loss of genetic variation.

The rapid development of molecular techniques offers technical approaches for population biologists interested in wide range of questions (Parker et al., 1998). For example, these tools can be used to determine individual reproductive success or to measure rates of genetic divergence among populations. Some major techniques are protein electrophoresis, nuclear and mitochondrial RFLPs (restriction fragment length polymorphisms), minisatellite and microsatellite VNTRs (variable number tandem repeats), RAPDs (random amplified polymorphic DNA) and DNA sequencing. For studies of population genetic structure, allozyme electrophoresis remains a powerful tool for most taxa, although the techniques based on nucleic acids are useful as well (Cruzan, 1998; Parker et al., 1998).

The distribution of genetic variation within plant species and the genetic structure of plant populations are dependent on the life history and other ecological traits (Loveless and Hamrick, 1984). Hamrick and Godt (1989) classified them into eight traits; major phyletic group, life form, geographic range, regional distribution, breeding system, seed dispersal mechanism, mode of reproduction and successional status. Geographical range accounts for the largest proportion of genetic variation at the species level, and breeding system in combination with geographic range accounts for the greatest proportion of variance in genetic diversity at the population level (Hamrick and Godt, 1989).

The genetic composition of an introduced, invasive species is influenced by its history of introduction as well as its life history characters (Pappert et al., 2000). The genetic composition of relatively recently established local populations of an invasive, colonizing species is of interest, since it may provide insights into the mode of local population establishment (Pappert et al., 2000).

Ageratina altissima (L.) R. King & H. Robinson (white snakeroot) is a terrestrial herb perennial reproducing by seeds and short rhizomes (King and Robinson, 1987). It is native to the eastern U. S. and Canada, found in woodlands, banks, damp or shady pastures, and fields (Muenscher, 1980). In Korea, it was first found in Mt. Namsan in Seoul by Lee (1987) and is recently receiving much attention because it is rapidly invading forested areas (Suh et al., 1997).

In this study, we have examined the allozyme variation of *A. altissima* in Seoul; to assess its genetic diversity within populations and genetic divergence among populations and to understand the effects of ecological factors.

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Materials and Methods

Plant sampling

A total of 280 leaf samples of *A. altissima* was collected from seven populations in Seoul, Korea (Table 1). The samples were collected randomly at intervals of greater than 2 m in each population in order to avoid biasing samples toward certain clones. Leaf samples were placed in plastic bags and stored on ice for transport to the laboratory. Samples were stored at 4℃ until enzyme extraction.

Electrophoresis

Leaf samples were cut finely and crushed with a mortar and pestle. A phosphate-polyvinylpyrrolydone extraction buffer (Mitton et al., 1979) was added to the leaf samples to facilitate crushing and to aid enzyme stabilization. The cellular extract was absorbed onto 4 ×6-mm wicks cut from Whatman 3 MM chromatography paper and stored at -70℃ until needed for electrophoretic analysis. Electrophoresis was performed using 12% starch gels. Gel, electrode buffers and staining procedures of Soltis et al. (1983) were used to assay 11 enzyme systems. Aconitase hydratase (ACO) and fluorescent esterase (FE) were resolved on "system 2". 6-Phosphogluconate (6-PGD), isocitrate dehydrogenase (IDH) and shikimic dehydrogenase (SKDH) were resolved on "system 6." Aldolase (ALD), glutamate dehydrogenase (GDH), leucine aminopeptidase (LAP), malic enzyme (ME), peroxidase (PER), and phosphoglucoisomerase (PGI) were resolved on "system 7."

Data analysis

A locus was considered polymorphic if two or more alleles were observed, regardless of their frequencies. Six genetic parameters were estimated using Popgene program (Yeh et al., 1997); allele frequency, percent polymorphic loci (*P*), mean number of alleles per locus (*A*), mean number of alleles per polymorphic locus (*AP*), observed heterozygosity (*Ho*) and Hardy-Weinberg expected heterozygosity (*He*). Observed heterozygosity was compared to Hardy-Weinberg expectations according to Wright's (1922) fixation indices (*F*). We also calculated genetic diversity statistics (H_S, H_T, G_{ST}) and Nei's genetic identity (Nei, 1972).

Table 1. Plant sampling data for seven populations of *Ageratina altissima* in Seoul

Population	Locality	N	Collection date
NS1	Mt. Namsan, south-facing slope	40	10 Sept. 2000
NS2	Mt. Namsan, north-facing slope	40	10 Sept. 2000
NS3	Mt. Namsan, east-facing slope	40	10 Sept. 2000
AS	Mt. Ansan	40	10 Sept. 2000
HC	National Memorial Cemetery	40	21 Sept. 2000
CD	Chungdam Recreational Park	40	12 Sept. 2000
ND	Nanji waste landfill	40	21 Sept. 2000

Table 2. Allele frequencies for two polymorphic loci among 11 loci of *Ageratina altissima* in Seoul

Locus/allele	Population*						
	NS1	NS2	NS3	AS	HC	CD	ND
Fe							
a	0.825	0.575	0.875	0.863	0.837	0.700	0.762
b	0.175	0.425	0.125	0.138	0.162	0.300	0.237
Per							
a	0.250	0.200	0.250	0.150	0.175	0.363	0.313
b	0.750	0.800	0.750	0.850	0.825	0.637	0.688

* Population codes correspond to those given in Table 1.

Results

Of the 11 loci examined, only two were polymorphic for all populations (Table 2). Percent of loci polymorphic (*P*), mean number of alleles per locus (*A*) and mean number of alleles per polymorphic locus (*AP*) were the same among the examined populations (Table 3). Observed heterozygosity (*Ho*) was not significantly different (P = 0.97) among populations. Mean expected heterozygosity within populations (*He*) was 0.063. *Ho* and *He* were not significantly different (P = 0.68). Genetic variation among populations (G_{ST}) was 0.042 (Table 4).

Analysis of fixation indices, calculated for two polymorphic loci, suggested that those populations approach conformance to Hardy-Weinberg expectations with a mean F_{IS} of -0.042 (Table 4). An excess of heterozygotes was found at the *Fe* locus (Table 4; P = 0.002). F_{IS} values of *Fe* locus at the level of population ranged from -0.739 to -0.143, and those of *Per* from -0.176 to 0.467.

Genetic diversity within populations and genetic variation among populations were very low as compared to those reported for other taxa with similar ecological traits (Tables 5, 6). Nei's genetic identity for pair-wise comparisons of populations ranged from 0.991 to 1.000 with a mean of 0.997.

Discussion

We have examined the allozyme variation of *A.*

Table 3. Allozyme diversity within seven populations of *Ageratina altissima* in Seoul

Population*	*P*	*A*	*AP*	*Ho*	*He*
NS1	18.2	1.2	2	0.064 (0.043)	0.061 (0.041)
NS2	18.2	1.2	2	0.105 (0.079)	0.074 (0.051)
NS3	18.2	1.2	2	0.041 (0.028)	0.055 (0.038)
AS	18.2	1.2	2	0.052 (0.035)	0.045 (0.030)
HC	18.2	1.2	2	0.048 (0.033)	0.052 (0.035)
CD	18.2	1.2	2	0.089 (0.061)	0.081 (0.055)
ND	18.2	1.2	2	0.073 (0.050)	0.073 (0.049)
Mean	18.2	1.2	2	0.067 (0.009)	0.063 (0.005)

* *Population codes correspond to those given in Table 1. SEs are given* in parenthesis. *P*, percent polymorphic loci; *A*, mean number of alleles per locus; *AP*, mean number of alleles per polymorphic locus; *Ho*, observed heterozygosity; *He*, Hardy-Weinberg expected heterozygosity.

Table 4. Genetic diversity and population differentiation of two polymorphic loci of *Ageratina altissima* in Seoul

Locus	H_T	H_S	G_{ST}[a]	F_{IS}[b]
Per	0.368	0.358	0.0268^{ns}	0.204^{ns}
Fe	0.347	0.327	0.0575^{ns}	-0.287^{**}
Mean	0.357	0.342	0.0417	-0.042

H_T, total genetic diversity; H_S, genetic diversity within populations; G_{ST}, proportion of the total genetic diversity partitioned among populations; F_{IS}, deviations of genotypic frequencies from Hardy-Weinberg expectations within individual populations.
[a] A x^2 test for allele frequency heterogeneity among populations; ns, not significant.
[b] Asterisks indicate F values significantly different from zero; ns, not significant; **, $P < 0.01$.

altissima in Seoul for 11 loci. Nine of 11 loci were monomorphic and *P*, *A* and *AP* were the same for all populations. *Ageratina altissima* is a long-lived perennial, predominantly outcrossed, insect-pollinated herbaceous dicot with dispersal by plumose seeds. The expected heterozygosity (*He*) and genetic variation among populations (G_{ST}) of *A. altissima* were 0.063 and 0.042, respectively, which were very low as compared to those reported for other taxa with similar ecological traits (Tables 5, 6). However, the expected heterozygosity within populations (*He*) of herbaceous and long-lived perennial plants was 0.084 (Hamrick and Godt, 1989). According to Loveless and Hamrick (1984), H_T, H_S and G_{ST} of long-lived plants were 0.221, 0.202 and 0.077, respectively, and those of plants with winged/plumose dispersal of seeds were 0.216, 0.196, and 0.079, respectively. It is indicated that the genetic diversity of *A. altissima* in Seoul is less than ecologically comparable species.

The relatively low level of genetic variation in *A. altissima* is consistent with several aspects of its biology. First, rapid invasion through one or even a few points of introduction and subsequent spread from these points may result in reduced levels of genetic diversity (Godt and Hamrick, 1991; Schierenbeck et al., 1995; Pappert et al., 2000). Naturalized populations of species that have been unintentionally introduced once or a few times often have little genetic diversity in their naturalized populations (Pappert et al., 2000).

Second, some events like founder effect might contribute to this severe loss of genetic variation of *A.*

Table 5. Comparison of genetic diversity at the population level between *Ageratina altissima* and summary data classified according to traits

Taxon/category	H_e*
A. altissima	0.063
Herbaceous, long-lived perennial (4 taxa)[a]	0.084
Widespread (85 taxa)[a]	0.159
Outcrossing-animal breeding system (164 taxa)[a]	0.124
Wind dispersal of seeds (105 taxa)[a]	0.123
Sexual/asexual reproduction (56 taxa)[a]	0.103

*Genetic diversity index. [a] From Hamrick and Godt, 1989.

Table 6. Comparison of genetic diversity among populations of species between *Ageratina altissima* and summary data classified according to traits

Taxon/categories	H_T	H_S	G_{ST}
A. altissima	0.357	0.342	0.042
Herbaceous, long-lived perennial (2 taxa)[a]	0.346	0.282	0.213
Widespread (87 taxa)[a]	0.347	0.267	0.210
Outcrossing-animal breeding system (124 taxa)[a]	0.310	0.243	0.197
Wind dispersal of seeds (121 taxa)[a]	0.292	0.241	0.143
Sexual/asexual reproduction (54 taxa)[a]	0.305	0.236	0.213
Winged/plumose dispersal of seeds (48 studies)[b]	0.216	0.196	0.079
Long-lived life cycle (48 studies)[b]	0.221	0.202	0.077
Widespread (52 studies)[b]	0.316	0.183	0.407

[a] From Hamrick and Godt, 1989; [b] From Loveless and Hamrick, 1984.

altissima populations in Seoul (Brown and Marshall, 1981; Clegg and Brown, 1983). Most introduced species in their new ranges usually possess little or no genetic variability (Barrett and Richardson, 1986; Gray, 1986; Barrett and Shore, 1989). In colonizing species, population establishment and migration frequently involve a small number of individuals (Barrett and Shore, 1989). Thus, we can assume that the invasion of *A. altissima* is accompanied by sampling of very small part of the entire gene pool.

In addition, a significant number of surveys of isozyme variation in weed species document depauperate amounts of genetic diversity both within and among populations, particularly following continental migration (Hamrick et al., 1979; Barrett and Richardson, 1986). Recent surveys of weed groups revealed highly homozygous populations or populations composed of a few genotypes, and these findings indicate that high levels of genetic diversity are not a prerequisite for a successful invading species (Barrett and Richardson, 1986).

Compared with other species introduced in Korea, *A. altissima* has lower genetic diversity than *Amorpha fructicosa* (H_{EP}=0.205, G_{ST}=0.156), which is also a predominantly outcrossed, insect-pollinated dicot (Huh and Huh, 1997). However, it was introduced about 70 years ago, compared with about 20 years of *A. altissima*.

However, assessment of the impact of founder effect should ideally include comparison of populations from both the introduced and native ranges, thereby giving a relevant gauge of a species' genetic variation in its new range (Novak and Mack, 1993). Because of the lack of consensus regarding the relation of genetic variability to the invasive ability of plants (Barrett and Richardson, 1986), first categorizing invasive species by their life history traits and looking for patterns through the comparison of taxonomically related non-invasive species may provide a new insight for the relative impact of invasion process on genetic divergence and the relationship of genetic variability to the invasive ability (Gray 1986; Schierenbeck et al., 1995). One of the major difficulties in the study of biology of invasions is the lack of information on the early stages of colonization (Barrett and Richardson, 1986). *Ageratina*

altissima provides a unique opportunity for further research into the actual dynamics and spread of invasive species. By including the peripheral genetic data of *A. altissima* after its wider spread and comparing them with populations from the native ranges, we could get more information on its general characteristics related to the invasive ability.

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