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Allozyme diversity in the endangered herb *Lycoris sanguinea* var. *koreana* (Amaryllidaceae)

Myong Gi Chung

Chung, M. G., Department of Biology, Gyeongsang National University, Chinju 660-701, The Republic of Korea

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Lycoris sanguinea Maxim. var. *koreana* (Nakai) Koyama (Amaryllidaceae), a sexually and clonally reproducing herbaceous perennial, is distributed in a few localities in the southern Korea and Japan (Kyushu and Tsushima Island). Seven Korean populations of the variety were analyzed by starch gel electrophoresis to measure genetic variation at 19 allozyme loci. Populations of *L. sanguinea* var. *koreana* maintain similar levels of allozyme diversity (percent of polymorphic loci, $P = 15\%$ and mean expected heterozygosity, $H_e = 0.052$) to values for its widespread congener *L. chinensis* Traub. Genetic divergence among populations of the variety was low (mean $G_{ST} = 0.092$). This might be due to recent divergence of populations in Korea. As nearly all genetic diversity in the variety is contained within populations, conserving on one Korean population of the variety would maintain most of the genetic diversity in the Korean populations as a whole.

Key words: allozyme, conservation, endangered species, genetic diversity, *Lycoris sanguinea* var. *koreana*

INTRODUCTION

Lycoris sanguinea Maxim. var. *koreana* (Nakai) Koyama (Amaryllidaceae) is narrowly distributed in a few localities in the southern Korean peninsula and in southern Japan (Kyushu and Tsushima Island) (Hsu *et al*. 1994). The variety reproduces sexually by seeds and asexually by rapidly formed bulbs (M. Chung, pers. obs.). *Lycoris sanguinea* var. *koreana* grows only on moist places in stony valleys. Stamens are shorter than the pistil and distinctly exceed the apricot-orange tepals (ca. 5–6 cm long), indicating an insect-pollinated, predominantly outcrossing breeding system. Each fruit (capsule), ca. 1.5 cm wide, contains several round seeds (ca. 5 mm wide), indicating that a large proportion of seeds may drop directly to the ground. Owing to its strong potential for vegetative reproduction and lack of specialized seed dispersal mechanism, small "mat-like" patches are scattered in natural habitats. In Korea, the number of individuals in natural habitats has rapidly de-

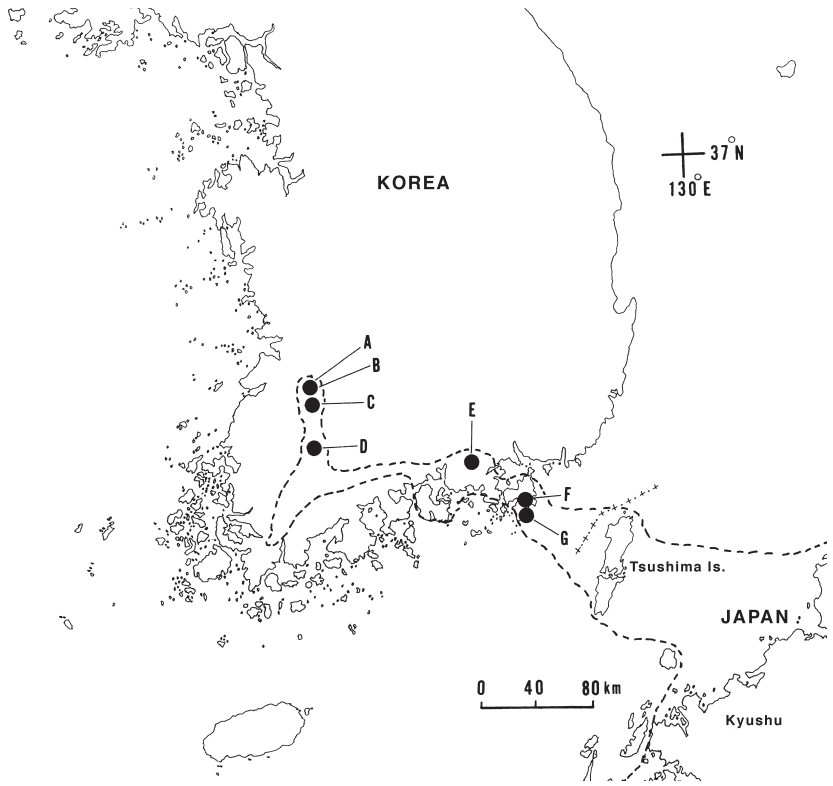

Fig. 1. The distribution (dashed line) and location of seven sampled populations (alphabetic codes as in Table 1) of *Lycoris sanguinea* Maxim. var. *koreana* (Nakai) Koyama in Korea.

creased, partly due to collection by plant enthusiasts and/or plant sellers. The plant only grows in a wet, stream valleys and has been listed in a catalogue of endangered plants in Korea (Ministry of Environment, Korea 1989). Bulbs are easily detached and could probably be transported by heavy runoff in the sloppy valley. This would affect the spatial distribution of clones and genetic variation in populations. Furthermore, the spatial distribution of clonal versus that of sexual reproduction should generally have differing effects on the levels of biparental inbreeding and selfing rate, produced via mating by proximity through limited pollen dispersal. For example, matings between clones, via spatial proximity, contribute to selfing. Chromosome number is 2n = 22 (Kurita 1988).

Survival of a species in the long term depends on the maintenance of enough genetic variation or allelic diversity within and among populations to track changing environmental conditions (Beardmore 1983, Huenneke 1991). In the short term, a decrease in genetic variation may indirectly affect individual fitness and population variability through increased levels of inbreeding (Barrett & Kohn 1991). Because a knowledge of levels and distribution of genetic diversity within and among populations of endangered species is thus important in designing conservation strategies, studies for this purpose using allozymes and molecular markers (e.g., RAPD, random amplified polymorphic DNA and AFLP, amplified fragment length polymorphism) have become increasingly common (e.g., Brauner *et al*. 1992, Lynch & Milligan 1994, Stewart & Excoffier 1996, Fisher & Matthies 1998, Gemmill *et al*. 1998, Godt & Hamrick 1998).

In this study, I describe amounts and patterns of genetic variation, clonal diversity, and genetic structure in populations of *Lycoris sanguinea* var. *koreana* using allozymes as genetic markers to provide baseline genetic information pertinent to the conservation of the plant.

MATERIAL AND METHODS

A total of 340 leaf samples from seven populations of *Lycoris sanguinea* var. *koreana* were collected in Korea (Fig. 1).

Except population C (40 individuals), 50 individuals per population were randomly sampled in 800–2 000 m^2 area, depending on population size. Because the variety exhibits extensive clonal growth, samples were collected at intervals of > 3 m within each population to avoid biasing samples toward certain clones. Leaf samples were placed in plastic bags wrapped with a wet paper towel, stored on ice, and transported to the laboratory. Samples were then stored at 4°C until protein extraction.

Leaf samples were cut finely and crushed with a mortar and pestle. A phosphate-polyvinylpyrrolidone extraction buffer (Mitton *et al*. 1979) was added to leaf samples to facilitate crushing and to aid enzyme stabilization. Enzyme extracts were absorbed onto 4 × 6-mm wicks cut from Whatman 3 mm chromatography paper, which were then stored at –70°C until needed.

Electrophoresis was performed using 10.5% starch gel. Nineteen loci from ten enzyme systems were resolved using two electrode and gel buffer systems. A Poulik buffer system, a modification (Haufler 1985) of system 6 of Soltis *et al*. (1983), resolved alcohol dehydrogenase (*Adh*), diaphorase (*Dia-1*, *Dia-2*, *Dia-3*, *Dia-4*), fluorescent esterase (*Fe-1*, *Fe-2*), Menadione reductase (*Mnr*), peroxidase (*Per-1*, *Per-2*), phosphoglucoisomerase (*Pgi-1*, *Pgi-2*), phosphoglucomutase (*Pgm-1*, *Pgm-2*), and triosephosphate isomerase (*Tpi-1*, *Tpi-2*). A modification (Chung & Kang 1994) of system 11 of Soltis *et al*. (1983) was used to resolve isocitrate dehydrogenase (*Idh-1*, *Idh-2*) and shikimate dehydrogenase (*Skdh*). All stain recipes were identical to those described by Soltis *et al*. (1983), except for diaphorase, which is given in Cheliak and Pitel (1984). The genetic basis of enzyme banding patterns was inferred from observed segregation pattern in light of typical subunit structure and subcellular compartmentalization (Weeden & Wendel 1989). Putative loci were designated sequentially, with the most anodally migrating isozyme designated '1', the next '2', etc. Likewise, alleles were designated sequentially with the most anodally migrating allele designated 'a'.

A locus was considered polymorphic if two or more alleles were detected, regardless of their frequencies. Four standard genetic parameters were estimated using a computer program developed by M. D. Loveless and A. Schnabel (pers. comm.): percent polymorphic loci (*P*) was calculated by dividing the number of loci polymorphic in at least one population by the total number of loci analyzed, mean number of alleles per locus (*A*) was determined by summing all the alleles observed and dividing by total number of loci. The effective number of alleles per locus (A_e) was calculated for each locus by $1/(1 - \Sigma p^2_i)$, where p_i is the mean frequency of the allele. Genetic diversity or expected heterozygosity (He) was calculated as the $H_e = 1 - \Sigma p^2_i$.

Before assessing the amount of clonal diversity within populations, the possibility (P_G) of randomly drawing two identical multilocus genotypes (Berg & Hamrick 1994), which is based on observed single-locus genotypic frequencies and assuming linkage equilibrium, was calculated. The total likelihood that two individuals could have identical multilocus genotypes when both are produced by sexual reproduction was substantially high ($P_G = 0.319$, Table 1), indicating that several identical multilocus genotypes in populations are produced by sexual reproduction. Thus, it is inappropriate to estimate the amount of clonal diversity within populations based on the present data set.

Nei's (1973, 1977) gene diversity formulae (H_T, H_S, D_{ST}, and G_{ST}) were used to evaluate the distribution of genetic diversity within and among populations. In addition, a χ^2-statistic was used to detect significant differences in allele frequencies among populations for each locus (Workman & Niswander 1970). Genetic divergence among populations was also estimated by calculating Nei's (1972) genetic identity and distance for all pairs of populations. In addition, we used NTSYS (Rohlf 1992) to conduct cluster analysis on genetic distances via the unweighted pairwise groups method using arithmetic average (UPGMA).

RESULTS

Nineteen putative allozyme loci were resolved from ten enzyme systems. Among them, only four loci were polymorphic in at least one population (Table 1). Estimates of genetic diversity are presented in Table 2. Mean H_e values of the seven

Table 1. Allele frequencies for four polymorphic loci in seven populations (A–G) of *Lycoris sanguinea* Maxim. var. *koreana* (Nakai) Koyama.

Locus	Allele	A	B	C	D	E	F	G
Dia-1	*a*	0.11	0.11	0.38	0.08	0.35	0.29	0.02
	b	0.89	0.89	0.62	0.92	0.65	0.71	0.98
Idh-1	*a*	1.00	1.00	1.00	1.00	0.99	0.95	1.00
	b	0.00	0.00	0.00	0.00	0.01	0.05	0.00
Idh-2	*a*	0.26	0.12	0.00	0.36	0.36	0.00	0.00
	b	0.74	0.88	1.00	0.64	0.64	1.00	1.00
Pgi-2	*a*	0.04	0.00	0.00	0.00	0.00	0.04	0.04
	b	0.43	0.44	0.60	0.49	0.31	0.53	0.59
	c	0.57	0.56	0.40	0.51	0.69	0.43	0.37

populations were not significantly different from each other (Wilcoxon signed-rank test, $p > 0.05$).

Korean populations of *Lycoris sanguinea* var. *koreana* were genetically similar, as indicated by the high mean genetic identity ($I = 0.992$). About 91% of the total variation in the variety is common to all populations. However, it should be noted that the high genetic identity is a consequence of the large numbers of monomorphic (15 of 19) loci (Berg & Hamrick 1997). Although the mean G_{ST} value was 0.092 (Table 3), statistically significant differences in allele frequencies were found among populations for all four loci (χ^2-tests; $p < 0.001$ in each case). Although population C is more similar to disjunct population F (Fig. 2), the low genetic distance suggests that all populations are nearly very similar.

DISCUSSION

A recent review of allozyme literature (Hamrick & Godt 1989) summarized the average genetic diversity statistics for populations of long-lived herbaceous perennials ($N = 4$, $P = 39\%$, $A = 1.44$, $A_e = 1.14$, $H_e = 0.084$), species with a narrow distribution ($N = 115$, 30.6%, 1.45, 1.13, 0.105), and species with seed dispersal mechanisms by gravity ($N = 199$, 29.8%, 1.45, 1.14, 0.101). For the populations of *Lycoris sanguinea* var. *koreana*, the mean values of these parameters were lower: 15% (P), 1.17 (A), 1.09 (A_e), and 0.052 (H_e).

Why was allozyme diversity in the populations of *Lycoris sanguinea* var. *koreana* low? The variety might have originated with low genetic diversity from its progenitor(s) followed genetic bottlenecks during its evolutionary history. During two years field survey, seedlings have been rarely encountered, though fruits are regularly produced (M. Chung, unpubl. data). It is probable that the clonal nature of *L. sanguinea* var. *koreana* could account for low levels of genetic variability within populations. If limited recruitment of seedling establishment is true for *L. sanguinea* var. *koreana*, the clonal nature of the variety may act as an enhancer of genetic drift by reducing the effective size of local populations in the near future. Comparisons of genetic diversity within a rare or endangered species with that of a closely related species with similar life history characteristics but a different geographic distribution can aid in interpreting the effects of rarity on genetic diversity (Karron *et al*. 1988). My ongoing allozyme study on Korean populations of *L. chinensis* Traub, distributed in China and Korea, also revealed low levels of allozyme variation within populations (P of 20% and H_e of 0.070; M. Chung, unpubl. data). As both *L. sanguinea* var. *koreana* and its widespread congener also have low levels of genetic diversity, it is difficult to conclude that populations of *L. sanguinea* var. *koreana* are genetically depauperate probably due to recent reductions in population sizes, habitat fragmentation, and reduced gene flow between discrete populations with a patchy distribution.

If reductions in population size and reduced gene flow cause loss of genetic diversity, it is expected that between-population differences be-

Table 2. Summary of allozyme variation for 19 loci within seven populations of *Lycoris sanguinea* Maxim. var. *koreana* (Nakai) Koyama[a].

Population	P	A	A_e	H_o (SE)	H_e (SE)	G	P_G
A	16	1.16	1.10	0.048 (0.007)	0.056 (0.033)	8	0.314
B	16	1.16	1.08	0.054 (0.007)	0.047 (0.029)	9	0.385
C	11	1.11	1.09	0.073 (0.009)	0.049 (0.034)	4	0.320
D	16	1.16	1.11	0.060 (0.008)	0.058 (0.035)	6	0.388
E	21	1.21	1.13	0.056 (0.007)	0.072 (0.038)	13	0.103
F	16	1.21	1.10	0.062 (0.008)	0.055 (0.034)	11	0.173
G	11	1.16	1.06	0.041 (0.006)	0.029 (0.027)	5	0.552
Mean	15.28	1.17	1.09	0.056 (0.003)	0.052 (0.013)	8	0.319

[a] Abbreviations: P = percentage of polymorphic loci; A = mean number of alleles per locus; A_e = effective number of alleles per locus; H_o = observed heterozygosity; H_e = Hardy-Weinberg expected heterozygosity or genetic diversity; G = number of multilocus genotypes; P_G = probability of randomly drawing two identical genotypes.

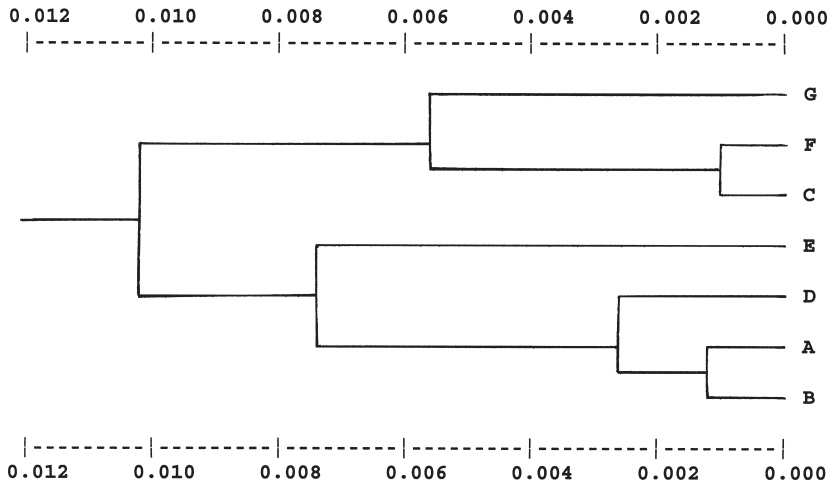

Fig. 2. Phenogram from UPGMA cluster analysis based on Nei's (1972) genetic distance among the seven populations of *Lycoris sanguinea* Maxim. var. *koreana* (Nakai) Koyama.

come large compared to within-population ones. This is not a case in this study. Of the total variation found in *Lycoris sanguinea* var. *koreana*, about 9% was due to differences among populations (mean $G_{ST} = 0.092$ vs. $H_s = 0.248$). Based on an allozyme literature reviewed by Hamrick and Godt (1989), long-lived herbaceous perennials, species with a narrow geographic range, species with a seed dispersal mechanism by gravity have mean G_{ST} values of 0.213 ($N = 2$), 0.242 ($N = 82$), and 0.277 ($N = 161$), respectively. Considering all information, it is suggested that Korean populations of *L. sanguinea* var. *koreana* might have diverged recently and/or been founded by individuals harboring similar genotypes probably by seed dispersal by birds. Low values of G_{ST} have also been observed in other Korean species. *Impatiens hypophylla* Miq. (Balsaminaceae), an annual herb, is restricted to a few locations in southwestern Japan and southeastern Korea. Two Korean populations that are about 50 km apart, with no intervening populations, differed little in gene frequencies (mean $G_{ST} = 0.059$; Chung & Kang 1996a). *Chimaphila japonica* Klenze (Pyrolaceae), a low evergreen subshrub, is widely distributed under pine-oak hillsides in northeastern Asia. Only 3% of the total variation found in eight Korean populations of *C. japonica* was due to differences among populations (mean $G_{ST} = 0.028$; Chung & Kang, 1996b). The mean G_{ST} value between Korean populations of *Adenophora grandiflora* Nakai, a perennial herb, restricted to the central and northern Korean peninsula, was 0.027 (M. Chung, unpubl. data).

As *Lycoris sanguinea* var. *koreana* in Korea maintains low levels of allozyme diversity, the recent increased destruction of natural habitats and reckless collection from several populations may further result in erosion of genetic diversity in the near future. The distribution of genetic variation among populations is of primary importance to the conservation of genetic diversity of plant species (Hamrick *et al.* 1991). The general formula for determining number of populations to be sampled or conserved to obtain 99% of the total genetic diversity is $0.99 = 1–(G_{ST})^n$ (Ceska *et al.* 1997), where n is the number of populations proposed for sampling or conserving. As the formula assumes that each population has equal levels of genetic diversity, I performed Wilcoxon signed-rank test for multiple comparison of means of the expected heterozygosities among seven populations. Means of the values were not significantly different ($p > 0.05$) for all pair-wise comparisons. Applying the formula to the present study, con-

Table 3. Nei's (1973, 1977) statistics of genetic diversity for four polymorphic loci in *Lycoris sanguinea* Maxim. var. *koreana* (Nakai) Koyama.

Locus	H_T	H_S	D_{ST}	G_{ST}
Dia-1	0.3019	0.2676	0.0343	0.1136
Idh-1	0.0175	0.0169	0.0006	0.0348
Idh-2	0.2649	0.2168	0.0481	0.1816
Pgi-2	0.5112	0.4913	0.0199	0.0390
Mean	0.2739	0.2481	0.0257	0.0923

serving just one Korean population of *L. sanguinea* var. *koreana* would maintain 99% of the genetic diversity in the Korean populations as a whole. Populations A–C and F lie in national parks and are relatively large, so preservation should focus on these populations. In addition, as *L. sanguinea* var. *koreana* is pollinated by bumblebees, these insects need also be conserved.

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