

# Notes on allozyme variation in *Lycoris radiata* (Amaryllidaceae) from Korea

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**Abstract.** *Lycoris radiata* (L'Her) Herb. var. *radiata*, a herbaceous perennial and sterile triploid ( $2n = 33$ ), grows in moist places in Japan (except Hokkaido), Korea, China, and Nepal. Several botanists have speculated that the taxon originated in China and was introduced into Japan. In Korea, the taxon is routinely found in the streamsides near temples in the southern regions. Allozyme analysis was conducted in eight Korean populations of *L. radiata* var. *radiata* as well as in one Korean population of *L. chinensis* Traub. ( $2n = 16$ ) to estimate levels of allozyme diversity. *Lycoris radiata* var. *radiata* was monomorphic at all 24 allozyme loci surveyed whereas *L. chinensis* was polymorphic at 19% of the 21 loci analyzed with 0.06 gene diversity. The results strongly indicated that only one or a few bulbs of *L. radiata* var. *radiata* were introduced from China or, secondarily, Japan to a temple in Korea and were then naturalized in the southern Korean peninsula via a strong vegetative reproduction by the rapid formation of new bulbs.

**Keywords:** Allozyme; Korea; *Lycoris chinensis*; *L. radiata* var. *radiata*; Naturalization; Sterile triploid.

## Introduction

Electrophoretic techniques provide genetic markers to recognize individual genotypes for asexually reproducing plants (e.g., Pleasants and Wendel, 1989; Murawski and Hamrick, 1990; Aspinwall and Christian, 1992; Lokker et al., 1994; Kim and Chung, 1995). This approach has made it possible to better understand the spatial distributions of clones and the genotypic diversity maintained within populations (e.g., Parker and Hamrick, 1992; Berg and Hamrick, 1994). Allozyme analysis has contributed in a variety of ways to better understanding of the origin, history of naturalization or domestication, and speciation of plant species (Crawford, 1989; Doebley, 1989).

*Lycoris radiata* (L'Her.) Herb. (Amaryllidaceae) consists of three varieties: *L. radiata* var. *radiata*, *L. radiata* var. *pumila* Grey, *L. radiata* var. *kazukoana* Yonezawa (Hsu et al., 1994). The former variety is the most widespread taxon of *Lycoris*. It is distributed widely in China, Japan (except Hokkaido), and the southern Korean peninsula. *Lycoris radiata* var. *pumila*, a fertile diploid ( $2n=22$ ), is endemic to eastern, central, and southern China (Hsu et al., 1994). *Lycoris radiata* var. *kazukoana* is endemic to Japan (Yonezawa, 1989).

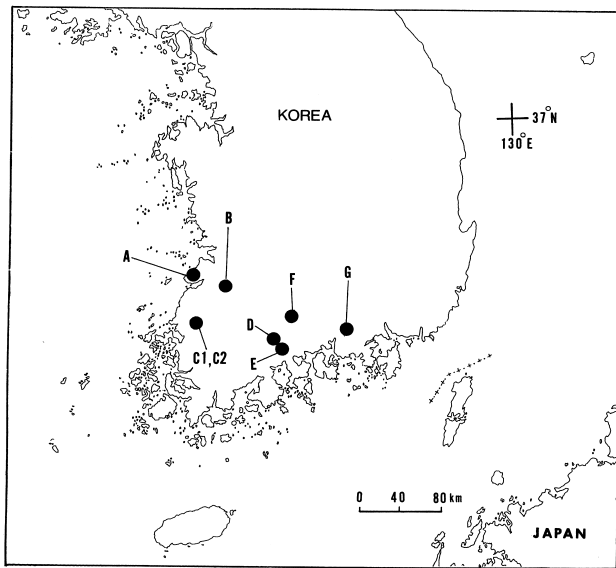
*Lycoris radiata* var. *radiata* is a sterile triploid ( $2n=33$ ) that propagates via strong vegetative reproduction by the rapid formation of new bulbs. It has been suggested, based on karyotype analysis, that this variety might have originated from the combination of an unreduced gamete

of a diploid with a normal gamete of a diploid *L. radiata* var. *pumila* (Liu and Hsu, 1989) because tetraploids have never been found in this species. It has also been suggested that *L. radiata* var. *radiata* originated in China and was introduced into Japan (Fukuda et al., 1980; Kurita, 1987) because the diploid *L. radiata* var. *pumila* has never been found in Japan or its adjacent regions (Hsu et al., 1994). *Lycoris radiata* var. *radiata* is routinely found in the streamsides near temples in southern Korea (Park et al., 1986b; M. Chung, pers. obs.).

In this study, allozyme analysis was conducted on eight Korean populations of *L. radiata* var. *radiata* as well as on one population from one known locality of *L. chinensis* Traub. ( $2n = 16$ ) in Korea to estimate levels of allozyme and clonal diversity and to gain ideas concerning the origin of this variety in Korea.

## Materials and Methods

Leaves were collected from eight populations of *L. radiata* var. *radiata* in Korea (Figure 1). Fifty individuals per population were randomly sampled in a 1,000–2,000 m<sup>2</sup> 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. In addition, twenty-two individuals of *L. chinensis* were collected from hillsides of Mt. Dosol, Kochang Gun, Pref. Chollabuk, Korea. Leaf samples were placed in plastic bags wrapped with a wet paper towel and stored on ice and transported to the laboratory. Samples were then stored at 4°C until protein extraction.



**Figure 1.** The location of the eight populations of *L. radiata* var. *radiata* in Korea examined in this study: A, near Seonun-sa ("sa" means a temple) in Mt. Dosol and 22 samples of *L. chinensis* were also collected in this population; B, near Naejang-sa in Mt. Naejang; C1, Bulgap-sa; C2, Yongch'eon-sa; D, Songkwang-sa in Mt. Jogae; E, Seonam-sa in Mt. Jogae; F, Ssanggae-sa in Mt. Chiri; G, near Okch'eon-sa in Mt. Yeonwha.

Leaf samples were cut finely, and crushed with a mortar and pestle. A phosphate-polyvinylpyrrolidone extraction buffer (Mitton et al., 1979) was added to the leaf samples to facilitate crushing and to aid enzyme stabilization. The crushed extract was absorbed onto  $4 \times 6$ -mm wicks cut from Whatman 3MM chromatography paper, which were stored at  $-70^{\circ}\text{C}$  until needed for analysis. Electrophoresis was performed using 10.5% starch gels. Twenty-four and 21 putative loci for *L. radiata* var. *radiata* and *L. chinensis* from ten enzyme systems, respectively, were resolved using two electrode and gel buffer systems. A Poulik buffer system, a modification (Hauffer, 1985) of Soltis et al. (1983) system 6, resolved alcohol dehydrogenase (*Adh*), diaphorase (*Dia-1*, *Dia-2*, *Dia-3*), fluorescent esterase (*Fe-1*, *Fe-2*, *Fe-3*, *Fe-4* only for *L. r.* var. *r.*), Menadione reductase (*Mnr*), peroxidase (*Per-1*, *Per-2*), phosphoglucosomerase (*Pgi-1*, *Pgi-2*, *Pgi-3* only for *L. r.* var. *r.*), phosphoglucosomutase (*Pgm-1*, *Pgm-2*, *Pgm-3*), and triosephosphate isomerase (*Tpi-1*, *Tpi-2*, *Tpi-3*). A modification (Chung and Kang, 1994) of Soltis et al. (1983) system 11 was used to resolve isocitrate dehydrogenase (*Idh-1*, *Idh-2*, *Idh-3* only for *L. r.* var. *r.*) 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). Putative loci were designated sequentially, with the most anodally migrating isozyme designated 1, the next 2, and so on. Likewise, alleles were designated sequentially with the most anodally migrating alleles designated a. Although the genetic bases of the loci were not documented by con-

trolled crosses, the isozymes expressed phenotypes that were consistent in subunit structure and genetic interpretation with most isozyme studies in plants, as documented by Weeden and Wendel (1989).

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: percent polymorphic loci (P), mean number of alleles per locus (A), effective number of alleles per locus ( $A_e$ ), and gene diversity ( $H_e$ ).

## Results and Discussion

For *L. var. radiata*, all 24 allozyme loci surveyed were monomorphic in all populations. On the other hand, a slightly higher level of allozyme variation was observed in a population of *L. chinensis* (P of 19.05%, A of 1.19,  $A_e$  of 1.09, and  $H_e$  of 0.060 based on 21 loci). Relative to mean values for short-lived herbaceous perennials at the population level ( $N=159$ , P of 28.0%, A of 1.40,  $A_e$  of 1.12, and  $H_e$  of 0.096), *L. chinensis* also has a lower level of variation. Individuals of *L. chinensis* grow on the moist slopes of hillsides in China (Henan, Jiangsu, Shaanxi, and Sichuan, and Zhejiang) and at a few localities (Mts. Dosol, Naejang, and Paekyang) in the southwestern Korean peninsula (Hsu et al., 1994; Tae and Ko, 1996). In other words, the Korean populations are located at the margin of the distribution of the species. Usually, marginal, isolated populations maintain less genetic diversity than continuously distributed, mainland populations (Chung and Kang, 1996; Godt and Hamrick, 1996; Godt et al., 1996; Gemmill et al., 1998). In this regard, a higher level of allozyme variation in *L. chinensis* would be expected, if more samples from China were added in the analysis.

The geographic range of a species accounted for the largest amount of genetic variation in population and species level genetic diversity (Hamrick and Godt, 1989). Usually, plant species with geographically widespread distributions tend to maintain higher levels of allozyme diversity than plants with more restricted ranges (Karron et al., 1988). Clonal reproduction could retard the loss of genetic diversity within populations because species with independent ramets could reduce the probability of genet death (Cook, 1983). *Lycoris radiata* var. *radiata* is the most widespread taxon of *Lycoris*. It is distributed widely in China, Japan (except Hokkaido), and the southern Korean peninsula (Hsu et al., 1994). As *L. radiata* var. *radiata* is a sterile triploid ( $2n=33$ ) and no seed-set was known, it reproduces extensively via vegetative reproduction by the rapid formation of new bulbs (Park et al., 1986a). The absence of variation in all 24 allozyme loci strongly suggests that one or more bulbs of the sterile *L. radiata* var. *radiata* were at one time introduced from China or Japan (i.e., by a monk) into a temple in Korea (probably a temple in the southwestern Korean peninsula). Due to its asexual reproduction, it is highly likely that the introduced bulbs escaped from a temple garden into nearby stream-

sides and were then spread by physical forces such as run-off into a muddy hillside valley. Usually, monks in Korean temples move into another temple every several years. It is also highly probable that one or more monks, attracted by *L. radiata* var. *radiata*'s showy, bright red flowers in autumn, carried several bulbs with them and planted them in a temple garden. These speculations are based on two lines of evidence: first, *L. radiata* var. *radiata* is commonly growing in all temple gardens located in southern Korea. Second, outdoor habitats are limited to wet, shaded places, and hillside valleys along streamsides near temples (Park et al., 1986b; M. Chung, pers. obs.). These activities would have enhanced the naturalization of *L. radiata* var. *radiata* in Korea for a long time. As *L. chinensis* maintains allozyme variation, though relatively restricted distributions in China and Korea, the monomorphism on all 24 loci found in Korean *L. radiata* var. *radiata* may be due to its introduction (only one or a few bulbs) from China or probably its secondary introduction from Japan and extensive asexual reproduction by lateral bulbs.

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## 韓國石蒜 *Lycoris radiata* (Amaryllidaceae) 之同功酶變異

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石蒜 (*Lycoris radiata* (L'Her) Herb. Var. *radiata*) 是三倍體不孕性的多年生草本，主要生長於日本 (除了 Hokkaido)、韓國、中國及 Nepal 之濕地，一些植物學者推想認為其起源於中國而後傳入日本，在韓國南部地區靠近廟旁的河流邊亦經常被發現。對生長於韓國 8 個 *L. radiata* var. *radiata* 及一個 *L. chinensis* Traub. ( $2n = 16$ ) 的族群進行 allozymes 歧異度的分析，在所有檢查的 24 個 allozyme 基因座 (loci) 中發現 *Lycoris radiata* var. *radiata* 都是單型性 (monomorphic)，然而 *L. chinensis* 於 21 個分析的基因座中，有 19% 的多型性 (polymorphic) 及 0.06 的基因歧異性。此結果強烈顯示只有一個或一些 *L. radiata* var. *radiata* 的球莖是從中國或其次從日本引入到韓國的廟宇，而後經由快速新球莖生成的營養繁殖方式而歸化於南韓半島。

關鍵詞：同功酶；*Lycoris chinensis*；石蒜；歸化；不孕性三倍體。