Allozyme Variation in *Ophiopogon xylorrhizus*, an Extreme Endemic Species of Yunnan, China

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Introduction

Ophiopogon xylorrhizus Wang et Tai is a narrowly distributed species in the family Liliaceae; it occurs only in the tropical area of southern China (Zhang 1991). A very few individuals found were recognized by Wang and Tai in 1978 in Mengla County of Yunnan Province (Wang & Tang 1978). Later expeditions to other regions from 1991 to 1994, southward to Laos and northward to central Yunnan, revealed another eight populations. The population at the type locality is now extinct. The extent of the current known range of the species is an area approximately 30 × 20 km (Fig. 1).

Ophiopogon xylorrhizus is a rhizomatous, herbaceous perennial that typically occurs in moist or damp mountain valleys by streams in the tropical rain forest in Mengla County. Although this region belongs to the national Xishuangbanna Reserve, our observations have revealed that one of the eight known populations (05 in Fig. 1) has recently been extirpated, and several other populations have gradually been reduced in size, mainly because of decades of destruction and degradation of habitat from agriculture, silviculture, and fire suppression. Because little is known about the biology and genetics of this species, developing a management plan to maintain or enhance the populations has been difficult.

Assessment of the level and distribution of genetic diversity within endemic plant species may contribute to knowledge of their evolutionary history and potential and is critical to their conservation and management in many cases (Schaal et al. 1991; Soltis & Soltis 1991; Soltis et al. 1992). Because allozyme analyses are extremely useful in determinations of the genetic structure of species and in comparisons among species (Hamrick et al. 1991; Schaal et al. 1991), they have been used to examine the genetic structure of rare and endemic plant species (Waller et al. 1987; Lesica et al. 1988; Soltis & Soltis 1991; Soltis et al. 1992; Cosner & Crawford 1994; Crawford et al. 1994; Richter et al. 1994; Godt et al. 1995). As part of a larger project that seeks to clarify the causes of the endangerment of *O. xylorrhizus* and to develop a more reasonable, scientific protection plan, we conducted analysis of the level and apportionment of genetic variation in this endemic species using isozyme electrophoreses. This information should contribute to a better understanding of the genetic diversity of the endemic tropical species and could then be used to develop plans to manage this endangered species.

Methods

Plants were collected from each of seven extant natural populations (Fig. 1) by random sampling and were subsequently maintained in greenhouse culture for further investigation on, for example, reproductive biology and mating systems. Collectors were careful to minimize potential damage to the populations. In some cases in which populations were exceptionally small, sample sizes were restricted accordingly (Table 1). Because only three individuals were collected from population 01, this population was excluded from the population analyses.

Young leaves were removed from each plant and ground immediately in the Tris-maleate grinding buffer with 15% polyvinyl pyrrolidone (PVP) and 0.2% v/v 2-mercaptoethanol (modified from Soltis et al. 1983). Enzyme electrophoresis generally followed the methods of Soltis et al. (1983). Three buffer systems were used for separating enzymes in 12% horizontal starch gels. System 1 was used to resolve leucine aminopeptidase (LAP), isocitrate dehydrogenase (IDH), and 6- phosphogluconate dehydrogenase (PGD). System 6 was used to resolve aspartate aminotransferase (AAT), phosphoglucoisomerase (PGI), and phoshoglucomutase (PGM). As-

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Paper submitted January 9, 1996; revised manuscript accepted May 16, 1996.

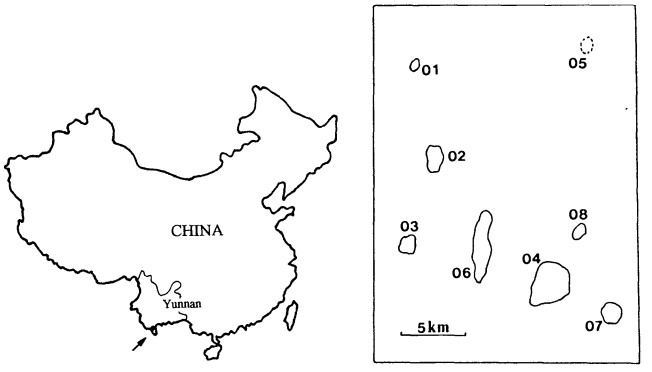

Figure 1. Distribution of Ophiopogon xylorrhizus. *Its entire range is indicated by the spot on the map of China. The locations of the seven extant populations and one extinct population (05) are shown at right. Numbers correspond to populations in Table 1.*

partate aminotransferase (AAT) (for *Aat-1*), diaphorase (DIA), and triosephosphate isomerase (TPI) were resolved on a modification of buffer system 11. Additional enzyme systems were initially surveyed but were discontinued because the banding patterns could not be adequately resolved. Staining procedures for all enzymes followed Soltis et al. (1983) and Wendel and Weeden (1989).

When more than one isozyme was observed for an enzyme, isozymes were numbered sequentially, with the most anodally migrating enzyme designated 1. Allelic variation at a locus was denoted alphabetically, with the most anodal as *a*. Interpretation of the genetic basis of enzyme banding patterns relied on knowledge of previously determined compositions of active subunits of the enzymes and numbers of isozymes expected in diploid angiosperms (Gottlieb 1982; Weeden & Wendel 1989).

Genetic diversity statistics, percentage of polymorphism (P), mean number of alleles per locus (A), observed heterozygosity (H_o) and expected heterozygosity (H_e), and deviation from Hardy-Weinberg equilibrium (fixation index, F) were calculated for each population. Nei's measures of genetic diversity, total genetic diversity (H_T), mean genetic diversity within populations (H_S), and the proportion of genetic variation that occurs among populations (G_{ST}) (Nei 1973) were also calculated for each polymorphic locus and were averaged to provide a single estimate for the species. The G_{ST} values were tested for significance by chi-square analysis (Hedrick 1985).

Results and Discussion

Approximately 21 enzyme loci were discovered for the eight enzyme systems, but only 13 putative loci were consistently interpretable and scorable. Of those *Aat-2*, *Aat-3*, *Dia-2*, *Idh-2*, *Pgd*, *Tpi-1*, and *Tpi-2* were monomophic. The other 6 polymorphic loci, *Aat-1*, *Idh-1*, and *Pgm* had three alleles, and the remaining loci, *Aat-1*, *Lap*, and *Pgi-1*, had two alleles. Allelic frequencies at the polymorphic loci for each population are available from S. Ge.

Parameters measuring levels of allozyme variability and the conformance of genotype frequencies to Hardy-Weinberg expectations in each of the six populations of *Ophiopogon xylorrhizus* are given in Table 1. These diversity values varied among populations, with P ranging from 23.1 to 46.2, A ranging from 1.231 to 1.538, H_e from 0.030 to 0.127; the means were P = 37.5, A = 1.353, and H_e = 0.091. Most populations showed a deficiency of heterozygotes, with positive fixation indices falling between 0.167 and 0.619 (Table 2). The only exception was population 07, with a negative fixation index (−0.100), which was probably a biased estimate due to the small sample size. There was a high proportion of total genetic variation (18.1%) among populations, indicating that almost one-fifth of genetic diversity occurs between populations in this species (Table 2). In addition, there were three unique, rare alleles, including *Dia-1a* and *Idh-1b* in population 02 and *Pgm-b* in population 04.

Table 1. Genetic variability and fixation indices at 13 loci for seven populations of *Ophiopogon xylorrhizus*.

Population no.	*Estimated population size*	*Sample size*	P	A	H_e	H_o	F
1	18	3	—	—	—	—	—
2	65	12	0.462	1.462	0.102	0.085	0.167
3	52	16	0.231	1.231	0.035	0.026	0.257
4	≈400	22	0.385	1.385	0.113	0.043	0.619
6	≈150	24	0.462	1.538	0.127	0.077	0.394
7	60	7	0.231	1.231	0.030	0.033	−0.100
8	35	12	0.385	1.385	0.101	0.071	0.297
Mean		15.5	0.375	1.353	0.091	0.055	0.337
Species	≈800	96	0.462	1.692	0.116		

Table 2. Gene diversity statistics for *Ophiopogon xylorrhizus*.[a]

Locus (no. of alleles)	H_T	H_S	G_{ST}	χ^{2b}
Aat-1 (2)	0.159	0.152	0.044	8.096*
Dia-1 (3)	0.154	0.140	0.091	33.488***
Idh-1 (3)	0.163	0.154	0.055	20.240*
Lap (2)	0.344	0.306	0.110	20.240***
Pgi-1 (2)	0.180	0.177	0.017	3.128
Pgm (3)	0.509	0.310	0.391	143.890***
Mean	0.116	0.095	0.181	

[a] *Means of* H_T *and* H_S *are averaged over all polymorphic and monomorphic loci (i.e., 13). Means for* G_{ST} *are computed from the mean values for* H_T *and* H_S.
[b] *Significance:* *$p < 0.05$; ***$p < 0.001$.

Several recent reviews of the literature on plant allozymes indicate that endemic and narrowly distributed plant species tend to maintain lower levels of genetic variation than more widespread species (Hamrick & Godt 1989; Hamrick et al. 1991). In extreme cases, some rare and endangered angiosperm species show no polymorphism at loci encoding soluble enzymes (Schwartz 1985; Waller et al. 1987; Lesica et al. 1988; Soltis et al. 1992; Crawford et al. 1994). By comparison, *Ophiopogon xylorrhizus*, maintains considerable levels of genetic variation both within and among populations. Even relative to the means found for endemic and narrowly distributed species (Hamrick & Godt 1989), *O. xylorrhizus* possesses high levels of genetic variation. For example, the mean genetic diversity values for about 100 endemic plant species are *Ps* = 40.0, *Pp* = 26.3, *Hes* = 0.096, and *Hep* = 0.063 (Hamrick & Godt 1989), whereas the values for *O. xylorrhizus* were *Ps* = 46.2, *Pp* = 37.5, *Hes* = 0.116, and *Hep* = 0.091.

This is noteworthy considering its restricted distribution with all known populations found in a narrow zone in a single county. In addition to *O. xylorrhizus*, however, several recent studies on rare and endangered species showed that endemics may actually maintain high levels of genetic variation even within extremely narrow distributions, such as with *Elmera racemosa* (Soltis & Soltis 1991), *Coreopsis pulchra* (Cosner & Crawford 1994), and *Delphinium viridescens* (Richter et al. 1994). These data reinforce previous observations that geographic distribution alone is not a reliable indicator of genetic variability (Hamrick 1983; Karron 1991; Soltis & Soltis 1991).

As has been demonstrated previously, geographic range plays almost no role in the distribution of genetic variation among populations (Hamrick & Godt 1989), so *O. xylorrhizus* has an amount of variation among populations comparable to long-lived perennial herbaceous (0.213) and animal-pollinated outcrossing species (0.197) (Hamrick & Godt 1989). Nevertheless, within the region of a few kilometers of each other, the differentiation of populations of *O. xylorrhizus* was relatively high. Although the pollination biology of the species has not been documented, based on its floral morphology and field observations, it appears that pollination is predominantly insect-mediated. A recent field survey indicated that only 15%–17% of the plants bloomed, a low percentage of seeds was produced in any given year, and the insect visitation was infrequent (Zhang et al. unpublished data). Consequently, self-fertilization may occur frequently, and gene flow is limited among the populations, as indicated by allozyme data with high fixation index and G_{ST} values.

The high levels of variability both within and among *O. xylorrhizus* populations revealed by our study have implications for its conservation. It is necessary in the short term to protect existing natural populations of this endemic species in order to preserve as much genetic variation as possible, especially in populations, such as population 02, which showed high variation and harbored rare alleles despite our small sample size.

In the long term, however, the most suitable strategy for the conservation of *O. xylorrhizus* is the protection of its habitat. As one of the well-recognized diploid species ($2n = 36$) within the genus *Ophiopogon*, *O. xylorrhizus* is apparently an ancient lineage, based on several lines of evidence including its primitive morphology and symmetrical karyotype. Furthermore, the species may be naturally rare because it may have ecological or reproductive adaptation to persistence in a small area with few populations. Because deforestation was intense over much of the tropical area of southern Yunnan before the Xishuangbanna Reserve was established in 1980, the loss of populations such as population 05 and decreased population sizes with subsequently lower levels of gene flow can probably be attributed to habitat loss and fragmentation. Detailed studies of the reproductive biology, population demography, and ecology of this species are currently under way and should yield valuable information for the conservation of *O. xylorrhizus*.

Acknowledgments

We thank Qing-jun Li of the Xishuangbanna Botanical Garden of Tropical Plants, and Yian Chen of the Laboratory of Systematic and Evolutionary Botany, Institute of Botany, for their assistance with greenhouse management and field work. This work was supported by grants from the National Natural Science Foundation of China.

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