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Short note

Genetic diversity of widespread *Ophiopogon intermedius* (Liliaceae s.l.): a comparison with endangered *O. xylorrhizus*

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Abstract

Allozyme electrophoresis was used to examine the levels and distribution of genetic diversity on *Ophiopogon intermedius*, a widespread perennial species. Based on allozyme variation at 17 putative loci, high levels of genetic variability were detected with 82.4% of the loci being polymorphic. The mean expected heterozygosity within population (*Hep*) and within species (*Hes*) were 0.352 and 0.426, respectively. Differentiation among populations was detected with $F_{ST} = 0.143$. The high levels of genetic diversity in *O. intermedius* in the present allozyme survey are consistent with the results from the study on morphological and chromosomal characteristics variation. The results from the comparison of the levels of genetic diversity between *O. intermedius* and *O. xylorrhizus*, an endangered congener, showed that the widespread *O. intermedius* maintained much higher levels of genetic diversity than *O. xylorrhizus* ($P = 46.2\%$, Hep = 0.091, Hes = 0.116; Ge, S., Zhang, D.M., Wang, H.Q., Rao, G.Y., 1997. Allozyme variation in *Ophiopogon xylorrhizus:* an extreme endemic species of Yunnan, China. Consservation Biology 11, 562–565). The present study seemly confirmed the general opinion that the widespread species maintains higher levels of genetic diversity than its rare and endangered congener with similar life history traits, while the latter does not necessarily have absolutely low genetic variation. Genetic drift in small populations and inbreeding could have contributed to the relative low genetic variation and gene flow within and among populations of *O. xylorrhizus* compared with it widespread congener.

Keywords: Genetic diversity; Population differentiation; Endangered plant; *Ophiopogon*

1. Introduction

Information on the levels and distribution of genetic diversity within rare and endangered plant species may contribute to the knowledge of their evolutionary history and potential, and is critical to their conservation and management (Schaal et al., 1991; Hamrick and Godt, 1996). Lower levels of genetic variability are expected for rare or geographically restricted plant species than the more widespread congeners (Wright, 1931), though several recent studies reported that rare and endangered species may actually contain high levels of genetic variation even within extremely narrow distribution (Soltis and Soltis, 1991; Cosner and Crawford, 1994; Lewis and Crawford 1995; Ge et al., 1997, 1999). Hamrick and Godt (1996) insisted that the accuracy of predicting genetic diversity in unstudied species based on such generalization is low because estimates of genetic diversity vary widely among species with similar life-histories. As a result, interest in population genetics and conservation biology has drawn attention to comparisons of widespread and restricted congeneric species (Lewis and Crawford, 1995; Smith and Pham, 1996; Ge et al., 1999).

Ophiopogon intermedius (Liliaceae s.l.) is one of the most widespread species in the genus *Ophiopogon*, ranging from east of the Himalaya, including Burma, Nepal, Vietnam, Thailand, to Southwest and South China (Wang and Tang, 1978). In contrast, *O. xylorrhizus* is extremely restricted in an area of 20×30 km, with about 1500 individuals (He, 1999; He et al., 2000). These two species are perennial herbs characterized with rhizome and mainly sexual reproduction (Zhang, 1998; He et al., 1998, 2000). Both are diploid ($2n = 36$), although infraspecific polyploids were occasionally

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detected in *O. intermedius* (Zhang, 1998). Because of the distinctive difference in distribution range, and similar life-history traits, they are well suited for the comparison of genetic diversity. Based on 13 allozyme loci, Ge et al. (1997) detected a high level of genetic variation and population differentiation in *O. xylorrhizus* relative to other narrowly distributed species. However, it is difficult to relate the genetic profile to the endangerment of this species without comparison with its more widespread congeners.

In this study, we report the genetic variation of *O. intermedius* using the same enzyme electrophoresis techniques. Particular emphasis was given to the comparison of the level and pattern of genetic diversity of the widespread *O. intermedius* and those of the endangered *O. xylorrhizus*. Such information may contribute to a better understanding of causes of the rarity of *O. xylorrhizus*, and thus help to develop management strategies for its conservation as well as shed light on the role of genetic diversity in the evolutionary process of plants.

2. Methods and materials

Eight populations of *O. intermedius* were studied (Fig. 1). More than 15 individuals were sampled from each population. The plants were subsequently maintained in a greenhouse. Enzymes were extracted from fresh leaf material by grinding in the extraction buffer with 5% PVP and 0.2% v/v 2-mercaptoethanol (modified from Soltis et al., 1983). The crude extract was absorbed onto 3×6 mm Xinhua-III filter paper wicks and stored in microtest plates at −70°C until needed for electrophoresis.

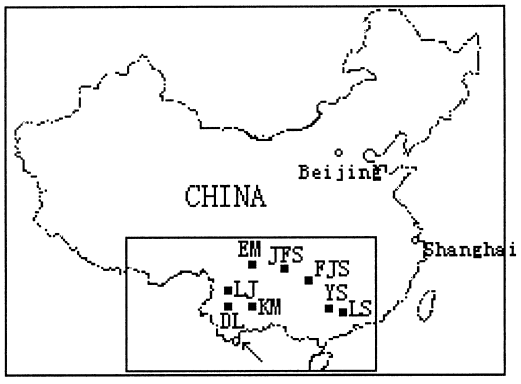

Fig. 1. Locations of the populations of *O. intermedius* sampled in this study. Alphanumeric codes of populations correspond to those in Table 1. Arrow pointed the distributed area of *O. xylorrhizus*. Area in black line square indicated the range of *O. intermedius*. KM: 102°45′ E, 25°10′ N, a.s.l. 2000 m; DL: 100°15′ E, 25°55′ N, a.s.l. 2450 m; LJ: 100°15′ E, 27°00′ N, a.s.l. 2380 m; EM: 103°00′ E, 29°00′ N, a.s.l. 850 m; FJS: 108°35′ E, 27°55′ N, a.s.l. 850 m; JFS: 106°10′ E, 29°00′ N, a.s.l. 1450 m; YS: 110°25′ E, 24°15′ N, a.s.l. 300 m; LS: 112°15′ E, 24°20′ N, a.s.l. 350 m.

Two buffer systems were used for separating enzymes in a 12% horizontal starch gel. The buffer numbers followed Soltis et al. (1993) System 1 was used to resolve aspartate aminotransferase (AAT, E.C. 2.6.1.1), leucine aminopeptidase (LAP, E.C. 3.4.11.1), glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.49), Hexokinase (HK, E.C. 2.7.1.1), Isocitrate dehydrogenase (IDH, E.C.1.1.1.41), Malate dehyogenase (MDH, E.C. 1.1.1.37), Phosphogluconate dehydrogenase (PGD, E.C. 1.1.1.44), Phosphoglucomutase (PGM, E.C. 5.4.2.2), and Shikimate dehydrogenase (SKD, E.C. 1.1.1.25). NAD(P)H-Diaphorase (DIA, E.C. 1.6.2.2), Phosphglucoisomerase (PGI, E.C. 5.3.1.9), and alcohol dehydrogenase (ADH, E.C. 1.1.1.1) were resolved on buffer system 6. Staining procedures for all enzymes followed Soltis et al. (1983).

When more than one allozyme was observed for an enzyme, allozymes were numbered sequentially, with the most anodally migrating allozyme designated 1. Allele variation at a putative locus was denoted alphabetically, with the most anodal designated a. Interpretation of the genetic basis of enzyme banding patterns follow Gottlieb (1982) and Weeden and Wendel (1989).

Levels of genetic diversity were calculated for each population. The parameters, including percentage of loci polymorphic (*P*), number of alleles per polymorphic locus (*A*), mean gene diversity per locus (= expected heterozygosity, *He*), observed heterozygosity (*Ho*), were calculated with BIOSYS-1 computer program (Swofford and Selander, 1989). Deviation from Hardy–Weinberg equilibrium (fixation index *F*) was calculated for each population. Genetic diversity among populations within a species (i.e. ratios of gene diversity of heterozygosity, F_{ST}) was calculated by Wright's (1965) *F*-statistics. Tests of significant differences in allele frequencies were performed using Chi-square method (Sokal and Rohlf, 1969).

3. Results

Seventeen putative allozyme loci were resolved with sufficient consistency and clarity for 12 enzyme systems. Of these, 14 loci were polymorphic, and the remaining three were monomorphic in *O. intermedius*. Data on allelic frequencies for each population is available from the first author.

High levels of genetic diversity were detected in the eight populations of *O. intermedius*. For the species as a whole, the percentage of polymorphic loci (*P*) was 82.4%, and mean number of alleles per polymorphic loci (*A*) and expected heterozygosity (*He*) were as high as 1.8 and 0.426, respectively. At the population level, high levels of variation were also maintained with *P*, *A* and *He* being 75.7%, 1.7 and 0.352, respectively. *F* values were positive or slightly negative with a mean of 0.082, indicating a slightly deficiency of heterozygotes

(Table 1). A mean of 50% (ranging from 33.3 to 66.6% in each population) of 14 loci significantly deviated from Hardy–Weinberg expectation (Table 2), indicating a slightly deviation from random mating and occasional inbreeding in populations.

The organization of genetic variation in *O. intermedius* was tested using *F* statistics of Wright (1965). Overall, mean F_{ST} was 0.143, varying from 0 to 0.367 for 14 polymorphic loci, which suggesting that 14.3% of the overall genetic variation exists among populations (Table 3).

4. Discussion

The present study demonstrated higher levels of genetic variability in *O. intermedius* than that found in *O. xylorrhizus* (Ge et al., 1997). In addition, the mean expected heterozygosity within population (*Hep*) and within species (*Hes*) in *O. intermedius* were much higher than the average reported for other long-lived perennials (Table 4). For *O. xylorrhizus*, *Hep* was slightly higher than the average reported, but *Hes* was rather lower than the average reported. Both *O. intermedius* and *O. xylorrhizus* maintained higher *Hes* and *Hep* than the mean *Hes* and *Hep* of 100 endemic plant species (Hamrick and Godt, 1989) (Table 4).

The high level of genetic variability in *O. intermedius* was not unexpected. Morphological study demonstrated great variation in vegetative traits and characteristics in *O. intermedius* (Zhang, 1998). Higher levels of genetic variability detected in *O. intermedius* fit the general opinion that widespread species have higher genetic variation than endangered and narrowly distributed species (Hamrick and Godt, 1989), although the latter do not necessarily contain very low levels of genetic diversity. High levels of genetic variation would bestow the population or species more flexibility in ability to fit a variable environment, or to occupy new ecological sites and new habitats, even for a species with more asexual reproduction (Huenneke, 1991). Theoretical simulation and empirical data revealed a positive relationship between the amount of genetic variation and evolutionary rate (Ayala and Valentine, 1979). Data on genetic variation would provide strong evidence for me to explore the evolutionary potential of a species.

Surprisingly, genetic differentiation among *O. intermedius* populations was lower than that among *O. xylorrhizus* populations ($F_{ST}=0.143$ in *O. intermedius* vs. $G_{ST}=0.181$ in *O. xylorrhizus*), given the fact that *O. intermedius* was sampled from six provinces while *O. xylorrhizus* was distributed in an area of approximately 30×20 km with six discrete small populations (He, 1999; He et al., 2000). Two genetic consequences of small population sizes are increased drift and inbreeding (Ellstrand and Elam, 1993). Genetic drift changes the distribution of genetic variation in two ways: (i) the decrease of variation within populations, and (ii) the increase of differentiation among populations (Ellstrand

Table 1
Genetic variability statistics of *O. intermedius*

Population	*n*	*P*	*A* (S.E.)	*He* (S.E.)	*Ho* (S.E.)	*F*
KM	16	76.5	1.8 (0.1)	0.349 (0.051)	0.313 (0.074)	0.085
DL	20	70.4	1.7 (0.1)	0.342 (0.057)	0.326 (0.088)	0.032
LJ	12	82.4	1.8 (0.1)	0.384 (0.047)	0.279 (0.077)	0.239
EM	17	64.7	1.7 (0.1)	0.302 (0.058)	0.273 (0.083)	0.077
JFS	15	82.4	1.9 (0.1)	0.393 (0.050)	0.392 (0.087)	−0.032
FJS	17	82.4	1.8 (0.1)	0.389 (0.049)	0.411 (0.091)	−0.018
YS	11	70.6	1.7 (0.1)	0.334 (0.056)	0.246 (0.086)	0.266
LS	10	76.5	1.8 (0.1)	0.325 (0.052)	0.347 (0.012)	0.009
Mean	15	75.7	1.7 (0.1)	0.352 (0.043)	0.323 (0.074)	0.082
Species level	118	82.4	1.8	0.426		

Table 2
Probabilities of Chi-square test with Hardy–Weinberg expectation for polymorphic loci of eight populations of *O. intermedius*

Loci	KM	DL	LJ	EM	FJS	JFS	YS	LS
AAT-1	0.036	0.000	0.014	0.530*	0.420*	0.000	0.005	0.000
AAT-2	0.045	0.002	0.003	0.000	0.000	0.015	0.000	0.075*
ADH	0.948*	0.000	0.880*	0.000	0.000	0.000	0.296*	0.003
DIA-1	0.839*	0.742.*	0.032	0.808*	0.906*	0.766*	–	0.208*
G6PD	0.291*	0.021	0.590*	0.111*	0.007	0.744*	0.024	0.530*
HEX	0.279*	0.001	0.423*	0.073*	0.073*	0.053*	0.009	0.000
IDH	0.000	0.000	0.001	0.294*	0.000	0.000	0.002	0.003
MDH-1	0.134*	0.214*	0.015	–	0.906*	0.130*	0.117*	0.489*
MDH-2	0.000	0.000	0.001	0.000	0.000	0.729*	0.002	0.003
LAP	0.042	0.456*	0.075*	0.000	0.808*	0.000	0.296*	0.208*
PGD	0.279*	–	0.423*	–	0.420*	0.193*	0.380*	–
PGM	–	0.001	0.000	0.001	–	–	0.001	0.000
PGI-3	0.002	0.313*	0.003	0.858*	0.007	0.766*	0.001	1.000
SKD	0.291*	–	0.003	–	0.000	0.521*	0.000	0.000

*Significant difference, $P>0.05$.

Table 3
F-statistics of the polymorphic loci in all eight populations of *O. intermedius*

Loci	F_{IS}	F_{IT}	F_{ST}
AAT-1	0.615	0.666	0.130
AAT-2	0.542	0.587	0.098
ADH	−0.596	−0.592	0.002
DIA-1	0.069	0.261	0.207
G6PD	0.279	0.308	0.040
HEX	0.477	0.528	0.098
IDH	−0.903	−0.899	0.002
MDH-1	0.128	0.306	0.204
MDH-2	−1.000	−1.000	0.000
LAP	0.357	0.433	0.117
PGD	0.371	0.592	0.350
PGM	0.568	0.709	0.326
PGI-3	0.054	0.151	0.103
SKD	0.541	0.709	0 .367
Mean	0.047	0.184	0.143

Table 4
Comparison of the amounts of genetic variation in *O. intermedius*, *O. xylorrhizus*, and the averages reported for long-lived perennial and endemic plant species

	Hes	*Hep*
O. intermedius	0.426	0.352
O. xylorrhizus[a]	0.116	0.091
Average reported for long-lived perennial[b]	0.213	0.084
Mean of 100 endemic plant species[b]	0.096	0.063

[a] Ge et al. (1997).
[b] Hamrick and Godt (1989).

and Elam, 1993). The result from our comparison seems to confirm this prediction. Populations of *O. xylorrhizus* with continually small effective population sizes would be especially susceptible to loss of variation and to limitation of gene flows between populations, which would increase the extent of differentiation among populations. Because *O. intermedius* was distributed in the entire eastern Himalaya with much larger and continuous populations, frequent gene flows and low differentiation among populations are expected. On the other hand, by studying the chromosome characteristics, Zhang (1998) asserted that *O. intermedius* would be a young lineage in the tribe of Ophiopogoneae because of its infraspecific polyploid and asymmetrical karyotype. Consequently, we may attribute the relative low population differentiation for a widespread species to its short evolutionary history. Unlike *O. intermedius* with occasional inbreeding (indicated by $F=0.082$), frequent inbreeding was detected in *O. xylorrhizus* with inbreeding rates as high as 0.071 (He et al., 1998) and F being 0.377 (Ge et al., 1997), which was partly responsible for the low genetic variation in *O. xylorrhizus* compared with its widespread congener. Inbreeding increases homozygosity, decreases the genetic variation within populations and influences fitness through inbreeding depression (Ellstrand and Elam, 1993). Karron (1989) detected high levels of inbreeding depression for seedling biomass in progeny of the restricted *Astragalus linifolius* with frequent inbreeding. A similar scenario was found in *O. xylorrhizus* and strong inbreeding depression for seed set was detected when the inbreeding rate was high (He et al., 1998). Moreover, small populations may suffer greater inbreeding depression than larger ones because deleterious recessive genes could become fixed by chance (Hedrick and Miller, 1992), which can be confirmed by many young seed abortions in *O. xylorrhizus* (He et al., 1998). It is likely that inbreeding depression would be an important factor decreasing the fecundity and viability of *O. xylorrhizus*.

Some researchers (e.g. Schemske et al., 1994; Hamrick and Godt 1996) have argued that populations and species more often go extinct for ecological and demographical reasons rather than for the lack of genetic variation. He (1999) conducted a study on reproductive characteristics and population ecology of the endangered *O. xylorrhizus* and found this species suffered from low reproductive ability. The low evolutionary potential and heavy inbreeding depression in *O. xylorrhizus* resulted in the failure of population recovery, and would be responsible for its endangerment. Compared with its successful congener *O. intermedius*, *O. xylorrhizus* contains less genetic variation and appears to in the process of losing further. Loss of genetic variation may decrease the potential to persist in the face of abiotic and biotic environmental change, as well the ability of a population to cope with short-term challenges such as pathogens and herbivores. Consequently, a specific conservation strategy is needed for the long time survival of *O. xylorrhizus*. The objective of conservation efforts is to maintain the evolutionary viability and to maximize chances for persistence in face of changing environment. Since *O. xylorrhizus* contain low genetic diversity compared with its widespread congener, conservation efforts including artificially promoting gene flowing within and between population are needed. On the other hand, it is difficult to conserve tropical forest plants ex situ for many reasons (Bawa and Ashton, 1991), and conservation in situ is the best way to protect this endangered species.

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