*Biochemical Genetics, Vol. 39, Nos. 3/4, 2001*

*Note*

# Genetic Structure and Heterozygosity Variation Between Generations of *Ophiopogon xylorrhizus* (Liliaceae s.l.), an Endemic Species in Yunnan, Southwest China

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*Received 5 Apr. 2000—Final 4 Oct. 2000*

## INTRODUCTION

Inbreeding depression and evolution of mating systems have been well discussed in the literature (Lande and Schemske, 1985; Schemske and Lande, 1985; Wolfe, 1993; Holsinger, 1988; Carr and Dudash, 1996). These studies all have a common assumption, i.e., homozygosity can cause deleterious recessive or partially recessive lethal alleles to show their effects on the phenotype, so heterozygotes are always more fit than homozygotes (Holtsford, 1996). This implies that inbreeding depression results from inbreeding and excessive homozygotes. Estimates of inbreeding depression usually depend on the fitness differences between selfed and outcrossed progeny. However, inbreeding depression has been generally underestimated, because it is difficult to measure at all possible stages of the life cycle (Charlesworth and Charlesworth, 1987). If inbreeding depression occurred in a species, it would lead to changes of homozygote frequency between seed populations and maternal populations or adult populations. The variation of homozygote frequency between generations may be a better parameter to describe inbreeding depression.

*Ophiopogon xylorrhizus* Wang *et* Dai is a herbaceous perennial, endemic to Mengla County, Yunnan Province, Southwest China. Fewer than 1500 individuals located in eight disjunct populations cover an area of ca. $30 \times 20$ km$^2$ and the

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0006-2928/01/0400-0093$19.50/0 

distribution area has tended to shrink. This species is considered a typical endangered plant in the understory of the tropical rain forest for its restricted distribution and limited number of individuals. *O. xylorrhizus* is thrips-pollinated; autogamous pollination and pollination between flowers have been found in field investigations (He *et al.*, 2000). *O. xylorrhizus* has floral traits favoring outcrossing such as a high pollen/ovule ratio, a long style with stigma above anthers, and some male sterility individuals. However, high selfing rates and strong inbreeding depression were demonstrated in this species (He *et al.*, 1998, 2000). No asexual clones were found in the field survey.

In this study, variation of genetic structure between seed and maternal populations was investigated by allozyme analysis. The causes of high genetic diversity in *O. xylorrhizus* populations are discussed. In addition, conservation measures to protect this endangered species are put forward.

## MATERIALS AND METHODS

Seeds of *O. xylorrhizus* were collected in November 1996 from three natural populations. More than 15 individuals were sampled randomly in each population. The details of the sampling populations are given by He *et al.* (1998) and Ge *et al.* (1997). All the seeds were collected from respective maternal individuals, and all the seeds from one maternal plant were referred to as a family. Seeds were returned to the laboratory and stored in a refrigerator for about 1 month until studied. A total of 383 seeds from 40 families was assayed.

Starch gel electrophoresis was used to estimate genetic structure. The methods of separating enzyme from embryo were the same as those of He *et al.* (1998). Nine enzyme systems resolving 13 putative loci were surveyed: aspartate aminotransferase (AAT), alcohol dehydrogenase (ADH), NAD(P)H-diaphorase (DIA), leucine aminopeptidase (LAP), isocitrate dehydrogenase (IDH), phosphogluconate dehydrogenase (PGD), phosphoglucomutase (PGM), phosphoglucoisomerase ( PGI), and triose-phosphate isomerase (TPI). Three buffer systems were used for separating enzymes in starch gels. ADH, PGI, and TPI were separated in modified buffer system I; AAT, LAP, DIA, PGM, and TPI were separated in buffer system 6; and PGD was resolved in buffer system 12. All buffer systems and staining programs followed Wang (1996).

Genotypes of maternal plants at each locus were obtained using Ritland's (1990) MLT computer program for a mixed mating model based on the methods of Ritland and Jain (1981). The MLT program assumes that progeny are derived from either random mating or self-fertilization. Genotypes of maternal plants in monomorphic loci were presumed to be the same as those of the seed family.

The following parameters were calculated to measure the genetic structure of three seed populations and three maternal populations (Wang, 1996; Nei, 1977):

percentage of polymorphic loci ($P$), mean number of alleles per locus ($A$), and gene diversity or expected heterozygosity ($H_e$). Observed heterozygosity ($H_o$) was measured directly from the observed frequency of heterozygotes in assayed loci. Wright's fixation index ($F$) for each polymorphic locus in every population and population differentiation index ($F_{ST}$) were calculated using the BIOSYS-1 computer program. Significant differences in genetic structure parameters between seed and maternal populations were examined by the $t$ test (Zar, 1984).

## RESULTS

High levels of genetic diversity were found in two generations, i.e., seed and maternal populations of *O. xylorrhizus*. The mean expected heterozygosity ($H_e$) was 0.292 in seed populations and 0.296 in maternal populations (Table I). No significant difference in the mean expected heterozygosity was found between two generations ($P > 0.05$). The two generations also exhibited the same level of polymorphism (0.615). The mean number of alleles per locus in seed populations ranged from 1.8 to 1.9 and was 1.8 in maternal populations (Table I).

A significant difference in observed heterozygosity ($H_o$) between seed and maternal populations was found in this species (Table I). The observed heterozygosity in seed populations ranged from $0.045 \pm 0.015$ to $0.146 \pm 0.060$, with an average of $0.091 \pm 0.051$, which was significantly lower than the mean expected heterozygosity (0.091 vs 0.292; $P < 0.05$). In contrast, no significant difference was detected between the observed and the expected heterozygosity for three maternal populations (0.242 vs 0.296; $P > 0.05$).

**Table I.** Genetic Variability and Fixation Indices at 13 Loci for Three Populations of *Ophiopogon xylorrhizus*

| Population | Mean sample size per locus | $A$ | $P^a$ | $H_e$ | $H_o$ | $F$ |
|---|---|---|---|---|---|---|
| | | (a) Seed populations | | | | |
| P3 | 89.6 (4.2)[b] | 1.8 (0.2) | 0.615 | 0.287 (0.068) | 0.146 (0.060) | 0.488 |
| P4 | 128.3 (5.1) | 1.9 (0.2) | 0.615 | 0.288 (0.067) | 0.083 (0.033) | 0.712 |
| P6 | 68.2 (2.4) | 1.8 (0.2) | 0.615 | 0.302 (0.071) | 0.045 (0.015) | 0.851 |
| Mean | 95.4 | 1.83 | 0.615 | 0.292 | 0.091 (0.051) | 0.688 |
| Species | 286 | 1.82 | 0.615 | 0.264 | | |
| | | (b) Maternal populations | | | | |
| P3 | 9.0 (0.0) | 1.8 (0.2) | 0.615 | 0.320 (0.074) | 0.308 (0.085) | 0.038 |
| P4 | 20.0 (0.0) | 1.8 (0.2) | 0.615 | 0.271 (0.064) | 0.208 (0.068) | 0.232 |
| P6 | 11.0 (0.0) | 1.8 (0.2) | 0.615 | 0.297 (0.070) | 0.210 (0.062) | 0.293 |
| Mean | 13.3 | 1.8 | 0.615 | 0.296 | 0.242 (0.057) | 0.182 |
| Species | 40 | 1.82 | 0.615 | 0.278 | | |

[a] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.
[b] Standard errors in parentheses.

Wright's fixation indices ($F$) in the seed populations were substantially high, ranging from 0.488 to 0.851, with a mean of 0.688, indicating a great deficiency of heterozygotes. More importantly, the $F$ values of three maternal populations, which ranged from 0.038 to 0.293 (mean, 0.182), were much lower than those of three seed populations (Table I). Of 13 loci examined, no or only 1 locus was in Hardy–Weinberg equilibrium for seed populations and 10–13 loci were in equilibrium for maternal populations (Table II).

Genetic differentiation among three seed populations was lower than that among three maternal populations ($F_{ST} = 0.028$ vs 0.062), which suggested that the genetic differentiation among populations increased from seed populations to maternal populations. No significant difference existed in allele frequency between seed and maternal populations, indicating that the allele frequency was stable throughout two generations.

## DISCUSSION

Significant differences were found in observed heterozygosity and Wright's fixation index between seed and maternal populations. The very low observed heterozygosity ($H_o = 0.091$) and the high fixation index ($F = 0.688$) detected in seed populations indicated an excess of homozygotes. He *et al.* (1998) demonstrated that selfing occurred frequently in natural population of this species, where high selfing rates, ranging from 0.533 to 0.909 (mean $= 0.706 \pm 0.190$), were detected. It is of interest that the frequency of observed heterozygotes increased in the maternal populations ($H_o = 0.242$, $F = 0.182$). This finding implies that a great number of homozygotes had been eliminated during the transformation from seeds to adult plants. When an individual self-fertilizes or mates with a relative, the lethal or highly deleterious recessive alleles are often made homozygous, and thus inbreeding depression occurs for an outcrossing species. It would represent a heavy inbreeding depression if excess homozygotes were removed from populations. Changes in the homozygote frequency may be a better parameter to measure inbreeding depression. It is common to consider that a low level of inbreeding depression is expected for predominantly selfing population (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987; Holtsford and Ellstrand, 1990; Barrett and Kohn, 1991). However, Holsinger (1988) argued that inbreeding depression was not sufficient to understand the evolution of selfing when there was no pollen discounting and suggested that selfing could evolve in an initially random mating population even with a rather high inbreeding depression, which depends on the fitness mechanism and mating history of the species.

High levels of genetic differentiation in *O. xylorrhizus* imply that protection measures must take all populations into account. Considering that it is difficult to conserve tropical forest plants *ex situ* for many reasons (Bawa and Ashton, 1991), conservation *in situ* is the most practical way to protect the abundant

**Table II.** Test for Deviation from Hardy–Weinberg Equilibrium in Three Populations of *O. xylorrhizus*

| Population | *Aat* | *Adh-1* | *Dia-1* | *Dia-2* | *Lap* | *Pgm* | *Pgi-1* | *Pgi-2* |
|---|---|---|---|---|---|---|---|---|
| | | | | (a) Seed populations | | | | |
| P3 | 64.876** | 0.053 | 4.800** | 7.906** | 107.971** | 172.151** | 15.626** | 10.595** |
| P4 | 45.140** | 4.564* | 210.640** | 309.992** | 48.796** | 208.021** | 51.255** | 51.730** |
| P6 | 51.312** | 62.031* | 41.504** | 44.145** | 84.187** | 189.049** | 26.002** | 41.485** |
| | | | | (b) Maternal populations | | | | |
| P3 | 1.171 | 2.228 | 1.026 | 1.171 | 6.904 | 6.381 | 1.818 | 2.228 |
| P4 | 4.222* | 0.218 | 44.444** | 4.886* | 0.924 | 24.163** | 16.889** | 0.440 |
| P6 | 1.939 | 8.000** | 0.892 | 2.000 | 7.924* | 5.316 | 2.537 | 0.128 |

* $P < 0.05$.
** $P < 0.01$.

genetic diversity of *O. xylorrhizus*. Other conservation efforts, such as increasing the chance of cross-fertilization by hand pollination and sowing seeds *in situ* to enhance the dispersal distance, should also be used.

## ACKNOWLEDGMENTS

This work was supported by grants from the Natural Science Foundation of China (Nos. 39391500 and 39870055).

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