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MATING SYSTEM OF *OPHIOPOGON XYLORRHIZUS* (LILIACEAE), AN ENDANGERED SPECIES IN SOUTHWEST CHINA

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The mating system of *Ophiopogon xylorrhizus* Wang *et* Dai was studied in three natural populations with allozyme electrophoresis. The outcrossing rate was estimated by assaying eight enzyme loci. A mixed mating system model was used, and outcrossing rates were estimated for populations and families. The multilocus outcrossing rates in three populations were 0.467, 0.323, and 0.091. The extent of outcrossing in populations depended on the plant density (r = 0.9998; $P < 0.02$; df = 1). The frequency distribution of family outcrossing rates was slightly bimodal. A mixed mating system with considerable complete selfing and complete outcrossing was found in this species. Positive correlations were observed between family outcrossing rate and maternal heterozygosity (r = 0.70–0.98) and between family outcrossing rate and fecundity (r = 0.971; $P < 0.0005$; df = 7). Inbreeding depression was examined in populations P3 and P4, both of which had high outcrossing rates. Inbreeding depression was expressed mainly in seed production. Mating system pattern contributed to the high genetic variation in this species. Because of high inbreeding depression and habitat destruction, this species is threatened with extinction. Conservation in situ by protecting the habitat is the best way to conserve this species.

Introduction

An important prerequisite for both understanding and manipulating the genetic structure of a species is knowledge of its breeding system (Brown et al. 1975). Because reproductive patterns in a population determine the genetic structure of the progeny generations, information on mating system and fertility variation in natural populations is needed to plan gene conservation programs and to manage breeding populations (Muona 1989; Morgante et al. 1991). Although much work on the mating systems of tree species in tropical rain forests has been conducted in recent years (O'Malley and Bawa 1987; O'Malley et al. 1988; Hamrick and Loveless 1989; Eguiarte et al. 1992), few of the understory plants, especially perennial herbs, have been studied. Moreover, information on mating mode and pattern of rare and endangered species may help to reveal their evolutionary history and potential, which is the basis of scientific and reasonable conservation management.

Ophiopogon xylorrhizus Wang *et* Dai, a herbaceous perennial endemic to Mengla county of Yunnan Province, China, is an endangered species. Its range covers an area ca. 30 km × 20 km. Fewer than 2000 individuals, located in eight disjunct populations, were found during expeditions from 1991 to 1997. Investigations over the last 2 yr indicated that two of the eight populations have become extinct, and another is on the brink of extinction because of the destruction and degradation of its habitat. Although *O. xylorrhizus* is perennial with rhizome, no asexual clone was found in our field investigations. The mean percentage of flowering plants in populations was found to be ca. 25.3%, and the flowering plant generally produced an inflorescence with 10–20 flowers each year with the characteristics of cleistogamy (T. H. He, G. Y. Rao, and R. L. You, unpublished data). However, not all cleistogamous flowers self-pollinated successfully; they could open and accept interfloral pollen occasionally. During anthesis, flowers were visited only by a kind of thrip (*Taeniothrips* sp.), whose flight ability is quite weak (T. H. He, G. Y. Rao, and R. L. You, unpublished data). Moreover, ca. 55.8% of the plants are male sterile (He et al. 1997). No other morphological correlates with the variation of mating system were found. Although *O. xylorrhizus* is a narrowly distributed species, high levels of genetic variability both within and among populations have been documented on the basis of the allozyme data (Ge et al. 1997). Ge et al. (1997) found a deficiency of heterozygotes and presumed that plants self-fertilize to some extent. Information on the mating system of *O. xylorrhizus* would provide insights into the reproductive ecology and management of this endangered species.

In this study, we examined the mating system of three natural populations of *O. xylorrhizus* in order to determine outcrossing rates among and within populations and to examine the relationships between outcrossing rate, maternal heterozygosity, inbreeding depression, and seed production.

Material and Methods

Field Sampling

Seeds were collected from 63 plants of *Ophiopogon xylorrhizus* randomly chosen in three natural populations in November, 1996 (table 1). Further details of the populations are provided by Ge et al. (1997). Since this previous study, populations P1 and P5 have disappeared from their habitats in 1996, and less than 10 individual plants in populations P2, P7, and P8 bore more than five seeds. As a result, these five populations were not used in the present study. For the three remaining populations, seeds of a single maternal parent, i.e., a plant, are referred to as a family and a seed as an

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Table 1 Sampling Amount (Family and Individual), Fecundity (Seeds/Plant), and Plant Density (Plants/m²) in Three Populations of *Ophiopogon xylorrhizus*

	P3	P4	P6
Family	16 (9)	27 (20)	20 (11)
Individual	187 (107)	342 (192)	109 (84)
Fecundity (seeds/plant)	11.68 ± 5.7	12.67 ± 7.9	5.45 ± 3.2
Density (plants/m²)	0.046	0.032	0.011

Note. The assayed amount of families and individuals in three populations is shown in parentheses.

individual. Most plants with more than five mature seeds in each population were sampled, and seeds were stored in a refrigerator for ca. 1 mo until assayed. The number of families examined in each population was less than the number collected, because insect larvae damaged some seeds (table 1).

Electrophoresis

The embryo of a single seed was stripped out, crushed, and ground in one drop (0.05 mL) of Tris-HCl buffer (Soltis et al. 1983), and the crude extract was absorbed onto Xinhua-3 filter paper wicks (7 mm × 3 mm). The starch gel electrophoresis followed the method of Soltis et al. (1983).

Embryo tissues were assayed for six enzyme systems: leucine aminopeptidase (LAP; EC 3.4.11.1), aspartate aminotransferase (AAT; EC 2.6.1.1), alcohol dehydrogenase (ADH; EC 1.1.1.1), NAD(P)H-diaphorase (DIA; EC 1.6.2.2), phosphoglucoisomerase (PGI; EC 5.3.1.9), and phosphoglucomutase (PGM; EC 5.4.2.2). Two buffer systems were used for separating enzymes in 12% starch gel. ADH and PGI were separated in modified buffer system 1 (Gottlieb 1981; 0.01M L-histidine HCl.H_2O in gel buffer), and AAT, LAP, DIA, and PGM were resolved in buffer system 6 (Mitton et al. 1979; 0.3 M boric acid in electrode buffer and 0.015 M Tris-citric acid in gel buffer). Enzyme staining procedures followed the methods of Soltis et al. (1983) and Wendel et al. (1989). Eight polymorphic loci were scored for the mating system analysis, *Aat, Adh, Dia-1, Dia-2, Lap, Pgm, Pgi-1,* and *Pgi-2*. For populations, single- and multilocus outcrossing rates, Wright's fixation indices, and their standard errors, and family maternal genotypes were estimated by using Ritland's (1990) MLT computer program for mixed mating model based on the methods of Ritland and Jain (1981). The estimates of outcrossing rates for families were also obtained. The MLT program assumes that progeny are derived from either random mating or self-fertilization and that the loci that are significantly correlated or substantially variable would be excluded from the analyses. Two hundred bootstaps were used to estimate the standard errors of the outcrossing rates.

Maternal genotype heterozygosity was calculated as the percentage of the heterozygotes at all analyzed loci. Fecundity of plants was the number of seeds produced by a single plant, and fecundity of populations was the mean seed set of sampled plants in a population.

Observed inbreeding depression (*ID*) in seed production was calculated according to the method of Lande and Schemske (1985):

$$ID = 1 - (W_{\text{selfed}}/W_{\text{outcrossed}}),$$

where $W_{\text{outcrossed}}$ is the mean value of a character for outcrossed progeny and W_{selfed} is the mean of the selfed progeny. The value of *ID* was calculated for three fitness components measured during seed production: the number of flowers that produced seeds every plant (flowers/plant), the number of seeds every flower produced (seeds/flower), and the number of seeds every plant produced (seeds/plant).

Expected inbreeding depression in whole life cycle was estimated following the method of Barrett and Kohn (1991):

$$ID = 1 - 2F(1 - s)/s(1 - F),$$

where F is the Wright's fixed index, t is the outcrossing rate, and s is the selfing rate ($s = 1 - t$).

Relationships between population parameters were examined with correlation analyses (Zar 1984). Because in population P4 and P6, a few flowers were sampled at an early stage for another study, some analyses of fecundity were not conducted for these two populations. Although some correlations between population parameters were derived, it is necessary to view these correlations with great caution because of the poor sample size, which is difficult to be resolved in the study of this endangered species with few reproductive plants.

Results

Outcrossing Rate Estimation and Its Distribution

Single- and multilocus estimates of outcrossing rate between populations were different (table 2). Single-locus outcrossing rates (mean of eight loci in populations P3 and P4; seven loci in population P6) varied from 0.058 (P6) to 0.285 (P3), whereas multilocus outcrossing rates ranged from 0.091 (P6) to 0.467 (P3). Biparental inbreeding, calculated as the difference between multi- and single-locus estimates, also occurred (table 2). Multilocus outcrossing rates of populations were positively correlated with individual plant density in populations ($r = 0.9998$; $P < 0.02$; df = 1) and slightly correlated with population fecundity ($r = 0.86$; $P < 0.35$; df = 1). Allowing for the relative high population selfing rate, the Wright's fixation indices of three populations were positive and high, which indicated a deficiency of heterozygotes. The distribution

Table 2 Inbreeding Parameter Estimates in Three Populations of *Ophiopogon xylorrhizus*

	P3	P4	P6[a]
Multilocus outcrossing rate	0.467 ± 0.174 (0.00–1.73)	0.323 ± 0.055 (0.00–2.00)	0.091 ± 0.037 (0.00–2.00)
Single-locus outcrossing rate	0.285 ± 0.131	0.210 ± 0.041	0.058 ± 0.034
Difference of outcrossing rate[b]	0.181 ± 0.065	0.113 ± 0.026	0.033 ± 0.017
Wright's fixation index F	0.140 ± 0.119	0.287 ± 0.134	0.374 ± 0.117

[a] Inbreeding parameter estimates of P6 population resulting from seven loci; LAP was excluded according to Ritland's program for its violent variance.

[b] The difference of outcrossing rates also represents biparental inbreeding (Barrett and Kohn 1991).

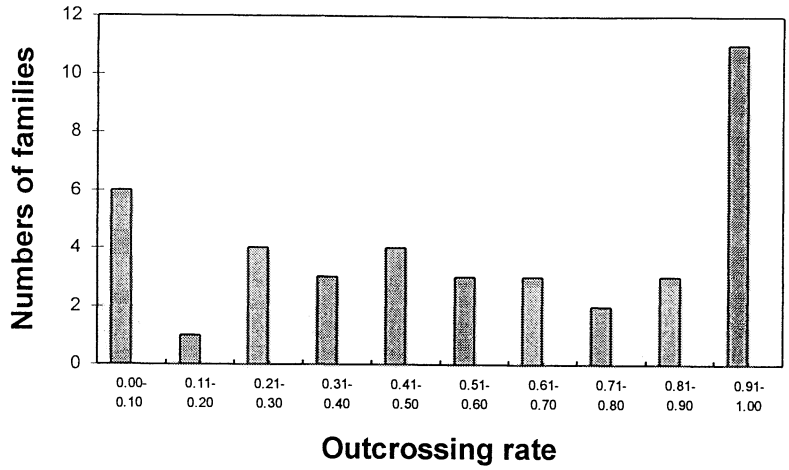

Fig. 1 Distribution of family outcrossing rates (t) in three populations of *Ophiopogon xylorrhizus*. If $t > 1$, it was regarded as 1.

of outcrossing rates of the 40 families is shown in figure 1. Outcrossing rates varied from 0 to 1 and appeared to be a slightly bimodally distributed, which indicates a mixed mating system with considerable complete outcrossing and complete selfing.

Family Outcrossing Rate, Maternal Heterozygosity, and Fecundity

The family outcrossing rates within populations varied from $t = 0$ (complete selfing) to $t = 1$ (complete outcrossing). The standard errors of the outcrossing rates of populations were high because the outcrossing rates of families in a population varied substantially. The outcrossing rates of families were slightly correlated with maternal heterozygosity ($r = 0.70$; $P < 0.30$; df = 2, in population P6; $r = 0.98$; $P < 0.10$; df = 2, in population P4; $r = 0.84$; $P < 0.30$; df = 2, in population P3 [table 3]). As maternal heterozygosity varied from 0 to 0.75 (or 0.60 in population P4), the family outcrossing rate increased from 0 to 1.

The population outcrossing rate and the mean population fecundity were slightly positively correlated ($r = 0.87$; $P < 0.30$; df = 1). Family outcrossing rates and family fecundity, however, were significantly correlated ($r = 0.97$; $P < 0.0005$; df = 7 [fig. 2]). In population P3, the family fecundity was 18 ± 2.8 (seeds/plant; $n = 2$) when $t = 1$, which was greater than the mean fecundity of the population (11.68 ± 5.7 seeds/plant; $n = 16$), whereas when $t = 0$, the fecundity was 6 ± 0.5 seeds/plant ($n = 2$). Moreover, a positive relationship ($r = 0.68$; $P < 0.10$; df = 7) between family fecundity and maternal heterozygosity was observed (fig. 3). Families with lower fecundity had lower maternal heterozygosity.

Inbreeding Depression in Populations

Observed and expected inbreeding values depression in three populations of *Ophiopogon xylorrhizus* are presented in table 4. A positive, but not significant, correlation between the observed depression of seed production and outcrossing rates of population was found ($r = 0.958$; $P < 0.2$; df = 1). In population P3, the outcrossing rate was 0.467, and the observed inbreeding depression was as high as 0.64. In contrast, a negative ($ID = -0.28$) inbreeding depression in fecundity was observed in population P6, in which the outcrossing rate was low ($t = 0.091$). Moreover, the number of flowers that produced seeds was influenced to the same extant by different outcrossing rates as the seed amount in each flower (table 4). In the three pop-

Table 3 Family Outcrossing Rate, Family Maternal Genotype Heterozygosity, and Their Correlation Coefficients in Three Populations of *Ophiopogon xylorrhizus*[a]

P3		P4		P6	
Maternal heterozygosity	Outcrossing rate	Maternal heterozygosity	Outcrossing rate	Maternal heterozygosity	Outcrossing rate
0.00 (1)	0.26 (—)	0.00 (1)	0.33 (—)	0.00 (1)	0.00 (—)
0.25 (1)	0.00 (—)	0.20 (7)	0.50 (0.27–0.68)	0.25 (5)	0.61 (0.00–2.00)
0.50 (4)	0.83 (0.58–1.07)	0.40 (7)	0.80 (0.29–0.69)	0.50 (2)	0.44 (0.29–0.58)
0.75 (3)	1.05 (0.73–1.07)	0.60 (5)	1.01 (0.67–2.00)	0.75 (1)	1.04 (—)
	$r = 0.84$*		$r = 0.98$**		$r = 0.70$*

[a] Data on outcrossing rates were the mean value of the families that had the same maternal heterozygosity. Data followed by dash in parentheses result from a single observation.

* $P < 0.30$.

** $P < 0.10$; df = 2.

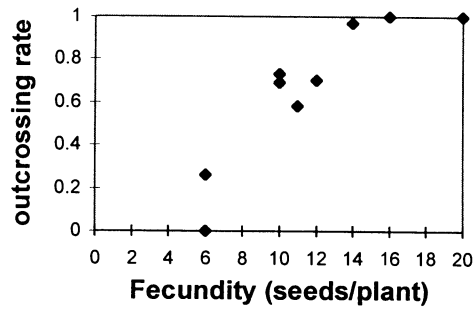

Fig. 2 Relationship between fecundity and multilocus outcrossing rates of families in population P3. If $t > 1$, it was regarded as 1 ($r = 0.97$; $P < 0.0005$; df = 7).

ulations, the degree of observed inbreeding depression of seed production was lower than that of the expected inbreeding depression (fig. 4).

Discussion

A mixed mating system with considerable selfing and outcrossing was detected in *Ophiopogon xylorrhizus* by analyzing the segregation of allozyme markers in 283 individuals and 40 families from three natural populations. The mating modes ranged from complete selfing to complete outcrossing. Factors responsible for the mixed mating system in this species are floral traits promoting selfing or outcrossing, the pollen movement between genets, and plant density of the population. The floral characteristics in favor of inbreeding or outcrossing (e.g., cleistogamy and male sterility) have been found in the populations, which result in complete selfing and complete outcrossing (T. H. He, G. Y. Rao, and R. L. You, unpublished data; He et al. 1998). However, cleistogamy was not strict in this species. Not all cleistogamous flowers self-pollinated successfully, because of the imperfect cleistogamy mechanism. The cleistogamous flowers could open and were able to accept the interfloral pollen after anthesis. These pollination characteristics would contribute to some degree of outcrossing in cleistogamous flowers (T. H. He, G. Y. Rao, and R. L. You, unpublished data). The distribution pattern of outcrossing rates in three populations was the same, but their mean outcrossing rates varied to a large extent, such as in

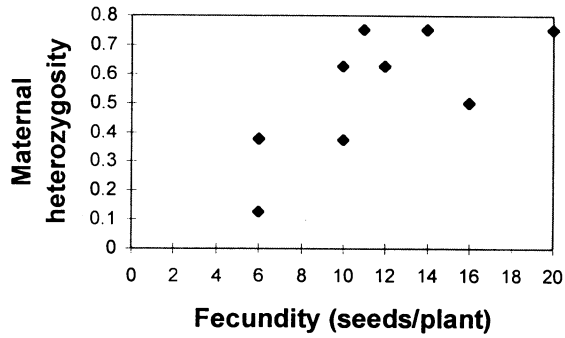

Fig. 3 Fecundity–maternal heterozygosity relationship in population P3 ($r = 0.68$; $P < 0.10$; df = 7).

Table 4 Coefficients of Inbreeding Depression in the Three Fitness Components of Seed Production in Three Populations of *Ophiopogon xylorrhizus*

	P3	P4	P6
Flowers/plant[a]	0.32	0.38	−0.04
Seeds/flower[a]	0.45	0.22	−0.25
Seeds/plant	0.64	0.54	−0.28

[a] Flowers that produced seeds.

the P3 population, where it was 0.467, while in P6 it was 0.091. Beside the mating history, these variations in outcrossing rate probably resulted from the possibility of pollination success between flowers from different families, which was largely influenced by the activity of pollinators and plant density of populations. Because of species scarcity and poor activity of pollinators and low plant density in populations of *O. xylorrhizus,* self-fertilization and biparental inbreeding frequently occurred. Lande and Schemske (1985) proposed that predominant selfing and predominant outcrossing should be alternative state of the mating in most plant populations. This can be expressed by the bimodal distribution of the outcrossing rate. Outcrossing rates of families in *O. xylorrhizus* displayed a slightly bimodal distribution. Intraspecific variation in outcrossing rate may be more valuable for identifying the factors in mating system changes. The cause of shifts in mating systems is interesting because of the important consequences that such changes have on the genetic structure of the population, selection response, and speciation (Barrett and Eckert 1990).

Inbreeding depression occurred to varying degree in the three populations of *O. xylorrhizus.* The most commonly assumed genetic basis for inbreeding depression is the presence of lethal or highly deleterious recessive allelles. When an individual self-fertilizes or mates with a relative, these alleles are often made homozygous, resulting in inbreeding depression. The low inbreeding depression in inbreeding populations is probably the result of the purging of lethal or highly deleterious recessive alleles following inbreeding for several generations (Barrett and Kohn 1991). In the

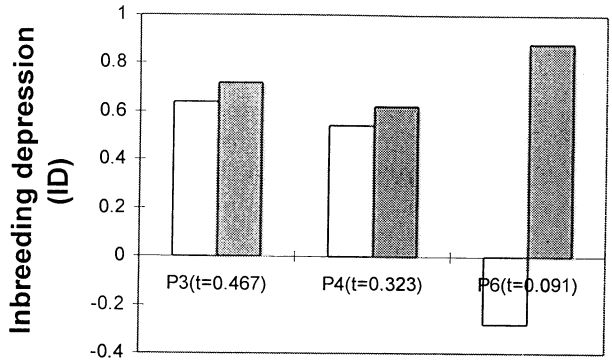

Fig. 4 Observed and expected inbreeding depression in three populations of *Ophiopogon xylorrhizus.* Open boxes = observed inbreeding depression; filled boxes = expected inbreeding depression.

present study, although the comparison of progeny derived from selfing and outcrossing to estimate inbreeding depression was not conducted, we compared the fecundities of plants with the alternative outcrossing rates ($t = 0$ vs. $t = 1$), which indicated that different inbreeding depression occurred in the three populations with varying outcrossing rates. Reasonably high inbreeding depression in seed production, for example, was found in population P3 with an outcrossing rate of 0.467, whereas the negative inbreeding depression was detected in population P6 with a relatively low outcrossing rate (0.091). It is not surprising that a negative inbreeding depression in seed production was detected in population P6 with a rather high expected inbreeding depression because the mean population fecundity of population P6 has decreased to the lowest level in comparison with other two populations (5.45 ± 3.2 vs. 11.67 ± 5.7 [P3] or 12.67 ± 7.2 [P4]). If our sampling or analyses errors do not affect the results substantially, it is reasonable to believe that low inbreeding depression in seed production in this population is the result of the purging of lethal or highly deleterious recessive alleles. Since the outcrossing rates varied from 0 to 1 in all three populations, these findings raise the question of why inbreeding depression varied between populations. Other studies have indicated that the magnitude of inbreeding depression may depend on the mating history of population (Stebbins 1950; Lande and Schemske 1985; Charlesworth and Charlesworth 1987). In order to resolve this question, further work on the population mating history of *O. xylorrhizus* is needed. Schemske and Lande (1985) proposed that the evolutionary direction of mating system depended primarily on the level of inbreeding depression, and if outcrossing rate were highly heritable, family selection on the mating system could lead to fixation of populations at either complete selfing or complete outcrossing. Although it is not clear without the quantitative genetic analyses whether the outcrossing rate of *O. xylorrhizus* is heritable, we do know that outcrossing rates of *O. xylorrhizus* depend on the materal heterozygosity to a great extent.

Ophiopogon xylorrhizus expressed inbreeding depression mainly at seed production. In the field investigation, little morphological difference between individual plants of *O. xylorrhizus* was observed, but great differences in fecundity between selfing and outcrossing families, the cleistogamous families and male sterile families, were found in population P3 and P4. In this study, a lower than expect observed inbreeding depression was found, probably because of the decreasing on fecundity only one of the main effects of inbreeding. It is evident from other studies that inbreeding depression can be expressed at any life-history stage, from seed maturation to seed production (Charlesworth and Charlesworth 1987; Kalisz 1989). An accurate estimate of total inbreeding depression must be based on several major life-history stages from zygote to adult (Schoen 1983). Positive relationships of individual heterzygosity with growth rate, but not with fecundity, have been found in some species (Mitton and Grant 1980, 1984; Ledig et al. 1983; Strauss 1986; Govindaraja and Dancik 1987; Shea 1987; Eguiarte et al. 1992). The lack of correlation between fecundity and individual heterozygosity may result from a greater effect of the environment on fecundity than on growth (Pinero and Sarukhan 1982). In some species, a decrease in fecundity has a greater effect on population development than does a low growth rate. In a stable environment, competition between individuals occurs to occupy an ecological niche and to establish new individuals. Moreover, a common finding of inbreeding depression studies in herbaceous plants is that inbreeding effects are often manifest most strongly at later life stages, namely, in flower or fruit production (Dudash 1990; Fenster 1991).

The protection of genetic diversity within species is the priority for conservation efforts, and the long-term objective is to maintain the evolutionary viability of a taxon and to maximize its chances for persistence in face of changing environment (Huenneke 1991). *Ophiopogon xylorrhizus* has high genetic variability among populations, and any protection measures must take all populations into account. Bawa and Ashton (1991) suggested that it was difficult to conserve tropical forest plants ex situ for many reasons, and conserving tropical forest plants may require large protected areas of hundreds of square kilometers. Considering the complex mating system and pollination mechanism of *O. xylorrhizus,* conservation in situ is the best way to protect its abundant genetic diversity.

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