Int. J. Plant Sci. 161(4):691–697. 2000.

1058-5893/2000/16104-0015$03.00

LOW LEVELS OF GENETIC DIVERSITY WITHIN POPULATIONS AND HIGH DIFFERENTIATION AMONG POPULATIONS OF A WILD RICE, *ORYZA GRANULATA* NEES ET ARN. EX WATT., FROM CHINA

Li-zhi Gao,[1] Song Ge, and De-yuan Hong

Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, Peoples Republic of China

To characterize genetic diversity within and among populations of *Oryza granulata* Nees et Arn. ex Watt., allozyme variation was assayed for 17 loci in 15 natural populations from Hainan and Yunnan provinces, China, using starch gel electrophoresis. A low level of genetic variability within populations (the mean A = 1.09, p = 6.33%, H_e = 0.016, and H_o = 0.009), but high genetic differentiation (F_{ST}) among populations was observed. The low amount of genetic variability of the species may be strongly affected by founder effect because of the marginal nature of the populations in China. The results also indicate that the restricted gene flow occuring between the two regions as well as the characteristics of *O. granulata* as a colonizing plant species are probably of significance in shaping the observed population genetic structure. Finally, an appropriate strategy for sampling more populations, but fewer individuals within populations, was proposed for the conservation of *O. granulata* in China.

Keywords: Oryza granulata, China, genetic diversity, population differentiation, conservation strategies.

Introduction

In recent decades, interests in taxonomy and phylogenetic relationships of species in the genus *Oryza* have increased (Tateoka 1963; Chang 1976; Second 1982, 1985; Ichikawa et al. 1986; McIntyre et al. 1992; Wang et al. 1992; Provan et al. 1997). However, up-to-date studies on population biology and ecology of wild rices that could help explain species relationships have been largely neglected (Vaughan 1989). Although many rice evolutionists have conducted studies on the ecological genetics of natural populations of *Oryza rufipogon* Griff. and strengthened our understanding of intraspecific variation in *Oryza* species (Morishima et al. 1961; Sano et al. 1980; Morishima et al. 1984; Barbier 1989*a*, 1989*b*; Morishima and Barbier 1990; Barbier et al. 1991), other species in this genus have not been adequately investigated. Considering that most wild *Oryza* species are seriously threatened (Vaughan and Chang 1992), knowledge of their genetic diversity will be crucially important. Studies on population genetics might provide valuable insights into the genetic relationships of those "difficult complexes" (Vaughan 1989) and clarify the taxonomy of the genus. Information about their genetic structure could help rice genetic conservationists develop effective strategies for conservation of wild rice species *in situ* and/or *ex situ*.

Oryza granulata Nees et Arn. ex Watt. is widely distributed in southern and southeastern Asia (Vaughan 1989, 1994) and is geographically isolated in three provinces of southern China—Yunnan, Hainan, and Taiwan. Because the species grows in the shade or partial shade of degraded primary or well-established secondary forests, on mainly sloping terraces, it has been severely threatened with most populations decreasing as a result of rapid human population growth and deforestation (Gao et al. 1996; Gao 1997). Accordingly, it is listed as a threatened species in China (Fu 1992). Because this species offers unique characteristics valuable to rice breeders in the future, such as the ability to live in dry land, tolerate shade, and resist bacterial blight, its germplasm conservation is of great importance. However, no studies on the population genetics of *O. granulata* have been reported, which makes it difficult to take effective conservation actions and resources management.

Allozyme analysis is a valuable technique to detect genetic variation in natural populations and has long been conducted to examine the genetic structure of natural populations of rare and endangered species (Soltis and Soltis 1991; Soltis et al. 1992; Ge et al. 1997). In this study, isozyme electrophoresis was conducted to explore the population genetics of *O. granulata* in China. The specific questions that we hoped to address are (1) How are levels and distribution of genetic variability within and among populations of *O. granulata*? (2) Where would be the possible center of genetic diversity of the species in China? (3) Does the degree of geographical isolation parallel

[1] Author for correspondence. Current address: Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences, 30 Baishiqiao Road, Beijing 100081, People's Republic of China; e-mail Gaolizhi@ihw.com.cn; telephone 86-010-62186621; fax 86-010-62186629.

Manuscript received June 1999; revised manuscript received December 1999.

Table 1

Sample Sizes and Localities of 15 Populations of *Oryza granulata* from China according to Number Assigned in the Data Analysis

Population no.	Locality	Sample size (*n*)
Yunnan:		
1	Gannanba Meibang, Jinghong Co.	27
2	Zuling, Simao City	21
3	Gongxing, Menglian Co.	8
4	Kiaoganlanba, Simao City	29
5	Mengkuang, Lancan Co.	20
6	Reshuitan, Lancan Co.	40
7	Tongchang, Lancan Co.	24
8	Zhichang, Lancan Co.	22
9	Mandan, Yuanjiang Co.	10
10	Gadong, Jinghong City	33
Hainan:		
11	Gongei, Dongfang Co.	13
12	Banqiao, Dongfang Co.	14
13	Chongpo, Ledong Co.	14
14	Juantian, Linshui Co.	17
15	Jianfeng, Ledong Co.	12

allozymic differentiation? and (4) What conservation management plan should we develop for the species based on allozymic data?

Material and Methods

Plants

Living samples were taken from 15 populations of *Oryza granulata* from Hainan and Yunnan provinces, China, in October 1994 (table 1; fig. 1). Because the species has high colonizing ability in the populations sampled, care was taken to prevent collecting multiple samples from a single individual. Live ratoons were randomly collected at intervals of at least 5 m in the field, numbered, transplanted to pots, and maintained in Xishuangbanna Tropical Botanical Garden (Mengla County, Yunnan) and South China Botanical Garden (Guangzhou City). Young leaves were collected individually in March 1995, stored in plastic bags on ice, and transported to the laboratory by airplane. In some cases where populations were small, all individuals were taken, including Gongxing (eight individuals) and Mandang (10). However, due to small population size, two populations from Yunnan Province, Erhaoqiao (six) (population 16) and Luchun (four) (population 17), were not included in this study. For each individual, 0.05 g of fresh leaf material was crushed in 100 μL of Tris-HCl buffer (pH 7.5; see Soltis et al. 1983). The extract was absorbed into 3 × 8-mm^2 paper wicks and stored at −70°C until electrophoresis was conducted.

Starch-Gel Electrophoresis

Twelve enzymes were resolved by using starch-gel electrophoresis (table 2). The electrophoretic methods followed Glaszmann et al. (1988) and Soltis et al. (1983) with 12% starch gels. A modification of buffer system 1 (S1#) was used to resolve 6PGD, MDH, and ME (electrode buffer was diluted twice before use); TPI, AAT, DIA, and PGI were resolved on buffer system 6 (S6); buffer system I of Glaszmann et al. (1988) (GI) was used to resolve PGM, SKD, G3PDH, ADH, and IDH. Staining procedures for all enzymes followed Soltis et al. (1983). When more than one isozyme were observed for an enzyme, isozymes were numbered sequentially with the most anodally migrating enzyme designated "1." Allelic variation at a locus was coded alphabetically with the most anodally migrating allozyme designated "a."

Data Analysis

Electrophoretic data were analyzed using the computer program Biosys-1, version 1.7 (Swofford and Selander 1989) for the IBM-PC. Data were entered as genotype numbers from which allele frequencies were calculated. Genetic variability, deviation from Hardy-Weinberg equilibrium (fixation indices), Nei's unbiased genetic identity (*I*) (Nei 1978), as well as *F* statistics were calculated.

Results

Loci and Alleles Scored

The electrophoresis clearly resolved 12 enzymes encoded by 17 putative loci (table 2). Of them, *Aat-1*, *Aat-3*, *Adh*, *Dia-2*, *G3pdh*, *Idh*, *Mdh-1*, *Me*, *Pgi-1*, *Tpi-1*, and *Tpi-2* were monomorphic, with all individuals from the 15 populations scored possessing a single enzyme band with identical mobility for each locus. All the other loci were polymorphic in at least one population; *Mdh-2*, *Mdh-3*, and *6Pgd* each had two alleles; and *Dia-1*, *Pgm*, and *Skd* each had three alleles. Although two isozymes of *Pgm* are typically present in diploid seed plants (Gottlieb 1982), only one *Pgm* isozyme was observed in *Oryza granulata* in this study. Two loci of G3PDH and PGD were typically reported (Second 1982), but only one was observed in this study. Although the banding patterns of PGI seemed to indicate more than one locus, only *Pgi-1* was used due to poor resolution in the other locus. Allele frequencies for all the loci in the 15 populations are presented in table 3.

Measures of Genetic Variability

The mean number of alleles per locus (*A*), percentage of polymorphic loci (*p*), observed heterozygosity (H_o), and expected heterozygosity (H_e) (table 4) varied among the populations, with *A* ranging from 1.0 in populations 11 and 13 to 1.2 in population 4; *p* from 0.0% in populations 11 and 13

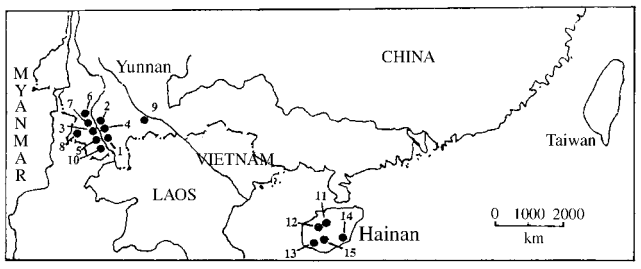

Fig. 1 Geographical localities of 17 populations of *Oryza granulata* from China.

Table 2

Enzyme Systems Assayed, Gel Buffers, and the Number of Loci Scored

Enzyme system	Abbreviation	EC No.	Gel buffer	No. of loci
Aspartate aminotransferase	AAT	EC 2.6.1.1	S6	2
Alcohol dehydrogenase	ADH	EC 1.1.1.1	GI	1
Diaphorase	DIA	EC 1.6.2.2	S6	2
Glutamate dehydrogenase	G3PDH	EC 1.4.1.2	GI	1
Isocitrate dehydrogenase	IDH	EC 1.1.1.42	GI	1
Malate dehydrogenase	MDH	EC 1.1.1.37	S1#	3
Malic enzyme	ME	EC 1.1.1.40	S1#	1
Phosphogluconate dehydrogenase	6PGD	EC 1.1.1.44	S1#	1
Phosphoglucoisomerase	PGI	EC 5.3.1.9	S6	1
Phosphoglucomutase	PGM	EC 2.7.5.1	GI	1
Shikimate dehydrogenase	SKD	EC 1.1.1.25	GI	1
Triosephosphate isomerase	TPI	EC 5.3.1.1	S6	2

to 16.7% in population 4; H_o from 0.000 in populations 1, 2, 3, 8–11, and 13 to 0.046 in population 12; and H_e from 0.000 in populations 11 and 13 to 0.029 in population 12. It is clear from table 4 that population 11 showed no genetic diversity ($A = 1.0$, $p = 0.000\%$, $H_o = 0.000$, and $H_e = 0.000$), while populations 3, 4, and 12 showed the highest levels. Generally, it seems that the level of genetic diversity in Hainan was almost as low as that in Yunnan. At the species level, genetic diversity with the values of $A = 1.53$, $p = 35.29\%$, and $H_e = 0.017$ was a bit higher than that at the population level, with total mean of $A = 1.09$, $p = 6.33\%$, $H_o = 0.009$, and $H_e = 0.016$.

Distribution of Genetic Variation

Wright's F statistics are a hierarchical series of fixation indices, where F_{IS} represents the deviation from Hardy-Weinberg expectations within populations (approximately equal to the mean F across populations), F_{ST} measures the fixation of different alleles in different populations, and F_{IT} measures devi-

Table 3

Allele Frequencies at All Loci in 15 Populations *Oryza granulata*

Allele	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Aat-1a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*Aat-3*a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Adh-a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Dia-1a	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.250
Dia-1b	1.000	1.000	1.000	1.000	1.000	0.988	0.833	1.000	1.000	0.970	1.000	0.583	1.000	1.000	0.750
Dia-1c	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.417	0.000	0.000	0.000
Dia-2a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Gdh-a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh-a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-1a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-2a	1.000	1.000	1.000	0.103	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-2b	0.000	0.000	0.000	0.897	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-3a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	1.000	1.000	1.000	1.000
Mdh-3b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
Me-a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgd-1a	0.852	0.941	1.000	0.966	1.000	0.875	1.000	0.895	1.000	0.697	1.000	1.000	1.000	1.000	1.000
Pgd-1b	0.148	0.059	0.000	0.034	0.000	0.125	0.000	0.105	0.000	0.303	0.000	0.000	0.000	0.000	0.000
Pgi-1a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgm-1a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000
Pgm-1b	1.000	1.000	0.375	1.000	1.000	1.000	1.000	1.000	0.900	1.000	0.000	0.000	0.000	0.000	0.000
Pgm-1c	0.000	0.000	0.625	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000
Skd-a	1.000	0.000	0.000	0.086	0.026	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	0.382	1.000
Skd-b	0.000	1.000	1.000	0.879	0.974	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.618	0.000
Skd-c	0.000	0.000	0.000	0.034	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Tpi-1a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Tpi-2a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table 4

Genetic Variability at All the 17 Loci and Mean Fixation Indices at All the Polymorphic Loci in 15 Populations of *Oryza granulata*

Population no.	A	p[a]	H_o	H_e[b]
1	1.1	5.6	0.000	0.014
2	1.1	5.6	0.000	0.006
3	1.1	5.6	0.000	0.028
4	1.2	16.7	0.002	0.027
5	1.1	5.6	0.003	0.003
6	1.1	11.1	0.001	0.014
7	1.1	5.6	0.019	0.16
8	1.1	5.6	0.000	0.011
9	1.1	5.6	0.000	0.011
10	1.1	11.1	0.000	0.027
Mean for Yunnan	1.11	7.81	0.003	0.016
11	1.0	0.0	0.000	0.000
12	1.1	5.6	0.46	0.029
13	1.0	0.0	0.000	0.000
14	1.1	5.6	0.036	0.027
15	1.1	5.6	0.028	0.022
Mean for Hainan	1.06	3.36	0.022	0.016
Total mean	1.09	6.33	0.009	0.016
Species level	1.53	35.29	...	0.017

[a] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99.

[b] Unbiased estimate (see Nei 1978).

ations from Hardy-Weinberg expectation across the population system as a whole. Statistical significance of F_{ST} values was tested for each locus by the χ^2-test, $\chi^2 = 2NF_{ST}(K-1)$, with $(K-1)(S-1)$ degrees of freedom, where N is the total sample size, K is the number of alleles per locus, and S is the number of populations (Workman and Niswander 1970) (table 5). In the populations under study, F_{IS} was 0.402, suggesting that most populations deviated from Hardy-Weinberg expectation within populations and were deficient in heterozygotes; F_{ST} was 0.859, indicating that 85.9% of the total genetic variation existed among populations. It agrees with the difference of allelic frequencies observed. For example, the genotype of *Pgm-a* existed in the populations from Hainan, while *Pgm-b* was found in those from Yunnan; most of the multilocus isozyme genotypes were unevenly distributed among the populations.

Genetic Identity Measures

Genetic identity values measure the similarity of allele frequencies between pairs of populations and range from 0, indicating no shared alleles between populations, to 1, indicating that the two populations have the same alleles in identical frequencies. Nei's (1978) unbiased genetic identities were computed to alleviate any bias caused by small sample sizes, for example, fewer than 50 individuals. Genetic identity values ranged from 0.769 between populations 5 and 12 to 1.00 between populations 2 and 6, 2 and 8, 6 and 8, as well as 11 and 13, with a mean of all pairwise comparisons of 0.901 (table 6). In Yunnan Province, genetic identity values ranged from 0.835 to 1.000, with a mean of all pairwise comparisons of 0.940, while in Hainan, they ranged from 0.970 to 1.000, with a mean of 0.988. In comparison, the mean genetic identity was merely 0.849 between the pairs of populations from the two regions, ranging from 0.769 to 0.949. Cluster analysis (UPGMA) produced a phenogram to show the genetic identity of all populations studied (fig. 2). It is apparent that the populations within Yunnan or Hainan each clustered together, respectively, before the populations from Yunnan and Hainan formed a cluster, indicating that there is differentiation between the two regions. For each region, the populations that are geographically nearer clustered closer to each other than those that are more distant.

Discussion

The electrophoretic data indicate significantly low levels of variability within populations and high genetic differentiation among populations in *Oryza granulata*. The mean values of p, A, and H are much lower than those with the same life-history and breeding systems previously published (Hamrick and Godt 1990). As far as the genus *Oryza* is concerned, these values of genetic diversity are much lower than the other wild rice species studied. For example, in his allozyme studies on *Oryza rufipogon* of Thailand, Barbier (1989*a*, 1989*b*) found the values of $A = 1.98$ and $H = 0.209$ for perennial populations, and $A = 1.58$ and $H = 0.099$ for annual ones. In addition, our recent allozyme survey showed that the other two Chinese wild rice species had relatively higher genetic variation, with $A = 1.3$, $p = 22.7\%$, and $H = 0.068$ for *O. rufipogon* Griff. (Gao et al. 2000*a*) and $A = 1.16$, $p = 16.20\%$ and $H = 0.056$ for *Oryza officinalis* Wall. ex Watt. (Gao et al. 2000*b*). An analysis of interpopulation differentiation using Wright's F statistics reveals a rather high level of genetic differentiation with mean $F_{ST} = 0.859$, which is much higher than the average values of both autogamous and gravity dispersed plants reported previously (Hamrick and Godt 1990).

A recent review (Hamrick 1989) based on allozyme studies claims that the characteristics of a species, such as geographically narrow distribution, short life, primarily selfing, or low lifetime fecundities, are usually associated with low genetic variation. *Oryza granulata* is a perennial and grows widely in pantropical regions of Asia. According to our field observation, it continuously produces flowers (Vaughan 1994; Gao et al. 1996) and thus gives rise to a large quantity of seeds (Gao et al. 1996). These characteristics of *O. granulata* should not lead to a pattern of low levels of genetic variation within a population and high genetic differentiation among populations. Several factors may explain the unexpected results.

Table 5

Summary of *F* Statistics at All the Polymorphic Loci of *Oryza granulata*

Locus	F_{IS}	F_{IT}	F_{ST}
Dia-1	−0.384	−0.011	0.269***
Mdh-2	1.000	1.000	0.944***
Mdh-3	...	1.000	1.000***
Pgd-1	1.000	1.000	0.143***
Pgm-1	1.000	1.000	0.914***
Skd	0.010	0.903	0.902***
$\overline{X}$	0.402	0.916	0.859***

*** $P < 0.001$.

Table 6

Matrix of Nei's (1978) Unbiased Genetic Identity Values among the Populations and Regions of *Oryza granulata*

Population no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	...	0.944	0.921	0.908	0.990	0.944	0.941	0.944	0.942	0.999	0.887	0.877	0.887	0.864	0.883
2		...	0.979	0.954	0.9441	0.000	0.999	1.000	0.888	0.940	0.833	0.822	0.833	0.879	0.828
3			...	0.932	0.922	0.978	0.977	0.978	0.872	0.916	0.845	0.834	0.845	0.892	0.840
4				...	0.999	0.954	0.952	0.954	0.852	0.904	0.797	0.785	0.797	0.836	0.792
5					...	0.943	0.943	0.944	0.835	0.885	0.780	0.769	0.780	0.824	0.775
6						...	0.998	1.000	0.886	0.942	0.831	0.821	0.831	0.878	0.827
7							...	0.998	0.886	0.937	0.831	0.827	0.831	0.877	0.828
8		0.940						...	0.887	0.941	0.832	0.821	0.832	0.878	0.827
9		(0.835–1.000)							...	0.938	0.949	0.940	0.949	0.928	0.946
10										...	0.882	0.873	0.882	0.859	0.879
11											...	0.9921	0.000	0.979	0.997
12												...	0.992	0.970	0.994
13													...	0.979	0.997
14		0.849									0.988			...	0.976
15		(0.769–0.949)									(0.970–1.000)				...

First, high genetic differentiation observed in this study stemmed mainly from the difference of allelic frequencies between Hainan and Yunnan, which is clearly shown in figure 2. Because Hainan Island is isolated from the mainland, restricted gene flow may result in high genetic differentiation between Hainan and other regions. Considering the fact that the species is also distributed in Vietnam and Laos, which geographically connect the two regions of China, and that populations from those regions are not included in this study, so incomplete a sampling may increase the genetic differentiation between the two regions of China. In addition, since F_{ST} is a relative value to estimate genetic differentiation among populations, a low genetic variability within the species may tend to overestimate the F_{ST}.

The second possible explanation for such a unique population genetic structure is that this species may be a colonizing one. According to our field observations (Gao et al. 1996), it possesses well-developed clonal propagation, which enables it to establish large populations in proper habitats. On one hand, in the course of migration mainly by means of clonal growth, various factors, including the frequency and directionality of spreading, as well as the genetic constitution of the source population and/or maternal plant, will affect the subsequent interpopulation differentiation; on the other hand, vigorous clonal propagation may enhance the mating among the relatives. As predicted by population genetic theory, inbreeding will lead to a loss of genetic variation within populations and increase genetic differentiation among populations (Hamrick 1989). Our results agree with the characteristics of depauperate levels of genetic variation and marked interpopulation differentiation commonly reported in colonizing species (reviewed in Brown and Marshall 1981; Rice and Jain 1985; Barrett and Richardson 1986; Barrett and Shore 1989).

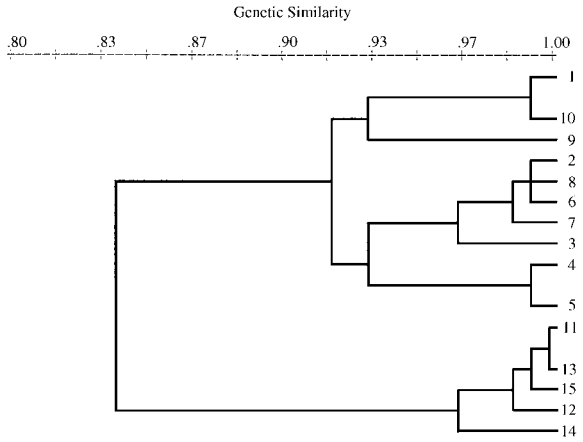

Fig. 2 Cluster analysis of 15 populations of *Oryza granulata* using unweighted pair group method and Nei's (1978) unbiased genetic identity values.

Finally, founder effect is also the most likely explanation for low genetic variability of *O. granulata* observed. In China, it is at the northeastern edge of its whole range and probably established by a few founders from southern Asia. As a result, low levels of genetic variability were maintained. However, there is no other evidence available to support this hypothesis and, therefore, more studies on dispersal and migration of the species are needed.

The results presented in this article are important in the conservation of *O. granulata* in China. As a rule, for a predominantly outbreeding perennial like this species, sampling fewer populations but more individuals within populations should be done. However, an estimation of $F_{ST} = 0.859$ indicates that 85.9% of the total genetic variation exists among populations. Therefore, sampling more populations but fewer individuals within each population should be adopted in Chinese *O. granulata*. Moreover, the lower mean genetic identity within Yunnan ($I = 0.940$) compared with Hainan ($I = 0.988$) indicates that the conservation of this species should include two regions and more populations should be sampled from Yunnan than Hainan. We should especially pay attention to populations 5 and 12, which showed the lowest value of $I = 0.769$, indicating that there are significant differences in allelic frequencies. Populations such as populations 3, 4, and 12,

which possess a higher amount of genetic variation, should be more attractive for both *in situ* conservation and germplasm collection.

We wonder whether population genetic structure of *O. granulata* in China is the nature of marginal populations or is typical of the species as the whole. As an upland wild rice, many factors, such as gravity-dispersed seeds with strong dormancy, may be significant in shaping its population genetic structure. In addition, the limitations of the use of allozymes to detect genetic variability and low sample sizes in this study may be the reasons for the observed population genetic structure. Further studies in comparison with other regions out of China, as well as detailed ecological studies, are needed. Hence, we suggest the following studies of *O. granulata* in the future: (1) to study population genetics of the *Oryza meyeriana* complex from other regions using molecular techniques such as RAPD, AFLP, and microsatellites; (2) to conduct detailed pollination biology and reproductive biology of the species, which should include estimates of outcrossing rates using genetic marker and progeny arrays, monitoring of germination rates of seeds in natural populations and seed dispersal distances; (3) to compare the patterns of allozymic variation and those of morphological variation in *O. meyeriana* complex, especially of spikelet length; and (4) to explore evolutionary relationships between the shade-loving species and sun-loving species in the genus.

Acknowledgments

We are grateful to Professor Cheng Kan-sen (Yunnan, AAS) for his valuable suggestions; to Dr. Zhang Shou-zhou (Institute of Botany, CAS), Professor Zhang Jiong-wei, Professor Luo Qing-yan (Simao Prefecture Institute of Agricultural Sciences), and Professor Wang Wen-hua (Xishuangbanna Prefecture Institute of Agricultural Sciences) for their help in collecting field materials; to Professor Wang Zhong-ren, Professor Pan Kai-yu, Dr. Zhou Shi-liang, Miss Wang Ke-qing, and Miss Zhang Fang for their various help in the laboratory; and to Mr. Lin Ru-sun (South China Botanical Garden, CAS), Professor Xu Zai-fu, Professor Tao Guo-da, Mr. Li Qing-jun, and Mrs. Xia Yong-mei (Xishuangbanna Tropical Botanical Garden, CAS) for their help in transplanting materials. We are also grateful to two anonymous reviewers for valuable comments on the manuscript. This research was supported by Chinese National Foundation for Sciences (39800013), International Foundation for Sciences (C/2738-1), and CAS Key project B "Studies on Evolutionary Biology of Some Important Endangered Plant Species" (KZ951-B1-102).

Literature Cited

Barbier P 1989*a* Genetic variation and ecotypic differentiation in the wild rice species *Oryza rufipogon*. I. Population differentiation in life-history traits and isozymic loci. Jpn J Genet 64:259–271.

——— 1989*b* Genetic variation and ecotypic differentiation in the wild rice species *Oryza rufipogon*. II. Influence of the mating system and life-history traits on the genetic structure of populations. Jpn J Genet 64:273–285.

Barbier P, H Morishima, H Ishihama 1991 Phylogenetic relationships of annual and perennial wild rice: probing by direct DNA sequencing. Theor Appl Genet 81:693–702.

Barret SCH, BJ Richardson 1986 Genetic attributes of invading species. Pages 21–23 *in* RH Gover, JJ Burdon, eds. Ecology of biological invasions. Australian Academy of Sciences, Canberra.

Barret SCH, JS Shore 1989 Isozyme variation in colonizing plants. Pages 106–125 *in* DE Soltis, PS Soltis, eds. Isozymes in plant biology. Dioscorides, Portland, Oreg.

Brown ADH, DR Marshall 1981 Evolutionary changes accompanying colonization in plants. Pages 351–363 *in* GCE Scudder, JL Reveal, eds. Evolution today. Hunt Institute for Botanical Documentation, Carnegie-Mellon University, Pittsburgh.

Chang TT 1976 The origin, evolution, cultivation, dissemination and differentiation of Asian and African rices. Euphytica 25:435–441.

Fu L-G, ed. 1992 Endangered plant species in China. Vol 1. Science, Beijing.

Gao LZ 1997 A study on genetic variation of three wild rices (*Oryza* spp.) in China and their conservation biology. PhD diss. Institute of Botany, Chinese Academy of Sciences, Beijing.

Gao LZ, S Ge, D-Y Hong 2000*a* Allozyme variation and population genetic structure of common wild rice *Oryza rufipogon* Griff., China. Theor Appl Genet (in press).

——— 2000*b* High levels of genetic differentiation of *Oryza officinalis* Wall. et Watt. from China. J Hered (in press).

Gao LZ, S-Z Zhang, Y Zhou, S Ge, D-Y Hong 1996 A survey of current status of wild rice in China. Chin Biodivers 4:162–166.

Ge S, D-M Zhang, H-Q Wang, G-Y Rao 1997 Allozyme variation in *Ophiopogon xylorrhizus*, an extreme endemic species of Yunnan, China. Conserv Biol 11:562–565.

Glaszmann JC, BG de los Reyes, GS Khush 1988 Electrophoretic variation of isozymes in plumules of rice (*Oryza sativa* L.): a key to the identification to 76 alleles at 24 loci. IRRI Res Pap Ser 134: 1–14.

Gottlieb LD 1982 Conservation and duplication of isozymes in plants. Science 216:373–379.

Hamrick JL 1989 Isozymes and the analysis of genetic structure in plant populations. Pages 87–105 *in* DE Soltis, PS Soltis, eds. Isozymes in plant biology. Dioscorides, Portland, Oreg.

Hamrick JL, MJW Godt 1990 Allozyme diversity in plant species. Pages 43–63 *in* ADH Brown, MT Clegg, AL Kahler, BS Weir, eds. Plant population genetics, breeding and genetic resources. Sinauer, Sunderland, Mass.

Ichikawa H, A Hirai, T Katayama 1986 Genetic analysis of *Oryza* species by molecular markers for chloroplast genomes. Theor Appl Genet 72:353–358.

McIntyre CL, B Winberg, K Houchins, B Appels, R Baum 1992 Relationships between *Oryza* species (Poaceae) based on 5S DNA sequences. Plant Syst Evol 183:249–264.

Morishima H, P Barbier 1990 Mating system and genetic structure of natural populations in wild rice *Oryza rufipogon*. Plant Species Biol 5:31–39.

Morishima H, HI Oka, WT Chang 1961 Directions of differentiation in populations of wild rice, *Oryza perennis* and *O. sativa f. spontanea*. Evolution 15:326–339.

Morishima H, Y Sano, HI Oka 1984 Differentiation of perennial and annual types due to habitat conditions in the wild rice *Oryza perennis*. Plant Syst Evol 144:119–135.

Nei TM 1978 Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Provan J, G Corbett, JW McNicol, W Powell 1997 Chloroplast DNA variability in wild and cultivated rice (*Oryza* spp.) revealed by polymorphic chloroplast simples sequence repeats. Genome 40:104–110.

Rice K, SK Jain 1985 Plant population genetics and evolution in dis-

turbed environments. Pages 287–303 *in* STA Pickett, PA White, eds. The ecology of natural disturbance and patch dynamics. Academic Press, New York.

Sano Y, H Morishima, HI Oka 1980 Intermediate perennial-annual populations of *Oryza perennis* found in Thailand and their evolutionary significance. Bot Mag Tokyo 93:291–305.

Second G 1982 Origin of the genetic diversity cultivated rice (*Oryza* spp.): study of the polymorphism scored at 40 isozyme loci. Jpn J Genet 57:25–57.

Second G 1985 Evolutionary relationships in the Sative group of *Oryza* based on isozyme data. Genet Sel Evol 17:89–114.

Soltis DE, CH Haufler, DC Darrow, GJ Gastony 1983 Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Am Fern J 73:9–29.

Soltis PS, DE Soltis 1991 Genetic variation in endemic and widespread plant species, examples from Saxifragaceae and *Polystiochum* (Dryopteridaceae). Aliso 13:215–223.

Soltis PS, DE Soltis, TL Tucker, FA Lang 1992 Allozyme variability is absent in the narrow endemic, *Bensonialla oregana* (Saxifragaceae). Conserv Biol 6:131–134.

Swofford DL, RB Selander 1989 BIOSYS-1: a computer program for the analysis of allelic variation in population genetics and biochemical systematics, release 1.7. Illinois Natural History Survey, Champaign.

Tateoka T 1963 Taxonomic studies of *Oryza*. III. Key to the species and their enumeration. Bot Mag Tokyo 76:165–173.

Vaughan DA 1989 The genus *Oryza* L.: current status of taxonomy. IRRI Res Pap Ser 138:1–21.

——— 1994 The wild relatives of rice: a genetic resources handbook. International Rice Research Institute, Manila, Philippines. 137 pp.

Vaughan DA, TT Chang 1992 *In situ* conservation of rice genetic resources. Econ Bot 46:368–383.

Wang ZY, G Second, SD Tanksley 1992 Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs. Theor Appl Genet 83:565–581.

Workman PL, JD Niswander 1970 Population studies on southwestern Indian tribes II. Local genetic differentiation in the *Papago*. Am J Hum Genet 22:24–49.