Biodiversity and Conservation (2005) 14:2821–2834
DOI 10.1007/s10531-004-0218-x

Patterns of allozyme variation at two stages of the life history in wild rice *Oryza rufipogon* and conservation genetic implications

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Received 17 September 2003; accepted in revised form 28 April 2004

Key words: Allozyme analysis, Conservation implications, Genetic diversity, Life history, *Oryza rufipogon* Griff., Population genetic structure

Abstract. *Oryza rufipogon* Griff. occurs widely in aquatic ecosystem of tropics and subtropics of monsoon Asia as well as Southern China. It is a vital gene source for rice breeding programs. Many populations of the species, unfortunately, have drastically diminished because of the disappearance of aquatic habitats as a result of human disturbance. In order to determine patterns of genetic variation at two stages of the life-cycle in the wild rice species, we investigated allozyme variation of four natural populations in China. Two southern populations have significant asexual reproduction while two other northern marginal populations show a mixed reproduction in China. At 22 allozyme loci, a significantly lower genetic diversity was observed in the ratoons than in the seeds of the two southern populations, whereas a significantly higher genetic diversity was found in the ratoons than in the seeds of the two northern marginal populations. The results suggest that the variation of reproductive system is probably associated with their patterns of genetic variation in the species. Moreover, a significantly higher genetic differentiation among populations found in the ratoons than in the seeds may stem from pollen-mediated gene flow among them. Finally, we propose suggestions for conservation management of the endangered species.

Introduction

As a member of about 21 wild species of the genus *Oryza* (Chang 1985; Vaughan 1989a), *O. rufipogon* Griff. is the most important gene source. It is commonly regarded as the progenitor of Asian cultivated rice *O. sativa* L. (Chang 1976; Oka 1988; Wang 1993), and thus many important traits of the species have been easily included in rice breeding programs. For example, annual populations of the species (synonymous with *O. nivara* Sharma et Sharstry) confer the gene for resistance to grassy stunt virus disease; the hybrid rice of China derived cytoplasmic male sterility from an individual of *O. rufipogon* (Wild Abortive) found on Hainan Island, China. The aquatic plant species is a genetically diverse diploid and widely distributed in tropics and subtropics of monsoon Asia (Vaughan 1989a) and Southern China (National

Exploring Group of Wild Rices 1984). It occurs extensively in a large ecosystem such as rivers, lakes, ditches, ponds, and marshes (Gao et al. 1998). Many populations of the species, however, have drastically diminished because of the disappearance of aquatic habitats as a result of human disturbance. Recent field survey revealed that it has been seriously threatened in China (Kiang et al. 1979; Hong 1995; Gao et al. 1996; Gao et al. 1998) and other southern Asian countries (Chang 1984; Vaughan 1989b; Vaughan and Chang 1992). Undoubtedly, the *in situ* and/or *ex situ* conservation management should give priority to such a species in the genus *Oryza*.

In the past decades, detailed studies on ecological genetics of the species in Thailand and India have greatly contributed to our understanding of its intraspecific variation (Oka and Morishima 1967; Oka 1988; Barbier 1989a, b; Morishima and Barbier 1990; Barbier et al. 1991). They provided valuable information for rice conservationists (Vaughan and Chang 1992). For example, Morishima and Barbier (1990) studied the variation of mating systems of *O. rufipogon* populations from Thailand. They predicted that reproductive system might be one of important factors in determining the genetic structure of the wild rice. More recently, we reported ecological differentiation of 31 natural populations representing the geographical distribution of the species in China. Our results suggested that the proportion of asexual reproduction tends to increase and that of sexual reproductions tends to decrease as latitudes increase. Perennial populations of the species in China are apparently characterized by a reproductive system ranging from almost asexual propagation of tropical populations to a mixed sexual–asexual propagation of northern marginal ones (Gao et al. 1999a). Although the dynamics of reproductive system in different populations as latitudes change has been observed in the species, the question of whether and how reproductive system has affected genetic structure of those populations located at different latitudes, is still unresolved. A study on temporal fluctuation of genetic diversity, such as the change of genetic variation at two stages of the life-cycle in natural populations may provide access to addressing how the variation is associated with population genetic structure in *O. rufipogon*. Such an endeavor should be helpful to improve our estimate of how genetic structure is shaped and to further determine what are the critical factors for bringing an evolutionary perspective to *in situ* conservation and germplasm management of the wild rice species.

Allozyme analysis may provide a good estimate of genetic variation in natural populations (Hamrick 1989; Hamrick and Godt 1989) and has been extensively used for studying population genetic structure of wild rice species (Morishima 1985; Barbier 1989a, b; Gao et al. 1999b; Gao et al. 2000a, b; Gao et al. 2001a, b, c; Gao et al. 2002). The goals of this paper are (1) to investigate the patterns of genetic diversity at two stages of the life-cycle in northern marginal and southern populations of *O. rufipogon*, and (2) to explore whether the reproductive systems affect its population genetic structure. The data here provide useful information towards developing genetic conservation management of the wild rice resources in the future.

Material and methods

Plant materials

Samples were taken from four natural populations. The Dongxiang population (No. 2; Jiangxi Province) and Guilin population (No. 1; Guangxi Province) are two northern marginal populations. They have been well isolated from rice fields; the other two from Chongpo (No. 3; Hainan Province) and Jinghong (No. 4; Yunnan Province) were collected from Southern China and are adjacent to rice fields (Figure 1; Table 1). The habitats of these four populations are either marshy ponds or streams. The seeds and ratoons were sampled from these populations at the same time in October-November, 1994. Because *O. rufipogon* populations have clonal growth habits and neighboring plants have a high probability of being a single genet, care was taken to prevent collecting duplicate samples. One or two seeds were randomly collected for an individual. Individual live ratoons were randomly collected at intervals of at

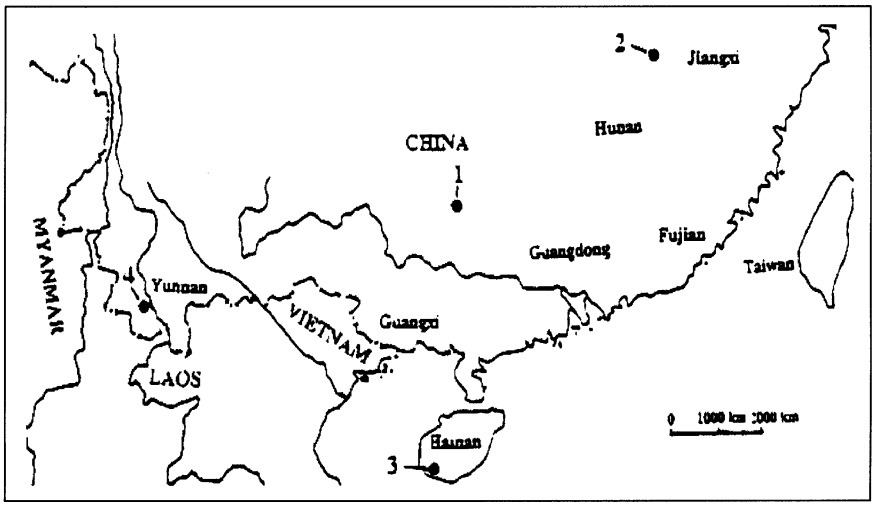

Figure 1. Geographical localities of four populations of *O. rufipogon* from China.

Table 1. The sample sizes and localities of four populations of *O. rufipogon* from China according to the number assigned in the data analysis.

Population no	Population locality	Sample size	
1	Zhoujiacun, Guilin City, Guangxi Province	45 (S)	15 (R)
2	Dongxiang County, Jiangxi Province	46 (S)	19 (R)
3	Chongpo, Ledong County, Hainan province	40 (S)	15 (R)
4	Gasa, Jinhong City, Yunnan Province	31 (S)	29 (R)

least 5 m in the field, numbered, transplanted to pots and maintained in Xishuangbanna Tropical Botanical Garden (Mengla County, Yunnan Province), South China Botanical Garden (Guangzhou City, Guangdong Province) and China Agricultural University (Beijing City).

Young leaves were individually collected from transplanted plants in March 1995, and stored in plastic bags on ice. Those sampled from two botanical gardens were transported to the laboratory by airplane. After dormancy was broken by a heat shock (1 week at 50–55 °C), seed samples were germinated in Petri dishes. The plumules and coleoptiles at 4–10 days after germination were used for enzyme extraction. For each individual, 0.05 g of fresh leaf material was crushed in 100 μl of Tris–HCl (pH 7.5; see Soltis et al. 1983). The extract was absorbed into 3×8 mm paper wicks and stored at -70 °C until electrophoresis was conducted.

Starch-gel electrophoresis

Fourteen enzymes were resolved and scored using starch-gel electrophoresis. The electrophoresis methods followed Glaszmann et al. (1988) and Soltis et al. (1983) with 12% starch gels. The buffer system 6 of Soltis et al. (1983) (S6) was used to resolve diaphorase (DIA, EC 1.6.2.2), aminopeptidase (LAP, EC 3.4.11.1), phosphogluco isomerase (PGI, EC 5.3.1.9), and triosephosphate isomerase (TPI, EC 5.3.1.1); phosphoglucomutase (PGM, EC 2.7.5.1), shikimate dehydrogenase (SKD, EC 1.1.1.25), glutamate dehydrogenase (G3PDH, EC 1.4.1.2), malate dehydrogenase (MDH, EC 1.1.1.37), alcohol dehydrogenase (ADH, EC 1.1.1.1), and isocitrate dehydrogenase (IDH, EC 1.1.1.42) were resolved on buffer system 1 of Glaszmann et al. (1988) (G1), while aspartate aminotransferase (AAT, EC 2.6.1.1), fructose-bisphosphate aldolase (FBA, EC 4.1.2.13), malic enzyme (ME, EC 1.1.1.40), and phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44) were resolved on buffer system 2 (G2). 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

Electrophoresis data were analyzed using the computer program Biosys-1 (Swofford and Selander 1989) version 1.7 for the IBM-PC. Data were entered as genotype numbers from which allele frequencies were calculated. The amount of genetic variation in each locus and population was indicated by the mean number of alleles per locus (A), the percent of polymorphic loci (P) and

observed and expected heterozygosities (H_o and H_e). Deviation from Hardy-Weinberg expectations and divergence among populations were measured using *F*-statistics of Wright (1977) and their significance was evaluated with a chi-square test following the method of Workman and Niswander (1970). In order to estimate the significance of the differences in genetic diversity parameters between two life stages, *t*-test was employed for two southern and northern marginal populations, respectively. The differences in genetic differentiation values (F_{IS} and F_{ST}) between two life stages were statistically examined for all four populations. The *t*-test was conducted by using the SAS system (Der and Everitt 2001).

Results

Allelic variation

Table 2 shows allelic frequencies in four populations at two stages of life-cycle. A total of 40 alleles at 22 isozyme loci could be identified in all individuals of four populations studied. *Aat-1*, *Dia-2*, *Fba*, *Gdh*, *Lap-1*, *Mdh-1*, *Mdh-2*, *Mdh-3*, *Me*, *Pgd-1*, *Pgi-1*, *Tpi-1* and *Tpi-2* were monomorphic, with all individuals from four populations scored possessing a single enzyme band with identical mobility for each locus, and other loci were polymorphic in at least one population. Although two loci of PGM isozyme are generally present in diploid seed plants (Gottlieb 1982), only one PGM isozyme was observed in *O. rufipogon*; two loci of G3PDH were reported in *Oryza* species (Second 1982), but only one was present in this study.

The differences in allelic frequency at most polymorphic loci between two stages of life-cycle were detected in four populations to a certain extent. No significant allelic variation was observed in the two northern marginal populations between the ratoons and seeds. For the Guilin population (No. 1), the rations, but not the seeds, had *Idh-a* (16.7%), *Pgd-2b* (44.4%) and *Pgi-3b* (22.2%), while the seeds but not the ratoons possessed *Idh-c* (2.2%), *Pgi-2c* (2.3%), *Pgi-3a* (1.1%) and *Pgi-3d* (12.5%); for the Dongxiang population (No. 2), *Dia-1a* (23.7%) appeared in the ratoons, while *Pgi-3b* (15.2%) was only detected in the seeds. However, more alleles were apparently observed in the seeds than in the ratoons of the two southern populations. Four alleles of *Adh-a* (42.5%), *Pgi-3a* (3.8%), *Pgm-c* (1.3%) and *Skd-c* (7.5%) were detected in the seeds but not in the ratoons for the Chongpo population (No. 3), while up to eight alleles of *Aat-3a* (3.1%), *Aat-3b* (15.6%), *Idh-a* (1.6%), *Pgi-2a* (18.0%), *Pgi-2c* (2.0%), *Pgi-3a* (4.0%), *Pgi-3b* (22.0%) and *Pgi-3d* (14.0%) were present in the seeds but not in the ratoons of the Jinhong population (No. 4). Nevertheless, there is an exception, that *Dia-1c* (5.0%) was merely possessed in the ratoons of the Chongpo population (No. 3) and *Pgm-a* (1.7%) in those of the Jinghong population (No. 4).

Table 2. Allele frequencies in four populations of *O. ruftpogon* at two stages of the life-cycle*.

Locus	Seeds				Ratoons			
	1	2	3	4	1	2	3	4
Aat-1a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Aat-3a	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000
Aat-3b	0.000	0.000	0.000	0.156	0.000	0.000	0.000	0.000
Aat-3c	1.000	1.000	1.000	0.813	1.000	1.000	1.000	1.000
Adh-a	0.000	0.000	0.425	0.000	0.000	0.000	0.000	0.000
Adh-b	1.000	1.000	0.575	1.000	1.000	1.000	1.000	1.000
Dia-1a	0.089	0.000	0.000	0.000	0.111	0.237	0.000	0.000
Dia-1b	0.911	1.000	1.000	1.000	0.889	0.763	0.950	1.000
Dia-1c	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000
Dia-2a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Fba-a	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
Idh-a	0.000	0.000	0.000	0.016	0.167	0.000	0.000	0.000
Idh-b	0.978	1.000	1.000	0.984	0.833	1.000	1.000	1.000
Idh-c	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lap-1a	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
Mdh-2a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-3a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Me-a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgd-1a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgd-2a	1.000	1.000	1.000	1.000	0.556	1.000	1.000	1.000
Pgd-2b	0.000	0.000	0.000	0.000	0.444	0.000	0.000	0.000
Pgi-1a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgi-2a	0.091	0.045	0.000	0.180	0.222	0.184	0.400	0.000
Pgi-2b	0.886	0.955	1.000	0.800	0.778	0.816	0.550	1.000
Pgi-2c	0.023	0.000	0.000	0.020	0.000	0.000	0.050	0.000
Pgi-3a	0.011	0.000	0.038	0.040	0.000	0.000	0.000	0.000
Pgi-3b	0.000	0.152	0.000	0.220	0.222	0.000	0.400	0.000
Pgi-3c	0.864	0.227	0.612	0.600	0.778	0.816	0.600	1.000
Pgi-3d	0.125	0.621	0.350	0. 140	0.000	0.184	0.000	0.000
Pgm-a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017
Pgm-b	0.733	1.000	0.988	1.000	0.889	1.000	1.000	0.983
Pgm-c	0.267	0.000	0.013	0.000	0.111	0.000	0.000	0.000
Skd-a	0.033	0.106	0.000	0.000	0.222	0.053	0.000	0.000
Skd-b	0.289	0.181	0.000	0.000	0.111	0.316	0.000	0.000
Skd-c	0.200	0.713	0.075	0.203	0.111	0.632	0.000	0.017
Skd-d	0.478	0.000	0.925	0.797	0.556	0.000	1.000	0.983
Tpi-1a	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

* Populations 1 and 2 are northern populations, and populations 3 and 4 are southern populations.

Amount 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) at two stages of

Table 3. Genetic variability and the mean fixation indices at 22 loci in four populations of *O. rufipogon* at two stages of the life-cycle[a].

Populations	*A*		*P*[b]		H_o		H_e[c]		*F*	
	S[d]	R[e]	S	R	S	R	S	R	S	R
1	1.5	1.4	27.3	31.8	0.013	0.015	0.078	0.119	0.833	0.874
2	1.2	1.2	13.6	18.2	0.042	0.050	0.049	0.068	0.143	0.265
t-test	$p = 0.3452$		$p = 0.0496$		$p = 0.0449$		$p = 0.0424$			
3	1.2	1.2	18.2	13.6	0.052	0.009	0.053	0.053	0.019	0.830
4	1.4	1.1	22.7	9.1	0.030	0.003	0.073	0.003	0.589	0.000
t-test	$p = 0.4236$		$p = 0.0487$		$p = 0.0374$		$p = 0.0394$			

[a] Populations 1 and 2 are northern populations, and populations 3 and 4 are southern populations.
[b] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99.
[c] Unbiased estimate (see Nei 1978).
[d] Seeds.
[e] Ratoons.

life-cycle in four populations are presented in Table 3. Among them, the Guilin population (No. 1) possessed the highest values for A, P and H_e in seeds as well as in ratoons. The Jinghong population (No. 4) had the lowest levels of genetic diversity in ratoons but not in seeds, which probably corresponds to the fact that it is composed of a few clones. The observed heterozygosity values (H_o)are generally lower than expected heterozygosity (H_e), indicating an excess of homozygotes. The result agrees with the positive F values observed in this study (Table 3). It is of interest to find a reverse pattern of genetic diversity between southern and northern populations when compared seeds with ratoons. Significantly lower levels of genetic diversity were observed in the ratoons than in the seeds of the two southern populations (A: $p = 0.4236$; P: $p = 0.0487$; H_o: $p = 0.0374$; H_e: $p = 0.0394$), while significantly higher levels of genetic diversity were found in the ratoons than in the seeds of the two northern populations (A: $p = 0.3452$; P: $p = 0.0496$; H_o: $p = 0.0449$; H_e: $p = 0.0424$).

Population genetic structure

Wright's F-statistics are a hierarchical series of fixation indices, where F_{IS} represents the deviation from Hardy-Weinberg expectation within populations (approximately equal to the mean F across population), F_{ST} measures the fixation of different alleles in different populations, and F_{IT} measures the deviation from Hardy-Weinberg expectation across the population system as a whole. The partition of genetic variation found in the seeds and ratoons into within- and between-population components by F-statistics is shown in Table 4. The differences of population genetic structure between seeds and

Table 4. Summary of *F*-statistics at all the polymorphic loci at two stages of life-cycle.

Locus	F_{IS}		F_{IT}		F_{ST}	
	S[a]	R[b]	S	R	S	R
Aat-3	1.000	–	1.000	–	0.126***	–
Adh	0.284	-0.056	0.539	0.048	0.357***	0.098***
Dia-1	1.000	0.600	1.000	0.652	0.068***	0.130***
Idh	0.579	1.000	0.584	1.000	0.012*	0.375***
Pgi-2	0.246	0.603	0.293	0.657	0.062**	0.135***
Pgi-3	0.167	0.673	0.317	0.726	0.180***	0.163***
Pgm	0.940	0.851	0.952	0.862	0.199***	0.072**
Skd	0.612	0.970	0.746	0.984	0.344***	0.473***
Mean	0.449	0.666	0.579	0.755	0.236***	0.267***
t-test	$p = 0.0451$				$p = 0.0372$	

*$p < 0.5$; **$p < 0.01$; ***$p < 0.001$.
[a] Seeds.
[b] Ratoons.

ratoons were observed. A higher value of F_{IS} was found in the ratoons ($F_{IS} = 0.666$) than in the seeds ($F_{IS} = 0.449$) at many loci ($p = 0.0451$), suggesting that populations at the juvenile stage more greatly deviated from Hardy-Weinberg expectation and had a greater deficiency of heterozygotes than at the seed stage. Fixation indices (Table 3) basically agree with a greater deficiency of heterozygotes in the ratoons than in the seeds except the Jinghong population (No. 4). Moreover, a significantly lower value of F_{ST} was observed in the seeds ($F_{ST} = 0.236$) than in the ratoons ($F_{ST} = 0.267$) ($p = 0.0372$), indicating that genetic differentiation among populations was slightly lower at seed stage than at juvenile stage.

Discussion

Genetic diversity and the variation of reproductive system

The non-random distribution of genetic variation within and among populations is often called the genetic structure of populations (Loveless and Hamrick 1984), which is primarily affected by selection, genetic drift, recolonization and gene flow. From the most recent and comprehensive review of allozyme literature, the modes of reproduction do not significantly affect genetic diversity within populations and proportion of variation among populations (Hamrick and Godt 1989). Therefore, we expected no significant differences of genetic diversity between seed-derived and juvenile-derived samples of these populations located at different latitudes. There also should be a similar genetic differentiation of the same populations at two stages of the life history under natural selection. Considering the data presented in this paper, however, there are three observations that may be of importance with respect to our questions

addressed. First, different allelic frequencies at most of the polymorphic loci between two stages of life-cycle were observed; second, lower levels of genetic diversity were detected in the ratoons than in the seeds of the two southern populations, while higher levels of genetic diversity were found in the ratoons than in the seeds of the two northern marginal populations; and third, slightly higher genetic differentiation was present in the ratoons than in the seeds.

The most plausible explanation for the results is the variation of reproductive system in *O. rufipogon*, which has been revealed by our recent studies on population ecology of the species (Gao et al. 1999a). We found that perennial populations in China are characterized by a reproductive system ranging from almost asexual to a mixed sexual–asexual propagation with the increase of latitudes. As far as the mating system is concerned, our recent estimate based on the value of the mean F over 21 natural populations showed that the outcrossing rate was 0.324 (Gao et al. 2000a), indicating that *O. rufipogon* has a typical mixed-mating system. Therefore, differences in the extent of asexuality among perennial populations studied may be closely correlated with the patterns of genetic diversity observed here.

The decrease of genetic diversity observed in the juvenile versus seed samples of two southern populations could be due to mainly asexual reproductive system. The Chongpo population, which is obviously propagated by clonal growth, was found in the marshy ponds with a relatively uniform water condition and comprised of about ten thousands of individuals (Gao, field observation). Because of only 15 ratoon individuals sampled, higher levels of genetic diversity in seeds collected from the population are readily explained by the idea that allozyme variation of pollen-mediated gene flow from spatially distant other genets was not included in the juvenile samples. No recruitment of seedlings was observed in this population, and thus the variation released by sexual recombination may be poorly represented in the ratoons. The Jinghong population is a surviving one found in a ditch close to rice fields, where almost all genotypes were collected and detected in the present study. Hence, another explanation should tentatively be sought. An input of alien alleles in seeds by pollen migration is unlikely because of almost complete sampling in this isolated population in the region, and therefore, genetic variation in seeds should reflect the original amount of genetic variation in parental plants of the population. A loss of genetic diversity could occur in one of the following ways. During the transition from the seed- to the juvenile-stages, few seeds produced may enter the soil seed bank, or be lost either by emigration out of the population, or due to seedling mortality after germination. The first author continuously visited the population in October of 1994, March of 1995, June of 1995 and August of 1996. Demographic data recorded showed no recruitment of seedlings observed (Gao, unpublished data). Hence, genetic diversity within the population may not be totally represented by the juvenile samples due to random loss within unsuccessful recruitment of seedlings. The results derived from these two southern populations agree well with those observations within perennial populations of *O. rufipogon* from Thailand (Barbier 1989a, b).

An increase of genetic variability observed in the ratoons of two northern marginal populations may be interpreted by the use of a mixed reproductive system as the sample sizes for the ratoons was much fewer than the seeds collected. Taking the Dongxiang population for an example, previous field investigations recorded that it was a perennial population with a mixed reproductive system, in which a relatively high fecundity was achieved through the production of both large numbers of reproductive tillers and high seed fertility (Jiang et al. 1988). Population ecological studies indicated that there were a considerable number of full seeds on the seed bank. Demographic data further showed that the percentage of seedling recruitment was up to 10% in 1993 (Zhou 1995). Therefore, sexual propagation appears important in affecting genetic variation within northern marginal populations. Most plants in the population are sexual individuals, and the amount of genetic variability harbored in the ratoons might somewhat represent levels of genetic diversity within the population before the occurrence of genetic random drift. A decrease of genetic diversity at seed stage can be explained by the fact that the Dongxiang population recently became small in size due to human disturbance. Inbreeding leads to the loss of genetic diversity that is detected in the seed samples (Gao et al. 1999a). A bit lower genetic differentiation in seeds is characteristics of outbreeding populations, with pollen-mediated gene flow between them. Nevertheless, pollen-mediated gene flow becomes effective only if seeds are recruited.

In conclusion, this study reveals that the reproductive system may be one of important factors in determining the genetic structure of wild rice. Considering most of derived results were marginally significant probably due to small sample sizes in the present study, however, further studies on more populations in larger sample size should help to outline a better picture of how population genetic structure is affected by the variation of reproductive system in the species.

Conservation genetic implications

O. rufipogon is a perennial plant with mixed sexual and asexual reproductive strategies (Barbier 1989a; Gao et al. 1999a). The allocation of asexual versus sexual reproduction may vary among populations at different aquatic habitats (Barbier 1989a). The results revealed in the present study are of significance in *ex situ* germplasm collection of the species. Vegetative reproduction is usually more important than seed reproduction for most of the perennial populations of *O. rufipogon* at natural habitats in the tropics (National Institute of Genetics 1987; Vaughan 1989a), resulting in a great difficulty in collecting seeds for *ex situ* conservation. Considering that lower levels of genetic diversity were observed in the ratoons than in the seeds for the tropical populations, seed samples are more attractive than juvenile samples as for germplasm collection in tropical regions. Therefore, the findings generally support our current germplasm preservation methodologies by collecting seeds into genebank rather than sampling live ratoons into field genebanks from wild populations.

However, a reverse strategy, in which ratoons, rather than seeds, are collected, should become efficient for those far away from the equator, e.g., northern marginal populations in this study, and thus genetic variation will be effectively collected. Based on the results observed, more genetic diversity can be rescued by means of collecting seeds than ratoons for those endangered populations with small size, such as the Jinghong population in the present study. In addition, the estimates of $F_{ST} = 0.236$ and $F_{ST} = 0.267$ for seeds and ratoons, respectively, suggest that a preservation plan by sampling fewer ratoons than seeds for a target population of wild rice may be adopted. Further studies on the dynamics of genetic diversity over time extending to those populations at a larger geographical scale should provide more information about the difference between seed and ratoon sampling strategies.

Knowledge of levels and dynamics of genetic diversity in the life-cycle of *O. rufipogon* has implications for establishing *in situ* conservation plans in the future. In the present study, different patterns of genetic variation at two stages of the life history in natural populations studied are closely related to reproductive systems and other factors such as population size and inbreeding in the species. Therefore, it is worthwhile to identify key biological factors that affect the dynamics of genetic diversity over time so as to find ways to enhance the conservation of biological diversity. Levels of genetic diversity and population genetic structure of the species can provide basic information to address questions such as how many populations should be involved and how much size should be taken at a large geographical scale for the purposes of *in situ* conservation plans (Gao et al. 2000a). However, long-term extensive micro-evolution studies on the dynamics of genetic diversity and the factors that affect those dynamics (e.g., environmental and biological) will be important for determining the principles of how to select target populations because the genetic diversity of *in situ* conserved populations must be maintained in changeable agroecosystems. A recent study on clonal structure in *O. rufipogon* suggested that, for instance, moderate disturbance and reduction of the water supply, gradually from sufficient to wet and finally to dry, at the stage of vegetative growth (from April to September of a year in China) could be helpful for the maintenance of genetic diversity within natural populations of the species (Xie et al. 2001). It is our belief that a deeper evolutionary understanding about the dynamics of genetic diversity over time in the species should help to develop better conservation strategies and continue their evolutionary processes in the aquatic ecosystem.

Acknowledgements

The authors are indebted to Dr Shou-zhou Zhang (Institute of Botany, Chinese Academy of Sciences), Prof Xiang-kun Wang (China Agricultural University), who were involved in the collection of the materials, and Mrs Li-ping Chang, who helped to type the manuscript. We are grateful an anonymous reviewer for his/her valuable comments to improve the manuscript. The research was

supported by International Foundation for Sciences (IFS) (C/2738-1) to GAO and the Grant of the President of Chinese Academy of Sciences to Hong.

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