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Allozyme variation and population genetic structure of common wild rice *Oryza rufipogon* Griff. in China

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Abstract In order to determine the genetic diversity and genetic structure of populations in common wild rice *Oryza rufipogon*, an endangered species, allozyme diversity was analyzed using 22 loci in 607 individuals of 21 natural populations from the Guangxi, Guangdong, Hainan, Yunnan, Hunan, Jiangxi and Fujian provinces in China. The populations studied showed a moderate allozyme variability (A=1.33, P=22.7%, Ho=0.033 and He=0.068), which was relatively high for the genus *Oryza*. The levels of genetic diversity for Guangxi and Guangdong were significantly higher than those for the other regions, and thus South China appeared to be the center of genetic diversity of *O. rufipogon* in China. A moderate genetic differentiation (F_{ST}=0.310, I=0.964) was found among the populations studied. Interestingly, the pattern of population differentiation does not correspond to geographic distance. An estimate of the outcrossing rate (t=0.324) suggests that the species has a typical mixed-mating system. The deficit of heterozygotes (F=0.511) indicates that some inbreeding may have taken place in outcrossing asexual populations because of intra-clone outcrossing events and "isolation by distance" as a result of human disturbance. In order to predict the long-term genetic survival of fragmented populations, further studies on gene flow among the remaining populations and the genetic effects of fragmentation are proposed. Finally, some implications for the conservation of endangered species are suggested.

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Introduction

As rice genetic resources are of great economic importance in developing countries, their conservation has become an active field throughout the world (Chang 1984; Vaughan and Chang 1992). China is one of original centers of cultivated rice *Oryza sativa* L. (Chang 1976; Oka 1988; Wang 1993). Common wild rice *Oryza rufipogon* Griff. (2n=2*x*=24) is widely distributed in eight provinces or regions in southern China: Guangxi, Guangdong, Hainan, Yunnan, Hunan, Jiangxi, Fujian (National Exploring Group of Wild Rices 1984) and Taiwan (disappeared in 1978, Kiang et al. 1979) and has proven to be a precious gene pool for rice genetic improvement. For example, the cytoplasmic male-sterility gene from an individual of common wild rice (Wild Abortive) found on Hainan Island has been used for the production of the hybrid rices of China; and two Chinese pest-resistant varieties have their genes derived from an *O. rufipogon* (*Oryza sativa* f. *spontanea*) population in Guangdong Province. Therefore, the potential uses of the genetic diversity of the wild species will play a critical role in rice production in the future.

Our recent field investigations suggest that human activities have led to the extinction of a large number of populations of the species (Hong 1995; Gao et al. 1996; Gao 1997; Gao et al. 1998). However, our knowledge of the genetic diversity of the few natural populations of *O. rufipogon* in China is rather limited (Wang et al. 1996; Cai et al. 1997), and a better understanding of its population genetics will be of great significance in in situ conservation, germplasm collection, and rice genetic improvement.

Allozyme analysis may provide a valuable estimate of genetic variation in order to examine the genetic structure of natural populations of endangered species (Hamrick 1989; Soltis and Soltis 1991; Gottlieb and

Edwards 1992; Soltis et al. 1992; Ge et al. 1997). In the present study, 21 populations of *O. rufipogon* were sampled in the major part of its range in China, and were genetically assayed by allozyme electrophoresis. The purpose of the present study was to provide answers to the following questions: (1) What are the levels and distribution of genetic variability within and among populations of *O. rufipogon*?, (2) what are the possible factors that explain the patterns and levels of genetic variation observed?, and (3) what genetic management plan should we develop for the species in the future?

Materials and methods

Plant materials

Samples were taken from the 21 populations of the seven provinces throughout (China (Table 1; Fig. 1). Seeds were collected in November–December, 1994 and 1995. One or two seeds were randomly collected from an individual panicle, and panicles were sampled at least 5-m apart to prevent collecting duplicate samples from the same genet. After dormancy was broken by a heat shock (1 week at 50–55°C), seeds 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^2 paper wicks and stored at –70°C until electrophoresis was carried out.

Starch-gel electrophoresis

Fourteen enzymes were resolved and scored by starch-gel electrophoresis (Table 2). The electrophoresis methods followed those of Glaszmann et al. (1988) and Soltis et al. (1983) using 12% starch gels. DIA, LAP, PGI and TPI were resolved on buffer system 6 (S6) of Soltis et al. (1983); ADH, G3PDH, IDH, MDH, PGM and SKD were resolved on buffer system 1 (G1) of Glaszmann et al. (1988), while AAT, FBA, 6PGD and ME were resolved on buffer system 2 (G2). Staining procedures for all enzymes followed Soltis et al. (1983). When more than one isozyme was observed for an enzyme, the isozymes were numbered sequentially with the most-

Table 1 The sample sizes and localities of 21 populations of *O. rufipogon* from China according to the number assigned in the data analysis

Population no.	Population localities	Sample size
1	Wulitang, Shiya, Laibing County, Guangxi	37
2	Maliaotang, Guigang City, Guangxi	40
3	Tengcum, Jiangxi, Nanning City, Guangxi	15
4	Henglin, Guigang City, Guangxi	27
5	Gongguan, Hepu County, Guangxi	21
6	Changtang, Daxuzheng, Guigang City, Guangxi	35
7	Xuanwang, Guiping County, Guangxi	50
8	Luxin, Wuxuan County, Guangxi	37
9	Fumian, Yulin City, Guangxi	19
10	Zhoujiacun, Guilin City, Guangxi	45
11	Litang, Bingyang County, Guangxi	38
12	Beidu, Tiandong County, Guangxi	28
13	Beipeng, Liaojiang County, Guangxi	18
14	Nongtang, Tengxian County, Guangxi	19
15	Boluo County, Guangdong	13
16	Dongxiang County, Jiangxi	46
17	Chongpo, Ledong County, Hainan	40
18	Gasa, Jinghong City, Yunnan	31
19	Jiangyoung County, Hunan	18
20	Chaning County, Hunan	15
21	Zhangpu County, Fujian	15

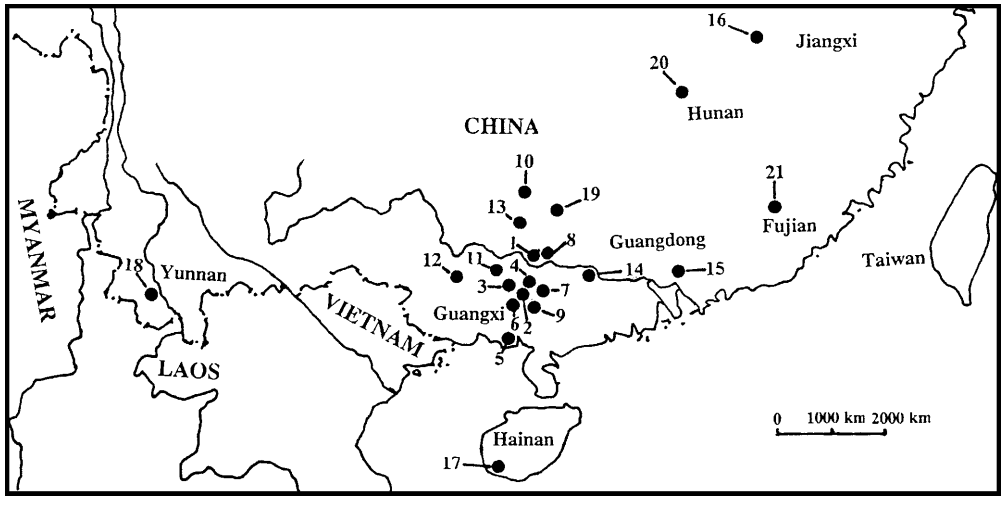

Fig. 1 Geographical localities of 21 populations of *O. rufipogon* from China

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	G2	2
Alcohol dehydrogenase	ADH	EC 1.1.1.1	G1	1
Diaphorase	DIA	EC 1.6.2.2	S6	2
Fructose-biphosphate aldolase	FBA	EC 4.1.2.13	G2	1
Glutamate dehydrogenase	G3PDH	EC 1.4.1.2	G1	1
Isocitrate dehydrogenase	IDH	EC 1.1.1.42	G1	1
Aminopeptidase	LAP	EC 3.4.11.1	S6	1
Malate dehydrogenase	MDH	EC 1.1.1.37	G1	3
Malic enzyme	ME	EC 1.1.1.40	G2	2
Phosphogluconate dehydrogenase	6PGD	EC 1.1.1.44	G2	1
Phosphoglucoisomerase	PGI	EC 5.3.1.9	S6	3
Phosphoglucomutase	PGM	EC 2.7.5.1	G1	1
Shikimate dehydrogenase	SKD	EC 1.1.1.25	G1	1
Triosephosphate isomerase	TPI	EC 5.3.1.1	S6	2

anodally migrating enzyme designated as 1. Allelic variation at a locus was coded alphabetically with the most-anodally migrating allozyme designated as a.

Data analysis

Electrophoresis data were analyzed using the computer Biosys-1 (Sworfford and Selander 1989) version 1.7 for the IPM-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. Outcrossing rate and fixation index are related by t=(1–F)/(1+F) (Weir 1990).

Results

Allele frequencies for all loci are presented in Table 3. A total of 47 alleles at 22 isozyme loci could be identified in 607 individuals of the 21 populations studied. *Dia-2*, *Fba*, *Gdh*, *Lap*, *Mdh-1*, *Me-1*, *Pgd-1*, *Pgd-2*, *Pgi-1* and *Tpi-1* were monomorphic, with all individuals from the 21 populations scored possessing a single enzyme band with identical mobility for each locus, and all the other loci were polymorphic in at least one population. *Aat-1*, *Adh*, *Dia-1*, *Mdh-2* and *Tpi-2* each had two alleles, *Aat-3*, *Idh*, *Mdh-3* and *Pgm* each had three, *Pgi-2* and *Pgi-3* each had four, and *Skd* had five. Although two isozymes of PGM are typically present in diploid seed plants (Gottlieb 1982), only one PGM locus was observed in *O. rufipogon*; two loci of G3PDH have been typically reported (Second 1982), but only one was observed in the present study.

Genetic variability

The mean number of alleles per locus (A), the percentage of loci polymorphic (P), the observed heterozygosity (Ho) and the expected heterozygosity (He) at all the 22 loci in the 21 populations are given in Table 4. All the values varied among populations, with A ranging from 1.1 in populations 20 and 21 to 1.6 in population 7; P ranged from 9.1% in population 21 to 31.8% in populations 2, 4, 6 and 7; Ho from 0.000 in population 21 to 0.060 in population 9, and He from 0.020 in population 19 to 0.108 in population 4. Clearly, populations such as 1, 4, 5, 6 and 7 from Guangxi as well as one population (population 15) from Guangdong possessed high levels of genetic diversity, while population 21 from Fujian had a rather low level of genetic variation. It is worth pointing out that the mean values for the population from Guangxi (A=1.37, P=25.6%, Ho=0.034, and He=0.074), as well as that from Guangdong (A=1.5, P=18.2%, Ho=0.059 and He=0.094), were higher than those from the other regions of China.

Conformance of genotype frequencies to Hardy-Weinberg expectations, and outcrossing rates

We used allozymic data to make a preliminary analysis of the mating system of the species. Deviations from Hardy-Weinberg expectation typically reflect a departure from random mating and can thus be used to assess the mating system of a population. These deviations can be measured by F, the fixation index, which can range from −1, indicating an excess of heterozygotes relative to Hardy-Weinberg expectation, to 1, indicating a deficiency of heterozygotes. All the populations seemed to show a deficiency of heterozygotes, with fixation indices varying from 0.019 to 1.000. Estimated by the value of the mean F as 0.511, the outcrossing rate of *O. rufipogon* was 0.324.

Distribution of genetic variation

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 populations), F_{ST} measures the fixation of different alleles in different populations, and F_{IT} measures deviations from Hardy-Weinberg expectation across the population system as a whole. In the 21 populations of *O. rufipogon* studied (Table 5) F_{IS} was 0.507, indicating that most deviated from Hardy-Weinberg expectation within populations and with a deficien-

Table 3 Allele frequencies at all loci in 21 populations of *O. rufipogon*

Locus	Population																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Aat-1a	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Aat-1b	1.000	1.000	1.000	1.000	1.000	1.000	0.980	1.000	1.000	1.000	1.000	1.000	1.000	0.900	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Aat-3a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000
Aat-3b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.156	0.000	0.000	0.000
Aat-3c	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	1.000	1.000	0.813	1.000	1.000	1.000
Adh-a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.425	0.000	0.000	0.000	0.000
Adh-b	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	1.000	0.575	1.000	1.000	1.000	1.000
Dia-1a	0.095	0.000	0.067	0.000	0.000	0.029	0.050	0.027	0.000	0.089	0.000	0.786	0.111	0.000	0.077	0.000	0.000	0.000	0.056	0.000	0.000
Dia-1b	0.905	1.000	0.933	1.000	1.000	0.971	0.950	0.973	1.000	0.911	1.000	0.214	0.889	1.000	0.923	1.000	1.000	1.000	0.944	1.000	1.000
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	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	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	1.000	1.000	1.000	1.000	1.000	1.000
Idh-a	0.135	0.200	0.000	0.071	0.738	0.171	0.010	0.095	0.395	0.000	0.090	0.036	0.944	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000
Idh-b	0.865	0.800	0.933	0.929	0.262	0.771	0.990	0.905	0.605	0.978	0.910	0.964	0.056	1.000	1.000	1.000	1.000	0.984	1.000	1.000	1.000
Idh-c	0.000	0.000	0.067	0.000	0.000	0.057	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	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	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	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-2a	0.000	0.025	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-2b	1.000	0.975	1.000	0.929	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	1.000	1.000
Mdh-3a	0.000	0.000	0.000	0.143	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-3b	0.000	0.025	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-3c	1.00	0.975	1.000	0.786	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	1.000	1.000
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	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	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1000	1.000
Pgd-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	1000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
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	1.000	1.000	1.000	1.000	1.000	1.000
Pgi-2a	0.029	0.100	0.000	0.188	0.095	0.047	0.122	0.000	0.053	0.091	0.051	0.000	0.000	0.053	0.125	0.045	0.000	0.180	0.000	0.000	0.000
Pgi-2b	0.971	0.900	1.000	0.813	0.905	0.797	0.844	0.971	0.947	0.886	0.808	0.964	1.000	0.842	0.583	0.955	1.000	0.800	0.972	0.550	1.000
Pig-2c	0.000	0.000	0.000	0.000	0.000	0.156	0.000	0.029	0.000	0.023	0.090	0.036	0.000	0.105	0.292	0.000	0.000	0.020	0.028	0.000	0.000
Pgi-2d	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.051	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.450	0.000
Pgi-3a	0.015	0.000	0.000	0.000	0.024	0.000	0.011	0.000	0.026	0.011	0.000	0.161	0.000	0.000	0.042	0.000	0.038	0.040	0.000	0.000	0.000
Pgi-3b	0.000	0.050	0.000	0.000	0.190	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.053	0.083	0.152	0.000	0.220	0.000	0.000	0.000
Pgi-3c	0.956	0.925	0.833	0.625	0.690	0.641	0.767	0.971	0.763	0.864	0.167	0.839	0.917	0.500	0.250	0.227	0.613	0.600	0.944	0.400	1.000
Pgi-3d	0.029	0.025	0.167	0.375	0.095	0.359	0.156	0.029	0.211	0.125	0.833	0.000	0.083	0.447	0.625	0.621	0.350	0.140	0.056	0.600	0.000
Pgm-a	0.324	0.000	0.000	0.000	0.000	0.014	0.050	0.608	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgm-b	0.676	0.893	0.267	0.643	0.735	0.986	0.950	0.392	1.000	0.733	0.857	1.000	0.667	1.000	1.000	1.000	0.988	1.000	0.972	1.000	0.867
Pgm-c	0.000	0.107	0.733	0.357	0.265	0.000	0.000	0.000	0.000	0.267	0.143	0.000	0.333	0.000	0.000	0.000	0.013	0.000	0.028	0.000	0.133
Skd-a	0.000	0.000	0.000	0.000	0.000	0.086	0.080	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.269	0.000	0.000	0.000	0.000	0.000	0.000
Skd-b	0.054	0.000	0.000	0.000	0.000	0.243	0.180	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.077	0.106	0.000	0.000	0.000	0.000	0.000
Skd-c	0.297	0.287	0.000	0.625	0.000	0.243	0.150	0.108	0.447	0.289	0.038	0.286	0.222	0.075	0.154	0.181	0.000	0.000	0.056	0.500	0.133
Skd-d	0.432	0.050	0.800	0.375	0.857	0.229	0.450	0.257	0.474	0.200	0.141	0.339	0.667	0.500	0.115	0.713	0.075	0.203	0.944	0.500	0.800
Skd-e	0.216	0.663	0.200	0.000	0.143	0.200	0.140	0.635	0.079	0.478	0.821	0.375	0.111	0.425	0.385	0.000	0.925	0.797	0.000	0.000	0.067
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	1.000	1.000	1.000	1.000	1.000	1.000
Tpi-2a	1.000	1.000	1.000	1.000	1.000	0.971	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-2b	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 4 Genetic variability at all the 22 loci and mean fixation indices at all the polymorphic loci in the 21 populations of *O. rufipogon*

Population no.	A	P[a]	Ho	He[b]	F
1	1.4	27.3	0.055	0.077	0.286
2	1.4	31.8	0.016	0.065	0.754
3	1.2	22.7	0.021	0.058	0.362
4	1.4	31.8	0.054	0.108	0.500
5	1.3	22.7	0.031	0.078	0.603
6	1.5	31.8	0.045	0.097	0.536
7	1.6	31.8	0.033	0.074	0.554
8	1.3	27.3	0.028	0.061	0.541
9	1.3	18.2	0.060	0.071	0.155
10	1.5	27.3	0.013	0.078	0.833
11	1.4	22.7	0.027	0.061	0.557
12	1.3	22.7	0.028	0.065	0.569
13	1.3	22.7	0.013	0.065	0.800
14	1.3	18.2	0.045	0.073	0.384
The mean for Guangxi	1.37	25.6	0.034	0.074	
15 (Guangdong)	1.5	18.2	0.059	0.094	0.372
16 (Jiangxi)	1.2	13.6	0.042	0.049	0.143
17 (Hainan)	1.2	18.2	0.052	0.053	0.019
18 (Yunnan)	1.4	22.7	0.030	0.073	0.589
19	1.2	22.7	0.010	0.020	0.500
20	1.1	13.6	0.023	0.071	0.676
The mean for Hunan	1.2	18.2	0.017	0.046	
21 (Fujian)	1.1	9.1	0.000	0.027	1.000
Total mean	1.3	22.7	0.033	0.068	0.511

[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)

Table 5 Summary of F-statistics at all the polymorphic loci

Locus	F_{IS}	F_{IT}	F_{ST}
Aat-1	1.000	1.000	0.081***
Aat-3	1.000	1.000	0.155***
Adh	0.284	0.580	0.413***
Dia-1	0.699	0.832	0.443***
Idh	0.505	0.750	0.495***
Mdh-2	0.212	0.256	0.055***
Mdh-3	1.000	1.000	0.144***
Pgi-2	0.103	0.238	0.151***
Pgi-3	0.334	0.519	0.278***
Pgm	0.582	0.726	0.345***
Skd	0.696	0.783	0.288***
Tpi-2	1.000	1.000	0.027*
Mean	0.507	0.660	0.310***

* $P<0.1$; *** $P<0.001$

cy of heterozygotes; F_{ST} was 0.310, indicating that 31.0% of the total genetic variation existed among populations.

Genetic relationships

Nei's (1978) unbiased genetic identities estimated among the pairs of the 21 populations studied, as well as seven regions of *O. rufipogon* over China, are presented in Table 6. Genetic identity values ranged from 0.914 between

Table 6 Matrix of Nei's (1978) genetic identity values among the populations of *O. rufipogon*

Population no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	*****	0.987	0.975	0.982	0.969	0.987	0.993	0.991	0.990	0.992	0.952	0.970	0.962	0.982	0.964	0.969	0.963	0.976	0.987	0.969	0.992
2		*****	0.959	0.974	0.960	0.987	0.985	0.983	0.984	0.996	0.968	0.964	0.954	0.982	0.971	0.956	0.981	0.990	0.967	0.960	0.976
3			*****	0.974	0.965	0.960	0.971	0.966	0.961	0.978	0.942	0.941	0.952	0.966	0.941	0.958	0.940	0.952	0.976	0.948	0.982
4				*****	0.958	0.985	0.985	0.962	0.986	0.986	0.960	0.950	0.950	0.980	0.972	0.979	0.950	0.963	0.973	0.984	0.979
5					*****	0.967	0.966	0.949	0.983	0.958	0.935	0.932	0.994	0.961	0.938	0.957	0.930	0.949	0.969	0.945	0.970
6						*****	0.995	0.970	0.993	0.989	0.976	0.963	0.957	0.993	0.990	0.984	0.972	0.983	0.978	0.984	0.981
7							*****	0.975	0.990	0.992	0.964	0.969	0.950	0.993	0.981	0.985	0.969	0.984	0.992	0.983	0.993
8								*****	0.966	0.984	0.953	0.951	0.941	0.970	0.952	0.945	0.965	0.974	0.963	0.944	0.971
9									*****	0.983	0.957	0.960	0.978	0.984	0.969	0.979	0.958	0.971	0.983	0.979	0.986
10										*****	0.971	0.972	0.949	0.988	0.976	0.968	0.978	0.988	0.978	0.971	0.985
11											*****	0.94	0.915	0.986	0.990	0.972	0.979	0.980	0.939	0.968	0.943
12												*****	0.929	0.960	0.950	0.944	0.947	0.958	0.962	0.944	0.962
13													*****	0.940	0.917	0.933	0.914	0.929	0.952	0.928	0.956
14														*****	0.991	0.992	0.980	0.990	0.983	0.985	0.983
15															*****	0.982	0.972	0.982	0.955	0.983	0.956
16																*****	0.955	0.967	0.978	0.988	0.976
17																	*****	0.986	0.949	0.950	0.955
18																		*****	0.966	0.963	0.970
19																			*****	0.970	0.999
20																				*****	0.969
21																					*****

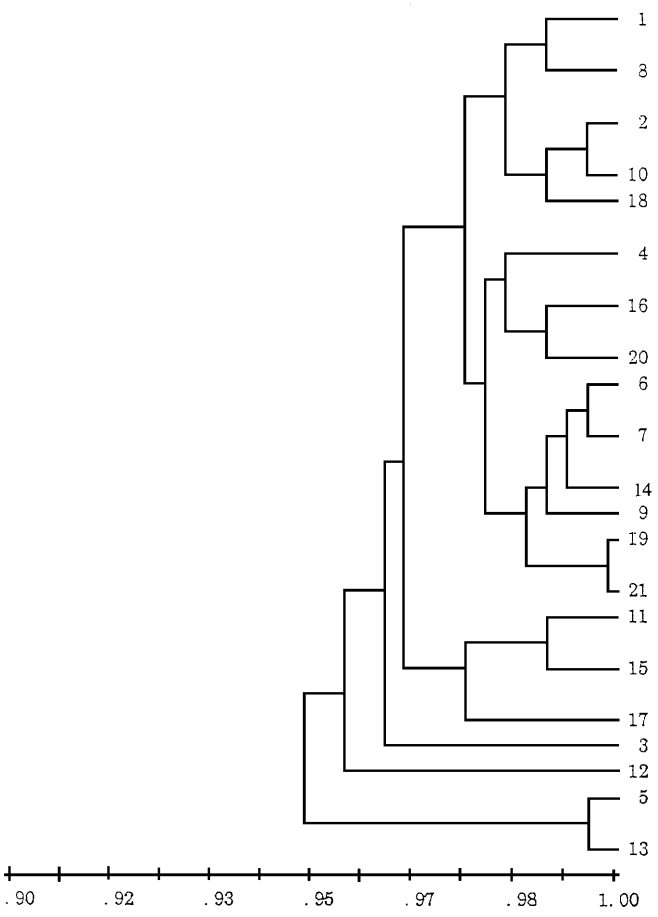

Fig. 2 Cluster analysis of 21 populations of *O. rufipogon* using the unweighted pair group method and Nei's (1978) unbiased genetic identity values

populations 13 and 17 to 0.999 between populations 19 and 21, with a mean of all pairwise comparisons of 0.964. Cluster analysis (UPGMA) was used to produce a phenogram and to show the genetic relationships of the populations studied (Fig. 2). The populations within a region did not cluster together before forming a cluster with any population of other regions, and thus genetic identify did not increase with geographic proximity; however, several populations that are geographically closer had higher genetic identities than those that are geographically more remote.

Discussion

Amount of genetic diversity

As compared with other seed plants with a similar life history and breeding system (Hamrick and Godt 1990), *O. rufipogon* may have a moderate allozyme variability with mean values of P=0.227 and He=0.068, which are slightly lower than those of short-lived perennial, herbaceous plants (*P*=0.280 and H=0.096). As far as the mating system is concerned, the levels of genetic diversity are almost within the reported range of self-crossing (P=0.200 and H=0.074) and wind-pollinated species (P=0.494 and H=0.148), which seems to be in accordance with mixed-mating of the species. Genetic variability in *O. rufipogon* is also moderate comparing with the other species of the genus *Oryza* studied. This species possessed a higher genetic diversity than the other two Chinese wild rices; for *Oryza officinalis* Wall. et Watt., A is 1.16, P is 0.162 and H is 0.036 based on eight populations (Gao et al. 2000a), and for *Oryza granulata* Nees et Arn. ex Watt., A is 1.08, P is 0.056 and H is 0.014 based on 15 populations (Gao et al. 2000b), our results, however, seem lower than those with mean values of P=0.538 and H=0.074 for the ten populations of *Oryza glumaepatula* distributed in the Amazon basin (Akimoto et al. 1998). On the other hand, the previous studies on *O. rufipogon* (Morishima 1985; Second 1985; Barbier 1989a) showed slightly higher levels of genetic diversity than those of the present study, which may stem from the larger number of polymorphic loci employed in their studies. For example, by using 24 loci (including 23 polymorphic loci), Second (1985) studied the genetic variation of 27 populations or strains from South China, with 13 from Guangxi, 10 from Guangdong (Boluo County) and 4 from Taiwan, and found much higher levels of isozyme variability (A=2.5 and H=0.19). Since the populations under the present study were randomly sampled from the wild with appropriate sampling, lower levels of genetic diversity in our case suggest that using more polymorphic loci leads to increased values of genetic variability. Barbier (1989a) studied four natural perennial populations from Thailand, and the levels of allozyme variability revealed (A=2.38 and H=0.310) are also higher than those of the present study. Among 15 loci scored, only six polymorphic loci (*Skd-1*, *Pgd-1*, *Pgi-1*, *Pgi-2*, *Est-2* and *Pox-1*) were used while the other monomorphic loci (*Adh-1*, *Cat-1*, *Gdh-1*, *Gdh-2*, *Got-1*, *Idh-1*, *Mdh-1*, *Mdh-2* and *Pgm-1*) were excluded in Barbier's study, which might lead to high values of genetic diversity. It is of interest to note that some of the monomorphic loci observed by Barbier, such as *Adh-1*, *Got-1*, *Idh-1*, *Mdh-1*, *Mdh-2* and *Pgm-1*, are somewhat polymorphic in our study (see Table 2), which probably suggests a reverse conclusion. Therefore, an overall picture of allozyme diversity in *O. rufipogon* needs extensive population sampling throughout South Asia using the same system of allozyme analysis. The levels of genetic diversity for Guangxi and Guangdong in South China are generally higher than those for the other regions, suggesting that South China may be the center of genetic diversity of *O. rufipogon* in China. Ting (1949) proposed that Asian cultivated rice originated in South China based on the fact that *O. rufipogon* is widely distributed in Guandong and Guangxi. Although this hypothesis was not accepted by later rice evolutionists (Wang 1993), our study shows that the relatively higher genetic diversity in this species seems to be in accordance with its wide distribution in these regions. In our preliminary study, a rather low genetic diversity (mean A=1.1, P=0.076, Ho=0.007 and

Table 7 Matrix of Nei's (1978) unbiased genetic identity averaged by region

Region	No. of pops.	1	2	3
1 Guangxi	14	0.969 (0.915–0.996)		
2 Guangdong	1	0.965 (0.917–0.991)	***** (*****–*****)	
3 Jiangxi	1	0.966 (0.933–0.992)	0.982 (0.982–0.982)	***** (*****–*****)
4 Hainan	1	0.959 (0.914–0.981)	0.972 (0.972–0.972)	0.955 (0.955–0.955)
5 Yunnan	1	0.971 (0.929–0.990)	0.982 (0.982–0.982)	0.967 (0.967–0.967)
6 Hunan	2	0.968 (0.928–0.992)	0.969 (0.955–0.983)	0.983 (0.978–0.988)
7 Fujian	1	0.976 (0.943–0.993)	0.956 (0.956–0.956)	0.976 (0.976–0.976)
Region	4	5	6	7
4 Hainan	***** (*****–*****)			
5 Yunnan	0.986 (0.986–0.986)	***** (*****–*****)		
6 Hunan	0.950 (0.949–0.950)	0.964 (0.963–0.966)	0.970 (0.970–0.970)	
7 Fujian	0.955 (0.955–0.955)	0.970 (0.970–0.970)	0.984 (0.969–0.999)	***** (*****–*****)

*****=no comparisons

He=0.011) was found in three surviving populations from Yunnan Province, known as one of the centers of genetic diversity of cultivated rice and a region historically important for rice cultivation and evolution, as compared to the levels of genetic diversity (mean A=1.2, P=0.241, Ho=0.045 and He=0.079) for five populations from other regions of China (Gao et al. 2000c). The results based on extensive population sampling from other regions of China in the present study further exclude Yunnan as a current center of genetic diversity of *O. rufipogon*, which implies that the current center of genetic diversity of cultivated rice is certainly not related to its wild progenitor. The populations from the Jiangxi, Hunan and Fujian provinces showed relatively low levels of genetic diversity, which may be related to their marginal nature.

Population genetic structure

The population genetic differentiation in the present study (F_{ST}=0.310) is not only much higher than the average for wind-pollinated plants (G_{ST}=0.099) but also slightly higher than those for perennial-herbaceous (G_{ST}=0.233) and gravity dispersed (G_{ST}=0.277) plants (Hamrick and Godt 1990). However, the value is much lower than those of the other two Chinese wild species, for *O. granulata* F_{ST}=0.870 (Gao et al. 2000b) and for *O. officinalis* F_{ST}=0.788 (Gao et al. 2000a), suggesting that *O. rufipogon* possesses only a moderate level of genetic differentiation within the genus *Oryza*. The main direction of differentiation in *O. rufipogon* is represented by that between a polycarpic out-crossing perennial and a monocarpic self-crossing annual type (Morishima et al. 1984). Our field observations showed that all the populations that we studied are perennial. A value of F_{ST}=0.310 in the present study is lower than that for 13 annual populations using six isozyme loci (G_{ST}=0.600) (Morishima 1985), indicating that perennial populations have a lower genetic differentiation than annual populations. These results strongly support the conclusion that inbreeding annual populations have a larger interpopulation genetic differentiation than outbreeding perennial populations (Morishima 1985; Barbier 1989a, b; Morishima and Barbier 1990). However, the value (F_{ST}=0.310) in the present study is a bit lower than that (G_{ST}=0.396) obtained using six isozyme loci for ten perennial populations (Morishima 1985; Morishima and Barbier 1990), but much higher than that (F_{ST}=0.033) for four perennial populations from a limited region of Thailand (Barbier 1989b; Morishima and Barbier 1990). The reason for these differences may stem from the geographical range of the populations sampled. The mean identity of 0.964 among populations of *O. rufipogon* in our study is higher than the 0.909 calculated among perennial populations from a limited region of Thailand (Barbier 1989a), and also much higher than that of 0.82 (0.57–1.00) from China and 0.780 (0.54–1.00) from Southern Asia (Second 1985). The ten monomorphic loci used in the present study may contribute to the high genetic identity ob-

served. We failed to find a significant correlation between geographical distance and genetic identity for the populations studied. First, although the populations of the species would be related to natural ecosystems rather than to agro-ecosystems, human impact has actually put them into an extensive agro-ecosystem. Therefore, they may be randomly dispersed due to human activities, as well as to numerous historical migration events. And second, the wild populations are introgressed by cultivated rice at a variable rate, and "genetic assimilation" leads to differences in their genetic differentiation.

An estimate of outcrossing rate (t=0.324) calculated from the mean fixation index agrees with previous results (Oka and Morishima 1967; Morishima and Barbier 1990), and implies that *O. rufipogon* has a typical mixed-mating system. The deficit of heterozygotes in *O. rufipogon* found in the present study (F=0.511>0, agrees with the observation on seed samples from Thailand (Morishima and Barbier 1990). Two of the causes for the deficiency of heterozygotes listed by Brown (1979) can be applied to common wild rice: first, in spite of allogamy, some inbreeding may occur in outcrossing asexual populations because of intra-clone outcrossing events (Morishima and Barbier 1990; Gao 1997; Gao et al. 2000d); and second, the "isolation by distance" hypothesis fits the species well, because most of the populations were isolated to a certain extent as a result of human disturbance in China (Gao et al. 1996, 1998).

Several important micro-evolutionary factors that determine the population genetic structure, such as mating systems, selection and reproductive systems, have been applied to an understanding of the genetic differentiation of common wild rice (Morishima 1985; Oka 1988; Barbier 1989a, b; Morishima and Barbier 1990). But it is rather difficult to rank them according to their significance. Due to the increase of population size and the rapid growth of the economy, the fragmentation of the huge population system of *O. rufipogon*, in which populations are isolated from each other, will reduce population size. As an outbredding perennial, the species may suffer from inbreeding as a result of the reduction in population size, with drift finally leading to a loss of genetic diversity and then to extinction. Therefore, in order to predict the long-term genetic survival of fragmented populations, priority should be given to the study of gene flow among the remaining populations, as well as to the study of the genetic effects of fragmentation.

Implications for conservation

Knowledge of the levels and distribution of genetic diversity is a pre-requisite for the establishment of effective and efficient conservation management. As the most endangered of the three Chinese wild rice species and the most useful gene pool for future rice breeding, *O. rufipogon* has attracted more and more attention in China. The population genetic structure revealed in the present study is instructive for making practical and effective conservation actions. First, an estimation of F_{ST}=0.310 suggests that 69.0% of the total genetic variation exists within populations and, therefore, for such a predominantly outbreeding perennial, a plan sampling less populations but more individuals within populations should be adopted. Because populations such as 1, 4, 5, 6, 7 and 15 from South China possessed a very large amount of genetic variation, they should be more attractive for both in situ conservation and germplasm collection. Second, in order to capture the considerable allelic variation haboured among populations, an appropriate strategy both for germplasm sampling and developing in situ conservation for those populations with a higher variation on behalf of the different geographic regions is needed. Populations 13 and 17, which showed the lowest value of I=0.914 and the most-significant differences in allelic frequencies, should be involved in conservation programs. Finally, because marginal populations 16, 19, 20, and 21 have been seriously threatened, and are on the verge of extinction due to human disturbance and strongly unfavorable ecological pressure (Gao et al. 1996), they should be given high priority for conservation practices because of their great significance in exploring valuable genes for rice genetic improvement and maintaining the genetic integrity of the gene pool of the species, although they have lower levels of genetic variation. Because the genetic diversity of in situ conserved populations should be dynamically maintained in changeable environments, the long term habitat protection is more important for preventing the species from further loss of genetic variation and a decrease in population size. However, as we postulated above, no genetic information on gene flow among the remaining populations and the genetic effect of their fragmented habitats is currently available. Beyond all doubt, however, such information is crucial for a deep understanding of genetic architecture and for making proper management decisions for the conservation of *O. rufipogon*.

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References

Akimoto M, Shimamoto Y, Morishima H (1998) Population genetic structure of wild rice *Oryza glumaepatula* distributed in the Amazon flood area influenced by its life-history traits. Mol Ecol:1371–1382

Barbier P (1989a) 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

Barbier P (1989b) 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

Brown ADH (1979) Enzyme polymorphism in plant populations. Theor Pop Biol 15:1–42

Cai HW, Wang XK, Morishima H (1997) Genetic diversity of Chinese wild rice populations. In: Wang XK, Sun CQ (eds) Origin and differentiation of Chinese cultivated rice. Agricultural University Press, Beijing, China, pp 154–156

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

Chang TT (1984) Conservation of rice genetic resources: luxury or necessity? Science 224:251–256

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

Gao LZ, Zhang SZ, Zhou Y, Ge S, Hong DY (1996) A survey of current status of wild rice in China (in Chinese with English abstract) Chinese Biodiversity 4:162–166

Gao LZ, Zhou Y, Ge S, Hong DY, Liang YM, Lin DH, Chen CB, Wu MS, Huang DA (1998) Current status of the genetic resources of *Oryza rufipogon* Griff. and its conservation strategies in Guangxi Province (in Chinese with English abstract). Acta Agric Sinica 31:32–39

Gao LZ, Ge S, Hong DY (2000a) High levels of genetic differentiation of *Oryza officinalis* Wall. et Watt. from China. The J Hered (in press)

Gao LZ, Ge S, Hong DY (2000b) 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. Int J Plant Sci (in press)

Gao LZ, Ge S, Hong DY, Lin RS, Tao GD, Xu ZF (2000c) Low allozyme variation and conservation genetics of common wild rice *Oryza rufipogon* Griff. from Yunnan, China. Euphytica (in press)

Gao LZ, Ge S, Hong DY (2000d) A preliminary study on ecological differentiation of common wild rice *Oryza rufipogon* Griff (in Chinese with English abstract). Acta Agron Sinica 26:210–216

Ge S, Zhang DM, Wang HQ, Rao GY (1997) Allozyme variation in *Ophiopogon xylorrhizus*, an extreme endemic species of Yunnan, China. Conserv Biol 11:562–565

Glaszmann JC, de los Reyes BG, Khush GS (1988) Electrophoretic variation of isozymes in plumules of rice (*Oryza sativa* L.) a key to the identification of 76 alleles at 24 loci. IRRI Research Paper Series No. 134:1–14

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

Gottlieb LD, Edwards SW (1992) An electrophoretic test of the genetic independence of a newly discovered population of *Clarkia franciscana*. Madrona 39:1–2

Hamrick JL (1989) Isozymes and the analysis of genetic structure in plant populations. In: Soltis DE, Soltis PS (eds) Isozymes in plant biology. Dioscorides Press, Portland, Oregon, pp 87–105

Hamrick JL, Godt MJW (1990) Allozyme diversity in plant species. In: Brown AHD et al. (eds) Plant population genetics, breeding and genetic resources. Sinauer, Sunderland, Massachusetts, pp 43–63

Hong DY (1995) Rescuing the genetic resources of wild rices in China (in Chinese). Bull Chinese Acad Sci 10:325–326

Kiang YT, Antonovics J, Wu L (1979) The extinction of wild rice (*Oryza perennis formosana*) in Taiwan. J Asian Ecol 1:1–9

Morishima H (1985) Habitat, genetic structure and dynamics of perennial and annual populations of the Asian wild rice *Oryza perennis.* In: Jacquard et al. (eds) Genetic differentiation and dispersal in plants. NATO ASI Series, vol G5. Springer, Berlin Heidelberg, pp 179–190

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

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

National Exploring Group of Wild Rices (1984) Investigation of resources of wild rice in China (in Chinese). Acta Agric Sinica 6:1–8

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

Oka HI (1988) Origin of cultivated rice. Japan Scientific Societies Press, Tokyo

Oka HI, Morishima H (1967) Variation in the breeding systems of a wild rice, *Oryza perennis.* Evolution 21:249–258

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 Sativa group of *Oryza* based on isozyme data. Genet Selec Evol 17:89–114

Soltis DE, Haufler CH, Darrow DC, Gastony GJ (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, Soltis DE (1991) Genetic variation in endemic and widespread plant species, examples from the Saxifragaceae and *Polystiochum* (Dryopteridaceae). Aliso 13:215–223

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

Sworfford DL, Selander RB (1989) Biosys-1, release 1.7. Illinois Natural History Survey, Champaign, Illinois

Ting Y (1949) Origin of cultivated rice in China (in Chinese). Special J Agric and Hort (Agricultural College, Zhongshan University) 7:11–24

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

Wang XK (1993) Origin, evolution and classification of the cultivated rice in China. In: Ying CS (eds) Rice germplasm resources in China (in Chinese). Chinese Agricultural Technology and Science Press, Beijing, pp 1–16

Wang ZS, Zhu LH, Liu ZY, Wang XK (1996) Gene diversity of natural populations detected by RFLP markers (in Chinese). J Agric Biotechnol 4:111–117

Weir BS (1990) Genetic data analysis: methods for discrete population genetic data. Sinauer, Sunderland, Massachusetts