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Intra-Population Genetic Structure of *Oryza rufipogon* Griff. in Yunnan, China

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In order to reveal the genetic subdivision within population of common wild rice *Oryza rufipogon* Griff., allozyme analysis was conducted using 22 loci on a typical population from Yunnan Province, China. Non-random distribution of genotypes and/or genetic variability was found among three subpopulations, and the result was further demonstrated by considerable genetic differentiation observed (F_{ST}=0.206) within the population. Microhabitat selection may not be an important factor in shaping intra-population genetic structure, and restricted gene flow (N_m=0.964<1) and genetic drift act together towards a genetic subdivision within the population. This genetic subdivision may enhance inbreeding and will ultimately lead to genetic depletion within the predominantly outcrossing (t=0.830) perennial population, and therefore, more attention should be paid to the conservation and genetic management of the population.

Key words: Allozyme analysis — Gene flow — Intra-population genetic structure — *Oryza rufipogon* — Yunnan

Population genetic structure has been considered as a major determinant of the rate of evolution (Wright 1932, 1977, 1978). Although intra-population genetic structure has been received far less attention than inter-population, there is some evidence to suggest that genes and genotypes are not distributed at random within populations (Christiansen and Feldman 1975, Turner *et al.* 1982, Loveless and Hamrick 1984, Epperson 1989, Chesser 1991, Huang 1994, Husband and Barrett 1995, Johannesen and Loeschcke 1996, Invarsson and Olsson 1997, Giles *et al.* 1998, Owuor *et al.* 1999). It is generally agreed that the distribution of genetic variation within a population is not likely to be random owning to the effect of factors such as founding events, mating systems, limited seed and pollen dispersal, and/or microhabitat selection (Wright 1943, Levin and Kerster 1968, Turner *et al.* 1982, Loveless and Hamrick 1984). These structure within populations clearly reflect major evolution and ecological processes affecting natural populations of plants (Epperson 1989), and thus understanding of intra-population genetic structure will be of significance in exploring the evolution and conservation of threatened plant species.

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Common wild rice *Oryza rufipogon* Griff., known as important gene sources for rice breeding programs, is widely distributed in tropics and subtropics in monsoon Asia (Morishima and Barbier 1990) and Southern China (Gao *et al.* 1996), and commonly considered to be the progenitor of cultivated rice *O. sativa* L. (Chang 1976, Oka 1988, Cheng and Cai 1993, Wang 1993). The species exhibits a large variation in life-history traits showing a differentiation into two ecotypes, polycarpic perennial and monocarpic annual types (Oka and Morishima 1967, Morishima *et al.* 1984, Oka 1988), and shows close sympatric distribution over a large geographical areas with cultivated rice. For a seriously threatened species like *O. rufipogon* (Vaughan and Chang 1992, Gao *et al.* 1996), increasing fragmentation of the landscape caused by human beings has reduced its population size, and thus the knowledge of its intra- and inter-population genetic structure is urgently needed for taking conservation and resources management. However, studies on genetic substructuring of the populations, as well as the assessment of gene flow have not been initiated. In the previous research works on inter-population genetic structure (Barbier 1989a, 1989b), the populations detected were almost located near the rice fields which have been frequently introgressed by the rice pollen. It is a drawback to estimate the real action of gene flow of *O. rufipogon* itself on population genetic structure, because gene flow of rice may affect outcrossing rates of the populations (Morishima and Barbier 1990) and further influence their population genetic structure. A few populations isolated well from cultivated rice in China (Wang 1993) may provide us an unique opportunity to study intra-population genetic structure and gene flow and enhance our understanding of evolutionary dynamics and conservation of the species.

Allozyme markers are ideal tool used to solve problems concerning the population genetic structure (Loveless and Hamrick 1984, Hamrick and Godt 1990), and have routinely been used to study population genetic structure of wild rice species (Barbier 1989a, 1989b, Gao 1997, Gao *et al.* 1999, 2000a, 2000b, 2000c). In the present study, we used allozyme electrophoresis to examine spatial variation of a typical population of *Oryza rufipogon* located in Yuanjiang County, Yunnan Province. This paper aimed to answer the following two questions: 1) How is genetic variation within the population spatially structured? and 2) which evolu-

tionary factors are probably significant in shaping its intra-population genetic structure?

Materials and Methods

Material sampling

The Yuanjiang population of *Oryza rufipogon*, 760 m alt., is located about 16 km away from Yuanjiang County (23°30′). It is surrounded by the sugarcane or shrubs, and remote from rice fields. There were at least four subpopulations reported before (Kansheng Cheng, pers. comm.), but only three of them were surviving in October, 1994 (see Fig. 1). SP1, SP2 and SP3 were about 60 m^2, 40 m^2 and 300 m^2, respectively. The first living samples were taken in October, 1994. For each subpopulation, 20 individuals were collected. Because *O. rufipogon* has high colonizing ability, care was taken to prevent collecting multiple samples from a single genet; mixed live ratoons were randomly collected at intervals of at least 1 meters in all the three subpopulations, numbered, transplanted to pots and maintained in Xishuangbanna Tropical Botanical Garden of Chinese Academy of Sciences (CAS) (Mengla County, Yunnan Province). Young leaves were individually collected in March, 1995, stored in plastic bags on ice and transported to the laboratory by airplane; the second living samples were randomly collected according to subpopulation sampling in July, 1996 (sampling methods followed the above). SP3, the greatest subpopulation in the past, was severely destroyed due to neibouring farmer's activities in March, 1995. As a result, only 10 individuals were found and collected. All samples were numbered in the field, transported by train and transplanted to the pots in the greenhouse of Institute of Botany, CAS (Beijing City). For each individual, 0.05 g of fresh leaf material was crushed in 100 ul 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 −70C until electrophoresis was conducted.

Starch-gel electrophoresis

Fourteen enzymes were resolved and scored using starch-gel electrophoresis (Table 1). 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, FBA, MDH and ME (Electrode buffer was diluted 2 times before use); TPI, AAT, DIA, LAP and PGI were resolved on buffer system 6(S6); PGM, SKD, G3PDH, ADH and IDH were resolved on buffer system I of Glaszmann *et al.* (1988) (GI). 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. Allelic variation at a locus was coded alphabetically with the most anodally migrating allozyme.

Data analysis

Electrophoretic data were analyzed using the computer program Biosys-1 (Swofford and Selander 1989) version 1.7 for the IBM-PC. For the samples collected in 1996, data were entered as genotype numbers from which allele frequencies were calculated; genetic similarities for each locus among the three subpopulations were estimated using Nei's (1978) identity. The levels of genetic variability within subpopulations were estimated using four variables: the mean number of alleles per locus (*A*), the percentage of polymorphic loci (*P*), the observed heterozygosity (*Ho*) and expected heterozygosity (*He*). The deviation from Hardy-Weinberg equilibrium and *F*-statistics (fixation indices) were calculated. Outcrossing rate (*t*) was estimated using the mean fixation index (*F*), and outcrossing rate and fixation index are related by $t=(1-F)/(1+F)$ (Weir 1990); a rough estimate of the quantity *Nm* (*N*=population size, m=migration rate) was also estimated using Wright's (1931) formula

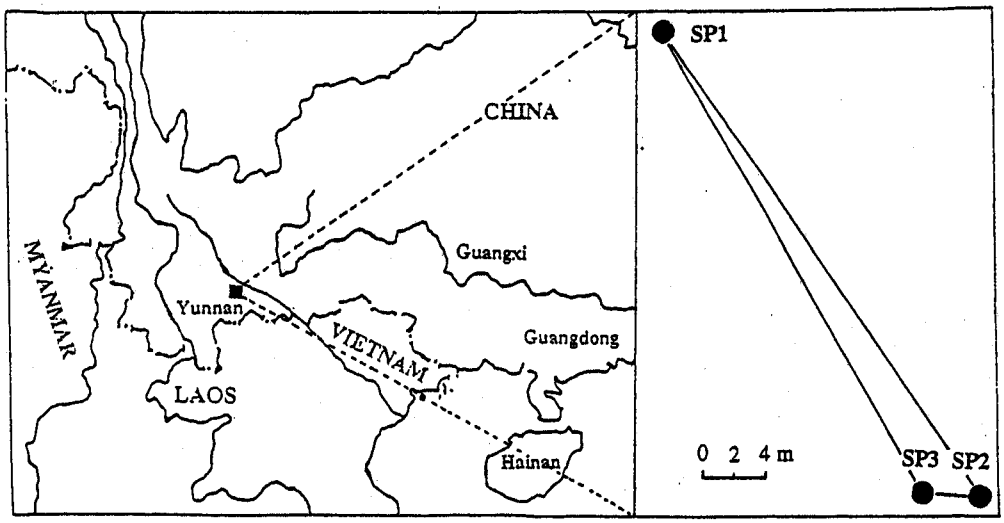

Fig. 1. Distribution map of the typical population of *Oryza rufipogon* located at Yuanjiang County, Yunnan Province. The circles indicate the locations of three subpopulations in the population, and the symbols Sp1, SP2, and SP3 show them as described in the text.

Table 1. 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	S_6	2
Alcohol dehydrogenase	ADH	EC 1. 1. 1. 1	G_r	1
Diaphorase	DIA	EC 1. 6. 2. 2	S_6	2
Fructose-bisphosphate aldolase	FBA	EC 4. 1. 2. 13	$S_1^\#$	1
Glutamate dehydrogenase	G3PDH	EC 1. 4. 1. 2	G_r	1
Isocitrate dehydrogenase	IDH	EC 1. 1.1. 42	G_r	1
Leucine-amino peptidase	LAP	EC 3. 4. 11. 1	G_r	1
Malate dehydrogenase	MDH	EC 1. 1. 1. 37	$S_1^\#$	3
Malic enzyme	ME	EC 1. 1. 1. 40	$S_1^\#$	1
Phosphogluconate dehydrogenase	6PGD	EC 1. 1. 1. 44	$S_1^\#$	2
Phosphoglucoisomerase	PGI	EC 5. 3. 1. 9	S_6	3
Phosphoglucomutase	PGM	EC 2. 7. 5. 1	G_r	1
Shikimate dehydrogenase	SKD	EC 1. 1. 1. 25	G_r	1
Triosephosphate isomerase	TPI	EC 5. 3. 1. 1	S6	2

$F_{ST}=1/(1+4Nm)$. For those collected in 1994, allele frequencies and the values of genetic variability within population (*A, P, Ho* and *He*) were calculated.

Results

Enzyme electrophoresis clearly resulted in 14 enzymes, encoded by 22 putative loci (Table 1). All enzymes migrated anodally. Although two isozymes of PGM are typically present in diploid seed plants (Gottlieb 1982), only one PGM isozyme was observed in this study. Two loci of G3PDH were typically reported (Second 1982), but only one locus was observed in this study; the banding patterns of *Pgi-2* and *Pgi-3* seemed to be apparent gene duplications. *Aat-1, Aat-3, Dia-2, Fba, G3pdh, Lap-1, Mdh-1, Mdh-2, Mdh-3, Me, Pgd-1, Pgd-2, Pgi-1, Pgi-2, Pgi-3, Pgm, Tpi-1* and *Tpi-2* were monomorphic, and all the other loci were polymorphic in at least one subpopulation; *Adh* and *Idh* had two alleles, and *Dia-1* and *Skd* each had three alleles.

A total of 28 alleles were identified for the 22 loci (1-3 per locus, with a mean of 1.27). There were 22 common alleles shared among three subpopulations (Table 2). However, *Adh-a* (0.058), *Dia-1a* (0.058) and *Dia-1c* (0.115) were observed only in SP1, and *Skd-a* (0.028) was observed only in SP2, and *Idh-a* (0.250) was observed only in SP3; in addition, *Skd-b* was found only in SP1 and SP2 at the frequencies of 0.288 and 0.556, respectively.

The levels of genetic diversity were variable among the three subpopulations (Table 3), indicating that allozymic variability was not randomly distributed within the population; a higher genetic diversity was found in the sample of 1996 ($A=1.2$, $P=13.6\%$, $Ho=0.024$, and $He=0.034$) than in that of 1994 ($A=1.1$, $P=9.1\%$, $Ho=0.010$, and $He=0,026$), showing the temporal fluctuation of genetic diversity.

The mean genetic identity ($I=0.992$) reveals that a slight genetic differentiation occurred among the subpopulations (Table 4). The two subpopulations (SP2 and SP3), which are closer to each other than other two pairs, possessed a bit

Table 2. Allele frequencies at all loci in three subpopulations of the Yuanjiang population of *Oryza rufipogon* in 1996

Locus	Subpopulations		
	SP1	SP2	SP3
Aat-1a	1.000	1.000	1.000
Aat-3a	1.000	1.000	1.000
Adh-a	.058	.000	.000
-b	.942	1.000	1.000
Dia-1a	.058	.000	.000
-1b	.827	1.000	1.000
-1c	.115	.000	.000
Dia-2a	1.000	1.000	1.000
Fba-a	1.000	1.000	1.000
G3pdh-a	1.000	1.000	1.000
Idh-a	.000	.000	.250
-b	1.000	1.000	.750
Lap-1a	1.000	1.000	1.000
Mdh-1a	1.000	1.000	1.000
Mdh-2a	1.000	1.000	1.000
Mdh-3a	1.000	1.000	1.000
Me-a	1.000	1.000	1.000
6Pgd-1a	1.000	1.000	1.000
6Pgd-2a	1.000	1.000	1.000
Pgi-1a	1.000	1.000	1.000
Pgi-2a	1.000	1.000	1.000
Pgi-3a	1.000	1.000	1.000
Pgm-a	1.000	1.000	1.000
Skd-a	.000	.028	.000
-b	.288	.556	.000
-c	.712	.417	1.000
Tpi-1a	1.000	1.000	1.000
Tpi-2a	1.000	1.000	1.000

Table 3. Genetic variability at 22 loci in three subpopulations of the Yuanjiang population

Subpopulation	*N*	*A*	*P**	*Ho*	*He***
SP1	26	1.2 (.1)	13.6	.023 (.016)	.038 (.023)
SP2	18	1.1 (.1)	4.5	.025 (.025)	.024 (.024)
SP3	10	1.0 (.0)	4.5	.023 (.023)	.023 (.023)
The population level (1996)		1.2 (.1)	13.6	.024 (.020)	.034 (.024)
The population level (1994)		1.1 (.1)	9.1 (.1)	.010 (.010)	.026 (.022)
The species level		1.2 (.0)	13.6 (.1)	.017 (.015)	.031 (.023)

* A locus is considered polymorphic if the frequency of the most common allele does not exceed. 99

** Unbiased estimate (see Nei 1978)

lower genetic identity (I=0.985) than others.

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 deviations from Hardy-Weinberg expectation across the population system as a whole. Statistical significance of F_{ST} values was tested for each locus by the chi-square test, $X^2=2NF_{ST}(K-1)$, 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 shows F-statistics in the Yuanjiang population. F_{IS} was 0.091, suggesting that most subpopulations slightly deviated from Hardy-Weinberg expectation within the population and a slight deficiency of heterozygotes; F_{ST} was 0.206 (P=0.00002), suggesting that 20.6% of the total genetic variation existed among subpopulations. Table 6 indicates that SP1 showed a deficiency of heterozygotes, SP2 almost subjected to Hardy-Weinberg expectation, while SP3 had an excess of heterozygotes. The results indicate that three subpopulations were different in Hardy-Weinberg expectations.

Table 4. Nei (1978) unbiased genetic identities at 22 loci

Locus	1-2	1-3	2-3
Aat-1	1.000	1.000	1.000
Aat-3	1.000	1.000	1.000
Adh	0.999	0.999	1.000
Dia-1	0.992	0.992	1.000
Dia-2	1.000	1.000	1.000
Fba	1.000	1.000	1.000
G3pdh	1.000	1.000	1.000
Idh	1.000	1.000	1.000
Lap	1.000	1.000	1.000
Mdh-1	1.000	1.000	1.000
Mdh-2	1.000	1.000	1.000
Mdh-3	1.000	1.000	1.000
Me	1.000	1.000	1.000
6Pgd-1	1.000	1.000	1.000
6Pgd-2	1.000	1.000	1.000
Pgi-1	1.000	1.000	1.000
Pgi-2	1.000	1.000	1.000
Pgi-3	1.000	1.000	1.000
Pgm	1.000	1.000	1.000
Skd	0.875	0.993	0.609
Tpi-1	1.000	1.000	1.000
Tpi-2	1.000	1.000	1.000
Mean	0.996	0.995	0.985

Discussion

In the typical population of *Oryza rufipogon*, the genotypes as well as allozyme variability, were not randomly distributed in the spatially isolated subpopulations. Theoretical studies on differentiation among local populations were pioneered by Wright (1931). The simplest model explored was an "island model" (Wright 1969), which assumes that a total population is divided into many isolated subgroups, within which mating is at random, except for a certain propotion of migrants draw at random from the whole. It is the case within common wild rice population studied due to non-random distribution of genotypes and/or genetic variability observed.

Non-random distribution of genetic diversity was demonstrated by genetic differentiation found among the subpopulations. The value of F_{ST}=0.206 in the present study is a bit lower than that of F_{ST}=0.302 estimated from all the three surviving populations from Yunnan, as well as that of F_{ST}=0.310 estimated from 21 populations with a large geographical scale over China (Gao 1997, Gao *et al.* 2000b, 2000c), suggesting that considerable genetic differentiation have taken place within the population.

The most possible explanation for genetic differentiation within the population is that gene flow may be too small to

Table 5. Summary of *F*-statistics at all polymorphic loci in the Yuanjiang population

Locus	F_{IS}	F_{IT}	F_{ST}	X^2	d.f.	*P*
Adh	−.061	−.020	.039	2.385	2	.30339
Dia-1	.872	.884	.094	7.674	4	.10429
Idh	−.333	−.091	.182	22.242	2	.00001
Skd	.028	.280	.260	11.226	4	.02414
Mean	.091	.278	.206			
Total				43.527	12	.00002

Table 6. Fixation indices at all polymorphic loci, mean fixation indices (*F*), in three subpopulations of the Yuanjiang common wild rice *Oryza rufipogon*

Locus	SP1	SP2	SP3	Mean
Adh	−.061	—	−0.333	−0.197
Dia-1	0.872	—	—	0.872
Skd	0.157	−0.075	—	0.041
Mean	0.323	−0.075	−0.333	0.093

restrict genetic divergence among the subpopulations. The two subpopulations (SP2 and SP3), which are spatially closest to each other among the three pairs, possessed the lowest genetic identity (I=0.985), suggesting that mating may not randomly occur among the subgroups. As a predominantly outcrossing species, mating seems random within a subpopulation because of a high density. Gene flow among the subpopulations may take place in three ways: the first involves pollen flow, the second occurs by migration of seeds, and the last is the dispersal of live ratoons. Because gene flow measured by direct methods is usually confined, an indirect approach (Slatkin 1985, Slakin and Barton 1989) to estimate the extent of gene flow (Nm= 0.964) within the population indicates a slightly low amount of gene flow. Wright (1931) established a rule of thumb when he showed that there can be significant genetic differentiation due to drift when $Nm<1$. Our result supports that limited gene flow may be one of the most important causes for high genetic differentiation within the Yuanjiang population. Hence, random genetic drift is probably a critical force in shaping spatial genetic structure of the population. Although microhabitat selection is usually regarded as an important factor in shaping intra-population genetic structure (Loveless and Hamrick 1984, Hamrick and Godt 1989), it may not play an important role in genetic differentiation within the population in the present study. According to our field observation, three subpopulations of the typical perennial population are only found in a microhabitat with relatively stable depths of water (about 20-30 cm), and thus lead to an environmental uniformity. In addition, F_{ST} is a relative value to estimate genetic differentiation of the populations. Few polymorphic loci will lead to an increased value of F_{ST}. A study on an tropical plant species *Kandelia candel* from Taiwan, China, provided a different pattern of intra-population genetic structure. There was no obvious differentiation (F_{ST}=0.043) occurred even between the subpopulations grown in the dry and wet microhabitats because of strong gene flow between them (Nm=5.66) (Huang 1994).

A very slight deficiency of heterozygotes was generally observed in the Yuanjiang population. In perennial population of *Oryza rufipogon*, some inbreeding may occur in outcrossing asexual populations because of intra-clone outcrossing events (Morishima and Barbier 1990), and thus enhance the deficiency of heterozygotes. The extent of inbreeding depression may determine the differences of three subpopulations conformed to Hardy-Weinberg expectations.

The outcrossing rate of the population was up to 83.0%, which is far higher than those previously reported (Oka and Barbier 1989b), indicating that the typical population of *Oryza rufipogon* may be predominantly outcrossing, while introgressed populations of the species are mixed crossing (Morishima and Barbier 1990). Obvious variation in outcrossing rates between families within introgressed population suggests that gene flow of rice probably influence outcrossing rates of wild populations and thus determine their population genetic structure (Morishima and Barbier 1990). The mating system has been identified as one of the major factors influencing levels of heterozygosity of individuals, genetic relationships among progeny with families, spatial genetic heterogeneity within populations and distribution of genetic diversity among populations within a species (Hamrick and Murawski 1990), and therefore, using more polymorphic loci and progeny arrays, a study on mating systems of the population comparing with those introgressed ones will help to gain an insight into intra-population genetic structure of *O. rufipogon*.

In conclusion, restricted gene flow and genetic drift may act together towards a genetic subdivision of the typical population of common wild rice, thereby increasing the possibility of non-random matings. This subdivision of intra-population genetic structure may enhance inbreeding (Sokal and Wartenberg 1983) and may lead to genetic depletion within the population, to which special conservation attention should be paid. In view of conservation, more attention to the conservation and genetic management of the population should be paid.

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