Comparison of population genetic structures of common wild rice (*Oryza rufipogon* Griff.), as revealed by analyses of quantitative traits, allozymes, and RFLPs

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We investigated genetic diversity among and within natural populations of Asian common wild rice, *Oryza rufipogon*, from three different classes of data: quantitative traits, allozymes, and restriction fragment length polymorphisms (RFLPs). The seven populations examined showed polymorphism to varying degrees. The amount of intrapopulation variability appeared to be influenced not only by breeding system but also by the evolutionary history of each population. Interpopulation differentiation was clear, but different classes of data elucidated different patterns. Quantitative traits revealed ecotype differentiation into

perennial and annual types of population, whereas allozyme and RFLP analyses revealed geographical differentiation among populations. These results suggest that the diversity patterns shown by quantitative trait analysis reflect mainly the occurrence of adaptive differentiation in response to habitat conditions and that those shown by allozyme and RFLP analyses reflect mainly the effect of isolation by distance. Population differentiation parameters (F_{ST}) were highly variable among loci in allozymes as well as in RFLPs. *Heredity* (2004) 92, 409–417, advance online publication, 3 March 2004; doi:10.1038/sj.hdy.6800435

Keywords: population genetic structure; wild rice; quantitative traits; allozyme; RFLP; population differentiation

Introduction

Population genetic structure or distribution of genetic variation among and within natural populations is a major concern in evolutionary genetics. In early studies, morphological traits were the only sources of evidence that could be used to elucidate population structure. However, during the last two decades molecular approaches have become a standard means of determining the genetic architecture of natural populations.

Phenotypic traits of either a continuous or discrete nature are substantially affected by natural selection, and their patterns of variation therefore largely reflect adaptive differentiation. On the other hand, it is usually assumed that a considerable portion of molecular variation is selectively neutral and that the molecular-clock hypothesis (Kimura, 1968) holds, although this assumption may not be always true, as argued by Koehn *et al* (1983) and Karl and Avise (1992). Differences between the patterns detected by allozymes and the use of various DNA marker techniques such as analyses of restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), and simple sequence repeats (SSR) might be of concern only at a level at which

genetic variation can be dissected: allozyme variation detects polymorphism at protein coding regions, whereas molecular variation reflects differences directly at the DNA level in both coding and noncoding regions.

Biosystematic and population studies conducted in various plant and animal species have shown that the results obtained by quantitative trait and molecular marker analyses are not always concordant (Podolsky and Holtsford, 1995; Bonnin *et al*, 1996; Latta and Mitton 1997; Reed and Frankham 2001). If the materials are exactly the same and the methods used are appropriate for their respective approaches, but the results differ, then any inconsistency suggests that the underlying evolutionary forces differ among different types of genetic variation. Therefore, there is a need to clarify which aspects of the entire genome can be revealed by using different types of genetic data.

The common wild rice, *Oryza rufipogon* Griff., is widely distributed in the tropical and subtropical regions of Asia and Oceania and is considered to be the progenitor of cultivated rice, *O. sativa* L. (see, for review, Oka, 1988). It contains a large amount of intraspecific genetic variability and tends to be differentiated into two ecotypes: partly outbreeding perennial and predominantly inbreeding annual. The two types are adapted to different habitat conditions and are characterized by contrasting morphologies, propagating methods and mating systems (Oka and Morishima, 1967; Sano and Morishima, 1982; Morishima *et al*, 1984). The population genetic structures of Indian and Thai populations of *O. rufipogon* have been studied by the analysis of quantitative traits (Morishima and Oka, 1970) or life-history traits and allozymes

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(Barbier, 1989; Morishima and Barbier, 1990). Chinese populations of *O. rufipogon* were investigated by the analysis of allozymes (Gao et al, 2000), RAPD (Ge et al, 1999), and SSR (Gao et al, 2002).

Our objective was to assess genetic diversity among and within natural populations of *O. rufipogon* collected from a broad area over the distribution range of this species in Asia. We compared the population structure as revealed by: (a) quantitative trait analysis, (b) allozyme analysis, and (c) nuclear DNA RFLP analysis. We discuss the evolutionary forces causing the different patterns of population differentiation resulting from the analysis of these three different classes of data.

Materials and methods

Plant materials

Seven population samples of *O. rufipogon* were taken from genetic stocks preserved at China Agriculture University, China, and the National Institute of Genetics, Japan. Samples were collected from China (3), Thailand (2), India (1), and Indonesia (1) (Figure 1). Basic information on each population is given in Table 1. From our field observations, population NE3 was a typical annual type propagating by seeds; NE88, W1981, and W120 seemed to be perennial, propagating mainly by vegetative means (clones); and YJ, DX, and GG seemed to be weakly perennial, propagating by both clones and seeds. In four populations (YJ, GG, W1981, and W120), the individuals examined were first-generation plants

derived from the original seeds collected at each site. In the other three populations (DX, NE3, and NE88), selfed progeny of first-generation plants derived from the original seeds collected on an individual plant basis were examined. Population GG (Guangxi Province, China) was adjacent to cultivated rice fields and was apparently susceptible to introgression by cultivar genes. Other populations were isolated from cultivated rice fields to varying degrees.

Quantitative trait analysis

Seeds of the seven populations were sown, and a total of 292 seedlings were transplanted into a paddy plot in a greenhouse at the National Institute of Genetics, Mishima, Japan, in 1994 July. The following 11 morphological and physiological traits were evaluated on an individual basis: plant height, panicle number, panicle length, spikelet number per panicle, awn length, seed fertility, shoot-regenerating ability, root-regenerating ability, dry weight of stem and leaf at maturity, dry weight of small tillers developed after heading, and heading date. Regenerating ability was assessed as follows: several basal stem segments with nodes were cut at the time of maturity and placed in moistened sand at 30°C. After 5 days, the degree of development of adventitious roots was given a value of 0, 1, 2, or 3 (root-regenerating ability), and the number of newly emerging shoots was counted (shoot-regenerating ability). The Indian population W120 was excluded from the trait analysis because the plants were grown in another greenhouse.

Allozyme assay

At the tillering stage, leaf samples were taken from each plant and analyzed for 29 isozyme loci. *Cat1*, *Sdh1*, *Amp1*, *Amp2*, *Amp3*, *Amp7(t)*, *Pgd1*, *Pgd2*, *Pgi1*, *Pgi2*, *Pgi3*, *Pox2*, *Est9*, and *EstS(t)* were assayed by a starch gel system (Ishikawa et al, 1989), and *Est1*, *Est2*, *Est3*, *Est5*, *Est10*, *Est12*, *Est14*, *Acp1*, *Acp2*, *Acp3*, *Acp5(t)*, *Acp6(t)*, *Mal1*, *Mal5(t)*, and *Mal6(t)* were assayed by the SDS-PAGE electrophoresis system (Cai et al, 1992). Newly identified loci (our unpublished data) are designated 'tentative' with a (t).

RFLP assay

Nuclear DNA was extracted with liquid nitrogen from fresh leaves sampled from each plant at the tillering stage and digested with four restriction enzymes, *HindIII*, *EcoRI*, *DraI*, and *BamHI*. DNA digestion, electrophoresis, and Southern blotting were carried out according to the method of McCouch et al (1988). DNA hybridization was performed with the ECL™ direct nucleic acid labeling and detection systems (Amersham,

Figure 1 Site map of the seven populations studied. Dotted line shows the distribution range of *O. rufipogon* in Asia.

Table 1 Basic information on the seven populations examined

Population code	No. of plants examined	Collection site	Distance from rice fields	Propagation system
DX	54	Dongxiang, Jiangxi, China	Far	Weakly perennial (ratoon and seeds)
YJ	14	Yuanjiang, Yunnan, China	Far	Weakly perennial (ratoon and seeds)
GG	68	Guigang, Guangxi, China	Adjacent	Weakly perennial (ratoon and seeds)
NE3	74	Saraburi, Central Plain, Thailand	ca. 200 m	Annual (seeds)
NE88	46	Hingkon, Central Plain, Thailand	ca. 100 m	Perennial (mainly ratoon)
W1981	22	Palembang, Sumatra, Indonesia	Far	Perennial (mainly ratoon)
W120	14	Cuttack, Orissa, India	ca. 100 m	Perennial (mainly ratoon)

Little Chalfont, Buckinghamshire, UK). Probes (RG# and G# – rice genomic clones; RZ# – rice cDNA clones; BCD# – barley cDNA clones, and CDO# – oat cDNA clones) were provided by Dr T Sasaki, Rice Genome Program, STAFF Institute, Tsukuba, Japan and Dr SR McCouch, Cornell University, Ithaca, NY, USA. These clones are known to correspond to loci mapped to 12 rice chromosomes. In all, 15 probe–enzyme combinations were used.

Data analysis

The amount of intrapopulation diversity for quantitative traits was represented by standard deviation. To assess the degree of population differentiation at the phenotype level, the variance component among populations divided by the sum of variance components among and within populations was computed for each trait (P_{ST}). For allelic diversity within populations in terms of allozymes and RFLPs, genetic parameters such as average gene diversity (H), proportion of polymorphic loci, average number of alleles per locus, and inbreeding coefficient (F_{IS}) were calculated from all loci examined. The population parameters H_T (gene diversity in whole population), H_s (gene diversity within a population) and F_{ST} (population differentiation) were estimated for each isozyme and RFLP locus. Further, the genetic distances (Nei, 1972) between the seven populations were computed. To elucidate the overall pattern of population differentiation, individual data on quantitative traits, allozymes, and RFLPs were analyzed by the same multivariate technique (factor analysis) using Statistica 3.0 software (StarSoft Technologies, Inc., Spokane, WA, USA).

Results

Quantitative traits

Means and standard deviations for the 11 quantitative traits in six populations (excluding W120 grown under different condition) are shown in Table 2. The annual population NE3 markedly differed from other perennial populations in many traits. It had shorter stature, more panicles, longer awns, higher seed fertility, lower shoot-regenerating ability, and less development of new tillers after maturity. The other five populations, in particular typical perennial populations (NE88 and W1981),

showed the opposite trend. These contrasting trait associations are characteristic of the annual and perennial types of *O. rufipogon*, respectively (Oka and Morishima, 1967; Sano and Morishima, 1982). The Chinese population DX, which was supposed to be weakly perennial, showed more vigorous development of new tillers after heading than the other perennial populations, suggesting its unique mode of vegetative propagation.

As shown by the standard deviations given in Table 2, the introgressed population GG and the perennial population NE88 showed greater within-population variation than the others in many traits. The parameters of population differentiation (P_{ST}) for various traits (Table 2) varied in a relatively narrow range from 0.262 to 0.426, with the exception of that for panicle length (0.023).

To elucidate the pattern of variation among and within populations on the basis of all the traits, data for all individuals were analyzed by factor analysis. The first and second factors explained 38.4 and 22.5%, respectively (cumulative contribution, 60.9%), of the whole multivariate variation. The traits mainly contributing to the first factor were shoot-regenerating ability (factor loading, 0.87), plant height (0.77), awn length (–0.71), and seed fertility (–0.65), and those contributing to the second factor were spikelet number per panicle (0.85), panicle length (0.74), and leaf and stem weight at maturity (0.73).

The scatter diagram of all individuals, as defined by the first and second factors, is shown in Figure 2. The plants belonging to each population tended to form respective clusters, although the three Chinese populations, DX, YJ, and GG, showed overlapping distributions. NE3 (annual type) and NE88 (perennial type) collected in the same locality in Thailand were clearly separated along the first factor axis, suggesting that this axis represents differentiation toward perennial and annual types. The three Chinese populations were scattered between the annual NE3 and perennial NE88, indicating that they possess intermediate characteristics between annual and perennial, in accordance with our field observations. Plants with larger scores for the second factor (scattered in the upper part) were supposed to have large seed productivity as well as vegetative vigor, judging from the traits contributing to the second factor. They were most probably the derivatives of natural hybridization with cultivated rice.

Table 2 Mean values and SD (in parentheses) of 11 quantitative traits in six populations, and a parameter for population differentiation (F_{ST})

Character	Population (propagating system*)						P_{ST}
	DX (WP)	YJ (WP)	GG (WP)	NE3 (A)	NE88 (P)	W1981 (P)	
Plant height (cm)	127 (21.8)	152 (25.3)	158 (26.4)	115 (13.1)	214 (42.0)	167 (33.2)	0.400
Panicle number per plant	17.3 (3.5)	4.9 (2.0)	6.1 (4.1)	13.5 (6.6)	4.6 (4.0)	3.7 (2.4)	0.294
Panicle length (cm)	17.3 (2.8)	17.5 (3.2)	19.0 (4.6)	18.0 (1.8)	20.1 (4.7)	17.5 (1.7)	0.023
Spikelet number per panicle	32.6 (12.0)	51.4 (12.4)	45.4 (32.7)	64.6 (11.5)	58.6 (24.5)	49.5 (11.8)	0.231
Awn length (mm)	4.9 (0.8)	5.5 (0.6)	6.1 (1.2)	9.3 (1.0)	5.9 (1.4)	4.3 (0.7)	0.426
Seed fertility (%)	24.1 (14.4)	15.6 (14.3)	9.5 (11.8)	43.5 (19.1)	17.5 (19.9)	6.7 (11.3)	0.278
Root-regenerating ability (index)	0.52 (0.5)	0.84 (0.6)	1.68 (0.8)	0.86 (0.5)	1.76 (0.8)	0.82 (0.6)	0.262
Shoot-regenerating ability (no. of shoots/stem cut)	0.60 (0.3)	0.90 (0.2)	0.80 (0.2)	0.10 (0.2)	0.90 (0.2)	0.80 (0.4)	0.418
Leaf and stem dry weight (g)	16.1 (8.0)	17.1 (8.7)	28.3 (27.4)	24.7 (11.9)	39.7 (29.5)	25.7 (16.4)	0.389
Dry weight of tiller developed after heading (g)	3.1 (1.7)	0.6 (0.6)	0.5 (0.6)	0.2 (0.5)	1.4 (1.1)	1.2 (1.0)	0.374
Days from seeding to heading	69 (7.3)	71 (5.0)	69 (6.7)	70 (3.9)	103 (7.3)	93 (2.8)	0.381

*WP: weakly perennial; P: perennial; A: annual.

Figure 2 Scatter diagram of the plants belonging to six populations on the first and second factor scores, based on quantitative traits.

Allozyme diversity

Of the 29 isozyme loci examined, *Acp1* and *Mal1* were monomorphic and the other 27 loci were polymorphic in the present materials. So far, we have detected polymorphism at *Acp5(t)*, *Acp6(t)*, *Mal5(t)*, and *Mal6(t)* only in *O. rufipogon* and not in *O. sativa* (our unpublished data). The allele frequencies for the 27 polymorphic loci in each population are given in Table 3, together with the population parameters H_T , H_S , and F_{ST} for each locus. At some loci, such as *Amp1*, *Cat1*, and *Est14*, the allele frequencies seemed to change dramatically between two (DX and YJ) or three (DX, YJ, and GG) of the Chinese populations and the remaining populations collected in tropical regions. Interestingly, *Cat1* (Second, 1982) and *Est14* (our unpublished data) are known as diagnostic loci for distinguishing Indica and Japonica types of *O. sativa*, and the alleles found in Chinese populations at high frequencies (*Cat1*² and *Est14*¹) are 'Japonica-specific alleles' (Second 1985, our unpublished data).

Estimates of genetic parameters of intrapopulation diversity, such as average gene diversity, proportion of polymorphic loci, and average numbers of alleles per locus, are given in Table 4, part (a) for each population, together with the inbreeding coefficient F_{IS} . Obviously, two Chinese populations, DX and YJ, showed less polymorphism than the others. The other Chinese population, GG, showed a high level of genetic diversity compared with the tropical perennial populations (NE88, W1981, and W120). The annual population NE3 showed a low level of polymorphism, as expected. The inbreeding coefficient F_{IS} was high in the annual NE3 population and low in the perennial NE88 and W120 populations.

H_T varied from 0.053 (*Acp6(t)*) to 0.674 (*Amp2*) and H_S from 0.012 (*EstS(t)*) to 0.365 (*Amp2*) (Table 3). The

parameter for population differentiation, F_{ST} , ranged from 0.075 (*Acp5(t)*) to 0.941 (*EstS(t)*), with an average of 0.431 (Table 3). F_{ST} values tended to be high at *Amp1*, *Cat1* and *Est14*, whose allele frequencies differentiated the Chinese populations from other populations. In addition, *Est1* differentiating the annual NE3 from other populations and *EstS(t)* differentiating the two South Asian populations (W120 and W1981) from the others showed high F_{ST} values (Table 3). The genetic distances between populations (Table 5) tended to be small within the three regions – China, Thailand, and South Asia (India and Indonesia) – and large between different regions.

To elucidate the overall variations among and within populations, we evaluated the allozyme genotypes of all individuals by factor analysis. Although only 28.1% of total variation was extracted by the first and second factors, individuals belonging to different populations were plotted as forming respective clusters (Figure 3a). The first factor separated Chinese populations from other populations collected in tropical Asia. The second factor separated the Indonesian population (W1981) from all others. In contrast to the result achieved by the analysis of quantitative traits, perennial (NE88) and annual (NE3) populations from the same locality in Thailand were closely located.

RFLP diversity

From the 15 probe-enzyme combinations, a total of 17 polymorphic loci were detected. The allelic frequencies of RFLPs for the seven populations are shown in Table 6, together with their population parameters. Intrapopulation polymorphisms at the RFLP level were detected in all populations examined. As in the allozyme analysis, we found that the Chinese populations shared some common RFLPs (G103², G249²) that were rarely found in other populations. This indicated that the Chinese populations tended to be genetically differentiated from the populations distributed in tropical countries.

Average gene diversity and other diversity parameters were calculated for each population (Table 4, part b). The annual population NE3 and the weakly perennial population YJ showed less polymorphism than some others. The inbreeding coefficient F_{IS} was high in the annual NE3 population and low in the perennial populations W1981 and W120. The population parameters H_T , H_S , and F_{ST} are shown in Table 6. F_{ST} values estimated for respective loci were widely distributed, ranging from 0.047 to 0.984, with an average of 0.430. The genetic distances calculated between the seven populations (Table 5) clearly showed genetic differentiation between the different regions.

RFLP data of all individuals were analyzed by factor analysis. Of the total variation, 35.7% was extracted by the first and second factors. Plants from the same population tended to form respective clusters reflecting their geographical distributions (Figure 3b). The pattern of variation proved to be essentially the same as that obtained from the allozyme data (Figure 3a): the first axis separated the Chinese populations and the others, and the second axis separated the south (India and Indonesia) and South-East Asian (Thailand) populations.

Table 3 Allele frequencies at 27 polymorphic isozyme loci, and the population parameters H_T , H_S and F_{ST}

Locus/allele	Chr.		Population (no. of plants examined)							H_T	H_S	F_{ST}
			DX (54)	YJ (14)	GG (68)	NE3 (74)	NE88 (46)	W1981 (22)	W120 (14)			
<i>Acp2</i>	1	12	0.056	0.357	0.063	0.838	0.522	1.000	0.357	0.492	0.239	0.514
	0		0.944	0.643	0.937	0.162	0.478		0.643			
<i>Acp3</i>	1	?	1.000	1.000	0.810	0.189	0.326	0.545	0.214	0.491	0.272	0.446
	0				0.190	0.811	0.674	0.455	0.786			
<i>Acp5(t)</i>	1	?	0.981	1.000	0.956	1.000	1.000	0.818	1.000	0.053	0.049	0.075
	2		0.019		0.044			0.182				
<i>Acp6(t)</i>	1	?	1.000	1.000	0.882	1.000	1.000	1.000	1.000	0.053	0.048	0.094
	2				0.118							
<i>Amp1</i>	1	2			0.044	0.459	0.957	0.500	0.429	0.596	0.226	0.621
	2					0.541			0.072			
	3		1.000	1.000	0.956		0.044	0.500	0.428			
<i>Amp2</i>	1	8	0.038		0.559	0.264	0.250		0.608	0.674	0.365	0.458
	2		0.963	1.000	0.426		0.250	1.000	0.393			
	3				0.015	0.730	0.087					
	4					0.006	0.413					
<i>Amp3</i>	1	6	0.981	1.000	0.470		0.355	0.273	0.500	0.579	0.320	0.447
	2		0.019		0.294	1.000	0.511	0.545	0.500			
	0				0.235		0.044	0.136				
<i>Amp7</i>	1	6	0.093		0.500		0.174	0.182		0.289	0.215	0.256
	0		0.907	1.000	0.500	1.000	0.826	0.818	1.000			
<i>Cat1</i>	1	6			0.964	1.000	0.978	1.000	0.750	0.381	0.045	0.882
	2		0.991	1.000	0.037				0.250			
<i>Est1</i>	1	7	0.926	1.000	0.985		0.870	0.818	1.000	0.424	0.090	0.788
	0		0.074		0.015	1.000	0.130	0.182				
<i>Est2</i>	1	6		1.000	0.162	0.973	0.348	0.182	0.572	0.551	0.250	0.546
	2				0.074		0.022	0.136				
	3					0.027	0.043		0.285			
	0		1.000		0.765		0.587	0.682	0.143			
<i>Est3</i>	1	9	0.722	1.000	0.809		0.761	0.545	0.286	0.496	0.260	0.476
	2		0.278		0.191	1.000	0.239	0.455	0.714			
<i>Est5</i>	1	1	1.000	1.000	1.000	0.892	0.870	1.000	0.429	0.139	0.108	0.223
	2					0.108	0.130		0.572			
<i>Est9</i>	1	7	0.915	0.465	0.287		0.011		0.072	0.386	0.158	0.591
	2		0.085	0.536	0.713	1.000	0.989	1.000	0.929			
<i>Est10</i>	2	1			0.007	0.048	0.011	0.045	0.179	0.157	0.138	0.121
	3				0.155			0.273				
	4		1.000	1.000	0.838	0.953	0.989	0.682	0.822			
<i>Est12</i>	1	9			0.250	0.689	0.261		0.214	0.407	0.273	0.329
	0		1.000	1.000	0.750	0.311	0.739	1.000	0.786			
<i>Est14</i>	1	?	0.981	1.000	0.588					0.464	0.120	0.741
	2		0.019		0.412	1.000	1.000	1.000	1.000			
<i>EstS(t)</i>	1	?	1.000	1.000	1.000	1.000	1.000	0.091		0.205	0.012	0.941
	2							0.909	1.000			
<i>Mal5(t)</i>	1	?			0.029	0.563				0.248	0.148	0.403
	2		1.000	1.000	0.971	0.437	1.000	1.000	1.000			
<i>Mal6(t)</i>	1	?			0.032	0.577				0.254	0.139	0.453
	2		1.000	1.000	0.968	0.423	1.000	1.000	1.000			
<i>Pgd1</i>	1	11	1.000	1.000	0.956	0.541	0.163	0.786	0.571	0.431	0.242	0.439
	2				0.037				0.286			
	3				0.008	0.459	0.838	0.214	0.071			

Table Continued...

Table 3 (Continued)

Locus/allele	Chr.		Population (no. of plants examined)							H _T	H _S	F _{ST}
			DX (54)	YJ (14)	GG (68)	NE3 (74)	NE88 (46)	W1981 (22)	W120 (14)			
Pgd2	1	6	0.991	1.000	1.000	1.000	0.956	0.387	0.929	0.110	0.060	0.455
	2		0.010				0.022	0.545	0.071			
	3						0.022	0.068				
Pgi1	1	3			0.044		0.229		0.107	0.154	0.140	0.091
	2		0.907	1.000	0.941	1.000	0.772	0.864	0.893			
	3		0.093		0.015			0.136				
Pgi2	1	6	0.907	1.000	0.948	0.886	0.554	0.364	1.000	0.304	0.220	0.276
	2		0.093		0.051	0.115						
	4						0.435	0.636				
Pgi3	1	?	0.981	1.000	0.833	1.000	0.891	0.864	1.000	0.130	0.120	0.077
	2		0.019		0.167		0.109	0.136				
Pox2	1	12	0.722	1.000	0.833	1.000	0.767	1.000	0.667	0.244	0.219	0.102
	2		0.278		0.136		0.233		0.333			
Sdh1	1	12		0.107	0.051	1.000	1.000	0.205	0.321	0.578	0.124	0.785
	2		0.898	0.893	0.919				0.678			
	4							0.796				
	5		0.102		0.030							

Table 4 Parameter values showing intrapopulation genetic diversity for the seven populations: (a) Allozymes (29 loci) and (b) RFLPs (17 loci), as estimated from allozyme and RFLP data

Parameter	Population (propagating system ^a)						
	DX (WP)	YJ (WP)	GG (WP)	NE3 (A)	NE88 (P)	W1981 (P)	W120 (P)
(a) Allozymes (29 loci)							
Average gene diversity	0.074	0.040	0.204	0.136	0.214	0.215	0.252
Proportion of polymorphic loci	0.55	0.10	0.83	0.41	0.69	0.55	0.59
Average no. of alleles/locus	1.55	1.10	2.10	1.45	1.97	1.72	1.76
Inbreeding coefficient (F_{IS})	0.736	0.872	0.725	0.912	0.670	0.917	0.476
(b) RFLPs (17 loci)							
Average gene diversity	0.113	0.049	0.209	0.099	0.208	0.118	0.109
Proportion of polymorphic loci	0.41	0.18	0.71	0.41	0.76	0.24	0.35
Average no. of alleles/locus	1.53	1.18	2.12	1.53	2.06	1.41	1.47
Inbreeding coefficient (F_{IS})	0.477	0.343	0.487	0.818	0.621	0.310	0.386

^aWP: weakly perennial; P: perennial; A: annual.**Table 5** Genetic distances between the seven populations, computed from allozyme (above diagonal) and RFLP (below diagonal) data

Population	China			Thailand		Indonesia	India
	DX	YJ	GG	NE3	NE88	W1981	W120
DX (China)		0.166	0.109	0.681	0.391	0.476	0.352
YJ (China)	0.141		0.196	0.377	0.343	0.444	0.285
GG (China)	0.107	0.140		0.435	0.207	0.328	0.215
NE3 (Thailand)	0.201	0.240	0.159		0.217	0.411	0.289
NE88 (Thailand)	0.227	0.243	0.167	0.049		0.224	0.204
W1981 (Indonesia)	0.249	0.287	0.403	0.250	0.273		0.220
W120 (India)	0.326	0.377	0.546	0.392	0.294	0.260	

Discussion

In *O. rufipogon*, annual types are predominantly inbreeding and perennial types are partly outbreeding (Oka and Morishima, 1967; Morishima and Barbier, 1990). Therefore, intrapopulation diversity is generally lower in

annual types than in perennial types (Barbier, 1989; Morishima and Barbier, 1990). In our study, the annual population NE3 was less diversified and had a high inbreeding coefficient, as expected. In contrast, the partly outbreeding perennial populations were found to preserve variable amounts of genetic diversity. It is difficult

Figure 3 Scatter diagram of the plants belonging to seven populations on the first and second factor scores, based on allozymes (a) and RFLPs (b).

to explain this intrapopulation genetic diversity only in terms of breeding systems, because we know little about the long-term evolutionary history of each population. The large amount of genetic variability found in the Chinese population GG is most probably due to the effect of introgression from cultivated rice growing nearby: this was evident from the fact that the GG population contained alleles such as *Est2²* and *Est10²*, which are rare in Chinese wild rice but are carried by cultivated rice. The relatively low diversity of YJ is probably because YJ is an isolated peripheral population. Thus, the amount of genetic diversity within the populations that we observed was undoubtedly influenced by both

the breeding system and the evolutionary history of each population.

A major difference in quantitative traits among our populations was ecotype differentiation towards either perennial or annual types (Figure 2), as has been repeatedly argued in this species (Oka and Morishima, 1967; Sano and Morishima, 1982; Morishima *et al*, 1984). As all of the plants we analyzed were grown in a common environment, we consider that the observed differentiation in quantitative traits has at least in part a genetic basis. This variation in pattern reflects the result of adaptive differentiation in life-history traits that has occurred within this species in response to different habitat conditions (Morishima *et al*, 1984).

In contrast, population differentiation revealed by allozyme and RFLP analysis more likely effects geographic variation due to genetic isolation (Figure 3). The approximate geographical distances among study sites were roughly estimated from the map of Asia. Rank correlations were calculated between the geographical distance matrix and two genetic distance matrices (Table 5). The rank correlation was $r = 0.529$ ($P < 0.05$) with the allozyme matrix, and $r = 0.660$ ($P < 0.01$) with the RFLP matrix. These significant correlations are probably due to increasing genetic distance with geographical distance.

The results of the allozyme and RFLP studies both suggest genetic differentiation among South Chinese (temperate) populations and South-East Asian (tropical) populations. This agrees with the results of our earlier allozyme study, which was based on a larger number of accessions collected over a broad area of Asia (Cai *et al*, 1996). Assuming that these allozymes and RFLPs are neutral, we can suggest that this genetic variation has resulted from long-range dispersal of this wild rice species, followed by geographical isolation (isolation by distance model, Slatkin, 1993) rather than as a result of adaptation to local environmental conditions. However, it cannot be ruled out that molecular markers may vary in relation to selection processes. Even if they themselves are neutral they can vary due to natural selection as a result of hitchhiking. Therefore, it is difficult to fully estimate the relative importance of selective and neutral processes in causing geographical differentiation. Raybould *et al* (1996, 1997) compared RFLPs and allozymes in their study on sea beet populations and found that isolation by distance was evident in RFLPs but not in allozymes. Latta and Mitton (1997) observed in limber pine populations that RAPD analysis showed a higher degree of divergence than allozymes. We failed to find marked differences between allozyme and RFLP markers in terms of differentiation pattern and population structure.

The measure of population differentiation, F_{ST} , can be influenced by various factors such as effective population size, genetic drift, migration rate (mating system), mutation rate, and selection (Podolsky and Holtsford, 1995). According to Hamrick and Godt (1990), who collected a large amount of information on allozyme diversity, mean F_{ST} (G_{ST}) is 0.197 for outcrossers, 0.084 for woody perennials, 0.099 for wind-pollinated species, 0.233 for perennial herbaceous species, and 0.397 for annuals. In *O. rufipogon*, Gao *et al* (2000) obtained $F_{ST} = 0.310$ for Chinese perennial populations. From studies of Indian and Thai populations,

Table 6 Allelic frequencies of RFLPs for the seven populations, and the population parameters H_T , H_S and F_{ST}

Locus/allele	Chr.	Population (no. of plants examined)								Population parameter		
			DX (40)	YJ (14)	GG (62)	NE3 (73)	NE88 (39)	W1981 (16)	W120 (9)	H_T	H_S	F_{ST}
RG214	1	4	1.000	1.000	0.645	0.822	0.564	0.531	0.889	0.396	0.324	0.181
	2				0.008	0.171	0.051	0.063				
	3					0.007	0.064					
	5				0.347		0.321	0.156				
	6							0.250				
RG181	1	8	1.000	0.857	1.000	1.000	0.744	1.000	0.444	0.125	0.085	0.321
	2			0.143			0.256		0.556			
RZ792	1	9	0.950		0.282	0.897	0.423	0.281	0.056	0.632	0.282	0.554
	2		0.050		0.024	0.014	0.013	0.719				
	3				0.363	0.089						
	4						0.526		0.778			
	5			1.000	0.331		0.038		0.111			
	6								0.056			
RZ141	1	11	1.000	1.000	1.000	0.986	1.000	1.000		0.076	0.001	0.984
	2					0.014			1.000			
BCD126	1	5	1.000	0.929	0.927	1.000	1.000	1.000	1.000	0.043	0.041	0.047
	3				0.040							
	4				0.071							
RZ561	1	1	0.663	1.000	0.734	1.000	0.577	1.000	0.111	0.360	0.199	0.446
	2		0.313		0.048		0.372		0.889			
	3		0.013		0.081		0.051					
	4		0.013		0.137							
CDO393	1	5	1.000	1.000	0.879	1.000	0.897	0.438	1.000	0.125	0.073	0.418
	2				0.121		0.013	0.563				
CDO127	1	11 or 12	1.000	1.000	0.565	0.61	0.654	1.000	1.000	0.397	0.289	0.272
	2				0.435	0.39	0.346					
RG345	1	1	1.000	1.000	0.935	1.000	1.000	1.000	1.000	0.031	0.029	0.056
	2				0.065							
BCD855	1	7	1.000	1.000	1.000	1.000	0.769	1.000	1.000	0.069	0.055	0.205
	2						0.231					
G103	1	9		0.643	0.032	0.986	0.974	1.000	0.944	0.495	0.12	0.758
	2		0.988	0.357	0.944	0.014	0.026		0.056			
	3		0.013		0.024							
G249	1	3	0.238		0.194	1.000	1.000	1.000	1.000	0.468	0.097	0.793
	2		0.763	1.000	0.806							
G187	1	8	0.025		0.048	0.329	0.051	0.063	0.389	0.304	0.192	0.368
	2		0.975	1.000	0.952	0.671	0.949	0.281	0.611			
	3							0.656				
G89-2a	1	1	1.000	1.000	0.790	1.000	0.974	1.000	1.000	0.105	0.088	0.161
	2				0.210		0.026					
G89-2b	1	10?				0.137	0.179			0.125	0.110	0.121
	2		1.000	1.000	1.000	0.863	0.821	1.000	1.000			
G282a	1	4	0.625				0.051	1.000	1.000	0.327	0.056	0.829
	2		0.375	1.000	1.000	1.000	0.949					
G282b	1	4	0.350		0.903	1.000	0.949			0.411	0.086	0.791
	2		0.650	1.000	0.097		0.051	1.000	1.000			

Morishima and Barbier (1990) reported 0.396 for perennials and 0.600 for annuals. The mean F_{ST} value obtained in our present study was 0.431 in allozymes and 0.430 in RFLPs.

The comparison of population differentiation parameters for different classes of traits or markers is a useful tool in the analysis of the causes of population variation. To describe differentiation among populations, quantita-

tive traits have lower resolving power than allozymes and RFLPs in our present study, although P_{ST} is not strictly identical to F_{ST} . Allozyme analysis showed a similar level of resolution to RFLPs, although the latter technique is much more expensive and time-consuming. Heterogeneity of F_{ST} values among loci was another feature of our study (Tables 3 and 6). In past studies, F_{ST} values estimated for allozymes have been relatively homogeneous across loci and lower than F_{ST} for quantitative traits (Podolsky and Holtsford, 1995) or RAPD (Latta and Mitton, 1997). It is difficult to conclude whether the heterogeneity of F_{ST} values that we found among loci is a reflection of a stochastic process, or whether indirect selection through hitchhiking is involved.

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