Theor Appl Genet (1992) 84:266–274

THEORETICAL AND APPLIED GENETICS

Indica-Japonica differentiation of rice cultivars viewed from variations in key characters and isozymes, with special reference to landraces from the Himalayan hilly areas

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Received August 2, 1991; Accepted December 19, 1991
Communicated by K. Tsunewaki

Summary. To assess the extent of differentiation into the Indica and Japonica types of Asian rice in its diversity center, we investigated landraces collected from the hilly areas of Nepal, Sikkim and Assam. We examined variations in four key characters and six isozyme loci known to be diagnostic for classifying Indica and Japonica types, and compared the results with those from a control set of rice cultivars representing the whole of Asia. The hill cultivars showed a high level of genetic diversity in key characters as well as in isozymes. A marked feature found in their character variation was the occurrence of various atypical cultivars that were intermediate between the Indica and Japonica types. With respect to isozymes, however, the hill cultivars could be classified mostly as either Indicas or Japonicas, although the patterns of allelic association were more random than in the control cultivars. Indica-Japonica variation in key characters and in isozymes corresponded well with each other in the controls, but not in the hill cultivars. This means that nonrandom association in characters as well as in genes (gametic disequilibrium) is not fully developed in the diversity center. Populations of hill cultivars were highly polymorphic genetically, but did not show a trend to Indica-Japonica differentiation within their populations. The process of Indica-Japonica differentiation is discussed in view of these observations.

Key words: Isozymes – Himalayan hill rice – Indica versus Japonica types – Gametic disequilibrium – Diversity center

Introduction

Varietal variation in Asian rice (*Oryza sativa* L.) has been studied by many workers using various morphological and physiological characters as well as hybrid sterility relationships. Those studies were aimed at investigating the genetic architecture of this rice species and finding out the keys for varietal classification. Since Kato et al. (1928) proposed that the Asian rice cultivars could be divided into two subspecies, *Indica* and *Japonica*, many researchers have followed this classification. But other systems of classification have also been proposed (Matsuo 1952; Chang 1976), and the concept of *Indica-Japonica* classification has become somewhat ambiguous. Through variation studies excluding "external criteria", Oka (1958) confirmed that two major varietal groups characterized by a particular pattern of character association exist as natural groups in Asian rice. In the present paper, we call the two varietal groups thus recognized the Indica and Japonica types, respectively.

Isozymic studies have shed a new light on the pattern of varietal differentiation in rice. Differential frequencies of alleles between Indica and Japonica types were reported for a few loci in the 1960's. Nakagahra et al. (1975) studied the geographical distribution of diverse genetic forms based on allelic combinations at three esterase loci. An analysis of 40 isozyme loci conducted by Second (1982) revealed that the cultivars of *O. sativa* used could be separated into the Indica and Japonica types. Glaszmann (1986, 1987) recognized six enzymatic groups in a large collection of Asian rice cultivars based on multilocus variation at 22 loci and discussed the relationships between these enzymatic groups and previously proposed classifications. The two major groups recognized in his study corresponded to typical Indica and Japonica types.

The area along the Himalayas and its associated ranges have attracted the interest of rice scientists because of its high level of genetic diversity (Nakagahra 1978; Glaszmann 1987) and the existence of cultivars which do not show F_1 sterility with both Indica and

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Japonica types (Morinaga 1968). Chang (1976) advocated, based on the multidisciplinary analysis of available information, that domestication of Asian rice must have occurred within the broad area ranging from the southeast foothill of the Himalayas to the mountainous region in mainland southeast Asia and southwest China. The purpose of the present study is to examine the extent to which the Indica-Japonica differentiation has taken place in the hilly area of the Himalayas, which would be a part of the diversity center of Asian rice cultivars. Using materials collected in the Himalayan hilly area, we examined isozyme variation and its relationship with Indica-Japonica differentiation as Indicated by previously used key characters and compared it with the data for control cultivars representing the whole Asia.

Materials and methods

Plant materials

A total of 101 accessions of rice cultivars, which were random samples from a large landrace collection from various Asian countries, were used as the control set to represent the whole of Asia. These accessions were repeatedly used by Oka and his colleagues to investigate varietal differentiation in Asian rice (Oka 1988). As representative material from the Himalayan hilly area, a total of 151 native cultivars was used; these had been collected by different explorers and have been preserved at the National Institute of Genetics, Japan (see Kihara and Nakao 1960; Morishima et al. 1980). The collection consists of 21 accessions from Nepal, 22 from Sikkim and 198 from Assam. In addition, eight populations sampled from farmers' fields in the hilly areas of Nepal and Northern Thailand were examined to assess intra-population diversity. For this population study, progeny lines derived from the original samples, each representing an individual of the mother population, were used.

Estimation of likeness to Indica or Japonica type by key characters

A discriminant function by which to classifiy Indica and Japonica types, constructed originally by Oka and Chang (1952), combines four diagnostic characters; this function was used in the present study. The formula was:

$$X_1 = K + 0.75C - 0.22H + 0.86P \text{ (standardized)},$$

where K is the potassium chlorate susceptibility of seedlings (log concentration of $KClO_3$ solution causing a certain degree of damage, with its absolute value used as $KClO_3$ susceptibility), C is the index of susceptibility to low temperature of seedlings shown by injury degree, H is apiculus hair length and P is phenol reaction of the hull (1 for positive and 0 for negative reaction). For the control cultivars, the data previously taken by Oka (1958) were used. For the materials from the hilly areas, characters were examined using the same methods as described by Oka (1958).

Estimation of Indica-Japonica variation in isozymes

For each allele at six isozyme loci (*Cat-1*, *Acp*-1, *Pgi-1*, *Pgi-2*, *Pox-2* and *Est-2*), discriminating parameter D_I was calculated as $D_I = F_I/(F_I + F_J)$, where F_I and F_J are the frequencies of a given allele in the Indica and Japonica groups of control cultivars classified by $X_1 = 4.0$ (described later), respectively. The average of D_I values over six loci representing the allelic constitution of a given accession, designated as X_2, was used for quantifying isozyme variation. The X_2 value shows the likeness of a given isozyme genotype to the typical Indica genotype.

Measure of nonrandom association between loci

The nonrandomness of multilocus association was measured in terms of the squared correlation coefficient of allelic frequencies, R^2 (Hill and Robertson 1968). For a two-locus (A, B) two-allele (1, 2) model, R^2 is defined as

$$R^2 = (P_{11} - pq)^2/p(1-p)q(1-q),$$

where p and q are the frequencies of alleles A^1 and B^1, respectively, and P_{11} stands for the frequency of the A^1B^1 gamete period. R^2 was used in the present study because this enables us to compare the degree of nonrandom association of alleles at different loci (gametic disequilibrium) between two populations of different sample sizes.

Isozyme assay

Six isozyme loci, *Cat-1*, *Acp-1*, *Pgi-1*, *Pgi-2*, *Pox-2* and *Est-2*, coding for catalase, acid phosphatase, phosphoglucose isomerase (2 isozymes), peroxidase and esterase, respectively, were assayed. These loci were chosen because they were known to show substantial variability within *O. sativa*.

Extracts were prepared from plumules of 5-day-old seedlings grown in a dark chamber at 30 °C. Two to three plants were assayed for each accession. When heterogeneity was observed within an accession, the predominant type was used for analysis. Samples were run on starch gels using the following system. Electrophoretic and staining procedures were modified after Cardy et al. (1980) and Second and Trouslot (1980). The gel contained 13% (w/v) starch and 0.8% (w/v) sucrose in a buffered solution composed of 9 parts gel buffer (pH 8.0, 0.032 *M* TRIS, 0.005 *M* citric acid) and 1 part electrode buffer (pH 8.0, 0.19 *M* boric acid, 0.032 *M* NaOH). Until the voltage reached 170 V, a constant current of 60 mA and then a constant voltage of 170 V (12.5 v/cm) was delivered to a horizontal gel (13 cm × 13.5 cm × 0.6 cm) at 2 °C for 5.5 h.

The staining solutions and procedures were as follows. (1) PGI: 75 ml 0.05 *M* TRIS-HCl pH 8.0, 40 mg D-fructose-6-phosphate, 5 mg NADP, 5 mg MTT, 1.5 mg PMS, 10 units G_6PDH; incubate for 60 min at 30 °C and rinse. (2) CAT: 100 ml 3% H_2O_2; pour onto the gel, leave for 3–5 min and rinse with distilled water. Add 5 ml 8% $FeCl_3$, 5 ml 8% $K_3Fe(CN)_3$, 90 ml H_2O, leave for 30 min and rinse. (3) ACP: 75 ml 0.1 *M* sodium acetate pH 5.0, 100 mg Fast Garnet GBC salt, 36 mg α-naphtyl phosphate; incubate for 60 min and rinse. (4) POX: 87 ml 0.05 *M* potassium acetate pH 5.0, 10 ml staining solution (2 drops eugenol, 10 mg 3-amino-9-ethyl carbazol, 10 ml acetone), 2 ml 0.1 *M* $CaCl_2$; just before use, add 1 ml 3% H_2O_2 (96 ml H_2O, 3 ml H_2O_2, 1 ml acetic acid), leave for 60 min and rinse. (5) EST: 10 ml 0.32 *M* Na_2HPO_4, 50 ml 0.2 *M* NaH_2PO_4, 40 ml H_2O, 2.5 ml *N*-propanol, 100 mg Fast Blue RR salt, 20 mg α-naphtyl acetate, 15 mg β-naphtyl acetate; incubate for 45 min and rinse.

Results

Variation pattern in Indica-Japonica diagnostic characters

Each accession was evaluated using a discriminant score combining four diagnostic characters (X_1) to classify them into the Indica and Japonica types. Control culti-

Table 1. Association between $KClO_3$ susceptibility (K), low temperature susceptibility (C), apiculus hair length (H) and phenol reaction (P)

Group	Number of cultivars	Correlation coefficient			*t* value		
		K-L	K-H	L-H	K-P	L-P	H-P
Control	101	0.60**	−0.38**	−0.45**	8.96**	7.20**	6.19**
Hill cultivars	151	0.17*	0.23**	−0.09	4.92**	0.98	0.95

*, ** Significant at 5% and 1% levels, respectively

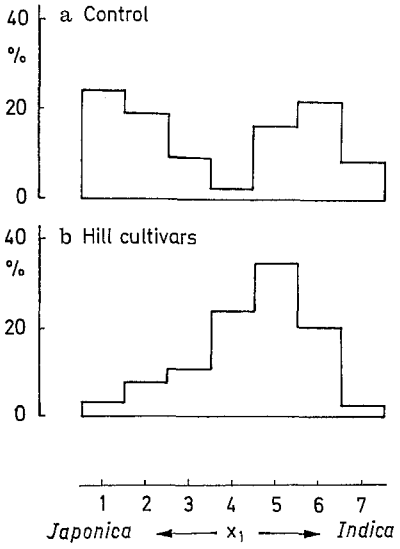

Fig. 1 a, b. Frequency distributions of Indica-Japonica discriminant scores (X_1) combining four character measurements

vars from various Asian countries were divisible into the two types as expected, although a few intermediates did remain (Fig. 1 a). In the following analysis, accessions with a discriminant score larger than 4.0 were classified as the Indica type and those with smaller scores as the Japonica type. Tropical Japonicas (so-called bulu or javanica) were grouped together with temperate Japonicas on this scale. In contrast, the hill cultivars showed a unimodal continuous variation as shown in Fig. 1 b. There was an array of intergrades between the typical Indica and Japonica types.

As is well known, the four characters used for the discriminant function are highly variable and intercorrelated among the control cultivars. Namely, high susceptibility to low temperature is associated with high $KClO_3$ susceptibility, short apiculus hair and positive phenol reaction (characterizing Indica type) and vice versa (Japonica type). In order to know to what extent such nonrandom character association occurs among hill cultivars, variation and covariation in these characters were examined. Three quantitative characters, $KClO_3$ susceptibility, low temperature susceptibility and apiculus hair length, showed a continuous variation covering the whole range found in the control cultivars. Of the hill cultivars 72% showed a positive reaction for phenol. Correlation coefficients between quantitative characters and *t*-values for the difference in their character measurements between phenol positive and negative groups are given in Table 1 together with the data for the control. The degrees of association were generally weak in the hill cultivars. $KClO_3$ susceptibility and apiculus hair length, which were negatively correlated in the control, were positively correlated in the hill cultivars.

Isozyme variation and relationships with key character variation

The allelic frequencies at six isozyme loci were computed separately in the Indica and Japonica types classified by character discriminant score X_1 for both control and hill cultivar sets. As shown in Table 2, in the control cultivars marked differences in allelic frequencies were found between the Indica and Japonica types at all of the loci examined. The Japonicas were nearly monomorphic showing a low average gene diversity, 0.133. In contrast, the Indicas were more polymorphic with a gene diversity of 0.318. In the hill cultivars, which were classified into 108 Indicas and 43 Japonicas by the same criterion (bordered by $X_1 = 4.0$), the difference in allele frequencies between the two groups was not conspicuous. Both the Indica and Japonica groups showed similar high average gene diversities (0.428 and 0.467, respectively).

The nonrandomness of the allele association between two given isozyme loci was examined by R^2 (Table 3). This measure ranges from 0 (random association) to 1 (complete association). Because the statistical significance of R^2 cannot be tested directly, the deviation from random association was tested by χ^2. R^2 is equivalent to χ^2/n, were n is sample number. In control cultivars, all of the combinations examined, except for that between *Est-2* and *Pox-2*, showed significant nonrandomness. This Indicates the occurrence of a particular association of alleles between these isozyme loci. The degree of nonrandomness thus revealed was not related to the linkage relation of the two loci concerned, which is given in Table 3. The R^2 values in the hill cultivars were also

Table 2. Frequencies of alleles at six isozyme loci, their relative frequency in the India control (D_I)[a] and average gene diversities (H)[b]

Locus	Allele[c]	Control			Hill cultivars			D_I
		Indica (48)	Japonica (53)	Total (101)	Indica (108)	Japonica (43)	Total (151)	
Cat-1	1	0.98	0.06	0.50	0.90	0.56	0.80	0.95
	2	0.02	0.94	0.51	0.10	0.44	0.20	0.02
Acp-1	1 (−4)	0.96	0.02	0.47	0.85	0.61	0.78	0.98
	2 (+9)	0.04	0.98	0.54	0.15	0.40	0.22	0.04
Pgi-1	1	0.79	0.00	0.38	0.60	0.26	0.50	1.00
	2	0.21	1.00	0.62	0.40	0.74	0.50	0.17
Pgi-2	1	0.33	0.96	0.66	0.34	0.54	0.40	0.26
	2	0.67	0.04	0.34	0.57	0.35	0.51	0.95
	4	0.00	0.00	0.00	0.08	0.12	0.09	
Pox-2	0	0.29	1.00	0.66	0.50	0.84	0.60	0.23
	1 (4C)	0.71	0.00	0.34	0.50	0.16	0.40	1.00
Est-2	0	0.27	0.81	0.55	0.19	0.30	0.22	0.25
	1	0.19	0.17	0.19	0.52	0.54	0.52	0.53
	2	0.54	0.02	0.27	0.30	0.16	0.26	0.97
H		0.318	0.133	0.493	0.428	0.467	0.471	

[a] $D_I = F_I/(F_I + F_J)$. F_I and F_J are the allele frequencies in the Indica and Japonica groups classified by X_I, respectively (see text)
[b] Average gene diversity, $H = 1/6 \sum (1 - \sum P_{ij}^2)$. P_{ij} stands for frequency of the *i*th allele at the *j*th locus
[c] Allele designations in the parenthesis are the ones formerly used

Table 3. Squared correlation coefficients (R^2)[a] for measuring the nonrandomness of allelic association between two isozyme loci

Control Locus	*Acp-1*	*Pgi-1*	*Pgi-2*	*Pox-2*	*Est-2*	Chromosomal location
Cat-1	0.815**	0.555**	0.518**	0.459**	0.253**	6
Acp-1		0.447**	0.462**	0.521**	0.251**	12, 24%-*Pox-2*
Pgi-1			0.195**	0.554**	0.127**	3
Pgi-2				0.262**	0.276**	6, 13%-*Est-2*
Pox-2		$R^2 = 0.382$			0.036	12
Est-2						6
Hill cultivars						
Cat-1	0.337**	0.189**	0.058**	0.117**	0.246**	
Acp-1		0.138**	0.066**	0.074**	0.035*	
Pgi-1			0.053**	0.244**	0.075**	
Pgi-2				0.008	0.016	
Pox-2		$R^2 = 0.115$			0.073**	
Est-2						

*, ** Significant at 5% and 1% levels, respectively
[a] $R^2 = (P_{11} - pq)^2/p(1-p)q(1-q)$ (after Hill and Robertson 1968, see text)

significant in most combinations, but much smaller than in the control.

Multilocus genotypes were then examined. Table 4 shows the frequency distribution of five-locus gametic genotypes (excluding *Est-2*) arranged by their discriminant score for characters (X_1). The relative frequencies of respective isozyme genotypes in percent of total number are shown together with their deviation from expected frequencies calculated as the product of observed single-locus allelic frequencies. In the control cultivars, only 13 genotypes were found among 32 possible genotypes. Genotype A, *Cat-1*^{1}, *Acp-1*^{1}, *Pgi-1*^{1}, *Pgi-2*^{2}, *Pox-2*^{1}, and its balanced opposite genotype V, *Cat-1*^{2}, *Acp-1*^{2}, *Pgi-1*^{2}, *Pgi-2*^{1}, *Pox-2*^{0}, showed a remarkable excess over the expected frequencies. Judging from their X_1 scores, these two isozyme genotypes were typical Indica and Japonica types, respectively. Genotypic diversity, as shown by $H' = -\Sigma p_i \ln p_i$ where p_i is the frequency of

Table 4. Frequency distributions of five-locus isozyme genotypes arranged by their Indica-Japonica discriminant score of key characters (X_1)

Genotype[a]	Control								Hill cultivars							
	Discriminant score								Discriminant score							
	1 (J)	2 ←	3	4 X_1	5	6 →	7 (I)	%[b]	1 (J)	2 ←	3	4 X_1	5	6 →	7 (I)	%[b]
A					3	13	4	19.8 (+18.8)			1	2	10	11	2	17.2 (+10.7)
B													1			0.7 (−0.7)
C					3	4	2	8.9 (+7.0)		1	1		10	6		11.9 (+6.8)
D					2	1		3.0 (+0.9)			1	4	2	1		5.3 (−4.3)
E							1	1.0 (−1.2)								
F										1				2		2.0 (+0.2)
G					2			2.0 (+0.4)				2	5	2		6.0 (−0.4)
H										1						0.7 (−0.9)
I						1		1.0 (−4.2)								
J					1	3		4.0 (+0.2)		1	3	3	9	2		11.9 (+4.4)
K							1	1.0 (−2.2)		1			1			1.3 (−3.7)
L				1				1.0 (+0.6)			1	5				4.0 (+1.3)
M				1	5	1		6.9 (+3.7)			4	13	6	1		15.9 (+6.5)
N						1		1.0 (−1.0)								
O				1				1.0 (−7.7)					1			0.7 (−1.4)
P												1				0.7 (−6.7)
Q													1			0.7 (+0.2)
R										1		1			1	2.0 (+0.5)
S													1			0.7 (+0.2)
T											2	2	3	1		5.3 (+4.9)
U													1			0.7 (+0.6)
V	26	16	8					49.5 (+42.1)	5	4	3	2	3	1		11.9 (+11.4)
W														1		0.7 (+0.6)
Total	26	16	8	3	16	24	8		5	10	16	35	54	28	3	

[a]	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
Cat-1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	1	1	1	1	2	2	2	2	2
Acp-1	1	2	1	1	2	2	1	1	2	1	1	2	1	1	2	1	2	1	2	1	2	2	2
Pgi-1	1	1	1	1	2	1	2	1	1	1	2	2	2	1	2	2	2	2	1	2	2	2	2
Pgi-2	2	1	1	2	2	2	2	2	1	1	1	2	2	1	1	1	4	4	1	4	4	1	4
Pox-2	1	1	1	0	1	1	1	1	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0

[b] Percentage of cultivar number: figures in the parenthesis indicate deviation from expected values estimated by the product of frequencies of component alleles

the *i*th isozyme genotype, was 2.47 for the Indica ($X_1 > 4.0$) and 0.69 for the Japonica ($X_1 < 4.0$) group.

In the hill cultivars, 20 different genotypes were found showing a genotypic diversity $H' = 3.04$ (for the control cultivars $H' = 2.21$). The two genotypes, A and V, had higher frequencies than was expected similar to the control. However, the deviations from expectation in respective genotypes were generally smaller than those in the control, suggesting that the isozyme genes tend to be randomly associated in the hill cultivars. The Indica and Japonica groups classified by X_1 (key characters) showed similar levels of diversity of isozyme genotypes ($H' = 2.97$ for Indica and $H' = 2.49$ for Japonica).

The discriminant scores X_2 based on six isozymes showed a bimodal distribution in the control (Fig. 2a). Cultivars having scores smaller than 0.4 were considered to be Japonicas and those having larger scores as Indicas. In the hill cultivars, the distribution of X_2 scores also showed a bimodality though not so distinct as in the control (Fig. 2b).

As shown in Table 4, Indica-Japonica variation in key characters as well as variation in isozymes corresponded well with each other in the control, but not in the hill cultivars. To look more closely into the relationships between variations in each isozyme and diagnostic character, differences in character values between carriers of different isozyme alleles were tested by *t*-values (or R^2 for isozymes versus phenol reaction). The results are given in Table 5. In the control, 29 out of 32 comparisons showed significant differences. In the hill cultivars, however, *t*-values and R^2 values were generally smaller than in the control, and only 16 out of 40 comparisons were signifi-

Table 5. Significance of association between isozymes and character measurements. *t*-values (χ^2 for phenol reaction) were tested

Alleles compared		Control character[a]				Hill cultivars character[a]			
		K	C	H	P	K	C	H	P
Cat-1	1:2	**	**	**	**	**	**	NS	**
Acp-1	1:2	**	**	**	**	**	NS	**	**
Pgi-1	1:2	**	**	**	**	**	NS	NS	**
Pgi-2	1:2	**	**	**	**	NS	NS	NS	NS
	1:4					NS	NS	NS	NS
	2:4					NS	NS	NS	NS
Pox-2	0:1	**	**	**	**	**	**	NS	**
Est-2	0:1	NS	**	**	*	NS	NS	NS	*
	0:2	**	**	**	**	NS	NS	**	**
	1:2	**	NS	NS	**	NS	NS	**	*

*,** Significant at 5% and 1% levels, respectively; NS, nonsignificant

[a] K, $KClO_3$ susceptibility; C, low temperature susceptibility; H, Apiculus hair length; P, phenol reaction

Table 6. Intra-population diversity observed in eight landrace populations in Indica-Japonica discriminant scores combining key characters (X_1) and isozymes (X_2)

Population	Altitude (m)	Character							Isozyme						r[c]
		J 2	← 3	X_1 4	5	→ 6	I 7	Concordance[b]	J 0.1	← 0.3	X_2 0.5	→ 0.7	I 0.9	R^2	
C9019	1900		1	8	4			0.03	4	14				0.00	0.21
C9003	1770	1	4	1				0.37	19					0.00	0.00
C9001	1750			2	6	4	2	0.33		15				0.02	0.05
C9002	1750		1	6	7	7		0.09		19	1	1		0.07	0.15
C9018	1500				3	26	3	0.08				31		0.20	0.07
C9050[a]	600	1	4		4			0.15				5	1	0.03	−0.69
C9043[a]	600	1	4	6		4		0.19				2	17	0.00	0.12
C9042	500			1	3	14	1	0.11				18	6	0.21	−0.53

[a] Upland field

[b] See text

[c] Correlation coefficient between X_1 and X_2 scores

cant. The carriers of *Acp-1*[1] tended to have longer apiculus hair than the *Acp-1*[2] carriers, showing an opposite tendency of association to that found in the control.

Within-populational diversity in the hill cultivars

Eight landrace populations studied for intra-populational variation showed polymorphism in varying degrees. To examine whether or not they show a trend of Indica-Japonica differentiation within each population, the same characters and isozymes as used for the cultivar survey were investigated using progeny lines, each line representing an individual in the farmers' fields. Two Indica-Japonica discriminant scores, X_1 and X_2, were then computed for each line. As shown in Table 6, each population showed a wide range of X_1 score (key characters). Their variation ranges overlapped, and the differences among populations were of a lower magnitude. The component characters did not show any specific association with each other, their correlations being insignificant. It is shown by average of all correlation coefficients (concordance) between four characters in each population in Table 6.

As judged from X_2 scores, the intra-populational variation in isozymes was relatively small. Individuals from the same population were either Indica-like or Japonica-like types. Within each population, though a certain degree of polymorphism exists, the alleles at different loci were not associated with each other in a particular manner as shown by small R^2 values. The two scores, X_1 and X_2, were not significantly intercorrelated with each other among individuals within the same population except for one population (C9042). The above results Indicate that Indica-Japonica variation in key characters and that in isozymes are not associated with each other among and within populations.

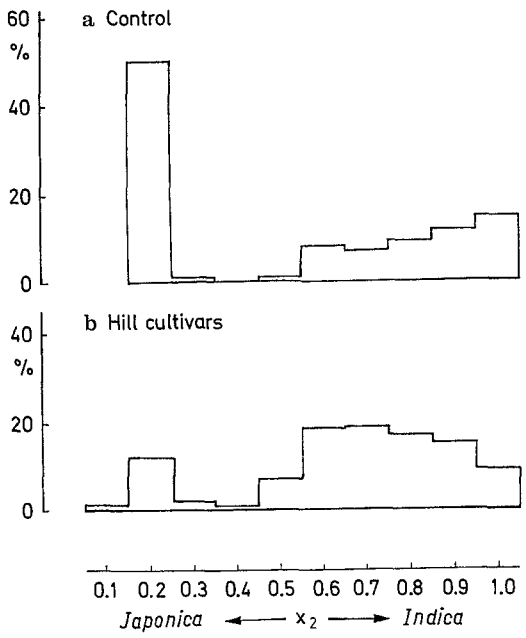

Fig. 2a, b. Frequency distributions of Indica-Japonica discriminant scores (X_2) combining six isozymes

As seen in Table 6, multilocus isozyme variation or X_2 score variation showed a clear altitudinal cline; Japonicas were found at higher altitudes and Indicas at lower altitudes (bordering each other at approximately 1500–1700 m altitude). In key characters, such a relationship was obscured. One from highland paddy (C9003) and two from upland fields at lower altitudes (C9043 and C9050) seemed to approach Japonica types, and others were intermediate or Indica-like.

Discussion

Multilocus analysis of isozyme variation in Asian rice cultivars Indicated that allele associations among loci are nonrandom and bring about a particular trend of gene combinations. Hedrick et al. (1978) used the term "gametic disequilibrium" instead of "linkage disequilibrium" for the nonrandom association of alleles at two or more loci since such phenomenon can occur even between unlinked loci. In fact, among the seven marker loci that showed strong associations in the present study (six isozymes and *Ph* for phenol reaction), only two pairs are known to be loosely linked: *Pgi-2* and *Est-2* with 13% (Sano and Barbier 1982) and *Acp-1* and *Pox-2* with 24% (Pai et al. 1975). The highest R^2 value was found between *Acp-1* and *Cat-1*, which are located on different chromosomes, 12 and 6, respectively. A major variation uncovered by multilocus isozyme studies corresponded well to a variation between the Indica and Japonica types that have been classified by key character association pattern (Second 1982; Glaszmann 1986, 1987; present study). The core issue to be argued is whether such nonrandom association observed in genes and characters is the product of some kind of selection (coadapted genetic change) or is it due to founder effect, or a combination of both. A possible role of gametic selection in forming nonrandom association was suggested by Sato et al. (1990). Yet, an understanding of the whole picture has to be left for future studies.

Cultivars collected in the Himalayan hilly areas showed a large amount of genetic variability comparable to that found among cultivars from broader areas of whole Asia. This result supports the view that the areas along the Himalayas and its associated ranges form a diversity center of Asian rice (Morinaga 1968; Vairavan et al. 1973; Nakagahra 1978; Glaszmann 1987). The genetic diversity of crops could have been accumulated in a particular region under various conditions, such as antiquity of cultivation, dissemination of cultivars by man, natural hybridization and isolation followed by selection under different climatic and ethnological conditions. The areas concerned seem to meet all these conditions. Thus, a diversity center is not necessarily the place of first domestication.

Whether Indica-Japonica differentiation has occurred after (monophyletic hypothesis) or before (diphyletic hypothesis) the start of domestication has been repeatedly argued. The monophyletic hypothesis is based on the potential of the wild progenitor to evolve both the Indica and Japonica types (Oka and Morishima 1982). Recent molecular genetic studies, however, seem to support the diphyletic hypothesis or independent origins of the Indica and Japonica types by demonstrating clear differences between the two types not only in nuclear DNA (RFLP: Wang and Tanksley 1989; Tanaka et al. 1989; rDNA: Sano and Sano 1990) but also in organellar DNA (mtDNA: Kadowaki et al. 1988; cpDNA: Ishii et al. 1988; Dally and Second 1990).

The geographical distribution and origin of the Indica-Japonica intermediate cultivars might be important to consider with respect to the differentiation process of the two types. The present survey Indicated that the cultivars distributed in the Himalayan hilly areas were highly diverse in characters as well as in genes. As to Indica-Japonica differentiation, however, key characters and isozymes revealed somewhat different variation patterns. For characters, the frequent occurrence of atypical cultivars which can not be classified either as Indica or Japonica type is a characteristics of these hilly areas. When ordered on the Indica-Japonica axis, many hill cultivars occupied intermediate positions. This is not always because they show intermediate measurements in each character, but often because they are recombined types of typical Indica and Japonica characteristics. On the other hand, for isozymes, most hill cultivars could be

classified either as Indicas or Japonicas, although non-randomness of allelic association was much lower than in the control. It should be noted that in a multivariate analysis of isozymes by Glaszmann (1987) four minor groups (II–V), which include many hill rice and deep-water rice, were either Indica-like of Japonica-like on the first axis and separated from typical Indica and Japonica groups only by the second axis. They were atypical, but not truly intermediate between the Indica and Japonica types.

It seems difficult to determine whether these intermediate or atypical cultivars are secondary derivatives from natural hybridization between the Indica and Japonica types, or transitional primitive types before differentiation, or whether they are differentiating along different lines other than that of Indica-Japonica variation. The role of natural hybridization between the Indica and Japonica types can not be ruled out to explain the occurrence of intermediate types, as asserted by Second (1982). Dally and Second (1990) found that intermediate cultivars mostly carry Japonica-specific plastotype, and they considered this fact as supporting evidence for hybridization in the past. There is also circumstantial evidence to support the possibility of natural hybridization between different cultivars in these areas; varietal dissemination through caravan routes from India to Tibet, traditional mix-planting of two or more different cultivars and coexistence of the Indica and Japonica types in the area. But to what extent natural hybridization can explain the origin of numerous "intermediate" cultivars widely distributed in a broad area remains unsettled.

Genetic architecture of the wild progenitor of the Asian cultigen (*O. rufipogon*) is an another important issue to be considered. This wild species is highly polymorphic among and within populations (Second 1985; Oka 1988 p. 67–72). In the context of Indica-Japonica variation, the wild populations are mostly intermediate types (Morishima and Gadrinab 1987). Further, particular isozyme alleles specifically carried by the landraces distributed in the diversity center, such as *Pgi-2*^{4} and *Amp-3*^{5}, are generally rare in the cultivars, but not rare in wild rice populations. It can be inferred that the intermediate cultivars were derived directly from the wild ancestor. Yet, this assumption does not necessarily exclude the independent origins of the Indica and Japonica types. It seems more plausible that domestication occurred at multiple sites.

The differential distribution of Indica and Japonica types in high and low altitudes as reported in this study is known in many other regions (Yunnan, Anonymous 1974; Northern Thailand, Oka and Chang 1963; Nepal, Sano et al. 1985; Bhutan, Morishima et al. 1991). In investigating the altitudinal cline of landraces, the significance of the ethnological factor of the growers and hydroedaphic condition (upland or lowland) should be taken into account. Such studies might uncover the relative importance of founder effect and selectional effect in Indica-Japonica differentiation.

Acknowledgements. We are grateful to Dr. H. I. Oka for critically reading the earlier manuscript.

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