Jpn. J. Genet. (1982) 57, *pp.* 25–57

Origin of the genic diversity of cultivated rice (*Oryza spp.*): study of the polymorphism scored at 40 isozyme loci[1)]

BY Gerard SECOND

Laboratoire de Génétique, Centre d'Adiopodoumé, ORSTOM, B.P. V51, ABIDJAN, Côte, d'Ivoire

(*Received July* 24, 1981)

ABSTRACT

An electrophoretic survey of up to forty presumed isozyme loci was carried out in Asian and African cultivated rice (*O. sativa* and *O. glaberrima*) and in weedy and wild forms of African *O. breviligulata* on a total of 1,948 strains. Hidden variability was checked by a test of heat stability at thirty isozyme loci in the same species.

The mean gene diversity index ("heterozygosity") was high (0.23) in *O. sativa*, medium (0.14) in wild *O. breviligulata* and low (0.06 and 0.03) in weedy *O. breviligulata* and *O. glaberrima*.

In contrast, a maximum of seven alleles at a single locus could be distinguished in wild *O. breviligulata* while only three at the most were found in *O. sativa* and two in *O. glaberrima* and the weedy forms of *O. breviligulata*.

Calculations of genetic distances showed that the cultivated, wild and weedy African species formed a genetic group distinct from *O. sativa*. Multivariate analysis of the data on an individual strain basis confirmed this fact and showed in turn that most varieties of *O. sativa* tended to cluster in two groups which correspond to the so-called *Indica* and *Japonica* subspecies. There was however a continuous array of intermediates between the two groups.

Multivariate analysis also showed that the endemic African strains with the most genetic affinity to *O. sativa* were certain strains of the weedy form of *O. breviligulata*.

Analysis of F_1 pollen sterility among 115 strains of *O. sativa* permitted the extraction of one small *Indica* and one small *Japonica* group of strains characterized by a high pollen sterility relationship but most strains were intermediate.

Each group had little gene diversity, with more than 80% of the loci fixed for one allele. Their isozyme patterns were remarkably complementary in that most of the various gametic associations of alleles found in the numerous intermediate strains could be explained by hybridization between varieties belonging to one and the other group. Consequently, these were assumed to represent the ancestral isozyme patterns of the *Indica* and *Japonica* subsp.

Similar genetic distances, which point to a divergence time of one to a few millions years ago were found between *O. glaberrima* and the ancestral types of *Indica* and *Japonica* in the three combinations.

Assuming the neutral theory of isozyme polymorphism, the data confirmed

1) This work was completed and the paper was written during a stay at the National Institute of Genetics, Mishima, Shizuoka-ken, 411 (Japan).

Present address: Laboratoire de génétique, ORSTOM, 70-74, route d'Aulnay 93140 BONDY (France).

that *O. glaberrima* was domesticated from *O. breviligulata* independently of *O. sativa*. They also suggest that, at the origin of *O. sativa*, the *Indica* and the *Japonica* types were also domesticated independently. The large diversity of *O. sativa* would result: (i) primarily from introgressions that occurred between the ancestral cultivated types, together with the selection imposed by man, and (ii) secondarily, following the dispersion of cultivars, from the introgression of genes of wild rice in various areas. Some of the weedy forms of *O. breviligulata* could have originated from natural hybrids between the two cultivated species, *O. sativa* and *O. glaberrima*.

1. INTRODUCTION

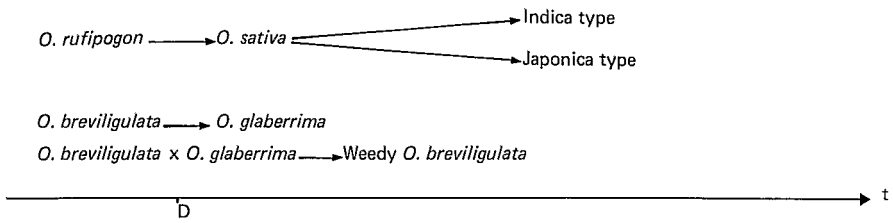
Cultivated rice includes two taxonomically distinct species: *Oryza sativa* L., found throughout the world and *O. glaberrima* Steud., whose economical importance is limited to West and Central Africa, from Sénégal to Sudan. With the wild species which have the diploid genome AA (Morinaga 1964), they form the *O. sativa* complex (Chang 1976a). *O. rufipogon* Griff. (=*O. perennis* Moench) in Asia is generally considered to be the ancestor of *O. sativa*, although the place(s) of origin and whether or not a particular form (annual or perennial) is the direct ancestor are not certain. On the other hand, *O. glaberrima* is closely related to the African species *O. breviligulata* A. Chev. et Roehr. (=*O. barthii* A. Chev.) (+) but their genetic affinity has been interpreted in quite different ways. According to Portéres (1950), Morishima *et al.* (1963), Oka (1974b) and Chang (1976b), *O. glaberrima* was domesticated from *O. breviligulata*. Others contend that *O. glaberrima* was introduced into Africa from Asia and *O. breviligulata* was a hybrid derivative either between *O. glaberrima* and *O. longistaminata* A. Chev. et Roehr (Richaria 1960) or between *O. glaberrima* and a more recent introduction of *O. sativa* in Africa (Nayar 1973). In order to remind the reader of the contradictory phylogenetic hypotheses proposed in the literature, Fig. 1 shows diagramatically three of the phylogenetic relationships previously proposed, along with the hypothesis that will be discussed in this paper.

The Chinese have traditionally distinguished "Keng" and "Hsien" rice but Kato (1930) was the first to recognize scientifically the two groups of varieties within *O. sativa* that he named *Japonica* and *Indica* subspecies. Terao and Mizushima (1939), Morinaga and Kuriyama (1958) showed later that no clear distinction between the *Indica* and *Japonica* types could be made since intermediates were found. Oka (1958) pointed out that no single character distinguishes the two types, but that strong character associations indicate a trend towards differentiation recognized by the above mentioned investigators.

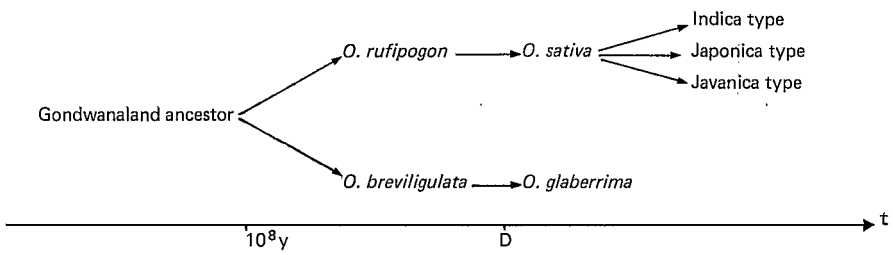
Further, Morishima (1969) showed that pathogenic races of *Piricularia*

(+) *O. barthii* A. Chev. may be the correct name (Clayton 1968) but has been used for many years to designate *O. longistaminata* A. Chev. et Roehr. I have kept the name used by Portères (1950) in order to avoid confusion.

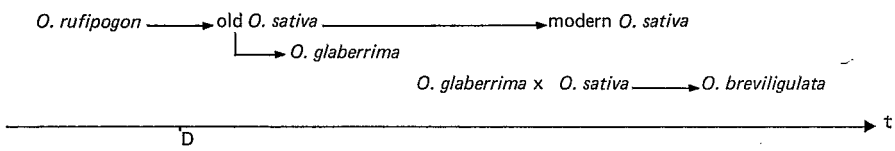
1) Adapted from Oka (1974) and Morishima *et al.* (1963). *O. sativa* may have been domesticated independently in various areas. Its differentiation is under selective forces in the course of domestication.



2) Adapted from Chang (1976b). The present distribution of wild rice is an outcome of the continental drift.



3) Adapted from Nayar (1973). *O. glaberrima* was introduced in Africa from Asia. *O. breviligulata* escaped from the cultivated fields following the introgression of genes of *O. sativa* into *O. glaberrima.*



4) Present paper. Three primary domestications. The differentiation of cultivated rice resulted in part from the geographical differentiation of races of *O. rufipogon.* Some of the weedy forms of *O. breviligulata* evolved through introgression of genes of *O. sativa* into *O. glaberrima.*

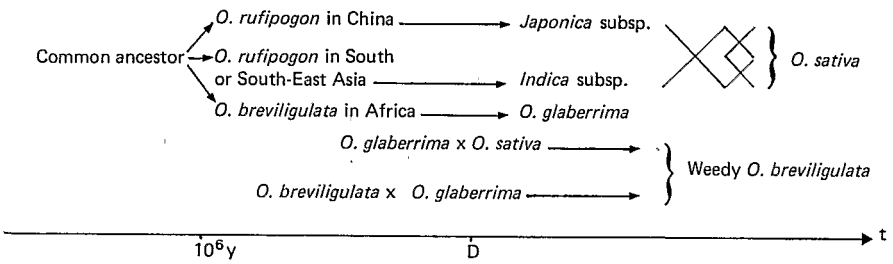


Fig. 1. Diagramatic representation of four proposed phylogenetic hypotheses for the material studied in this paper (*O. longistaminata* not considered). Brief mentions of the main controversial points are given for each of them. The horizontal axis stands for time in years (with no fixed scale). D represents the unknown time at which domestication occurred. ($D=3\times10^8$ to 10^5 years?)

oryzae were also differentiated into two groups according to the subdivision of *O. sativa* cultivars. In addition to this main subdivision of the species, numerous agroecotypes have been identified within *O. sativa* in its various regions of cultivation. They are grown in different areas, conditions or seasons (Chang 1976b).

Within *O. glaberrima*, on the contrary, no such classification into *Indica* and *Japonica* subsp. can be made and only two agroecotypes were clearly identified (Bezançon *et al.* 1977; Oka 1977): "floating" and "non floating". The former is found in inundated areas with a water level up to 3 or 4 m high, the latter is found in moderately inundated lowlands, irrigated fields or upland (rainfed) conditions.

O. breviligulata is endemic in Africa. It is found both as a weed in cultivated fields or recent fallows (in the area of distribution of *O. glaberrima* only) and as a wild species in the savanna zones from Sénégal to Botswana. Its usual habitat is in temporary (rainfed) pools or marshes, generally isolated from cultivated fields.

This report is part of an investigation on genetic polymorphisms of wild and cultivated rice using an isozyme survey which was initiated in order to look into the relationship between African and Asian cultivated rice and the origin of *O. breviligulata*, as well as to furnish a rationale for the conservation and utilization of rice genetic resources.

The present paper aims to suggest: (i) the domestication of *O. glaberrima* from *O. breviligulata*, independently of *O. sativa*, (ii) the origin of *O. sativa* in two independent domestications in Asia, and (iii) the introgression of *O. sativa* into *O. glaberrima* to derive some weedy forms of *O. breviligulata*.

2. MATERIALS AND METHODS

Samples were obtained from collections of genetic stocks kept by the Office de la Recherche Scientifique et Technique Outre-mer (ORSTOM) in Abidjan, Ivory Coast, and by the National Institute of Genetics (NIG) in Mishima, Japan. They are of various origins: First, 515 strains of *O. glaberrima* and 965 strains of *O. breviligulata* were observed. Most of them were the original seeds collected in wild populations or traditional fields during the field surveys and collections conducted in numerous countries by ORSTOM and the Institut de la Recherche Agronomique Tropicale (IRAT) from 1974 to 1979, and by the team led by Dr. H.I. Oka (NIG), in Nigeria in 1977. A few of them are from the collections of the NIG including the countries of Guinea and Sierra Leone. They represent 133 populations of wild *O. breviligulata*, 137 weedy populations of *O. breviligulata* in fields or fallows and 152 populations from traditional fields in which *O. glaberrima* was cultivated in pure stand or mixed with *O. sativa*. Second, 468 strains of *O. sativa* were examined. Ninety-five of them

were upland cultivars of the IRAT collection in Ivory Coast, they represented all groups classified by M. M. Jacquot (IRAT) and all geographical origins in this collection; 20 were from the IRAT collection in Madagascar; 22 were from the temperate countries collection of the Institut National de la Recherche Agronomique (INRA), Montpellier, France; 18 were from a collection of the National Institute of Agricultural Sciences (NIAS), Tsukuba, Japan, chosen to represent the eleven genotypes identified in a study of 1,095 native cultivars at three loci coding esterase isozymes (Nakagahra 1977); 70 were taken at random from a collection of the NIAS from Europe, the Middle-East and Central Asia; 170 were from the genetic stocks of the NIG, of which 158 were the strains used as testers by Oka (1958); 73 were from original seeds collected in various African countries.

Most of the electrophoretic and hidden variability surveys took place in the ORSTOM laboratories at Abidjan from 1975 to 1979. Additional surveys of some strains from the NIG and NIAS collections, and the distinction between the esterase bands B^1 and B^2 were made at the NIG in Mishima.

The experimental procedures followed at Abidjan were described in detail by Trouslot and Second (1980). They can be summarized as follows:

Plants were individually grown in pots. Depending on the locus surveyed, chlorophylliferous or non-chlorophylliferous parts of a growing leaf or the blade of a developed leaf were sampled from a plant at the tillering or flowering stage. Crude extracts were obtained by homogenization in a bit of distilled water, absorbed in filter paper strips and immediately subjected to horizontal starch gel electrophoresis at 10°C, the plates being covered with ice. The following 13 enzymes were studied: alcohol dehydrogenase (ADH), glutamate dehydrogenase (GDH), malate dehydrogenase (MDH), isocitrate dehydrogenase (ICD), 6 phosphogluconate dehydrogenase (PGD), catalase (CAT), peroxidase (POX), glutamate-oxaloacetate transaminase (GOT), phosphoglucomutase (PGM), esterase (EST), acid phosphatase (ACP), leucine amino peptidase (LAP) and phosphoglucose isomerase (PGI). For ACP, flag leaves were used and the buffer system was as described in Pai *et al.* (1975). For other enzymes, a discontinuous histidine-sodium citrate buffer was used, at pH6.0 or 8.0 depending on the enzyme examined (Histidine HCl 5mM, 2.5mM NaCl added, pH adjusted with NaOH in the gels; Citric acid 0.4M, pH adjusted with NaOH in the trays). Electrophoresis was conducted for 5 to 6 hours with a constant voltage of 8.5V/cm.

For the examinations made at Mishima, the following modifications were adopted: for pH 8.0 buffer in the gels, NaCl was not added and pH was adjusted with Tris (hydroxy) aminomethane (Tris) to pH 7.5 at 27°C (about pH 8.0 at 2°C). In the trays, Tris 0.4M was adjusted to pH 7.5 with citric acid; 15% gels were prepared with starch hydrolyzed in Dr. T. Endo's laboratory. Similar results were obtained with overnight (16h) migrations using

the same buffer 3 times more concentrated (and no NaCl added) in the gels and a constant current so as to have a voltage of 3 or 4V/cm at pH 6.0 and 8.0, respectively, at the starting time. The electrophoresis was conducted at 2°C. For the surveys of ADH, GDH, MDH, ICD, PGD, CAT, GOT-A and C, PGM, EST-E, LAP-E, PGI bands in Mishima, fresh extracts of coleoptile and plumule from 3 to 5 days old seedlings were used. Three to 6 slices, 1.5mm thick, were used out of a 6 to 12mm thick starch plate. For the survey of EST-B^1 and B^2 bands at pH6.0, extracts from fully developed leaves (tillering stage) preserved at −20°C for 2 months were used. EST-B^1 and B^2 phenotypes were double banded. B^1 was slightly faster than B^2.

Hidden variability was revealed by testing the heat stability of the isolated isozymes. The same extracts were inserted in 4 to 6 plates and electrophoresis was conducted in the usual way. Only the bottom halves of the plates were used. Before staining, they were placed in polyethylene bags and immersed for 20 minutes in a range of water baths with temperatures varying by 3°C steps. Only clear differences in band intensities between plates treated by different temperatures were recorded. As shown by dilution tests they could not be accounted for by small differences in the initial activity. LAP, GDH and GOT were not surveyed for heat stability for technical reasons.

The genetic interpretation of the bands was established by direct comparison of the zymograms revealed with autogamous and allogamous rice species; the principles previously outlined (Second and Trouslot 1980a) were followed. The system of nomenclature is one capital letter for the locus and one superscript number for the electromorph. The same nomenclature was used for the presumed homomeric bands and their relative electromorphs. Roman capitals were used to denote the bands and italics to denote the genetic locus.

The theoretical distributions of single-locus heterozygosity for the observed values of the mean heterozygosities were computed from the distributions given in Fuerst *et al.* (1977 Table 1). The observed mean heterozygosities fell between the values for which theoretical distributions were generated. As the theoretical expectations appeared to be nearly proportional to the mean heterozygosity they were linearly extrapolated from the two adjacent distributions given in Fuerst *et al.* (1977).

F_1 pollen fertility was measured by staining with an iodine solution. The original data compiled by Oka (1958) were made available through the courtesy of Dr. Oka. Factorial correspondence analysis (Benzecri 1973) and principal component analysis were performed with computer programs. Correspondence analysis is a principal component analysis in which the use of a distance of χ^2 in place of correlation coefficients leads to give much weight to rare states of a character as well as to the cases in which a character is found in a rare association. The computations are made on the basis of qualitative states of characters. Apart from that, graphic representations of both analyses are

interpreted in the same way (Pernès 1975). Genetic identity will be read in terms of proximities, keeping in mind that representations on the planes are projections from a multidimensional space.

3. RESULTS

1. *Electrophoretic data*

A total of 40 presumed enzyme loci were identified. All of them were reported to be polymorphic among the *O. sativa* sp. complex and all observed zymograms have been previously described, with an indication of their frequencies (Second and Trouslot 1980a). Seven loci were monomorphic in the present material (*Adh-A, Gdh-B, Mdh-B, Pgd-B, Pgm-A, Lap-B* and *D*). One was polymorphic in heat stability only (*Mdh-C*). The remaining 32 were polymorphic for electrophoretic mobility and 6 of them (*Mdh-A* and *-B, Cat-A, Est-F, Pgi-A* and *B*) were also polymorphic for heat stability. One to four active electromorphs (class of alleles with a common phenotype in electrophoresis, King and Ohta 1975) were identified at each locus with an average of 2.0 per locus. Fourteen (35%) of the loci had a presumed silent electromorph.

At 13 of the 40 presumed loci, Mendelian segregations were observed: *Acp-B* and *C* loci are identical to *Acp-1* and *2*, respectively, as identified by Pai *et al.* (1975). *Est-D, E* and *J* are assumed identical to *Est-1, 2* and *3*, respectively, as identified by Nakagahra (1977), because the same bands were found in the same varieties although the electrophoretic procedures were different. Mendelian segregations in F_2 populations of 80 to 360 plants were observed (Second and Trouslot, unpublished data; Second and Morishima 1981b) for the following loci and electromorphs; *Mdh-A*^{1} and *A*^{2}, *Cat-A*^{1} and *A*^{2}, *Pox-B*^{1} and *B*^{2}, *Est-D*^{1} and *D*^{2}, *H*^{1} and *H*^{2}, *I*^{1} and *I*^{2}, *Lap-E*^{1} and *E*^{2}, *Pgi-A*^{1} and *A*^{2}, *A*^{2} and *A*^{3}, *B*^{1} and *B*^{2}.

For all the remaining loci and the most frequent electromorphs, heterozygotes were observed in artificial F_1 hybrids. Active electromorphs were found to be codominant whereas silent electromorphs were recessive. In the case of bands PGI-A^1 and A^3 which differ by a slight mobility difference, the heterozygote was observed in F_1 hybrids (male sterile) between *O. glaberrima* and *O. sativa*; as expected, there was a single intermediate band, more diffused than the parental one. The PGI-A^3 band, never found in native cultivars of *O. sativa*, has been observed in an isogenic line of *O. sativa* with a sterility factor introgressed from *O. glaberrima* (Second and Sano 1981).

The material surveyed here was most frequently found homozygous as it would be expected from its predominantly self-pollination reproductive system. Presumed intergenic complementation (Scandalios 1979) was observed with hybrid isozymes (heteromers) in homozygous individuals in 3 cases: be-

tween *Mdh-A* and *B* loci and *Pgi-A* and *B* loci, with one hybrid band, and between *Gdh-A* and *B* loci with 5 hybrid bands, in accord with the presumed dimeric or hexameric secondary structure of these enzymes found in several other plant and animal species.

Electromorphs with a single-band phenotype in the homomeric state (no secondary isozyme), showed a heterozygote phenotype with 2, 3 or 5 bands according to the secondary structure of the enzyme, presumed or known in other species: monomeric (EST-E and J, LAP-E, PGM-A), dimeric (ADH-A, MDH-A, B and C, POX-E, GOT-A, PGI-A and B), or tetrameric (CAT-A).

2. *Gene diversity and genetic distances*

On the basis of the taxonomic characters of the plants and the agroecological situation of the original populations, 4 groups were distinguished *a priori* in our material, as follows.

Group:	Taxonomic characters:	Agroecological situation:
O. glaberrima (G):	Short and truncate ligule, weak or no spontaneous shedding	In cultivated fields, sometimes in recent fallows
Weedy *O. breviligulata* (Ba):	Short and truncate ligule, spontaneous shedding, long awn	In cultivated fields or recent fallows
Wild *O. breviligulata* (Bs):	*ib.*	In the wild, generally in temporary pools
O. sativa (S):	Long, acute ligule, no spontaneous shedding	In cultivated fields, rarely in recent fallows

Table 1 presents for each locus surveyed: 1/ the number of active electromorphs and whether or not a silent electromorph was scored, 2/ a gene diversity index ($\overline{H}$=one minus sum of squares of electromorphs frequencies= "heterozygosity") computed in the four groups of strains (hidden variability not included). 3/ Presence or absence of hidden variability revealed by heat stability and in which group.

O. sativa appeared to be the most diverse group ($\overline{H}$=0.23) followed by wild *O. breviligulata* ($\overline{H}$=0.14), weedy *O. breviligulata* ($\overline{H}$=0.06) and *O. glaberrima* ($\overline{H}$=0.03).

The distributions of single-locus gene diversity within groups were compared to their expectations under the mutation drift hypothesis as computed in Fuerst *et al.* (1977). They are shown in Fig. 2 for *O. sativa* and the wild *O. breviligulata* group with a high average heterozygosity. For the three African

Table 1. *Number of active electromorphs, whether or not a null electromorph and hidden variability were revealed, and gene diversity at 40 loci in 4 ecotaxonomic groups of rice strains*

Locus	A.E.	N.E.	H.V.*	Gene diversity: H**			
				G	Ba	Bs	S
Adh-A	1	−	−	0 (121/ 62)	0 (87/ 14)	0 (346/110)	0 (70)
Gdh-A	2	−	/	0 〃	0 〃	0 〃	.02 〃
B	1	−	/	0 〃	0 〃	0 〃	0 〃
Mdh-A	3	−	+ (S)	0 (220/124)	0 (256/107)	.04 (518/129)	0 (303)
B	1	−	+ (Bs)	0 〃	0 〃	0 〃	0 〃
C	1	−	+ (Bs)	0 〃	0 〃	0 〃	0 〃
Idh-A	2	−	−	0 (40/ 40)	0 (40/ 40)	0 (30/ 15)	.04 (61)
Pgd-A	3	−	−	0 (10/ 10)	.25 (12/ 12)	.50 (25/ 25)	.36 〃
B	1	−	−	0 〃	0 〃	0 〃	0 〃
Cat-A	2	−	+ (Bs)	0 (15/ 15)	0 (15/ 15)	0 (32/ 32)	.50 (315)
Pox-A	2	−	−	0 (515/152)	.02 (473/137)	.06 (479/120)	0 (181)
B	4	−	−	.04 〃	.18 〃	.16 〃	.41 〃
C	1	+	−	0 〃	.01 〃	0 〃	.13 〃
D	2	−	−	0 〃	0 〃	.01 〃	.02 〃
E	2	−	−	0 〃	0 〃	.29 〃	0 〃
Got-A	2	−	/	0 (78/ 41)	0 (63/ 35)	.11 (180/ 66)	0 (181)
B	2	+	/	0 〃	0 〃	.14 〃	.18 (109)
C	2	−	/	0 〃	0 〃	.04 〃	0 (181)
Pgm-A	1	−	−	0 (12/ 12)	0 (12/ 12)	0 (32/ 32)	0 〃
Est-A	1	+	−	0 (515/152)	.13 (473/137)	.39 (479/120)	0 (181)
B	2	+	−	0 (10/ 10)	0 (12/ 12)	0 (12/ 12)	.56 (75)
C	2	+	−	0 (515/152)	.01 (473/137)	.10 (479/120)	.47 (181)
D	3	+	−	0 〃	.12 〃	.06 〃	.37 〃
E	2	+	−	0 〃	.01 〃	.01 〃	.62 (357)
F	2	+	+ (S)	0 (120/ 60)	0 (130/ 70)	0 (75/ 50)	.50 (41)
G	1	+	−	0 (515/122)	0 (473/137)	0 (479/120)	.48 (181)
H	2	+	−	.48 〃	.38 〃	.44 〃	.45 (94)
I	3	+	−	.22 〃	.42 〃	.38 〃	.54 〃
J	2	+	−	0 (10/ 10)	0 (12/ 12)	0 (12/ 12)	.52 〃
Ca	2	−	−	0 (515/152)	0 (473/137)	0 (479/120)	.24 (181)
Acp-B	3	−	−	0 (80/ 80)	0 (35/ 35)	.18 (60/ 60)	.48 (190)
C	1	+	−	.03 〃	0 〃	.48 〃	.47 〃
E	3	−	−	0 (10/ 10)	.28 (12/ 12)	.60 (12/ 12)	0 (41)
Lap-A	1	+	/	0 (107/ 69)	0 (169/ 66)	0 (227/ 58)	.50 (130)
B	1	−	/	0 〃	0 〃	0 〃	0 〃
C	2	−	/	0 〃	0 〃	0 〃	.18 〃
D	1	−	/	0 〃	0 〃	0 〃	0 〃
E	3	−	/	.12 〃	.10 〃	.15 〃	.21 〃

(to be continued)

Table 1. *Continued*

Locus	A.E.	N.E.	H.V.*	Gene diversity: H**			
				G	Ba	Bs	S
Pgi-A	4	−	+ (Bs)	.18 (78/ 41)	.37 (74/ 52)	.50 (165/ 83)	.48 (319)
B	4	−	+ (Bs)	0 〃	0 〃	.39 〃	.45 〃
Mean:	2.0	.35 (+)		.03	.06	.14	.23
Variance				.008	.014	.038	.052

* A.E.: Number of active electromorphs per locus; N.E.: presence or absence of null electromorph; H.V.: presence or absence of hidden variability revealed (for all loci tested, at least the 60 strains described in paragraph III were analysed, the group in which H.V. was revealed is indicated in parentheses).
+: present; −: absent; /: not tested.

** H=1 minus sum of squares of electromorph frequencies (hidden variability not included). G=*O. glaberrima*, Ba=weedy *O. breviligulata*, Bs=wild *O. breviligulata*, S=*O. sativa*. In parenthesis, number of strains analysed per number of original populations (for *O. sativa*, each strain represents a different collection sample).

groups, the distributions remarkably well corresponded with the theoretical expectations. On the other hand, for *O. sativa*, the number of loci with a heterozygosity close to 0.5 was larger than expected. To test the significance of this deviation, the expected and observed number of loci within classes of heterozygosity values were computed in Table 2. Adjacent classes were combined so that all expected classes but the last one were in excess of 5. The

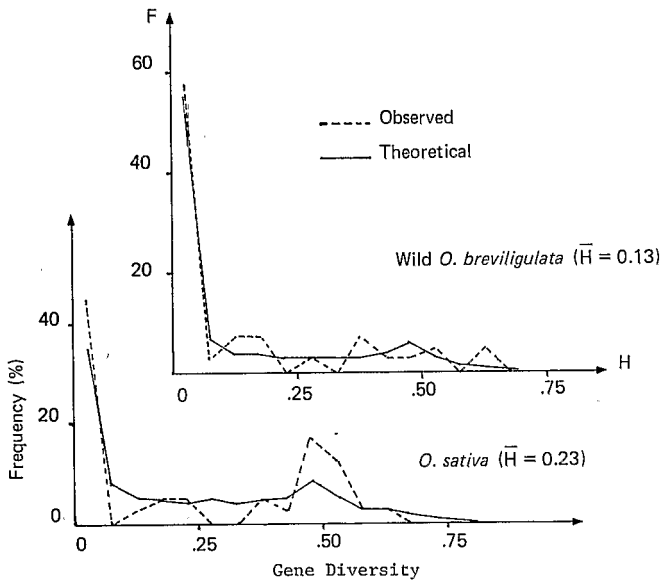


Fig. 2. Frequency distributions of single locus heterozygosity for *O. sativa* and *O. breviligulata*. The theoretical distributions correspond to those calculated by extrapolation from the values given in Fuerst *et al.* (1977).

Table 2. *Comparison of the observed distribution of single locus gene diversity for O. sativa with the theoretical expectations under the mutation-drift hypothesis with a constant rate of mutations per locus*

Gene diversity (h)	Expected Number of loci	Observed Number of loci
$0 < h < 0.05$	13.9	18
$0.05 < h < 0.15$	5.7	1
$0.15 < h < 0.30$	5.6	4
$0.30 < h < 0.45$	5.8	3
$0.45 < h < 0.55$	5.4	12
$h > 0.55$	3.7	2

$\chi^2 = 15.7$, significant at $P = 0.01$

The theoretical expectations are calculated by extrapolation for $\overline{H} = 0.23$ from the values given in Fuerst *et al.* (1977) for $\overline{H} = 0.20$ and 0.30.

Table 3. *Probability of identity of 2 randomly chosen electromorphs (J_{XY}, above diagonal) and genetic distance (D; Nei 1975, below diagonal) between 4 groups of rice classified on an ecologic and taxonomic basis*

J_{XY} / D	G	Ba	Bs	S
G: *O. glaberrima*		.955	.896	.690
Ba: Weedy *O. breviligulata*	.003		.891	.684
Bs: Wild *O. breviligulata*	.026	.017		.657
S: *O. sativa*	.225	.218	.217	

value of the χ^2 was found to be highly significant.

The amount of variation between the 4 groups was analysed using Nei's (1975) measurements of gene diversity. The matrces of Nei's probability of identity and genetic distance are given in Table 3. A dendrogram summarizing the genetic relationships was constructed from the genetic distances as shown in Fig. 3.

While the three African groups (wild, weedy and cultivated) were genetically close (D=0.026 or less), *O. sativa* appeared to be relatively distant from them (D=0.22). The group of weedy forms of *O. breviligulata* was very close to *O. glaberrima* (D=0.003) but, at 8 loci, it showed rare electromorphs not found in *O. glaberrima*. These could be classified in 3 classes as follows.

Class 1, electromorphs also found in wild *O. breviligulata* but not in *O. sativa*: *Pox-A*^{2}, *Est-A*^{1} and *D*^{2}, *Acp-E*^{2}.

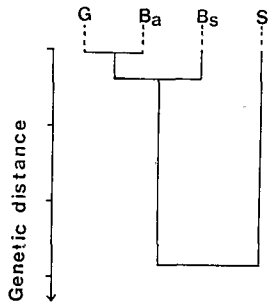


Fig. 3. Dendrogram constructed from the genetic distances found among four groups of rice strains classified on a taxonomic and ecologic basis. G=*O. glaberrima*. Ba= weedy *O. breviligulata*. Bs=wild *O. breviligulata*. S=*O. sativa*.

Class 2, electromorphs also found in *O. sativa* but not in wild *O. breviligulata*: *Pox-C*^{0}, *Est-C*^{2}.
Class 3, electromorphs also found in both *O. sativa* and wild *O. breviligulata*: *Pgd-A*^{3}, *Est-E*^{2}. Class 2 suggests the introgression of genes of *O. sativa* into weedy *O. breviligulata*.

3. *Hidden variability as detected by heat stability*

Sixty strains, including 41 *O. sativa*, 6 *O. glaberrima*, 7 weedy *O. breviligulata* and 6 wild *O. breviligulata* of various origins were chosen to represent maximum electrophoretic variability and the available agroecological and geographical origins. They were analysed with regard to the heat stability of the electrophoretic bands.

As shown in Table 1, electrophoretically hidden variability was detected at 7 out of 30 loci surveyed. For various reasons it must be considered as a minimal estimation useful for comparison between groups.

The genetic basis of the differential heat stability was the observation of F_1 heterozygotes and segregations among 5 to 15 F_2 plants (for *Pgi* and *Mdh* loci only). In all cases, the bands of the F_1, as well as the heteromeric bands in heterozygotes for electromorphs or in cases of intergenic complementation had an intermediate heat stability, as compared to the homomeric bands in homozygotes. Segregation into parental and F_1 phenotypes occurred in the F_2 generation. This suggests that the electrophoretic mobility and the heat stability are coded by the same loci and justifies the use of the term "electrothermomorph".

More strains were examined at the only loci at which the hidden variability had been found. A total of 27 electrothermomorphs were characterized at the 7 loci scored. Table 4 shows their distribution in the four ecotaxonomic groups with a total of 252 strains scored. No hidden variability was detected in *O.*

Table 4. *Distribution, in number of strains scored, of 27 electrothermomorphs among 4 groups of rice classified on an ecological and taxonomic basis*

Electrothermomorphs*	Number of strains per group**			
	G	Ba	Bs	S***
Est-F^{2}53°	—	—	—	13
Est-F^{2}50°	—	—	—	7
Cat-A^1 and *A*^{3}47°	32 (*A*^{1})	30 (*A*^{1})	65 (*A*^{1})	41 (*A*^{1} and *A*^{2})
Cat-A^{1}44°	—	—	3	—
Mdh-A^{1}62°	32	30	66	40
Mdh-A^1, *A*^{2} and *A*^{3}56°	—	—	2 (*A*^{2} and *A*^{3})	1 (*A*^{1})
Mdh-B^{1}62°	32	30	67	41
Mdh-B^{1}59°	—	—	1	—
Mdh-C^{1}56°	32	30	66	41
Mdh-C^{1}53°	—	—	2	—
Pgi-A^1, *A*^{2} and *A*^{3}59°	26 (*A*^{3})	24 (*A*^{3})	25 (*A*^{3})	122 (*A*^{1} and *A*^{2})
Pgi-A^2 and *A*^{4}56°	6 (*A*^{2})	6 (*A*^{2})	29 (*A*^{2} and *A*^{4})	—
Pgi-A^{1}56°	—	—	9	—
Pgi-A^1 and *A*^{2}53°	—	—	4	—
Pgi-A^{1}47°	—	—	1	—
Pgi-B^1, *B*^{2}, *B*^{3} and *B*^{4}56°	32 (*B*^{1})	30 (*B*^{1})	67 (*B*^{1} and *B*^{4})	122 (*B*^{1}, *B*^{2} and *B*^{3})
Pgi-B^{4}53°	—	—	1	—

* Electrothermomorphs are symbolized according to the electromorphs and the highest temperature (in °C) tolerated without noticeable loss of activity. (Only one electrothermomorph found in one group means no hidden variability found in it.)

** In parentheses: the electromorph concerned, when ambiguous.

*** G=*O. glaberrima*, Ba=Weedy *O. breviligulata*, Bs=Wild *O. breviligulata*, S=*O. sativa*.

glaberrima or weedy *O. breviligulata* while some were found at 6 loci in wild *O. breviligulata*. In contrast to its higher level of electrophoretic polymorphism, *O. sativa* had relatively little hidden variability, revealed at two loci only. As shown in Table 4, a maximum of nine electrothermomorphs could be distinguished at one locus; *Pgi-A*. Two were peculiar to *O. sativa*. The remaining seven were found in wild *O. breviligulata* and only two in *O. glaberrima* and weedy *O. breviligulata*. No electrothermomorph was shared by the African groups and *O. sativa* at the *Pgi-A* locus. In every case, the most common electrothermomorph within a species was also the most thermoresistant.

The hidden variability increased the genetic distance between the African species and *O. sativa* and the gene diversity for *O. sativa* and wild *O. breviligulata* (A=0.02 and 0.09 increase for $\overline{H}$, respectively, as calculated from the data of Table 3). On the whole, it did not alter the relative genetic identity between groups. The fact that *O. sativa* had a relatively small amount of hidden variability merits attention.

4. *Multivariate analysis of inter-group relationships*

Taking each strain as a taxonomic unit and the 40 loci with multiple alleles as 40 characters with different qualitative states, the scores of the 60 strains described in the previous paragraph 3 were treated by correspondence analysis (Benzecri 1973).

Calculations were made first including all polymorphic loci and secondly with the 25 loci polymorphic in cultivated rice only. Both led to essentially the same results and the representations with all loci involved will be presented here. Calculations were also made using all 60 strains or the 41 *O. sativa* strains only. The first three vectors (axes) extracted in both cases involved all loci listed in Table 6 and represent the isozymic differentiation of cultivated rice.

The distributions of the strains in the plane defined by the axes 1 and 2: 49.5 and 60.3% of the total variation in the case with all strains or with the *O. sativa* strains, respectively, are shown in Fig. 4 and 5. Most of the strains clustered in 3 groups: one group including *O. breviligulata* and *O. glaberrima*, and 2 groups comprising *O. sativa*. As shown in the figures, the phenol reaction (Oka 1958), of the strains suggested that the 2 groups in *O. sativa* correspond to the *Indica* and *Japonica* subsp. although there were numerous intermediates. Strains of *O. sativa* from the same geographical area did not always cluster together. In the *O. breviligulata*/*O. glaberrima* group, the phenol reaction was not associated with isozymic variation. One strain of weedy *O. breviligulata* was close to the *Indica* cluster. It has electromorph *Est-C*^{2} found otherwise only in *Indica* subsp. Both the wild and weedy groups of *O. breviligulata* appeared more variable than *O. glaberrima*. It must be remembered however that these few strains studied were chosen in order to account for maximum genetic diversity in each group.

Figure 5, with the *O. sativa* strains only, better shows the distribution of the intermediate strains between the *Indica* and *Japonica* clusters. Many strains belonging to the so-called Javanica type (from Indonesia, Philippines, Africa and America) and Asian type (from Southern China) fell in the intermediate area but were closer to the *Japonica* or *Indica* cluster, respectively. In the low values of axis 2, 2 varieties from Africa were found and show a particular band (PGI-B^3) common in *O. longistaminata*. This perennial African wild species was growing abundantly in the original fields of these *O. sativa* varieties in the inland delta of the Niger river. Although the same band was also found in *O. rufipogon* and in one cultivar from China, it is possible that the African cultivars absorbed some genes from *O. longistaminata*. Interestingly, the variety Chinsurah Boro II, from India, with a cytoplasm inducing male sterility in many Japanese cultivars (Shinjyo 1975), was close to these African cultivars although with a different band combination. Opposite to these in the high values of axis 2, were found one upland variety from Sri Lanka and two leading upland varieties from Ivory Coast (traditional variety

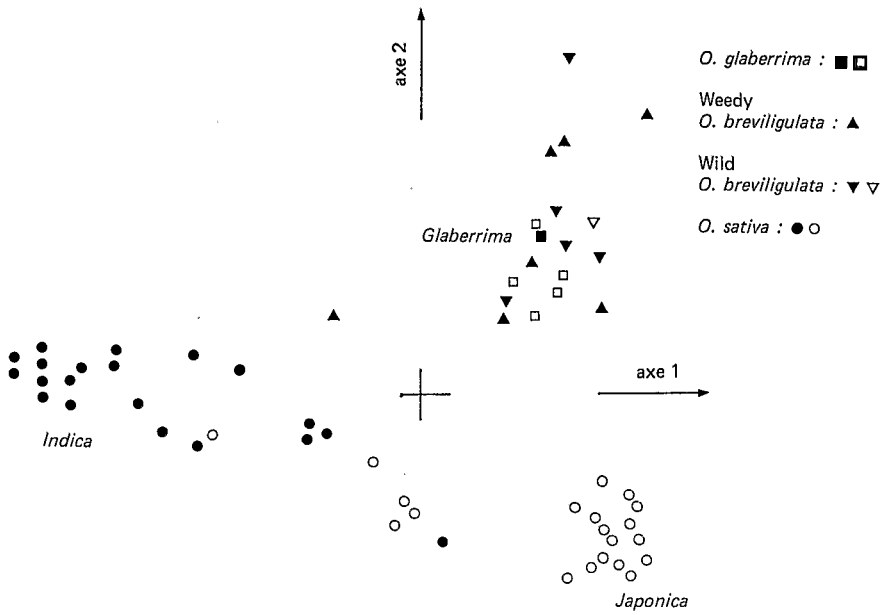


Fig. 4. Sixty strains of rice plotted in the plane defined by the axes 1 and 2 of a correspondence analysis of the genic diversity scored at forty isozyme loci. Their classification is indicated by a conventional sign. An open sign indicates the negative phenol reaction of the strains and allows the distinction of an *Indica* and a *Japonica* group among *O. sativa*.

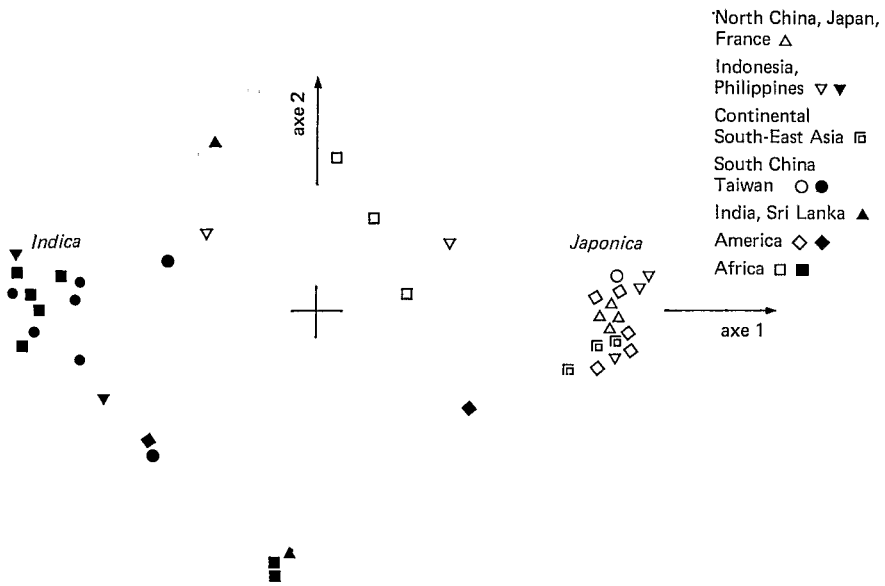


Fig. 5. Forty one strains of *O. sativa* plotted in the plane defined by the axes 1 and 2 of an analysis of correspondences of the genic diversity scored at forty isozyme loci. Their geographic origin is indicated by a conventional sign. An open sign indicates the negative phenol reaction of the strains and allows the distinction of an *Indica* and a *Japonica* group.

Moroberekan and recently released IRAT 13 which had a similar isozyme pattern with variety IRAT Acc No. 63.83). The 3 strains had different allelic combinations.

It appeared that axis 1 distinguished the *Indica* group from all other strains. Axis 2 distinguished the *O. breviligulata/O. glaberrima* group from *O. sativa*. In its higher values the strains of *O. breviligulata* were found with some electromorphs peculiar to them. Axis 3 in the case with all strains pooled had a similar loadings of characters as the axis 2 when only the *O. sativa* strains were analysed. They distinguished particular associations not directly related to the main differentiation in the three groups. Within *O. breviligulata*, axis 3 distinguished some weedy strains which are closer to *O. sativa* than is *O. glaberrima*.

5. *Relation of isozyme identity with F_1 pollen sterility and the search for the ancestral isozyme patterns within O. sativa*

Oka (1958) studied the percentage of sterile pollen of the F_1 hybrids between 115 strains of *O. sativa* and 7 of them taken as test-strains. All the strains were also classified on the basis of various quantitative measurements and the phenol reaction test. Dr. H. I. Oka kindly made available the original data and the strains for this study. I examined *Pgi-A*, *Pgi-B*, *Cat-A* and *Est-E* loci polymorphism in this material and analysed the original data on pollen sterility by the principal component analysis method.

Three of the four loci studied (i. e., *Pgi-A*, *Pgi-B* and *Cat-A*) are genetically independent (Second and Morishima 1981b). However, among the 24 ($2\times2\times2\times3$) possible homozygous associations between the 9 frequent ($>.05$) electromorphs ($2+2+2+3$), only 7 were commonly found and these represented 75% of the total. When, for the locus *Est-E*, the sole presence or absence of bands was considered (Electromorphs *Est-E*^{1} and *E*^{2} were pooled as *Est-E*$^+$), 4 frequent combinations of electromorphs remained, as follows: 121+, 111+, 212+, 2120 (loci in the order: *Pgi-A*, *Pgi-B*, *Cat-A*, *Est-E*). They represented 21%, 7%, 6% and 41%, respectively of the total observed combinations. The first two and last two combinations were generally found among the varieties classified by Oka (1958) as *Indica* and *Japonica*, with most often a phenol reaction positive and negative, respectively. Pooling all the strains with a rare ($<.05$) combination of characters in one category, 5 groups of strains were recognized as shown in Table 5. The associations N°1 and 4 of the most numerous groups were called "parental" in the sense that, if rare electromorphors (namely *Pgi-B*^{3} and *B*^{4}) are discarded, all the other associations found can theoretically be obtained by hybridization between the strains of the two groups. Conversely, other associations were called "hybrid": 32 strains (28%; 22 *Indica* and 10 *Japonica*) were pooled in association N°5 which regrouped 16 different combinations with 1 to 6 strains observed for each.

F_1 pollen sterility data for 103 strains (12 strains had missing data), including the 7 test strains, were subjected to the principal component analysis. With 37.4% and 21.1% of the total variation, only the first two vectors extracted had a contribution superior to the mean contribution of a single test-strain (100: 7=14%). Vector 1 had relatively large absolute values of loading on all testers although with large differences among them, the plus or minus signs represented the *Indica* and *Japonica* classification among the testers. Accordingly, this vector represented the *Indica-Japonica* differentiation of the strains observed. Vector 2 gave weight only to the *Indica* and to the "tropical" *Japonica* testers, all with positive values. Accordingly, it differentiated, within the *Indica* strains, those partly sterile with all testers and, among the *Japonica* strains, it represented the tropical-temperate differentiation. Thus, the "tropical" *Japonica* strains were generally more fertile with the *Indica* testers than the "temperate" *Japonica*.

Figure 6 shows the distribution of the 103 strains in the plane defined by vectors 1 and 2 (53% of the variation). The strains are symbolized according to the associations of their characters outlined in Table 5. The geographical origin of the strains and the position of the test strains were indicated. No cluster was clearly recognized and the geographical origins were scattered. Strains with any combination of characters are mixed although "parental" associations tended to segregate on both axes.

Another representation was made using only the data of the test-strains the furthest along both vectors. These were Acc 108, an *Indica* strain from the lowlands of Taiwan, and Acc 563 and 521, two *Japonica* strains from the lowlands of Japan. The test-strains Acc 563 and 521 were very close on both vectors although the pollen sterility in crosses between them and the same strain varied in some cases. The mean values of the pollen sterility with both *Japonica* testers were calculated. 115 strains were scattered in the plane defined by the pollen fertility with the *Indica* tester and the mean pollen fertility with the two *Japonica* testers. They are shown in Fig. 7 with the symbols used in Fig. 6.

Most strains had a pollen fertility of more than 60% on one axis at least and their distribution could be delimited by a seemingly hyperbolic curve. As in the distribution defined by the principal component analysis of the data with 7 testers, strains with any combination of characters were mixed. However, the segregation of the "parental" associations appeared clearly in the two asymptotic areas of the distribution and two homogeneous group of 8 strains were selected. They are shown by the Acc. numbers of the strains written in italics in Fig. 7. They had nearly 100% pollen fertility on one axis, between 12 and 50% of pollen fertility on the other axis and only the "parental" association of characters. They were presumed to represent the "ancestral

Table 5. *Five types of associations between electromorphs scored at 4 loci, phenol reaction and the classification in Indica and Japonica previously established (Oka 1958) among 115 strains of O. sativa, with the number of strains observed*

Association number and type	Classification of the strain	Phenol reaction	Electromorphs				Number of strains
			Pgi-A	*Pgi-B*	*Cat-A*	*Est-E*	
1. Presumed parental "I"	*Indica*	(+)	A^1	B^2	A^1	E^1 or E^2	23
2. Presumed hybrid "I"	*Indica*	(+)	A^1	B^1	A^1	E^1 or E^2	11
3. Presumed hybrid "J"	*Japonica*	(−)	A^2	B^1	A^2	E^1 or E^2	1
4. Presumed parental "J"	*Japonica*	(−)	A^2	B^1	A^2	E_0	42
5. Presumed any hybrid	Any combination between the characters in the associations 1 to 4.						32

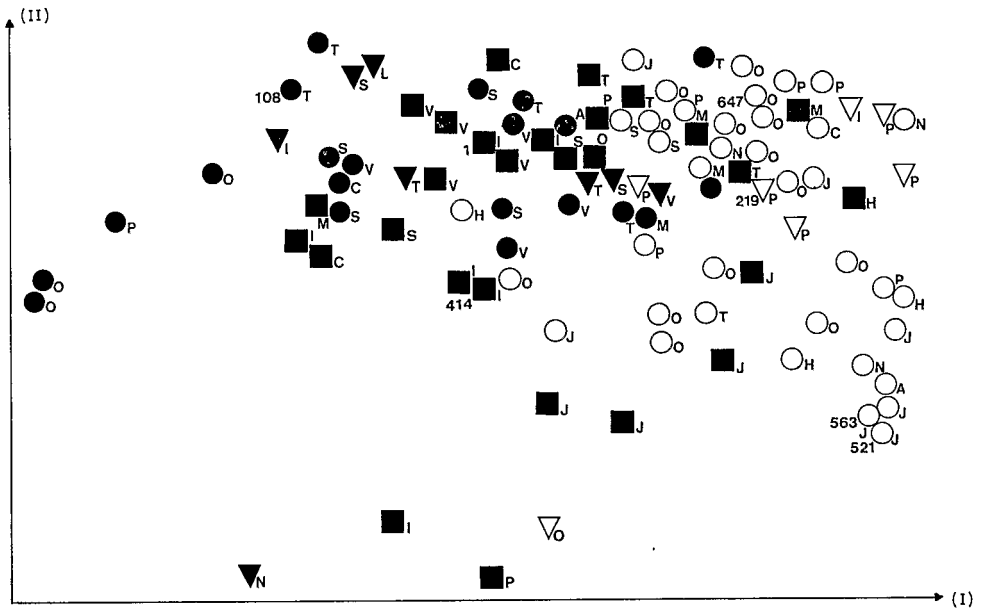


Fig. 6. Scatter diagram showing the distribution of 104 strains in the plane defined by the vectors I and II of a principal component analysis of F_1 pollen fertility with 7 tester strains. Key: The strains are indicated by a conventional sign expressing the type of association of electromorphs, phenol reaction and classification by Oka (1958) as outlined in Table 5: full circle=parental I, full triangle=hybrid I, open triangle=hybrid J, open circle=parental J, square=any hybrid association. The test-strains are identified by their Acc. numbers and the geograghical origins of all strains are given by a letter on the right of the respective symbol: A: Thailand, B: Burma, C: Central China, H: Hai-Nan island, I: India, J: Japan, L: Sri-Lanka, M: Mountains of Taiwan, N: North China, O: Indonesia, P: Philippines, S: South China, T: Lowlands of Taiwan, V: Vietnam.

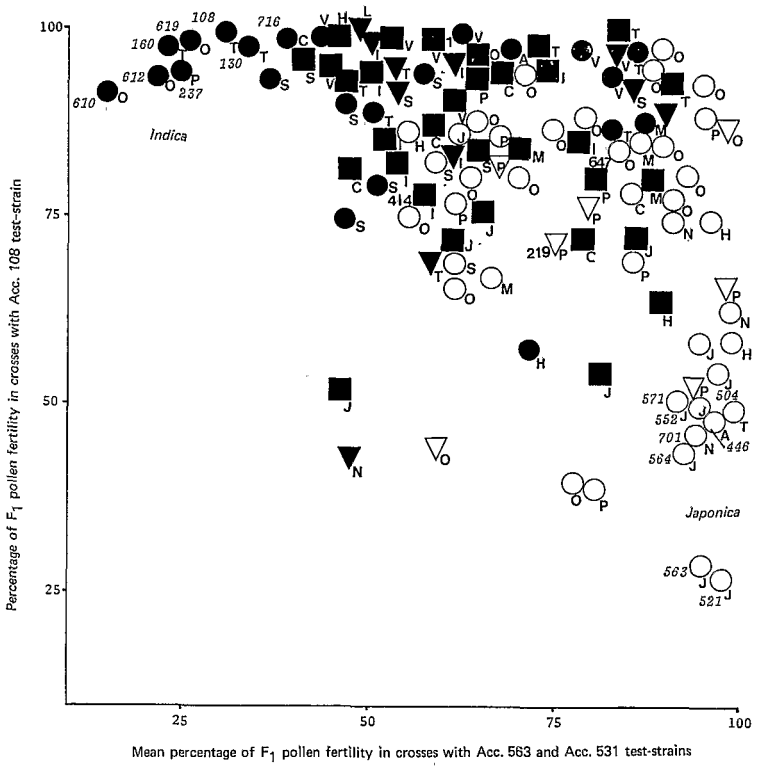


Fig. 7. Scatter diagram showing the relation between specific markers of the *Indica* and *Japonica* types and the F_1 pollen fertility (in percent) with one *Indica* (Acc 108) and 2 *Japonica* (Acc 563 and Acc 521, mean value) test-strains. Each strain is characterized in the same manner as in Fig. 6. Acc. numbers of the strains presumed to represent the ancestral patterns of isozymes of the *Indica* and *Japonica* types are indicated in italics.

Indica" and "ancestral *Japonica*" subspecies for isozyme patterns and a complete analysis of the 40 enzyme loci was performed on them.

In Table 6, the frequent (>.05, about) electromorphs found within the three types of cultivated rice (*Indica, Japonica* and *O. glaberrima* as classified in Fig. 4 or by Oka 1958) and the electromorphs found in the "ancestral *Indica*" and "ancestral *Japonica*" varieties defined above are indicated. It was remarkable that most of the frequent electromorphs characteristic of *O. sativa* were found in the 16 "ancestral" *Indica* and *Japonica* varieties. Exceptions included the null electromorph *Est-D*^{0} found to be frequent in the tropical form of *Japonica* also called Javanica ecotype (Nakagahra 1978) and rarer electromorphs *Lap-E*^{2} and *Est-B*^{0}. *LAP-A*^{0} and *A*^{1} bands were faint and were not scored in this experiment.

The actual forms of *Indica* and *Japonica* appeared to be more polymorphic

Table 6. *Frequent (>5%, about) electromorphs (indicated by their conventional numbers and listed in the order of their frequencies) found whithin the three types of cultivated rices for the 21 most polymorphic loci. For the Indica and Japonica types, the electromorphs found in the presumed ancestral varieties as determined through the F_1 pollen sterility relationships are indicated. When ambiguous, the electrothermomorph is specified in parentheses*

Loci	*Indica*		*Japonica*		*Glaberrima*
	actual	"ancestral"	actual	"ancestral"	
Pgd-A	1, 2 and 3	1, 2 and 3	1 and 3	1	1
Cat-A	1	1	2 and 1	2	1
Pox-B	4 and 3	4 and 3	3 and 4	3	1 and 2
E	2	2	2	2	1
Got-B	1	1	1 and 0	0	1
Est-B	2 and 0	2 and 0?	1 and 0	1 and 0?	1
C	2	2	0	0	0
D	1 and 0	1 and 0?	1 and 0	1 and 0?	1
E	1, 2 and 0	1 and 2	1 and 0	0	1
F	2	2	0 and 2	0	1
G	0 and 1	0	1	1	1
H	?* and 0	?*	1 and ?*	1	1 and 0
I	0	0	2	2	1 and 0
J	2	2	1	1	0
Ca	1 and 2	1 and 2	2	2	1
Acp-B	2	2	1	1	1
C	1	1	0	0	1
Lap-A	1 and 0	?	1 and 0	?	1
C	1 and 2	1 and 2	1 and 2	1 and 2	1
E	1	1	1 and 2	1 and 2?	1 and 3
Pgi-A	1 and 2 (59°)	1	2 (59°)	2 (59°)	3 and 2 (56°)
B	1 and 2	2	1	1	1

* Overlaps with *Est-F*[2]

than *O. glaberrima* in terms of number of alleles distinguished at one locus because many electromorphs were common to both types of *O. sativa*. On the other hand, the "ancestral" *Indica* and *Japonica* patterns had a polymorphism comparable to *O. glaberrima* with 33 to 35 loci out of 40 fixed for one electromorph and a maximum of 2 to 3 alleles distinguished at one locus.

The number of discordances between the electrophoretic patterns of "ancestral" *Indica*, "ancestral" *Japonica* and *O. glaberrima* were counted as 1 or 1/2 for complete or partial discordance, respectively. The distances D of Nei (1975) were calculated by arbitrarily counting a frequency of 0.25 for the least fre-

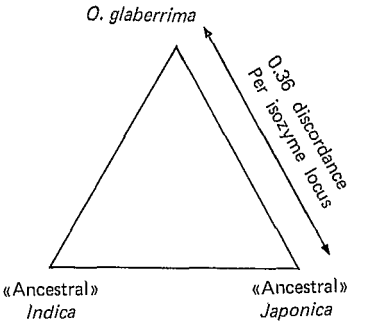


Fig. 8. Distances between the electrophoretic patterns, scored at 40 loci, of *O. glaberrima* and the "ancestral" *Indica* and *Japonica* subspecies in terms of the proportion of discordances per locus.

quent electromorphs at the polymorphic loci.

They were as follows:

Types compared	Number of discordances in the electrophoretic patterns	Distance D
"ancestral" *Indica* and "ancestral" *Japonica*	15.5/40=.39 per locus	.51
"ancestral" *Indica* and *O. glaberrima*	14/40=.35 per locus	.35
"ancestral" *Japonica* and *O. glaberrima*	14/40=.35 per locus	.34
Mean:	.36 per locus	.40

The differences between the three comparisons cannot be taken as significant. It is remarkable that similar distances can be estimated on this basis between the three types of cultivated rice, as symbolized in Fig. 8.

Also, numerous rare (<.05 electromophs were found among the varieties of *O. sativa*: *Gdh-A*^{3}, *Lap-E*^{3}, *Icd-A*^{2}, *Est-I*^{1}, *I*^{3} and *J*^{0}, *Pgi-B*^{3} and *B*^{4}. Most were found in varieties of *Indica* type or in rather intermediate types as classified by Oka (1958) or in Fig. 5. Such electromorphs were also found in *O. rufipogon* (Second, in preparation).

4. DISCUSSION

As argued by Lewontin (1974), reliable estimates of the genetic variation in natural populations require examination of a large number of randomly selected loci. The genetic distance is not affected much by the sample size: because of the considerable variation in polymorphisms found between and within loci (Fuerst *et al.* 1977; Chakraborty *et al.* 1978, 1980), a large sample of loci is often much more important than a large sample of individuals. In the present survey, although the number of individuals sampled varied

greatly according to the enzymes studied, they always represented a wide range of geographical and ecological origins. This may be a guarantee of their representativity of the total variation (Brown 1978).

As far as possible, the genetic interpretation of electrophoretic patterns should be based on inheritance experiments. In rice, this is difficult because various crossing barriers (Chu *et al.* 1969) do not allow in many cases to obtain large F_2 populations. Certation factors and pseudo-linkage due to sterility genes distort the mendelian ratios or the gene recombinations. (Oka 1957, 1974a; Nakagahra *et al.* 1974).

However, one should not refrain from using such data for the analysis of genetic structure and of distances between and within species, or forms of cultivated and wild rice. So far, all examinations of genetic determinisms (13 loci among the most important for the conclusions) have confirmed the presumed determinisms based on the comparison of the observed zymogram patterns among strains of predominantly inbreeding or predominantly self-incompatible species (*O. longistaminata*) of the *O. sativa* sp. complex (Second and Trouslot 1980). Segregations at three independent loci are shown for example in Fig. 9. Our estimate of the number of loci is conservative. For example, one invariant band (ACP-D) was not considered because its isozymic nature could be questioned; bands EST: F^1 and F^2, which were never found in the same inbred individual, were assumed to be coded by different alleles of the same locus although there was no direct evidence for it. Apart from this, any of the observed bands could be determined by more than one locus. In particular, the absence of a band could be the effect of a regulatory gene (generally recessive) at an additional locus. However, our estimations of the mean gene diversity index should be on the same level of accuracy as those made on other species by similar techniques and a similar set of isozymes studied.

More difficulties in interpretation come from disagreements concerning the extent to which allozyme polymorphisms are neutral to natural selection (Kimura 1979; Selander 1976). Adepts in the neutral theory interpret the extent of polymorphism and the genetic distances based on isozyme data in terms of population size, time since divergence and migration between populations, relevant to the history of the populations. On the other hand, selectionists explain the same observations in terms of selective forces dependent on the environment. They admit a possible genetic convergence at the isozyme level, rendering impossible inferences about the history of populations from their polymorphism.

Secondly, although the loci bearing on this question may be considered independently of the chromosomes segments to which they belong in large and stable outbreeding populations (Ohta and Kimura 1975), to what extent this remains true in small, subdivided and inbreeding populations subjected to extreme reductions in the population size, is not clear. In other words, it is

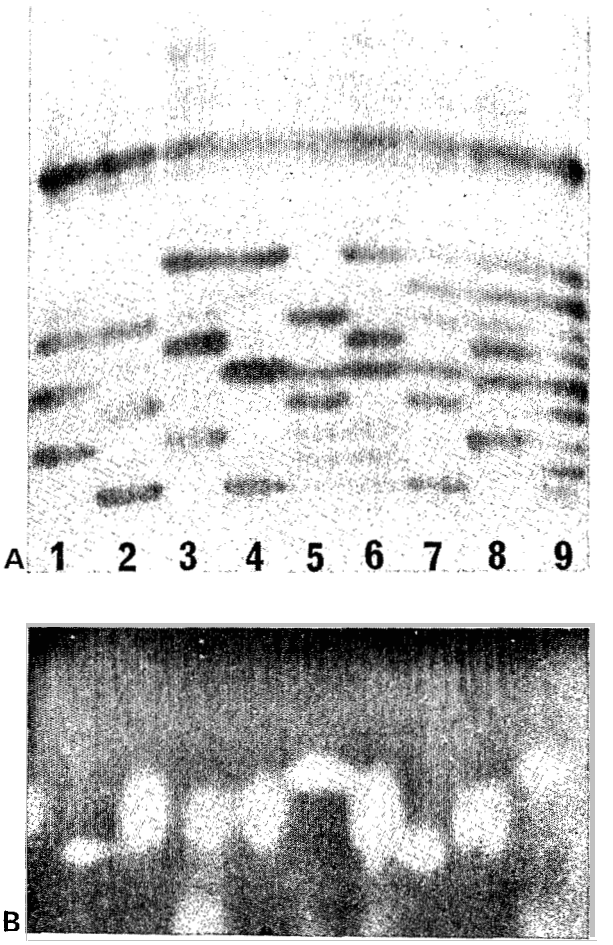


Fig. 9. A) Nine phospho-glucose isomerase zymograms (Z.) segregating in an F_2 population between a *Japonica* (Z. type N°=1, bands A^2 and B^1) and an *Indica* (Z. type N°=4, bands A^1 and B^2) parent. The direction of migration is from bottom to top. Band B is faster than band A. The fastest band with no variation in this material corresponds to band PGD- B^1. Z.1 to 4: four homozygotes, Z.5 to 8: four simple heterozygotes, Z.9: double heterozygote. They are found in the ratio: 1:1:1:1:2:2:2:2:4, expected for two independent loci with two alleles each.

B) Segregation for catalase A^1 (slow) and A^2 (fast) bands in the same material as above. The heterozygote figure comprises 5 bands. Only the three bands intermediate to the parental ones are strongly stained. A ratio 1:1:2 for the parental and hybrid phenotype was observed. The electrophoresis were performed at the germination stage with an overnight migration.

not possible at the moment to prove definitively that any interpretation of isozyme polymorphism is correct. The neutralist and selectionist points of view are not entirely exclusive and intermediate situations with neutral genes "hitchhiked" by selected genes are likely to occur in our material.

With remarkable similarity to the case of *O. sativa*, Kahler and Allard (1981) showed that only a relatively small number of gametic allele combinations were commonly found among 4 *Est* loci in a world collection of barley, resulting in 2 groups of populations, namely "Western" and "Asiatic". Nevo *et al.* (1979) found statistically significant gametic associations of alleles in wild barley *Hordeum spontaneum* L. in Israel. Wild barley in this area however is known to be sometimes conspicuously weedy (Harlan and Zohary 1966). Allard *et al.* (1978) and Kalher *et al.* (1980), similarly found associations between the isozyme phenotypes and the environment in slender wild oats in Israel and California. This cannot be taken as direct evidence, however, for the action of natural selection acting on isozyme phenotypes (Hamrick and Holden 1979).

Turning to the isozyme polymorphism revealed on rice, it appeared that the geographical and ecological pattern of variation observed cannot be explained in a simple way if electromorphs have been subjected to direct selection. On the contrary, we will tentatively assume that: (i) isozymes are generally neutral markers that can be used as tracers to draw phylogenetic relationships and (ii) genetic recombination occurs between neutral and selected loci, even if various mechanisms reduce the rate of recombination. Considering such recently evolved taxa as cultivated species, and under the neutral theory, it may be postulated that the presumed ancestral forms must show most—if not all—of the isozyme polymorphism of the derived ones. The occurrence of rare alleles in one cultivated species may *suggest* the introgression of genes from another species in which they are frequent, if: (i) field observations showed that such introgressions are feasible, and (ii) the same alleles are not found in the presumed ancestor. Electromorph diversity being much richer in wild rice than in cultivated rice (Shahi *et al.* 1969; Pai *et al.* 1973, 1975; Second et Trouslot 1980a) independent domestications should produce cultivars with different sets of electromorphs. This paper will retain the simplest hypothesis compatible with all data so far available to the author.

(i) On the origin of wild O. breviligulata

Considering its greater allelic polymorphism compared with *O. glaberrima* and its large genetic distance from *O. sativa*, it appears improbable that this species has escaped from cultivated fields. From other data (see the zymograms in Second et Trouslot 1980a), one may also doubt of its direct relation with the perennial African species *O. longistaminata*. The present observations thus fully corroborate Portères's (1950) hypothesis that *O. breviligulata* is a wild species endemic in Africa.

Nei's measurement of genetic distance can be used for crude estimates of the time since divergence (Nei 1975, p. 193), assuming there was no migration. The genetic distance between *O. breviligulata* and *O. sativa* (see Table

3) corresponds to a time of divergence of one million years as a provisional estimate. Further details on the isozyme polymorphism found within this species will be presented elsewhere.

(ii) On the origin of weedy O. breviligulata

Weedy *O. breviligulata* was distinguished on the basis of the site of collection. It had a greater genetic identity with *O. glaberrima* than with wild *O. breviligulata* but it also showed rare electromorphs absent in *O. glaberrima* and specific to wild *O. breviligulata* or *O. sativa*. This suggests various origins. Some plants may be considered as intermediates between wild *O. breviligulata* and *O. glaberrima* as discussed by Morishima and Oka (1970) and Morishima *et al.* (1963). Others might rather be of hybrid origin between *O. glaberrima* and *O. sativa* with backcrossing on *O. glaberrima* according to the hypothesis set forth by Nayar (1973). Such hybrid swarms have been repeatedly observed in nature (Second 1975; Borgel et Second 1978) and make this hypothesis credible. It may explain the rare cases of pollen sterility found in crosses between some weedy strains of *O. breviligulata* and *O. glaberrima* (Chu *et al.* 1969). The hypothesis set forth by Portères (1950) and Nayar (1973) on the origin of *O. breviligulata* were not exclusive. Both receive support from the present data.

The appellation *Stapfii* has often been used to designate some forms of weedy *O. breviligulata* but no specific characteristic has so far been presented. It could designate those forms which taxonomically belong to *O. breviligulata* but may have arisen through introgression between independently domesticated African and Asian rices. The multivariate analysis of multiloci isozyme data seems likely to be able to distinguish *O. stapfii* from *O. breviligulata* to some extent although more data are necessary. Such an evolutionary path for a weed could be an interesting case. It emphasites the well-founded suspicion that some weedy presumed progenitors of a crop may not be representative of the wild forms existing before domestication occurred.

(iii) On the origin of the variability of O. glaberrima

A drastic reduction of the isozyme polymorphism accompanied the domestication of *O. glaberrima* from wild *O. breviligulata* with a gene diversity declining to 0.03 from 0.14 and with no hidden variability found. All electromorphs specific to *O. glaberrima* were very frequent in wild *O. breviligulata*.

The bottleneck effect (Nei *et al.* 1975; Maruyama and Kimura 1980) through the selection of useful mutations during the domestication can explain this fact.

The gene diversitiy of *O. glaberrima* was small but still about half of the value of *O. sativa* in terms of number of alleles per locus. The genetic dis-

tance between *O. glaberrima* and *O. sativa* was not greater than that between the presumed "ancestral" *Indica* and *Japonica* subsp. An "*Africa*" type of cultivated rice could probably be generated by the introgression of genes of *O. sativa* into *O. glaberrima*.

The case of a strain of *O. glaberrima* with an electromorph specific to *O. longistaminata* was previously reported (Chu and Oka 1967). It is interesting to find that the same strain also had a silent band ACP-C^0 ($=Fa^-$) which was never found in other strains of *O. glaberrima*. It confirmed the possible introgression of genes of *O. longistaminata* into that strain.

(iv) *On the origin of the genic diversity of O. sativa*

In contrast to *O. glaberrima* and *O. breviligulata*, the gene (electromorph) diversity was large ($\overline{H}=0.23$) in *O. sativa*. The examination of the hidden variability revealed little polymorphism within electromorphs in this species compared with *O. breviligulata* so that the electromorph diversity may be taken as a reasonable estimate of the true gene diversity of *O. sativa* for isozyme loci. A maximum of three alleles per locus was detected in *O. sativa*, compared with seven in *O. breviligulata*. The distribution of single locus heterozygosity was distorted in *O. sativa* with an excess of loci with a heterozygosity close to 0.5 in comparison to the theoretical curve generated under the mutation-drift hypothesis. This was not the case for *O. breviligulata* and there was an indication that *O. sativa* is composed of at least two divergent lineages. Multivariate analysis of the isozyme diversity showed that most strains of *O. sativa* clustered into two groups corresponding to the classical *Indica* and *Japonica* subspecies. The analysis of the F_1 pollen sterility relationships among varieties permitted the extraction of two small groups of *Indica* and *Japonica* strains with a high F_1 pollen sterility relationship. All of the isozyme diversity of *O. sativa* could be explained by hybridization between varieties of these two groups with the sole exception of one very frequent null electromorph and a few rarer active electromorphs. In other words, the strains pollen fertile with all testers were no more polymorph than the others.

Oka (1974b) proposed a monophyletic origin of the *Indica* and *Japonica* types. That is, in a given geographical area, the two types became differentiated under selective pressures in the course of domestication. This process could have occurred several times in different areas. He proposed supporting evidence from the intermediate wild-cultivated plants collected in the Jeypore Tract in India (Oka and Chang 1962l). Assuming this hypothesis, the isozyme pattern of the cultivars should reflect the polymorphism of the wild rice in the various areas of domestication. The cultivated and intermediate strains from the Jeypore Tract showed, however, isozyme patterns essentially identical to the other varieties of *O. sativa* while *O. rufipogon* of the same area

frequently had some different isozymes (Shahi *et al.* 1969; Endo *et al.* 1971; the author's unpublished data for EST, POX, LAP and PAC bands). How independent domestications resulted in the selection of various cultivars with convergent isozymes bands would appear to be mysterious unless isozymes were subjected to direct selection. As recognized by Oka (1981), there could however, be a simpler alternative interpretation in the possible introgression of genes between wild and cultivated rice.

Observing that the F_1 pollen sterility relationships between the *Indica* and *Japonica* types were complex and did not discriminate between the two types, Oka (1981) proposed that the differentiation in F_1 sterility might have followed differentiation in other characters. Noting that the F_1 pollen sterility was often weaker between wild and cultivated strains than between the cultivars alone (Hinata and Oka 1962), the same author (Oka 1974) assumed that numerous pollen sterility mutations had accumulated during the course of domestication as they were selected through certational advantage for the ability of pollen to fecundate. Under this hypothesis, the varieties with fertile pollen in crosses with all testers would be the most "primitive". They should be expected to show more isozyme polymorphism than the differentiated varieties because of genetic drift. Various lineages of differentiation for sterility genes should be accompanied by the loss of different alleles. This was not observed. On the contrary, it was found that the F_1 pollen sterility relationship permitted the extraction of two small groups of varieties with reduced but complementary isozyme polymorphisms. The F_1 pollen sterility between the *Indica* and *Japonica* subsp. could have existed before domestication because *O. rufipogon* as observed now is not necessarily representative of the direct ancestors of *O. sativa* for both genetic structure and geographic distribution.

On the other hand, Chang (1981) assumed that the temperate *Japonica* most probably became differentiated from the tropical *Indica*. That is not supported by isozyme data.

Among the various phylogenetic relationships between rice varieties put forward in the literature, only the hypothesis of the independent domestication of the *Indica* and the *Japonica* types, proposed by Chou (1948), fit the observed pattern of isozymic variation in a simple way. Agreement alone is not proof but we wish to support this hypothesis because it seems compatible with all data so far published, even though alternative interpretations have been considered, and because it has several important specific implications for the collection, the evaluation and the utilization of rice genetic resources which are worth considering.

Discordances in isozyme patterns between genetically isolated groups may be used as well as the genetic distance D of Nei (1975) to estimate the time since divergence (Maruyama 1973). The proportion of 0.36 discordance per

locus and a D of 0.22 correspond to a crude estimation of a divergence time of 300,000 to 2 millions years, respectively between the three presumed original domesticates of rice, "ancestral" *Indica*, "ancestral" *Japonica* and *O. glaberrima*. If this estimate is correct, it points to the late tertiary epoch, which quaternary has witnessed extensive shifts in the distribution of animals and plants. This means that the observed differentiation preceded domestication.

Further agreement with this hypothesis comes from the observation of a geographic pattern of variation for some of the 24 isozyme loci scored in *O. rufipogon* (Second and Morishima 1981; Second, in preparation). The populations of *O. rufipogon* from South China, including Taiwan, were found to be clearly differentiated from the populations of other geographic origins, with respective genetic affinity to the *Japonica* and *Indica* subsp. It could be that the Himalaya mountain ranges which extend as a barrier to the land migration of wild rice from the Hindukush to South-East Asia has allowed such differentiation. The *Japonica* type would have been domesticated in China and the *Indica* type in other places of tropical Asia.

The genetic structure of *O. sativa* at the isozyme level, suggests that most ecotypes of this species may have been selected after introgressive hybridization between the ancestral *Indica* and *Japonica* subspecies. The fact that numerous rare electromorphs are found mainly in the *Indica* varieties could rather be interpreted in terms of introgressions of genes from the wild rice since *Indica* varieties are more often grown than *Japonica* varieties in the area of distribution of *O. rufipogon*.

Following the extension of rice cultivation, human migration or the opening of routes, the two types of Asian rice would have been brought together (probably more than 2000 years ago). Hybridization would have occurred between them and also with the wild rice resulting in a progressive loss of reproductive barriers and building up a great amount of diversity. Numerous agroecotypes might have been selected at that time which, in turn, allowed a wide dispersion of rice cultivation in geographically, ecologically and anthropologically diverse environments.

Introgressions of genes from wild rice in various areas have, undoubtedly contributed to the genetic diversity of cultivated rice since hybridization of cultivated varieties with wild rice can also be artificially directed to produce highly adaptive and valuable varieties (Ting 1953; Chen *et al.* 1980). It seems to occur naturally in Africa from *O. longistaminata* to both *O. sativa* and *O. glaberrima*.

One wonders why so few truly intermediate *Indica-Japonica* are found if their differentiation were related to founder effect. When the progeny of an *Indica-Japonica* hybrid was propagated without deliberate selection, plants with parental combinations of independent genes tended to increase as com-

pared with those with recombination (Oka 1981). This return to the parental type seemed to be due to the environment: predominantly toward the *Indica* type in Taiwan (Oka 1964, p. 170) and toward the *Japonica* type in Japan (Nagamatsu and Omura 1960). The latter authors however claimed that they had experimentally produced a few intermediate types from *Japonica-Indica* hybrids. Further, a recent survey of *Acp* loci among 3,748 collection samples of *O. sativa* (Katayama and Chern 1982) showed that the frequencies of electromorphs specific of the *Indica* and *Japonica* types in a given collection had possibly changed during repeated seed multiplication according to the environmental conditions of the preservation sites. Some kind of natural selection is evidently responsible in part for the maintenance of the *Indica-Japonica* differentiation, and neutral genes may be "hitchhiked" in the process. Most, if not all, genes subjected to natural selection may have been present in the wild rice geographically differentiated. On the other hand many genes selected by man accounted for the *Indica-Japonica* differentiation: the eating quality (amylose content), the aspect, the growing habit *etc*... They may have been selected since domestication occurred and super-imposed onto the original differentiation because selection was made by different people in different areas. Coadapted gene systems are likely to account for such characters with one locus subjected to selection together with some modifier loci as modeled by Endler (1977), for example. After hybridization with a distantly related variety, coadapted systems can be reassociated more easily through a return to one of the parental type.

One may argue that, if the hypothesis of only two independent "primary domestications" of *O. sativa* were true, multiple genes for such characters as the absence of awn, shedding *etc*... should not be expected. However, supposing a chromosome segment was introgressed from one variety to another, there might well be a return to a wild phenotype for one or another character because of gene complementation between cultivars domesticated independently. Another mutation may then be selected for the same character in a "secondary domestication". This, with other examples such as the possible introgression of multiple adaptive alleles from the wild rice, suggests that the genetic structure for adaptive genes of breeding value cannot be expected to be as simple as for isozyme loci. In this respect, it was observed that the differentiation of varieties of *O. sativa* was more evident at the isozyme level (Fig. 5) than at the F_1 pollen sterility level (Figs. 6 and 7). The above mentioned geographic structure of *O. rufipogon* could be still evident only because isozyme markers may be neutral to selection while sterility genes could be counter-selected following hybridization between and within *O. sativa* and *O. rufipogon*.

Rather than mutations occurring since domestication, the main source of variability of cultivated rice could be the differentiation of wild rice before

domestication. It should be urgent to look for more strains of truly willd *O. rufipogon* in Asia and for more cultivars genetically closer to the presumed ancestral forms of *Indica* and *Japonica* because they may represent the true genetic resources of cultivated rice in Asia.

This work was initiated under the directorship of Dr. J. Pernès. It was completed during a stay in the National Institute of Genetics, Mishima. Drs. T. Endo, S. Iyama, H. Morishima and H. I. Oka kindly received me in their laboratories and helped me in many ways. Messrs M. Arraudeau, M. Jacquot and R. Marie, Drs. M. Nakagahra and Y. Sano supplied some of the seed material. Mr. P. Trouslot at Abidjan did much in setting up and realizing a great part of the electrophoretic analysis. Mrs. R. Sano conducted part of the electrophoresis work made in Mishima. Mrs. E. Nguyen Van of CNRS ran the computer program for the analysis of correspondences.

Hearty thanks are due to them all.

I am also grateful to Drs. J. Harlan, T. Maruyama, H. I. Oka, H. Morishima and J. Pernès for their critical reading and constructive comments on the first draft.

REFERENCES

ALLARD, R. W., MILLER, R. D. and KAHLER, A. L. (1978) The relationship between degree of environmental heterogeneity and genetic polymorphism. In *Structure and functionning of plant populations* (ed. A. H. J. Freysens and J. W. Woldendrop), pp. 49–73. North-Holland Publishing Company.

ANDERSON, E. (1949) *Introgressive hybridization*. Wiley, J. and Sons. New York Chamman Hall. London.

BEZANÇON, G., BOZZA, J., KOFFI, G. and SECOND, G. (1977) Genetic diversity of *O. glaberrima* and *O. breviligulata* shown from direct observation and isozyme electrophoresis. In *Meeting of African rice species* (ed. IRAT-ORSTOM, Paris), pp. 15–46. In French and English translation.

BENZECRI, J. P. (1973) *L'analyse des données*, tome II "*L'analyse des correspondances*". Dunod, Paris.

BORGEL, A. et SECOND, G. (1978) Prospection des variétés traditionnelles et des espèces sauvages de riz au Tchad et Cameroun. Mimeogr. Report, 15p. ORSTOM, Paris.

BROWN, A. H. D. (1978) Isozymes, plant population genetic structure and genetic conservation. *Theor. Appl. Genet.* **52**, 145–157.

CHANG, T. T. (1976a) Rice. In *Evolution of crop plants* (ed. N. W. Simmonds). Longman, London and New York.

CHANG, T. T. (1976b) The origin, evolution, cultivation, dissemination and diversification of Asian and African rices. *Euphytica* **25**, 425–441.

CHANG, T. T. (1981) The origins and early cultures of the cereal grains and food legumes. In *The Origins of Chinese Civilization* (ed. D. Keightley). University of California, Berkeley Press.

CHAKRABORTY, R., FUERST, P. A. and NEI, M. (1978) Statistical studies on protein polymorphism in natural populations. II. Gene differentiation between populations. *Genetics* **88**, 367–390.

CHAKRABORTY, R., FUERST, P. A. and NEI, M. (1980) Statistical studies on protein polymorphism in natural populations. III. Distribution of alleles frequencies and the number of alleles per locus. *Genetics* **94**, 1039–1063.

CHEN, W. G., LIANG, N. and YU, J. R. (1979) A brief report on the investigation of wild rice in Boluo county. *Rep. Intern. Rice. Res. Workshop* (ed. IRRI), pp. 22–26.

CHOU, C. L. (1948) "China is the place of origin of rice" *J. Rice Soc. China* 7(5), 53–54 (In Chinese).

CHU, Y. E., MORISHIMA, H. and OKA, H. I. (1969) Reproductive barriers distributed in cultivated rice species and their wild relatives. *Jpn. J. Genet.* **44**, 207–223.

CHU, Y. E. and OKA, H. I. (1967) Comparison of variations in peroxidase isozymes between *Perennis-Sativa* and *Breviligulata-Glaberrima* series of *Oryza*. *Bot. Bull. Acad. Sinica.*

VII, sp. Number.

CLAYTON, W. D. (1968) Studies in *Graminae*: XVII, West African wild rice. *Kew Bulletin* **21**, 487–488.

ENDO, T. E., SHAHI, B. B. and PAI, C. (1971) Genetic convergence of the specific acid phosphatase zymograms in *Oryza sativa*. *Jpn. J. Genet.* **46**, 147–152.

ENDLER, J. A. (1977) *Geographic variation, speciation and clines*. Princeton University press. New Jersey.

FUERST, P. A., CHAKRABORTY, R. and NEI, M. (1977) Statistical studies on protein polymorphism in natural populations. I. Distribution of single locus heterozygosity. *Genetics* **86**, 455–483.

GOVINDASWAMI, S., KRISHNAMURTY, A. and SASTRY, N. S. (1966) The role of introgression in the varietal variability in rice in the Jeypore Tract of Orissa. *Oryza*, sp. issue, 74–85.

HAMRICK, J. L. and HOLDEN, L. R. (1979) Influence of microhabitat heterogeneity on gene frequency distribution and gametic phase disequilibrium in *Avena barbata*. *Evolution* **33**, 521–523.

HARLAN, J. R. and ZOHARY, D. (1966) Distribution of wild wheats and barley. *Science* **153**, 1074–1080.

HEDRIC, P. W. (1980) Hitchhiking: a comparison of linkage and partial selfing. *Genetics* **94**, 791–808.

KAHLER, A. L. and ALLARD, R. W. (1981) Worldwide patterns of genetic variation among four esterase loci in barley (*Hordeum vulgare* L). *Theor. Appl. Genet.* **59**, 101–111.

KAHLER, A. L., ALLARD, R. W., KRZAKOWA, M., WEHRHAHN, C. F. and NEVO, E. (1980) Associations between isozyme phenotypes and environnement in the slender wild oat (*Avena barbata*) in Israel. *Theor. Appl. Genet.* **56**, 31–47.

KATAYAMA, T. and CHERN, J. L. (1982) Genetic analysis and geographical distribution of acid phosphatase isozymes in cultivated rice, *Oryza sativa* L. *Jpn. J. Genet.* (In press).

KATO, S. (1930) On the affinity of the cultivated varieties of rice plants, *Oryza sativa* L. *J. Dpt. Agr., Kyushu Imp. Univ. 2.* **9**, 241–276.

KIMURA, M. (1979) The neutral theory of molecular evolution. *Scient. Amer.* **241**, 98–126.

KING, J. L. and OHTA, T. (1975) Polyallelic mutational equilibria. *Genetics* **79**, 681–691.

LEWONTIN, R. C. (1974) *The genetic basis of evolutionary change*. Columbia Univ. Press, New York.

MARUYAMA, T. (1973) Some theoretical aspects of isozyme polymorphism viewed from the standpoint of population genetics. *Seiken Ziho* **24**, 75–88.

MARUYAMA, T. and KIMURA, M. (1980) Genetic variability and effective population size when local extinction and recolonization of subpopulations are frequent. *Proc. Natl. Acad. Sci. USA.* **77**, 6710–6714.

MORINAGA, T. (1964) Cytogenetical investigations on *Oryza* species. In *Rice genetics and cytogenetics* (ed. IRRI), pp. 91–103. Elsevier, Amsterdam.

MORINAGA, T. and KURIYAMA, H. (1958) Intermediate type of rice in the subcontinent of India and Java. *Japan. J. Breed.* **7**, 253–259.

MORISHIMA, H. and OKA, H. I. (1970) A survey of genetic variations in the populations of wild *Oryza* species and their cultivated relatives. *Jpn. J. Genet.* **45**, 371–385.

MORISHIMA, H., HINATA, K. and OKA, H. I. (1963) Comparison of modes of evolution of cultivated forms from two wild rice species, *O. breviligulata* and *O. perennis*. *Evolution* **17**, 170–181.

NAGAMATSU, T. and OMURA, T. (1960) Sexual affinity between the lines derived from the intersubspecific hybrids and the ecotypes of rice plant. *Japan. J. Breed.* **10**, 205–208. (in Japanese with English summary).

NAKAGAHRA, M. (1977) Genic analysis for esterases isoenzymes in rice cultivars. *Japan. J. Breed.* **27**, 141–148.

NAKAGAHRA, M. (1978) The differentiation, classification and center of genetic diversity of cultivated rice (*Oryza sativa* L.) by isozyme analysis. *Tropical Agriculture Research Series*, No. **11**, Japan.

NAKAGAHRA, M., AKIYAMA, T. and HAYASHI, K. I. (1975) Genetic variation and geographic cline of esterase isozymes in native rice varieties. *Jpn. J. Genet.* **50**, 373–382.

NAKAGAHRA, M., OMURA, T. and IWATA, N. (1974) New certation gene on the first linkage group

found by inter-subspecific hybridization on cultivated rice. *J. Fac. Agr. Kyushu Univ.* **18**, 157–167.

NAYAR, N. M. (1973) Origin and cytogenetics of rice. *Adv. Genet.* **17**, 153–292.

NEI, M. (1975) *Molecular population genetics and evolution.* North-Holland, Amsterdam.

NEI, M., MARUYAMA, T. and CHAKRABORTY, R. (1975) The bottleneck effect and genetic variability in populations. *Evolution* **29**, 1–10.

NEVO, E., ZOHARY, D., BROWN, A. H. D. and HABER, M. (1979) Genetic diversity and environmental associations of wild barley, *Hordeum spontaneum*, in Israel. *Evolution* **33**, 815–833.

OHTA, T. and KIMURA, M. (1975) The effect of selected linked locus on heterozygosity of neutral alleles (the hitchhiking effect). *Genet. Res., Camb.* **25**, 313–326.

OKA, H. I. (1975) Genic analysis for the sterility of hybrids between distantly related varieties of cultivated rice. *Jpn. J. Genet.* **55**, 397–409.

OKA, H. I. (1958) Intervarietal variation and classification of cultivated rice. *Ind. J. Genet. Pl. Breed.* **18**, 79–89.

OKA, H. I. (1964) Considerations on the genetic basis of intervarietal sterility in *Oryza sativa*. In *Rice genetics and cytogenetics* (ed. IRRI), pp. 158–174. Elsevier, Amsterdam.

OKA, H. I. (1974a) Analysis of genes controlling F_1 sterility in rice by the use of isogenic lines. *Genetics* **77**, 531–534.

OKA, H. I. (1974b) Experimental studies on the origin of cultivated rice. *Genetics* **78**, 475–486.

OKA, H. I. (1977) The ancestors of cultivated rice and their co-evolution. In *Meeting on African Rice Species* (ed. IRAT and ORSTOM). Paris.

OKA, H. I. (1978) Phylogenetic differentiation of cultivated rice. XXI. The sporophytic pollen sterility: its genetic basis and intervarietal relationships as shown by F_2 sterility. *Jpn. J. Genet.* **53**, 397–410.

OKA, H. I. (1981) The *Indica-Japonica* differentiation of rice cultivars. A review. IV SABRAO Congress, Kuala Lumpur.

OKA, H. I. and CHANG, W. T. (1962) Rice varieties intermediate between wild and cultivated forms and the origin of the *Japonica* type. *Bot. Bull. Acad. Sinica* **3**, 109–131.

PAI, C., ENDO, T. and OKA, H. I. (1973) Genic analysis for peroxidase isozymes and their organ specificity in *Oryza perennis* and *O. sativa*. *Can. J. Genet. Cytol.* **15**, 845–853.

PAI, C., ENDO, T. and OKA, H. I. (1975) Genic analysis for acid phosphatase isozymes in *Oryza perennis* and *O. sativa*. *Can. J. Gent. Cytol.* **14**, 637–650.

PERNÈS, J. (1975) Organisation évolutive d'un groupe agamique: la section des *Maximae* du genre *Panicum* (Graminées). *Mémoires* ORSTOM, 75, ORSTOM Ed., Paris.

PORTÈRES, R. (1950) Vieilles agricultures de l'Afrique intertropicale. Centres d'origine et de diversification variétale primaire et berceaux d'agriculture antérieurs au XVI° siècle. *L'agronomie tropicale*, **V**, 489–507.

RICHARIA, R. H. (1960) Origins of cultivated rices. *Indian J. Gen. Pl. Breed.* **20**, 1–14.

SANO, Y., CHU, Y. E. and OKA, H. I. (1979) Genetic studies of speciation in cultivated rice. I. Genic analysis for the F_1 sterility between *O. sativa* L. and *O. glaberrima* Steud. *Jpn. J. Genet.* **54**, 121–132.

SCANDALIOS, J. G. (1979) Control of gene expression and enzyme differentiation. In *Pysiological Genetics*, pp. 63–107. Academic press.

SECOND, G. (1975) Prospection des espèces Africaines de riz au Sénégal et en Gambie. Mimeogr. Report, 30p. ORSTOM, Paris.

SECOND, G. and MORISHIMA, H. (1981a) Geographical and ecological pattern of variation of *O. perennis* at 7 isozyme loci. *Natl. Inst. Gen. Mishima. 1980 Annual Report.* pp. 95–96.

SECOND, G. and MORISHIMA, H. (1981b) Mendelian segregation analysis at 3 isozymes loci in rice cultivars. *Ibid.* pp. 117–118.

SECOND, G. and SANO, Y. (1981) An introgressed electromorph found in an isogenic line of *O. Sativa* with a sterility gene from *O. glaberrima*. *Ibid.* p. 118.

SECOND, G. et TROUSLOT, P. (1980) Polymorphisme de treize zymogrammes observés parmi diverses espèces sauvages et cultivées du genre *Oryza*. In *Electrophorèse d'enzymes de riz* (*Oryza* Spp.), Travaux et documents 120, pp. 50–88. ORSTOM, Paris.

SELANDER, R. K. (1976) Genic variation in natural populations. In *Molecular evolution* (ed.

J. Ayala), pp. 21–45. Sinauer associates. Sunderland.

SHAHI, B. B., MORISHIMA, H. and OKA, H. I. (1969) A survey of variations in peroxidase, acid phosphatase and esterase isozymes of wild and cultivated *Oryza* species. *Jpn. J. Genet.* **44**, 303–319.

SHINJYO, C. (1975) Genetical studies of cytoplasmic male sterility and fertility restoration in rice, *O. sativa* L. *Bull. Coll. Agr. Univ. Ryukyus.* **22**, 1–55.

TERAO, H. and MIZUSHIMA, U. (1939) Some considerations on the classification of *Oryza sativa* L. *Japan. J. Bot.* **10**, 213–258.

TING, Y. (1933) Wild rice of kwangtung and new variety bred from the hybrids of wild rice with cultivated rice. *Coll. Agric. Sun Yatsen Univ., Agron. Bull.* **3**, 1–22.

TROUSLOT, P. et SECOND, G. (1980) Technique d'électrophorèse en gel d'amidon appliquée à l'étude du polymorphisme de 14 enzymes de riz. In *Electrophorèse d'enzymes de riz* (*Oryza* Spp.), Travaux et documents 120, pp. 7–48. ORSTOM, Paris.