THE STUDY OF ISOZYMES IN RELATION TO THE DISTRIBUTION OF THE GENUS *ORYZA* IN THE PALAEOENVIRONMENT AND THE SUBSEQUENT ORIGIN OF CULTIVATED RICE

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Introduction

The present distribution of wild species of rice includes tropical monsoonal and equatorial Asia, intertropical Africa, New Guinea, Northern Australia and intertropical America. The genus *Oryza* belongs to that group of Gramineae considered to have retained some primitive features (Stebbins, 1956, Tateoka, 1957). The chromosomes are small with a basic number n = 12. Some 20 species are recognised : 3 of them are distinctive but others, whose delimitation is often a matter of opinion, occur in complexes. Two species complexes with a nearly pantropical distribution will be mainly studied in this paper: 1) the *O. sativa* complex including all cultivated rices and their closest spontaneous relatives. 2) the *O. latifolia* complex with no domesticated form. The isolated species *O. australiensis* will be considered in association with the *O. latifolia* complex for reasons indicated below.

It is known (Portères, 1950) that rice was probably domesticated independently in Asia (*O. sativa*) and in Africa (*O. glaberrima*) but the place of domestication in Asia has not been settled since de Candolle (1883) suggested that India and South China were probable places of origin.

This paper is based mainly on extensive isozyme surveys; the objective is to propose or to confirm the following points:

(1). The distribution of wild species closely related to cultivated rice may have been restricted to the Old World and Australia before their recent introduction in America.

(2). Three main areas of differentiation of the two complexes with a present pantropical distribution are recognised in the Old World: Africa, South Asia and China. They may be related to geographic barriers to the land migration of rice which arose since the glaciations. Australia and New Guinea represent a fourth area of differentiation which could be related to temporary migration between Australasia and Asia during the Tertiary. The latter area corresponds to the greatest differentiation.

(3). The distribution of the ancestors of cultivated rice may have extended beyond that of the present day, at the margins of their distribution in the west of monsoon Asia (Pakistan in particular) and in central (and northern ?) China, because they have been part of the ecosystems in these areas for a very long time.

(4). At the time of domestication in Asia, the differentiation between annual and perennial life forms was probably more pronounced than at present.

(5). The two main types of common Asian rice, the *indica* and the *japonica* types of *O. sativa,* may probably be explained by two independent domestications in South Asia and China respectively.

Not all the original isozyme data on the basis of which these points are made have yet been published. This presentation is made in the hope that discussion with specialists in the study of the palaeoenvironement may help to clarify the interpretation of the isozyme data.

1. The electrophoretic technique

The technique used is an electrophoresis on starch gel of direct extracts of seedlings a few days old or of leaves. Thirteen different enzyme systems are stained. The bands are estimated to be determined by at least 40 loci of which 15, among the most important for the conclusions, have been demonstrated on the basis of Mendelian segregations. In addition, a technique was developed to study the thermo-stability of the isolated isozymes. Details on the technique and the data obtained can be found in Second and Trouslot (1980) and Second (1982, 1984).

2. The genetic structure of cultivated rice

A multivariate analysis of the isozyme diversity studied at forty loci in cultivated rice is shown in Fig. 1. The spontaneous annual African species, *O. breviligulata,* with wild and weedy origins and a close affinity to the African cultivated rice, *O. glaberrima,* are included in Fig. 1. Distinction between *indica* and *japonica* types of *O. sativa* is based on the phenol reaction test shown to possess a low probability of misclassification in the two types (Morishima and Oka, 1981). Although the number of strains plotted is small, it represents more than 2,000 strains observed in preliminary isozyme analysis. Great care has been taken to include types of as many geographical and ecological origins as possible. A dot represents a single or many individual varieties.

The presence or absence of isozymes on the zymograms, their electrophoretic mobility and their thermostability are considered. The area covered by a group of strains or a species is associated with their overall isozyme diversity while the distances in Fig. 1 are related to isozyme genetic distances.

It appears that, while *O. sativa* is much more polymorphic than *O. glaberrima,* its isozyme diversity is clearly associated with the *indica/japonica* differentiation. The small diversity of *O. glaberrima* is included within the larger polymorphism of its spontaneous counterpart, *O. breviligulata*. Remarkably, some weedy strains of the latter had a closer genetic affinity with *O. sativa* than both *O. glaberrima* and the wild strains of *O. breviligulata*. On the basis of field and artificial plot observations of hybrid swarms and multivariate analysis of data of visible characters, such weedy strains are assumed to be escapes from introgressive hybridization of *O. sativa* by *O. glaberrima*.

The observed genetic structure fits the hypothesis of domestication of *O. glaberrima* from *O. breviligulata,* independently of *O. sativa*. The relation between *O. glaberrima* and *O. breviligulata* may then be tentatively taken as a model to exemplify the domestication of rice: the diversity of the wild form largely exceeds that of the cultivated one; the origins of weedy forms of rice are complex and include hybridization between wild and cultivated forms and a return to wild characters through introgressive hybridization between independently domesticated cultivars. This emphasizes the ambiguity of the term "wild" rice.

Another characteristic permitting some distinction between *indica* and *japonica* is the pollen sterility of the F_1 hybrids. Fig. 2 shows the distribution of 115 strains of *O. sativa* in the plane defined by the mean percentage of pollen fertility in F_1 hybrids with, axis 1: two *japonica* strains, and axis 2, one *indica* strain. The two groups of 8 strains each with the extreme F_1 pollen fertility relations were analysed at the 40 isozyme loci. They appeared each to have a low diversity but to be complementary in that the greater part of the polymorphism of *O. sativa* could be explained by introgressive hybridization between them. An "actual" and an "ancestral" pattern of isozymes of *indica* and *japonica* was thus defined as the isozymes frequently found in the whole groups, or the restricted groups with the extreme sterility relations defined above, respectively. They are presented in table 1 along with the pattern of *O. glaberrima* for the 21 most polymorphic loci in cultivated rice. On first approximation, the three patterns are at a similar genetic distance in the three pair comparison as shown on Fig. 3.

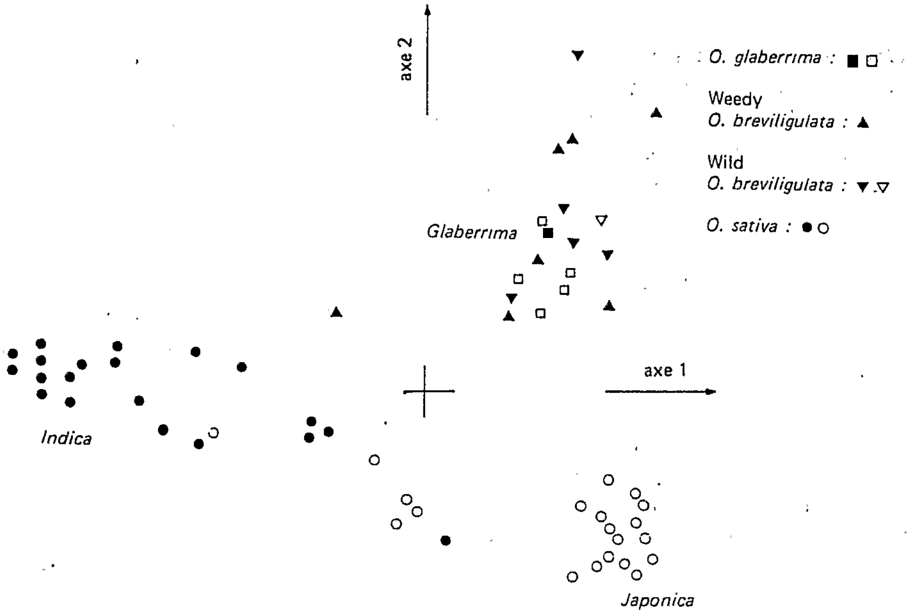


Fig. 1. Sixty strains of rice plotted in the plane defined by the axes 1 and 2 of a correspondence analysis of the genic diversity scored at forty isozyme loci. Their classification is indicated by a conventional sign. An open sign indicates the negative phenol reaction of the strains and allows a distinction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

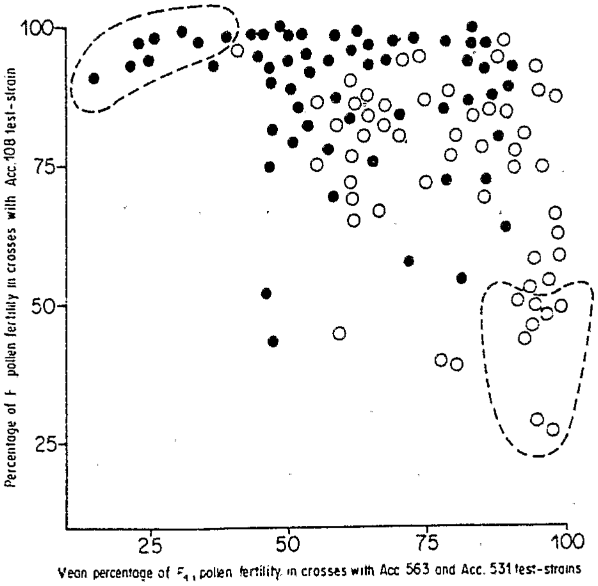


Fig. 2. Scatter diagram of 115 strains of *O. sativa* showing the relation between F_1 pollen fertilities with an *indica* test strain (108) and two *japonica* test strains (563 and 521, mean value). An open sign indicates the negative phenol reaction as in Fig. 1. The two groups presumed to represent the ancestral pattern of isozymes were encompassed with dotted lines (The F_1 pollen fertility data were kindly communicated by Dr. H.I. OKA).

Table 1. Frequent (5 per cent, about) electromorphs (indicated by their conventional numbers and listed in the order of their frequencies) found within 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 relations 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	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]

On the basis of these results, which are presented in greater detail in Second (1984), a hypothesis for an independent origin of *indica* and *japonica* is proposed.

This view is not shared by Oka (1974), who proposed that the two types differentiated from a common ancestor in the course of domestication. Moreover, on that matter, there is considerable difference of opinion among research workers. It was thus hoped that a study of the spontaneous relatives of *O. sativa* would throw some light on its origin. As shown above with *O. breviligulata*, a "wild" form may be an escape from cultivars. Human interference in the evolution of spontaneous rice has, no doubt, been serious and the situation may be expected to be complex. This is discussed in the next section.

3. The genetic structure of the spontaneous forms of the *O. sativa* complex and a proposed interpretation.

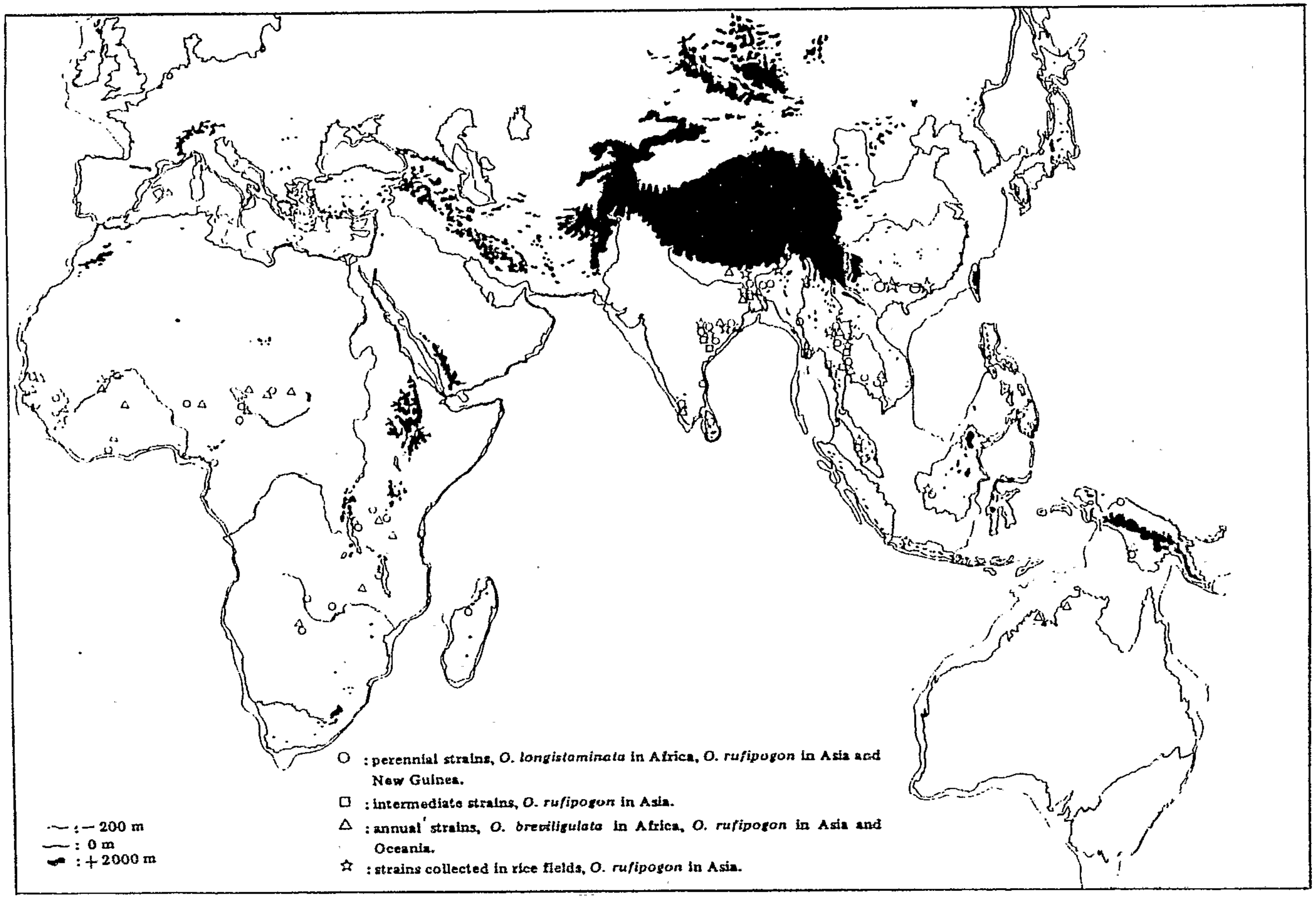
The *O. sativa* complex shares only one genome: AA. All species are diploid. A geographical axis of morphological variation with Asian, New Guinean, Australian, African and American forms (Morishima 1969) and an ecological axis with annual, perennial and intermediate life forms (Oka and Morishima, 1967) have been recognised. Typical habitats for the annual life forms are the temporary pools found in dry savannahs during the rainy season; the intermediate or perennial life forms are more often found in more humid savannahs or open areas in inundated equatorial forests, in pools, calm river flats or deltas and flood plains. In Asia, they are, more often than not, found in or close to rice fields. Although they are commonly found in a great part of monsoonal and equatorial Asia, Africa and Northern Australia, they are more difficult to locate in tropical America (H.I. Oka, personal communication). In Africa, both life forms are found over vast areas still relatively little disturbed by agricultural activities.

One hundred and eighty strains were chosen to represent as equally as possible all geographical and ecological origins available in collections (including collections in France, Japan and the Philippines) as shown on Map 1.

They were studied at 24 loci, those which expressed a most stable polymorphism independent of growing conditions and life stage. A multivariate analysis of the data based on the genetic distances of Nei (1975) in a comparison of pairs of strains is shown in Fig. 4A. The 3 "ancestral" isozyme patterns of cultivated rice defined above are included for comparison.

Three groups may be clearly distinguished:

(a) A large cluster including all Asian and American strains and some New Guinean strains (all belonging to *O. rufipogon*) and the African



Map. 1 A map of the Old World and Australia showing the - 200 m. isobathic line and the areas more than 2000 m. above sea level. Geographical origins of the spontaneous strains of the *O. sativa* complex are shown by conventional symbols according to their classification. A dot may correspond to several strains extracted from different populations.

annual *O. breviligulata*. The three types of cultivated rice are included in this group.

(b) The African perennial species *O. longistaminata*.

(c) The Australian annual form and some New Guinean strains belonging to *O. rufipogon*.

Among the large cluster, *O. breviligulata* may be distinguished from all others on the basis of one specific frequent isozyme. On the contrary, no frequent isozyme is peculiar to the American strains which had counterparts in Asia, particularly in Indonesia. It is interesting that (Fig. 4B) the *japonica* type of isozyme pattern is to be found only among some Chinese strains which are distinct from all others. These strains did not show, at the morphological level, any trace of hybridization with cultivated rice. Other Chinese strains, including some bearing evidence of hybridization with cultivated rice, are found to be intermediate with types of south Asian and south-east Asian origin. The *indica* type of isozyme pattern is found on the opposite side to the Chinese strains, on the margin of the cluster but close to the south or south-east Asian group in which no specific geographical or ecological trend could be clearly distinguished. In fact, a distinctive feature of the strains of Asian origin is their greater diversity. In comparison, both annual and perennial African species and the Australian species have a small diversity, although, in view of their habitat and distribution, they had more reasons to be representative of the situation in the palaeoenvironment. This observation fits the hypothesis (Chang, 1976) that before domestication in Asia there existed annual and perennial life forms with no intermediates.

Moreover, it seems that the Himalaya acted as a barrier to the land migration of rice, enhancing the differentiation between Chinese and South Asian types. The great diversity of Asian spontaneous rice could result simply from the breakdown of most of their reproductive geographical, ecological and genetical barriers through the transportation of seeds and the reclamation of the natural habitat of rice for cultivation.

It is believed that, assuming no migration of genes, the isozyme genetic distances may be related to the time of divergence (Wilson, Carlson and Thomas, 1977). The large isozyme genetic distances found between the Australian types and the other (D of Nei of the order of magnitude of 0.8 in pairs of strains comparisons) give an estimated time of divergence of roughly 10 to 20 million years on the basis of the calibration of the clock made by Sarich (1977). It could correspond to the epoch when migration was possible at least temporarily between Australia and Asia, an event which is believed to have affected many grass genera (Clifford and Simon, 1981) and the diversity of the tropical vegetation of the area (Walker, 1982). The differentiation of the Australian representatives of the *O. sativa* complex is also perceptible at a morphological level as described in Ng *et al.* (1981). If such an estimate of a remote migration to Australia is accepted, it means that the *O. sativa* complex existed at a time when the climate was more hot and humid and probably more suitable for rice over vast areas up to the mid-latitudes. The present geographical structure within the Old World could have arisen when, following cooling of climate, rice retreated toward the tropics with the separation by oceans, deserts or high mountains of three large areas: Africa, South Asia and China.

As judged from the isozyme genetic distances between the annual and perennial life forms in Africa (D of the order of magnitude of 0.5 in pairs of strains comparisons) their separation could be as early as the end of Miocene time. Whether this dating would mean that the separation into life forms is as ancient, or that the annual African form represents a more recent introduction from Asia, is an interesting question. The fact that the three isozyme patterns of cultivated rice appear to be relatively close to each other (D = 0.3) and to the African annual *O. breviligulata,* compared with the available diversity within the *O. sativa* group, supports the hypothesis put forward by Whyte (1972) that they were all domesticated from an annual ancestor but points to a time of divergence during Pliocene time (See section 5).

Palaeontologists may possibly decide in which epoch there might have existed arid, hot savannahs suitable for the land migration of annual wild rice, along with animals or the ancestors of Man and by steps represented by temporary pools. In this connection it may be noted that present representatives of rodents which arrived in North Africa during the Miocene from Eurasia are also characteristic for most of the arid and semi-arid zones (Jaeger 1975: 131). Aridification of the Sahara began in late Miocene time with more or less arid oscillations; its desertification began in early Pliocene (Maley, 1980).

In any case, a remarkable finding is that, under the same assumptions of isozyme evolution, types of American origin represent recent introductions, hence due probably to human agency.

To test the validity of the proposed interpretation, a study of the *O. latifolia / O. australiensis* group is in progress. Preliminary results are given in the next section.

4. The genetic structure of the *O. latifolia / O. australiensis* group of wild species:

The *O. latifolia* complex includes seven taxonomic species (Tateoka, 1962) which share the genomes BB, CC and DD at the diploid level and BBCC and CCDD) at the tetraploid level. At the diploid level, the BB and CC genomes are more frequent in Africa and south Asia respectively. The only Chinese strain available to me corresponds, at isozyme level, to the DD genome (obviously, that point must be confirmed, as it is

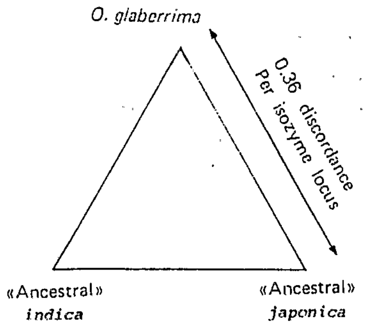
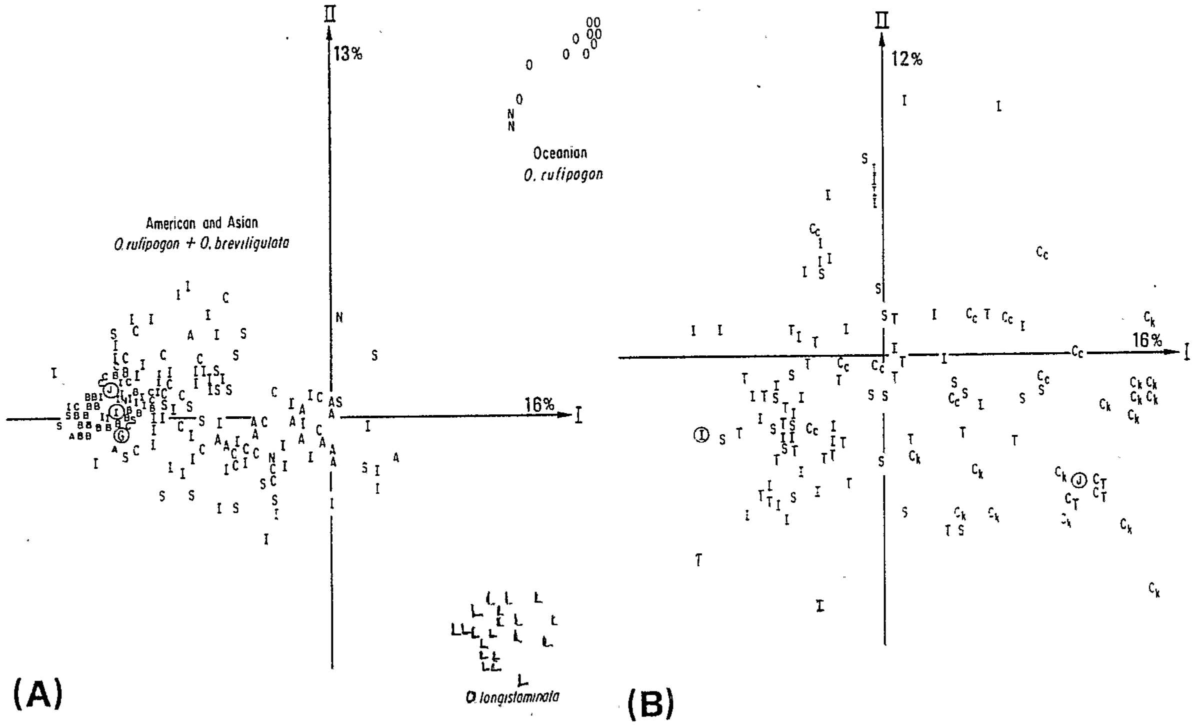


Fig. 3. Distances between the electrophoretic patterns, scored at 40 loci, of *O. glaberrima* and the "ancestral" *indica* and *japonica* subspecies in terms of the proportion of discordances per locus.



A: South America, Cuba; B: *O. breviligulata;* C: China (C_k: Kwangsi, C_c: Kwangtung) C_T: Taïwan;
I: India, Sri-Lanka, Nepal, Bangladesh; T: Burma, Thailand, Kampuchea; L: *O. longistaminata;*
N: New Guinea; O: Australia; S: Malaysia, Indonesia, Philippines.
Ⓖ: *glaberrina;* Ⓘ: *indica;* Ⓙ: *japonica.*

Fig. 4. A principal coordinate analysis of the genetic distances of Nei based on 24 isozyme loci among spontaneous strains of the *O. sativa* complex
A) 180 strains representative of the whole complex,
B) 110 strains representative of Asian origins only. The geographical origins are indicated by a conventional sign. The "ancestral" patterns of *indica, japonica* and *glaberrima* types of cultivated rice are included.

central to the following argument). The tetraploïds BBCC are found mainly in Asia but also in Africa, and the tetraploïds CCDD constitute the great diversity of forms found in tropical America. Compared to the *O. sativa* complex, the habitat of the *O. latifolia* complex is found under shade, often in forests. Most forms or species are perennial, except for the diploïd BB representatives in Africa which are annual and found in temporary pools, sometimes under shade.

The distinct species *O. australiensis* is close to the *O. latifolia* complex on the basis of spikelet morphology. It is a diploïd with the genome EE which was suggested to be closely related to the DD genome (Katayama, 1982). *O. australiensis* and the *O. latifolia* complex also share the characteristic of larger chromosomes than the *O. sativa* complex (see Nayar, 1973). Consequently, they are considered here as belonging to the same biological species complex.

At the isozyme level, only preliminary studies at seven loci have been made in some 150 strains of various origin. An interesting parallel between the two complexes appeared: in both, the American origins had homologues in Asia, at least at isozyme level if not at the morphological level which is thought to evolve sometimes rapidly (Anderson and Stebbins, 1954). Thus, they may be assumed to have been introduced recently. The very distant relative *O. subulata (= Rhynchoryza subulata)* may be the only species of *Oryza* (?) endemic to America.

A distinctive feature in the *O. latifolia* complex is that each genome has a low diversity, more comparable to the diversity of a single entity as the African annual or the African perennial species of the *O. sativa* group. This point confirms the view that the large heterogeneity of the Asian representatives of the *O. sativa* group shown in the preceding section may be due rather to a recent heterogeneity through human interference than to their spontaneous evolution. However, the total diversity of the *O. latifolia* complex largely exceeds that of the *O. sativa* complex. These points being made, it seems reasonable to assume on the basis of the present distribution of the diploïds that, in the palaeoenvironment, each diploïd genome, B, C, D and E, corresponded to one of the basic areas of differentiation recognised in the *O. sativa* complex: Africa, south Asia, China and Australia respectively. Recent migration would explain that, at present, more than one genome is found in a single continent (except in Australia ?). The appearance of allotetraploïds BBCC and CCDD would then be recent, as confirmed by the fact that their zymograms seemed to represent the simple sum of the zymograms of their putative ancestors. A main difference in the evolution of the two groups may be that, in the *O. sativa* complex, migration between continents resulted only in the introgression of genes from different geographic origins, while in the *O. latifolia* complex allotetraploïds arose. That would fit the result of the overall smaller diversity of the *O. sativa* complex compared to the *O. latifolia* complex.

The postulate that migration of rice between continents through long distance dispersal is a recent phenomenon fits the thesis of Whyte (1972 and 1981) that there has been considerable migration and transport of grasses through human agency within the Old World (see also the "Africanization" of the New World tropical grasslands, Parsons, 1970).

These preliminary results show that the isozymic genetic distance between *O. australiensis* and the diploïd representatives of the South Asian species of the *O. latifolia* complex does not exceed that found between some Australian and Asian representatives of the *O. sativa* complex. That would fit the postulates that: 1) the application of the molecular clock hypothesis to the data is reasonable, and 2) that the geographic structure revealed is due rather to common climatic or orogenic causes that to rare, long distance, spontaneous dispersal. In turn, it implies that the differentiation into genomes has been more rapid in the *O. latifolia* than in the *O. sativa* complex. Assuming the larger DNA content of the first group genomes, this is not unreasonable. The "extra DNA" may fix mutations more rapidly leading to the loss of chromosome homology, as was proposed by Flavell (1982) in a different approach to the study of genomes of the temperate world cereals.

5. The origin of common rice:

It is beyond the scope of this study to discuss whether the origin of rice lies in Gondwanaland as postulated by Chang (1976) and which Plate may have borne it because the junction of the Plates making up the Old World may have been accomplished by the time of the described differentiation. It is however possible to argue for an introduction of rice species to Africa posterior to its junction with Laurasia. The only diploïd African *Oryza* species adapted to the shade of humid forest is *O. eichingeri* which has a CC genome and a close relation with Asiatic *O. officinalis*. All other diploïd species (*O. longistaminata* and *O. breviligulata* with AA *genome, O. punctata* with BB genome and *O. brachyantha* with FF genome) are adapted to savannas. It appears that, besides *O. eichingeri* whose introduction in Africa would be more recent (hence, not by means of land migration) the African species might have migrated to Africa through an arid environment during the end of the Tertiary epoch. On the other hand, the *O. officinalis, O. meyeriana* and *O. ridleyi* complex in Asia are adapted to forest environments. Consequently, palaeo-Eurasia could be the centre from which some *Oryza* species migrated to Africa and Australasia. Among the genus *Oryza,* the *sativa* and the *latifolia* complex are the most closely related but the *sativa* complex is strictly adapted to savannahs while the *latifolia* one is generally found under shade, particularly in Asia. Their differentiation may have occurred when open spaces became widespread during the Tertiary, probably in a coevolution of vegetation and vegetarian mammals (see Gabunia and Chochieva, 1982).

As suggested by observations made in Africa, the evolution of the large grain type, characteristic of the *O. sativa* complex, might have been enhanced by the gathering of large mammals around inundated areas as their feet sunk the rice seeds deep into the mud. Grains deep in the soil are protected from their parasites but need more energy to germinate. The larger seeds are found in the annual life forms, at least those which are adapted to temporary pools on silt compared to those found on hard pan with little soil.

The evolution of annual life forms might have occurred from perennial life forms when the duration of the dry season increased and temporary pools replaced large areas flooded for a longer period. It may have occurred independently in Australia and in Asia but land migration within the Old World for the annual life forms might have been possible until the end of Pliocene time between Africa, south Asia and China as suggested by the genetic distance of D = 0.3 found between the three types of cultivated rice. Such possibilities of migration might have been stopped about contemporarily at the end of the Pliocene, due to desertification of the Sahara, cooling of climate and the rise of the Himalaya as a barrier to the land migration of animals not older than 2 or 3 million years (see Liu and Ding, this volume).

How does the study of isozymes fit the various hypotheses concerning the domestication of Asian rice ? Oka and Chang (1962) proposed that the Asian ancestors of cultivated rice have a potentiality to differentiate into *indica* and *japonica* which would have been revealed in the course of domestication. Such a view is corroborated in several papers by H.I. Oka and collaborators but alternative interpretations are rarely considered. It seemed to be confirmed by the study of hybrids between cultivated rice and a single strain of spontaneous rice (Oka and Morishima, 1982); however the characters analysed related more to certain characters which might have been selected by Man rather than to the basic genetic diversity. On the other hand, this hypothesis does not agree with the data of isozyme studies as analysed by the present author. Isozyme data support rather an hypothesis of the independent domestication of *indica* and *japonica*, followed by their introgression of genes reciprocally and from the local wild rice in the various areas of their distribution. It is assumed that coadapted gene complexes including some directly selected by Man but superimposed on the spontaneous genetic structure are involved in the maintenance of the differentiation. Neutral isozyme markers would be "hitch-hiked" in the process (Second, 1982). It was not possible, however, to design an experiment to "prove" the validity of either opinion.

An attempt has been made to show that, most probably, the isozymic differentiation characteristic of *indica* and *japonica* was established by the time of domestication and that it corresponded to the south Asian and Chinese areas respectively. This rather fits the opinion of the present author and points to the two areas of initial domestication. A study of the presupposed third independent domestication, that of

O. glaberrima, could then possibly lead to an understanding of the primary steps in the domestication of rice.

Nevertheless, supporters of the opinion of H.I. Oka could argue, for example, that the separation between south Asian and Chinese areas has never been absolute for rice, or that land migration of wild rice may have been particularly enhanced through Indochina during the maximum glaciation, when tropical forest may have retreated further south. It could have created a centre of diversity of wild rice in south-east Asia. Domestication could have started in this area involving, since its origin, a complex heterogeneity of ancestors. Evidently, convergence from different fields of study is necessary to throw light on this problem.

6. Conclusion: Are isozymes relevant to a reconstruction of the palaeoenvironment?

In conclusion, it is desirable to explain the reasons why isozyme markers may be relevant to phylogenetic studies in the state of the great lack of material evidence concerning this subject of research. Several problems encountered in the interpretation of isozyme data will be mentioned.

First, the interest in isozymes lies in the fact that they are universally distributed among living organisms and that their genetic determinism is generally simple. Second, these simple markers may be neutral to direct Darwinian selection (see Kimura, 1979). This has two important consequences:

(a). Isozymes may evolve at a constant rate, thus being a molecular clock.

(b). The amount of polymorphism within an isolated population built up as a function of only the mutation rates and the reproductively effective size of the population. In other words, the neutral theory permits predictions. For example, a low polymorphism means a small effective size of the population or a recent bottleneck in the population size. A high polymorphism would mean either the reciprocal proposition, large population for a very long time, or, when bottlenecks are frequent, it would suggest a recent heterogeneity due to the mixing of two divergent lineages.

In addition to the fact that it is difficult to prove that isozymes are effectively neutral to direct selection, several problems remain:

(a) - Neutral genes may be "hitch-hiked" by a selected gene borne on the same unit of genetic recombination. The effect of this is not clear in transient populations that are likely to be studied.

(b) - Because of the relatively small number of genes that we are technically able to study, the statistical variance of all estimations is very large. Moreover, the samples of genes studied cannot represent a random sample of the genomes.

(c) - Functional constraints responsible for the elimination of deleterious genes - no doubt in an overwhelming majority of the non-neutral mutants - may vary according to the environment and the structure of the genomes (duplicated genes, etc.). The concept of neutrality could thus be relative to the environment, at least in some cases.

(d) - Migration between populations may enhance their divergence to an unknown degree when genetic reproductive barriers are weak. To complicate this, an observed strong genetic barrier may have become established in the course of an extensive migration.

(e) - It is difficult to calibrate the presumed isozyme clock. It is not clear whether the rate of evolution may vary according to the organisms considered but it is known that the rate of fixed mutations depends on the loci considered and that the observed differentiation depends on the electrophoretic technique utilized. An isozyme clock is certainly a stochastic one in which the variance of error is large. Depending on the isozyme loci surveyed, it may be applied tentatively in the range of a few hundred thousand years to perhaps 40 million years (Sarich, 1977).

The model of evolution proposed for rice with four geographical areas isolated by tectonic or climatic events and recent considerable migration between them through human agency should be an appropriate example in which to apply the neutral theory of isozymes and the molecular clock hypothesis. Independent methods to test the model and a strengthening of the theoretical basis on which the interpretation is constructed are then essential. Artifacts can represent one possibility. Fossil records are rare for *Oryza* or related genera. *O. exasperata* is reported from the Miocene strata of Switzerland by Heer, who described and illustrated supposed anthoecia (Heer, 1855, table XXV, Fig. 5a, b). Unfortunately the illustrations do not provide convincing evidence for their placement in the genus *Oryza*. *Archaeoleersia nebraskensis* found in north America is the first undoubted close parent of the genus *Oryza* to have been reported from late Miocene strata. It is interesting to note that the fossil spikelets resemble some of the tropical species of the modern genus *Leersia* which are now found only in south America or in Africa. (Thomasson, 1980).

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ROBERT ORR WHYTE

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