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PACHYTENE ANALYSIS IN THE GENUS *ORYZA*

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ABSTRACT

Pachytene analysis is in the genus *Oryza* is the most fruitful line of approach to the elucidation of evolutionary problems. Cultivated species of this genus have more asymmetric karyotypes. The cell to cell variation in absolute lengths of the chromosomes in *O. perennis* var. *barthii* was shown to be due to general condensation differences and hence the data on relative lengths can be employed in the identification of the karyotype. Sterility in *japonica-indica* rice hybrids is largely due to chromosome structural hybridity. Occurrence of 24I at metaphase I of the F_1 hybrid *O. sativa* × *O. officinalis* is duc to desynapsis rather than lack of homology between the constituent genomes. The F_1 hybrid *O. sativa* × *O. australiensis* exhibits distinct timing imbalance in meiosis, the chromosomes of *O. australiensis* being earlier in condensation as well as migration. Pairing at pachytene is normal in the F_1 hybrid *O. perennis* var. *balunga* × *O. perennis* var. *cubensis*. The F_1 hybrid *O. sativa* × *O. perennis* exhibited several abnormalities in pairing at pachytene. The F_1 hybrid *O. sativa* (4x) × *O. perennis* var. *longistaminata* exhibited a high frequency of frying-pan-shaped trivalents, indicating segmental homology between the genomes of the parents.

Pachytene analysis in the genus *Oryza* was initiated by Yao, Henderson and Jodon (1958) for demonstration of structural hybridity in inter-varietal rice hybrids. Shastry, Ranga Rao and Misra (1960) employed this method for karyomorphological studies and indicated the potentialities of using this technique for various problems. The cytological studies in the past two years bore out the expectations and indicated that the genus *Oryza* is eminently suited for pachytene analysis. Published and unpublished data on rice cytology obtained by me and my collaborators at Indian Agricultural Research Institute are discussed below:

1. Interspecific variation in the karyotypes: The karyotypes of *O. australiensis* Domin., *O. perennis* Moench., *O. officinalis* Dall., *O. sativa* var. *fatua* Prain., *O. stapfii* Roschev., *O. breviligulata* Chev. et Roer., *O. glaberrima* Steud. and *O. sativa* Linn. (sub sps. *japonica* and *indica*, Kato) were investigated by pachytene analysis. The karyotypes of these species exhibit distinct variation not only in symmetry, but also in the appearance of the bivalents at pachytene.

The pachytene bivalents of *O. australiensis* are highly heterochromatic, darkly stained segments alternating with lightly stained fibrillae, a feature noted exclusively in this species (Shastry and Mohan Rao, 1961). The bivalents of *O. officinalis* and *O. perennis* are not as heterochromatic as those of *O. australiensis*, but are thicker than those of *O. sativa* and exhibit no chromomeric details. The bivalents of *O. sativa*, *O. glaberrima*, *O. spontanea*, *O. stafii* and *O. breviligulata* are seriated by several micro- and macro-chromomeres all along their length, interrupted by darkly stained segments in some places (Shastry and Mohan Rao, 1961, Gopakumar and Shastry, in press, Sharma and Shastry in press.) The appearance of bivalents itself suggests a parallel evolution of *O. perennis* and *O. officinalis* from stocks resembling *O. australiensis* and this view is further supported by the meiotic studies of the F_1 hybrids to be discussed later.

It is well known that in several plant genera, primitive taxa are characterized by symmetric karyotypes. Two criteria-predominance of isobrachial chromosomes and limited variation in length of the chromosomes—are employed as a measure of symmetry. Employing these criteria, Stebbins (1958) suggested 12 groups within which the karyotypic diversities can be expressed. According to his classification, the karyotypes belonging to the group 1a are most symmetric while those belonging to 4c are most asymmetric. The karyotypic data of the *Oryza* sp. so far secured are presented in Table 1, following the method adopted by Stebbins. It would be obvious that *O. perennis* var. *barthii* and *O. perennis* from Assam are included in the group 1b while *O. perennis* var. *balunga* in the group 2b. The inclusion of *O. perennis* var. *balunga* in group 2b is due to single chromosome which had an arm ratio less than 0.5. The analysis of more forms of *balunga* will help to decide whether this belongs to a distinct group or whether other forms will be included in group 1b. In any case, the forms of *O. perennis* have the most symmetric karyotypes in the genus which supports the concept that this species is one of the most primitive members (Das and Shastry, in press).

The group 2b is most frequent, being represented by *O. sativa* var. *fatua*, *O. australiensis*, *O. perennis* var. *balunga*, *O. officinalis*, *O. stafii* and *O. sativa* sub. sp. *indica*. A single variety of *O. glaberrima* is included in group 3b while another of *O. sativa* sub. sp. *japonica* (Norin 6) is included in the group 3c. A general conclusion may therefore be justified that the cultivated species of *Oryza* have more asymmetric karyotypes than the wild species. The apparent diversity between the subspecies, *indica* and *japonica*, remains to be verified by the study of a

larger number of varieties. It is quite likely, however, that the large spectrum of morphological variability evident in *O. sativa* might well be reflected in karyotypes as well.

Comparison of karyotypic data secured by pachytene analysis with that secured by the analysis of somatic metaphases reveals some interesting points. The size variation between the extreme members of the complement is in general agreement. The observation of Mitsukuri *et al* (1958) that *O. perennis* has more median and *O. sativa* has more submedian and subtelocentric chromosomes is confirmed. The valuable additional data from pachytene analysis are regarding the linear differentiation of the chromosomes (eu- and heterochromatic segments) and the detection of minor differences in arm ratios—both of which are undetectable by metaphase analysis. Hu (1958, 1960 a and b) studied the karyotypes of *O. sativa* and *O. glaberrima* at somatic metaphase and of *O. granulata* at pachytene and concluded that general relations in homologies are probably maintained in these species, since he observed great agreement in the individual chromosomes of these species. This conclusion is, however, open to question since the data on *O. sativa* and *O.glaberrima* are secured by somatic metaphases where minor differences are undetectable and the analysis of *O. granulata* appears to have been undertaken with PMCs at late pachytene (as evident from his photographs). The present study reveals wide verietal differences in arm ratios, although the relative values of the chromosomes might be in agreement. This observation points out the need for standardization of methods of analysis.

2. Evaluation of the pachytene analysis for karyotypic studies: Karyotypic studies involve the measurement of lengths of total chromatin, of the individual chromosomes and of the arms. Since the chromosomes are coiled at all stages of cell cycle and the degree of coiling might be subject to variation from cell to cell because of intrinsic differences as well as due to the differential effect of the fixation and staining, absolute lengths of the chromosomes have limited value unless checked by an independent method of approach such as DNA estimation by microspectrophotometry. To obviate or minimize the cell to cell variation in condensation of the cromosomes, Tjio and Hagberg (1951) introduced "relative value", which is the percentage of the length of individual chromosomes to the total chromatin length. The relative values have their own limitation, since they minimize the general condensation differences but not differential condensation differences, if the latter were to exist.

The relative values of individual chromosomes were studied in 19 PMCs of *O. perennis* var. *barthii* wherein the full complement was analysed in each cell (Shastry and Das, unpub.). In these PMCs, the total chromatin length varied from 208.5 to 350.0 μ and hence the PMCs analysed represent various stages of condensation from early to late pachytene. When the absolute lengths of individual chromosomes are ranked in descending order, comparable chromosomes exhibit discordant lengths. When the relative lengths are compared, the individual chromosomes of the complement are identifiable and their relative lengths are comparable. To test whether the cell to cell variation is due to general or differential condensation differences between the cells, correlations between the absolute lengths of the chromosome and the total chromatin lengths of the cell from which the data on length of the chromosome were taken were calculated. It was observed that the 12 correlations so obtained were not only highly significant, but among them the 'r' values were homogeneous. This observation will indicate that cell to cell variation in total lengths is due to general condensation differences and hence the comparison of the karyotypes by relative lengths is valid. Analysis of the same data by X^2 confirmed this conclusion (Pershad, Shastry & Das, in press).

3. Chromosome pairing at pachytene in species of Oryza: One of the basic tenets of the theory of meiosis is that homology is a prerequisite for synapsis and that the former is the property of chromosomal segments rather than of whole chromosomes or genomes. Failure of pairing between homologous chromosomes or segments, and pairing between non-homologous chromosomes or segments is the result of superimposed factors which obliterate the general tendencies of attraction between strictly homologous sites on the chromosomes. With the above reservations, any deviation from the normal pairing at pachytene may therefore be attributed to structural hybridity. Further, homozygosity for genes as well as chromosomal segments is a rule in self-fertilized plants and hence most of the species of *Oryza* are expected to exhibit normal pairing at pachytene. This is what was observed in *O. sativa, O. glaberrima, O. perennis* (var. *balunga* and collection from Assam), *O. officinalis* (Bangkok) and *O. australiensis.*

The pairing at pachytene in different forms of *sativa* var. *fatua,* however, was highly abnormal (Gopakumar and Shastry, in press). Extensive differential segments were noted in the entire complement of this species. Some of the bivalents were heteromorphic and some regions were loosely paired. The high degree of pairing abnormalities in *O. sativa* var. *fatua* might be cited as evidence for hybrid origin of these forms as postulated by Sampath and Rao (1951), although it is not possible to exclude the possibility of their origin as a result of high frequency of out-pollination in a stable pure species. The wide difference in symmetry between the karyotypes of *O. sativa* and *O. perennis* and the intermediate position of *O. sativa* var. *fatua* in this respect might well be interpreted either in favour of hybrid origin of the last species or in the sequence of origin of *O. perennis*→*O. sativa* var. *fatua*→*O. sativa.* Sampath and Govindaswamy (1958)

considered the latter possibility at least for some forms of cultivated rices from Jeypore (Orissa).

A new form of abnormality was recorded in the pachytene bivalents of *O. perennis* var. *barthii*. In the entire complement, several segments were observed wherein the constituent chromosomes are not relationally coiled but paired side to side. This may be considered as a form of loose pairing, but is different from the segments with hazy outlines observed by Shastry and Misra (1961b) in *japonica-indica* rice hybrids. These segments probably represent regions which are not strictly homologous but which have a tendency for secondary association and hence when such segments are flanked by fully homologous ones, the pairing of the type observed is expected. This structural hybridity is most probably related to the cross pollinated habit of this species. Diakinesis and metaphase I stages, exhibited great reduction in chiasma frequency in this species. Several cases are known in literature where the cross pollinated species have lower chiasma frequency in comparison to the related self-pollinated species (Grant, 1958) and hence the observed structural hybridity and reduced chiasma frequency might be attributed to the breeding system of this species. It is pertinent to refer the observations of Richharia (1960) that *O. perennis* var. *barthii* is semi-sterile under open pollinated conditions and that seed setting was nil when bagged and of Sampath (1961) who suggested that this species may even be self-sterile.

4. Chromosomal sterility in japonica-indica rice hybrids: The existing controversies regarding the causes for sterility in *japonica-indica* rice hybrids are evident from the works of Mello-Sampayo (1952), Sampath and Mohanty (1954), Yao *et al* (1958), Sampath (1959), and Henderson, Exner and Jodon (1959). All the above workers postulated chromosomal causes—inversions, translocations, and cryptic structural hybridity while, Oka (1957) considered that the causes for sterility are due to gametic development and duplicate fertility genes. Erratic inheritance for sterility in these hybrids, rarity of some recombinant types, disturbances in genetic ratios are all interpreted as due to genic causes by Oka (1957). This controversy is largely because of adherence to observed normal pairing at metaphase I, inadequate proof of anaphase bridges as a result of heterozygosity for inversions and cursory cytological analysis at pachytene. Some of the hybrids were therefore investigated at diplotene and pachytene stages by Shastry and Misra (1961a and b) whose results are discussed below.

At diplotene, in a low percentage of cells, quadrivalents were observed in 2 of the 4 F_1 hybrids studied. At pachytene, 10 out of the 12 bivalents in one F_1 hybrid, T-21 (India) x A-18 (Japan), exhibited one or more of the abnormalities *viz.*, loose pairing, heteromorphicity, reversed repeats and differential segments. Similar abnormalities were recorded in several PMCs of the other 3 hybrids as well, although at a lower frequency. The rarity of inversion loops at pachytene, in the hybrids studied, indicates that mechanism of genetic differentiation between these sub-species is to be sought in other structural changes. While heterozygosity for inversion will lead to sterility only following a cross over within the inverted segment, the translocation heterozygosity can lead to sterility, irrespectively of whether a quardrivalent is formed or not, by the independent assortment of the chromosomes resulting in duplication-deficiencies in the gametes. On this basis, the above authors hypothesized that most of the differential segments represent cases of small reciprocal translocations. Venkataswamy (1957) and Sampath (1959) considered the role of translocations in the differentiation of these sub-species, but heir views were not supported by adequate cytological evidence.

Sterility in *japonica-indica* F_1 hybrids and its erratic inheritance in the following selfed generations, suppression of some recombinant types, sporadic occurrence of chlorophyll deficient (*albina*) plants in F_2 to F_6 generations, viable 'mutations' such as—'narrow leaf' 'dwarf-sterile' and 'long glume' —can all be re-interpreted in the light of the cytological data secured from pachytene analysis. G. D. genes of Oka might indeed represent the homologous segments located on non-homologous chromosomes. Erratic inheritance for sterility might be related to the frequency of occurrence of crossing over between the centromeres and differential segments. Chlorophyll and viable mutations may be due to haploviable deletions resulting from crossing over in a structural hybrid. In the light of high degree of chromosomal differentiation, as evidenced from the present tusdy, it is superfluous to postulate purely genic models in accounting for sterility. Evolutionary diversification between the sub-species—*japonica* and *indica* may therefore be considered to have been achieved largely by small (equal or unequal) reciprocal translocations.

5. Desynapsis vs. Genomic differentiation: Pioneering work on the genome analysis in *Oryza* was done by Morinaga and his collaborators. In this study as well as in other crop plants, only data from metaphase pairing were available. At the diploid level, the pairing in the F_1 hybrid, *O. sativa* x *O. officinalis* was minimal (24I in majority of PMCs) which led to the designation of different genome symbols for the chromosome complements of these two species (Morinaga, 1959 and Richharia, 1960). When this hybrid was reinvestigated by Shastry, Sharma and Rao (1960, 1961) they observed that the anaphase disjunction was exceedingly normal (59% PMCs), an observation which is least expected in an intergenomic hybrid with total asynapsis. This hybrid was, therefore, reinvestigated for pachytene, diplotene, diakinesis and metaphase I stages. The data on metaphase I secured from 148 PMCs were

in accord with those of earlier workers (Ramanujam, 1937; Gopalakrishnan, 1959), but good congression on equitorial plate was noted in some PMCs. At diakinesis, likewise, considerable pairing was observed. Complete pairing was observed in several PMCs at pachytene.

Complete pairing at pachytene, variable pairing at later stages of prophase and complete univalent formation at metaphase I, indicate that the major cause for apparent non-pairing at metaphase I is desynapsis rather than lack of homology. This interpretation of meiosis in the hybrid has far reaching implications on (a) the subdivision of the section, Sativa of Roschevicz (1931) into two sections, Sativa and Officinalis (Ghose *et all*, 1956; Richharia, 1960), (b) on the genomic symbolization of the species, *O. sativa* and *O. officinalis* and (c) on the role of *O. officinalis* in the origin of cultivated rices which was excluded largely on the basis of non-pairing in the F_1 hybrid, *O. sativa* × *O. officinalis*. Since *O. perennis* (carrying the same genome as *O. sativa*) and *O. officinalis* are sympatric species, desynapsis in the hybrid might have served as an effective isolation barrier, thereby channelizing divergent evolution between these species and their descendents.

6. Timing imbalance in the F_1 hybrid, O. sativa × O. australiensis: The karyotypic studies of *O. sativa* and *O. australiensis* described earlier, indicated that the pachytene bivalents of the latter species are heterochromatic. In this context, the data of Gopalakrishnan (1959) that this hybrid exhibited 8 bivalents at metaphase I and the conclusion of Gopalakrishnan (1959) and Richharia (1960) that *O. australiensis* originated by hybridization between the members of the sections, Sativa and Officinalis seemed unlikely. This hybrid was therefore reinvestigated by Shastry and Ranga Rao (1961), whose results are discussed below.

The meiotic data on the F_1 hyrid *O. sativa* × *O. australiensis* was secured by the analysis of pachytene, diplotene, diaknesis and metaphase I stages. At pachytene, unpaired threads which were interpreted to be univalents were observed. At diplotene, only 12 univalents were observed which were interpreted to belong to *O. australiensis*. At diaknesis, the number of visible univalents varied from cell to cell and those of *O. australiensis* are clearly identifiable from those of *O. sativa* by their larger size and darker staining. The distinction between metaphase I and anaphase I stages was not clear due to high frequency of univalents. In PMCs at meta-anaphase I, true bivalents were only those of *O. sativa*, which were autosyndetic. The inter-genomic pairing (allosyndesis) is exclusively in the form of non-chiasmatic, end-to-end, pseudobivalents which ranged from 0-7 per PMC. Further, the univalents of *O. australiensis* migrated to poles earlier than those of *O. sativa*. The above data thus indicate that, in this hybrid, meiotic cycle of the chromosome complement of *O. australiensis* is advanced in comparison to that of *O. sativa*. Although no true allosyndetic bivalents were observed in this hybrid, this may be either due to lack of homology between these genomes or due to timing imbalance preventing the pairing between homologous chromosomes.

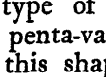
Richharia's (1960) inference of hybrid origin of *O. australiensis* and the inclusion of this species in an intermediate position between the sections, Sativa and Officinalis is based upon the meiotic data of Gopalakrishnan (1959) that a maximum of 4 and 8 bivalents are formed in the F_1 hybrids, *O. sativa* × *O. officinalis* and *O. sativa* × *O. australiensis*. Neither of the observations of Gopalakrishnan are confirmed. The intermediate morphological appearance of *O. australiensis* may therefore be reinterpreted to suggest that *O. australiensis* represents an isolate from pre-*Sativa* and pre-*Officinalis* stock. Restricted distribution of *O. australiensis* (to Australia) renders such a possibility very likely. Further proof for this view, however, can be secured only from the study of F_1 hybrids, *O. perennis* × *O. australiensis* and *O. officinalis* × *O. australiensis*, which are being attempted.

7. Genetic differentiation between O. perennis and O. sativa: Normal pairing in the F_1 hybrid *O. sativa* × *O. perennis* observed by Madhavan Nayar (1958) and Gopalakrishnan (1959) led to the conclusion that chromosome complements of these species belong to the same genome (P_1 Richharia, 1960). The only chromosomal difference indicated by the above workers is the occurrence of a single quadrivalent in 20-30 per cent of the PMCs at metaphase I. A. hybrid between *O. sativa* × *O. perennis* (from Madhya Pradesh) was analysed at pachytene for a detailed study of chromosomal differentiation between these species (Shastry and Misra, unpublished) and the results are reported below:

The pairing at pachytene in the F_1 hybrid *O. sativa* × *O. perennis* was highly abnormal. Differential segments, unequal terminal regions, loosely paired regions and reversed repeats were observed in all the 12 bivalents of this hybrid. Although pairing occurs between all the chromosomes of the one species with all the chromosomes of the other species, the homologous regions are limited in size. Even at diplotene, the heteromorphicity of the nucleolar bivalent was clearly analyzable. As was observed in the inter-varietal rice hybrids, the inversion loops are rare in this hybrid as well. The differential segments might therefore owe their origin to small reciprocal translocations. Structural differentiation between the chromosome complements of these species is as wide as expected from the distinct morphological differences.

The analysis of meiosis in the triploid F_1 hybrid, *O. sativa* (4x) × *O. perennis* var. *longistaminata* (2x)

revealed a high degree of chromosomal differentiation by way of translocations between the chromosome complements of these species. Although the data on this hybrid were not from pachytene, they are reported here as supporting evidence. One hundred and sixteen PMCs at metaphase I were analysed which exhibited 0-2 VIs, 0-1 Vs, 0-2 IVs and 2-12 IIIs per PMC, in addition to bivalents and univalents. The occurrence of full complement of trivalents confirms that the genomes of the parental species are homologous. The shapes of the associations gave a significant picture of importance. Since the hybrid is composed of two identical genomes of *O. sativa* and one of *O. perennis* var. *longistaminata*, if the chromosome complements of these species are completely homologous, the triploid must behave like an autotriploid. It is evident from the analysis of Upcott (1935), in autotriploid tomato, that random pairing between three chromosomes usually leads to a chain configuration. With structural hybridity, however, this relationship is bound to change. In the hybrid under study, a maximum of 10 frying-pan-shaped trivalents were observed.

The frying-pan-shaped trivalents are interpreted to originate from preferential pairing of *Sativa* chromosomes and limited but highly localized (segmental) homology of the chromosomes of *O. perennis* var. *longistaminata* with those of *O. sativa*. Ring-shaped trivalents, indicating secondary trisomic condition for some chromosome segments were observed in 20 per sent of the PMCs. The quadrivalents were mostly ring shaped which represent autosyndetic pairing between the chromosomes of *O. sativa*. An exceptional shape,  of quadrivalent confirms the hypothesis on structural hybridity leading to the origin of frying-pan type of trivalents. Dumb-bell shaped  penta-valents were observed in some PMCs and this shape is indicative of tertiary trisomic condition.

Structural hybridity revealed by the metaphase analysis of the allotriploid reveals the following conclusions:

(1) In general, the genomes of *O. perennis* var. *longistaminata* and *O. sativa* exhibit segmental homology.

(2) Autosyndetic pairing is more prevalent in *O. sativa* than in *O. perennis* var. *longistaminata*.

(3) The chromosomes of *O. sativa* are differentiated from those of *O. perennis* var. *longistaminata* by a series of reciprocal translocations affecting fairly large size and some of these might be duplicative translocations.

and (4) Normal bivalent formation at diploid level is misleading as far as genetic differentiation is concerned.

8. Inter-varietal genetic differentiation in O. perennis: Studies on genetic differentiation between different taxonomic varieties of *O. perennis* assume special significance for the evaluation of the hypotheses on mono- and polyphyletic origin of cultivated rices. Inclusion of *O. barthii*, *O. longistaminata* and *O. cubensis* which were described as distinct species by different workers into one taxonomic species, *O. perennis* Moench. (Chatterjee, 1948, Sampath and Rao, 1951 and Richharia, 1960) will be justified only when it is conclusively demonstrated that genetic (not morphological) differentiation between them is not considerable. As a preliminary study of this problem, the karyotypic studies of *O. perennis* var. *balunga*, *O. perennis* var. *barthii* and a collection of *O. perennis* from Assam were undertaken which indicated that these forms are comparable if not identical in their karyotypes. The next step was to study the meiosis in the hybrids involving these different varieties and the data from pachytene analysis on a single hybrid, *O. perennis* var. *balunga* × *O. perennis* var. *cubensis* are available (Shastry and Misra, unpublished).

The pairing at pachytene in the F_1 hybrid, *O. perennis* var. *balunga* × *O. perennis* var. *cubensis* is complete. Some abnormalities in the shapes of nucleoli were observed, but their significance is not clear. The pachytene bivalents in several regions, however, exhibited regions which are paired side-to-side with no relational coiling. Since similar observations were made in *O. perennis* var. *barthii*, accompanied by a great reduction in chiasma frequency, it was interpreted that these loosely paired regions represent structural hybridity. Pairing of this type was also observed at pachytene in haploid *Antirrhinum* (Rieger, 1957). The study of meiosis in the F_1 hybrid, *O. perennis* var. *balunga* × *O. perennis* var. *cubensis* justifies the inclusion of both the parents in one species.

9. Conclusions on the evolution in the genus Oryza: The present study indicates that *O. australiensis* originated from pre-*Sativa* and pre-*Officinalis* stock which might have given rise to 3 forms—*O. australiensis*, retaining most of the primitive characters and *O. perennis* and *O. officinalis* with only some. This differentiation would have involved, chromosomally, the loss of several heterochromatic segments and morphologically, channelization of a complex of characters—(lax panicles —long grain—long anthers—solitary panicle branches) in *O. perennis* and (lax panicles—small grain—small anthers—whorling of panicle branches) in *O. officinalis*. The relationship of *O. granulata* and other members of the section Granulata in the phylesis cannot yet be determined since no hybrids involving this section are studied. Within the section Sativa, the present evidence strongly supports that different varieties of *O. perennis* constitute the most primitive members.

The origin and evolutionary diversification of

diploid species of the section Sativa is probably traceable to *O. perennis*. Even so, whether the cultivated rices, *O. sativa* and *O. glaberrima* are derived directly from *O. perennis* or through another species or complex, bearing resemblance to *O. sativa* var. *fatua* cannot yet be decided with certainty. The evolutionary diversification between *O. sativa* and *O. perennis* is due to several equal or unequal, duplicative and reciprocal translocations and as a consequence of these structural changes, the primitive characters—laxity of the panicle, rhizomatous stem, perennating habit and shattering are probably lost. The genetic differentiation between *O. sativa* and *O. glaberrima* is not yet critically investigated, although, from the data on sterility in several of these interspecific hybrids, it might be expected that several chromosome structural changes differentiate these species as well. The different, geographically separated, taxonomic varieties of *O. perennis* are also differentiated by chromosome structural changes. The genetic differentiation between the sub-species of *O. sativa* covers a broad spectrum—some inter-varietal hybrids exhibiting low sterility and low chromosome structural changes while others, high sterility and high chromosome structural changes. Chromosome structural changes, therefore, seems to have played significant role in the differentiation of these sub-species although differentiation of some characters might be due to point mutations.

Mechanisms of reproductive isolation appear to be variable in the genus *Oryza*. At the level of the varieties of the same species and of the related species, the chromosome structural hybridity offers severe restriction on the recombination. At the level of intersectional hybrids, desynapsis seems to play an important role as between *O. sativa* and *O. officinalis*. Still a different mechanism is by the timing imbalance in the meiosis, whereby the constituent genomes of the interspecific hybrid, *O. sativa* × *O. australiensis* remain unpaired.

The present study cautions against hasty conclusions on the origin of a species or relationships between different taxa—all of which are better understood when meiotic data on all the possible hybrids are available from the most dependable techniques of analysis.

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TABLE I

Classification according to Stebbins (1958) of Karyotypic asymmetry in Oryza Sp.

Ratio Larges/ smallest	*Proportion of chromosomes with arm ratio 2 : 1*			
	0.00	0.01-0.50	0.51-0.99	1.00
2.1	1a	2a	3a	4a
2 : 1-4 : 1	1b *O. perennis* (Assam) *O. perennis* var. *barthii*	2b *O. perennis* var. *balunga* *O. sativa* var. *fatua* *O. australiensis* *O. stapfii* *O. sativa* (*Indica*)	3b *O. glaberrima*	4b —
4 : 1	1c	2c	3c *O. sativa* (*japonica*)	4c

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