CHROMOSOME MORPHOLOGY OF *ORYZA SATIVA* AND *ORYZA AUSTRALIENSIS* AND THEIR PAIRING IN THE F_1 HYBRID AT EARLIER MEIOSIS[1]

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(Received Mar. 10, 1964)

Chromosomes association in the diploid hybrid of *Oryza sativa* × *Oryza australiensis* were studied by many workers (Morinaga *et al.* 1960; Nezu *et al.* 1960; Shastry *et al.* 1961 & Li 1963). However, most of them devoted their efforts in studying stages of meiosis after early prophase. At these stages, univalents are mostly found. Therefore, the chromosomes of these species are given two genomic symbols, A and E for *sativa* (Morinaga *et al.* 1960) and *australiensis* (Li 1963) respectively. The univalents observed at diakinesis or metaphase I of interspecific hybrids were conventionally interpreted as a criterion of non-homology of parental chromosomes. But this might not always be true. Menzel (1962) reported that univalents at metaphase I in a intergeneric hybrid (*Lycopersicon esculentum*×*Solanum lycopersicoides*) resulted not from failure of synapsis or lowered chiasma frequency but from an unbalanced distribution of chiasmata in the arms of chromosomes. Technique for evaluating rice pachytene chromosomes was improved recently and it might be practicable to analyze the pachytene chromosome complements of the F_1 hybrid of *Oryza sativa*×*Oryza australiensis* and its parents. The present study deals with their morphological and synaptic relationships at earlier prophase.

Material and Methods

F_1 hybrid (2n=24) of *Oryza sativa*×*Oryza australiensis* used in this study was crossed, examined at Taichung. In crossing, Taichung 65 (a *japonica* variety of *O. sativa*) and *O. australiensis* (brought by Dr. Oka from CRRI, India) were used. Suitable panicles of both F_1 and its parents were fixed in 1:3 acetic alcohol for at least 24 hours at room temperature. Propiono-carmine and Fe $(OH)_3$ (trace) were employed for staining and 45% acetic acid was used for

(1) Paper No. 28 of the Scientific Journal Series, Institute of Botany, Academia Sinica. This research is partly supported financially by USDA grant FG-Ta-102.

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differentiation. Not too much difficulty was encountered in tracing pachytene chromosomes of *O. sativa* but not in *O. australiensis* and their F_1 hybrid. Therefore, the analysis of pachytene chromosomes of *O. sativa* was based on whole cells in which twelve bivalents could be easily traced. Whereas that of *O. australiensis* and F_1 hybrid the analysis was made mainly within those chromosomes singled out of the crowd. In addition, detail studies of diplotene chromosomes were made in analyzing the pairing condition of F_1 hybrid.

Results

Karyotypes of the chromosomes of both parents, *O. sativa* and *O. australiensis,* were examined and worked out semidiagrammatically as shown in figure 1 and 2. Their total lengths, relative lengths, lengths of centromeres and arm ratios are presented in table. 1. In the complement of *O. australiensis,* chromosomes showed a marked resemblance to the pachytene chromosomes of *L. esculentum* (Brown, 1949) and *Plantago ovata* (Hyde, 1953). They were thick and characterized by their larger and prominent centromeres which were always flanked by heterochromatic segments on both sides (Fig. 4). The localized heterochromatic segments were, in contrast with the euchromatic ones, heavily stained and became granulated when condensation took places at late pachytene and early diplotene stage. The long arm of chromosome VIII, and the short arms of chromosomes IX, X and XII were entirely heterochromatic which made themselves recognizable among others. On the other hand, chromosomes of *O. sativa* were thin and the amount of heterochromatic segments were far less than those of *O. australiensis,* even though the short arms of chromosomes V, VII, X and XII were also heterochromatic. Chromosome VIII was almost euchromatic in both arms (Fig. 3). In both parental complements, the short arm of chromosome X was always attached to the nucleolus (or nucleoli) and it was reasonable to assign it as the nucleolar chromosome. Minute nucleoli which were absent in the complement of *O. australiensis* could be found in the microsporocyte of *O. sativa* (Fig. 4) but they did not attach to any chromosome at pachytene stage.

Pachytene chromosomes in the F_1 hybrid were intermediate in appearence and they inherited the character from the paternal parent that they were not easy to be well spread out in the preparation of slides. Minute nucleoli could also be found but less prominent. Pairing of F_1 chromosomes at pachytene stage was more or less complete (Fig. 5) and mostly allosyndetic. The allosyndetic nature was not so clear at pachytene and very early diplotene (Fig. 6) but it was evident at later stages (Figs. 7 & 8) in which the two constituent members came from different parents were well differentiated in staining. Not only the bivalents found were allosyndetic (Figs. 9 & 10, 11–13, 14–16) but were hetero-

Table 1 *Measurements of pachytene chromosomes of O. sativa and O. australiensis*

	1	2	3	4	5	6	7	8	9	10*	11	12	
O. sativa (Taichung 65)													
Total length	46.17	38.88	36.72	30.02	28.73	27.49	26.19	23.81	23.11	22.52	20.47	18.20	342.31μ
Relative length		1.10	1.26	1.54	1.61	1.68	1.76	1.94	2.00	2.05	2.26	2.54	
Length of centromere	1.18	1.03	0.81	1.24	1.13	0.81	0.54	0.81	0.81	0.81	0.81	0.81	μ
L/S ratio	1.40	1.60	1.20	1.90	2.50	1.91	2.70	1.30	1.40	2.70	1.60	2.70	
O. australiensis													
Total length	65.34	55.73	46.44	44.39	42.28	34.29	30.24	26.62	25.22	24.30	23.81	22.41	441.07μ
Relative length		1.17	1.41	1.47	1.55	1.91	2.16	2.45	2.59	2.69	2.74	2.92	
Length of centromere	2.21	2.43	2.16	1.76	1.62	1.43	1.88	1.89	1.46	1.08	1.30	1.08	μ
L/S ratio	1.12	1.67	1.17	1.70	1.00	1.90	1.30	1.60	4.00	2.00	1.30	1.60	

* The tenth chromosome was the nucleolar chromosome.

morphic as well (Figs. 9, 11, 13 & 16). In the PMC's studied at mid- and late-diplotene stages, variable numbers of allosyndetic bivalents were found. In one cell, as many as nine such pairs were observed. Most of them were paired in an end-to-end fashion. However, some were of the side-by-side type. Furthermore, two to three allosyndetic bivalents with 3–4 chiasmata were also noted in several mid-diplotene cells (Fig. 8). It might be inferred from these observations, therefore, that some true allosyndetic bivalents were formed in this F_1 hybrid at earlier stage. Autosyndetic bivalents of *sativa* were rare.

At metaphase I, only few cells with most chromosomes arranged in the region of the equatorial plane could be found. In these cells bivalents were allosyndetic too.

Both allo- and autosyndetic bivalents existed at anaphase I stage.

Discussion

Shastry is the first cytogenetist to work on the karyotype of *sativa* (Shastry 1960) and the other species, *australiensis, glaberrima* and *stapfii* (1961). It is well known that centromeres of the chromosomes of the species, even though the preparation is very good and the chromosomes are well spread, can not be located for sure in many instances. With a better technique adopted by us the heterochromatic regions as well as the euchromatic regions of the chromosomes of *sativa* and *australiensis* can be well differentiated. Since the centromeres are flanked on both sides by heterochromatic segments, the localization of centromere can be assured with clear differentiation. The chromosomes can be identified also by this differentiation. If the location of centromere can be assured, the arm ratio can be more or less made with certainty. Unfortunately, the chromosomes of *australiensis* are not spread good enough to make the measurement of all the chromosomes in one cell possible, the karyotype of this species is obtained only from the measurement of more than 137 singled chromosomes. More studies should be done to verify the conclusions so obtained. The karyotype of *sativa* is based upon the measurements of well prepared slides. We are quite certain that it can represent the karyotype of the variety with which we studied.

Shastry (1961) conjectured that the end-to-end allosyndetic bivalents are pseudobivalents. Li (1963) found that some chromosomes at diplotene with multiple chiasmata and chromosomes with multiple chiasmata also are found in diakinesis and metaphase I. These chromosomes found with multiple chiasmata in late prophase and metaphase would signify that this allosyndetic bivalents are true bivalents. From our observation, we found that the chromosomes are more or less completely paired at pachynema. As meiosis proceeds, there is an increase of dissociation of the pairing condition of bivalents and this

dissociation is complete at late diakinesis. Since the chromosomes can be differentiated by their staining ability, the chromosomes can be identified from mid-prophase on. It was observed, therefore, that the pairing would involve chromosomes of the intergenomic nature. Even if the chromosomes are more or less separated but they would stay near each other or paired in an end-to-end fashion or they are connected by chromatic thread. Furthermore, in diplonema we found several chromosomes which have multiple chiasmata (Fig. 8). With all these observations, they would put up a strong arguement in favor of the conclusion that these allosyndetically paired bivalents are true bivalents rather than pseudobivalents.

Concerning the homology of these two species we may put up two alternatives:

(1) If these two genomes would be distantly related, the pairing condition as we observed in early prophase would indicate that the pairing is then of the enforced nature as McClintock pointed out (1933, Vide from Brachet & Mirsky P. 41, 1961).

(2) On the contrary, if these two species are closely related to each other, they may have a common ancestor to start with. It is well known that the native place of *sativa* is in the South-east Asia and that of the *australiensis* is

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Figs. 1 & 2 Karyotypes of *O. sativa* and *O. australiensis* respectively, showing the semidiagrammatically chromosomes in natural order at pachytene stages.

Figs. 3 & 4 PMCs of *O. sativa* and *O. australiensis* respectively at pachytene stage in which 12 bivalents can be traced. Centromeres are denoted by arrows. Nucleolar chromosomes are indicated by an arrow followed by the letter N. "st" indicates the euchromatic segment stretched × 900.

Figs. 5-8 inclusive. PMCs in the F_1 hybrid of *O. sativa* × *O. australiensis*, × 900.

Figs. 5 At pachytene stage the chromosomes are more or less well paired. Note the less prominent minute nucleoli (indicated by arrows).

Figs. 6 At very early diplotene stage, some chromosomes (denoted by arrows) are separating while others (indicated by double arrows) are still paired.

Figs. 7 At mid-diplotene stage, the allosyndetic nature is clear. Most chromosomes are still partly paired and contracting but one allosyndetic bivalent (indicated by an arrow) is already contracted.

Figs. 8 At late diplotene stage, two allosyndetic bivalents with multiple chiasmata are shown.

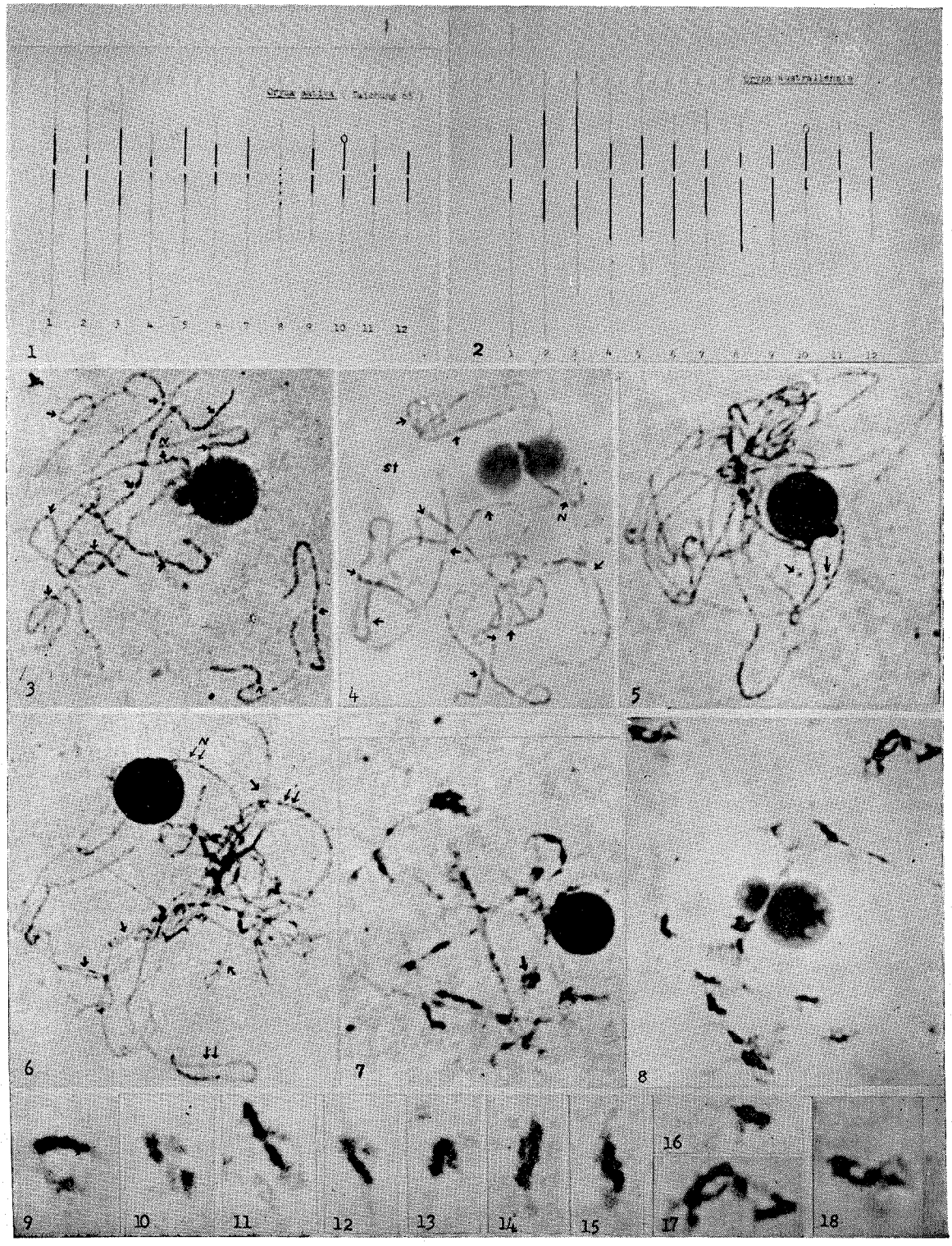
Figs. 9-18 inclusive. Chromosomes of F_1 at late diplonema, they are contracted further, some with multiple chiasmata. The allosyndetic nature of each pair is clearly visible, × 1350.

Figs. 9 & 10. The allosyndetic bivalents are picked out from one late diplotene PMC. The bivalents are heteromorphic.

Figs. 11, 12 & 13. Showing three allosyndeitc bivalents picked from another late diplotene cell. The heteromorphic nature is clear.

Figs. 14, 15 & 16. Three allosyndetic and heteromorphic bivalents from another cell.

Figs. 17 & 18. Two allosyndetic bivalents with multiple chiasmata as in Fig. 8.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in Australia. If these widely separated geographical niches are the results of continental drift separating Australia from the continent of Asia, these two species would be well isolated from each other in the course of evolution. There would be genic changes as well as structural changes altering the genetic constitution of the original ancestral species from which these two species are derived. When the chromosomes of these two species are brought together again in crossing, there are enough homologous segment on each chromosomes so that they would pair with each other even allosyndetically. However, in the course of evolution genic changes probably would arise as to cause dissociation of chromosomes at later stages after the initial pairing of the chromosomes. The nature of this gene or genes may be similar to the desynapsis gene found in Zea mays (Beadle 1933), Sorghum (Krishnaswamy & Meenakshi, 1957) and rice (Chao, 1960, 1961) only the gene or genes concerned is dominent rather than recessive.

Summary

Karyotypes of two *Oryza* species, *sativa* and *australiensis* were worked out semidiagrammatically. They were different in general appearence, total chromatic length, size of centromeres, degree of symmetry and their distribution of heterochromatic segments. Pairing of chromosomes in the F_1 hybrid of *O. sativa* ×*O. australiensis* was analyzed at earlier stages of meiosis. Evidence on hand showed that pairing was more or less complete in pachynema and mostly allosyndetic but the paired chromosomes dissociated from each other from the diplotene stage on. Bivalents with multiple chiasmata at mid- and late diplotene stages proved that these allosyndetically paired bivalents were true bivalents. The degree of homology between the complements of these two isolated species was discussed.

栽培稻 (*O. sativa*) 澳洲野生稻 (*O. australiensis*) 粗絲期核型及其雜種第一代早期減數分裂的研究

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稻粗絲期（pachytene）染色體的製片技術甚爲困難。過去數年中，作者等全力以赴，已經解決若干部份。茲將 *O. sativa*（臺中65號）及 *O. australiensis*（得自印度中央稻作試驗場）粗絲期染色體的長度、相對長度、中心體大小、長短臂比例等，列如表一（參看圖1, 2,

3 及 4)。大致上，此兩稻種核型外觀上不盡相同，然連於核仁上的，則似均爲第十染色體的短臂。

雜種第一代粗絲期染色體的配對極爲完美(圖 5)，並大部爲栽培稻與澳洲野生稻之間的相互配對 (allosyndetic 圖 9-18)。十二對二價體中，有兩至三對各具 3-4 個 chiasmata (圖7 及 8)。此等二價體於減數分裂中期及後期猶保持其二價，其他各對，或因 chiasmata 數的不足，至分裂前期之末，已散爲單價體。此現象與玉米，高粱及栽培稻等作物中所見的二價體分散爲單價體的現象 (desynapsis) 相似，僅是其控制因子應爲顯性。兩種染色體組同質程度 (homology) 的大小曾經討論。

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