RICE GENETICS AND CYTOGENETICS

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FOREWORD

Rice is the principal food of more than 60 percent of mankind. Asia produces and consumes more than 93 percent of all rice grown, yet in the tropics of that region, rice yields are consistently among the lowest in the world.

These and related facts led the Ford and Rockefeller Foundations to establish jointly, in cooperation with the Government of the Philippines The International Rice Research Institute as a world center for the study and improvement of rice.

Negotiations between the two Foundations and the Government of the Philippines were started in 1959. The lnstitute was legally incorporated in April, 1960, and dedicated on February 7, 1962. In recognition of its scientific and humanitarian character, the Institute has been exempted, by congressional action, from the payment of all Philippine taxes and duties.

The Institute is located adjacent to the College of Agriculture, University of the Philippines, at Los Baños, Laguna, 65 kilometers southeast of Manila.

The primary purpose of The International Rice Research lnstitute is to conduct basic and applied research on the rice plant, with the objectives of improving rice quality and quantity. In addition, the Institute maintains a Library and Documentation Center and an Office of Communication for the collection and dissemination of research results; conducts regional projects in the agricultural sciences in Asia, and has a resident training program in rice research methods and techniques.

The Ford Foundation contributed funds for the land, buildings, and initial equipment. It also has supported the regional training and cooperative research programs of the Institute. The Rockefeller Foundation provides funds annually to operate and maintain the Institute, and has assigned eight of its staff members to the Institute.

The professional staff of the Institute includes scientists of seven nationalities* who conduct research in plant breeding, genetics, taxonomy, chemistry, agronomy, soil chemistry, microbiology, plant physiology, plant pathology, entomology, agricultural economics, agricultural engineering, communication, and statistics in fully equipped, modern laboratories. The Institute's Plant Experimentation Center is equipped for growing plants under controlled and semicontrolled environmental conditions.

The adjacent 80-hectare experimental farm has an underground irrigation and surface drainage system to enable any plot to be flooded or drained independently of the others, at any time of the year.

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* Australia, China (Taiwan), Ceylon, India, Japan, Philippines and United States.

The Institute's training program provides opportunities for young scientists from the rice-producing countries to learn and do research under the guidance of competent scientists. By October 1, 1963, 49 research scholars and fellows were enrolled at the Institute, and, in most cases, also were enrolled as graduate students at the nearby College of Agriculture of the University of the Philippines. When this program is fully developed, it is expected that up to 60 research scholars at any one time will be receiving training in research techniques.

A vital facility of the Institute is the Library and Documentation Center, where a comprehensive collection of the world's technical rice literature is being assembled. By late 1963, a 10-year (1951–1960) bibliography of technical literature about rice was compiled, and essentially all of the 10,000 citations microfilmed and placed in the library. An up-to-date bibliography of Japanese rice literature is maintained, and significant scientific articles are being translated into English. The library receives, on a continuing basis, more than 800 scientific journals and periodicals,

The Institute actively encourages cooperation among the world's rice research workers, particularly on problems of international significance. Its auditorium and seminar rooms will accommodate international conferences, symposia, and seminars on topics of importance to rice-producing nations.

The founders and staff hope that The International Rice Research Institute will provide an environment dynamically conducive to new attacks on the age-old problems of rice culture.

ROBERT F. CHANDLER, JR.
Director

October 15, 1963

Mail address:

The International Rice Research Institute
Manila Hotel
Manila, Philippines

PREFACE

This Symposium, the first of a series of international conferences sponsored by the International Rice Research Institute on broad areas in rice research, was also the first international conference solely devoted to the discussion of rice genetics, cytogenetics, and taxonomy.

The aims of the Symposium were, to determine the status of research achievements in rice genetics, cytogenetics, and taxonomy, to review areas of agreement and disagreement, and to identify problems most needing research. Consultations with workers in India, Japan, Taiwan, the U.S.A., and with the representative of the International Rice Commission preceded the planning of the meeting.

The sessions, moderated by Dr. H. H. Kramer of the University of Nebraska, were attended by 102 persons from 26 institutions and organizations of 9 nations. The program consisted of 24 invitational papers, plus formal discussions, a formal resumé, and an exhibit of *Oryza* species.

The Institute gratefully acknowledges the financial support of the Ford Foundation which made this Symposium possible.

Drs. T. T. CHANG and PETER R. JENNINGS, with the help of Drs. H. I. OKA and S. SAKAMOTO, edited invitational papers prior to the meetings, Dr. CHANG and Dr. MERLIN T. HENDERSON summarized the discussions. Dr. CHANG served as technical editor for the final papers before transmitting them to the Institute's Office of Communication for final editing and publication arrangements.

STERLING WORTMAN
Associate Director
The International Rice Research Institute

October 15, 1963

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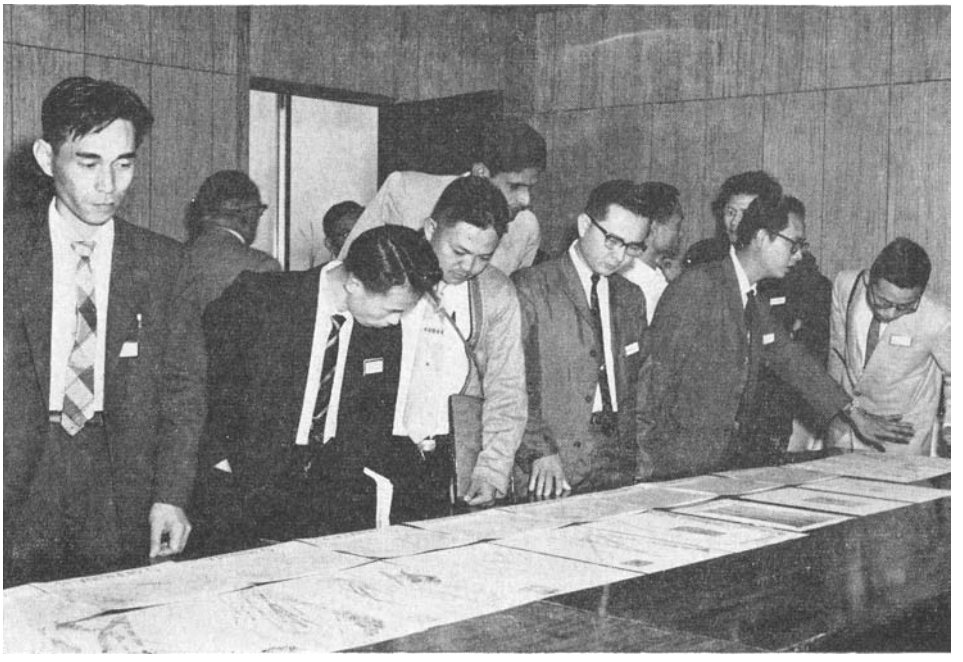
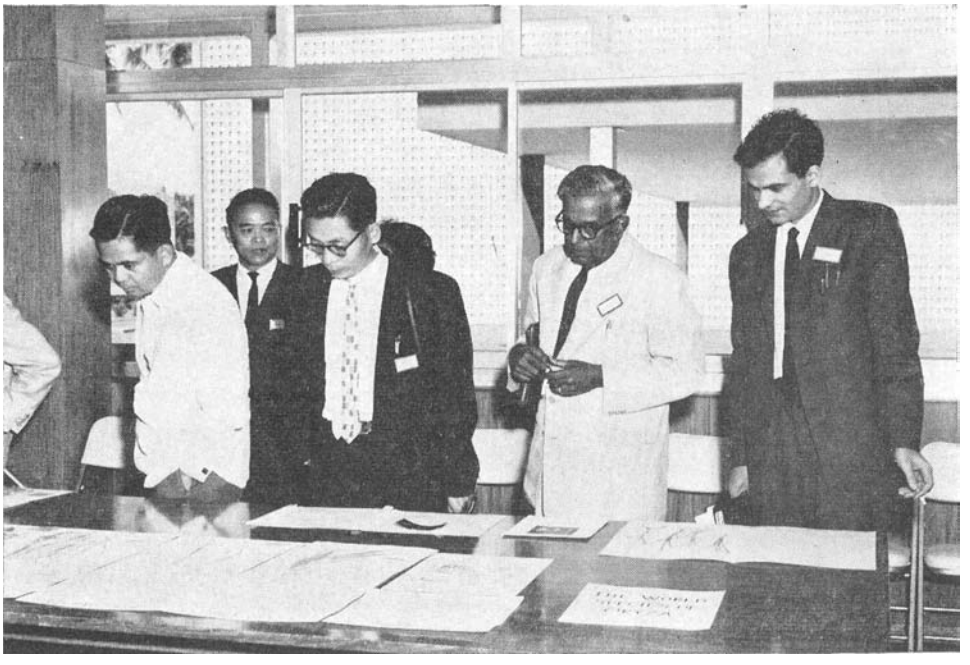
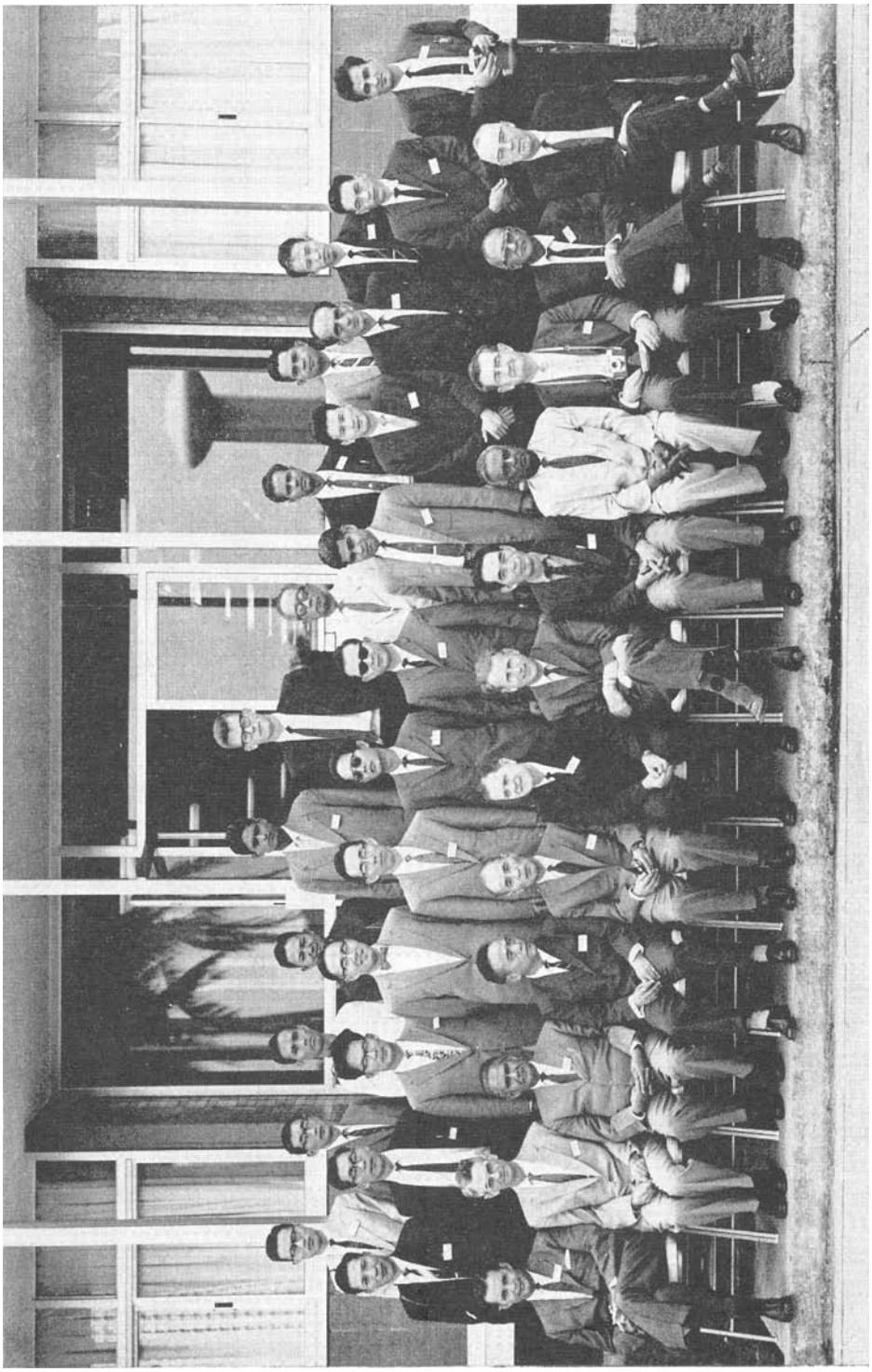




Participants and observers examining specimens of *Oryza* species.



Participants and observers in open discussion during one of the sessions.

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OPENING ADDRESS

NEED FOR STANDARDIZATION OF GENETIC SYMBOLS AND NOMENCLATURE IN RICE

HITOSHI KIHARA

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The International Rice Research Institute, Manila (Philippines)

INTRODUCTION

Many authors have stressed the need for unified gene symbols, including YAMAGUCHI, who in 1926 proposed gene symbols for 43 characters. The following year he published a paper in the German language with the names of 43 genes given mostly in German. There was no alteration in the gene symbols. Later, YAMAGUCHI (1939) proposed quite different symbols (130 loci in all), using Latin for the respective characters. Accordingly, many of the gene symbols had to be changed [e.g., *m* = mochi, the Japanese term for glutinous, became *am* (amylacea)]. YAMAGUCHI believed that Latin should be adopted as the universal language for naming characters.

KADAM AND RAMIAH of India also proposed gene symbols as early as 1938, revising them in 1943.

The National Committee of Genetics and Breeding of the Japan Science Council was established in 1949. One of its functions was to establish unified genetic symbols for important plants and animals.

After 6 years of study, Japanese geneticists proposed recommendations for gene symbolization in plants and animals when the International Genetics Symposia were held in Tokyo and Kyoto in 1956. At the same time, when the Japanese recommendations were prepared, the gene symbols for rice were revised in accord with the system. With some modifications, the system was adopted by the International Committee of Genetic Symbols and Nomenclature (ICGS, 1957). These symbols differing considerably from those of the Indian authors, complicated the situation. Fortunately the International Rice Commission (IRC) took the matter in hand and appointed a working committee on rice symbolization consisting of three scientists, Dr. R. SEETHARAMAN representing Dr. K. RAMIAH, Dr. M. TAKAHASHI representing Dr. S. NAGAO, and Mr. N. E. JODON. They tabulated the results obtained in their respective countries, and the IRC Newsletter in 1959 published a list of IRC-recommended gene symbols for rice.

GENE SYMBOLS

Tables 1 to 4 provide some examples of the gene symbols of rice. On the right side of these tables, the guiding principles abstracted from the *Recommended rules of symbolization* (ICGS recommendation) are presented.

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TABLE 1

EXAMPLES OF GENE SYMBOLS IN RICE

Characters	Symbols (IRC)	Recommended rules (ICGS)
albino (rec.)	*al*	Write symbols in Roman letters (preferably in italics) and as short as possible. (Symbols may be based on a key word of an adjective-noun combination.)*
brittle culm (rec.)	*bc*	
Awned (dom.)	*An*	
Black hull (dom.)	*Bh*	

* This sentence is taken from IRC.

TABLE 2

SYMBOLS FOR ALLELIC SERIES

Characters	Symbols (IRC)	Recommended rules (ICGS)
Allelic anthocyanin-activator genes	A, A^{d}, a	Use literal or numerical superscripts to represent the different members of an allelic series.
Allelic basic genes for anthocyanin color	$C, C^{B}, C^{Bp}, C^{Bt}, C^{Br}, c$	

TABLE 3

SYMBOLS FOR NON-ALLELIC LOCI HAVING SIMILAR EFFECTS

Characters	Symbols		Recommended rules (ICGS)
erectoides (barley): basic symbol *ert*	non-allelic	*ert-a*^{6}... etc. (allelic) *ert-b*^{2}... etc. („) *ert-c*^{1}... etc. („)	Designate non-allelic loci* having phenotypically similar effects by a common basic symbol. An additional letter or Arabic numeral either on the same line after the hyphen or as a subscript.
necrosis (wheat): basic symbol *Ne*		Ne_1 Ne_2 Ne_3	

* No examples were given by IRC in the paper on gene symbols for rice.

TABLE 4

USE OF THE + SIGN

Characters	Recommended rules (ICGS)
The IRC recommendation gives no examples. For cultivated plants it is hardly possible to distinguish a standard or wild type from its mutants. However, for normal traits contrasted with abnormal mutants, the plus sign might be used.	Designate standard or wild type alleles by the gene symbols with + as a superscript or by + with the gene symbol as a superscript. In a genotype formula the + alone may be used.

The list of IRC-recommended gene symbols for rice includes 88 loci. Many genes not yet thoroughly studied are excluded.

In general, we are satisfied with this recommendation and appreciate the efforts of the committee in unifying the gene symbols for rice. However, in Table 5 I suggest a few modifications.

TABLE 5

NAMES OF CHARACTERS AND THEIR GENE SYMBOLS

IRC recommendation		Suggested modification	
Names of character	Symbol	Symbol	Character
(1) dwarf	*d*	*dw*	
(2) deep water paddy	*dw*	*fl*	floating
(3) glabrous	*gl*	*gb*	
(4) grain length (kernel length)	*kl*	—	kernel length (grain length)
(5) open	*o*	*op*	
(6) waxy endosperm	*wx*	*gl*	glutinous

The suggested modifications (1–5) are intended to clarify the symbols representing respective traits.

The term "waxy" seems inadequate for the endosperm character (6). As early as 1793, Loureiro described glutinous rice as an independent species, *Oryza glutinosa* LOUR. It is also known as a variety of *Oryza sativa.* As far as I am aware, we have never heard of waxy rice.

In maize and barley the term "waxy" is unanimously used for glutinous endosperm. In wheat, "waxy" is used only for foliage character (WATKINS, 1927). A survey of literature shows that the gene symbols for this character in rice have been revised many times. The examples below reflect the situation.

TABLE 6

GENE SYMBOLS FOR GLUTINOUS ENDOSPERM

Name	Symbol	Author
Mochi* (glutinous)	*m*	YAMAGUCHI, 1918
Uruchi* (non-glutin ous)	*U*	TAKAHASHI, 1923
amylacea	*am*	YAMAGUCHI, 1927
glutinous	*gl*	CHAO, 1928
glutinous	*g*	ENOMOTO, 1929

* Japanese term.

Data in the table indicate that the glutinous locus was named after a recessive

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character (mochi) studied by one author and after a dominant character (uruchi) by another. As it is almost sure that the character glutinous was noted after man had taken the wild ancestor (non-glutinous) into cultivation, the plus sign should be applied to the non-glutinous allele and the gene symbol should be named after the mutant character (glutinous).

On the basis of the list prepared by the IRC, the genetic analysis of characters in rice is still limited. It shows that the genetic research of this crop should be conducted more extensively in the future.

LINKAGE GROUPS AND CHROMOSOMES

The ICGS recommended that linkage groups and corresponding chromosomes should be designated by arabic numerals. For example, linkage group 1 should correspond to chromosome 1.

Hexaploid wheat offers a unique example, because of its allohexaploidy. The genome formula of common wheat is AABBDD. A normal plant invariably has 21 bivalents. Nullisomics, where 20 bivalents instead of 21 are found, can be obtained in many ways. They are usually highly sterile and dwarfish. Since the first nullisomic (g-dwarf) was obtained from the offspring of a pentaploid hybrid between *Triticum polonicum* and *T. spelta* in 1924, it took almost 30 years to establish a complete set of 21 different nullisomics (KIHARA, 1924; SEARS, 1944, 1954). At the beginning, nullisomics were designated by the missing chromosomes such as a-dwarf, b-dwarf, etc. (KIHARA, 1924; KIHARA AND MATSUMURA, 1942) or nulli-I, nulli-II, etc. (SEARS, 1954). After elaborate studies by SEARS and his associates, it became clear that the 21 wheat chromosomes can be divided into seven groups of three homoeologous chromosomes. Each of three chromosomes within a group was ascribed to either the A-, B- or D-genome, and, accordingly, symbols for the 21 wheat chromosomes were completely revised. For instance, three homoeologous chromosomes of group 1 are designated as 1A, 1B and 1D, where A, B and D denote the affiliated genomes. The revised system is given in Table 7.

TABLE 7

ASSIGNMENT OF THE 21 WHEAT CHROMOSOMES TO 7 HOMOEOLOGOUS GROUPS

Homoeologous group	Genome						
	A		B		D		
1	1A	XIV	1B	I	1D	XVII	c
2	2A	XIII	2B	II	2D	XX	e
3	3A	XII	3B	III	3D	XVI	g
4	4A	IV	4B	VIII	4D	XV	d
5	5A	IX	5B	V	5D	XVIII	f
6	6A	VI	6B	X	6D	XIX	b
7	7A	XI	7B	VII	7D	XXI	a

a ~ g: used by KIHARA AND MATSUMURA, 1942.

According to HUSKINS' terminology (1941), two chromosomes are homoeologous when they are homologous in part (SEARS, 1954). Now we find that a missing chromosome of any homoeologous group can be complemented by one of the two other members. So the function of the three chromosomes of a given group is similar. Therefore, we may consider that the homoeologous chromosomes originated from one common ancestor-chromosome in the course of evolution.

As for the symbols of homoeologous chromosomes, it seems that the symbols 1_A, 1_B, 1_D, etc. fit the ICGS recommendations better than 1A, 1B, 1D, etc.

When the corresponding relationship between linkage groups and chromosomes is not sufficiently determined, it may be necessary to modify numeral symbols to avoid confusion. For instance, roman letters will be used for chromosomes, and eventually linkage groups can be represented by their well-known loci, as is already in common usage.

Twelve linkage groups should be identified in rice. This was not possible until 1960 when NAGAO AND TAKAHASHI proposed a chromosome map of 10 linkage groups and two independent single genes. Recently they have found other genes linked to those two genes. Thus 12 linkage groups are now established,

The history of linkage studies reveals that two apparently independent groups can be found on the same chromosome. In barley, seven linkage groups were established in 1941 by ROBERTSON *et al.* However, two of them were found to be in one chromosome (KRAMER *et al.,* 1954; TSUCHIYA, 1956). Later investigations by the conventional method showed that eight groups were demonstrated to be superficially independent of each other (RAMAGE *et al.,* 1958; TAKAHASHI *et al.,* 1957). However, two of them were combined into one group. This correction was made possible by the use of trisomics and also by translocation analysis (KRAMER *et al.,* 1961; TAKAHASHI *et al.,* 1962; Table 8).

TABLE 8

CORRESPONDENCE OF TRANSLOCATION INTERCROSS DESIGNATION, LINKAGE GROUPS, TRISOMIC TYPES, AND TYPE GENE PAIR TO THE CHROMOSOME KARYOTYPE IN BARLEY

Morphological chromosome number	Translocation intercross designation	Previous linkage group	Type gene	Primary trisomic type
1	b	III, VII	*N, n*	bush
2	f	I	*V, v*	slender
3	c	VI	*Uz, uz*	pale
4	e	IV	*K,k*	robust
5	a	II	*B, b*	pseudonormal
6	g	—	*O,o*	purple
7	d	V	*R, r*	semi-erect

It is recommended that the 12 linkage groups of rice proposed by NAGAO and his associates be tested by trisomics or translocation analysis. In rice, trisomics and translocation lines are not yet fully established. It is necessary to produce all trisomics and translocation lines.

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GENOME SYMBOLS

The rules of ICGS do not mention genome symbols. However, most workers use capital letters (not italics) for genome symbols.

Consciously or unconsciously, we have adopted two systems in genome symbolization. One is the ABC system, in which the genome symbols are given to different species in alphabetical order. The other can be called the "initial-letter" system. Here the first letter of a given species is used as its genomic symbol.

Gossypium is a good example of the first system, and *Nicotiana* is a well-known case of the second. Namely, S represents the genome of *N. sylvestris* and T is the genome of *N. tomentosiformis.* The genome type of the allotetraploid species, *N. tabacum,* is SSTT.

Although the initial-letter system seems to enable users to remember the symbols, its use still has minor disadvantages. For instance, there is a group of diploid species with identical genomes on one hand, while two different species may have the same initial letter on the other. Above all, there is a danger that the specific names might be altered because of taxonomic revisions.

Triticum aestivum L. is a good example: We were accustomed to use *T. vulgare* Vill. as the species name of our common wheat. However, quite recently we returned to the old name of *T. aestivum.* In rice, *Oryza perennis* Moench was widely used as the wild-growing putative ancestor of *Oryza sativa.* But application of this species name is thought doubtful. Accordingly, some authors believe that *Oryza rufipogon* Griff. should be used.

Before going into rice genomes, I want to relate my experience with *Triticum* and *Aegilops.*

The genome of Einkorn wheat *(T. aegilopoides* and *T. monococcum)* is A in the haploid phase, and that of Emmer wheat is AB. The genome A is common to these two groups of wheat. The third group of wheat, Dinkel, has one more genome, which I designated in 1924 as D. The symbol was taken from Dinkel, to indicate the genome special to Dinkel wheat. That was the beginning of my symbolization of genomes in this group of cereals. For instance, C stands for *Aegilops caudata,* S for *Sitopsis* (a section including four closely related species: *Aegilops speltoides* (SS), *bicornis* (S^bS^b) and *longissima* (S^lS^l) or *sharonensis).*

In *Oryza,* the A, B, C... symbols (originally a, b, c...) were adopted by MORINAGA (1939). This was followed by many authors. YEH AND HENDERSON (1961) adopted a slightly different system for the *Sativa* group. However, RICHHARIA (1960) has proposed a new system, namely:

Oryza Species	RICHHARIA	Conventional
perennis	P^1P^1	AA
sativa	P^2P^2	AA
glaberrima	P^3P^3	AA
officinalis	O^1O^1	CC
minuta	$O^1O^1M^1M^1$	BBCC
malampuzhaensis	$O^1O^1O^3O^3$	BBCC
latifolia	$O^1O^1O^2O^2$	CCDD

As the genomes of two Asian tetraploid species (*minuta* and *malampuzhaensis*) are identical (BBCC) and the American tetraploid species (*latifolia, alta* and *grandiglumis*) share the same genome type (CCDD), the symbols following RICHHARIA'S system will be rewritten as follows for the Section *Sativa* Roschev.

PP	*perennis, sativa, glaberrima,* etc.
OO	*officinalis*
OOMM	*minuta* (Asian tetraploid species)
OOLL	*latifolia* (American tetraploid species)

(Superscripts for distinguishing slight genome modifications are omitted here.)

Genome symbolization is strikingly confused in a voluminous paper on interspecific hybrids of *Oryza* by BOUHARMONT (1962). The author studied four *Oryza* hybrids, namely, *sativa* × *glaberrima, sativa* × *stapfii, sativa* × *officinalis* and *sativa* × *schweinfurthiana* ($2n = 48$).

The last combination is worth mentioning. In this combination he used two kinds of *sativa* (one is diploid and the other is autotetraploid). Accordingly, the hybrids were triploid and tetraploid.

Chromosome pairing was low (1.7%) in a triploid hybrid as expected. However, the meiosis of the tetraploid hybrid was more irregular. The bivalents were rare (0.4%). Abnormalities often were found. It is expected that the hybrid should have 12 bivalents, as there are two homologous genomes in its genome constitution. A wheat hybrid with similar genome constitution (ABDD) obtained from a cross between *Triticum polonicum* (AABB) and an autotetraploid *Aegilops squarrosa* (DDDD) showed clearly $7_{II} + 14_I$ in metaphase I.

BOUHARMONT'S symbols, although tentative, do not throw any light on the genome relationships of *Oryza.* They are given below (see p. 10).

His assumption that *Oryza punctata* could be synonymous to *Oryza schweinfurthiana* might be right. However, his idea to place *schweinfurthiana* in the group of American tetraploid species (*latifolia*) is improbable. A taxonomical revision of African tetraploid species and their cytogenetic studies is needed.

Recently a hybrid of two *Oryza* species (*sativa* and *brachyantha*) belonging to two different sections, *Sativa* and *Coarctata,* was produced by LI *et al.* (1961). No chromosome pairing was found in the metaphase I of the hybrid. This is the first report concerning intersectional hybridization in rice. The success of this hybridization is mainly related to the embryo culture of the hybrid. This technique should be applied for other cross combinations to permit analysis of species outside the *Sativa* section.

Rice cytogeneticists agree that the genome of *officinalis* is present in *Oryza minuta.* However, they disagree about the other genome of *minuta.* One group (GOTOH AND OKURA, 1933; NANDI, 1936) maintains that the genome A is included in *O. minuta,* as the hybrid between *sativa* and *minuta* shows $12_{II} + 12_I$ in meiosis. On the other hand, MORINAGA (1940) considers that the A genome can not be present in *minuta* as the hybrid of the same combination in his studies showed only univalents. Results obtained by NEZU *et al.* (1960) are different. They found 0–9 bivalents in the first division of the PMC.

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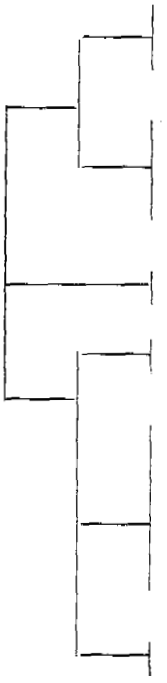
Tentative genome symbolization of *Oryza* by BOUHARMONT (1962)



sativa	24	AA
fatua	24	AA
glaberrima	24	AA
stapfii	24	A_1A_1
breviligulata	24	A_1A_1
australiensis	24	
officinalis	24	OO
alta	48	OOEE
minuta	48	BBCC
latifolia	48	CCDD
schweinfuthiana	48	FFGG*
punctata	48	
eichingeri	48	

* Genomes of FG could be identical with B, C or D (BOUHARMONT, 1962, p. 121).

At present we can not decide what is the second genome of *minuta.* However, NANDI'S drawings of the first metaphase of the hybrid between *sativa* and *minuta* clearly indicate that the second genome of *minuta* might be homologous to the genome A of *sativa.*

Considering that the genome of *Oryza officinalis* is included in the polyploid species distributed in Asia and America, the A genome of *sativa* might have taken a part in the formation of polyploid species. The artificial hybridization of *sativa* and *officinalis* is easy, and the hybrid is morphologically similar to *minuta.*

If we assume that *Oryza minuta* is an allotetraploid species of *sativa* and *officinalis,* then we have to explain why the sativa genome in some hybrid combinations did not form 12 bivalents with the second genome of *minuta.*

In wheat, we encounter similar difficulties in identifying the B genome of tetraploid species. Many authors believe that species belonging to the section *Sitopsis* (*Aegilops speltoides,* etc.) might have contributed to the formation of tetraploid species. However, the chromosomes of the S genome of *Sitopsis* and B genome of wheat do not form normal bivalents.

According to OKAMOTO (1957) and SEARS AND OKAMOTO (1958), the chromosome 5B (V) of tetraploid wheat has a gene or genes inhibiting the pairing of chromosomes. This assumption was made from the evidence that haploid common wheat without 5B had many more bivalents than the other haploids. OKAMOTO'S finding explains the

lack of chromosome pairing among chromosomes of B and S in a hybrid (AABS), which was obtained from Emmer wheat (AABB) × synthesized AASS. Such a mutation taking place in the course of evolution was presumed, when we had investigated synthesized CCC^uC^u (= *Aegilops caudata* × *umbellulata*). It was expected that this amphidiploid should have 14 bivalents. However, many multivalents were observed in the maturation division of PMC's, whereas, *Aegilops triuncialis* with identical genome (CCC^uC^u) has only 14_{II} (Table 9). We have assumed that in the course of evolution such genic changes might have occurred in the naturally synthesized CCC^uC^u (KIHARA AND KONDO, 1943).

TABLE 9

CHROMOSOME CONFIGURATION OF CCC^uC^u

KIHARA AND KONDO 1943

Chromosome configuration	Frequency (%)
$1_{IV} + 12_{II}$	24
$1_{III} + 12_{II} + 1_I$	29
$13_{II} + 2_I$	12
$1_{IV} + 11_{II} + 2_I$	12
$1_{III} + 11_{II} + 3_I$	10
$12_{II} + 4_I$	2
$1_{IV} + l_{III} + 10_{II} + 1_I$	2
$2_{III} + 10_{II} + 2_I$	2
$1_{IV} + 1_{III} + 9_{II} + 3_I$	2
$1_{III} + 10_{II} + 5_I$	4
$1_{IV} + 1_{III} + 8_{II} + 5_I$	2

In rice no amphidiploids, except *indica-japonica* autotetraploids (CUA, 1952), have been produced yet. This remains a vast area for exploration.

CONCLUSIONS

I draw these brief conclusions:

1. Unification of gene symbols might not be difficult, if we are willing to cooperate with each other, The principles are set.
2. Few traits have been thoroughly investigated by rice geneticists. We must know more about the inheritance of rice, of which more than several thousand varieties are now cultivated.
3. A complete set of trisomics and a set of translocation lines should be established. With these tools the 12 linkage groups of NAGAO AND TAKAHASHI should be tested.
4. Genome symbols should be revised. Eventually a new system should be established.
5. For the unification of genome symbols of rice, we need further investigations on interspecific hybrids.

It also is necessary to reexamine the taxonomy of *Oryza.*

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SESSION I

TAXONOMY

TAXONOMIC STUDIES OF THE GENUS *ORYZA*

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INTRODUCTION

Since the early part of the century, the taxonomy of the genus *Oryza* has been intensively studied (PRODOEHL, 1922; ROSCHEVICZ, 1931; CHEVALIER, 1932; CHATTERJEE, 1948; SAMPATH, 1962; etc.). ROSCHEVKZ (1931) published a comprehensive study of 19 species which provided a basis for later taxonomic studies in the genus. Following ROSCHEVICZ, CHATTERJEE (1948) listed 23 species. Recently, SAMPATH (1962) presented a revised list, enumerating 23 species of *Oryza.*

Despite these contributions, the taxonomy of *Oryza* species still involves many problems. As the genus *Oryza* is distributed throughout the tropics, and the specimens are scattered in many herbaria of different countries, it is difficult to study exhaustively specimens of all known species. Further, the flora of unexplored areas in tropical countries is only partly known. Expeditions to certain tropical countries would likely discover new varieties of known species and also new species of this genus.

Because of these difficulties, we presently may draw only tentative conclusions. Because of the confusion in nomenclature, however, it is essential to attempt a revision of *Oryza* species on the basis of available evidence. A standardization of species names would greatly assist workers in this genus. Some species of *Oryza* occur as complexes. These often invite different opinions as to species delimitation. It is desirable to clarify the morphological distinctions among closely related species.

In 1961, I visited various herbaria in the United States and Europe and examined many specimens, covering almost all the reported type-specimens. I also discussed nomenclature problems with experts associated with these herbaria. The results are given in a paper to be published in the Botanical Magazine, Tokyo. Now, certain important points will be selected from this paper, with particular reference to the species closely related to cultivated rice.

Oryza sativa COMPLEX

Taking wild species only into consideration, this species-complex is widely distributed throughout the tropics. In the taxonomic literature, the Asiatic forms are generally listed as *fatua* or *rufipogon,* and the American ones are called *perennis.* The African forms are called *barthii* by most taxonomic workers, although CHEVALIER (1932) and CHATTERJEE (1948) applied *perennis* to both the African and American plants. It is

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generally assumed that these taxa differ in the lengths of spikelet and awn, in the presence or absence of rhizomes, and in the perennial *vs.* annual habit.

The results of investigations of spikelet and awn lengths in the samples obtained from the three continents are shown in Table 1.

TABLE 1

SPIKELET AND AWN LENGTHS OF WILD PLANTS IN THE *Oryza sativa* COMPLEX
(figures below the class-range indicate the number of samples observed)

Spikelet length (mm)	6.75 —	7.25 —	7.75 —	8.25 —	8.75 —	9.25 —	9.75 —	10.25 —	10.75 —	11.25
Asiatic samples	5	20	27	17	6	2	1	0	0	
American samples	2	1	5	6	15	11	7	2	1	
African samples	1	18	31	32	26	13	4	1	1	

Awn length (cm)	2 —	3 —	4 —	5 —	6 —	7 —	8 —	9 —	10 —	11 —	12 —	13 —	14 —	15 —	16
Asiatic samples	0	25		5	12	14	18	7	5	4	2	0	0	0	
American samples	0	13		2	2	3	2	5	7	6	8	5	3	1	
African samples	5	19	24	28	29	18	4	5	0	0	0	0	0	0	

The Asiatic, American, and African samples did not differ markedly in spikelet length. But, the Asiatic samples had a smaller mean spikelet length than the American. The African samples had shorter awns than the American. Roughly, the Asiatic samples appeared to be intermediate between the American and the African samples in awn length. Nevertheless, the data in Table 1 indicate that the Asiatic, American, and African samples cannot be clearly distinguished by these characteristics.

Rhizomes usually are lacking in the Asiatic and the American forms. The lower internodes of their culms, usually immersed in water, are elongated and geniculate, and the nodes produce rootlets. Such geniculate or stoloniferous culms are not identical to true rhizomes which usually develop underground. But at least some of the Asiatic and American forms seem to produce rhizomes when they grow in swamps which are not constantly immersed in water. I have observed a plant growing at the fringe of Lake Apo, Musuan, Mindanao, the Philippines, having rhizomes about 6 cm. long. On the other hand, the African forms usually have well-developed rhizomes. The presence of an extensive system of creeping and branched rhizomes in the African plants was reported by PORTÈRES (1949), CHIPPINDALL (1955), and others. It also was confirmed by experimental cultivation of many strains at the National Institute of Genetics, Japan.

Perennial *vs.* annual habit has been adopted at times as a characteristic discriminating among the taxa of this species-complex found in Asia. However, it now seems unadvisable to use this characteristic to distinguish the species of this complex. They inhabit swampy areas in wet tropics, and their life span, or the development of new

shoots, must be considerably affected by the conditions of the habitat. It is well known that *Oryza sativa* is not strictly an annual and lives longer than one year, if the environment is favorable. Although both the so-called *'spontanea'* type and the *'perennis'* type are found in Asia, they may hardly be separated at the species level. This view agrees with the opinions of BACKER (1946), BOR (1960), and others.

The nomenclature of the Asiatic forms has been confused. The name *Oryza fatua* was adopted by some authors, but this is a *nomen nudum* (naked name). The name *perennis,* which has been widely used in recent cytogenetic or genetic literature, also cannot be adopted. BOR (1960) and I agree that the correct specific name for the Asiatic plants is *Oryza rufipogon* Griff. Before going further, I should discuss details of the problems regarding the application of the name *perennis.*

Oryza perennis Moench (Meth., p. 197, 1794) was described from a plant cultivated in the botanical garden at Marburg, Germany. In his original description, Moench stated that the plant grows in "in frigidario" (at moderately cool countries), and commented that Tab. 296 in TOURNEFORT's (1719) "Institutiones" resembles *Oryza perennis* except for awn traits. The plant appearing in Tab. 296 has broadly oblong spikelets and is unlike the tropical plants to which the name *perennis* has been applied by CHEVALIER (1932), HITCHCOCK (1936), CHATTERJEE (1948), and others, In fact, it resembles a form of *Oryza sativa.* ROSCHEVICZ (1931) regarded *perennis* a synonym of *sativa.* This view seems to be correct, but no evidence can be obtained from the description.

In 1878, BALANSA AND POITRASSON published an amended description for *Oryza perennis.* Their description, however, clearly shows that the plant they saw was a form of *Oryza alta:* "Ligule courte, arrondie, ciliée-velue, au bord, brune – Epillets lineaires oblongs, longs de 8 à 9 mm." It seems rather doubtful that their *O. perennis* is identical with that of MOENCH. PARODI (1933) interpreted *O. perennis* according to BALANSA'S description, so the plants called *O. perennis* by PARODI are the same as those generally known now as *O. latifolia* (a close relative of *O. alta*). CHEVALIER (1932) also interpreted *O. perennis* according to BALANSA'S description, but curiously adopted it as the correct name with priority for the tropical African and American wild rice with long and glabrous ligules.

Unfortunately, specimens of MOENCH'S herbarium, including the type of *Oryza perennis,* have been missing for a long time (according to a personal communication from the curator of the botanical garden at Marburg), and it seems most probable that none of recent taxonomists have examined the type of *perennis.* In my opinion, *perennis* should be treated as a name of uncertain application, and should be abandoned,

So far as the present examinations have confirmed, the American forms of the *sativa* complex cannot be clearly distinguished morphologically from the Asiatic *rufipogon.* Nevertheless, the spikelets of the American plants are generally longer than those of the Asiatic plants (*cf.* Table 1); their awns are usually more erect and stronger than those of the Asiatic plants. Scientists at the National Institute of Genetics, Japan, especially Dr. H. I. OKA and his associates, are studying the differences in morphology and in some other characteristics. The results of this work, when completed, may influence

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the taxonomy of this complex group. From our present knowledge, we wish to keep the Asiatic and American forms in one species. Thus, the species *Oryza rufipogon* covers the American forms.

As mentioned earlier, the African plants are distinguished from *O. rufipogon* by the presence of well-developed rhizomes. The awns of the African plants are usually shorter and more flexuous than those of *rufipogon.* These differences support the view that the African plants may be separated from *rufipogon* at the species level. The African plants have been listed as *barthii* A. Chev. in most of the literature, and it seems that the application of this name is correct. From our present knowledge, we wish to treat the African plants as an independent species under this name. Recently, SAMPATH (1962) also adopted a similar treatment.

Literature and specimens indicate that *Oryza rufipogon,* which lacks well-developed rhizomes, is not distributed in Africa. But it should be noted that a few collections from Africa did not produce rhizomes when cultivated at Misima, Japan (in a greenhouse or in an artificial short-day chamber). It is possible that the same plants produce rhizomes in their natural habitat. Further expeditions to tropical Africa will indicate whether plants lacking rhizomes exist in Africa.

CHEVALIER (1932) described *Oryza perennis* subsp. *madagascariensis* (= *O. madagascariensis*) from the specimen, Perrier de la Bathie No. 11239. In his original description, he described the ligules as short. This type specimen, preserved in the National Museum of Natural History in Paris, has a culm base and upper parts of several culms with four panicles and a few leaves. The ligules of these leaves are shorter than one cm. The same plant might have had longer ligules on the lower leaves, because the lower leaves of this species-complex generally have longer ligules. I examined a number of specimens of *O. madagascariensis* in Paris, but I could not find any morphological difference between *barthii* and *madagascariensis.* For this reason, I consider *Oryza madagascariensis* a synonym of *Oryza barthii.*

Thus, two species are recognized among the wild-growing plants of this *Oryza* species-complex: *rufipogon* and *barthii.* But, it should be emphasized that this conclusion is tentative and subject to further studies. Various problems related to the taxonomy of this species-complex remain unsolved. To my knowledge, no one has tried to determine whether the Australian forms of *O. rufipogon* are closer to the Asiatic or to the American forms. Also, the nomenclature of intraspecific taxa covered by *O. rufipogon* requires careful study.

Oryza glaberrima COMPLEX

ROSCHEVICZ (1931) and CHATTERJEE (1948) recognized three species in this species complex: *glaberrima, stapfii,* and *breviligulata. Oryza stapfii* is superficially intermediate between *glaberrima* and *breviligulata.* CHEVALIER (1932, 1937), PORTÈRES (1956), and others discussed the taxonomy of these species. Opinions regarding the taxonomic treatment of *stapfii* are split three ways: (1) that the plant named *Oryza stapfii* may be

a form of the cultivated species, *glaberrima;* (2) that *stapfii* represents a wild species which is separated from *breviligulata* by smaller spikelets (up to 9 mm long *vs.* 10–11 mm long in *breviligulata*) and shorter sterile lemmas (2–2.5 mm long *vs.* 3–4 mm long in *breviligulata*); (3) that *stapfii* is united with *breviligulata.*

The writer carefully studied the type specimen of *Oryza stapfii,* preserved in the Kew Herbarium, and found it unidentical to any of the cultivated forms; in fact, the type recalls a dwarf form of *Oryza breviligulata.* Ths indicates that *Oryza stapfii* should not be united with *Oryza glaberrima,* thus disproving the first opinion.

The writer's observation also failed to support the second view that both *stapfii* and *breviligulata* represent distinct wild species. Table 2 shows the variation in the lengths of spikelets and sterile lemmas found among collections belonging to either *stapfii* or *breviligulata.* The data clearly indicate that the supposed distinctions are practically nil.

TABLE 2

LENGTHS OF SPIKELETS AND STERILE LEMMAS OF WILD PLANTS IN THE *Oryza glaberrima* COMPLEX

Spikelet length (mm)	8.0 – 8.2	8.2 – 8.4	8.4 – 8.6	8.6 – 8.8	8.8 – 9.0	9.0 – 9.2	9.2 – 9.4	9.4 – 9.6	9.6 – 9.8	9.8 – 10.0	10.0 – 10.2	10.2 – 10.4	10.4 – 10.6
No. of samples	2	3	1	2	5	7	6	7	7	6	4	5	3

Length of sterile lemmas (mm)	2.4 – 2.6	2.6 – 2.8	2.8 – 3.0	3.0 – 3.2	3.2 – 3.4	3.4 – 3.6	3.6 – 3.8	3.8 – 4.0	4.0 – 4.2	4.2 – 4.4	4.4 – 4.6	4.6 – 4.8	4.8 – 5.0	5.0 – 5.2	5.2 – 5.4	5.4 – 5.6
No. of samples	2	4	7	9	6	6	11	8	4	0	0	0	0	0	0	1

It has also been stated that *Oryza stapfii* has roots at the lower nodes, but that in *Oryza breviligulata* roots appear only at the base. However, this statement is not correct. In the present study, no correlation was found between the rooting condition and the lengths of spikelets and sterile lemmas. Thus, *Oryza stapfii* can reasonably be referred to *breviligulata* as a synonym, as PORTÈRES (1956) suggested. SAMPATH (1962) reached the same conclusion in his classification of this species-complex,

TAXONOMY OF THE GENUS *Oryza* IN GENERAL

According to the results of the present work, 22 species are enumerated in the genus *Oryza* (Table 3). The table also compares the writer's classification with that of CHATTERJEE (1948). Further explorations in the tropics might uncover new species and new varieties of known species.

Several points of interest which warrant further study are:

(1) Wild plants closely related to *Oryza sativa.* – As already discussed, various problems remain.

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TABLE 3

LIST OF *Oryza* SPECIES COMPARING TATEOKA'S CLASSIFICATION WITH CHATTERJEE'S (1948)

CHATTERJEE (1948)	REVISED CLASSIFICATION (TATEOKA)
schlechteri Pilger	*schlechteri* Pilger
granulata Nees et Arn. ex Watt	*meyeriana* (Zoll. et Mor. ex Stcud.) Baill.
meyeriana (Zoll. et Mor. ex Steud.) Baill.	
coarctata Roxb.	*coarctata* Roxb.
ridleyi Hook. f.	*ridleyi* Hook. f.
	longiglumis Jansen (described in 1953)
sativa L.	*sativa* L.
sativa L. var. *fatua* Prain	*rufipogon* Griff.
perennis Moench	
Asiatic	
American	
African	
glaberrima Steud.	*barthii* A. Chev.
stapfii Roschev.	*glaberrima* Steud.
breviligulata A. Chev. et Roehr.	*breviligulata* A. Chev. et Roehr.
australiensis Domin	*australiensis* Domin
eichingeri A. Peter	*eichingeri* A. Peter
punctata Kotschy ex Steud.	*punctata* Kotschy ex Steud.
minuta J. S. Presl ex C. B. Presl	*minuta* J. S. Presl ex C. B. Presl
officinalis Wall. ex Watt	*officinalis* Wall. ex Watt
latifolia Desv.	*latifolia* Desv.
alta Swallen	*alta* Swallen
grandiglumis (Doell) Prod.	*grandiglumis* (Doell) Prod.
brachyantha A. Chev. et Roehr.	*brachyantha* A. Chev. et Roehr.
	angustifolia C. E. Hubbard (described in 1950
perrieri A. Camus	*perrieri* A. Camus
tisseranti A. Chev.	*tisseranti* A. Chev.
subulata Nees	*Rhynchoryza subulata* (Nees) Baill. (excluded from *Oryza*)

(2) *Oryza latifolia, alta* and *grandiglumis,* – SAMPATH (1962) recently suggested to lump these three species under the name *Oryza latifolia.* My examinations indicate that the intermediates between *alta* and *latifolia* are not found in the collections from the West Indies, Central America, and the northernmost parts of South America. But, in the collections from the southern and eastern parts of South America, the intermediates between these two species are rather common, and their differences are obscure. A similar relation also was found between *alta* and *grandiglumis.* Considering our limited knowledge, it might be a matter of choice whether the *alta* and the *grandiglumis* types should be treated as species or subspecies (or varieties) of *latifolia.* I have followed the taxonomic treatment which is generally adopted, and have treated these taxa as different species. Sampath's suggestion can be verified by further studies.
(3) *Oryza officinalis* and its close relatives distributed in the Old World. – *Oryza officinalis* and its relatives constitute the most intricate complex in the genus *Oryza.* According to the results of the present work, four species are enumerated: *officinalis,*

minuta, punctata and *eichingeri.* But further studies may revise this classification. The tetraploid species, *Oryza malampuzhaensis,* reported from Coimbatore, is morphologically close to the diploid species, *officinalis;* I failed to find any clear distinction between them. Dr. H. KIHARA and his associates (unpublished) found that the genomes of *malampuzhaensis* are similar to those of another tetraploid species, *minuta,* distributed in the Philippines, But, *minuta* and *malampuzhaensis* are clearly different morphologically as well as phytogeographically, and *officinalis* may be regarded as an intermediate between the two species insofar as morphological characters and geographical distribution are concerned.

In Africa, two species of this complex, *eichingeri* and *punctata,* are found and both are known to be tetraploids. But among the collections gathered by Dr. K. FURUSATO in his expedition to tropical Africa in 1959, both diploid and tetraploid plants which morphologically fall under *punctata* have been found (TATEOKA AND KATAYAMA, unpublished). The tetraploid forms are close to the type specimens of *Oryza punctata,* which is obviously a tetraploid species. Accordingly, it should be noticed that certain diploid plants distributed in tropical Africa are closely related to *Oryza punctata.* The diploid plants differ morphologically from the Asiatic diploid species, *officinalis.*

If more strains of this species-complex are collected and studied from the morphological, cytogenetical and phytogeographical viewpoints, our knowledge of the taxonomy of this species-complex will be further advanced.

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SUGGESTIONS FOR A REVISION OF THE GENUS *ORYZA*

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The genus Oryza is small. Most of the species have been collected and grown, and it should now be possible to complete a revision of the genus. The recent study by TATEOKA (1963) of *Oryza* collections in all the important herbaria permits a clear understanding of its taxonomy. This study supplements previous work and supersedes that by the author (SAMPATH, 1962). In this context, some suggestions are offered regarding the delimitation of species, as well as the recognition of subspecies.

The revision of taxonomy is of use to workers who study the cytogenetics of the genus, and it has to be accepted by them. Therefore, the revision should incorporate findings from work on interspecific hybrids and should help in tracing evolution and interrelationships of species in the genus. If this objective is accepted, some problems arise in the nomenclature of the various taxa.

An important instance is the group of wild species closely related to the cultivated rice, *Oryza sativa.* Hitherto the specific names, *perennis* Moench, *sativa* var. *fatua* Prain, *cubensis* Ekman, *barthii* A. Cheval. and *rufipogon* Griffith, have been applied to different populations having fully or partly homologous genomes in a diploid constitution. From TATEOKA'S study, it is apparent that the widely used specific name *perennis* is of uncertain application and that the taxonomy of the group needs revision. If the rules are to be rigidly applied, the name *Oryza rufipogon* Griffith would be valid for the Asian and American populations of this group. Experimental work has shown that Asian and American populations are genetically differentiated and that the Asian populations include two different taxa. One is a primitive species with slender spikelets and long anthers, and the other is a complex hybrid swarm having coarser spikelets as well as shorter anthers. Separation of the two into taxonomic categories is difficult, particularly because segregation accompanies natural crossing. However, in tracing the origins of cultivated rices, it is necessary to recognize the difference between the two species. Designating the different groups as subspecies of a cosmopolitan, major species with an acceptable specific name, recognizing continuous variation might help solve the problem.

A parallel instance can be cited from the intensively studied species sativa. In this cultivated species, division into three subspecies, *indica, japonica,* and *javanica,* has been proposed. Such a division is difficult to maintain on the basis of morphological characters alone, and is therefore taxonomically invalid. There are at least three well-

defined groups among the large number of cultivated varieties, and distinctions can be made by using a group of genetical characters, and by applying biometrical techniques in analysis.

A different kind of taxonomic problem is presented by the group of American tetraploid species, presently classified as *Oryza latifolia* Desv., *alta* Swallen and *grandiglumis* (Doell) Prod. Experimental work at Cuttack shows that the species alta and *grandiglumis* can be hybridized, and that the F_1 is moderately fertile. The character used for discriminating the two species, namely the length of sterile lemma or glume, is inherited in a Mendelian manner. These observations suggest that the two species can be merged as *alta* and that the varietal name *grandiglumis* be used for the long glumed plants. This revision would help bring out the relationship between the two taxa and would facilitate tracing their evolution. It is probable that they evolved by polyploidy from the same two diploid species, one of which had the genomes of *officinalis* Wall. The American species *latifolia* also probably had a similar evolutionary origin, and further work may show its close relationship to *alta.*

A different kind of problem arises in delimiting the African *Oryza* species *eichingeri* Peter and *punctata* Kotschy ex. Steud. TATEOKA *(loc. cit.)* has shown that morphological distinctions, although not conspicuous, can be recognized. He infers that *punctata* includes diploid as well as tetraploid forms and that the Sinhalese species generally listed as *latifolia* may be classiffed as *eichingeri.* Study at Cuttack of one collection of these Sinhalese species shows it to be a diploid ($2n = 24$). It would follow that *eichingeri* includes both diploid and tetraploid forms. Such a situation is unsatisfactory as it is desirable to distinguish tetraploid forms from diploids. It is established that *eichingeri* is not an autopolyploid and that its genomes are cytologically differentiated. Further cytogenetic work is needed to solve this taxonomic problem.

This writer's collection of *Oryza barthii* A. Cheval. from Madagascar had longer ligules than those in the Sudan collection of this species. It is not true that longer ligule is characteristic of *barthii* subsp. *madagascariesis* A. Cheval. TATEOKA'S study shows that the subspecies should be characterized by short ligules, and that such a subspecies has not been clearly delimited. These observations indicate the genetic variability present in *barthii* in Madagascar. Such natural variability may possibly also be found in the related Asian species. Further study is required before their taxonomy can be established.

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REPORT OF A POLL ON *ORYZA* SPECIES

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In rice literature the taxonomic status of the genus *Oryza* is unsettled. The number of recognized species varies from 13 (SASAKI, 1935) to 23 (CHATTERJEE, 1948; SAMPATH, 1962), depending on the particular classification scheme followed. Recently, a few more species have been added. Rice workers also show no rigid adherence to any particular classification scheme, adding confusion to the recognition of the plant material referred to in the literature. Errors in nomenclature are often noted (OKA AND CHANG, 1960; SAMPATH, 1961). For these reasons, and probably because of mis-identification, the chromosome count of a number of *Oryza* species has not been clearly defined (KIHARA, 1959).

The current interest in the interrelationships among *Oryza* species and in the origin and evolution of the cultivated rices necessitates a thorough review and revision of the taxonomic status of the genus. Unfortunately, the information derived from the cytogenetical studies of interspecific hybrids has not been utilized fully to resolve the taxonomic problems. We anticipate that some positive suggestions will evolve from this Symposium. The International Rice Research Institute will encourage efforts leading to a workable and unified scheme of classification, applying available knowledge from taxonomy and cytogenetics.

A questionnaire was sent November 14, 1962, to 32 rice workers, including taxonomists, geneticists, cytogeneticists, and breeders, to prepare for the present discussion on *Oryza* species. The following questions were asked:

(1) Which are the species of *Oryza* which you recognize as valid?

(2) Which are the other species that would require further study by taxonomists and cytologists to establish their validity?

(3) What criteria should be used in establishing valid species?

(4) Do you consider it worthwhile to reappraise the taxonomic status of *Oryza* species and to work toward a revised scheme of classification acceptable to both taxonomists and cytogeneticists?

By mid-January 1963, 15 scientists had responded to our query: 4 had no opinion to offer, 1 indicated that the problems involved are too complicated for simple answers, and 10 answered one or more of the four questions.

For the first question on valid species, the voting on recognized species is tabulated in Table 1.

TABLE 1

Oryza SPECIES RECOGNIZED BY SEVEN WORKERS

Recognized Species	Number of Votes
1. *alta* Swallen	3
2. *angustifolia* Hubbard	3
3. *australiensis* Domin	7
4. *barthii* Chev.	4
5. *brachyantha* Chev. et Roehr.	6
6. *breviligulata* Chev.	7
7. *coarctata* Roxb.	6
8. *eichingeri* Peter	6
9. *glaberrima* Steud.	7
10. *grandiglumis* Prod.	3
11. *granulata* Nees et Arn.	3
12. *latifolia* Desv.	6
13. *longiglumis* Jansen	3
14. *malampuzhaensis* Krish. et Chand.	3
15. *meyeriana* Baill.	4
16. *minuta* Presl	6
17. *officinalis* Wall.	6
18. *perennis* Moench	5
19. *perrieri* Camus	4
20. *punctata* Kotschy	4
21. *ridleyi* Hook.	6
22. *rufipogon* Griff.	3
23. *sativa* L.	7
24. *sativa* L. *spontanea* Roschev.	1
25. *schlechteri* Pilger	4
26. *subulata* Nees	4
27. *tisseranti* Chev.	4
28. *ubanghensis* Chev.	1

It can be seen above that only four species (*australiensis, breviligulata, glaberrima* and *sativa*) are recognized by all of the seven workers.

For the second question regarding species of doubtful validity and for which further studies are needed, the following 16 species have been named with the number of nominating workers (5) given in parentheses:*angustifolia* (l), *breviligulata* (1), *eichingeri* (1), *fatua* (2), *granulata* (1), *malampuzhaensis* (l), *meyeriana* (1), *minuta* (1), *officinalis* (3), *perrieri* (l), *punctata* (2), *rufipogon* (l), *schlechteri* (2), *schweinfurthiana* (1), *tisseranti* (1), and *ubanghensis* (1).

With regard to the third question on those criteria which should be used in establishing valid species, various criteria proposed by eight workers are summarized in Table 2.

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TABLE 2

CRITERIA FOR ESTABLISHING VALID SPECIES

Criterion	Number of Votes
Geographical distribution	6
Morphology of vegetative and floral organs	7
Morphology of caryopses, morphology and anatomy of embryos	1
Physiological and/or ecological behavior	4
Chromosome number	6
Karyotype	2
Crossability	6
Chromosome homology	5
Fertility relationship	6

To the fourth question, about the desirability of reappraising the taxonomic status of *Oryza* species and working toward a revised scheme of classification based on cyto-taxonomic criteria, all eight workers who responded answered positively. One scientist suggested adopting CHATTERJEE'S 1948 scheme as a working basis for improvement. Another worker urged the use of the numerical-taxonomic approach in studying species relationships. Three workers (one English, one Indian, and one Japanese) indicated their desire to collect species in Africa, especially, the west coast and Madagascar.

DISCUSSION IN SESSION ON TAXONOMY

Most of the discussion about the taxonomy of *Oryza* concerned the types of information that should be used in classifying the genus and in determining the relative importance of each type. The conferees stressed that systematic treatments of *Oryza* by traditional taxonomists have been based almost entirely on gross morphological characteristics. Several conferees felt that the considerable amount of accumulated genetic and cytogenetic evidence pertaining to many of the wild and cultivated forms also should be considered in taxonomy that the classification might provide reliable indication of phylogenetic relationships, while "pigeon-holing" the various forms. This phase of the discussion dealt particularly with classification of what might be called the *perennis-fatua* complex. There was serious disagreement over the systematics of this group. The discussion led to the appointment of a committee, composed of T. TATEOKA (Japan), S. SAMPATH (India) and M. T. HENDERSON (U.S.A.), to study the subject further and to report its deliberations on the final day.

It was suggested that other fields, in addition to morphology and cytogenetics, could contribute to taxonomy of the genus and should be investigated. Biochemistry was mentioned specifically. It was cited that the so-called *fatua* or *spontonea* form, presumed to be a hybrid, has appeared in collections from Africa. The African strains of *fatua* were assumed to be of recent origin.

It was suggested that The International Rice Research Institute prepare and publish color diagrams and descriptions of various parts of the rice plant in order to aid rice workers, including those dealing with systematics, in attaining uniformity of terminology.

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SESSION II

GENE SYMBOLIZATION AND NOMENCLATURE

EARLY HISTORY OF GENIC ANALYSIS AND SYMBOLIZATION IN RICE

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Improvement of the rice crop by selection in natural populations has been practiced with satisfactory results in many countries of Asia since the beginning of this century. With the rediscovery of Mendelism early in this century, plant breeders in the West started to use it in planning their breeding program. Except in Japan, crop improvement work in all Asian countries was in the hands of foreign scientists trained in the West. Genetic studies on rice were initiated somewhat later. Such studies were only incidental and secondary to the main work of crop improvement by breeding. These early genetical studies were mostly conducted to determine if Mendelian principles applied to inheritance of characters in rice. These studies dealt mostly with such easily recognizable morphological features, as the presence and distribution of anthocyanin pigment in different parts of the plant, color in the lemma-palea, and color in the rice pericarp.

Mendelian segregation of any particular character may not be exciting in the present day, but the author, four decades ago, considered the experience thrilling when his F_2 analysis confirmed any of the Mendelian ratios. Another exciting experience was his discovery that a hybrid between two rices, one with glutinous and another with starchy endosperm, had both types of grains in the same panicle, roughly in the proportion of three starchy to one glutinous. This finding led to the publication of a note on the detection of segregation by examination of the rice pollen (PARNELL, 1921). The first recorded instance of Mendelian segregation in rice was by VAN DER STOK (1908) in Indonesia. Instances of linkage and cross-over mainly with anthocyan color were first published from Coimbatore, India (PARNELL *et al.*, 1917, 1922).

These earlier studies on easily recognizable characters were later extended to quantitative and physiological characters such as size and shape of grain, height of plant, period of maturity, etc., where both simple and polygenic inheritance were recorded. Up to 1930 in India, genetic association or correlations between quantitative characters and between morphological characters on one hand and quantitative or physiological characters on the other, were observed and recorded. Japan and the United States also contributed to the genetics of rice. Some early workers whose contributions have been outstanding are KATO, AKEMINE, IKENO, HOSHINO, NAGAI, MOFUNAGA, YAMAGUCHI, and MIYAZAWA, all from Japan, JONES from the U.S.A. and CHAO from China.

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This paper concerns the history of rice genetics, and mentions some of the salient results that have followed the earlier work in India. With regard to anthocyan genetics, it was recognized that besides other genes responsible for localizing, intensifying, diluting, and inhibiting the development of pigment, there were two basic genes the simultaneous presence of which was necessary for development of pigment in any part of the plant. Critical information on the interrelationship of these genes was not collected. RAMIAH (1945) showed that the presence of pigment in different morphological parts appeared in definite patterns or groups different for the collections in India (all *indica's*) and those in the U.S.A. (mostly *japonica's*), indicating perhaps the presence of different loci for anthocyan genes. This has since been confirmed in recent linkage studies. The work in India, particularly in Coimbatore, indicates that there are three linkage groups, each having a varying number of genes responsible for the development of pigment in particular parts. A multiple allelic series was recognized with regard to genes controlling color (non-anthocyan) in lemma-palea.

It was indicated that the observed association of presence of color in different parts may be due either to multiple allelomorphism-cum-pleiotropy or to closely linked genes. Types with patterns different from the parental ones did occur in small numbers whenever a sufficiently large population was grown. These types are mutations on the former hypothesis or cross-overs on the latter hypothesis, Earlier work in Coimbatore seemed to support the linkage hypothesis since the two complementary cross-over types occurred in approximately equal proportions, while the work in Bengal (HECTOR, 1916, 1922) supported the multiple allele hypothesis. Work in Japan demonstrates the multiple allele theory with regard to apiculus locus, but there is no record of mutations having occurred. This may be due to the fact that large populations were not grown. A critical appraisal of the position by a program of suitable crosses, growing large populations and testing the new combinations genotypically, may be worth undertaking. Preliminary work done in Coimbatore on some of the suspected mutants did confirm their mutational origin. It appears that in rice cross-overs as well as mutations did occur with regard to anthocyan genes.

In some of the earlier genetical studies in Coimbatore, involving such quantitative characters as length of spikelet and length of culm or a physiological character as period of maturity, a clear bimodal curve was obtained in F_2, indicating a definite monohybrid segregation, but types corresponding to the parents were never recovered even with large populations indicating major gene action with minor modifiers. The segregation for the dark purple color of the rice pericarp also behaved in the same way. Several instances of close association between morphological and quantitative or physiological characters also have been recorded. It is possible that by using this material in suitable crosses the major genes controlling the valuable characters could be assigned to particular linkage groups.

Since 1930, however, the rapid development in sister sciences, such as taxonomy, cytology, physiology, and biometry, have influenced further development of formal genetic studies on rice. The volume of inheritance studies on rice published after 1930 was significantly greater than in the earlier period. By 1940, nearly 300 genes were

recognized, including about 50 major characters (KADAM AND RAMIAH, 1943). Although studies on rice genetics were pursued in several centers in India, workers had few opportunities to compare their results. Each worker described plant characteristics, particularly those relating to color, in his own way, and others had great difficulty in understanding the characteristics involved.

Use of genic symbols was even more confusing. Often no symbols were used in designating particular genes. Usually, the first two or three letters of the English alphabet were used, and only in a few specific cases were intelligible symbols used. In the last case, however, letters were used without reference to alphabets already used by other workers to denote the same characteristics. The general result was utter confusion. Lack of contact of workers in India with those in other countries and language difficulties also contributed to this confusion.

The Indian Council of Agricultural Research, established in the middle thirties, encouraged rice research at several centers by providing financial help. To eliminate the confusion in naming plant parts and assigning symbols controlling particular characters, the Council appointed in 1937 a special committee, with the author as convener, to draw up a standardized botanical and genetic nomenclature of rice. The report of this Committee with appropriate tables and color charts was published in 1938 (HUTCHINSON AND RAMIAH). This committee worked to bring about a common understanding regarding use of genic symbols. KADAM and RAMIAH, members of this committee, undertook a survey of the existing position with regard to the use of genic symbols, and drew up a rational system acceptable to all rice workers. The plan was first discussed and approved by the special committee, and later sent to workers outside India, through the Imperial Bureau of Plant Breeding and Genetics, Cambridge, to obtain comments and suggestions for improvement. Abstracts in English of the results of genetical studies on rice in various parts of the world, published periodically by the Commonwealth Bureau, was a landmark, and helped workers everywhere to understand what was happening.

At the time this plan of genic symbolization was drawn up, maize was the only crop on which extensive genic analysis was available. An understanding already existed among maize geneticists. The genic symbolization of maize was kept as a model, therefore, and the symbols were assigned mainly on the lines recommended by DE HAAN (1932). In many cases similar letters as used in maize were adopted to denote similar characteristics. The author served on a Special Committee of the International Genetics Congress which met in Paris, 1939, to draw up general principles for genic nomenclature. The proposals drafted in India conformed with the principles laid down by this committee. Useful and constructive suggestions for improving the proposals were received, and these were incorporated in the final plan. Japan was the only country for which the proposals were entirely unacceptable. In the meantime, World War II intervened, and for several years after its termination in 1945, conditions were such that no progress was possible in reconciling objections raised by the Japanese geneticists. To avoid aggravating the existing confusion in genic nomenclature of rice, the new plan incorporating the useful suggestions received was published in 1943 (KADAM AND

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RAMIAH). This paper covers all literature on rice genetics up to 1942 and provides complete information on genes used by various workers to denote particular characteristics, an alphabetical list of proposed basic symbols and information on inheritance of characteristics in rice with the recommended designation of the genes. Rice geneticists the world over, except Japan, have since adopted these proposals. The Indian Council of Agricultural Research also published a scientific monograph on Rice Breeding and Genetics (RAMIAH AND RAO, 1953) including the genetic results of rice published up to 1950 and using the genic nomenclature proposed by KADAM AND RAMIAH. This monograph contains not only results available in India but also those in other countries, including Japan, within reach of the authors.

In 1954, the International Rice Commission, realizing the importance of solving the existing difficulties and of arriving at a scheme that would be acceptable to rice geneticists all over the world, constituted a special committee of three with Mr. JODON of U.S.A. as convener and two other members, one from Japan and one from India. We are grateful to Mr. JODON for his keen interest, these last few years, in meeting the objections of the Japanese geneticists against the original plan and in bringing out a new plan acceptable to all. We are particularly glad that the International Genetic Congress has approved all the proposals. The IRC Working Party meetings on Rice Production and Protection held in December 1959, and December 1961, discussed the new proposals and recommended that rice geneticists adopt them in their future publications. The IRC Newsletter, Vol. VIII, No. 4 published these proposals. To bring about uniformity of genic nomenclature in the future, the IRC meeting in Delhi also established certain procedures, which The International Rice Research Institute has been designated to monitor among rice geneticists everywhere.

The foregoing is a brief history of the present position of genic nomenclature in rice, and as the oldest rice geneticist present at the meeting, the author is happy with the satisfactory progress made. We are thankful, particularly to Mr. JODON and his committee, who have helped achieve this progress. Rice geneticists should be reminded that no plan, however perfect, can be completely satisfactory. The present plan may not be absolutely final, and further intensification of genetical studies in rice, undoubtedly, may require changes, but we should continue using the plan until a change is found absolutely necessary. As the law of constitution in politics, the genic symbolization for geneticists should not be interfered with to satisfy individual whims or fancies.

Lastly, great advances are being made in understanding the nature and behavior of genes. Geneticists have new and valuable tools to produce mutations. This will help intensify genetic studies of rice. Rapid progress might be expected, particularly in fields dealing with the genetics of the rice plant with respect to its resistance or susceptibility to pests, diseases, and unfavorable conditions. By applying genetic principles, future rice breeders may evolve the most suitable varieties for particular environmental conditions. In many Asian countries, the genetic study of rice is only a side line to the main work of breeding, and often the formal study of genetics is discouraged. Any fundamental work done in earlier years at any center depended solely on the initiative and competence of a particular scientist, and usually was discontinued when the

scientist left. RAMIAH AND PARTHASARATHY (1938) were the first, as early as 1933–34, to produce artificially in the field a large number of mutations by X-raying rice seed in Coimbatore. Genetical and cytological studies of some of these mutations also were initiated and the results were published from Coimbatore. Unfortunately, they had to leave Coimbatore. Not only was the work stopped, but shortly after, the research material also was lost.

Central institutes which undertake genetic study have been established recently. The IRC (FAO) had stressed the importance of fundamental research on the rice plant which can be undertaken most effectively in such institutes. Lack of funds, facilities and trained personnel have limited the scope of research in these institutes. It is in this context that one has to think of an international institute such as the one that has sponsored this Symposium. This Institute undertakes all aspects of basic research on rice and trains scientists of the rice-growing countries. It has been a pleasure to visit this Institute and take part in the Symposium. May the Institute rapidly increase its sphere of activities and usefulness to the rice-growing countries of the world.

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FAO'S INTEREST AND ROLE IN GENE SYMBOLIZATION AND NOMENCLATURE

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FAO's interest in technical matters connected with rice dates back to 1947 when it recognized the urgent necessity of promoting national and international action to increase the world's rice supply. The low level of production in the densely populated tropical countries of Asia, which are the main areas of production as well as consumption, has necessitated the pooling of technical knowledge on this important cereal and taking concerted action to advance research to improve rice. The International Rice Commission of FAO, founded in 1949, is the first international body concerned with the technical problems relating to "production, conservation, distribution and consumption of rice." The IRC functions through three Working Parties, namely:

(1) Working Party on Rice Production and Protection; previous to 1958 termed Working Party on Rice Breeding.
(2) Working Party on Rice Soils, Water and Fertilizer Practices; previous to 1958 termed Working Party on Fertilizer.
(3) Working Party on Engineering Aspects of Rice Production, Storage and Processing.

These Working Parties and IRC meet once in two years. The meetings have functioned as forums for discussion and recommending improvements in rice production in various countries. As a result of these efforts, the countries have realized that the following major aspects of rice production require their increasing attention: Regional trials to reduce the existing large number of varieties in the countries to a few with wider adaptability, allowing more efficient seed multiplication programs; development of varieties with higher yield response to fertilizers; a change from long-growing to short-growing varieties with low sensitivity to photoperiodism for double-cropping of rice as well as diversification; breeding for short and stiff straw, non-shattering, uniformly ripening varieties for high fertilization and mechanization; development of varieties resistant to the blast disease; control of insect pests and diseases.

IRC recommendations resulted in cooperative projects such as: a rice hybridization project for evolving varieties with high fertilizer response; international variety trials of superior native varieties with wider adaptation in the country of origin; uniform blast nursery trials, which have just been initiated.

The IRC has emphasized fundamental research on rice. Although significant progress is being made on the physiology and genetics of rice in Japan, only a few countries in the tropical region can afford rice research. Most lack funds, facilities, and trained

personnel. They felt a keen interest in the establishment of an international rice research body to undertake fundamental research and training of personnel. The establishment of The International Rice Research Institute, therefore, is cause for great satisfaction. In convening the present Symposium on Rice Genetics and Cytogenetics, the Institute is providing leadership to advance knowledge fundamental to the improvement of rice.

Considering the importance of rice in the world economy, the progress of genetical studies on this crop has been slow compared to the advancement made in similar research on other cereals such as maize, wheat, and barley. In the early decades of this century, the reports on genetic analysis of characters resulted mainly from the initiative of rice breeders whose primary objective, however, was to evolve better varieties by selection and hybridization. The governments gave pratically no support to systematic genetical studies. The universities showed little interest because of a lack of research facilities.

There were, therefore, no continued and systematic studies of inheritance in rice, and the few analyses reported lacked uniformity in the use of gene symbols. When such reports accumulated, this led to confusion, and the Indian Council of Agricultural Research in 1937 appointed a committee to survey the situation and to submit proposals for a uniform and common nomenclature.

This action led to the publication by KADAM AND RAMIAH in 1943 wherein many conventions used in maize, cotton, and *Drosophila* were adopted for designating rice gene symbols. The recommended symbols, along with those used by various others, and relevant references were given. This symbolization was accepted in the United States and India, but the rice geneticists of Japan continued to use a different set. This was the situation when the subject of symbolization was reopened by the IRC Working Party on Rice Breeding at its Sixth Session in Penang, Malaya, in December 1955.

In connection with its function of promoting genetical studies on rice as fundamental to breeding, the IRC Working Party on Rice Breeding at its Fifth Session in Tokyo, October, 1954, examined the progress of linkage studies and requested Dr. S. NAGAO, Japan, to present a working paper on "Linkage Groups in Rice" at its next meeting. The gene symbols used in the working paper contributed by him at the Sixth Session of the meeting drew attention to the need for a standardized and universally acceptable genetic nomenclature to accelerate the progress of linkage studies. Cooperative linkage studies had to be deferred until a commonly accepted genic symbolization was established. The Working Party decided, therefore, that usage of symbols by the rice geneticists of Japan and elsewhere should be examined and a unified system be developed. For this purpose, it appointed a small committee with N. E. JODON of the U.S.A. as convener.

At about the same time, world geneticists also were discussing the question of gene symbols and nomenclature. The rapid development of genetics and genetic analysis of characters in a wide array of plants and animals, and man, stimulated the Permanent International Committee for Genetics Congresses to nominate, in 1954, a small committee to draft rules for genetic symbols and nomenclature. This International Committee on Genetic Symbols and Nomenclature met under the auspices of the

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International Union of Biological Sciences at Zurich in August, 1957, and based its proposals on the recommendations of a group of Japanese geneticists in consultation with some participants in the International Genetics Symposia held in Tokyo and Kyoto in 1956.

The Committee felt that adherence to some standard system would lessen the confusion and greatly facilitate communication among specialists in different areas in genetics. However, the Committee would leave the work involving details of symbols for individual organisms to small groups specializing in this kind of study. This was later accomplished in rice by the IRC Committee on gene symbolization.

The report of the International Committee of 1957, adopted by the Tenth International Genetics Congress in Canada in 1958, was timely and provided the necessary authoritative guidelines for the rice committee in preparing a list of gene symbols for rice. The International Committee believed that "standardization of symbols and adoption of common rules, although they cannot, and should not, be compulsory are highly desirable whenever possible." Based on established practices, their recommendations were broad enough for use in diverse situations. Mr. JODON will report on the critical work he and his colleagues did in this field and will present a list of proposed symbols for all the genes reported before the IRC Working Party meeting on Rice Production and Protection in Ceylon in December 1959. In this report the rules laid down by the International Committee on gene symbols indicate their suitability to the system proposed for rice. The proposed gene symbols were adopted with a few minor revisions and, in accord with the recommendation made at the meeting, were published in the December issue of the IRC Newsletter (Vol. 8, No. 4, pp. 1–7).

A more comprehensive report, containing tabulations of recommended and original symbols together with bibliographies, according to Mr. JODON, is already in press.

In their future publications, rice geneticists should conform to the usage recommended in the committee's report as published in the IRC Newsletter. It also is necessary that symbols given to new genes in the future should conform with the rules adopted at the Tenth International Genetics Congress. It is felt, however, that compulsion to adhere to the recommended system is not practical.

For greater uniformity in the future, the following procedures were suggested at the IRC Working Party on Rice Production and Protection held at New Delhi during December 1961:

(1) Genetic literature is to be reviewed periodically so that the symbols used in unreviewed papers can be recorded. Symbols conforming to the adopted rules may be registered; if they do not conform, proper symbols may be designated and published along with the symbols used by the authors.

(2) Such reviews and designations of new symbols are to be published in the IRC Newsletter or other journals generally read by rice geneticists.

(3) The International Rice Research Institute could be the ideal center for exercising the above functions.

(4) Until the Institute assumes these duties, Mr. JODON will act as advisor.

The Working Party, therefore, recommended that the above procedures be followed,

and it is gratifying to note that the IRRI has scheduled this item of symbolization for panel discussion at this symposium. In this connection, the Eighth Session of the IRC Working Party on Rice held at Colombo in December 1959, heard a suggestion to publish complete morphological and histological diagrams of rice properly labelled in English. This would allow common reference to the plant parts in which gene expression occurs.

With its excellent library facilities and expert staff, the Institute, it is hoped, will carry on these advisory and documentary functions to promote the advancement of genetical research.

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TOWARD UNIFORMITY OF GENE SYMBOLIZATION IN RICE GENETICS

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INTRODUCTION

The use of symbols in dealing with genetic data is so familiar that we seldom reflect how cumbersome the subject would be without them. Genetic symbols afford brevity as well as convenience. They serve much the same purpose as chemical symbols, the representation of elements in their intricate combinations. The use of symbols dates back to Mendel, who, however, apparently based his usage on algebra rather than chemistry. Present usage has affinities with both.

Unless there is uniformity of usage, symbols may confuse as much as simplify the presentation of inheritance study results. To quote YAMAGUCHI (1927) in his early attempt at unifying symbolization, "One should strive for uniformity in genetic articles if one wishes to secure the reader's best possible comprehension." In spite of YAMAGUCHI's admonition, some subsequent reviewers ignored symbolization, and, unfortunately, the tendency among workers to display individuality in the use of gene symbols still persists.

YAMAGUCHI listed 40 characters to which he assigned symbols. His list was included in MATSUURA's first edition (1929) but was omitted from the second. Interestingly, 4 of YAMAGUCHI's 40 symbols remain the same in the recommended list of the International Rice Commission adopted in 1959. These are *C* for chromogen, *d* for dwarf, *Pl* for purple leaf, and *Ps* for purple stigma.

IKENO (1927), in a review published about the same time as YAMAGUCHI's, used no symbols. MATSUURA (1933), in his second edition, listed characters according to number of genes involved, using no symbols. In the 1936 United States Department of Agriculture yearbook, JONES (1936) also omitted symbols in listing character segregation. Haphazard usage continued through the 1930's.

The symbols used by CHAO (1928), in one of the earliest intensive studies of rice genetics in the United States, were patterned after the usage of the corn geneticists. He did not cite YAMAGUCHI.

Results of studies carried out by J. W. JONES in California in the late 1920's were published "sans symbols." RAMIAH and other Indian workers used symbols that seemed appropriate to them individually and probably the Japanese workers did likewise. Sometimes A, B, and C were used as convenient temporary symbols.

The lack of uniformity of usage in India came to the attention of botanists in the Imperial Council of Agricultural Research, and a committee headed by Dr. K. RAMIAH

was appointed in 1937 to draw up a standardized agronomicand genetic nomenclature. Dr. B. S. KADAM was given a mandate to survey the situation and submit proposals for the symbolization of genes in rice. A preliminary draft of their paper was circulated and some suggestions by S. W. JONES and the writer regarding symbols and the inclusion of segregation ratios were adopted. The final proposals were published in 1943 (KADAM AND RAMIAH, 1943).

The symbolization proposed by KADAM AND RAMIAH was comprehensive and workable. It was accepted and used in India and the United States, but one or two of its features seemed bothersome. The first was that basic letters were assigned to plant parts for all characters these letters might express, obscuring pleiotropic effects and requiring in some instances more letters per symbol than seemed necessary. The other awkward feature was that the noun preceded the adjective where symbols were based on adjective and noun.

RENEWED HOPE FOR AGREEMENT

Unfortunately, the symbolization of KADAM AND RAMIAH appeared at a time when there was no mood for cooperation. Nevertheless, I am sure that Dr. RAMIAH continued to hope for eventual agreement among all rice research centers, and it was at his instigation that the matter was brought up for consideration by the IRC at the sixth meeting of the Working Party on Rice Breeding in 1955.

Dr. K. RAMIAH has reviewed the history of the early efforts to establish nomenclature and gene symbols, and Dr. N. PARTHASARATHY has explained the concern of the IRC in the establishment of standard symbolization. How the IRC committee functioned and finally carried through on the rather formidable assignment given it by the Working Party remains to be recounted.

On his return from the 1955 IRC meeting, Mr. H. M. BEACHELL told me informally of the recommendation of the Working Party for the standardization of gene symbols and of its appointment of Dr. S. NAGAO, Dr. N. PARTHASARATHY, and me as a committee to work toward that end. At the time, however, there seemed to be no starting point and no basis for an acceptable compromise. No progress was made until the Tenth International Congress of Genetics had convened and the Report of the International Committee on Gene Symbols and Nomenclature (TANAKA, 1959) became available. The report laid down ground rules, the authoritative status of which afforded the needed basis for bringing about eventual general agreement among rice geneticists.

The applicable rules of the International Committee are few and simple. They do not differ materially or seriously conflict with the "conventions" of KADAM AND RAMIAH (1943) as is apparent from the following parallels: (see page 42).

At first, it appeared that the best approach might be to begin with a limited recommended standard list of symbols, including the best known loci. Thus the symbolization of other genes according to the International Rules would have to be revised by rice geneticists as their research progressed. It was eventually decided, however, that it would be opportune to organize, as completely as possible, a set of symbols applicable to all reported Mendelian segregations.

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RULES OF THE INTERNATIONAL CONGRESS OF GENETICS	CONVENTIONS OF KADAM AND RAMIAH
2. Symbols should be as short as possible.*	(brevity not mentioned)
4. Use literal or numerical superscripts to distinguish allels of a series.	1. Use literal superscripts only.
6. Use basic symbols for genes with phenotypically similar effects. Use numerical subscripts for non-allelic genes.	2. and 3. Use literal subscripts for complementary. Use numerical subscripts for polymeric genes**.
7. Use prefixes for modifying genes: Inhibitors = I- Suppressors = Su- Enhancers = En-	Inhibitors = I (no dash)

Suggestions of IRC Committee:

* Symbols should be based on a key word or on an adjective-noun combination.

** These conventions should be followed.

Once accomplished, this objective necessitated the compilation of all available data on inheritance studies in rice, including the reported segregations and the symbols applied. Fortunately, it was necessary to refer back only to as far as 1942 and bring up-to-date the tabulation in KADAM AND RAMIAH'S 'Symbolization of Genes in Rice'. In their publication they had summarized the accumulated information in tabular form under the headings: characters, recommended symbols, original symbols, segregation ratio, and authority.

Bringing the record up-to-date, however, was practically impossible for any one person to accomplish because of the scattered distribution of publications and the language barrier. It required workers familiar with the literature in each country to do the job. Not all of the original members of the committee were in a position to assume the necessary detailed work. In India, Dr. RAMIAH had retired from active work, Dr. PARTHASARATHY had changed positions, and Dr. KADAM was no longer engaged in rice research. Fortunately, however, a staff member of the Central Rice Research Institute at Cuttack, Dr. R. SEETHARAMAN, who was then in residence at Louisiana State University and about to return home, agreed to undertake the tabulation of the Indian work. Fortunately also, Dr. MAN-EMON TAKAHASHI, an associate of Dr. NAGAO, had spent a year in Louisiana and willingly took the responsibility for the Japanese literature.

Dr. TAKAHASHI, who thus acted as Dr. NAGAO'S deputy on the committee, had the latter's advice in tabulating the Japanese work. Dr. SEETHARAMAN acted for Dr. PARTHASARATHY and with the approval of Dr. RAMIAH tabulated the work done in India. Personal acquaintance with these men made my part, organizing a coordinated report, much easier. We corresponded by airmail for many months to clarify points and resolve differences.

The work progressed well enough so that we were able to present a report and list of symbols at the eighth meeting of the now renamed IRC Working Party on Rice Production and Protection in 1959. The report was accepted and the proposed symbols recommended for standard usage. The basic tabulations were not fully completed and were not included in the IRC report (Anonymous, 1959).

The committee felt that a full report including complete tabulations of recommended and original symbols, citations, and literature lists should be published. The Working Party directed the publication of such a report. The airmail "conversations" were continued; the symbols list was rounded out, and the literature lists were completed. The completed manuscript was referred to Dr. D. W. ROBERTSON, of barley genetics fame, and to Dr. WARREN H. LEONARD, who includes rice genetics in his broad field of interest. They suggested that wherever symbols had been previously applied, recommended symbols should be provided, and this was done. Our symbolization differs in some respects from the barley model, but does conform to the International Rules. A suggestion that was not acted upon was to organize the tabulation into one table and the literature lists into one bibliography. This would have meant better organization but would have further delayed publication; besides, the presentation by countries seems useful despite some duplication.

Dr. C. ROY ADAIR arranged to have the report printed in the U.S. Department of Agriculture, Agriculture Research Service Report Series (ARS 34–28)to bring about publication as promptly as possible. All literature citations were checked in the U.S. Department of Agriculture Library.

In making up the list of symbols the committee largely disregarded precedent and priority. The aim was to make each symbol brief and distinct and to provide a pattern for future use conforming with the International Rules. The committee did not aspire to set up a complete or final list of symbols, nor were the symbols listed for complex characters intended as complete genetic formulae. Symbolization was left to future workers where no symbols were given by the author. For certain complementary genes such as some of those for color, and polymeric genes such as those for dwarfing, information was lacking to detail the subscripts that should be used. Cross references were inserted where there might be confusion between characters, duplication, or possibly different names for characters having similar or the same sort of effects (for example, *Ef, Lf, Se*). The committee suggested that until the new symbolization becomes established it would be helpful if workers would indicate in their publications the previously used symbols.

MONITOR NEEDED

Sa far as I am aware the only publications to date in which the new symbols were employed is a linkage report and one other publication by Dr. NAGAO and Dr. TAKAHASHI. It is obvious from other recent publications that so far there is no strong trend toward uniformity of symbolization.

Is the promulgation of the IRC-recommended symbolization sufficient in itself to assure the attainment of essential uniformity? Dr. WARREN H. LEONARD has emphasized

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that the effort may be wasted unless some central referee is established. He wrote Dr. ADAIR as follows: "The paper on 'Rice Gene Symbolism and Linkage Groups' (ARS 34–28) is a heroic effort to bring order out of chaos with respect to gene symbols for rice. International standardization of symbols has been long overdue. However, I would caution that the Chairman of the International Committee, or some person appointed by the Committee, should have the responsibility for the assignment of gene symbols for new genetic factors in the future. Otherwise, chaos will continue as additional research is conducted on rice genetics beyond the scope of this report." Let me cite an example from the experience of geneticists working with another crop (tomatoes) in their effort to "minimize confusion and to improve gene naming and symbolization." In view of the report of the International Committee on Genetic Nomenclature the group recently published some clarifying rules, one of which is especially revealing; "Much confusion will be avoided by clearing names and symbols with the chairman of either the Gene List Committee or Coordinating Committee of the Tomato Genetics Cooperative. Such action will assist the investigator by assigning a reasonably permanent symbol to his mutant" (CLAYBERG *et al.,* 1960).

The need for a monitor to advise and assist in the assignment of gene symbols in particular studies was recognized by the IRC Working Party at the ninth meeting in 1961. The concensus was that new gene symbols should be registered with IRC Headquarters and published in the Newsletter and that The International Rice Research Institute should be delegated to serve as advisor-referee. I was asked to serve temporarily until the Institute becomes sufficiently organized, but to date I have not been called upon in this capacity. Perhaps the Newsletter should publish periodically summary tabulations of recent investigations, which would be one way of calling repeated attention to the current degree of conformity or lack of it. The help of all those interested in the advancement of rice genetics is needed in placing copies of the Gene Symbolization report in all centers where rice studies are being conducted and in various other ways, making their colleagues aware of the need for uniformity.

THE INTERNATIONAL RICE RESEARCH INSTITUTE AS AN INFORMATION AND COMMUNICATION CENTER FOR RICE GENETICS, CYTOGENETICS AND BREEDING

TE-TZU CHANG

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Speaking in behalf of The International Rice Research Institute, we were pleased to receive from the International Rice Commission the responsibility of implementing the IRC-recommended gene symbols and rules of nomenclature. We also note with pleasure that the IRC has suggested the assistance of Mr. NELSON E. JODON of the United States Department of Agriculture in the capacity of an adviser. With the effort already made, our role will be less arduous.

To make the standardized system truly workable, cooperation from all parties concerned is indispensable. The IRC-recommended gene symbols comprise a basic set to which new symbols can be added and which can be revised when needed. The time which has elapsed between the publication of the IRC recommendations and the present should indicate the response of the rice geneticists to the system and the workability of the system itself. We have felt that this Symposium is an appropriate occasion to study the best means of implementing the system, especially as we have among our participants Dr. RAMIAH, Mr. JODON, Dr. PARTHASARATHY, Dr. TAKAHASHI, and Dr. SEETHARAMAN, all of whom have contributed much to the formulation of the present system.

Dr. KIHARA, Dr. PARTHASARATHY and Mr. JODON have pointed out that there is need to monitor new gene symbols, revisions in the rules of nomenclature and certain symbols, and a medium (the IRC Newsletter or another) to disseminate genetic information to workers. I trust that others present will comment on the subject and will propose to include the subject of genomic symbols, too. On a trial basis, I propose that, with Mr. JODON'S concurrence and cooperation, Mr. JODON and I study all the points covered in this discussion and prepare a revised list of gene symbols, including new ones which have been reported since the publication of the IRC recommendations in 1959 and which are concordant with or amenable to the IRC's rules of nomenclature. The above information will be incorporated with the rules of nomenclature in the form of an article to be carried in the IRC Newsletter and in other accessible channels. Standardized genome symbols also may be included in this paper, if agreement on this subject is reached in this Symposium. For additional genome and gene symbols appearing in publications subsequent to the above, I propose that an annual listing be included in an international rice genetics newsletter.

In my contacts with rice workers of various countries and from my recent survey of published information on rice genetics and cytogenetics, I have noticed that the

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information appearing in various publications is often not accessible to all. other workers in the same field. Some of the known barriers in communication are language differences, the scattered distribution of papers in a large number of periodicals, and the time lapse between the submission of a manuscript and its appearance in published form. Other barriers prevent scientists from subscribing to periodicals from a politically hostile country or to conduct person-to-person communication with individuals in certain other countries. We are fortunate to have the IRC Newsletter which aids in the dissemination of scientific information on a wide and international basis. However, for rice genetics, cytogenetics and breeding, all of which involve a large number of workers who frequently publish experimental data, there appears to be ample room for an additional literary channel.

To provide an outlet for wide and rapid dissemination of information on current research activities and experimental material available for exchange, announcements of new gene symbols and genome symbols, and an annual listing of publications in breeding, genetics and cytogenetics, I propose the initiation of an international rice genetics newsletter, fashioned after the newsletters of maize, wheat, barley, oats and tomato, to be published annually in English. The newsletter will include the following categories of information:

(1) Brief and concise reports of current research activities about fundamental studies on breeding methods and materials (excluding descriptions on the routine development of varieties), genetics, cytogenetics and taxonomy, to be submitted by contributors on an institutional basis with the names of workers concerned mentioned at the end of each research project. The information included will be presented only as preliminary notes and will be distinct from a conventional publication and should not be cited in publications without the written consent of the authors concerned.

(2) Announcements of new gene and genome symbols reported during the past year, with IRRI acting as monitoring agency.

(3) Announcements of *Oryza* species, mutants, genetic testers and stocks available for exchange – IRRI already has a collection of 990 genetic stocks (species, mutants and testers) and 7,545 varietal stocks.

(4) Reports of technical committees on cooperative projects, e.g. linkage studies and genome analysis.

(5) A listing of newly released improved varieties with brief notes on the pedigree and agronomic traits of special significance.

(6) A listing of publications in the above fields which have appeared during the past year.

With the encouragement, cooperation and support of workers in genetics, cytogenetics, breeding, and taxonomy, the first issue of the international rice genetics newsletter can be published before the end of 1963. Mimeographed copies will be distributed to all agricultural institutions concerned and to individual scientists who are active contributors.

To make the above proposal a reality, spontaneous cooperation and contribution

in terms of both information and funds from all parties concerned are indispensable. I shall appreciate receiving comments and suggestions from you about implementing this proposal.

This also appears to be an appropriate occasion to report that a comprehensive bibliography on world rice literature for the period of 1951–60 will be made available by IRRI within a few months. Additional bibliographies will be compiled for periods preceding and following this period. Every paper listed in the 1951–60 bibliography will be copied on microfilm and filed in the IRRI library. For interested scientists, either Thermofax copies from the microfilm or Copyrapid duplicates from originals will be furnished at cost upon request. Our Library will perform similar services for papers not present in our own collection but available elsewhere. In providing such library services and assistance in the publication of a genetic newsletter, we hope that IRRI will serve rice workers of the world as an international center for information on rice genetics and cytogenetics.

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DISCUSSION IN SESSION ON GENE SYMBOLIZATION AND NOMENCLATURE

The question of using the plus sign (+) in designating certain alleles was raised. The gist of the discussion is as follows:

(1) In the IRC-recommended rules, the use of + is allowed to designate a standard or wild type allele when used either as a superscript to the gene symbol or with the gene symbol as a superscript. In formulae, the + alone may be used, especially in linkage groups. The usage of the sign + is, therefore, a matter of personal preference.

(2) In the case of rice genetics, the use of the + sign will not always imply that the character represented is dominant or of the wild type.

The use of the symbol *wx* also was raised. It was explained that the term "waxy" was adopted after usage in maize genetics and indicates the opaque appearance of the endosperm. On the other hand, the term "glutinous" was used to describe its cooking behavior. Neither one of the two terms is correct from a biochemical standpoint. In conformity with the IRC recommendation, the use of *wx* is urged for the time being.

Discussion was continued on the use of terms related to certain plants parts, e.g. node and growth habit. Differences in terminology pointed to the need for standardizing and unifying terms, preferably in a booklet form which will describe various plant parts and mutant traits. The conferees urged The International Rice Research Institute to undertake this assignment.

Ensuing discussion was concerned with the problem of implementing the IRC-recommended gene symbols and rules of nomenclature. All conferees present favored a uniformity in gene symbolization. The following points were agreed upon:

(1) Conformity with the IRC recommendation by rice workers is urged. There already is some indication that the IRC-recommended system is gaining acceptance among workers.

(2) Greater publicity for the above system should be given to insure broader acceptance.

(3) The IRC-recommended rules of nomenclature and gene symbols, thought subject to revision, will not be modified for the time being. Time and usage will prove its usefulness or inadequacy.

(4) The International Rice Research Institute will assume the responsibility of monitoring gene symbols. Rice workers who are planning to publish information involving new gene symbols or new alleles are urged to communicate with the geneticist of the Institute for verification on such points. This will facilitate the assigning of new symbols or allelic designations. Meanwhile, the various gene symbols which have appeared in publications since 1959 will be reviewed and redesignated with the appropriate symbols in conformity with the IRC recommendations. Periodic compilations of monitored symbols appearing in current literature also will be made. The geneticist of the Institute will make these compilations in consultation with NELSON E. JODON, and also with M. TAKAHASHI and R. SEETHARAMAN, if needed. The above reports will be carried in the Newsletter of the IRC and other available literary channels.

SESSION III

CHROMOSOME MORPHOLOGY IN *ORYZA* SPECIES

FURTHER STUDIES ON THE CHROMOSOME MORPHOLOGY OF *ORYZA SATIVA* L.

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Since KUWADA'S (1910) first investigation, many workers have studied the chromosomes of *Oryza sativa.* But the karyotype is not yet well defined. In recent years the author studied chromosome morphology and secondary association of meiotic chromosomes. Lately, he found that different species of *Oryza* show almost the same pattern of secondary association (Hu, 1962). The question is whether morphologically similar chromosomes exist in the haploid set.

The following information may be considered :

(1) The same chromosome number, n = 12, is found in different varieties of *Oryza sativa,* as well as in other related species.

(2) Varieties of *Oryza sativa* can be divided into two or three groups, viz., subspecies *indica* and *japonica* (KATO, 1930), A, B and C types (MATSUO, 1952), and Continental and Insular groups (OKA, 1953d). The F_1 hybrids between groups show partial sterility, but normal pairing of chromosomes.

(3) NANDI (1936) reported that the length of somatic chromosomes ranged from 0.7 to 2.8 microns, all having median constrictions. Later, RAMANUJAM (1938), MORINAGA (1939), YASUI (1941), Hu (1958), ISHII AND MITSUKURI (1960) made more detailed observations. However, it is difficult to obtain distinct figures of chromosome configurations because of their small size.

(4) Pachytene chromosomes may be suitable for detailed observation. In *Oryza sativa,* however, they do not always stain well, and the position of the centromere cannot clearly be distinguished (YAO *et al.,* 1958; SHASTRY *et al.,* 1960). The mitosis of pollen nuclei as attempted by BHADURI *et al,* (1958), may also be a good stage for observation, but a better technique is required for examining the morphology of chromosomes at this stage.

To solve these difficulties, the writer chose haploid plants as his experimental material, since in haploids each chromosome is represented once and relatively distinct figures can be obtained. He found that seven to eight different types of chromosomes could be distinguished in a set, and that *Oryza sativa* and *Oryza glaberrima* showed almost the same karyotype (Hu, 1958, 1960b). Comparing the data from haploid plants with those from diploids, he further suggested that the various species of *Oryza* have similar karyotypes (Hu, 1961). He found that somatic prometaphase is suitable for observation. According to recent reports by SHASTRY and his co-workers (1960),

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the positions of the centromeres at pachytene do not correspond to those observed by the writer in somatic chromosomes. Therefore, he continued his investigations of somatic chromosomes at late prophase to metaphase in a haploid plant recently obtained from a variety of *Oryza sativa*, Taichung 65.

MATERIALS AND METHODS

For cytological study, the root tips of a spontaneous haploid plant of Taichung 65 found in the writer's experimental field were fixed by the low-temperature method (4°–5° C, 20–24h) as described by Hu (1958). Root tips collected at 3–4 p.m. were put in distilled water in fixation tubes, and kept in a refrigerator. After 20 to 24 h, they were hydrolyzed at 60° C in 1 *N* HCl for 7 to 8 min, stained with 1% aceto-orcein, squashed and examined. By this technique, well-stretched configurations of chromosomes could be obtained.

RESULTS OF OBSERVATIONS

The somatic chromosomes of *sativa* generally stain well with aceto-orcein, except for the constrictions. In late prophase or early metaphase, several large chromosomes have constrictions at the position of the centromere and are also darkly stained at other segments near the centromere. However, small chromosomes were deeply stained throughout. The lengths of the chromosomes and long arm-short arm ratios measured at this stage are given in Table 1.

TABLE 1

LENGTHS OF INDIVIDUAL CHROMOSOMES (in μ)* AND THE PERCENTAGE OF LONG-ARM LENGTH IN 21 CELLS

	Chromosome No. (Hu's system)														
Cell	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	Total	Average	S.E.
1	11.0	7.8	7.2	6.9	6.3	4.2	6.7	3.9	5.7	3.6	3.5	3.3	70.1	5.84	2.29
	59.0	56.5	53.6	50.5	62.2	67.4	65.9	78.2	65.1	60.8	52.8	66.7			
2	7.2	5.8	5.9	5.1	4.6	4.5	4.5	2.6	5.2	4.2	3.6	3.0	56.2	4.68	1.28
	62.0	68.2	54.3	56.2	69.5	66.6	64.5	71.2	71.5	61.5	66.2	64.0			
3	5.3	5.0	4.5	4.1	3.5	3.5	3.4	3.1	3.9	3.1	3.2	2.5	45.1	3.76	0.83
	57.2	60.0	55.8	53.2	61.2	64.8	59.0	–	71.7	61.0	51.2	64.0			
4	4.2	3.6	3.5	3.0	3.6	3.7	3.1	2.3	3.0	3.3	3.2	2.3	38.8	3.23	0.55
	64.6	56.0	57.0	53.3	64.2	57.7	64.7	86.5	78.0	54.5	54.0	73.0			
5	4.1	3.5	3.5	3.0	3.3	3.0	3.0	2.6	3.4	2.9	2.9	1.8	37.0	3.08	0.57
	68.8	76.8	59.6	51.4	61.2	60.7	65.0	74.1	73.3	58.6	50.2	62.6			

* The length of satellites is not included in the chromosome length.

Table 1. (Continued – 1)

Cell	*Chromosome No.* (Hu's system) 1	2	3	4	5	6	7	8	9	10	11	12	Total	Average	S.E.
6	4.7	4.3	3.0	3.0	3.0	3.0	3.0	2.3	2.9	2.4	2.4	1.8	35.8	2.98	0.81
	60.0	68.8	55.2	55.8	58.8	67.8	72.2	82.2	78.3	63.5	62.5	66.7			
7	4.1	3.4	3.1	2.5	3.1	2.8	3.0	2.2	2.7	2.2	2.3	2.1	33.5	2.79	0.59
	63.3	58.5	54.5	51.0	56.9	56.9	74.7	72.3	73.2	61.4	59.5	65.0			
8	3.7	3.4	3.1	3.4	2.9	2.3	2.0	1.6	2.6	1.8	2.1	1.7	30.6	2.55	0.74
	63.8	67.3	54.3	50.3	58.3	62.2	67.8	72.4	75.5	61.0	58.6	70.2			
9	4.2	2.8	3.0	2.0	2.6	2.7	2.2	2.1	2.5	2.2	2.0	1.8	30.1	2.51	0.65
	66.3	70.3	56.7	51.3	72.0	70.0	62.8	65.5	68.0	65.3	50.0	61.1			
10	4.4	2.6	2.7	2.2	2.3	2.4	1.8	1.7	2.8	2.1	2.3	1.8	29.1	2.43	0.72
	67.0	57.6	55.5	51.2	66.5	66.8	51.6	72.2	64.2	56.2	55.7	69.6			
12	4.2	3.5	2.7	2.3	2.7	2.2	1.9	2.0	2.7	1.7	1.7	1.2	28.8	2.40	0.83
	65.5	61.5	56.2	52.6	64.5	65.6	57.2	75.0	72.7	63.2	56.0	79.2			
12	3.4	3.3	2.9	2.4	2.3	2.3	2.5	1.6	2.5	2.0	2.0	1.5	28.7	2.39	0.59
	56.8	58.5	52.6	50.0	65.2	63.8	50.5	–	67.5	62.5	55.0	56.8			
13	3.8	3.6	2.8	2.2	2.4	2.1	2.4	1.9	2.0	1.7	2.0	1.5	28.4	2.37	0.71
	64.9	61.0	51.8	52.3	61.3	69.1	58.2	–	75.2	63.1	60.0	–			
14	4.0	3.5	2.9	2.7	2.0	2.0	2.1	1.5	2.1	1.8	2.0	1.5	28.1	2.34	0.78
	62.5	52.5	53.0	52.8	67.3	63.7	55.9	–	71.7	71.6	51.6	–			
15	3.7	3.1	3.1	2.2	2.5	2.2	2.3	1.5	2.2	2.0	2.0	1.3	28.1	2.34	0.68
	72.0	63.2	55.6	53.0	61.7	62.8	66.7	84.5	53.0	54.5	53.0	68.0			
16	3.4	3.7	2.6	2.7	2.3	2.4	2.1	1.4	1.6	1.6	1.6	1.5	26.9	2.24	0.76
	59.8	61.2	54.8	54.6	65.5	62.4	65.9	–	74.0	56.2	56.2	71.7			
17	3.5	3.0	2.4	2.0	2.3	2.2	1.8	1.9	2.4	1.9	1.9	15	26.8	2.23	0.55
	65.0	65.1	63.8	52.4	76.4	58.0	50.5	72.3	80.0	77.5	55.0	66.3			
18	2.8	3.2	2.1	2.5	2.2	2.0	1.9	1.9	2.2	1.8	1.9	1.2	26.3	2.19	0.54
	64.3	72.3	65.6	50.0	65.6	64.5	61.6	63.5	56.8	59.2	56.6	61.2			
19	3.3	2.9	2.5	2.3	2.4	2.1	2.2	1.3	2.1	2.0	1.6	1.5	26.2	2.18	0.57
	70.0	56.9	54.4	50.5	62.0	55.3	80.5	79.0	82.8	64.0	–	83.5			
20	2.9	2.7	2.3	2.1	2.0	1.7	1.5	1.5	1.8	1.8	1.8	1.5	23.5	1.97	0.46
	58.5	55.7	61.2	50.5	54.4	55.9	66.7	–	71.7	61.2	56.4	66.7			
21	2.8	2.6	2.2	2.0	1.8	1.6	1.3	1.2	2.0	1.3	1.2	1.2	21.2	1.77	0.56
	56.8	63.7	54.9	53.7	51.4	56.4	57.7	70.5	67.8	54.0	53.0	62.2			
Total length	90.1	77.3	68.6	60.6	60.1	54.9	54.7	42.1	58.3	47.4	47.2	37.5	699.4		
Average	4.32	3.68	3.27	2.89	2.86	2.61	2.61	2.00	2.78	2.26	2.25	1.79		2.77	
S.E.	1.82	1.22	1.19	1.19	1.03	0.79	1.19	0.65	1.04	0.74	0.66	0.57			
% of ong-arm	68.0	60.1	56.7	52.2	63.1	67.6	67.6	74.8	71.1	61.5	55.7	67.8			

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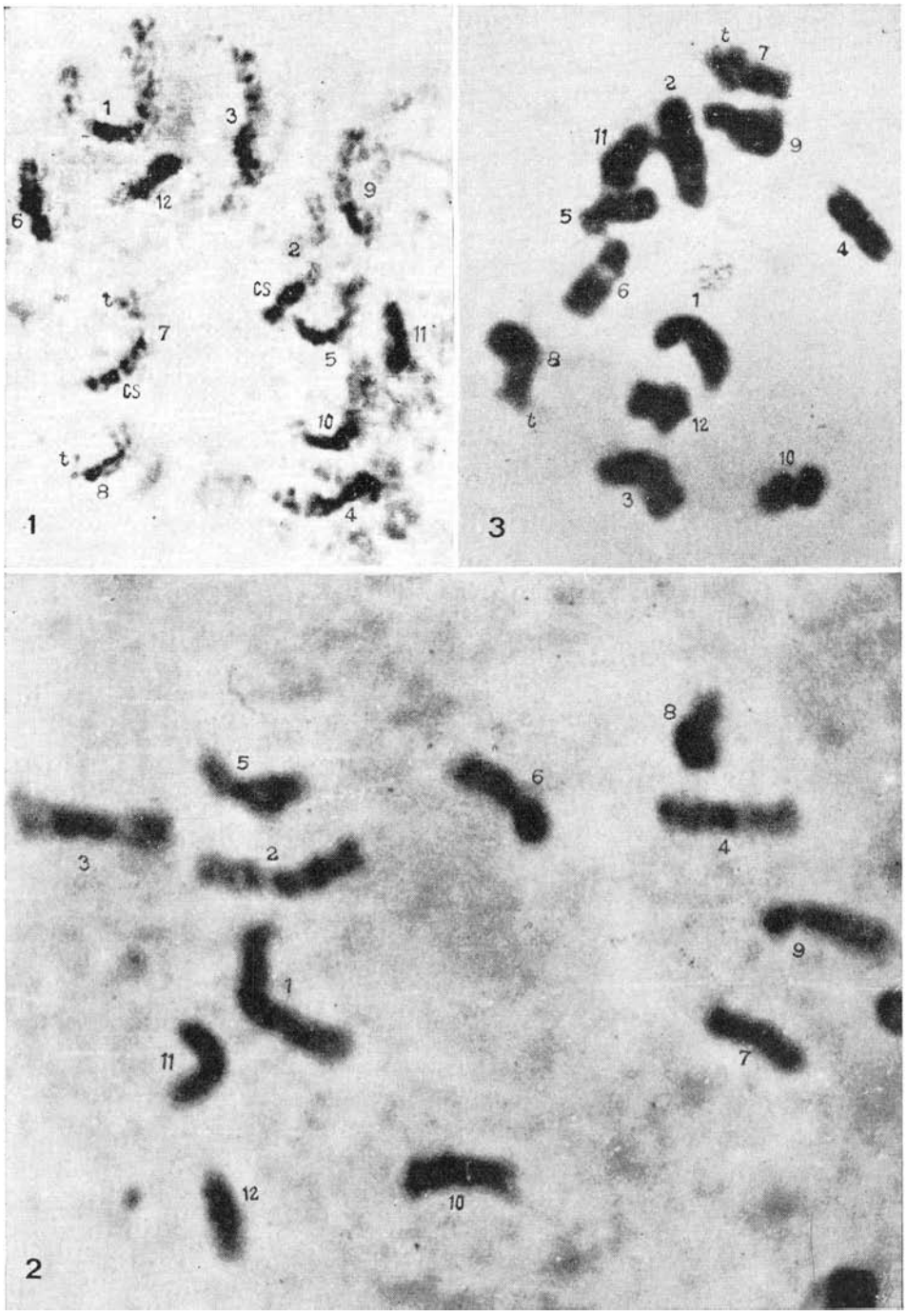
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3
t
7
2
11
9
5
4
6
1
8
t
12
10
3
2
5
6
8
4
3
9
1
7
11
12
10

The 12 chromosomes are arranged according to the number previously assigned by the writer, in the order of their length (Hu, 1958, 1960 b). The 21 cells observed also are arranged in the order of total chromosome length. The variation in chromosome length among cells might be due to contraction of chromosomes which varies with the mitotic stage. Each of the 12 chromosomes is characterized as follows:

Chromosome 1. The longest chromosome. Sub-median. In late prophase, the distal end of the long arm was slightly stained in some cells, but the central part of the chromosome near the constriction was darker stained: this region might be heterochromatic.

Chromosome 2. Submedian. A prominent secondary constriction appears in the middle of the long arm. At prometaphase, the long and short arms appear to be of nearly the same length, but at metaphase the primary and secondary constrictions in the long arm divide the chromosome into three equal parts. Usually, the middle part is more broadly stained than the other two. From observation of metaphase figures, the writer previously considered this chromosome to be subterminal.

Chromosome 3. Median. In the central region there is frequently a heterochromatic segment extending over one-third of the total length. At the end of the long arm there was found a satellite-like granule in some cells, though it disappeared at metaphase.

Chromosome 4. Median. Slightly shorter than chromosome 3.

Chromosomes 5 and 6. Submedian. It is difficult to distinguish between them.

Chromosome 7. Submedian. The writer previously considered this chromosome to be subterminal. This chromosome has one secondary constriction and a satellite.

Chromosomes 8. Subterminal. It has a prominent satellite.

Chromosomes 9. Subterminal. Previously considered to be submedian. It is difficult to distinguish it from chromosomes 5 and 6 in metaphase figures, but at prometaphase chromosome 9 can be identified by the two arm lengths and a darkly stained short arm.

Chromosomes 10 and 11. Submedian to median. They are small and darkly stained even in late prophase and prometaphase. One of them appears to be median at metaphase in the form of a V-shaped chromosome.

Chromosome 12. The smallest chromosome. Submedian. The chromosome is so small that it is often difficult to find the position of the centromere.

EXPLANATION OF MICRO-PHOTOGRAPHS

Somatic chromosomes in root tip cells of a haploid plant. Numbers 1–12 are the writer's previous numbering. The symbol "t" indicates satellite and "cs" denotes secondary constriction.

Fig. 1 (Cell 1 in Table 1) Late prophase (× ca. 3000). *Chromosomes 1* is submedian, heterochromatic at the constriction region. *Chromosome 2* has a submedian constriction. The long arm of this chromosome is darkly stained and a secondary constriction is shown. *Chromosomes 3 and 4* are median. *Chromosomes 5 and 6* are submedian. *Chromosomes 7 and 8* have a satellite. *Chromosome 9* appears to be subterminal, and the short arm darkly stained. *Chromosomes 10 and 11* are submedian or median. The whole body of *chromosomes 11* is darkly stained, and a terminal knob is seen. Chromosome 12 is submedian.

Fig. 2. (Cell 3 in Table 1) Prometaphase (× ca. 6000). The above-mentioned features are all recognized in this figure, except for *chromosomes 8* which is not lined on the same plane as others.

Fig. 3. (Cell 12 in Table 1) Metaphase (× ca. 6000). Each chromosome can be recognized by its length and constriction. The two SAT-chromosomes of nos. *7* and *8* are distinct.

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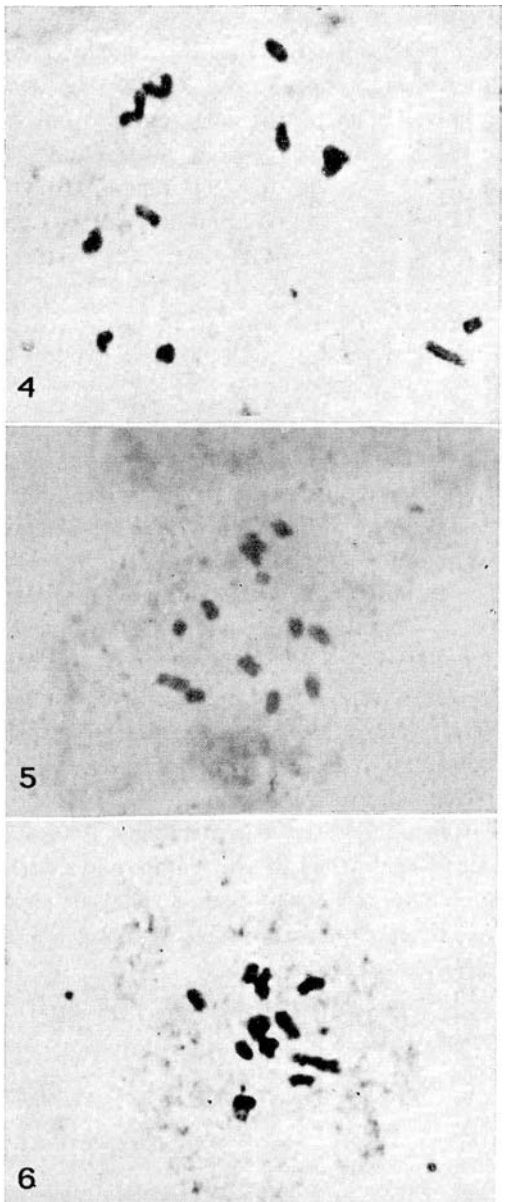

Figs. 4–6. Chromosomes are in somatic pairing. (× ca. 2000).

Fig. 4. A pattern of four groups of two (a large chromosome and a small one form a pair, two V-shaped chromosomes form another pair), and one group of three (of which two are overlapped).

Fig. 5. Another pattern of somatic pairing.

Fig. 6. Two groups of a large one and a small one form a pair.

It may be stated that the haploid plant of Taichung 65 has a karyotype similar to that found in other strains of *sativa* and *glaberrima.* This agrees with the writer's previous observation in metaphase cells. Photomicrographs of cells at late prophase to metaphase are shown in Figs. 1–4. The above-described characteristics of chromosomes may be partly recognized in these figures.

Regarding the variation in chromosome length, it was found that if the chromosomes are arranged in the order of average length based on the 21 cells listed in Table 1, the order will be *1, 2, 3, 4, 5, 9, 6, 7, 10, 11, 8, 12.* Variance analysis of the lengths of individual chromosomes showed that, as given in Table 2, both the differences between chromosomes and between cells were highly significant. By using the "Studentized multiple range tests," the 12 chromosomes were divisible into six classes of different lengths, i.e., *1, 2, 3, 4–5–9–6–7, 10–11–8* and *12* (Table 5A). As to the variation among cells, the data in Table 1 show that cells numbered 1, 2 and 3 in late prophase had greater chromosome lengths than other cells. The nearer the stage to metaphase, the less the total chromosome length. However, it seems that the rate of shortening may differ according to individual chromosomes. Small chromosomes seem to have a lower contraction rate.

TABLE 2

ANALYSIS OF VARIANCE OF THE LENGTH OF INDIVIDUAL CHROMOSOMES

Source of variation	d.f.	Mean squares	F value
Chromosomes	11	10.76	46.99**
Cells	20	11.02	
Error	220	0.22	

** Significant at 1% point.

Using the data in Table 1, variance analysis of the percentages of long-arm lengths (transformed into angular values) was made. The results are given in Table 3.

TABLE 3

ANALYSIS OF VARIANCE OF THE PERCENTAGES OF LONG-ARM LENGTH (in angular values) OF INDIVIDUAL CHROMOSOMES IN 14 CELLS

Sourceof variation	d.f.	Mean squares	F value
Chromosomes	11	187.85	16.54**
Cells	13	16.49	
Error	143	11.36	

** Significant at the 1% point.

By using the "Studentized multiple range test" in the same manner as for the lengths of individual chromosomes, the 12 chromosomes were found to be divisible into three classes, i.e., *4–11–3* (median), *10–7–6–1–2–12* (submedian), and *9–8* (subterminal)

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(Table 5B). Combining this classification with that by chromosome lengths, 10 different types could be distinguished as shown in Table 4. SAT-chromosomes can be distin-

TABLE 4

CLASSIFICATION OF SOMATIC CHROMOSOMES BY THE LENGTH AND ARM RATIO

% of long-arm	Order of length (1)	(2)	(3)	(4–5–9-6–7)	(10-11-8)	(12)
Median			*3*	*4*	*11*	
Submedian	*1*	*2*+		*5, 6, 7**+	*10*	*I2*
Subterminal				*9*	*8**	

* Satellite, + Secondary constriction.

guished from others further. It may then be concluded that all the 12 chromosomes, except for numbers *5* and *6,* can be morphologically distinguished from one another, if a sufficient number of cells are observed in a haploid plant.

TABLE 5

MULTIPLE RANGE TEST FOR CHROMOSOME LENGTH AND ARM RATIO OF CHROMOSOMES IN ROOT-TIP CELLS

A: 1% significance of chromosome length (μ)

Chromosomes	*1*	*2*	*3*	*4*	*5*	*9*	*6*	*7*	*10*	*11*	*8*	*12*
Mean	4.32	3.68	3.27	2.89	2.86	2.78	2.61	2.61	2.26	2.25	2.00	1.79

B: 1% significance of long-arm ratios (in %)

Chromosomes	*4*	*11*	*3*	*10*	*7*	*6*	*5*	*1*	*2*	*12*	*9*	*8*
% of long-arm	52.4	55.9	56.9	60.8	63.1	63.1	63.5	64.2	64.7	66.8	69.9	74.3

In a single cell, since the distinctions between chromosomes *10* and *11,* between chromosomes *5–6* and *9,* and the satellite of chromosome *7,* are often difficult, it may be said that, taking the presence or absence of satellites and secondary constrictions into consideration, nine types are distinguishable.

The writer has previously pointed out that the chromosomes of haploid rice tend to pair somatically (Hu, 1958). This also was found in the present material: 20 out of about 100 metaphase cells showed one to four pairs of chromosomes. In some, a large chromosome and a small one were found to lie near each other, as shown in Figs. 4–6. In two cells a group of three and four groups of two chromosomes were observed, and in another cell two groups of three and three groups of two.

DISCUSSION

To describe the karyomorphology of the haploid set of chromosomes, we must determine length, position of constrictions, presence or absence of satellites, and other characteristics. Previous findings for *Oryza sativa* are compared with the results of the present study.

Regarding the lengths of chromosomes, RAU (1929) and SETHI (1937) reported that among the 12 pairs of the diploid set, five were large, four medium and the remaining three small, the large ones being about twice as long as the small ones. Nandi (1936) assumed that the 12 chromosomes could be divided into five groups, three groups consisting of two chromosomes and other two groups of three chromosomes of equal length. YASUI (1941) found that the relative length of the chromosomes in root tip cells of haploid plants varied in ratio from about 5.0. to 2.0. The writer (1958) indicated that the lengths of metaphasic chromosomes in haploid plants were 1 to 3 microns in both *sativa* and *glaberrima*, and that the two species might have the same karyotype (Hu, 1960b). ISHII AND MITSUKURI (1960) considered that haploids should have larger chromosomes than diploids, although they did not explain the variation among cells of the same plant and the difference in karyotypes between haploid and diploid plants of the same variety. In the present study, the variances of chromosome length and the percentage of long-arm length were analyzed; the results proved that the variations due to chromosomes, as well as those among cells, were highly significant, and that the 12 chromosomes of the haploid set could be divided into six classes of different lengths and three classes of different arm ratios.

In addition to these investigations of somatic chromosomes, SHASTRY *et al.* (1960) observed the chromosomes at pachytene stage, and reported that there were one large, three medium, and eight small chromosomes. Mr. H. K. Wu of the Institute of Botany, Academia Sinica, Taiwan, engaged in the same line of work, showed the writer some figures of pachytene chromosomes. According to his observations, the relative lengths of chromosomes are similar to those found from metaphase cells by the writer. The data obtained by the above five workers are summarized in Table 6. It may be concluded that, except SHASTRY and his co-workers' data, the longest chromosome is 12–13% of the total length of chromosomal material, while the shortest is 4–5%, and the other chromosome lengths vary between the two extremes.

Regarding the position of centromere and constrictions, NANDI (1936) reported that all somatic chromosomes were V-shaped, or median types. However, PATHAK (1940), YASUI (1941), Hu (1958) and ISHII AND MITSUKURI (1960) consistently re-

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cognized that three to four chromosomes are of the median type, while the remaining ones have submedian or subterminal constrictions. SHASTRY *et al.* (1960) observed that only two chromosomes could be regarded as median. The arm ratios of the so called median chromosomes, however, differed according to workers, as shown in Table 6. In addition, the writer (HU, 1958) and ISHII AND MITSUKURI (1960) recognized secondary constrictions in a large chromosome (no. 2) and in another of medium size (no. 7).

Regarding the presence or absence of satellites, NANDI (1936) reported that there were two pairs of medium size which had a terminal knob and were attached to a nucleolus. YASUI (1941) showed that only a small chromosome had a satellite, while several chromosomes were linked to the nucleolus at prometaphase. The writer (1958) showed that there is a SAT-chromosome with a subterminal constriction (no. 8) and a less prominent SAT-chromosome (no. 7). In contrast, ISHII AND MITSUKURI (1960) reported that the SAT-chromosome was relatively large (no. 4 in their order). SHASTRY *et al.* (1960) observed that chromosomes 3 and 4 were attached to the nucleoli in pachy-

TABLE 6

COMPARISON OF THE DATA OBTAINED BY DIFFERENT INVESTIGATORS REGARDING CHROMOSOME LENGTH AND POSITION OF CENTROMERE

Author	Material		Order of chromosome length												Total
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	length
YASUI	R-T	length(ca.)	5.0	4.5	4.1	3.8	3.7	3.5	3.1	3.0	2.9	2.6	2.3	2.0	40.5
(1941)	hapl.	class.	SM	M	M	M	SM	SM	SM	SM	M	ST*	SM	SM	
		% of total	12.2	11.0	10.6	9.4	9.1	8.6	7.6	7.4	7.1	6.4	5.7	4.9	100.0
HU	R-T	length (μ)	2.4	2.2	2.2	1.9	1.7	1.6	1.6	1.6	1.5	1.5	1.4	1.2	20.8
(1958)	hapl.	class.	SM	ST	M	M	SM	SM	ST*	ST*	SM	M	M	SM	
		% of total	11.5	10.6	10.6	9.0	8.2	7.7	7.7	7.7	7.2	7.2	6.7	5.8	100.0
ISHII &	R-T	length (μ)	4.2	3.9	3.4	3.2	3.1	2.8	2.7	2.5	2.4	2.3	2.1	1.9	34.5
MITSUKURI	hapl.	class.	SM	M	SM	ST*	SM	M	ST	SM	SM	SM	M	SM	
(1960)		% of total	12.2	11.3	9.8	9.3	9.1	8.1	7.8	7.2	7.0	6.7	6.1	5.5	100.0
idem	R-T	length (μ)	3.5	3.2	2.9	2.6	2.4	2.4	2.3	2.2	2.0	2.0	1.9	1.6	29.0
	dipl.	class.	SM	SM	SM	ST	SM*	SM	SM	SM	SM	SM	SM	M	
		% of total	12.0	11.0	10.0	8.9	8.3	8.3	7.9	7.6	6.9	6.9	6.6	5.5	100.0
HU	R-T	length (μ)	4.3	3.7	3.3	2.9	2.9	2.8	2.6	2.6	2.3	2.3	2.0	1.8	33.5
	hapl.	class.	SM	SM	M	M	SM	ST	SM	SM*	SM	M	ST*	SM	
		% of total	13.0	11.1	9.8	8.7	8.6	8.3	7.8	7.8	6.8	6.7	6.0	5.4	100.0
SHASTRY	PMC	length (μ)	79.0	47.5	47.0	38.5	30.5	27.5	26.5	23.0	21.0	21.0	20.5	18.0	400.0
et al.	dipl.	class.	SM	SM	M*	SM*	SM	ST	M	SM	SM	ST	SM	SM	
(1960)		% of total	19.7	11.9	11.8	9.6	7.6	6.9	6.6	5.8	5.2	5.2	5.1	4.5	100.0
WU	PMC	length (μ)	49.9	45.8	42.0	33.6	31.6	28.9	27.7	26.0	23.7	21.8	19.4	17.5	367.9
(unpubl.)	dipl.	class.	SM	M	SM	SM	SM	SM	M	ST	SM	SM*	M	M	
		% of total	13.4	12.4	11.4	9.1	8.6	7.9	7.5	7.1	6.5	5.9	5.3	4.8	100.0

* SAT-chromosome.

tene nuclei. Wu, however, showed the writer a clear microphotograph of a pachytene nucleus indicating that the nucleolar chromosome is small (no. 9 or 10, in his order). In the present work, it was found that the second smallest one (chromosome 8) and a medium one (chromosome 7) showed a satellite from late prophase to metaphase, and that chromosome 3 had a terminal knob in prometaphase.

The association of somatic chromosomes in rice was first pointed out by PARTHASARATHY (1938). He also noticed this phenomenon in the figures given by KUWADA (1910), and considered that the maximum number of pairs might be 10. As assumed by PARTHASARATHY, somatic pairing may be compared with secondary association of meiotic chromosomes. They differ in that somatic pairing occurs between homologous chromosomes while secondary association might occur between supposedly homoeologous ones, representing the homology relationships in the ancestral species. In haploid plants, however, these two phenomena can be of the same nature, since each chromosome is represented only once. The types of somatic pairing found by the writer, one group of three and four groups of two chromosomes, and two groups of three and three groups of two chromosomes, are quite consistent with the pattern of secondary association in meiosis. It was further found by the writer that the same pattern of secondary association also occured in other species of *Oryza* (Hu, 1962). The somatic pairing found in haploid plants, which may be regarded as a case of intragenomic pairing, thus seems to support the hypothesis that the basic chromosome number of *Oryza* might be five.

SUMMARY

In a haploid plant derived from a variety of *Oryza sativa,* Taichung 65, the morphology of root tip chromosomes at late prophase to metaphase was investigated. The results obtained largely agreed with the writer's previous findings based on metaphase readings. From variance analysis of the data for the lengths, and the percentages of long-arm lengths of individual chromosomes, it was found that the chromosomes could be divided into six classes of different lengths represented by chromosomes *1, 2, 3, 4–5–6–7–9, 8–10–11* and *12,* and into three classes of different arm ratios, *3–4–11* (median), *1–2–5–6–7–10–12* (submedian), and *9–8* (subterminal) respectively. It was also confirmed that two relatively small chromosomes have a satellite each. These findings were compared with the results reported by previous workers. Furthermore, most chromosomes seem to have heterochromatic segments. Somatic pairing was found at metaphase; the pattern was consistent with that of secondary association of meiotic chromosomes.

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A NEW APPROACH TO THE STUDY OF RICE KARYOMORPHOLOGY

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Species with small chromosomes are difficult to analyze for karyotype from metaphase plates in root tips. It is not surprising, therefore, that most of the critical data on the evolution of karyotypes come from plants with large chromosomes such as *Crepis, Fritillaria, Vicia,* and the members of the *Triticinae.* Studies on pollen mitosis offer the next advance, but even this method is of limited application in cereals for reasons discussed by MORRISON (1953). By far the most fruitful, yet least exploited, method of approach to karyomorphology in species with small chromosomes, is pachytene analysis, The work of RHOADES AND MCCLINTOCK (1935) and LONGLEY (1952) in *Zea,* ERNST (1939) in *Antirrhinum,* HYDE (1953) in *Plantago,* LIMA-DE-FARIA (1952) in *Secale,* GOTTSCHALK AND PETERS (1954) in *Solanum,* BARTON (1950) and MENZEL (1962) in *Lycopersicon,* and SHASTRY, SMITH AND COOPER (1960) in *Melilotus* have extended considerably the scope of cytological analysis and improved our understanding of the underlying processes in the evolution of respective genera. This paper reports recent studies in *Oryza* employing this technique.

YAO, HENDERSON AND JODON (1958) introduced the technique of pachytene analysis in rice cytology to clarify the nature of hybrid sterility in intervarietal hybrids. Later, SHASTRY, RANGA RAO AND MISRA (1960) employed this technique for karyotypic studies. The advantages of this technique are: high degree of attenuation of the chromosomes and the avoidance of matching the homologues.

Species of *Oryza* so far investigated by pachytene analysis are *sativa, glaberrima, stapfii, sativa* var. *fatua, perennis, barthii, breviligulata, australiensis, officinalis,* and *granulata.* Except the last named, which was reported by Hu (1960a), all the species were investigated by my colleagues at the Indian Agricultural Research Institute. The above experience, along with that of some uncompleted studies on *brachyantha* and *subulata,* indicates that all the diploid species of the genus eminently suit this technique which yields satisfactory to excellent preparations for analysis. The tedium of analysis, inadequate clarity of centromeres in comparison to *Zea* (YAO *et al.,* 1958) and the lack of such diagnostic markers as knobs and heterochromatic segments, as in *Zea* and *Plantago,* are not serious limitations, since karyotypic data are more dependable than data obtained from somatic metaphases. It is surprising, therefore, that few investigators use this technique for karyotypic analysis.

The morphology of pachytene bivalents differs significantly among the species of

Oryza. The karyotype of *Oryza australiensis* is seriated by small darkly stained segments alternating with lighter ones and the centromeres in this species are the most distinct of all so far studied (SHASTRY AND MOHAN RAO, 1961). Furthermore, this species exhibits a high degree of heterochomatinization of bivalents in comparison to all others. This accounts for the apparent largeness of the chromosomes of this species in somatic metaphases as reported by HU (1961) and in the *Oryza* hybrid *sativa* × *australiensis* (GOPALAKRISHNAN, 1959; SHASTRY AND RANGA RAO, 1961). In the opinion of the author, the karyotype of *Oryza granulata* (as evident from the photographs of HU, 1960a) shares the features of *australiensis* with reference to centromeres and heterochromaticity, although the cells shown by HU are probably not strictly at the mid-pachytene stage. The karyotypes of *perennis* and *officinalis,* in significant contrast, are not as heterochromatic but are seriated by uniformly dense chromeric patterns (DAS AND SHASTRY, in press; SHASTRY AND RANGA RAO, unpublished). The karyotypes of most of the varieties of *sativa* and *glaberrima* studied so far by us (SHASTRY, RANGA RAO AND MISRA, 1960; MISRA, 1960; SHASTRY AND MOHAN RAO, 1961 ; MISRA, unpublished), exhibit a seriation of several micro- and macro-chromomeres occasionally interrupted by darkly stained segments of limited size. In combination with other criteria, the morphology of pachytene bivalents might serve as a useful criterion to trace the evolution of chromosome structure in the genus *Oryza.*

Karyotypic studies involve length measurements of chromosomes and their arms. Since chromosomes are coiled at all stages of cell division and the degree of coiling is inversely related to the lengths measured, the cell-stage constitutes a major source of error in all measurements of total lengths. The best way to overcome this is to have an independent measurement, such as an estimation of DNA content by microspectrophotometry. Progress in this field is limited by poor-quality preparations with Feulgen-nuclear reaction in rice (BHADHURI, NATARAJAN AND MOHANTHY, 1958). In the absence of such data, statements such as one species "having larger chromosomes" than the other are bound to be abstract. NANDI (1938) and HU (1961) reported that the chromosomes of *Oryza officinalis* are larger than those of *sativa* but in the F_1 hybrid *sativa* × *officinalis* size differences between the univalents were not distinct according to SHARMA (1960), and SHASTRY, SHARMA AND RANGA RAO (1961).

A simple procedure to avoid error in measurement arising from differences in cell-stage was introduced by TJIO AND HAGBERG (1951). It expressed the length of individual chromosomes as percentage of total chromatin length. Few rice workers have employed relative lengths for avoiding error due to cell to cell variation in condensation. Early data on karyotypes by NANDI (1936), SETHI (1937), and RAMANUJAM (1938) are therefore of little value for comparison. The utmost level of perfection in karyotypic studies is the identification of individual chromosomes in the complement. This has so far been possible in rice, with some reservation, only by pachytene analysis (SHASTRY *et al.,* 1960; DAS AND SWASTRY, 1963; SHARMA AND SHASTRY, in press). When the most elementary unit of evolution is neither the genome nor the chromosome, but a chromosomal segment, pachytene analysis for detecting small differences becomes valuable.

Karyotypes may be classified on the basis of their symmetry. It is well established

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by the pioneering investigations of LEVITSZKY (1931), BABCOCK (1947), and MCKELVEY AND SAX (1933) that primitive species are characterized by symmetric karyotypes and the advancedspecies by asymmetric ones. STEBBINS (1958b)furthersuggestedaclassification of karyotypes employing two criteria: variation in lengths between extreme members of the complement and the preponderance of submedian and subtelocentric chromosomes. The classification of STEBBINS (1958b) has been employed by us in karyotypic studies of *Oryza* species. In general, the wild species exhibit more symmetric karyotypes than the cultivated *Oryza sativa* and *glaberrima* (SHASTRY, 1961). Furthermore, the *japonica* varieties appear to be more asymmetric in their karyotypes than the *indica* varieties (MISRA, 1960), although this observation is not certain. Karyotypic data on the species so far investigated are presented in Tables 1–b.

TABLE 1

CLASSIFICATION OF KARYOTYPIC ASYMMETRY IN *Oryza* SPECIES (as per STEBBINS, 1958b)

Ratio of largest/ 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 –
2:14:1	1b *perennis* (Assam) *perennis* var. *barthii*	2b *perennis* var. *balunga* *spontanea* *australiensis* *stapfii* *sativa* (indica)	3b *glaberrima*	4b –
>4:1	1c –	2c –	3c *sativa (japonica)*	4c –

Although pachytene is the most “stable” stage in meiosis and for this reason is being employed for karyotypic studies, considerable variation in condensation occurs in *Oryza* species, even in PMCs at mid-pachytene. For this reason, analysis of incomplete chromosome complements in some PMCs and matching the chromosomes from different PMCs may lead to serious errors in the construction of karyotypes and the identification of bivalents. For this reason, the lengths of individual chromosomes are of little value. If the cell to cell variations in the lengths of bivalents are largely due to “general condensation differences” (i.e., if the condensation factor is homogeneous for different regions of the karyotype), the lengths of the individual chromosomes will bear a direct relationship with the total length of the chromosome complement in the PMCs from which the former measurements are made. If, on the other hand, differential condensation occurs, this relationship would be disturbed. DAS (1961) investigated this relationship in *Oryza perennis* var. *barthii* and reported that not only the correla-

TABLE 2

KARYOTYPIC DATA BASED UPON PACHYTENE ANALYSIS IN *Oryza* Spp.

A. Relative lengths

Chromosome number	*perennis* (Das and Shastry, in press)			*sativa* f. *spontanea* (Gopakumar, 1941)			*brevili gulata* (Sharma & Shastry, in press)	*sativa* (Misra, 1960)		
	balunga	Assam	*barthii*	Orissa	Assam I	Assam II	Andhra	Norin 6	N.P. 130	N. 32
1	14.4	13.8	13.1	13.5	16.0	13.4	12.6	13.7	19.75	13.1	18.5
2	11.6	11.5	11.8	11.6	12.7	11.9	11.0	12.3	11.9	11.2	12.8
3	10.3	9.8	10.4	10.8	11.4	11.2	10.8	11.8	10.5	12.5	12.8
4	8.9	8.7	9.1	9.7	8.8	9.9	9.1	9.1	9.6	10.2	9.9
5	8.2	8.4	8.2	8.5	7.7	8.9	9.7	8.0	7.6	9.3	7.8
6	7.6	7.9	8.0	8.4	7.7	8.0	7.5	7.6	6.9	8.5	7.7
7	7.2	7.7	7.4	7.8	1.3	7.2	6.9	7.6	6.6	7.5	7.7
8	7.1	7.4	7.2	7.0	6.3	7.0	7.5	6.9	5.8	7.5	7.1
9	6.8	6.9	6.7	6.4	6.3	6.6	7.0	6.8	5.3	6.8	6.3
10	6.5	6.3	6.4	5.9	6.4	5.8	6.4	6.2	5.3	6.1	5.8
11	6.2	5.8	6.1	5.9	5.6	5.4	5.8	5.8	5.1	5.3	4.8
12	5.4	5.7	5.5	5.0	4.4	4.6	5.1	5.2	4.5	4.4	3.9
B. Arm ratios											
1	0.60	0.73	0.83	0.50	0.35	0.67	0.77	0.89	0.58	0.48	0.98
2	0.83	0.54	0.64	0.77	0.38	0.83	0.73	0.46	0.46	0.69	0.44
3	0.80	0.84	0.82	0.88	0.56	0.61	0.84	0.81	0.81	0.19	0.44
4	0.47	0.56	0.64	0.78	0.79	0.23	0.55	0.51	0.43	0.34	0.71
5	0.82	0.77	0.86	0.49	0.32	0.80	0.84	0.81	0.49	0.89	0.76
6	0.57	0.62	0.67	0.93	0.77	0.57	0.92	0.63	0.25	0.66	0.55
7	0.89	0.90	0.67	0.27	0.53	0.43	0.24	0.42	0.96	0.69	0.80
8	0.58	0.58	0.84	0.70	0.93	0.67	0.46	0.42	0.59	0.91	0.74
9	0.66	0.82	0.63	0.88	0.67	0.80	0.92	0.62	0.31	1.00	0.85
10	0.79	0.64	0.80	0.84	0.28	0.78	0.57	0.80	0.17	0.64	1.00
11	0.60	0.83	0.68	0.48	0.67	0.88	0.84	0.42	0.64	0.41	0.47
12	0.80	0.61	0.73	0.90	0.53	0.15	0.80	0.75	0.33	0.44	0.64

TABLE 3

CORRELATIONS BETWEEN MEASURED LENGTHS OF INDIVIDUAL CHROMOSOMES AND TOTAL CHROMATIN LENGTH OF THE POLLEN MOTHER CELLS AT PACHYTENE STAGE IN *Oryza perennis* Moench. var. *barthii*

Chromosome number	Number of observations (n)	Correlation* coefficient (r)
1	19	0.8002
2	19	0.8407
3	19	0.6064
4	19	0.8231
5	19	0.8950
6	19	0.8689
7	19	0.9687
8	19	0.9234
9	19	0.8521
10	19	0.8349
11	19	0.8452
12	19	0.8578

* All these correlation coefficients exceed the 1% level of significance, 0.5751 (d.f. = 17). They were also found to be homogeneous by c^2 test.

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tions between the lengths of individual chromosomes and the total length were highly significant but also that they were homogeneous (Table 3). This observation validates the procedure of employing the relative values in the comparison of karyotypes. This analysis is further refined by PERSHAD, SHASTRY AND DAS (unpublished) by testing the homogeneity of c^2 values for condensation in three varieties of *perennis.* This analysis confirmed that the rate of condensation of individual chromosomes was of the same order within as well as among the varieties (Table 4).

TABLE 4

HOMOGENEITY OF CHI-SQUARE TESTS FOR CONDENSATION OF INDIVIDUAL CHROMOSOMES FOR THREE VARIETIES OF *Oryza perennis*

Varieties	1	2	3	4	5	6	7	8	9	10	11	12	Within Varieties df	Within Varieties c^2
barthii														
Observed	37.3	33.8	29.6	26.2	23.4	22.8	21.3	20.6	19.2	18.4	17.5	15.7	227	36.83
Expected	39.3	33.3	29.1	25.6	23.6	22.3	21.2	20.6	19.4	18.3	17.3	15.7		
balunga														
Observed	46.0	37.0	33.0	28.4	26.3	24.3	22.9	22.8	21.6	20.7	19.8	17.0	95	20.46
Expected	44.0	37.2	32.6	28.6	26.4	25.0	23.7	23.1	21.7	20.5	19.3	17.6		
Assam														
Observed	26.2	21.8	18.6	16.7	16.1	15.0	14.8	14.1	13.3	11.9	10.8	11.0	71	5.22
Expected	26.2	21.1	19.4	17.0	15.7	14.9	14.1	13.7	12.9	12.2	11.5	10.4		
	8.83	5.84	10.62	6.75	3.52	3.02	1.81	2.20	3.58	4.91	4.53	6.90		

Calculated c^2 (df = 35) among varieties = .50

In conclusion, it can be said that karyotypic data obtained from the analysis of the complete chromosome complement from even a limited number of PMCs at pachytene would be more valuable and dependable than extensive data collected from metaphasic cells of root tips where difficulties in staining, size measurement and clarity most seriously hinder critical analysis. Furthermore, the pachytene is not only useful for the study of chromosome morphology but also for chromosomal pairing. Hence, structural hybridity is readily identifiable and is easily localized by employing this method of analysis.

SUMMARY

A high degree of attenuation of the chromosome, homologous pairing and juxtaposition of the homologous regions render pachytene analysis an ideal tool for the study of not only chromosome morphology but also of chromosome pairing. Employing this method, smaller differences between the karyotypes, which are not detectable at

metaphase, can be revealed. The following conclusions are made by employing this method in *Oryza* species:

(1) The morphology of bivalents differs between the species. The karyotype of *Oryza australiensis* is highly heterochromatic.

(2) Wild species have more symmetric karyotypes than cultivated species.

(3) The rate of condensation (with the advance of prophase) of individual chromosomes is of the same order both within and among three varieties of *Oryza perennis.*

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DISCUSSION IN SESSION ON CHROMOSOME MORPHOLOGY IN *ORYZA* SPECIES

The discussions were largely concerned with the technique of pachytene analysis and the interpretation of observations made at pachytene from various *Oryza* species and *indica–japonica* hybrids. The conferees agreed that pachytene analysis is potentially promising in establishing karyomorphology in various forms and is also useful in analyzing pairing behavior. Metaphase analysis in haploids and pachytene analysis could well supplement each other. It also was agreed that further refinement in technique is needed to allow the inclusion of the entire chromosome complement in a large number of analyzable cells.

To enhance the usefulness of pachytene analysis, it was agreed that:

(1) A uniform standard of designating the various prophase stages is essential.

(2) The problem of differential condensation during the progress of meiosis needs to be studied.

(3) The relation between condensation and terminalization should be further elucidated.

(4) Aberrant behavior of chromosomes at pachytene should be compared with metaphase behavior.

(5) Larger numbers of strains and more observations per strain are needed to establish valid karyotypes.

It also was pointed out that there are certain intrinsic difficulties with pachytene analysis. For instance, chromosome homology in maize is not necessarily indicated by region-by-region pairing. Chromosomes carrying translocations or inversions are more readily identified when these aberrations are located near the ends of the chromosomes.

SESSION IV

GENETIC AND CYTOGENETIC EVIDENCE FOR SPECIES RELATIONSHIPS

PATTERN OF INTERSPECIFIC RELATIONSHIPS AND EVOLUTIONARY DYNAMICS IN *ORYZA*

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How to define a species in the genus *Oryza* is a most puzzling problem. We may recognize "species" as various taxonomic workers have designated. ROSCHEVICZ (1931) listed 19 species and divided them into the sections *Sativa, Granulata, Coarctata* and *Rhynchoryza,* while CHATTERJEE (1948) enumerated 23 species. More than 60 latin names have been given to different specimens. Recently, TATEOKA (1962a, 1962b, 1963) considers that the genus *Oryza* comprises 22 species which can be divided into six sections, as follows:

Section *Schlechterianae... schlechteri* Pilger
Section *Granulatae... meyeriana* Baill.
Section *Coarctatae... coarctata* Roxb.
Section *Ridleyanae... ridleyi* Hook. and *longiglumis* Jansen
Section *Oryzae... sativa* L., *rufipogon* Griff., *barthii* A. Chev., *glaberrima* Steud., *breviligulata* A. Chev. et Roehr., *australiensis* Domin, *eichingeri* A. Peter, *punctata* Kotschy, *officinalis* Wall., *minuta* Presl, *latifolia* Desv., *alta* Swallen and *grandiglumis* Prod.
Section *Angustifoliae... brachyantha* A. Chev. et Roehr., *angustifolia* Hubbard, *perrieri* A. Camus and *tisseranti* A. Chev.

Dealing with systematic problems, we should further draw a distinction between "taxonomic" and "phylogenetic" viewpoints. From the former the present status of organic variations is investigated, while from the latter the phylogenetic hierarchy of a given group is considered. Although these two lines often overlap, they may be compared with topographic survey and geology, respectively.

Our present knowledge of the genus *Oryza* apparently is insufficient to allow describing either the variation patterns or the phylogeny and evolutionary mechanisms. In this paper, considerations on these matters are presented with the hope of summarizing various findings and pointing out problems to be solved.

METHODS OF SURVEYING INTERSPECIFIC RELATIONSHIPS

Different methods of evaluating interspecific relationships are given below. Some of these methods have been used in *Oryza.*

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(1) Survey of morphological and physiological differences. This has been done by many workers. Biochemical methods also may be used, although they have not been attempted in *Oryza;* for instance, ALSTON AND TURNER (1962) compared paper-chromatographic patterns of leaf extracts of *Baptisia* species.

(2) Comparison of ecological and distributional features. Two given species do or do not occur in the same geographical area. If found in the same area, they may be sympatric or allopatric, and other ecological characteristics can be studied. The distribution areas of various *Oryza* species have been located on the world map by a number of workers (SASAKI, 1929; ROSCHEVICZ, 1931; CHATTERJEE, 1951; TATEOKA, 1962a; etc.), but more detailed information on a smaller scale is lacking. The writer has observed in Thailand that *officinalis* was sympatric with *ridleyi* in a forest, and with *granulata* (= *meyeriana*) in another forest. He also found that *perennis* and *grandiglumis* were sympatric in a stream in the basin of the Amazon river. Such distributional relationships between wild and cultivated species will be mentioned later.

(3) Possibility and frequency of natural hybridization, introgression and establishment of hybrid swarms between two given species. Natural hybridization between *perennis* and *sativa* has been reported in India by ROY (1921), BHALERAO (1928) and others. The breeding systems and population dynamics of the species have important bearings in these respects.

(4) Crossability between two given species. This may differ according to environmental conditions. For instance, to hybridize *australiensis* with *sativa* is difficult in Misima, but it succeeds easily in Taichung. It is well known that when fertilization is normal, but a disharmonious interaction between embryo and endosperm interrupts the development of embryos, the technique of embryo culture may be used advantageously. In *Oryza,* this has been demonstrated by LI *et al.* (1961, 1962).

(5) Inviability or weakness, sterility and breakdown of hybrids between two given species. These phenomena found in various organisms have been reviewed by STEBBINS (1958a) with many examples available. In *Oryza,* except for intervarietal F_1 sterility in *sativa,* little is known. The writer and his colleagues are engaged in a survey of these hybrid abnormalities.

(6) Evaluation of differences in chromosome structure. Genome analysis and karyotype analysis represent methods for comparing the chromosome structure between species. Studies of *Oryza* genomes have been reported by GOTOH AND OKURA (1935), MORINAGA and his co-workers (1940, 1941, 1943, 1956, 1957), JONES AND LONGLEY (1941), NEZU *et al.* (1960), LI *et al.* (1961, 1962) and others. Based on the mode of pairing of chromosomes in F_1 hybrids, they designated a number of genomes, named A to F, which are distributed in different species. On the other hand, karyotype analysis in *Oryza* was reported by HU (1958, 1960b), ISHII AND MITSUKURI (1960), and others. SHASTRY AND MOHAN RAO (1961) placed particular emphasis on the morphology of pachytene chromosomes.

(7) Comparison of gene arrangement and action of particular genes. As it has been partly worked out for *Gossypium* (PHILLIPS AND GERSTEL, 1959; STEPHENS, 1961; etc.), investigations in these respects may yield substantial knowledge on speciation.

Results obtained by these methods vary in significance. Morphological, physiological, and biochemical characters show developmental sequences of the given genotype. If two species are similar in these characters, we may consider that they have similar genotypic constitutions. Difficulty in crossing, as well as inviability or weakness, sterility and breakdown of hybrids, may be attributed to interaction between two given genotypes. Whether the interaction is harmonious or disharmonious, it will serve as a measure of differentiation, as these phenomena may sometimes be regarded as isolating barriers which promote differentiation. Ecological and distributional features are determined by interaction between genotype and environment, though they might have been affected by historical incidences. Natural hybridization and other population-genetic features may indicate the results of interactions between two or more co-existing genotypes and the environment. Knowledge in this field is important for evaluation of evolutionary dynamics. Furthermore, studies of chromosome structure and gene arrangement will display the sequence of speciation in genetic materials. The internal mechanisms of speciation may be inferred from these and other findings.

Thus, when data obtained from different sources are inconsistent, it is difficult to relate them and establish a simple criterion for evaluating species relationships. However, we hope to become acquainted with the systematic constitution of a given species-group by properly combining information from various sources.

QUANTITATIVE REPRESENTATION OF INTERSPECIFIC RELATIONSHIPS

We may find a cross section of the present variation by investigating variations in characters. The characters often used by taxonomists in working with *Oryza* species are: presence or absence of sterile lemmas, the shape of sterile lemmas being linear or setaceous, ligule length, spikelet length, awn length, leaf-blade width, perennial vs. annual habit, the microscopic structure of epidermal tissue of spikelets, etc.

An observation of certain "important" characters may sometimes provide a practical way for approaching the system of organic variation. However, we have no biologically objective reason for considering a certain character as particularly important. The writer is therefore inclined, to consider that the more characters we observe, the more reliable would be the conclusion reached. Observing many characters, we will find different variation patterns and we will have to consider how to synthesize them into an integrated picture. For distinguishing between Caucasians and Orientals, it is accepted that eye color is more important than the color of overcoats. However, it is usually difficult to find such a criterion in organic variation for evaluating the relative importance of characters. A character relative to the breeding system or isolating mechanism might have played an important role in evolution, but if we want to illustrate the present cross section of variation, we had better simplify our standpoint. A character determined by many genes might be more important than that determined by a few genes. But it is difficult to estimate the number of relevant genes in species crosses. In general, internal biochemical processes will be reflected in morphological characteristics; a minor character may be an expression of an important biochemical

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reaction. Therefore, as pointed out by MICHENER AND SOKAL (1957), we may assign equal importance to all recognizable characters.

Based on these considerations, the writer and his colleagues have attempted a survey of interspecific variation in *Oryza* by numerical-taxonomic methods (MORISHIMA AND

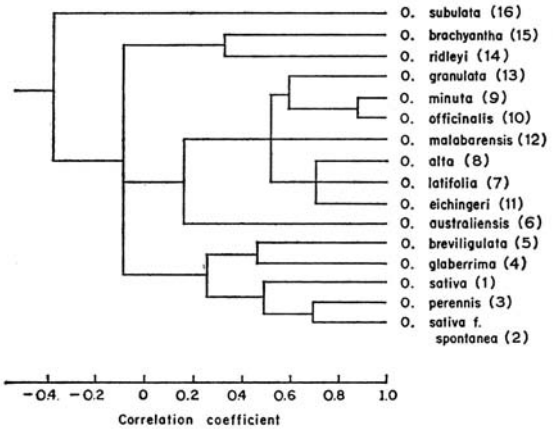
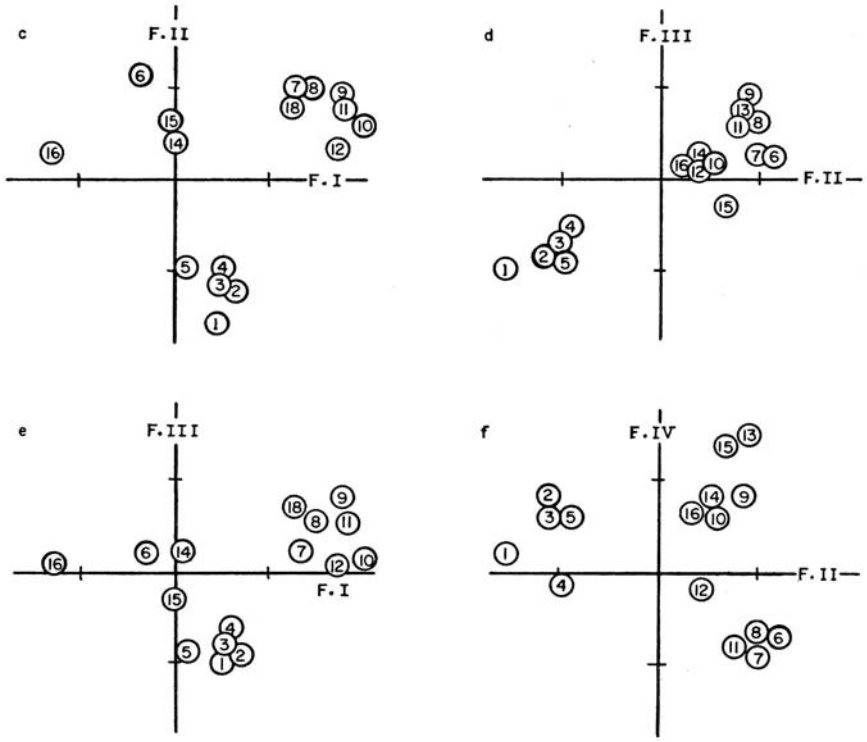


Diagram of relationships obtained from correlation matrix II (based on 42 characters).



Distributions of factor loadings on two-dimensional spaces.

Fig. 1. Diagrams showing interspecific relationships, obtained by SOKAL's method (above) and factor analysis (below). (From MORISHIMA AND OKA, 1960)

OKA, 1960). Sixteen species, each represented by one to five strains, were investigated regarding 42 characters, of which 20 were quantitative and the remaining 22 qualitative. The measurements of quantitative characters were standardized with respect to interspecific variation; qualitative characters were recorded in code numbers. Then, correlation coefficients between species were computed in all combinations of the 16 species. The correlation matrices thus obtained were analyzed first by Sokal's weighted variable-group method (SOUL AND MICHENER, 1958), and then by the technique of factor analysis (averoid method).

These computations proved that the results obtained by the two methods were quite consistent with each other, as shown in Fig. 1. It was pointed out that the species belonging to ROSCHEVICZ'S section *Sativa* could be divided into two groups, one comprising *Oryza sativa, perennis, sativa* f. *spontanea, glaberrima* and *breviligulata* (called *Sativa* Group) and the other comprising *Oryza officinalis, minuta, eichingeri, latifolia, alta,* etc. (called *Officinalis* Group), though *Oryza australiensis* was outside these two groups. Species belonging to other sections of ROSCHEVICZ were distributed away from the above two groups.

The *Sativa* Group consists of species which are known to have the genome A. Since two of them, *sativa* and *glaberrima,* are cultivated, this species-group may be regarded as particularly important for students of the origin of cultivated rice. On the other hand, the *Officinalis* Group comprises *officinalis* (diploid) and its related species which are known to be tetraploid. As they closely resemble one another in morphological features, they have shown high correlations.

The same set of data used for the above work was analyzed further by TAKAKURA (1962) by the quantification method, earlier developed by HAYASHI (1952). In TAKAKURA'S computation, records of individual strains, instead of species means, were analyzed with an electronic computer. The method, though its mathematical procedure is tedious, is to transform code numbers or measurements of quantitative characters into weighted variables so as to maximize the between-strain variances relative to within-strain ones,

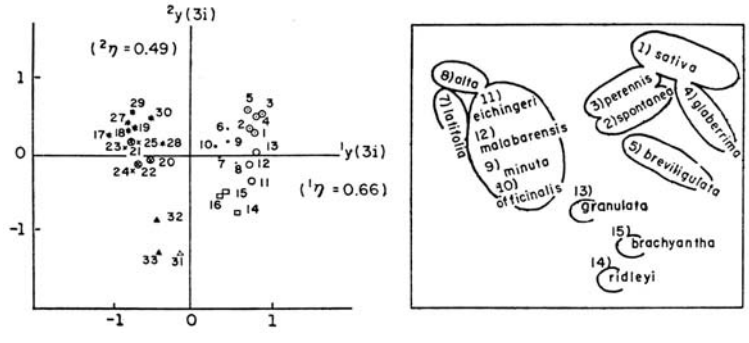


Fig. 2. Strains belonging to 14 *Oryza* species scattered by two latent vectors of a "quantification" matrix (From TAKAKURA, 1962)
1–5: *sativa,* 6-10: *perennis,* 11–13: *glaberrima,* 14–16: *breviligulata,* 17–19: *latifolia,* 20: *malabarensis,* 21–22: *minuta,* 23–25: *officinalis,* 26: *australiensis,* 27–28: *eichingeri,* 29–30: *alta,* 31: *brachyantha,* 32: *granulata* and 33: *ridleyi.*

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and to combine them according to the formulas thus obtained. The results were, as an example in Fig. 2 shows, well consistent with those of the writer. In this case, it was found that strains belonging to a species were located near one another or formed a cluster representing the species. Theoretically, the quantification method may be said to be more advanced than factor analysis. The results obtained by TAKAKURA, however, suggest that, as pointed out by SNEATH AND SOKAL (1962), different methods of analysis generally give similar results, if the same original data are used. It is especially important, therefore, to prepare a proper set of data. It also is known that if the number of characters investigated is large enough, an addition of new characters does not much modify the results (SNEATH AND SOKAL, 1962). This implies that interspecific variations can be correctly estimated by recording many characters and applying multivariate analysis.

SPECIES RELATIONSHIPS IN THE *Sativa* GROUP

As mentioned in the previous chapter, *Oryza sativa* L., *perennis* Moench, *glaberrima* Steud. and *breviligulata* A. Chev. et Roehr. compose a distinct group. Forms described by other names, seemingly belonging to this group, will fall into any of the four species. Namely, *Oryza stapfii* Roschev. may be regarded as synonymous with *breviligulata* (TATEOKA, 1962b; MORISHIMA *et al.,* 1963). *Oryza barthii* A. Chev. may be considered as an African form of *Oryza perennis* (SAMPATH AND RAO, 1951; SAMPATH, 1961), and *sativa* f. *spontanea* Roschev. (= *sativa* var. *fatua* Prain., *fatua* Konig, and *rufipogon* Griff.) is an Asian annual form of the same species (MORISHIMA *et al.,* 1961). In view of the ambiguous original description for *perennis,* however, TATEOKA (1963) recommends the use of the species name *rufipogon* in place of *perennis.*

Oryza sativa is distributed in all rice-growing countries, and *perennis* in most tropical countries of the world, while the distribution of *glaberrima* and *breviligulata* is limited to West Africa. It is known that in some regions these species grow sympatrically, and naturally cross with one another. Regarding these mixed populations, we have the following evidence:

TABLE 1

A BRIEF DESCRIPTION OF HABITATS OF WILD RICE IN ASIA, AFRICA AND AMERICA

Species and type	Water level in early dry season			Distance from nearest rice field			
	dry	swampy (< 50 cm)	deep (> 50 cm)	very close (< 2 m)	close (2–20 m)	distant (> 20m)	isolated (> 1 km)
perennis:				(from rice field of *sativa)*			
Asian *perennis*		3	11	3	5	5	1
Intermediate		11	3	4	7	1	
spontanea	6	13		4	10	4	1
African forms	4	7	4	3	4	4	4
American forms	1	3	9	1*			12
breviligulata:				(from rice field of *glaberrima)*			
(in West Africa)	6	6	1	3	1	9	

* A population in Cuba; rice fields were recently reclaimed in the proximity.

(a) *perennis–sativa.* As shown in Table 1, wild populations of *perennis* in Asia mostly are found in swamps near rice fields, while those in tropical America are found to be isolated from cultivated rice. It seems that natural hybrids between these two species frequently occur in tropical Asia, sometimes giving rise to hybrid swarms (OKA AND CHANG, 1961). An exceptional hybrid swarm between these species was also found in Cuba (OKA, unpublished),

(b) *breviligulata–glaberrima.* Four out of 13 populations of *breviligulata,* observed by Dr. K. FURUSATO of the National Institute of Genetics, Japan, who visited West African countries, were near rice fields in which *glaberrima* was grown, and in some places the two species were mixed. Natural hybridization may occur frequently, though no valid observation is available.

(c) *perennis–breviligulata.* Mixture of these two wild species in the same population was found in two out of 14 populations observed by Dr. FURUSATO, in which *O. perennis* was predominant. We do not have enough evidence of the occurrence of natural hybridization.

TABLE 2

PERCENTAGES OF OUT-CROSSING ESTIMATED IN WILD AND CULTIVATED RICE SPECIES

Species and type	No. of populations	Place of estimation	Result (%)	Method	Reference
perennis:					
spontanea (?)	1	India	7.9	From segregation	ROY, 1921
perennis (Taiwan)	1	Taiwan	30.7	Progeny test for a marker gene	OKA, 1956
perennis (Thailand)	1	Thailand	44.0	Frequency of glutinous gene in a hybrid swarm	OKA AND CHANG, 1961
perennis (India)	1	Taiwan	41.2–44.2	From variances of heading date	OKA AND CHANG, 1959
spontanea (India)	1	Taiwan	25.2–30.8	idem	idem
perennis (Ceylon)	2	Ceylon	22.4–26.5	From variances of spikelet width	SAKAI AND NARISE, 1959
spontanea (Ceylon)	4	Ceylon	7.4–50.7	idem	idem
spontanea (India)	3	Ceylon	16.6–33.9	From variances of spikelet width	SAKAI AND NARISE, 1960
breviligulata	2	Taiwan	3.2–19.7	From variances of heading date	MORISHIMA *et al.,* 1963
sativa (examples):					
34 varieties of India		India	0.0–6.8	Progeny test for a marker gene	BUTANY, 1957
2 varieties of Africa		Sierra Leone	0.0–1.1	idem	ROBERTS *et al.,* 1961
4 indica varieties		Taiwan	0.1–0.3	idem	OKA (unpubl.)
5 japonica varieties		Taiwan	0.6–3.9	idem	idem
A variety of India		Taiwan	0.0–8.0	From variances of heading date	OKA AND CHANG, 1959
A variety of Ceylon		Ceylon	3.6	From variances of spikelet width	SAKAI AND NARISE, 1960

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(d) *sativa–glaberrima.* Dr. H. D. JORDAN of the West African Rice Research Station, Rokupr, Sierra Leone, wrote that these two cultivated species are often mixed in West Africa, since the local people cannot always distinguish between them. According to Dr. FURUSATO, plants supposedly of hybrid origin are ravely found. From these observation records it may be tentatively concluded that these four species can occur sympatrically, and exchange of genes also may take place among them. The frequency of self- vs. cross-pollination in the wild species has been estimated by different methods, i.e., test of the progeny of mixed plants for a certain marker gene (OKA, 1956a), investigation of the frequency of glutinous gene in a hybrid swarm (OKA AND CHANG, 1961), and biometrical estimation from the ratio of between-plant to within-plant variances of certain quantitative characters (OKA AND CHANG, 1959; SAKAI AND NARISE, 1959, 1960; MORISHIMA *et al.*, 1963; cf. SAKAI AND IYAMA, 1957). It was found rather consistently that the Asian forms of *perennis* might be 20% to 45% cross-pollinated, while *breviligulata* might have a somewhat lower percentage, as shown in Table 2. In contrast, cultivated rice species are almost completely self-pollinated. It may be that the gene flow proceeds from cultivated to wild plants when a wild and a cultivated population come in contact. Though hybridization may thus occur rather frequently, it seems that hybrid swarms can be established only under certain conditions, possibly in disturbed habitats (OKA AND CHANG, 1961).

Crossing experiments between these four species have been made by many workers. The writer and his colleagues have obtained F_1 hybrids of various cross combinations using a number of strains belonging to each of these species. It is said that the African forms of *Oryza perennis* (*barthii*) cannot be easily crossed with other forms of the same species or other species. However, as shown in Table 3, the percentages of success in

TABLE 3

RATES OF SUCCESS OF ARTIFICIAL POLLINATION IN DIFFERENT CROSS-COMBINATIONS (in %)

Maternal parent	Pollen parent					
		forms of *perennis*				
	sativa	Asian	American	African	*brevilig.*	*glaberr.*
sativa	44	35	39	54	33	47
perennis						
Asian forms	26	35	40	36	37	33
American forms	54	50	50	56	75	
African forms	24	40	19	80	67	25
breviligulata	26	45	21	29		70
glaberrima	34	40	48	31	50	45

crossing *barthii* strains were not lower than those in other cross-combinations, when pollen-fertile plants were used. It is found that some plants of African as well as Asian *perennis* strains, which are capable of vegetative propagation, have a low pollen fertility and are partly self- as well as cross-sterile. It may then be concluded that

except for those partially fertile ones, plants of these wild and cultivated species can be easily crossed with one another. It was also found that the F_1 seeds germinated well, if dormancy had been overcome (Table 4),

TABLE 4

GERMINATING CAPACITY OF F_1 SEEDS, INCIDENCE OF F_1 WEAKNESS AND POLLEN FERTILITY VARIATION OF F_1 HYBRIDS, IN INTRA- AND INTER-SPECIFIC CROSSES

Cross-combination	Germin. capacity (%)	Crosses showing F_1 weakn.	Total no. of crosses	Pollen fertility (%)	
				Mean	Range
Within Series *Sativa:*					
sativa × *sativa*	96.6	2	815	74.1	99–1
Asian *perennis* × Asian *perennis*	62.6	–	60	89.2	99–35
sativa × Asian *perennis* (& recip.)	78.1	–	353	80.1	99–1
sativa × African *perennis* (& recip.)	35.1	1	9	33.0	90–0
Asian *peren.* × African *peren.* (& recip.)	17.1	2	6	75.7	99–6
Between Series *Sativa* × *Glaberrima:*					
sativa × *glaberrima* (& recip.)	86.7	–	295	0.6	9–0
sativa × *breviligulata* (& recip.)	97.4	–	59	0.4	4–0
Asian *perennis* × *glaberrima* (& recip.)	72.4	4	85	4.6	23–0
Asian *perennis* × *brevilig.* (& recip.)	63.1	2	31	4.5	19–0
African *perennis* × *glaberrima* (& recip.)	16.1	6	9	12.1	30–0
African *perennis* × *brevilig.* (& recip.)	33.3	3	5	1.0	3–0
Within Series *Glaberrima:*					
glaberrima × *glaberrima*	91.2	1	56	94.1	99–83
breviligulata × *brevilig.*	71.3	5	17	95.9	99–91
glaberrima × *brevilig.* (& recip.)	53.1	8	58	96.6	99–85

THE OUTCOME OF SPECIATION AS SHOWN BY HYBRIDIZATION EXPERIMENTS

When different species or races are crossed, the hybrid plants often show various deterioration phenomena which STEBBINS (1958a) has classified as weakness or inviability, sterility and breakdown according to the order of alternation of generations. In general, these phenomena result from a disharmonious interaction of parental genotypes, or of genotype and cytoplasm. Hybrid sterility also can be classified into haplontic and diplontic.

In the crosses made by the writer and his colleagues, hybrid weakness was occasionally found, as shown in Table 4. In those cases, the growth of the F_1 plants slowed down after several leaves were produced, and the plants, if protected from competition, developed small panicles with a few seeds. According to AMEMIYA AND AKEMINE (1960), the root of weak F_1 plants of *Oryza sativa* does not develop well, possibly due to lack

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of some enzyme controlling the respiratory system. This phenomenon was found to be due to a set of complementary dominant lethals (OKA, 1957c). Such weak plants may die in natural habitats. The F_1 weakness may then be regarded as an effective isolating barrier. The data in Table 4 show that this phenomenon occurs relatively frequently when *breviligulata* or *glaberrima* is crossed with the African *perennis.*

The sterility of F_1 hybrids between and within species of the *Sativa* Group may result from a recombination of duplicate factors maintaining the development of gametes; accordingly, it is haplontic as will be discussed in the writer's other paper presented at this Symposium. The ranges of F_1 pollen fertilities found in different cross combinations also are shown in Table 4. The data show that almost all *sativa,* Asian *perennis* and African *perennis* strains produced highly or partly fertile F_1 hybrids, while American *perennis* strains generally showed a high sterility with their Asian and African relatives. Further, it is found that *glaberrima* and *breviligulata* strains, which produced fertile F_1 hybrids, gave a high F_1 sterility with *sativa* and *perennis* strains. The four species under consideration may then be divided into two series separated by a sterility barrier, which MORISHIMA *et al.* (1963) called Series *Sativa* comprising *Oryza sativa* and *perennis,* and Series *Glaberrima* comprising *Oryza glaberrima* and *breviligulata.*

The two species series are distinguished by certain characters: compared with Series *Sativa,* Series *Glaberrima* is characterized by short and tough ligules with roundish tips, death of the plants after maturity, low resistance to drought, and other characters. It must be noticed, however, that in Series *Sativa* distantly related forms give partly sterile F_1 hybrids, but Series *Glaberrima* is a completely intrafertile group. It is interesting that some F_1 hybrids between *perennis* and *breviligulata,* as well as those between *perennis* and *glaberrima,* tend to show partial fertility, the highest pollen fertility reaching 30%. There may or may not exist in nature wild-growing forms which show higher F_1 fertilities than this value so far obtained in hybrids between the two species series. If such plants are found, they may be regarded as approaching the common ancestor of both species series, as will be discussed later.

The tendency for a hybrid to breakdown, appearing as weakness or partial sterility in F_2 segregants, is often found in intervarietal crosses of *Oryza sativa.* The genetic basis of these phenomena could be explained by assuming duplicate factors whose double recessive combinations bring about weakness or diplontic sterility (OKA, 1957c; OKA AND DOIDA, 1962). Whether or not these phenomena are common in the species of *Sativa* Group has to be investigated in the future; these might be of the same value as the F_1 sterility as criteria for estimating phylogenetic relationships.

VARIATIONS BETWEEN WILD AND CULTIVATED SPECIES

Oryza perennis comprises several geographical and ecological races. The writer and his colleagues have investigated a number of strains of this species collected from India, Thailand and other tropical Asian countries regarding variations in various characters (MORISHIMA *et al.,* 1961). From the results of multivariate analysis of the data, they concluded that the Asian forms of *perennis* vary between *perennis (balunga;* perennial,

growing in deep swamps) and *spontanea* (*fatua;* annual, in temporary swamps) types, displaying a continuous array of intergrades. The African forms *(barthii)* are distinguished from these Asian forms by well-developed rhizomes. In Central and South America, various forms of this species occur, one of which is known as *cubensis,* while the rest are in South America and are mostly floating types. As shown in Table 5, the American forms seem to be characterized by large spikelet size and some other characters. However, investigations of their variations and hybrid-sterility relationships are still under way.

TABLE 5

GEOGRAPHICAL VARIATIONS IN CHARACTERS OF *Oryza perennis*

Character	Asian type		American	African
	perennis	*spontanea*		
Spikelet length (mm)	8.39 ± 0.60	8.21 ± 0.39	9.43 ± 0.98	8.37 ± 0.58
Spikelet width (mm)	2.28 ± 0.24	2.58 ± 0.19	2.65 ± 0.32	2.47 ± 0.25
Lth/width ratio	3.71 ± 0.30	3.21 ± 0.22	3.58 ± 0.19	3.44 ± 0.40
Apiculus hair length (0.1 mm)	5.09 ± 0.85	5.02 ± 0.80	6.69 ± 1.19	4.48 ± 0.95
Awn length (cm)	5.84 ± 2.01	6.27 ± 2.39	6.05 ± 2.10	4.88 ± 1.95
Ligule length (cm)	2.03 ± 0.65	1.50 ± 0.55	2.33 ± 0.74	2.34 ± 0.47
Anther length (mm)	4.17 ± 0.96	2.75 ± 0.58	4.55 ± 0.56	5.24 ± 0.78
Panicle length (cm)	18.6 ± 2.13	16.5 ± 4.05	18.8 ± 3.99	20.6 ± 2.91
Rachis no./panicle	6.5 ± 1.69	6.9 ± 1.91	7.3 ± 1.88	7.1 ± 1.70
Spikelet no./panicle	44.9 ± 15.3	53.7 ± 18.2	49.8 ± 27.9	46.6 ± 17.8
100 grain wt. (gm)	1.81 ± 0.38	1.85 ± 0.19	1.85 ± 0.48	
Seed dormancy index[1]	2.11 ± 0.34	1.97 ± 0.30	1.92 ± 0.26	
Regenerating ability[2]	3.24 ± 0.68	2.64 ± 0.86	1.82 ± 0.50	3.00 ± 0.52
No. of strains observed	37	31	15	20

* Mean measurements and standard deviations for inter-strain variation are shown.
[1] Refers to OKA AND CHANG (1962)
[2] Degree of adventitious root development from stem cuttings, in index-numbers ranging from 1 to 3.

Cultivated varieties of *Oryza* sativa show partial F_1 sterility in varying degrees. However, as shown in Tables 4 and 6, Asian forms of *perennis* were interfertile, and tended to produce fertile F_1 hybrids with various *sativa* varieties (HINATA AND OKA, 1962a), In contrast, the African forms generally produced partly sterile hybrids and the American forms highly sterile hybrids with Asian forms and *sativa* varieties. The Asian forms of *perennis* are sympatric and naturally hybridized with *sativa,* as mentioned before. These facts indicate that sativa might have arisen from the Asian forms of *perennis.*

It is a matter of dispute whether *Oryza sativa* evolved from the *perennis (balunga)* type or from the *spontanea (fatua)* type. The writer considers that the *perennis* type might be the progenitor of *sativa* varieties for the following reasons:

(1) the *perennis* type contains in its populations a larger amount of genetic varia-

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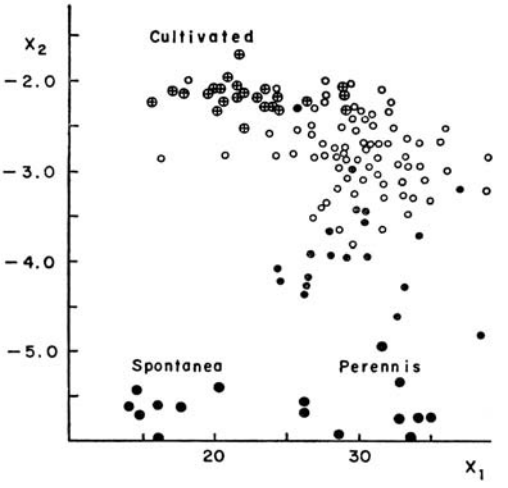

Fig. 3. Wild controls, Jeypore strains, and cultivated controls scattered according to the scores given by two discriminant formulas, one (abscissa) for classifying *perennis* and *spontanea* types, and the other (ordinate) for wild and cultivated forms. (From OKA AND CHANG 1962).

⊕ Cultivated control ○ Jeypore, cultivated
● Jeypore, wild ● Wild control

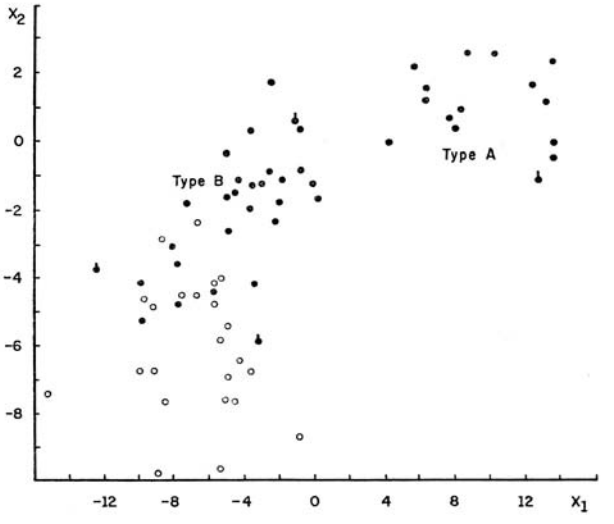

Fig. 4. Populations of *Oryza breviligulata* and *glaberrima* scattered in the p lane defined by two facto axes, X_1 and X_2 (From MORISHIMA *et al.,* 1963).

● *breviliguluta,* wild ● labelled as *stapfii* ○ *glaberrima,* cultivated

bility, and accordingly might have a larger evolutionary potentiality, than *spontanea* (MORISHIMA *et al.*, 1961);
(2) materials collected from the Jeypore Tract, Orissa, India, exhibited various intermediates between wild and cultivated forms. Those approaching wild forms were of the *perennis* type, as shown in Fig. 3; they can be considered as a bridge connecting wild forms with cultivated varieties (OKA AND CHANG, 1962).

TABLE 6

DISTRIBUTIONS OF AVERAGE F_1 POLLEN FERTILITY OF ASIAN *perennis* AND *sativa* STRAINS WITH FIVE TEST-STRAINS OF *Oryza sativa*

Species and type	% of good pollen											Number of strains
	95	90	85	80	75	70	65	60	55	50	45	
perennis:												
spontanea type	2	5	5	3	2	1		1	1			20
perennis type		2			1	1	2	1				7
sativa:												
Continental (*indica*)			3	4	11	10	8	8	1	1	2	48
Insular (*japonica*)												
Tropical		1	7	8	10	4	1	3	1			35
Temperate			4	3	4	3	3	1				18

These facts suggest further that the *spontanea* (*fatua*) type also might have been derived from the *perennis* (*balunga*) type. It is interesting to note, as pointed out by STEBBINS (1958b), that plants growing in "stable habitats" are generally long-lived, cross-pollinated and rich in variability, and can be regarded as the progenitor of related species which occur in "unstable habitats". Our comparisons between the *perennis* and *spontanea* types seem to fit this theory well, as the deep swamps in which the former type occurs may be regarded as stable, while the temporary swamps of the latter type may be unstable.

It may be likewise assumed, in view of their similarity in characters and high F_1 fertility, that *Oryza glaberrima* might have evolved from *Oryza breviligulata.* It was found that populations of *breviligulata* collected from the suburb of Segou, Mali Republic (French Sudan), formed a continuous array connecting this wild species with *glaberrima,* as shown in Fig. 4. This indicates further that the origins of *sativa* and *glaberrima* are independent. It is interesting that the above-mentioned place, Segou, is near the inland delta of the Niger River which PORTÈRES (1956b) has envisaged as variation center of these species.

Comparing the modes of evolution of cultivated forms from the two wild species, *perennis* (Asian *perennis* or *balunga* type) and *breviligulata,* MORISHIMA *et al.* (1963) have pointed out certain similarities and differences. In both species series, the wild and cultivated forms differ in the same characters, a latent variation toward cultivated forms can be detected in wild populations from the results of multivariate analysis of

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the data, and a continuous array of intergrades connecting wild and cultivated species is found in a particular region. On the other hand, major differences are, first, that Asian *perennis* populations contain a large amount of genetic variation mainly in a heterozygous state, while *breviligulata* populations vary in a wide range; and secondly, that *sativa* varieties differ greatly in certain characters as they are differentiated into *indica* and *japonica* types, and the variation appears to become wider with the approach to cultivated forms, while *glaberrima* varieties are not differentiated into such types as the *indica* and *japonica* (MORISHIMA *et al.,* 1962). Thus, it seems that a part of the variants of *breviligulata* might have followed the evolutionary route toward cultivated forms. MORISHIMA *et al.* (1963) considered that some of these differences could be attributed to the difference in breeding system between the wild progenitors, i.e., Asian *perennis* being partly vegetatively propagated, while *breviligulata* is annual.

Summarizing the above considerations, the relationships among the species mentioned may be shown by a hierarchic diagram as presented in Fig. 5. The evolutionary dynamics bringing about such a pattern of differentiation contain various problems of scientific interest.

INTERNAL MECHANISMS OF SPECIATION IN *Sativa* GROUPS

It is well known that *sativa* varieties can be divided into *indica* and *japonica* types, which have different character complexes and ecological requirements. They are often considered to be intrafertile groups which produce partly sterile F_1 hybrids, although this does not always happen, The putative wild progenitor of *Oryza sativa,* the Asian *perennis,* is not differentiated into *indica* and *japonica;* its strains do not vary so widely in characters differentiating the two types, e.g., potassium-chlorate resistance, low-temperature resistance, apiculus hair length, etc., and as mentioned already, they generally give fertile F_1 hybrids with *sativa* varieties of different types (cf. Tables 6 and 7). OKA AND CHANG (1962) showed that among the intermediate plants between wild

TABLE 7

DISTRIBUTION OF DISCRIMINANT SCORES COMBINING F1 POLLEN FERTILITIES WITH FIVE TEST-STRAINS SO AS TO MAXIMIZE THE DIFFERENCE BETWEEN *indica* AND *japonica* TYPES

Species and type	*indica* 70	50	30	10	–10	–30	*japonica* –50	–70	Number of strains
perennis:									
spontanea type			4	6	8	2			20
perennis type	1		1	2	1	2			7
sativa:									
Continental (*indica*)	3	3	15	16	8	3			48
Insular (*japonica*)									
Tropical				4	13	11	3	5	36
Temperate					4	4	5	4	17

and cultivated forms of Jeypore Tract, the *indica–japonica* differentiation might be advanced as they approach cultivated forms, as shown in Fig. 6. This suggests that the *indica* and *japonica* types are monophyletic and have appeared in the course of evolution toward cultivated forms. It was suggested further that the differentiation in character complexes might proceed ahead of that in F_1 sterility barriers. It may be that the differentiation starts from a differential response of genotypes to some environmental factor involved in cultivated conditions. These indicate that the genotypes of wild rice have a potentiality of differentiation into various types.

For looking into this problem, we may take up, first, the hypothesis that the genus *Oryza* is a secondary balanced polyploid, which is supported by the pronounced secondary association of meiotic chromosomes observed by various workers (SAKAI, 1935b; NANDI, 1936; PARTHASARATHY, 1938; OKUNO, 1944). Recently, HU (1962) found that different species of *Oryza* show similar patterns of secondary association.

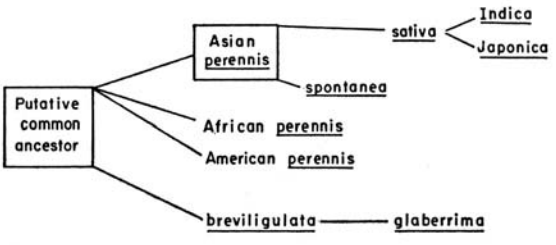

Fig. 5. A diagrammatic representation of species relationships in the *Sativa* group.

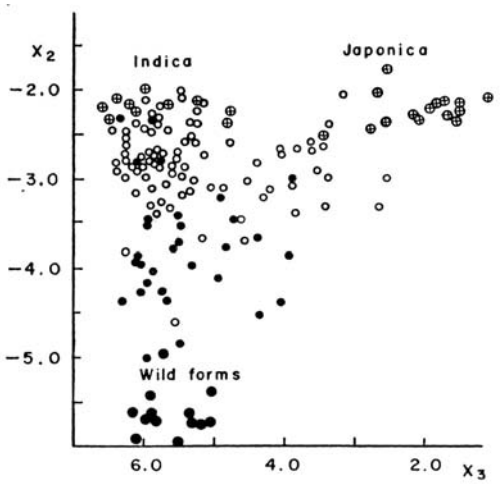

Fig. 6. Wild controls, Jeypore strains, and cultivated controls scattered according to the scores given by two discriminant formulas, one (abscissa) for classifying *indica* and *japonica* types, and the other (ordinate) for wild and cultivated forms. (From OKA AND CHANG, 1962).

⊕ Cultivated control ○ Jeypore, cultivated
• Jeypore, wild ● Wild control

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Secondly, as demonstrated by the present writer (OKA, 1957a; OKA AND DOIDA, 1962) the haplontic as well as diplontic sterilities of intervarietal hybrids in *sativa* can be accounted for by assuming sets of duplicate genes. Then, the fact that the Asian forms of *perennis* produce fertile F_1 hybrids with various *sativa* varieties may be well explained by assuming that the wild forms have dominant alleles at many duplicated loci, and mutations or deficiencies at one or the other of the loci have brought about mutually partly sterile varieties.

It was found that Asian *perennis* populations, though they generally produce fertile F_1 hybrids with various *sativa* varieties, contained many sterility factors of haplontic as well as diplontic effects, but that the sterility found in some plants of those *perennis* populations was mainly due to diplontic sterility factors (HINATA AND OKA, 1962a). In agreement with this, it was suggested from mathematical computations that combinations of duplicate genes causing diplontic sterility would occur in populations more frequently than those causing haplontic sterility, if the plants are partly cross-pollinated and heterozygotes have a selective advantage; however, if the plants become self-pollinated, plants separated by both haplontic and diplontic sterility barriers will be released from the populations (HINATA AND OKA, unpublished). This will partly account for the occurrence of sterile plants in populations of *perennis,* and for the origin of intervarietal sterility in *sativa.*

We have found that *sativa* and *glaberrima* might have independently evolved from their respective wild progenitors, *perennis* and *breviligulata,* However, they have the same genome, A. According to HU (1960b), the two species have almost the same karyotype and show the same pattern of intragenome pairing of chromosomes in haploid plants, so that they might not be much differentiated in chromosome structure. They must have a common ancestor.

It is difficult to imagine what features the common ancestor has had. Some Indian workers (cf. RICHHARIA, 1960) assume that *perennis* might be the ancestral form for both *sativa* and *glaberrima,* and *breviligulata* might be a hybrid derivative from *perennis* and *glaberrima.* Considering that mutations and deficiencies at duplicated loci produce intervarietal sterility, we may assume as a working hypothesis that the sterility of F_1 hybrids between Series *Sativa* and *Glaberrima* might be largely of the same nature as found between *sativa* varieties. In terms of pollen and embryo-sac development, we find no particular difference between the two cases of F_1 sterility. It is then expected that the ancestral plants common to *perennis* and *breviligulata* might be capable of producing fertile F_1 hybrids with both of them. We have found that some Asian forms of *perennis* show a partial fertility with strains belonging to Series *Glaberrima.* This suggests that such forms of *perennis* might be relatively close to the common putative ancestor of *perennis* and *breviligulata.*

To throw more light on this problem, however, we have to look into the genetic basis of sterility between the two species series, and to search for wild plants which show a high fertility with both species series. Data recently obtained by the writer indicate that the African forms of *perennis* are of two types, one with a small number of panicles per plant, a small number of spikelets per panicle and high pollen fertility, and the other

with the same characters in opposite. The selfed progeny of the latter type segregated into various forms, some of which had relatively short and tough ligules partly similar to those of *breviligulata.* Their sterility relationships are now being observed. If intermediate plants between Series *Sativa* and *Glaberrima,* approaching their putative common ancestor, were found in some place in Africa, they would serve as a key to trace the origin of these species.

The above discussions are based on the hypothesis that both wild and cultivated species of *Oryza* have many duplicate genes due to their secondary polyploid origin. Though the writer is inclined to consider that the evolutionary process might be mainly genic, he does not reject the possibility that differentiation in chromosome structure is involved. It is possible, though cytological evidence so far reported does not indicate, that not only mutations and deficiencies but also translocations and inversions might have contributed to the evolution of these species. In addition, genes of complementary effect might have been substituted in the process. To evaluate the amount of structural differentiation present between *sativa* and *glaberrima,* the writer is now investigating hybrids between tetraploid strains of the two species. Regarding the relative importance of gene substitution, the tendency of hybrids to break down must be investigated in more detail.

EVOLUTIONARY DYNAMICS OF CULTIVATED FORMS

With regard to the breeding system of the Asian *perennis* type of wild rice, the following aspects may be pointed out (cf. HINATA AND OKA, 1962b):

(1) The wild plants are 20% to 45% cross-pollinated. They have a several-minute interval between flower opening and pollen emission, longer lasting viability of pollen grains emitted from the anther than the cultivated rice, and other characters which increase the opportunity for cross-pollination.

(2) The seeds generally show pronounced dormancy, though a variation in degree is found among strains.

(3) The seeds can remain alive in moist soil for more than two years.

(4) They have a strong ratooning ability. The rate of vegetative propagation in natural habitats, though it is difficult to estimate, might be higher in populations containing more sterile plants.

However, in *Oryza breviligulata,* the putative progenitor of *glaberrima,* it seems that the rate of outcrossing is somewhat lower than that of *perennis* and Vegetative propagation does not take place.

It is known from theoretical studies that (1) in partly crossbreeding populations, mutant genes may advance relatively easily (BODMER AND PARSONS, 1960), (2) partial selfing tends to increase both heterozygotes as well as homozygotes for many independent genes in proportion to partly heterozygous and homozygous plants (BENNETT AND BINET, 1956; KIMURA, 1958), and (3) overlapping ofgenerations due to vegetative propagation, as well as to seed dormancy, tends to favor hetcrozygotes against homozygotes insofar as hetcrozygotes have a selective advantage (HINATA AND OKA, 1962b).

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Thus, the breeding system of wild rice may be suitable for accumulating genetic variation. Further, introgressive hybridization may increase the amount of variation present in populations.

Actually, the populations of the *perennis* (*balunga*) type of wild rice were found to contain a considerable amount of genetic variability in various characters, mainly in the heterozygous state (MOHISHIMA *et al.,* 1961). Investigations of their hybrid swarms with cultivated rice showed further that they contained a great amount of variability, which, if released, could cover the range from wild to cultivated forms (OKA AND CHANG, 1961). On the other hand, populations of the *spontanea* (*fatua*) type, and of *breviligulata,* contained a relatively small amount of variability. This suggests that the capacity to store up genetic variation might be a function of the breeding system.

In contrast to wild species, cultivated rice is almost completely self-pollinated. We may infer that with the evolutionary change toward cultivated forms, the wild plants might have gradually become self-pollinated. Theoretically, it is expected, as worked out by CROSBY (1949) and BODMER (1960) with *Primula* populations in England, that if plants with a high and a low selfing rate coexist in a population, the former will pollinate the latter more frequently than being pollinated by the latter, so that the population will eventually become self-pollinated unless counteracted by another force which favors heterozygotes (HINATA AND OKA, 1962b). Actually, in hybrid swarms between wild and cultivated forms, the frequency of heterozygotes was found higher than expected (OKA AND CHANG, 1961). For instance, in northern Thailand, glutinous rice covers almost all paddy fields, while wild rice growing in them is essentially non-glutinous. A hybrid swarm found there contained the glutinous gene at a 28.3% frequency; from this gene frequency and the frequency of non-glutinous grains produced by natural hybridization in glutinous plants, the rate of outcrossing was estimated to be 44%. It was pointed out that heterozygotes were more numerous than expected from this rate of outcrossing, as shown in Table 8.

However, when those plants were tested in the conditions of experimental fields, the homozygotes showed no tendency of inbreeding depression (cf. Table 8). In general, selfed progenies of wild rice grow normally in the experimental field. It seems that heterozygotes have a wider range of adaptability to different environments and are

TABLE 8

FREQUENCIES OF HOMO- AND HETEROZYGOTES FOR THE GLUTINOUS GENE IN A HYBRID SWARM (SAMPATOON POPULATION) AND THEIR CHARACTERS IN AN EXPERIMENTAL CONDITION

Item	+ +	+ *gl*	*gl gl*	Total
Expected frequency	0.593	0.248	0.159	1.0
Expected number	62.8	26.3	16.9	306.0
Observed number	55	42	9	106
				(Mean)
Spikelet no./panicle	69.9 ± 19.6	86.9 ± 23.0	92.4 ± 19.1	79.6
Plant height (cm)	151.4 ± 18.9	146.4 ± 13.2	142.6 ± 11.4	14.83

more advantageous in a wild habitat thn homozygotes. In contrast, homozygotes, especially those for cultivated characters such as self-pollination, might be eliminated from wild habitats.

FRYXELL (1959) discussed advantages and disadvantages of self-pollination; as it increases reproductive potentiality, selfing will be advantageous if the genotype is adapted to a given environment. When the environment changes, crossbreeding populations will have an advantage owing to their maintenance of a large potential variability and a wide adaptive range. In this balanced condition, wild rice populations might fluctuate around an optimum selfing rate. When the habitat is controlled by man and resembles the conditions of cultivated fields, however, relatively homozygous plants will become advantageous and the population will tend toward selfing. Those populations containing a large amount of genetic variation thus will respond to selection due to the habitat, giving rise to various forms which are partly adapted to cultivated fields.

It is interesting to find that a semi-wild form existing as a weed in rice fields in India appeared to have an outcrossing rate (10−20%) intermediate between those estimated for truly wild and cultivated forms. Those plants might have arisen from introgressive hybridization between wild plants of the *spontanea* type and a cultivated variety grown in the same area, while their establishment as weeds might be due to selection for an adaptability to the conditions of rice fields which the writer has called "cultivation pressure" (OKA AND CHANG, 1959). In such a population, an outcrossing rate lower than that of truly wild forms might be adaptive. The seeds of those plants also showed an intermediate type of dormancy.

The evolutionary change from wild to cultivated forms might be a gradual and continuous process. We have found for both *sativa* and *glaberrima* a continuous array of intermediate plants which connects the cultivated forms with their respectively wild progenitors in a particular region, Ten rice varieties ranging from wild (*spontanea* type) to improved cultivated forms were tested under different growing conditions. The results showed that the nearer the genotype approached improved cultivated varieties, the higher would become the response to fertilizer application, weeding and transplanting, which may be regarded as elements of intensive rice culture in Asia (OKA AND CHANG, 1964). This suggests that the evolution of genotype and that of agriculture might proceed in parallel.

We may then infer that for the origin of cultivated forms there must be a series of intermediate habitats which enable semi-cultivated plants to be established. Investigations of ecological factors involved in such habitats seem necessary to understand the evolutionary mechanisms of cultivated plants.

SUMMARY

There are different methods of estimating species relationships. This paper reviews the relationships among *Oryza* species investigated by some of those methods and discusses the evolutionary-dynamic forces in cultivated rice. Principal conclusions follow:

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(1) The pattern of interspecific variations was investigated by numerical taxonomic methods. Different statistical techniques consistently showed that the species belonging to Roschevicz's Section *Sativa,* could be sorted into two groups, called the *Sativa* and *Officinalis* groups.

(2) *Oryza sativa* (cultivated), *perennis* (wild), *glaberrima* (cultivated) and *breviligulata* (wild), which form the Sativa Group, grow partly sympatrically in different localities, and can be easily hybridized with one another. Based on hybrid-sterility relationships and differences in some characters, these four species can be divided into Series *Sativa (sativa* and *perennis)* and *Glaberrima (glaberrima* and *breviligulata).* Weakness of F_1 hybrids was frequently found in crosses between these species series.

(3) *Oryza perennis* comprises various geographical and ecological races. Its Asian forms vary between *perennis (balunga;* perennial, in deep swamps) and *spontanea (fatua;* annual, in temporary swamps) types showing an array of intergrades. They generally produce fertile F_1 hybrids with various *sativa* varieties. On the other hand, the African forms *(barthii)* produce partly sterile, and the American forms highly sterile F_1 hybrids with the Asian forms and *sativa* varieties.

(4) Continuous series of intermediates between wild and cultivated forms, connecting Asian *perennis* with *sativa,* and *breviligulata* with *glaberrima,* were found existing in particular regions in India and in Africa, respectively. These may indicate the evolutionary pathway of cultivated forms. The Asian *perennis (balunga)* type of wild rice is then considered to be the progenitor of *sativa,* and *breviligulata* to be the progenitor of *glaberrima.* The origins of *sativa* and *glaberrima* are thus considered to be independent of each other.

(5) Comparing the mode of evolution of cultivated forms between Series *Sativa* and *Glaberrima,* certain differences and similarities were pointed out. One of the differences is that the varieties of *sativa* are differentiated into *indica* and *japonica* types, but those of *glaberrima* are not. Investigations of intermediate plants between *perennis* and *sativa* showed that the *indica* and the *japonica* types are monophyletic and the differentiation might have proceeded in parallel with the evolution to cultivated forms.

(6) On the basis of the hypothesis that the genus *Oryza* is a secondary polyploid, it was deduced that as a speciation mechanism mutations and deficiencies at one or the other of the duplicated loci might bring about intersterile groups. The differentiation of sativa varieties into partly intersterile groups could be well accounted for by this theory. Along this line of thinking, it was speculated that the common ancestor for the species series *Sativa* and *Glaberrima* might have been a strain that showed high F_1 fertilities with both.

(7) Wild rice assumed to be the progenitor of cultivated forms is partly cross-pollinated and has a large capacity to store up genetic variation in its populations. The change of the pollinating system toward selling, which might proceed with the adaptation to cultivated conditions, might have played an important role in the evolution of cultivated rice.

CYTOGENETICAL INVESTIGATIONS ON *ORYZA* SPECIES

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Artificial hybridization of rice under controlled conditions was first successfully undertaken in 1898 by TAKAHASHI who compared the characteristics of the hybrid with those of its parents (MORINAGA, 1957). This may have been the beginning of rice genetics in Japan. It was about 10 years later that KUWADA (1909, 1910) reported the development of the pollen grain, embryo sac, and the formation of endosperm of rice. He determined the number of chromosomes of rice as $n = 12$ and $2n = 24$, and described in detail the behavior of chromosomes during meiosis. He observed that some of the chromosomes in the second division of metaphase presented a paired arrangement, showing a tendency to form a group of more than two. He also observed such a paired arrangement in the somatic metaphase of the nucellar tissue. He could not determine, however, any relation between the racial differentiation of rice and the number and size of the chromosomes.

Since then a number of workers observed cytologically several mutant strains and varieties belonging either to the *japonica* or *indica* subspecies, but they failed to detect any differences in chromosome number or size among those strains or varieties. Many sterile plants proved, in the meantime, as factor or gene mutations. A mutant type of nearly 50% gametic sterility often produced in the next generation sterile and fertile plants in nearly equal numbers. Such mutant was often assumed as an interchanged heterozygote, but sometime later, the author actually observed a ring of four chromosomes and counted 25 chromosomes in the dwarf and sterile segregates (MORINAGA, 1939), They noticed no abnormal behavior of chromosomes in the meiosis of the partly sterile intersubspecific hybrids of *japonica* and *indica* varieties (KATO, 1928, 1930; MORINAGA, 1954). Thus the present author postulated that exaggerated gene or gene pattern differences, allowing small structural chromosomal differences to exist without affecting the meiotic behavior, caused the sterility of intersubspecific hybrids (MORINAGA, 1939, 1942). Cytogenetics in rice seemed rather unpromising until 1931.

Since 1931, haploid, triploid, tetraploid and aneuploid plants, which stimulated the development of cytogenetical studies on rice, were discovered in rapid succession in Japan, India, and U.S.A. (JONES AND LONGLEY, 1941; RAMIAH AND RAO, 1953).

The author and his co-workers (MORINAGA AND FUKUSHIMA, 1934) found, during 1931–33, seven haploid plants, five in varietal hybrid progenies and two in common

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paddy fields. They found in 1932 one triploid plant in a hybrid progeny. In 1933 they found 45 triploid plants in common paddy fields (MORINAGA AND FUKUSHIMA, 1935) and one tetraploid plant in a hybrid progeny. In 1934 they found two sterile diploid plants which segregated into three main types: normal diploid, sterile diploid and tetraploid (MORINAGA and FUKUSHIMA, 1937). One highly sterile, abnormal strain under cultivation since 1922 also was shown to be a tetraploid strain. Most of the individuals of the strain had 48 chromosomes, though some exceptional ones possessed 47 or 49 chromosomes. One plant treated by chloral hydrate produced highly sterile tillers in 1933, and one plant out of a few which were raised from the seed of those tillers was also tetraploid.

In a similar manner, RAMIAH *et al.* discovered a haploid plant as a twin in a normal pure line (RAMIAH AND RAO, 1953). Kawakami found triploid and haploid plants in twin seedlings of rice (KAWAKAMI, 1943). The occurrence of haploid, triploid and tetraploid plants in twin seedlings is more striking in common wheat than in rice (NAMIKAWA AND KAWAKAMI, 1934). As to the mechanism of polyploid formation, the experiments of ICHIJIMA (1934) also are suggestive. He induced mutations in rice by means of X-ray, ultraviolet light and variation in temperature, and the mutant characters practically covered all the types of spontaneous mutation previously reported in rice. He classified the artificial mutations into two groups: (1) the mutant characters appeared in the first generation as the direct effect of the treatment and (2) the mutant characters occurred in the second or third generation. All gene mutations belonged to the first group; in the second group, heteroploid, tetraploid and haploid plants appeared and triploids were most common. He observed that in F_2 generation more than 31000 individuals of different series exhibited sterility in various degrees. These results agree with the author's observations that sterility often precedes the occurrence of polyploidy and that triploids occur most frequently. The occurrence of triploid through the cross of a diploid and a tetraploid is, however, hardly conceivable in nature. OKURA cross-pollinated 18 flowers of a tetraploid with the pollen grains of diploid plants and obtained 11 seeds, of which eight germinated, producing five diploid plants and three tetraploid plants (OKURA, 1940). The author and KURIYAMA repeatedly tried in 1943 the reciprocal cross between the diploid and tetraploid, but no hybrid was obtained. They tried again in 1948, using various ways of emasculation and pollination, with the diploid exclusively used as the maternal parent. After pollinating more than 22000 flowers, they obtained only 28 triploid plants (MORINAGA AND KURIYAMA, 1959a).

The polyploid and haploid plants were studied from various points of interest by many workers.

The degree of sterility in those polyploids studied by the author was as follows:

Haploid I:1212 individuals obtained by vegetative propagation produced 41 seeds in the field, the calculated fertility being 0.0022%. When the normal pollen grains of diploid plants were applied artificially, however, the percentage of seed set increased to 2.41%. The seeds obtained under field conditions germinated, but the seedling did not develop beyond the plumule stage. All the seed obtained by the cross pollination

produced diploid plants of normal appearance and fertility. The haploid plants occasionally produced diploid tillers giving good seeds.

Triploid: The average fertility of 19 triploid plants in the field was estimated as 1.58%, and the percentage was not improved noticeably by the artificial application of normal pollen grains.

Tetraploid: The degree of seed fertility differed considerably in tetraploids of different origin, the fertility being loss than 6% for the lowest line and 38% for the highest. In the course of 10 generations the fertility of the lowest lines was slightly improved. The tetraploid strain which has a history of nearly 15 years showed about 70% sterility. In certain cases, hybridization slightly improved the fertility of tetraploids (MORINAGA AND KURIYAMA, 1946).

The author and his co-workers observed somatic divisions, microsporogenesis, megasporogenesis and embryo-sac formation in these polyploids. The mode of association of chromosomes in the first division of meiosis was as follows:

In the haploid, 98 microsporocytes in metaphase and early anaphase showed distinctly 12 univalents, and in 37 sporocytes two or rarely four chromosomes appeared in contact. Such chromosomes were observed near the pole as well as near the equator. Some of the pairs near the equator were no doubt true bivalent chromosomes. At anaphase, 6–6 distribution of univalents to each pole was observed most frequently; 5–6 and 4–8 distributions followed in that order. The entire complement of 12 chromosomes were rarely found in the same pole region.

In triploids, 36 chromosomes were usually arranged in 12 groups of 3 each at diakinesis. In metaphase I usually 12 or more chromosomes are counted, the largest number observed being 18 ($6_{III} + 6_{II} + 6_{I}$). In the following anaphase, the trivalent chromosomes disjoin to the 3 components, and one travels to one pole and the other two to the opposite pole.

In tetraploids, 13–20 chromosomes were counted at diakinesis, the average number being 16.04. In metaphase I, quadrivalents and bivalents were easily identified and the maximum and minimum numbers of quadrivalents observed were 12 and 6 respectively, the average number being 9.38. In the following anaphase, the chromosomes disjoined into four homologues, which, as a rule, were evenly distributed to each pole.

The cross, haploid × diploid, was repeated by TAKAHASHI (1936), but the hybrid plants obtained were exclusively diploid, showing that male gametes containing less than 12 chromosomes, for instance 5 or 7, were not functional. The author and his coworkers found in 1936 a sterile and extremely dwarf mutant in a presumably pure line originated from the haploid. The plant had 12_{II} chromosomes, showing no irregularities at meiosis (MORINAGA, KURIYAMA AND AOKI, 1942).

In 1934, the author and his coworkers (YUNOKI AND MASUYAMA, 1945) obtained 150 plants raised from seed sets on the triploid plants, of which 26 were abnormal. Four abnormal plants produced only normal plants, and the other abnormal plants produced simple trisomics ($2n = 25$) of various types, double trisomics ($2n + 1 + 1$) and simple tetrasomics ($2n + 2$), in addition to normal diploids. The trisomic plant often produced fewer trisomic progenies than theoretically expected. The percentage of sterility of the

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simple trisomic plant was variable according to the type of trisomic, and the sterility percentage of the simple tetrasomic plant was always very low.

The author made crosses between autotetraploid lines to ascertain the segregation ratio of the characters in F_2 (MORINAGA, 1951). The glume tip color segregated in a ratio close to 20.8:1, which approaches the theoretical ratio under the random assortment of chromatids. In the F_3 generation, however, the ratios approximated more the theoretical ratios under the random assortment of chromosomes.

The author will now refer to a few of the important studies subsequently done in Japan on the problems hitherto untouched.

NAGAMATSU (1956) classified abnormal derivatives discovered among the progeny of rice plants collected in Nagasaki several months after the atomic bomb explosion into three categories by their genetic behavior. Non-genetic type, which contained various teratological plants, appeared in earlier generations. Gene mutations for high sterility, miniature plant form, large or coarse grain, long outer glumes, zebra striping in chlorophyll development, etc., appeared also in earlier generations. Chromosomal aberrants, the third category, were easily subdivided into structural hybrids, heteroploids and polyploid series. The structural hybrid appeared most frequently among all abnormal derivatives of earlier generations, and the heteroploids were found among the descendants of such structural hybrids. Haploid and polyploid series appeared spontaneously with a low frequency in rather early generations.

A related phenomenon was reported by KATAYAMA (1941). He obtained asynaptic plants of normal appearance by X-ray irradiation. No pairing of chromosomes was seen at zygotene and pachytene stages. The number of paired chromosomes at diakinesis and MI varied from 0 to 12. The average number of chiasmata per bivalent was 1.03 for the asynaptic plant, and 2.21 for the normal when all chromosomes, paired and unpaired, were taken into consideration. A single recessive gene is responsible for this asynaptic character.

NISHIMURA (1961) found in the progeny of rice subjected to atomic bomb explosion and X-ray irradiation 224 semi-sterile strains of which 153 were confirmed cytologically as strains involving reciprocal translocations. He selected from those only the strains homozygous for the translocated chromosomes and made hybrids of various combinations among them to determine, by the mode of chromosomal association, the mutual relation of the translocated chromosomes. Then, he crossed certain selected translocation homozygotes and cytologically normal strains, and studied the linkage relation between the character and sterility to identify the chromosome on which the gene is located. He deduced that, the gene for a short culm character was located on his chromosome IV, and six other genes were located on five other chromosomes.

In 1941, YASUI (1941) reported that the ratio of the square root of the average area of haploid cells to that of diploid is ca. 1:1.24, which is very near to $\sqrt[3]{2}$, in other words, the 2x cells have about 2 times the volume of 1x cells.

For the chromosome sizes, the following was given:

Chromosome No.	Relative length of the chromosome	Ratio of the short arm to the whole chromosome
1	ca. 5.0	ca. 2/5
2	4.4	1/2
3	4.1	1/2
4	3.8	1/2
5	3.7	2/5
6	3.5	2/5
7	3.1	2/5
8	3.0	1/3
9	2.9	1/2
10	2.6	1/6 (SAT)
11	2.3	2/5
12	2.0	3/8

CUA (1952) made cytological observations on diploid F_1 and colchicine-induced tetraploid F_1 of the cross Sekitori (*japonica*) × Konanto (*indica*). The pollen fertility of the former was 50.24% and that of the latter, 94.5%, the percentage of seed-setting being 11.38% and 70.32% respectively. At meiotic metaphase in the diploid F_1 hybrid, the regular 12-bivalent types of plates were frequently observed. In the meiotic metaphase of the tetraploid F_1 hybrid, bivalents and varying numbers of polyvalents were observed. The number of quadrivalents per cell ranged from 12 to none (24 bivalents), 7 or 6 quadrivalents occurring most frequently. Besides quadrivalents, trivalents and univalents were occassionally observed. An octavalent, probably a secondary association of two quadrivalents, was observed only once in the 88 perfectly clear cells studied. The intersubspecific tetraploid hybrid had less quadrivalents per cell in the first metaphase of meiosis than "autotetraploid" varieties, either spontaneously produced or colchicine induced, being 6.06 as against 9.38 (MORINAGA AND FUKUSHIMA, 1937) and 8.93 (CUA, 1952). Previously, CUA (1951a) reported that the sterility of the intersubspecific F_1 hybrid was reduced when the whole chromosomes were doubled. In the progenies of such intersubspecific tetraploid hybrids, he found strains with more than 80% fertility.

Later, OKA *et al.* (1954) reported that the average number of quadrivalent and bivalent chromosomes per cell (in tetraploid) differed with the variety or hybrid and ranged from 5 to 9 or from 5 to 12 respectively. It was generally found that in intervarietal hybrids the number of quadrivalent and univalent chromosomes was less than in "autotetraploid" varieties. However, hybrids between remote varieties and between closely related ones showed no significant difference in this tendency. Hybrids were generally higher in fertility, but among the hybrids or the parental varieties, no significant correlation was found between the number of quadrivalent or univalent chromosomes and fertility. The improvement of chromosome behavior in hybrids may be due either to selective pairing of chromosomes derived from the same parent or to a complementary effect of parental genes.

In 1936, SAKAI (1935b) concluded that 12 is not the basic chromosome number of the species, *Oryza sativa,* in the strict sense, but that 5 is the primary number from which

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the 12 were derived. In other words, *Oryza sativa L.* is a double hexasomic tetraploid, which may be represented as follows:

$n = 12$

A_1	A_2	
B_1	B_2	
C_1	C_2	C_3
D_1	D_2	D_3
E_1	E_2	

Cytological data supporting the above conclusion were: (1) quadrivalent association of chromosomes at diakinesis and metaphase I was observed in the normal diploid, (2) secondary association of the meiotic chromosomes was very distinct; the maximum association being two groups of three, and three groups of two. Average associations in PMI, MI and MII were 4.83, 3.73 and 4.37 respectively,

OKUNO (1944) observed in meiotic division of *Oryza sativa* the following 13 types of secondary associations: 2(3) + 3(2), 2(3) + 2(2) ± 2(1), 2(3) + 1(2) + 4(1), 1(3) + 4(2) + 1(1), 1(3) + 3(2) + 3(1), 1(3) + 2(2) + 5(1), 1(3) + 1(2) + 7(1), 5(2) + 2(1), 4(2) + 4(1), 3(2) + 6(1), 2(2) + 8(1), 1(2) + 10(1), and 12(1). Associations involving two groups of three and three groups of two were most common. Associations of different sized bivalents were often observed.

TAKENAKA, HU AND TATEOKA (1956) found only univalents in 64% of pollen-mother cells of haploid plants, one bivalent in 33% of the cells and two bivalents in 2% of the cells. Cells with three bivalents or one or two trivalents were very rare. They also observed "secondary association" of chromosomes. Taking both kinds of association together, the chromosome configurations varied from 12(1) to 5(2) + 2(1) with the mode at 2(2) + 8(1) during diakinesis, from 12(1) to 2(3) + 3(2) with the mode at 1(3) + 3(2) + 3(1) during diakinesis-metaphase, and from 12(1) to 2(3) + 3(2) with the mode at 3(2) + 6(1) during metaphase-anaphase. The highest association found was 5(2) + 2(1) at diakinesis. Assuming the secondary association to be due to residual homology, they suggested the chromosome set of rice a b c d e a' b' c' d' e' a" b"

Hu (1957) also investigated the mode of chromosome association in haploid rice. The primary as well as secondary association was most apparent at the transitional stage from late diakinesis to MI. The mode of association was 1(3) + 3(2) + 3(1), and the maximum association was 2(3) + 3(2). He said the primary and secondary associations in haploid rice might suggest the presence of some residual homology among the twelve chromosomes of the present genome of rice. Hu (1958) observed a tendency of somatic pairing at the mitotic metaphase of haploid rice. The maximum pairing consisted of four groups of two and one group of three chromosomes. From the results of observations he assumed the karyotype of rice as follows:

$A_1^{sm} + A_2^{sm} + csB^{st} + C_1^{m} + C_2^{m} + 2D^{sm} + cs^{t}E_1^{st} + {}^{t}E_2^{st} + F^{sm} + 2G^{m}$									
(1)	(12)	(2)	(3)	(4)	(5, 6)	(7)	(8)	(9)	(10, 11)

ISHII AND MITSUKURI (1960) suggested the karyotypes and numerical designations as follows:

Diploid *Oryza sativa* L. (Norin No. 8)

$$K = 2n = 24 = A_1^{sm} + csA_2^{sm} + B^{sm} + C_1^{st} + {}^tC_2^{sm} + 2C_3^{sm} + D_1^{sm} + 2D_2^{m} + E_1^{sm} + E_2^{m}$$

Haploid *Oryza sativa* L. (Norin No. 8)

$$K = 2n = 12 = A_1^{sm} + A_2^{m} + B_1^{sm} + {}^tB_2^{st} + B_3^{sm} + C_1^{m} + csC_2^{st} + D_1^{sm} + 2D_2^{sm} + D_3^{m} + E^{sm}$$

According to them, the karyotype of *Oryza sativa* has three or four pairs of median chromosomes, and six or seven pairs of subterminal or submedian chromosomes. They observed one pair of chromosomes with secondary constrictions and SAT-chromosomes,

With respect to the number of nucleoli appearing in the somatic telophase, SAKAI (1938) found two distinct types of rice. The subspecies *indica* had four nucleoli and four nucleolar chromosomes (quadrinucleolar type) and the subspecies *japonica* had two nucleoli and two nucleolar chromosomes (binucleolar type). An autotetraploid plant of *japonica* showed four nucleoli and four nucleolar chromosomes. In the meiotic prophase of F_1 hybrid between indica and japonica, ten ordinary and two nucleolar bivalents were found.

According to OKA AND KAO (1956), about two-thirds of the continental and a half of the tropical-insular varieties were of the quadri-nucleolar type. In the former, the mean number of nucleoli ranged from 2.2 to 3.6. All Japanese lowland varieties were of the binucleolar type, while both bi- and quadrinucleolar types were found in different proportions in other regions. They said that rice might have two pairs of nucleolar chromosomes, and the nucleolus-forming power of the second pair might vary continuously among varieties.

SHINOHARA (1962) examined the number of nucleoli of *Oryza* species having AA genomes, and found that the variation in nucleolar number is continuous. If strains with more than 2.1 nucleoli per cell are taken as the quadrinucleoli, and those with less than 2.1 as binucleolar type, all the species investigated can be said to have both types. It is found, however, that strains belonging to *Oryza perennis* and *sativa* f. *spontanea,* as well as those of the *indica* type of *Oryza sativa* are mostly of *quadri-nucleolar* type, while strains of *Oryza breviligulata* and *glaberrima* are binucleolar. Another point of interest was that plants collected from adjacent sites, belonging actually to the same population, showed a marked difference.

Since 1933, study of the wild species of rice, and their hybrids with cultivated rice, became increasingly important in Japan. In 1933, GOTOH and OKURA reported the somatic number of chromosomes of *Oryza cubensis* Ekman, *latifolia* Desv, and Oniine (later identified as *Oryza sativa* f. *spontanea;* – cf. HARA, 1954) as 24, 48 and 24 respectively. The next year, the author (MORINAGA, 1934) reported the chromosome number of *Oryza minuta* Presl as $n = 24$ and $2n = 48$. In 1935, GOTOH AND OKURA (1935), confirming the gametic number of chromosomes of *cubensis, latifolia* and Oniine as 12, 24 and 12, described briefly the hybrids, *sativa* × *cubensis* and *sativa* × *latifolia.* Meiosis progressed normally in the former hybrid, which was highly sterile and resembled *cubensis;* in the latter, which showed an intermediate appearance, no pollen-mother cells could be found in division. In 1937, the author (MORINAGA, 1937)

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reported on the microsporogenesis of three kinds of interspecific hybrids, *sativa* × *minuta, minuta* × *latifolia* and *sativa* × *latifolia.* The F_1 hybrid of *sativa* × *minuta* showed 36 univalents at diakinesis, and the same number of univalents were also found usually in metaphase I, though a few bivalents were sometimes found. The F_1 hybrid of *minuta* × *latifolia* showed ca. 36 chromosomes of both univalent and bivalent nature at diakinesis. In metaphase I, ca. 12 bivalents arranged themselves regularly on the equatorial plane, the corresponding number (12) of univalents surrounding them in a circle. The F_1 hybrid of *sativa* × *latifolia* showed 36 univalents in diakinesis and ca. 36 univalents in metaphase I. From these observations the author postulated that the genome of *sativa* and those of *minuta* and *latifolia* were of remote affinity with each other and that the two di-genomic species *minuta* and *latifolia* contained only one genome in common.

At the same time, HIRAYOSHI (1937) reported on the F_1 hybrid of *sativa* and *latifolia.* We described that at the metaphase I of the hybrid, 36 univalent chromosomes appeared near the equatorial plane. Occasionally, he saw two or three loosely associated bivalents but they usually moved to either one of the poles without separation. He concluded that perhaps neither of the two genomes of *latifolia* was homologous to the genome of sativa.

Just then OKURA (1937) also reported on the F_1 hybrid of *sativa* and *minuta.* The results of observations on the configuration of chromosomes in first prometaphase, metaphase and anaphase were summarized by him as follows:

Chromosome elements	22	23	24	25	26	Total
Configuration	2III + 10II + 10I	lIII + 11II + 1II	12II + 12I	1III + 10II + 13I	11II +14I	10II + 16I	
PMCs observed	27	6	3	15	2	3	56
%	48.2	10.7	5.4	26.7	3.6	5.4	100

From the above observations he considered that the hybrid contained 12 sets of homologous chromosomes, and one or two sets rarely fail to conjugate.

The author and his co-workers (MORINAGA AND AOKI, 1938; MORINAGA AND KURIYAMA, 1943) described in 1938 and 1943, the crossing, morphology and sterility of the hybrids, *sativa* × *minuta, sativa* × *latifolia, minuta* × *latfolia* and *sativa* × *cubensis.* In the crossing experiments, *sativa* × *officinalis, minuta* × *officinalis, latifolia* × *officinalis, officinalis* × *cubensis* and *minuta* × *cubensis,* they could not obtain until that time any true adult F_1 plants. In 1940 (MORINAGA, 1940), the author described the hybrid *sativa* × *minuta.* The PMCs at diakinesis, which were difficult to obtain, contained about 36 chromosomes, showing no regular formation of bivalents. To decide on the degree of bivalent formation, if any, the author carefully examined mote than 40 pollen-mother cells in the metaphase or early anaphase of the first meiotic division. Though the exact counting of the whole chromosomes was fairly difficult, the total number of chromosomes was always 36 or close to 36. Occasionally, one, and

infrequently, two or three clear bivalents, were noticed in those stages. As already mentioned, however, such bivalent formation was also occasionally noticed in metaphase I or anaphase I of the haploid plant of *sativa.* Thus, the author concluded that the chromosomal set of *Oryza sativa* L. was not homologous to either one of the two sets composing the chromosome complex of *Oryza minuta* Presl.

In 1941, the author (MORINAGA, 1941) reported in detail on the hybrid, *sativa* × *latifolia.* Degeneration of archesporial and pollen-mother cells occurred in this hybrid. In the first meiotic division, no regular bivalent formation occurred. Counting occasional bivalent formation (1–3), as autosyndesis, the author also concluded that, the chromosome set of *Oryza sativa* L. was not homologous to either one of the two sets comprising the chromosome complex of *Oryza latifolia* Desv.

In 1943, the author (MORINAGA, 1943) reported on the hybrid, *minuta* × *latifolia.* At the full metaphase of the first division, the bivalent chromosomes, 12 in number, arranged themselves regularly on the equatorial plate, and most of the 24 univalents came close to the equator and surrounded the group of bivalents. Thus the author concluded that one chromosomal set in *Oryza minuta* Presl was similar to a set in *Oryza latifolia* Desv., the other set of each species being different from each other.

To satisfy the above mentioned relations that the chromosomal set of *sativa* is dissimilar to either one of those two chromosomal sets of *minuta* or *latifolia* and that *minuta* and *latifolia* contain only one chromosomal set in common, the genomic constitutions of those three species are formulated by the author as follows:

Oryza sativa L.	($2n$ = 24)	AA
Oryza minuta Presl	($2n$ = 48)	BBCC
Oryza latifolia Desv.	($2n$ = 48)	CCDD

In 1956, MORINAGA AND FUKUSHIMA published the results of observations on the backcross progenies of two interspecific hybrids, namely, (*sativa* × *minuta*) × *sativa* and (*sativa* × *latifolia*) × *sativa,* or (A × BC) × A and (A × CD) × A according to the genomic constitution postulated above. These backcrossed plants invariably possessed 48 (36 + 12) somatic chromosomes. This suggests that, of the gametes produced by the F_1 plants ABC (hybrid of AA and BBCC) and ACD (hybrid of AA and CCDD), only those unreduced ones with all 3 genomes can produce viable zygotes upon mating with the gamete from AA plant. Thus, the genomic constitutions of the backcrossed hybrids treated in this report are presumed as ABC + A and ACD + A. As a matter of course, then, in their sporocytes *Drosera* scheme first meiotic divisions with 12 bivalents (2A) and 24 univalents (B and C or C and D), will be expected. The results of cytological observations correspond with this expectation.

In 1956 and 1957, MORINAGA AND KURIYAMA reported in detail on the hybrids, *sativa* × *cubensis* and *sativa* × *glaberrima.* Though both of these hybrids were very highly sterile, the meiosis appeared normal showing 12 bivalent chromosomes. In the former hybrid the authors observed, as a rare exception, a few univalents in anaphase I (MORINAGA AND KURIYAMA, 1956, 1957b).

In 1957 and 1960, the author and his co-workers (MORINAGA AND KURIYAMA, 1957a; MORINAGA, KURIYAMA AND ONO, 1960) reported briefly on the hybrids, *sativa* × *bre-*

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viligulata and *sativa* × *australiensis.* In the former hybrid they observed 12 bivalent chromosomes exclusively, and in the latter hybrid they usually found 24 univalent chromosomes, in both cases during metaphase I. The chromosomes of *australiensis* were clearly larger than those of sativa.

Reporting more recently on 12 interspecific hybrids: *sativa* × *perennis, sativa* × *officinalis, glaberrima* × *breviligulata, sativa* × *paraguaiensis, sativa* × *eichingeri, glaberrima* × *eichingeri, officinalis* × *latifolia, officinalis* × *paraguaiensis, minuta* × *officinalis, eichingeri* × *minuta, latifolia* × *paraguaiensis* and *minuta* × *paraguaiensis,* we postulated (MORINAGA, 1959; MORINAGA and KURIYAMA, 1960) the genomic constitution of the 11 species involved as follows :

I.	AA group	*sativa, cubensis, glaberrima, breviligulata, perennis*
II.	CC group	*officinalis*
III.	BBCC group	*minuta, eichingeri*
IV.	CCDD group	*latifolia, paraguaiensis.*

The genomic constitution of *australiensis* is not quite decided yet, but it is clearly dissimilar to AA.

Adding new observations on the hybrid *officinalis* × *australiensis, minuta* × *australiensis* and six other hybrids between *grandiglumis* and other species, the experimental results obtained by the author and his co-workers are compiled in Table 1 to show the mode of chromosome conjugation. Table 2 shows the genomic constitution postulated for those 12 species involved. The constitution of *australiensis* is not clear yet, as

TABLE 1

CHROMOSOME PAIRING IN PARENTS AND INTERSPECIFIC HYBRIDS OF *Oryza*

Hybrid		Pairing in F_1	
		Mode	Occasionally
	(2*n* × 2*n*)		
sativa (12 II)	× *cubensis* (12 I)	12 II	few I
,,	× *glaberrima* (12 II)	12 II	
,,	× *breviligulata* (12 II)	12 II	
,,	× *perennis* (12 II)	12 II	
glaberrima (12 II)	× *breviligulata* (12 II)	12 II	
sativa (12 11)	× *officinalis* (India) (12 II)	24 I	1–2 II
,,	× *officinalis* (Ceylon) (12 II)	24 I	
,,	× *australiensis* (12 II)	24 I	
officinalis (Burma) (12 II)	× *australiensis* (12 II)	24 I	1–3 II
	(2*n* × 4*n*)		
sativa (12 II)	× *paraguaiensis* (24 II)	36 I?	
,,	× *grandiglumis* (24 II)	36 I	
,,	× *latifolia* (24 II)	36 I	1–3 II
,,	× *latifolia* (Guiana) (24 II)	36 I	1–5 II
,,	× *eichingeri* (24 II)	36 I	
sativa (12 II)	× *minuta* (24 II)	36 I	1–3 II
glaberrima (12 II)	× *eichingeri* (24 II)	36 I	
,,	× *grandiglumis* (24 II)	36 I	
minuta (24 II)	× *australiensis* (12 II)	36 I	1–3 II

Table 1 (continued)

Hybrid		Pairing in F_1 Mode	Occasionally
officinalis (Ceylon) (12 II)	× *latifolia* (M II)	12 II + 12 I	only 11 II
officinalis (India) (12 II)	× *latifolia* (24 II)	12 II + 12 I	only 11 II
officinalis (Ceylon) (12 II)	× *paraguaiensis* (24 II)	12 II + 12 I	
	× *grandiglumis* (24 II)	12 II + 12 I	
minuta (24 II)	× *officinalis* (Ceylon) (12 II) ($4n \times 4n$)	12 II + 12 I	
eichingeri (24 II)	× *minuta* (24 II)	24 II	
latifoliu (24 II)	× *paraguaiensis* (24 II)	24 II	
latifolia (Guiana) (24 11)	× *paraguaiensis* (24 II)	24 II	
	× *grandiglumis* (24 II)	24 II	
grandiglumis (24 II)	× *latifolia* (24 II)	24 II	
paraguaiensis (24 II)	× *grandiglumis* (24 II)	24 II	
rninuta (24 II)	× *paraguaiensis* (24 II)	12 II + 241	
”	× *latifolia* (24 II)	12 II + 24 I	
”	× *latfolia* (Guiana) (24 II)	12 II + 24 1	
”	× *grandiglumis* (24 II)	12 II + 241	

TABLE 2

GENOMIC CONSTITUTION OF *Oryza* SPECIES

Species	Chromosome Number	Genomic Constitution	Native place
sativa L.	12 II	AA	
cubensis Ekman	12 II	AA	Cuba
gluberrima Steud.	12 II	AA	West Africa
breviligulata Chev. et Roehr.	12 II	AA	West Africa
perennis Moench	12 II	AA	Tropical America, West Indies, Tropical Africa
officinalis Wall.	12 II	CC	Burma, Ceylon
australiensis Domin	12 II	Not A, B or C	Australia
latifolia Desv.	24 II	CCDD	Middle, South America
paragwiensis Wedd	24 II	CCDD	South America
grandiglumis Doell.	24 II	CCDD	South America
minuta Pres1	24 II	BBCC	Philippines
eichingeri Peter	24 II	BBCC	East Africa

the hybrid between this species and CCDD species did not provide the opportunity to study the microsporocytes satisfactorily.

KIHARA and co-workers reported the results of their extensive studies on the crossability and chromosomal affinity among 17 species of *Oryza.* No intra- and intersectional cross was succesful except those between species within the section *Sativa* Roschev. They established two intrafertile but intersterile groups among diploid species possessing the A genome. One group consists of three Asian species, *sativa, sativa* var. *spontanea* and *perennis,* while to the other belong three African species, *glaberrima, bre-*

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viligulata and *stapfii,* A similar case of two fertility groups concerns the American tetraploid species with the genome formula CCDD, namely *latifolia* and *alta.* KIHARA *et al.* suggested that differentiation of genes and structural changes of chromosomes took place in the course of evolution from the ancestral species (KIHARA, 1959; NEZU *et al.,* 1960).

KIHARA *et al.* also made clear the genomic constitution of 12 species, including *stapfii* (AA) and *alta* (CCDD), which the author had not studied. They concluded that the B genome of *minuta* and *eichingeri* is partially homologous to A (NEZU *et al.,* 1960; KIHARA *et al.,* 1961).

In the field of rice cytogenetics there are still many important problems that remain unsolved.

CYTOGENETIC STUDIES AT THE LOUISIANA AGRICULTURAL EXPERIMENT STATION OF SPECIES RELATIONSHIPS IN *ORYZA*

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The research reported here was performed by Dr. BIRDIE YEH while a graduate student at Louisiana State University. Most of the studies involved have been published (YEH AND HENDERSON, 1961, 1962) but one of the publications has been off the press for less than a month. The investigations were supported in part by a grant from The Rockefeller Foundation. Assistance of Dr. C. R. ADAIR, ARS, United States Department of Agriculture, in obtaining the various species of *Oryza* included in the study is gratefully acknowledged.

For too many years only a limited amount of research was conducted in the field of cytogenetic relationships among the diploid species of *Oryza* in comparison to the extensive research of this type in such other genera as *Triticum, Gossypium,* and *Nicotiana.* Within the past few years, however, research of this nature has been initiated in *Oryza* at several locations. With this encouraging development, the deficiency of fundamental information in species relationships in *Oryza* should be corrected in time. Because of the wide distances separating the personnel conducting this research and other factors, there is an apparent lack of uniformity in the criteria adopted for differentiating species and chromosome genomes. This already is leading to unfortunate differences among investigators in treatment of the genus, differences which are barriers to progress in rice research.

SPECIES INCLUDED IN STUDIES

All of the species investigated were diploid, In some instances a species has been designated even in recent literature by two or more somewhat different scientific names. Consequently, in order to establish the identity of each species with certainty, the following information is supplied concerning each:

Oryza sativa. – Altogether, 10 varieties of *sativa* were used in crosses with the other *Oryza* species. These were selected to represent wide genetic diversity in the species, including *japonica* and *indica* types and varieties developed in the United States possessing germ plasm from both of these types.

Oryza glaberrima. – Three varieties of this cultivated species native to tropical west Africa were among the hybrids studied. All were typical of the species described in the literature. There have been no problems in classification of this species. However, of four

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strains from Africa supplied the Station for this research under the designation *glaberrima*, one strain had the distinguishing characteristics of *sativa* instead and did not behave like the other *glaberrima* strains in hybrids. It appears that occasionally, *Oryza sativa* varieties are collected in tropical west Africa under the assumption that they belong to the *glaberrima* species. This confuses experiments.

Oryza sativa var. *fatua.* – Apparently this group of highly variable, annual wild rices, closely resembling cultivated *sativa*, was first recognized by KOENIG in 1839 as a distinct species and assigned the name *fatua.* In 1903, PRAIN considered this group of rices to be a botanical variety of the principal cultivated species and assigned the name *sativa* var. *fatua.* In 1931, ROSCHEVICZ referred to these rices as *sativa* f. *spontanea.* In recent years, workers in Asia have tended to adopt the name *spontanea.* I am unaware of any logical reason to reject the name *fatua*, which merely means a wild form, for this group. Rices of this type occur commonly in cultivated fields of rice in the United States, where they are known as "red rice".

Oryza sativa var. *formosana.* – This distinct type from Taiwan was originally described in 1935 by SUZUKI AND MASAMUNE as a separate wild species with the binomial *Oryza formosana.* In 1942, HARA reclassified it as *Oryza sativa* var. *fatua.*

Oryza perennis subsp. *cubensis.* – This wild form, native to the West Indies and found primarily in Cuba, was described and assigned the name *perennis* by MOENCH in 1794. It apparently represents the original type of the species, I wish to emphasize this fact. Before other wild forms of *Oryza* are assigned this species designation, it should be established that they bear a sufficiently close relationship to the West Indian type to justify their inclusion in the species *perennis.*

Oryza perennis subsp. *barthii.* – This wild form is native to tropical Africa. It was described first by CHEVALIER in 1910 as a distinct species, *barthii.* In 1914, however, CHEVALIER AND ROEHRICH renamed it *longistaminata*, Then in 1932, CHEVALIER pointed out that this species fits in many respects the description of *perennis* and suggested that it be recognized as a subspecies of *perennis.* I have reservations concerning this classification but not sufficient evidence to warrant changing the recommendation of CHEVALIER.

Oryza balunga (*perennis* var. *balunga* of SAMPATH AND GOVINDASWAMI). – It is apparent that this wild, perennial form has been described in earlier publications by several workers as *Oryza sativa* var. *fatua.* However, RAMIAH AND GHOSE (1951) distinguished it from *fatua* and referred to it as an Asiatic form of *perennis.* In 1958, SAMPATH AND GOVINDASWAMI gave a more complete description and adopted the name *Oryza perennis* var. *balunga.* Reasons for treating this form as a distinct species will be given later.

Oryza breviligulata. – There has been no confusion about this wild annual form, native to tropical west Africa, since it was described and named by CHEVALIER AND ROEHRICH in 1914.

Oryza glaberrima var. *stapfii.* – ROSCHEVICZ in 1931 treated this wild African form of *Oryza* as a separate species with the designation *stapfii.* Most workers have adopted this classification. The limited research reported here tends to confirm the conclusion

of CHEVALIER, however, that this *Oryza* form can be considered a botanical variety of *glaberrima.* Additional research is needed.

CRITERIA USED IN THE STUDY OF SPECIES RELATIONSHIPS

As yet, various workers in this phase of rice research have not developed and adopted uniform procedures for a classification of the genus *Oryza* that would be based primarily on cytogenetic evidence. All previous attempts at classification of *Oryza* have been based solely on the morphological characters of conventional taxonomy. These classifications of the genus have been useful in rice cytogenetics, but it is obvious that a systematic treatment based primarily on cytogenetic evidence, or at least one which strongly considers this type of evidence, is badly needed.

The research being reported here attempted to adapt to *Oryza,* in the most feasible manner, the procedures and criteria used in cytogenetic studies with other plant genera. There is no intent to imply that others should adopt these criteria, but it is felt that these criteria represent a step in the desirable direction.

The types of information obtained and used in the investigation of cytogenetic relationships reported here included crossability of species, chromosome behavior during meiosis in hybrids, fertility of hybrids, morphological characters, geographic distribution and previous taxonomic classifications of the species studied. Since this was primarily a cytogenetic study, greatest attention and weight were given to the first three of these criteria.

With respect to crossability of species, records were kept of the percentage of emasculated and pollinated florets which set seed in each cross, and the results were compared with those from intervarietal crosses within *sativa* and *glaberrima.* Studies of chromosome behavior during meiosis involved pairing at diakinesis and metaphase I, occurrence of various abnormalities at anaphase I and more limited studies of meiosis II. Chromosome behavior in homozygous varieties and intervarietal hybrids was used for comparison.

In most genera of plants, sterility does not occur in intervarietal hybrids. Consequently, the occurrence of sterility, whether complete or only partial, in hybrids between distantly related forms is usually taken as sufficient evidence of a cytogenetic barrier to warrant recognition of the parents as separate species. However, since sterility is characteristic of many intervarietal hybrids within *sativa,* the mere occurrence of partial sterility in hybrids between distantly related forms of *Oryza* obviously does not constitute valid evidence for placing the parents in separate species. On the other hand, the consistent occurrence of complete sterility, or a markedly higher degree of partial sterility than characteristic of intervarietal hybrids within *sativa,* should be considered in differentiating *Oryza* species.

RESULTS OF THE CYTOGENETIC EXPERIMENTS

Crossability. – All crosses were made in the greenhouse but were spread over more

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than 2 years and were done during different seasons. All attempts at hybridization among the seven wild and two cultivated species described earlier were successful. Although wide differences were found among the crosses in percentage of pollinated florets that set seed, all combinations were within the range of crossing percentages obtained among varieties of *sativa* under similar conditions. Consequently, it was concluded that the various species in the study could be hybridized about as readily as varieties of cultivated rice and that the data on crossability did not show any differentiation among the species. The form designated as *Oryza perennis* subsp. *barthii* from Africa represented an exception to this rule. Numerous attempts to cross it with varieties of *sativa* resulted in only one F_1 plant. This wild type proved completely self-sterile, however, and it is felt that the low success in crosses did not necessarily indicate lack of relationship.

Chromosome Behavior in Meiosis and Fertility of Hybrids. – Based on chromosome behavior and fertility of the hybrids, the nine species studied could be divided into three groups. One group included *sativa, sativa* var. *fatua, sativa* var. *formosana* and *balunga* (*perennis* var. *balunga*). A second group was composed of *perennis* subsp. *cubensis* and *perennis* subsp. *barthii.* The third group consisted of *glaberrima, glaberrima* var. *stapfii,* and *breviligulata.*

No cytogenetic differentiation was found among the members within each group of species. Chromosome behavior in meiosis and fertility of hybrids within the groups were as normal and regular as in intervarietal hybrids of *sativa.* On the other hand, irregularities of various types were found in hybrids between members of the different groups.

The four members of the first group of species, which included *sativa,* are all native to southeast Asia and differed only in respect to certain morphological and physiological traits, with no evidence of any cytogenetic barrier between them. Because of the close cytogenetic relationship and the distinct similarity in key morphological characters of the wild forms *sativa* var. *fatua* and *sativa* var. *formosana* to cultivated rice, *sativa,* the results were interpreted as confirming the previous classification of these wild forms as botanical varieties of *sativa.* The perennial wild form from southeast Asia which was designated *balunga* in these studies was also indistinguishable cytogenetically from *sativa.* However, it did differ appreciably from cultivated rice in several morphological features and, on this basis, should more properly be considered a separate, though very closely related, species.

In the second group, composed of the wild types *perennis* subsp. *cubensis* from the West Indies and *perennis* subsp. *barthii* from Africa, only a single F_1 plant was obtained from attempts to hybridize the two and it died after heading but before data on seed setting could be obtained. Consequently, the Cytogenetic evidence of the relationship, is too meager to permit reliable conclusions. Only slight irregularities were found in meiosis of the hybrid and 100% of pollen grains examined were stainable. However, the two forms show considerable morphological differences and have obviously been separated in geographical distribution for a long period. Until more critical evidence

is available, the proposal of CHEVALIER that these two forms be considered subspecies of *perennis* has been adopted.

The relationship between *sativa* and *perennis* subsp. *cubensis* was investigated intensively in hybrids involving seven cultivated varieties crossed with the wild form. In these seven hybrids, meiosis was essentially regular. However, none of the F_1 plants of the seven hybrids set any seed and only 10–15% of the pollen grains of each hybrid were stainable. The consistent occurrence of virtually complete sterility in all hybrid combinations was interpreted as indicating a cytogenetic barrier of some nature between *sativa* and *perennis* subsp. *cubensis* that is markedly greater than within *sativa* or between *sativa* and the other forms placed in the same group in the previous discussion. Unquestionably, the recognition of *sativa* and *perennis* as distinct species is justified.

Special attention is directed to the relationship between *perennis* subsp. *cubensis* and the southeast Asiatic perennial form that also has been considered in most of the recent publications to be a botanical variety of *perennis.* A large body of detailed evidence of an indirect nature places some doubt on the validity of this classification. Among the hybrids involving five varieties of *sativa* crossed with the southeast Asiatic form, three hybrids were completely fertile and the other two had approximately 80% of stainable pollen. When this high fertility is contrasted with the evidence of almost complete sterility in seven hybrids of *sativa* varieties crossed with *perennis* subsp. *cubensis,* the evidence suggests strongly that it would be more logical and useful to place the southeast Asiatic form in a separate species from *perennis.* These two forms are definitely not related equally to *sativa* from the cytogenetic viewpoint. In fact, since the relationship of *sativa* to the southeast Asiatic perennial form is much closer than that of *sativa* to *perennis* subsp. *cubensis,* it seems illogical to place the two wild types in the same species without including *sativa* with them.

A direct study of the relationship between *Oryza perennis* subsp. *cubensis* and the southeast Asiatic form confirmed the tentative conclusion from indirect evidence. Although both types have long ligule and long anthers and are perennial, traits used for recognizing them as members of the same species, they still differ greatly in many morphological and physiological traits. Furthermore, the hybrid between these forms was completely sterile in somewhat limited tests and 21% of PMCs had univalents at metaphase I. On the basis of this indirect and direct evidence, it was proposed that the southeast Asiatic perennial form be recognized as a separate species, for which the binomial *Oryza balunga* was adopted tentatively.

Only one hybrid plant was obtained from numerous attempts to cross *perennis* subsp. *barthii* with varieties of *sativa.* The F_1 plant was a vigorous perennial and meiosis was essentially regular. Only 5% of the florets on the F_1 plant set seed, though an appreciable percentage of the pollen grains were stainable. As pointed out earlier, the data from *perennis* subsp. *barthii* were too meager to permit reliable conclusions concerning its relationships.

Species of the third group, made up of *glaberrima, glaberrima* var. *stapfii* and *brevili-*

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gulata, are annuals and are indigenous to tropical west Africa. As in the two previous groups, hybrids between members within this group were either completely fertile or had only slight degrees of sterility and meiosis was normal. It is apparent that these three annual African diploid types compose a group of closely related, interfertile forms comparable to the first group described earlier.

The behavior of hybrids between *sativa* varieties and the three African forms is of interest. Altogether, 13 hybrid combinations of this sort were studied in great detail. All 13 combinations gave highly consistent results. The behavior of the seven hybrid combinations between *glaberrima* and *sativa* varieties exemplifies the results of the entire 13. All F_1 plants were completely self-sterile. Not a single seed was found on any plant. More significantly, however, meiosis was distinctly irregular in all hybrids. Although in four of the seven hybrids more than 50% of PMCs contained 12 bivalents, the number of PMCs with univalents ranged from a low of 26% to a high of 75%. The number of univalents per PMC varied from two to the maximum number possible of 24. In two of the crosses, more than 25% of PMCs had 24 univalents. All of these hybrids also showed abnormalities at anaphase I in the form of lagging univalents and unequal disjunction, Abnormalities were found also at telophase I and in meiotic division II.

Because of the irregularities in chromosome behavior during meiosis in the hybrids, it was concluded that the three African annual species are more sharply differentiated from *sativa* than are the other species included in the study and more so than has generally been assumed. The relationship between the African annual species and *perennis* subsp. *cubensis* and subsp. *barthii* was not investigated but there was indirect evidence that meiosis should also prove irregular in hybrids of this type,

GENOME DESIGNATIONS

The letter A was designated as the symbol for the genome of *sativa* by MORINAGA. From the results of the studies reported herein, it was concluded that all forms of the first group of species — *sativa, sativa* var. *fatua, sativa* var. *formosana* and *balunga* — possess this genome in an undifferentiated form. The genome (or genomes) of *perennis* subsp. *cubensis* and *perennis* subsp. *barthii* is very closely related to that of *sativa* and use of the symbol A for this genome also appears to be warranted. However, because of the much more marked sterility in hybrids between the two groups, it was felt that the genomes should be differentiated by subscript numbers—A_1 for the first group and A_2 for the second. Based on the consistent occurrence of irregularities during meiosis in hybrids between species having the A_1 genome and the African annual forms, it was felt that the genomes were sufficiently distinct to warrant separate letter symbols. Since B, C and D had already been assigned as symbols for genomes in other species of *Oryza,* the symbol E was suggested for the genome of the African species.

The genome symbols proposed are summarized below:

Oryza species	Genome
sativa	A_1
sativa var. *fatua*	A_1
sativa var. *formosana*	A_1
balunga	A_1
perennis subsp. *cubensis*	A_3
perennis subsp. *barthii*	$A_?$
glaberrima	E
glaberima var. *stapfii*	E
breviligulata	E

ORIGIN OF THE CULTIVATED SPECIES

The results appear to support the proposal of SAMPATH AND RAO (1951) and SAMPATH AND GOVINDASWAMI (1958) that *Oryza sativa* was derived directly or indirectly from the wild perennial Asiatic species designated *balunga* in the present studies. It is also suggested that primitive types which would now be classified as *sativa* var. *fatua* probably constituted intermediate steps in the development of *sativa,* and consequently, types identifiable as *sativa* var. *fatua* might have been the immediate progenitor of *sativa.*

The results tended to support the earlier conclusion of CHEVALIER that the cultivated species *glaberrima* originated from *breviligulata* and, thus, had an origin independent of *sativa.*

ADDENDUM

At the time of the Symposium, I believed that the cytological data reported in the preceding paper showing chromosome irregularities in hybrids between *Oryza sativa* and the three African annual species *Oryza glaberrima, breviligulata,* and *stapfii* were the only recorded examples of this nature. In several other studies of these hybrids, meiosis was found to be regular and, in the paper presented by LI at the Symposium, unpublished data of Hu were cited, showing regular pairing of chromosomes at metaphase I in five hybrids between *sativa* and *glaberrima.*

After the Symposium, I learned that BOUHARMONT (1962) had recently reported that among six hybrid combinations between varieties of *sativa* and *glaberrima,* meiosis was regular in three, but in the other three combinations more than 50 percent of pollen mother cells contained univalents at metaphase I. There was an exceptionally high frequency in the latter hybrids of cells with 24 univalents. Thus, the results found by BOUHARMONT agree in part with those of YEH and HENDERSON, which formed the basis for the paper presented by the writer at the Symposium.

The conflicting results found by different investigators relevant to chromosome behavior during meiosis in hybrids between *sativa* and *glaberrima* are puzzling. However, the most probable explanation for the variable results is the effect of the en-

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vironment under which the hybrids are grown. Apparently, under most environmental conditions chromosome pairing and chiasma formation are regular, leading to normal behavior throughout meiosis. On the other hand, when the hybrids are grown under certain environmental conditions, pairing and/or chiasma formation will be reduced, giving rise to univalents at metaphase I and consequent irregularities in anaphase I and meiosis II.

Apparently the reduction in pairing or chiasma formation under certain conditions is genically controlled by, rather than due to, structural differences in chromosomes. In the experiments of YEH and HENDERSON and of BOUHARMONT, there was a marked tendency in many hybrids for pollen mother cells to contain either 12 bivalents or 24 univalents. For example, in one of the hybrids studied by the former workers, 25 percent of pollen mother cells contained 12 bivalents while 39 percent had 24 univalents.

The results of BOUHARMONT further emphasize the conclusion drawn by YEH and HENDERSON that *Oryza glaberrima* is probably differentiated to a greater degree from *Oryza sativa* than has usually been assumed.

CHROMOSOME STRUCTURAL DIFFERENTIATION, ISOLATING MECHANISMS AND SPECIATION IN *ORYZA*

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The concept of species has two facets–the first is taxonomical which notes morphological differentiation and satisfies diagnosis; the other is evolutionary which probes into causal mechanisms by cytogenetical approach. Either of these aspects will be incomplete and misleading when treated independently. Earlier literature on the taxonomy of ORYZA is confusing, such as the mis-identifications of *Oryza officinalis* for *Oryza latifolia* (RAMIAH, 1934) and *Oryza perennis* for *Oryza alta* (MORINAGA, pers. comm.; KIHARA, 1959), the mis-prediction of chromosome numbers of *alta* and *meyeriana* (SAMPATH AND RAO, 1951), the synonymy and distinctness of *granulata* and *meyeriana* (HAINES, 1928; BACKER, 1946; CHATTERJEE, 1948), the validity of the American tetraploid species (CHATTERJEE, 1948; SAMPATH, 1962), and the merging of several species into perennis (SAMPATH AND RAO, 1951) and a subsequent separation of one valid species, *barthii* (SAMPATH, 1961) – all originating from the overpowering influence of morphology in taxonomy. Recently, experimental taxonomy has been greatly emphasized and this may end the present confusion. This paper concerns the cytogenetical mechanisms in the genus *Oryza* which operate at sub-specific, specific, and sectional levels, and attempts to highlight the most predominant mechanism of speciation in the genus.

Since the days of ROSCHEVICZ (1931), the morphological diversity of *Oryza* species permitted its classification into different sections. The original classification of ROSCHEVICZ into four sections, namely: *Sativa, Granulata, Coarctata* and *Rhynchoryza,* was not satisfactory, however, and was replaced by a new classification with three sections– *Sativa, Officinalis* and *Granulata* by CHOSE, GHATGE AND SUBRAHMANYAN (1956). The species which ROSCHEVICZ previously included in the section *Sativa* are classified into two sections *Sativa* and *Officinalis* on the basis of spikelet and anther sizes. MORISHIMA AND OKA (1960) supported the splitting of ROSCHEVICZ'S section *Sativa* based upon available morphological evidence. The data from the genome analysis of MORINAGA (1959) and his associates supported the classification scheme of GHOSE *et al.* (1956). LI, WENG, CHEN, AND WANG (1962) also supported this classification. KIHARA and his co-workers (KIHARA, 1959; NEZU, KATAYAMA AND KIHARA, 1960), however, followed ROSCHEVICZ'S classification. As in any other classification, the position of several anomalous species *(subulata, coarctata, schlechteri)* is not clear, and the intermediate forms such as *australiensis* and *minuta* are recognized both by RICHHARIA (1960) and SAMPATH (1962). This paper, despite these limitations, follows

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classification of GHOSE *et al.,* which is an improvement over that of ROSCHEVICZ.

The section *Sativa* Ghose is represented by two Asian forms, *Oryza sativa* and *sativa* var. *fatua,* three African species, *breviligulata, stapfii* and *glaberrima,* and one species, *perennis,* common to Asia, Africa, and America. Extensive data on hybridization indicate that all the species are crossable and that the fertility is high in intracontinental, and low in intercontinental hybrids (MADHAVAN NAYAR, 1958; GOPALAKRISHNAN, 1959; NEZU *et al.,* 1960; HAKIM, 1962; SAMPATH, pers. comm.). In all the interspecific hybrids within this section, the above workers reported 12 regular bivalents with the following exceptions: YEH AND HENDERSON (1961a) recorded some meiotic abnormalities in the hybrids between Asian and African species, and for this reason, postulated a distinct genome symbol E for the African species. Several other workers (NEZU *et al.,* 1960; LI *et al.,* 1962; SAMPATH, 1962), however, do not share this view. Recent work at the Central Rice Research Institute has indicated that in the hybrids involving *glaberrima,* genic factors are responsible for the low bivalent frequency (SAMPATH, pers. comm.), and in this context, the separate genome symbolization for African species cannot be supported.

Despite extensive data on chromosome pairing at metaphase I, critical data on pairing during prophase stages are meager in the intrasectional hybrids of *Sativa.* SHASTRY AND MISRA (unpubl.) analyzed the pachytene stages of the hybrids *perennis* var. *balunga* × *perennis* var. *cubensis* and *sativa* × *perennis* (a strain from Madhya Pradesh, India). In the former hybrid, although the pachytene bivalents appeared normal on cursory cytological examination, several loosely paired segments were located in the entire complement. The loosely paired regions were characterized by side-to-side association of the segments with no relational coiling. Segments of this type were observed only in *perennis* var. *barthii* which was highly self-sterile (SAMPATH, 1961) and they were believed to result from structural hybridity (DAS AND SHASTRY, in press). In the hybrid *perennis* var. *balunga* × *perennis* var. *cubensis,* the high sterility probably results from the structural hybridity as reflected in loosely paired segments. By analogy with the data on subspecific hybrids of *Oryza sativa* (SHASTRY AND MISRA, 1961), these could represent small translocations. The bivalents attached to the nucleolus observed in the PMCs of this hybrid at diplotene confirmed the presence of extensive structural hybridity. While in both parents of this cross only two bivalents were attached to the nucleolus, as many as five to six were attached to the nucleolus in the hybrid. Most probably, the nucleolus-organizing sites are not homologous in the two subspecies.

The cytological studies of the hybrid *sativa* × *perennis* (the strain from Madhya Pradesh) are not complete, but the analysis so far suggests extreme structural hybridity in the form of differential segments, unequal bivalents, and loosely paired segments at pachytene (SHASTRY AND MISRA, unpubl.). The earlier observation of MADHAVAN NAYAR (1958) that quadrivalents occur at metaphase I are not confirmed either in our studies or in the studies of NEZU *et al.* (1960) and LI *et al.* (1962). We may conclude, therefore, that the chromosomal differentiation between *sativa* and *perennis* is cryptic and that it can be revealed only by pachytene analysis.

Interspecific hybrids, where one of the parents is an autotetraploid and the other a basic diploid, offer excellent material for the study of chromosome differentiation. If the chromosomes of the two species are completely homologous, the hybrid would behave as an autotriploid in the frequency and shapes of trivalents. If, on the other hand, the genome of one of the species is segmentally homologous, every multivalent formed would result from pairing between two completely homologous and one segmentally homologous chromosome and hence the distribution is likely to be skewed in favor of a particular shape. This point is well illustrated in the meiotic data of the F_1 hybrid of *Oryza sativa* (4 x = 48) × *Oryza perennis* var. *longistaminata* (2 x = 24) studied by SHASTRY and MISRA (unpubl.). Their data on this hybrid is summarized as follows:

(1) In every PMC, trivalents occur and their number per PMC ranges from 2–12. Trivalents are mostly of the frying pan type (Type 9 of DARLINGTON, 1931) followed by chain, Y and ring types. Further, the distribution of the frying pan type is closest to that of summation of trivalents, thereby proving that the potential pairing in this hybrid is by the trivalents of the frying pan type, which results from pairing between identical chromosomes of *sativa* and segmentally homologous chromosomes of longistaminata. An exceptional quadrivalent with the shape (⌐◇—) of a ring and two arms supports the postulate on the segmental homology.

(2) Bivalents are mostly of the ring type (434 out of 446) with at least two chiasmata per pair which are interpreted to be due either to failure of pairing or terminalization of the segmentally homologous chromosomes of *Oryza longistaminata* from a potential frying pan-shaped trivalent.

(3) Quadrivalents are largely due to "autosyndetic" pairing of *sativa* chromosomes.

(4) Pentavalents of a dumb-bell shape (○—○) indicate a tertiary trisomic condition of the hybrid for some chromosomes. Either failure of pairing or terminalization of the constituent chromosomes can lead to a ring bivalent and a frying pan-shaped trivalent.

(5) Hexavalents due to "autosyndetic pairing" in *sativa* (⌐◇◇—) and quadrivalents due to "autosyndetic pairing" in *longistaminata* chromosomes (◇— —) indicate that segmental reduplications occur in both species.

In autotriploids, with three identical sets of chromosomes, random pairing between segments may result largely in symmetric associations. The cytogenetic evidence of STEERE (1932), MUNTZING (1933) and UPCOTT (1935) in triploid *Petunia*, *Solanum* and *Lycopersicon* supports this assumption. A high frequency of the frying pan-type trivalents in the triploid *Oryza* hybrid is highly significant and constitutes conclusive evidence for the differentiation of the chromosomes of the species by large translocations as reported in interracial hybrids of *Datura* (BLAKESLEE, 1934). Persistent associations of this type indicate that the size of the translocated segments is sufficiently large to initiate pairing and for the chiasmata to persist until metaphase I. The pairing at the diploid level can at best be reflected only by a reduction in chiasma frequency at metaphase I or by differential segments at pachytene. Hence, the value of triploid pairing data is self-evident.

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In the light of the pachytene data on the limited number of diploid hybrids studied and the metaphase pairing in the above triploid hybrid, we may conclude that, on account of normal chromosome pairing in diploid hybrids, extensive chromosomal differentiation between the species of the section *Sativa* has escaped detection. Similar differences have been demonstrated at the subspecific level in *Oryza sativa* (SHASTRY AND MISRA, 1961). In light of these findings, it is difficult to support the view of HU (1960b) that the predominant form of differentiation between the species of *Oryza* is by gene mutations.

One of the earliest reported interspecific hybrids is *sativa* × *officinalis* (RAMIAH, 1934). It was analyzed by NANDI (1938), RAMANUJAM (1937), GOPALAKRISHNAN (1959), MORINAGA AND KURIYAMA (1959b, 1960), and NEZU *et al.* (1960). All these workers report that pairing at metaphase I was extremely limited. A re-investigation by SHASTRY, SHARMA, AND RANGA RAO (1961) revealed, that although metaphase pairing was limited, the anaphase distribution of univalents was nearly equal. This is difficult to visualize in allogenomic (A and C, according to MORINAGA AND KURIYAMA, 1959b) hybrids of this type. In the re-investigation, it was found that pachytene pairing in this hybrid was nearly complete and normal and that the intermediate prophase stages reflected varying degrees of pairing. The data were interpreted to mean that the chromosomes of these species were fairly homologous, but desynapsis was responsible for the high frequency of univalents at metaphase I. This finding has far-reaching implications on the designation of distinct genome symbols for *officinalis,* on our understanding of the origin of cultivated rices, on the validity of subdivision of original *Sativa* section of ROSCHEVICZ into two sections *Sativa* and *Officinalis* by GHOSE *et al.,* and on a basic problem of genetic differentiation in relation to isolating mechanisms. We now understand more clearly that isolating mechanisms may originate with limited chromosomal differentiation and that freely hybridizing sympatric taxa might compound chromosomal hybridity by irregular meiosis and chance back-crossing to parents (in *Melilotus,* SHASTRY, SMITH and COOPER, 1960).

In the light of the cytogenetical data above on the F_1 hybrid of *sativa* × *officinalis,* extensive morphological differentiation between the species of the sections *Sativa* and *Officinalis* may have originated by gene mutations, more probably by "macro-mutations" as GOLDSCHMIDT (1955) and STUBBE (1959) have postulated, than by chromosomal changes. Desynapsis would have played an important role in initiating differentiation.

The meiotic data of the F_1 hybrid of *sativa* × *australiensis* illustrate a novel mechanism of reproductive isolation. Although this hybrid was studied by GOPALAKRISHNAN (1959), a re-investigation was considered because of the report that a maximum of eight bivalents were found in this hybrid. This was surprising since the parents of this cross differed significantly in heterochromatinization of the chromosomes (SHASTRY AND MOHAN RAO, 1961; SHASTRY, RANGA RAO AND MISRA, 1960). The data and conclusions of SHASTRY AND RANGA RAO (1961) are summarized as follows:

(1) No regular bivalent formation occurred in this hybrid; all the associations which GOPALAKRISHNAN (1959) described as bivalents were only terminally associated non-

chiasmatic pseudo-bivalents. MORINAGA AND KURIYAMA (1960), NEZU *et al.* (1960), and LI *et al.* (1961) likewise considered that no bivalents were formed in this hybrid.

(2) A more interesting feature of the meiosis in this hybrid is the distinct timing imbalance in condensation and migration of the chromosomes of the two species at prophase and anaphase. All of the earlier workers missed this observation, but it was evident in the photographs of NEZU *et al.* (1960) and the drawings of GOPALAKRISHNAN (1959). The chromosomes of *australiensis* showed earlier condensation at prophase and earlier migration to poles at anaphase in comparison with those of *sativa.* Timing imbalance constitutes an important isolating mechanism in this case and whether all the species of the section Sativa behave similarly on hybridization with *australiensis* should be investigated.

It may be concluded that structural differentiation ofchromosomes play an important role at the subspecific level between *japonica* and *indica* rices of *Oryza sativa.* High sterility of several intervarietal hybrids of *Oryza officinalis* (MORINAGA, pers. comm.; SAMPATH, 1962; GOPALAKRISHNAN, unpubl.), likewise, may well be caused by chromosomal differentiation. The limited data on pachytene analysis from interspecific hybrids of the section *Sativa* Ghose also indicate the operation of the same mechanism in the differentiation of *Oryza* species. At the level of higher taxa, as between the sections *Sativa* and *Officinalis,* desynapsis and timing imbalance play significant roles as isolating mechanisms.

SUMMARY

The observations on pachytene analysis in the F_1 hybrids of *sativa* × *officinalis, sativa* × *australiensis, sativa* × *perennis* and *perennis* var. *balunga* × *perennis* var. *cubensis* and on the metaphase analysis of a triploid hybrid, *sativa* (4 x) × *perennis* var. *longistaminata* (2 x), are reported. It is concluded that the predominant mechanism of speciation in *Oryza* is by chromosome structural differentiation at varietal and specific levels, and by desynapsis and timing imbalance at the level of higher taxa.

APPENDIX

EVIDENCE ON INTERSPECIFIC RELATIONSHIPS FROM GENETIC STUDIES

Variation in Asian wild rice, *Oryza sativa* var. *fatua* (syn. *Oryza sativa* var. *spontanea)*

SAMPATH AND RAO (1951) postulate that this taxon is of hybrid origin between *Oryza sativa* and *perennis.* This view was substantiated by the segregation of *fatua* types in the experimentally produced crosses (SAMPATH AND GOVINDASWAMI, 1958). Extensive morphological studies from our laboratory (SHARMA, unpubl.) indicate that the ramification of the panicle is a dependable criterion of classification and that, on

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this basis, wild rice not belonging to *perennis* and classifiable as *sativa* var. *fatua* can be divided into two types. The first type is extremely short, fertile, and exhibits a limited number of secondary branches on the panicle and normal pairing at pachytene. This form occurs in regions of India where *perennis* does not occur, as in Gujerat, Andhra, Punjab and Uttar Pradesh. The second type is heterotic (tall), semi-sterile, has secondarily branched panicles comparable to *sativa* and exhibits abnormalities in pairing at pachytene.

While the hypotheses of SAMPATH AND RAO (1951), RICHHARIA (1960) and SAMPATH AND GOVINDASWAMI (1958) provide for the forms of the first type as a result of segregation and homozygosity, we believe that this type is probably a distinct taxonomic entity and partakes in hybridization with *perennis* producing forms like the second type which might also be produced by a direct cross between *perennis* and *sativa.* For convenience, I suggest that type 1 be referred to as *sativa* var. *fatua* and type 2 as *sativa* var. *spontanea.*

A comparison of the panicle morphology, grain shape and leaf characters of *perennis, sativa* and *officinalis* revealed that all characters which are absent in *perennis* and present in *sativa,* are manifested to a high degree in *officinalis* (SHARMA AND SHASTRY, 1962). Consequently, a rigid monophyletic scheme of origin of *sativa* as postulated by SAMPATH AND RAO (1951) and RICHHARIA (1960), I believe, may require modification so as to include *officinalis* (or a similar taxon) as a donor in introgression to confer the high density in the panicles and a slight flattening of the caryopsis in comparison to *perennis.* Accumulation of mutations might have transformed *perennis* into a taxon close to *sativa* var. *fatua* (type 1 as above), but at this stage it seems likely that introgression of *officinalis* is necessary to produce forms similar to *sativa.* (Original objections to including *officinalis* in the plan for origin of *sativa* are fewer after the study of SHASTRY, SHARMA AND RANGA RAO, 1961). A hypothetic scheme of interrelationships is presented in Fig. 1.

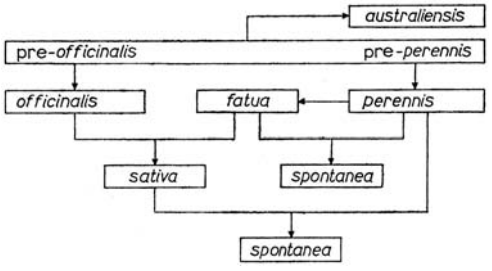

Fig. 1

Interrelationship between the sections of *Oryza*

A general observation that, in the *Gramineae,* reduction in floral parts and diminution in size are an indication of evolutionary complexity, greatly influenced the hypotheses on the interrelationships of the different sections of *Oryza.* SAMPATH

AND RAO (1951) and more recently RICHHARIA (1960) consider that the *Sativa* section is most primitive, and that the sections *Officinalis* and *Granulata,* in which pigmentation, pubescence and size of floral parts are greatly reduced, are more advanced, in the order indicated. Two cytological findings from our laboratory indicate that the morphological distinctness between sections *Sativa* and *Officinalis* is illusive and that these sections are probably more closely related. The first is the data on pairing of the F_1 hybrid of *sativa* × *officinalis* (SHASTRY, SHARMA AND RANGA RAO, 1961). Second is the data on the F_1 hybrid of *sativa* × *australiensis* which indicate that the species *australiensis,* which combines the characters of both the sections, *Sativa* and *Officinalis,* is probably a progenitor rather than a hybrid derivative (SHASTRY AND RANGA RAO, 1961).

Genetic evidence on the dominance in intersectional hybrids further supports the conclusion that section *Officinalis* is more primitive than the section Sativa. In all the intersectional F_1 hybrids of the two sections, the panicle morphology of the *officinalis* type is dominant. LI *et al.* (1961) recorded that the triploid hybrid *paraguaiensis* × *brachyantha* resembled more the male parent. It is surprising that even against the tetraploid parent, the diploid species of the *Granulata* section manifested the dominance of characters. A newly recorded hybrid, *latifolia* × *australiensis,* resembles more the diploid male parent than the female parent (GOPALAKRISHNAN, unpubl.). These observations may mean that the succession of the different sections of *Oryza* is: *Granulata* → *Officinalis* ⇢ *Sativa,* and not the reverse, as RICHHARIA (1960) indicates. This view, however, needs to be confirmed by further studies.

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STUDIES ON GENETIC AND CYTOGENETIC EVIDENCE FOR SPECIES RELATIONSHIPS IN THE REPUBLIC OF CHINA

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INTRODUCTION

The genomes of the genus *Oryza* were first studied by MORINAGA (1943) who assigned symbols for three species, viz., *sativa, minuta* and *latifolia,* Since then, genome constitutions of many species were supplemented by the investigations of MORINAGA and his co-workers (MORINAGA, 1956; MORINAGA AND KURIYAMA, 1960). Their proposed designations are in Table 1. According to them, all diploid species within the section *Sativa* Ghose have the same genome, AA.

TABLE 1

GENOMIC SYMBOLIZATION OF RICE SPECIES BY DIFFERENT AUTHORS AS OF 1962

Oryza species	MORINAGA *et al.*	RICHHARIA	YEH AND HENDERSON	LI *et al.*
sativa	AA	P^2P^2	A_1A_1	
sativa var. *fatua*	AA		A_1A_1	
sativa var. *formosana*	AA		A_1A_1	
sativa var. *spontanea*	AA			
balunga			A_1A_1	
perennis	AA	P^1P^1		
perennis var. *barthii*			A_2A_2	
perennis var. *cubensis*	AA		A_2A_2	
glaberrima	AA	P^3P^3	EE	
breviligulata	AA	P^3P^3	EE	
stapfii	AA*	P^3P^3	EE	
austroliensis	Not, A, B, C			GG
officinalis	CC	O^1O^1		
latifolia	CCDD	$O^1O^1O^2O^2$		
paraguaiensis	CCDD			
alta	CCDD*	$O^1O^1O^2O^2$		
minuta	BBCC	$O^1O^1M^1M^1$		
eichingeri	BBCC			
malampuzhaensis		$O^1O^1O^3O^3$		
brachyantha				FF

* Designated by KIHARA *et al.,* 1961.

RICHHARIA (1960) indicated that the five diploid species of the section *Sativa* Ghose,

carry identical or similar genomes. He used the symbol P^1 for *perennis* (this species as archetype) and *breviligulata;* P^2 for *sativa;* and P^3 for *glaberrima* and *stapfii*. This symbolization implied that chromosomal differentiation accompanied speciation. As for the genome constitutions of the section *Officinalis* Ghose, RICHHARIA (1960) used a system of symbols different from that originally used by MORINAGA and his associates. RICHHARIA designated *officinalis* as O^1O^1, *alta* and *latifolia* $O^1O^1O^2O^2$, *malampuzhaensis* $O^1O^1O^3O^3$, and *minuta* $O^1O^1M^1M^1$.

YEH AND HENDERSON (1961) assigned the genomes of *sativa, sativa* var. *fatua, sativa* var. *formosana* and *balunga* as A_1, and *perennis* as A_2. The three African species, *glaberrima, breviligulata* and *stapfii,* were given the symbol E. This assignation was based on a cytogenetic study of these diploid species and their hybrids. They assigned the same genome to all species which showed no cytogenetic differentiation. They considered species having regular chromosome behavior, but showing abnormally high sterility in hybrids, as having the basic genome. To these, they assigned the same letter, but different subscript numbers. To species showing significant irregularities in chromosome behavior and abnormally high sterility in hybrids, and which they believed had different genome constitutions, they assigned different letters,

By using the embryo culture method, LI *et al.* (1961, 1963) and Wuu *et al.* (1963) obtained several intersectional hybrids, some of which have not been obtained by other rice cytogeneticists. Among these hybrids, the most important are: *paraguaiensis* × *brachyantha, sativa* × *brachyantha, minuta* × *brachyantha, paraguaiensis* × *australiensis, australiensis* × *alta, minuta* × *australiensis,* and *sativa* × *australiensis.* Since the genomes of *sativa, paraguaiensis, alta,* and *minuta* were already known, the new symbols F and G, were assigned to *brachyantha* and *australiensis,* respectively, according to the mode of pairing of the chromosomes in these hybrids.

RECENT STUDIES IN TAIWAN, CHINA

Two institutions in Taiwan, China, are working on the cytogenetical problems dealing with rice species. They are the Institute of Botany, Academia Sinica, and the Laboratory of Genetics, Taiwan Provincial Chung-Hsing University.

In the Institute of Botany, Dr. H. W. LI and his associates started this work in 1960. Dr. H, I. OKA supplied most, if not all, of the species. In the first two crossing seasons, the hybridization work was conducted in Tainan where the climate and environment were ideal. Crosses involving intersectional hybridization were tried on a large scale, and the young embryos were artificially cultured on White's medium. *Oryza brachyantha* was successfully crossed with *Oryza paraguaiensis* in 1960, and with *sativa* and *minuta* the following season. Crosses were also made with species in the section *Sativa.* The full report has been published (LI *et al.,* 1962).

Drs. H. I. OKA, W. T. CHANG and C. H. HU conducted the work in Taichung. They are all affiliated with the project dealing with the origin of rice being undertaken by the National Institute of Genetics, Japan. Since publications covering the work of Drs. OKA and CHANG are readily available, no special mention is necessary here.

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TABLE 2

RESULTS OBTAINED BY C. H. HU IN INTERSPECIFIC CROSSES OF *Oryza*

Oryza hybrids	Plant number	cells observed	Chromosome pairing at MI IV	III	II	I
sativa × *glaberrima*	504 × W 039	199	–	–	10–12 (11.91)	0–4 (0.16)
sativa × *glaberrima*	108 × W 039	161	–	–	11–12 (11.95)	0–1 (0.10)
glaberrima × *sativa*	KY × PM	163	0–1 (0.006)	–	10–12 (11.85)	0–4 (0.23)
glaberrima × *sativa*	KY × GT	228	0–1 (0.035)	–	10–12 (11.79)	0–4 (0.28)
glaberrima × *sativa*	KY × NT	222	0–1 (0.013)	–	9–12 (11.88)	0–4 (0.18)
sativa × *officinalis*	NT × W 002	54	–	0–1	0–1	22–24
sativa × *australiensis*	504 × W 008	192	–	0–1 (0.01)	0–5 (1 .W)	14–24 (21.68)
sativa × *australiensis*	563 × W 008	105	–	–	0–5 (0.83)	14–24 (22.34)
glaberrima × *minuta*	W 040 × W 045	15	–	–	0–3	30–36

However, some of the results obtained by Dr. Hu, not published before, are summarized in Table 2.

UNPUBLISHED RESULTS OBTAINED BY THE INSTITUTE OF BOTANY, ACADEMIA SINICA

The following pages highlight the results obtained from the crosses of *brachyantha* and *australiensis* with other species.

(1) CROSSES INVOLVING *Oryza brachyantha.*

Three parent species, *sativa* L. ($2n = 24$), *minuta* Presl ($2n = 48$) and *brachyantha* Chev. et Roehr. ($2n = 24$) were used in these crosses.

TABLE 3

RESULTS OF INTERSPECIFIC CROSSING OF RICE BY USING ARTIFICIAL CULTURE OF THE IMMATURE EMBRYOS

Cross combinations	numbers of pollinated florets	embryos cultured	seedlings transplanted	seedlings died after transplanting	true hybrids obtained	Cross-ability*
sativa × *brachyantha*	3201	57	48	5	1	0.03%
minuta × *brachyantha*	713	33	10	–	5	0.70%

* Crossability = $\frac{\text{number of true hybrids}}{\text{number of pollinated florets}} \times 100.$

(a) *Crossability of the parent species.* – ROSCHEVICZ classified three parent species under two different sections. The chances of a successful hybridization were poor. Table 3 shows the crossability of these three species.

These data clarify two facts. First, an intersectional hybrid was much more difficult to obtain than an intrasectional one (this comparison being made with our former results). Second, by comparison, tetraploid *minuta* might be more successful as the female parent in hybridization than diploid *sativa.* Of 3201 florets pollinated in the combination of *sativa* × *brachyantha,* only one true hybrid was obtained. On the other hand, of 713 pollinated florets of *minuta* × *brachyantha,* five hybrids were produced. The ratio of success of hybridization of the former direction to the latter was only 0.043.

(b) *Cytological observations of the hybrids.* – Of the three species used in this experiment, two are diploid species with 24 chromosomes, namely, *sativa* (KUWADA, 1909) and *brachyantha* (MORINAGA AND KURIYAMA, 1954c, KRISHNASWAMY *et al.,* 1954). The other species, *minuta,* is a tetraploid with 48 chromosomes (MORINAGA, 1934). Since 1934, MORINAGA has carried out an extensive program on the studies of interspecific crosses and genomic analyses in the genus *Oryza.* Through hybridization of *sativa* with seven other diploid forms, namely, *glaberrima, breviliguluta, perennis, cubensis, sativa* var. *fatua, sativa* var. *spontanea* and *formosana,* and based on the normal chromosome behavior at first metaphase in these hybrids, he concluded that all these forms have the same genomic constitution and proposed the designation of genome A.

OKURA (1937) crossed *sativa* with *minuta.* Since then, several other workers such as MORINAGA (1940), NANDI (1938), CAPINPIN AND MAGNAYE (1951) and others used the same cross or its reciprocal as material for their cytogenetic studies. In spite of much confusion in their respective reports, MORINAGA (1956) finally designated the genomic symbol of BBCC for *minuta.*

According to MORINAGA AND KURIYAMA (1960), *paraguaieinsis* has genomes CCDD. LI *et al.* (1961), through meiotic analysis of *paraguaiensis* × *brachyantha,* found that 36 univalents were most frequently observed at MI. Based on the failure of crossing *brachyantha* with *sativa, officinalis, minuta* and others, these authors concluded that *brachyantha* has a genome different from any previously known genomes, namely, A, B, BC and CD, so they designated F for the genome of *brachyantha.*

From this brief review, we know that the three species used in our experiment differ in genomic constitution.

In the *sativa* × *brachyantha* hybrid, 350 microsporocytes were studied at MI. Nearly all of the cells had 24 univalents (Fig. 1.3), the average number of bivalents was only 0.03 (Table 4). There were nine cells with one bivalent and 22 univalents (Fig. 1.2). The highest bivalent number found in any one cell was two. Since the chromosomes of the two parents (at MI in particular) are not appreciably different in size, it is not known whether the paired chromosomes are the result of autosyndesis or allosyndesis.

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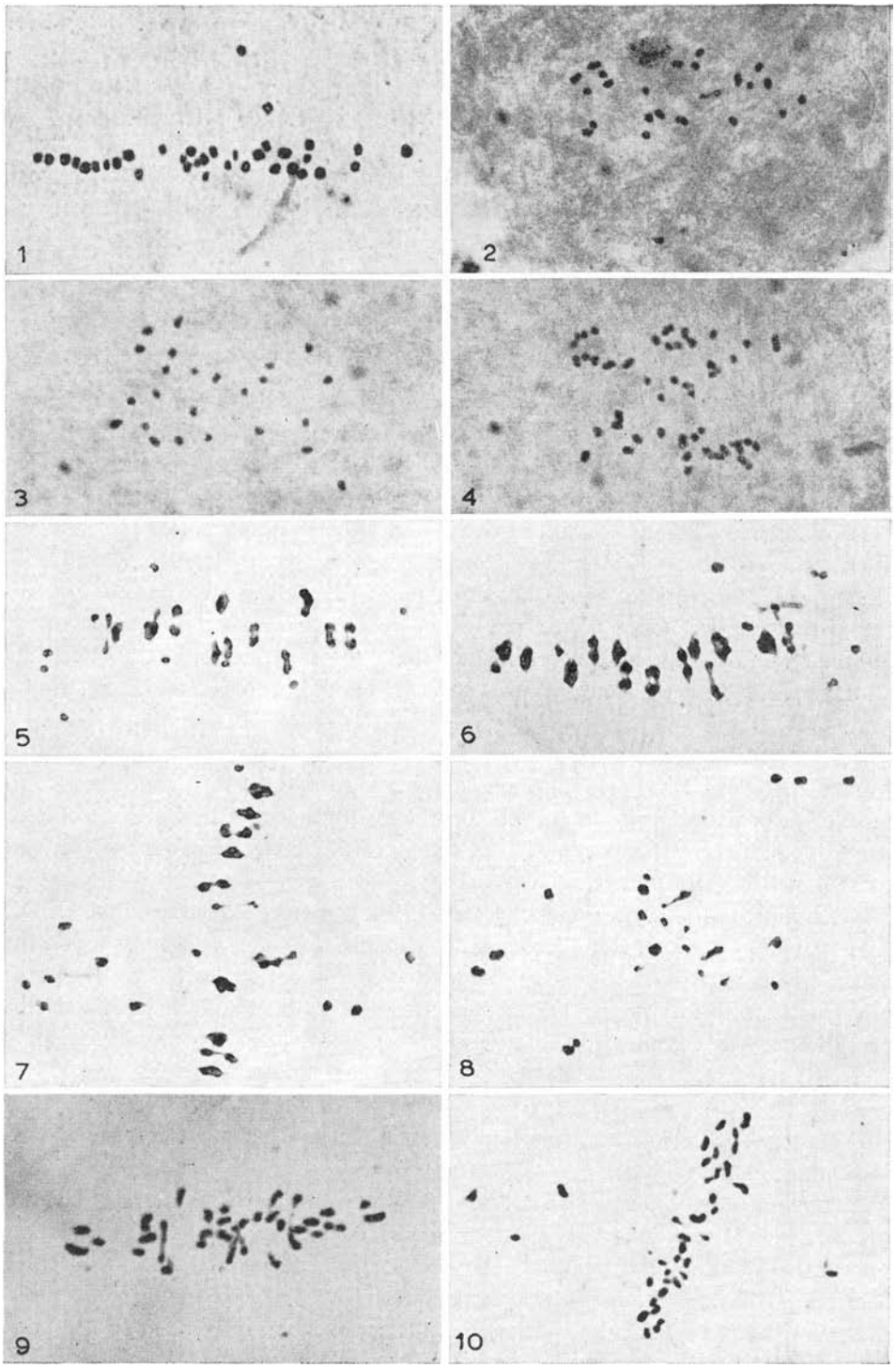
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TABLE 4

MEIOTIC OBSERVATIONS OF CHROMOSOME PAIRING IN TWO INTERSPECIFIC HYBRIDS OF *Oryza*

Cross combinations	No. of cells observed	Chromosome configuration				
		V Range (Mean)	IV Range (Mean)	III Range (Mean)	II Range (Mean)	I Range (Mean)
sativa × *brachyantha*	350				0–2 (0.03)	20–24 (23.94)
minuta × *brachyantha*	130	0–1 (0.008)	0–1 (0.008)	0–1 (0.015)	0.7 (1.439)	23–26 (33.007)

In addition to the data presented in Table 4, there were four cells in which chromosomes numbered 48 instead of 24 (Fig. 1.4).

In the *minuta* × *brachyantha* hybrid, a much more complicated pattern of chromosome associations was observed at MI. Of 130 cells observed, 47 cells had 36 univalents, 82 cells had variable numbers of bivalents (Fig. 1.1), ranging from one to seven. Multiple chromosome associations, such as pentavalents, tetravalents as well as trivalents, were also found in different cells.

As Hu (1961) previously pointed out, *brachyantha* had smaller chromosomes than other species of *Oryza.* We also observed that there were about 12 small and 24 large chromosomes in the microsporocytes of the hybrids. Accordingly, bivalents were roughly divided into three groups, viz.,

(i) pairing of the chromosomes between those from *brachyantha,*
(ii) pairing of the chromosomes between those from *minuta,*
(iii) pairing of the chromosomes between those from *minuta* and *brachyantha.*

The frequency of these three groups was 22 (11.77%), 155 (82.89%) and 10 (5.34%, respectively, in a total of 187 cells observed. Apparently, pairing between chromosomes of *brachyantha* and *minuta* was not a common phenomenon. The mean number of bivalents was only about 0.08 per cell, indicating little homology between the chromosomes of *minuta* and *brachyantha.* Most of the bivalents originated, it seemed, from autosyndesis of the chromosomes of *minuta.*

Pollen grains of these hybrids were stained with iodine and examined microscopically. No normal pollen was found. All of them were wrinkled in shape and light-stained in color. Seed set also was found nil.

←

Fig. 1. Chromosome pairing at different stages of first meiosis in *Oryza* hybrids (1115 ×), 1 – *minuta* × *brachyanta.* MI, $1^{II} + 34^{I}$. The bivalent is supposed to come from the autosyndesis of the chromosomes of *minuta;* 2 – *sativa* × *brachyantha,* MI, $1^{II} + 22^{I}$; 3 – *sativa* × *brachyantha,* MI, 24^{I}: 4 *sativa* × *brachyantha,* AI, showing 48 chromosomes; 5 – *sativa* × *australiensis* (3*n*) – 1^{III} (AAS) + 12^{II} (11AA + 1SS) + 9^{I} (S); 6 – *sativa* × *australiensis* (3n) – 14^{II} (12AA + 2SS) + 8^{I}(S); 7 – *sativa* × *australiensis* (3*n*) – 12^{II} (11AA + 1SS) + 12^{I} (2A + 10S); 8 – *sativa* × *australiensis* (2n)–4^{II} (3AS + 1SS) + 16^{I} (9A + 7S); 9 – *minuta* × *australiensis* – 6^{II} (1AM + 5 MM) + 24^{I} (11A + 13M); 10 – *minuta* × *australiensis* – 3^{II} (1AM + 2MM) + 30^{I} (11A + 19 M).

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(c) *Morphological descriptions of the hybrids.* – Hybrid *sativa* × *brachyantha:* – the female parent *sativa* was Taichung No. 65, a *japonica* variety characterized by its intermediate height, broad and awnless spikelets, oblong and intermediate sized empty glumes, long, acute, split ligules and colorless stigmas. *O. brachyantha* was characterized by short, slender culms, long and narrow spikelets with straight, stout and lengthy awns, linear-lanceolate empty glumes, minute ligules and purple stigmas. The vegatative parts of the F_1 hybrid, such as plant height, length and width of leaf blade and shape of ligule, were apparently more like *sativa* than *brachyantha.* The panicle characters resembled *brachyantha* more closely. It had long and narrow spikelets with the same length of awns as that of the male parent. Empty glumes were lanceolate but slightly longer and wider than those of *brachyantha.* The color of the stigma was dark purple.

Hybrid *minuta* × *brachyantha:* – *Oryza minuta* was characterized by its procumbent growth habit, slantly round ligules, small spikelets with short, soft awns, semi-lax panicle, and black, minute, triangular empty glumes. The F_1 hybrid strongly resembled *minuta* morphologically except for compact panicles and narrower and longer spikelets. The length of awn was intermediate between that of the two parents.

(d) *Discussion.* – Up to the present, LI and his co-workers successfully hybridized *brachyantha* with *sativa, minuta* and *paraguaiensis* in Taiwan during 1960–1962. The present study supports the designation of F for the genome of *brachyantha.* CHEVALIER (1932, reviewed by YEH, unpubl.) believes *brachyantha* should be taken out of the section *Coarctata* Roschev. and placed under section *Sativa* Roschev. He also divided *Oryza* into four sections, i.e., *Euoryza, Padia, Sclerophyllum* and *Rhynchoryza,* corresponding to *Sativa, Granulata, Coarctata* and *Rhynchoryza* of Roschevicz's scheme except for the species *brachyantha.* GHOSE *et al.* (1956) divided the genus *Oryza* into *Sativa, Officinalis* and *Granulata* sections. They believe that *brachyantha* could be listed in the section *Granulata* together with *granulata, ridleyi* and *coarctata.* The success of crossing *brachyantha* with three other species belonging to section *Sativa* Roschev. perhaps further supports the postulate of CHEVALIER. However, the different aspects of this problem should be considered before a definite conclusion can be made.

TABLE 5

THE RESULTS OF INTERSPECIFIC CROSSES IN THE HYBRIDS OF *sativa* × *australiensis* AND *minuta* × *australiensis*

Crosses	Pollinated florets	Embryos cultured	Seedlings transplanted	True hybrids obtained	Adult plants raised	Cross-ability*
sativa × *australiensis*	1173	22	8	1	1**	0.00058
minuta × *australiensis*	1263	331	many	29	27	0.02137

* Crossability = $\frac{\text{number of true hybrids}}{\text{number of pollinated florets}} \times 100$.

** This hybrid was cytologically triploid. See next paragraph.

NEZU *et al.* (1960) conducted an extensive study of interspecific crosses in *Oryza.* They obtained no hybrids in crosses involving *brachyantha* crossed by *granulata, coarctata, ridleyi,* and *subulata.* The number of pollinated florets of these crosses, however, was rather small to warrant any definite conclusion.

(2) CROSSES INVOLVING *Oryza australiensis.*

The parents used were *sativa* L. ($2n = 24$), *minuta* Presl ($2n = 48$), and *australiensis* Domin ($2n = 24$).

(*a*) *Results of crossing.* – A large scale crossing involved two combinations. Most of the hybrid seeds of *minuta* × *australiensis* were characterized by their degenerated size, about one-third of the normal grain of *minuta.* By comparing the pollinated florets to the true hybrids obtained in two crosses, we found that *minuta* × *australiensis* was more successful than *sativa* × *australiensis.* The crossing results are listed in Table 5.

TABLE 6

CHROMOSOME ASSOCIATIONS AT MI IN THE HYBRID OF *Oryzasativa* × *australiensis* (triploid)*

IV			III	II			I		Frequency
AAAA	AAAS	AASS	AAS	A	SS	AS	A	S	
				12	1			10	15
				11	1		2	10	10
			1	11	1			9	9
				12	2			8	7
				12				12	7
			1	10	1		2	9	7
				11			2	12	4
				12	3			6	4
				11	2		2	8	2
			1	10	3		2	5	2
		1		10	1		2	8	1
1				10	1			10	1
	1		1	9			1	10	1
		1		10			2	10	1
		1	1	10	1			7	1
		1		11	1			8	1
			2	9	1		2	8	1
			1	11	2			7	1
			1	10			2	11	1
			1	10	2			9	1
				10	1		4	10	1
				11	3		2	6	1
				11		2		10	1
Total 1	1	4	25	893	90	2	65	746	80
Mean 0.01	0.01	0.05	0.31	11.16	1.13	0.03	0.81	9.36	
Range 0–1	0–1	0–1	0–2	9–12	0–3	0–2	0–4	5–12	

* A and S represent the chromsomes of *australiensis* and *sativa* respectively.

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(b) *Cytological studies of the hybrids.*

(i) *Oryza sativa* × *australiensis.* — In the *sativa* × *australiensis* hybrid, 80 cells in MI were studied. This plant was a triploid from the fusion of unreduced male gamete of *australiensis* with a normal egg of *sativa* (see discussion).

Table 6 shows the extremely variable chromosome association at MI in this triploid hybrid. Univalents, bivalents, and multivalents were all observed; however, the bivalents were the predominant ones. Most of these were closed types of large sizes. Occasionally, however, there were some bivalents made of much smaller chromosomes and were of the open type and furthermore they were apt to disjoin precociously at this stage. Since, outwardly, this triploid hybrid looks very much like the male parent, *australiensis,* the diploid gamete must have originated from the male parent.

Some outstanding morphological differences between the parents, *sativa* and *australiensis,* and their diploid and triploid hybrids are presented below:

australiensis–australiensis is a tall plant with lax inflorescences and long and narrow leaves, culm somewhat prostrate and spreading, ligules short, auricles absent, awn of the lemma about 4–5 cm long, the tip of the palea extended with an apiculus elongation.

sativa — The culm and leaf-blades are shorter than those of *australiensis,* culm erect, ligules long and acute, auricles small, panicle dense and drooping, spikelet awnless, the tip of the palea without an apiculus elongation.

sativa × *australiensis* — Diploid–Plant morphology is intermediate between the parents, culms erect, leaf-blade as long as *sativa,* ligules intermediate between its parents, auricles smaller than those of *sativa,* panicles erect, rachis much shorter than *australiensis.*

Triploid–Plant morphology is more similar to *australiensis* than to *sativa* in plant height, shape of panicle, length of leaf-blade, spreading culms; the awn and the apiculus elongation of the palea are longer than those of *australiensis;* ligules as long as those of *sativa* or even longer, auricles slightly larger than those of *sativa.*

If the diploid gamete did come from the male parent, then we could assume that the bivalents (about 12) were the doubled chromosomes of *australiensis,* the small ones were those of *sativa,* and the precociously dividing smaller bivalents were the chromosomes of *sativa.* In Table 6, the chromosomes were classified according to size, and A and S were assigned to represent the chromosomes of *australiensis* and *sativa* respectively.

Of the bivalents observed, the mean frequency of the doubled *australiensis* chromosomes was 11.16 per cell, Pairing of the non-homologous chromosomes of *sativa* was 1.13. Of the univalents observed, naturally, most of them would belong to *sativa,* and the mean frequency was 9.36. However, a mean frequency of 0.81 univalents was found, presumably to be of *australiensis* origin. In many cells studied, one trivalent was observed comprising two large chromosomes and one small one.

Dr. C. H. Hu of Chung-Hsing University furnished a hybrid plant *sativa* × *australiensis,* having 24 chromosomes. The results of our cytological study are in Table 7.

Table 7 indicates that the bivalents found per cell were 2.4 and 19.2 for univalents

TABLE 7

CHROMOSOME ASSOCIATION AT MI IN THE HYBRID OF *Oryza sativa* × *australiensis* (diploid)*

III			II			I	Frequency
AAS	ASS	SSS	AA	AS	SS	A + S	
						24	18
				1		22	14
			1			22	2
					1	22	11
1						21	1
				2		20	16
				1	1	20	12
					2	20	2
			1	1		20	2
				3		18	10
				2	1	18	10
			2		1	18	1
			2	1		18	1
			1	1	1	18	4
			1		2	18	1
				1	2	18	2
			1	2		18	2
		1		2		17	1
				2	2	16	1
				3	1	16	8
1	1			1		16	1
				4		16	5
			1	1	2	16	1
				2	2	16	1
			1	3		16	2
			1	2	1	16	4
			2	1	1	16	1
				5		14	2
			1	4		14	2
			1	3	1	14	2
				4	1	14	3
			1	2	2	14	1
	1			2	2	13	1
			1	4	1	12	2
			2	4	1	10	1
			2	2	3	10	1
				6	1	10	1
Sub-total	2	2	1	35	248	83		
Tota1		5			366		2853	150
Sub-mean	0.01	0.01	0.007	0.23	1.66	0.55		
Mean		0.033			2.4		19.02	
Sub-range	0–1	0–1	0–1	0–2	0–6	0–3		
Range		0–2			0–7		10–24	

* A and S represent chromosomes of *australiensis* and *sativa* respectively.

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and there was an occasional trivalent or two, indicating that the genomes of *sativa* and *australiensis* were different. From the triploid hybrid it was learned that the larger chromosomes were those of *australiensis.* Accordingly, in the diploid hybrid of *sativa* and *australiensis* the 12 larger chromosomes were assumed to belong to *australiensis.* Generally, the chromosomes of *australiensis* were two to four times as large as those of *sativa* as measured at MI. Thus the chromosomes of *sativa* and *australiensis* were easily classified. In some cells, however, the largest chromosome of *sativa* in comparison with the smallest of *australiensis* make a clear-cut classification confusing. In some such cells, 13 instead of 12 large chromosomes were counted. When cases like this were found, there were two chromosomes more or less of the same size and of intermediate size. In a few cells, however, three chromosomes were of such intermediate size. Altogether 150 cells were studied, and since there were 24 chromosomes in cach cell, the error was only 1.2% (45/24 × 150).

Since the identity of the chromosomes could be ascertained almost with certainty, the partners of the bivalents might be also identified. From 150 cells studied, there were altogether 366 bivalents found. Of these,

AA: 35/366 = 9.6%
AS: 248/366 = 67.8%
SS: 83/366 = 22.7%

TABLE 8

CHROMOSOME ASSOCIATION AT MI IN A F_1 HYBRID OF *Oryza minuta* × *australiensis*

	IV	III	II	I	Frequency
			0	36	1
			1	34	3
			2	32	12
			3	30	11
		1	2	29	1
			4	28	26
		1	3	21	3
			5	26	23
	1	1	4	25	2
			6	24	16
			4	24	1
		1	5	23	3
			7	22	16
		1	6	21	1
			8	20	6
			9	18	1
		1	8	17	1
			10	16	1
Total	1	11	601	3359	128
Mean	0.007	0.085	4.70	26.24	
Range	0–1	0–1	0–10	16–36	

From these results, we can see that about two-thirds of the bivalents were the result of allosyndesis, the other one-third of autosyndesis. It seems that there was more pairing between the chromosomes of different genomes rather than that of an intra-genomic nature.

Because of the appreciable difference in site of the chromosomes making up the AS bivalent, they were extremely heteromorphic. These bivalents were generally found to be open types. On the contrary, the AA bivalents were also heteromorphic in most of the cases. About 50% of them were found to be the closed type. Similarly, the SS bivalents were also heteromorphic in most of the cases and they were all of the open type (Fig. 1. 8).

In many cells studied, the bivalents would remain on the equator with the chromosomes of *sativa* at MI, whereas the chromosomes of *australiensis* seemed to get to the poles ahead of the chromosomes of *sativa.* But this was not a constant phenomenon.

(ii) *Oryza minuta* × *australiensis.* – In the *minuta* × *australiensis* hybrid, there were 128 cells examined (Table 8).

It was assumed that there was little affinity between the chromosomes of *minuta* and *australiensis,* though the number of bivalents ranged from 0–10, with a mean of 4.70 per cell. The chromosomes of *australiensis* were seemingly larger than those of *minuta,* but the difference was not as clear-cut as that between *sativa* and *australiensis.* These bivalents were rather of a mixed nature, i.e., they were the results of: (1) pairing between the non-homologous chromosomes of *australiensis,* (2) pairing between the chromosomes of *minuta* and *australiensis,* and (3) pairing between the non-homologous chromosomes of *minuta.* Through detailed cytological observations we obtained roughly the frequencies of 6.32%, 21.46% and 72.21% for the three kinds of pairing, respectively, as mentioned above. The majority of bivalents came from autosyndesis of the genomes BC of *minuta.* In *minuta* × *brachyantha* (Wuu *et al.,* 1963), the chromosomes of genomes B and C have some affinity among themselves. Multivalents were found, but rarely. Neither pollen fertility nor seed fertility were found in these two hybrids.

(c) *Discussion.* – Most of the rice workers, such as ROSHEVICZ (1931), CHEVALIER (1932), GHOSE *et al.* (1956) and others, classify the species *sativa and australiensis* as members of the section *Sativa.* GOPALAKRISHNAN (1959, cited by RICHHARIA, 1960) studied cytologically the hybrid *sativa* × *australiensis* and found that there was no homology between the chromosomes of these two species. For this reason, he postulates that *australiensis* should be removed from the section *Sativa.* MORISHIMA AND OKA (1960) adopted the techniques of factor analysis and matrix of correlation to study the degree of resemblance among 16 species in the genus *Oryza.* Based on the calculation of 42 characters, they constructed two tree-like diagrams (from correlation matrix 1 and 2). *O australiensis* was linked with *subulata* from the correlation matrix 1 diagram. It also appears, however, related to the *Officinalis* group from the correlation matrix 2 diagram. They finally postulated that section *Sativa* Roschev. might be divided into

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three sections, viz., *Sativa*, *Officinalis* and *Australiensis*, on condition that *Oryza australiensis* was ranked as a section.

NEZU *et al.* (1960) studied hybrids of *sativa* × *australiensis* and *officinalis* × *australiensis* cytologically. The metaphase figures of these two hybrids examined were 0.2 II + 23.6 I and 0.5 II + 23 I, respectively. Since chromosome pairing rarely occurred in these hybrids, they conclude that *australiensis* has neither the A nor the C genome. MORINAGA *et al.* (1960b) crossed *minuta* with *australiensis* and found 36 univalents in this hybrid. Since the genomes of *minuta* are BBCC, *australiensis* has neither genome B nor C.

LI *et al.* (1961) reports that in the hybrids *paraguaiensis* × *australiensis* and *australiensis* × *alta*, more bivalents were found at MI. The means per cell were 7.81 (range 2–12) and 6.175 (range 2–11) respectively. From these results, *australiensis* is partially homologous to one of the genomes of *paraguaiensis* or *alta.* However, our later studies (LI *et al.*, 1962), show that the mean bivalent number per cell at MI in *sativa* × *latifolia* was 6.26 (range 0–11) in one cross and 4.64 (range 1–8) in another. The mean for *paraguaiensis* × *sativa* var. *spontanea* was 3.55 (range 0–8). All these involved the crossing of species carrying genome A with CD. NEZU *et al.* (1960) also found similar results in the hybrids of *sativa indica* × *minuta;* the mean bivalent number at MI was 4.5 (range 0–9) per cell and it was 4.7 (range 1–8) when *japonica* was used instead of *indica.* These crosses involved the genomes A and BC. It was reported earlier that there was a size difference in the chromosomes of the two parents concerned. More than two-thirds of the heteromorphic bivalents of *minuta* × *australiensis* presumably were from the pairing of B and C genomes. The pairing between chromosomes of C and D genomes in a hybrid might possibly be as frequent as the chromosomes of genomes B and C. If this is true, then the majority of the bivalents found in the hybrids of *Oryza paraguaiensis* × *australiensis* or *australiensis* × *alta* might result from pairing between the chromosomes of genomes C and D. Unfortunately, there seemed to be no appreciable size difference between the chromosomes of *australiensis* and those of *paraguaiensis* or *alta.* Therefore, a detailed analysis of the pairing condition could not be made.

We found that the genomes of *australiensis* may differ from any other known genomes, i.e., A, C, BC, DC, with the exception of genome F of *brachyantha*, which until now has not been crossed successfully with *australiensis*, and from E (YEH AND HENDERSON, 1961a), the genome of the three African species. All of these species were crossed with *australiensis* by NEZU *et al.* (1960), but none was successful. Because of their difference in chromosome size, in plant morphology as well as in the failure to produce hybrids, the genome of *australiensis* should be considered different from F. Consequently, *australiensis* may be assigned a separate genome. We suggest G.

(d) Suggestions. — It is rather difficult to suggest future studies to be undertaken in connection with species relationships. Nevertheless, the following topics are suggested:

— 1 — Extensive collections of *Oryza* species by rice experts from various districts of the world on an internationally cooperative basis.

– 2 – A germ plasm bank for *Oryza* species should be organized, serving as the center for the exchange or provision of the species stocks.

– 3 – As there is considerable confusion in the nomenclature of the *Oryza* species, perfection or standardization of this should be undertaken, with the results published.

– 4 – Both intensive and extensive studies should be applied to the hybridization of species in the sections of *Granulata, Coarctata,* and *Rhynchoryza* with the species in section *Sativa* and with each other. An improved crossing technique should be worked out and many crosses must be attempted with the improved technique.

– 5 – Synthesis of new species by chromosome doubling should be attempted. New tetraploids cm be produced as well as hexaploids. Genetical studies in the polyploidy level should be undertaken to help further elucidate the interrelationships of these species. Perhaps, these polyploids produced can be examined from the standpoint of practical application.

– 6 – Lastly, an exchange center should be maintained for the published literature pertinent to the investigations dealing with rice in various laboratories of the world. If it is altogether possible, a newsletter dealing with rice should be published. This would facilitate communication and offer help to all investigators.

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SUPPLEMENTARY NOTES

I. DIFFERENTIAL CONDENSATION AND CHROMOSOME PAIRING IN THE HYBRID OF *ORYZA SATIVA* × *ORYZA AUSTRALIENSIS*

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Since the senior author completed his paper for this Symposium (cf. LI, see p. 118), we have studied the F_1 diploid hybrid of *Oryza sativa* × *australiensis*. The full text of our findings will be published elsewhere (LI *et al.*, 1963). This paper summarizes some of the salient features of our recent investigation, as these features concern the mechanism of chromosome pairing in the hybrid.

(1) *Pachytene stage.*

In both the parental strains of *australiensis* and *sativa*, we easily distinguished 12 pairs of chromosomes (Fig. 2.1, 2 and 2a). It was difficult, however, to identify the centromere in some chromosomes of *sativa* but not those of *australiensis*. The salient feature mentioned here is that most, if not all, of the chromosomes of *australiensis* were prominently heterochromatic. One chromosome was almost entirely composed of heterochromatin. In others, the heterochromatic material was found in the proximal region or the distal end or ends. One of these chromosomes seemed to be made up mostly of euchromatin (Fig, 2.2a). Our observation seems to agree with those of SHASTRY AND MOHAN RAO (1961). On the contrary, the chromosomes of *Oryza sativa* were made up mainly, if not entirely, of euchromatin. Generally, the chromosomes of *australiensis* were much thicker and more darkly stained than those of *sativa* at this stage, even though there seemed no appreciable difference in the length of the chromosomes of these two species (SHASTRY AND MOHAN RAO, 1961; H. K. WU, unpubl.).

In the F_1 hybrid, we were unable to obtain preparations with readily identifiable chromosomes. The chromosomes were clumped tightly together. Many of them were recognizably paired (Fig. 2.3, 3a, 2.4, 4a), at least in the region which stood out from the rest of the clump. Loops and bumps indicative of structural segmental differences characterized these paired chromosomes. The relational coiling of these paired chromosomes was recognizable and the ends of the two paired chromosomes were usually widely separated, indicating that synapsis occurred only between certain segments. Unfortunately, these paired chromosomes could not be identified to indicate whether pairing was autosyndetical or allosyndctical. Other chromosomes apparently were univalent. Some chromosomes, presumably belonging to *australiensis*, were darkly stained and highly impregnated with heterochromatin, and they were definitely thicker. Others, presumably the *sativa* chromosomes, were lightly stained and thinner.

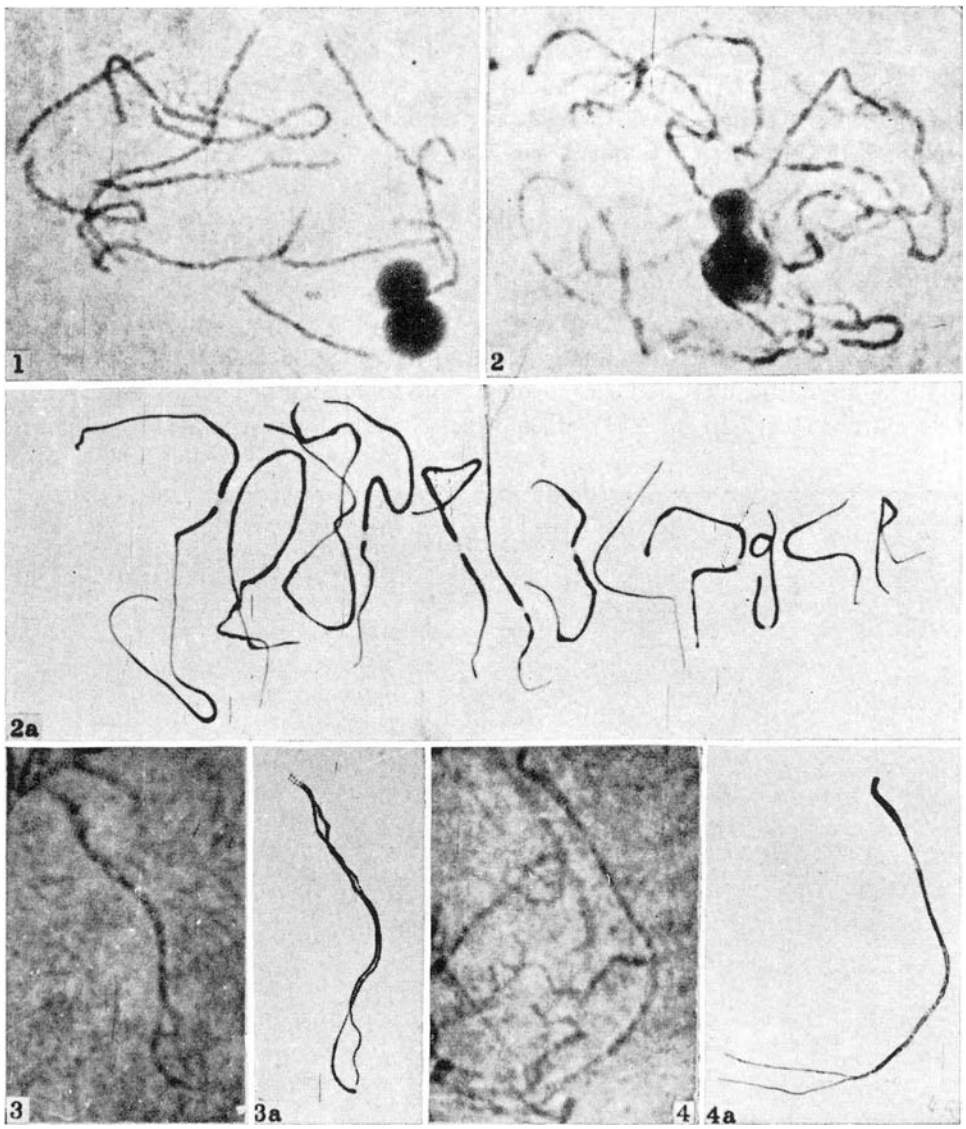

Fig. 2.1–4a. Pachytene stage of the parents and their F_1 hybrid.
1 – *Oryza sativa.* Note that there are no observable heterochromatic regions in these chromosomes: 2 – *Oryza australiensis.* Note the heterochromatic regions in almost all of the chromosomes; 2a – Camera lucida drawing of the individual chromosomes, showing noticeably heterochromatic regions in different chromosomes. One of them seems to be devoid of any heterochromatin; 3 – One segment of a bivalent of the F_1 hybrid, showing the loose nature of synapsis. The identity of these paired chromosomes is not recognized; 3a – Camera lucida drawing of the same; 4 – Another paired segment of the F_1 hybrid; 4a – Camera lucida drawing of the same segment.
(1, 2 and 2a = × 1570; 3 and 4 = × 2500; 3a and 4a = × 2250).

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(2) *Diplotene stage*

With propiono-carmine, the chromosomes of *O. sativa* at mid-diplonema at times did not stain readily (H. K. WU, unpubl.) as shown in Fig. 2.5. On the other hand, the chromosomes of *O. australiensis* were deeply stained and had loops and nodes characteristic of the presence of chiasmata (Fig. 2.4).

The chromosomes of the F_1 hybrid were rather sticky, so that they were more or less clumped together. The darkly stained, thicker chromosomes of *australiensis* were rather conspicuous at this stage. But the faintly stained, thinner chromosomes of *sativa* also were discernible (Fig. 2.7). However, we could not easily identify the individual chromosomes of these two species, particularly the *sativa* chromosomes. At this stage, most of the bivalents were paired end-to-end (Fig. 2.8 and 2.9). We attempted to find allosyndetically paired chromosomes with more than one chiasma and finally detected two (Fig. 2.10 and. 2.11), one of which could easily be separated by its degree

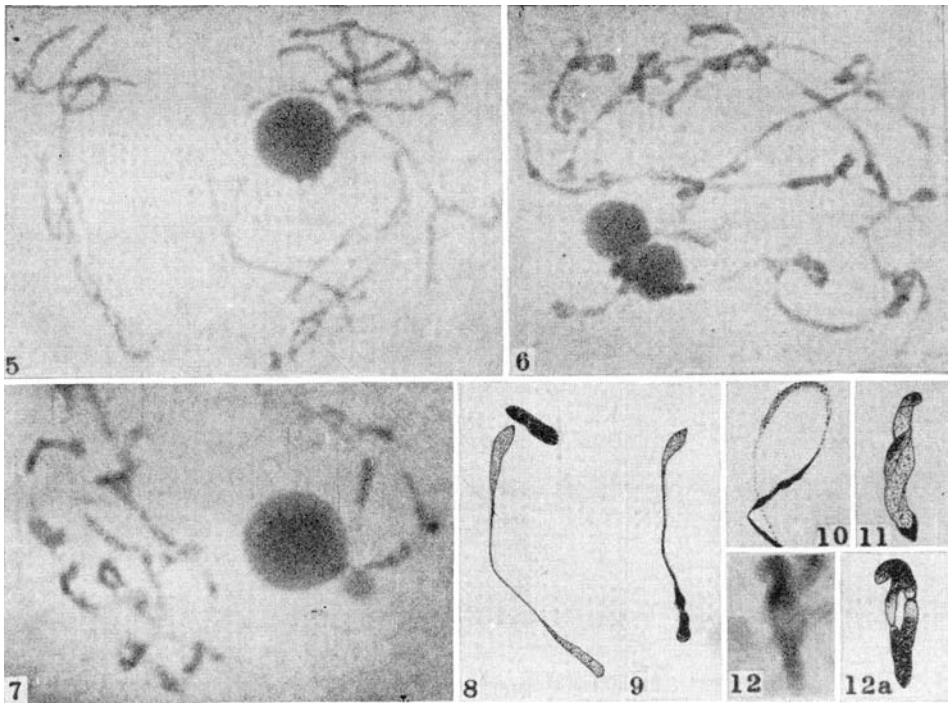

Fig. 2.5–12a. Diplotene stage of the parents and their F_1 hybrid.
5 – *Oryza sativa:* 6 – *Oryza australiensis;* 7 – F_1 hybrid, showing about 24 elements. The *australiensis* chromosomes are darkly stained in the heterochromatic regions, whereas the *sativa* chromosomes are faintly stained at this stage; 8 – Camera lucida drawing of a pair of *sativa* chromosomes of the F_1 hybrid. In contrast, a darkly stained, more condensed *australiensis* univalent is shown in the top portion of the drawing; 9 – Camera lucida drawing of an allosyndetic rod-shaped bivalent; 10 – Camera lucida drawing of an allosyndetic ring-shaped bivalent at early diplonema showing the existence of two chiasmata; 11 – Camera lucida drawing of an allosyndetic bivalent showing the existence of three chiasmata; 12 – An autosyndetic ring-shaped *australiensis* bivalent of the F_1 hybrid; 12a – Camera lucida drawing of the same as Fig. 2, 12. Note the hetero- and eu-chromatic regions.

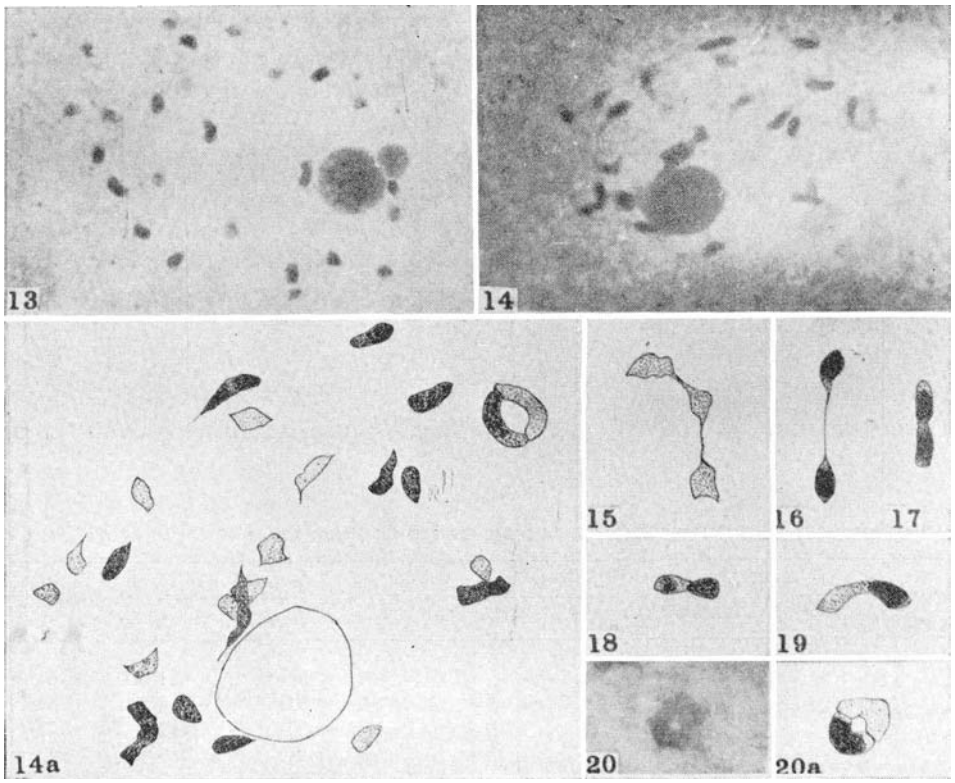

Fig. 2.13–20a. Diakinesis of the F_1 hybrid.
13 – Late diakinesis showing 24 elements. Twelve of them are darkly stained whereas the remainder, lightly stained. Note that the size of these two different sets of chromosomes is more or less the same; 14 – Mid-diakinesis showing one allosyndetic ring-shaped bivalent; 14a – Camera lucida drawing of the same bivalent; 15 Camera lucida drawing of a *sativa* trivalent; 16 and 17 – Camera lucida drawings of one rod-shaped *australiensis* bivalent; 18 and 19 – Camera lucida drawing of one rod-shaped allosyndetic bivalent; 20 – A ring-shaped allosyndetic bivalent. Note that the size of these two chromosomes is almost the same: 20a – Camera lucida drawing of the same bivalent.
(5–6 = × 1570; 7 = × 1910; 8–11, 12a, 14a–19 = × 2250; 12, 20 = × 2500: 20a = × 3000; 13–14 = × 1230).

of staining (Fig. 2.10). The other had three distinct chiasmata at late diplonema (Fig. 2.11), and was the only one of its kind in our studies so far. Apparently, the chromosomes of *australiensis* contracted more at this stage than its paired *sativa* chromosome (Fig. 2.10). Heterochromatic connections (thin threads) linked many chromosomes together.

(3) *Diakinesis*

We studied many PMCs at early and late diakinesis in the F_1 hybrid, and found that the chromosomes of both *australiensis* and *sativa* contracted further but at a different rate, resulting in the dark staining of chromosomes of *australiensis.* At this stage, the chromosomes of *australiensis* and *sativa* were more or less the same in size. In some

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PMCs, however, the chromosomes of *sativa* were only faintly stained and were difficult to recognize. With careful scrutiny, however, we identified them (Fig. 2.13). Table 9 contains the results of attempts to study the association of the chromosomes at diakinesis.

Table 9 shows the similarity between the association of the chromosomes of *australiensis* and *sativa* and that found at MI-AI (Table 7, p. 127).

Most of the bivalents showed end-to-end configurations at MI-AI. Again, we tried to determine whether there were the so-called allosyndetic pairs (AS). Several such bivalents were ring-shaped, signifying the existence of two chiasmata per bivalent (Fig. 2.14, 14a and 2.20, 20a).

(4) *Metaphase* I – *Anaphase* I.

From the study of 150 PMCs, we found that AA associations were about half as

TABLE 9

CHROMOSOME ASSOCIATION AT DIAKINESIS IN THE HYBRID OF *Oryza sativa* × *Oryza australiensis*

III	II			I		Frequency
ASS*	AA	AS	SS	A	S	
		1		11	11	6
		2	1	10	8	5
				12	12	4
		3		9	9	4
		2		10	10	4
	1			10	12	3
			1	12	10	2
		2	2	10	6	2
	1	2		8	10	2
	1		1	10	10	2
		1	2	11	7	1
			2	12	8	1
	1	1	2	9	7	1
	2	3		5	9	1
		1	1	11	9	1
		3	1	9	7	1
	1	1		9	11	1
	1	1	1	9	9	1
		2	2	10	6	1
	1	4		6	8	1
			3	12	6	1
	1	1	3	9	5	1
		1	3	11	5	1
	1	2	1	8	8	1
1	1	3	1	6	5	1
	2		2	8	8	1
Total 1	18	68	37	495	456	50
Mean 0.02	0.36	1.36	0.74	9.9	9.12	
Range 0–1	0–2	0–4	0–3	5–12	5–12	

* A and S represent chromosomes of *australiensis* and *sativa* respectively.

many as the SS associations. But this observation differed from the findings of SHASTRY AND RANGA RAO (1961) who found almost no AA association. Studying 50 PMCs, we observed the same proportion of AA and SS associations at diakinesis (Table 9). With AS association, there were 1.66 such bivalents per PMC at this stage as against 1.36 at diakinesis.

In our study, with the AA bivalents, I7 were of the closed type (Fig. 2.22) and 18 open types (Fig. 2.21) in a total of 35 such bivalents found at this stage. Of 83 SS bivalents found, all but one were the open type (Fig. 2.23). With AS bivalents, most of them were of the open type (Fig. 2.24), but the proportion of open and closed ones was not recorded. Reexamination indicated there were some bivalents of the closed type (Fig. 2.25, 25a and 2.26, 26a) at this stage, clearly indicating the existence of two chiasmata (Fig. 2.25, 25a). It is inferred that the open-type bivalents have one chiasma.

In previous studies, we found that the *sativa* chromosomes were about one-fourth to one-half the size of *australiensis* chromosomes. At diakinesis (especially the late stage), the chromosomes of A and S were about equal in size. Definite comparisons could be made from the paired bivalents of the AS type (Fig. 2.20, 20a). The two chromosomes were about the same size. However, at metaphase, the members of paired AS bivalent differed greatly in size (Fig. 2.24, 2.25, 25a and 2.26, 26a), indicating that the chromosomes of *sativa* with mostly euchromatic constitution contracted further at this stage. Contraction was slower at early stages but was completed at metaphase. On the contrary, the chromosomes of *australiensis,* made up of hetero-

TABLE 10

FREQUENCY DISTRIBUTION OF *sativa* AND *australiensis* CHROMOSOMES AT BOTH OF THE POLES AT MI-AI IN F_1 HYBRID OF *Oryza sativa* × *australiensis*

sativa	*australiensis*													Tota1
	0	1	2	3	4	5	6	7	8	9	10	11	12	
0	1		2		3	1	1	1						9
1		1	1	2	1	3	3	4	5		1			21
2	1		1		2	1	5			2	1	1	1	15
3		1			4	2	6	2		3	2	1	1	32
4					2	1	1	5	4	5				18
5					1	2	3	3	1	2	2			14
6			1			1		2	3	1	1			9
7						1		1	1	1	1			5
8									1	2	2			5
9										1				1
10								1						I
11														
12													2	2
Total	2	2	5	2	13	12	19	19	15	17	10	2	4	122

A test of independence was calculated. With $c^2 = 171.26$, $P < 0.01$, and 110 df., the test indicates that the hypothesis of independent migration of chromosomes of *O. sativa* and *O. australiensis* to the two poles was not valid.

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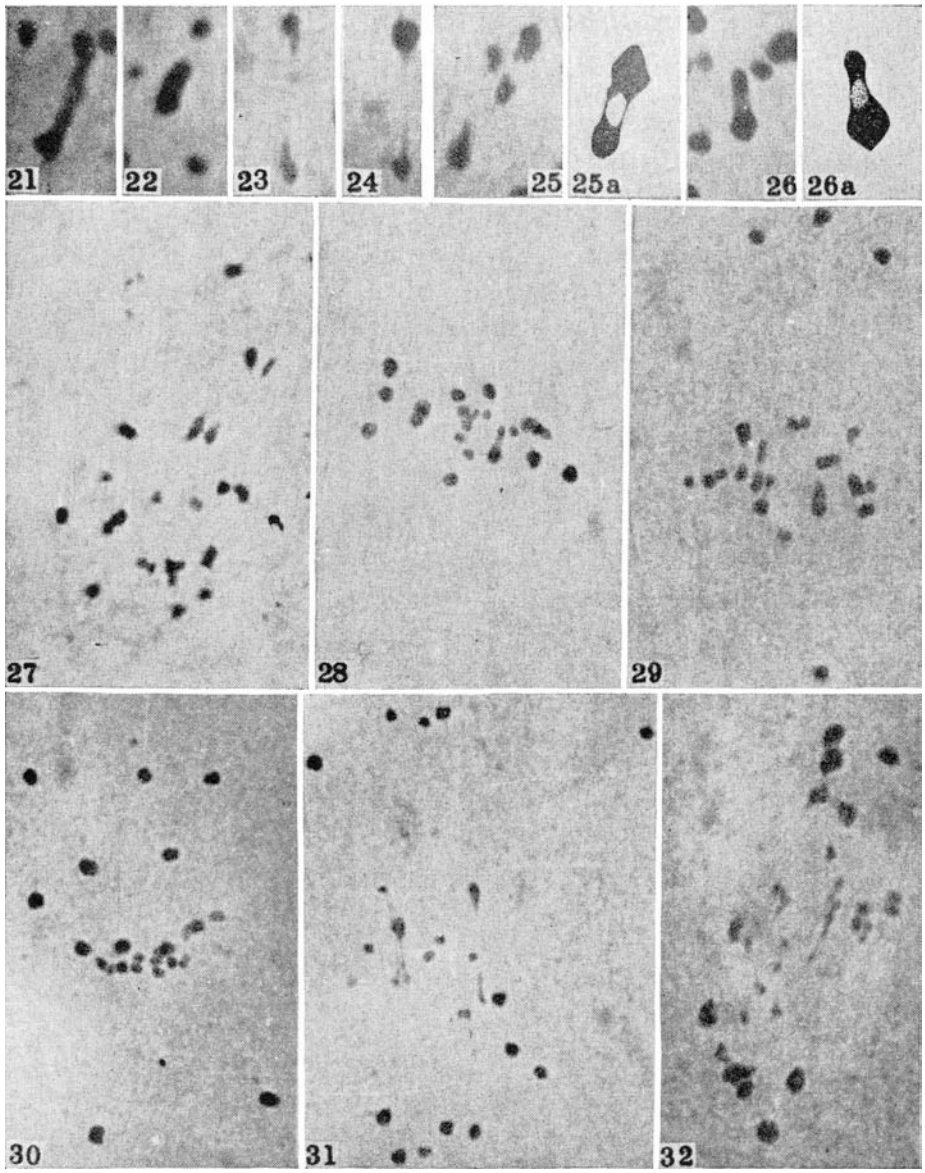

Fig. 2.21–32. MI-AI in the F_1 hybrid.
21 – A rod-shaped autosyndetic *australiensis* bivalent; 22 – A ring-shaped autosyndetic *australiensis* bivalent; 23 – A rod-shaped autosyndetic *sativa* bivalent; 24 A rod-shaped allosyndetic bivalent; 25 – An allosyndetic bivalent on the right showing the existence of two chiasmata; 25a – Camera lucida drawing of the same bivalent; 26 – Another allosyndetic bivalent with two chiasmata; 26a – Camera lucida drawing of the same bivalent: 27 – Prometaphase showing chromosomes are distributed all over the cell; 28 – The congression of the chromosomes at the equator; 29 and 30 – The differential migration of the chromosomes of the two complements; 31 – The bivalents as well as the *australiensis* univalents are moving to the respective poles: 32 – All of the chromosomes are dividing at this stage. (21–25 and 26 = × 1910; 25a, 26a = × 3375; 27–32 = × 2417).

chromatin and euchromatin, contracted faster in the earlier stages and stopped contracting early, perhaps by late diplonema or diakinesis.

We observed some differences in the stainability of the chromosomes of *australiensis* and *sativa* in some PMCs at this stage (Fig. 2.26). But in many other PMCs studied, except for the size difference, the chromosomes of both complements stained almost identically.

The *australiensis* chromosomes tended to migrate to the poles ahead of those of *sativa.* Table 10 shows the results of our counts which approximated the data obtained by SHASTRY AND RANGA RAO (1961).

Considering the movement of chromosomes at this stage, we must take into account that (1) there are chromosomes of two separate complements, and (2) there are mostly univalents and none or few bivalents or multivalents.

At prometaphase, the chromosomes were distributed all over the cell (Fig. 2.27). Presumably, congression had taken place (Fig. 2.28). The migration of the *australiensis* univalents occasionally divided at the poles, with the *sativa* univalents dividing at the equator after the chromosomes of the bivalents reached their respective poles (Fig.2.32).

If, the chromosomes of all the PMCs follow the pattern described, then we can safely conclude that the *australiensis* chromosomes migrated ahead of the *sativa* chromosomes and even ahead of the bivalents which were bound together by chiasma or chiasmata, In *Bromus* hybrids, WALTERS (1958) found that during metaphase the numerous univalents moved to the poles, then returned to the equator, and the spindle increased and decreased in length. Anaphase might begin at any time during the return of the univalents to the equator. In the hybrid studied, the movement of chromosomes did not behave as that in *Bromus* hybrids.

SUMMARY

The chromosomes of *Oryza australiensis* were made up partly of heterochromatin, whereas those of *sativa* were mostly euchromatin.

In the F_1 hybrid of *sativa* × *australiensis* there was differential condensation in these two morphologically different types of chromosomes. The *australiensis* chromosomes with partly heterochromatin and partly euchromatin condensed early, beginning presumably from pachynema until diakinesis. The *sativa* chromosomes seemingly started their condensation later but condensed more completely at first metaphase. Thus, before diakinesis, the *australiensis* chromosomes were stained darker and were 2–4times the size of the *sativa* chromosomes at MI-AI.

At either diakinesis or MI-AI, about two bivalents could be found per PMC. Differences in size or staining at diakinesis separated these bivalents into the following two types at MI-AI: (1) autosyndetic bivalents, and (2) allosyndetic (multivalents were also found, but rarely) ones.

All these proved to be authentically true bivalents. The evidences are:

– 1 – Loosely paired chromosomal segments were repeatedly observed in many PMCs at pachynema.

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– 2 – At diplonema, most allosyndetically paired bivalents were found to have one chiasma, or two or more chiasmata, in some PMCs.

– 3 – At diakinesis, these allosyndetically paired bivalents were found to be ring-shaped or end-to-end.

– 4 – Closed allosyndetic bivalents with two chiasmata were frequently observed at MI-AI.

Pairing of the homologous segments in these allosyndetic pairs may have occurred at the euchromatic regions of the two chromosomes concerned. Presumably, these euchromatic regions of the chromosomes from two different species might have the same rate of condensation at various stages of meiosis.

II. PACHYTENE STUDIES OF THE HYBRID *ORYZA SATIVA* × *ORYZA OFFICINALIS*

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From a pachytene analysis of the F_1 hybrid between *sativa* (PTB-10) and a Ceylonese form of *officinalis,* SHASTRY and his co-workers (SHASTRY *et al.,* 1961) concluded that there was complete pairing between the chromosomes of the two constituent species. They postulated that desynapsis, not lack of homology, caused complete univalent formation in the hybrid at metaphase I.

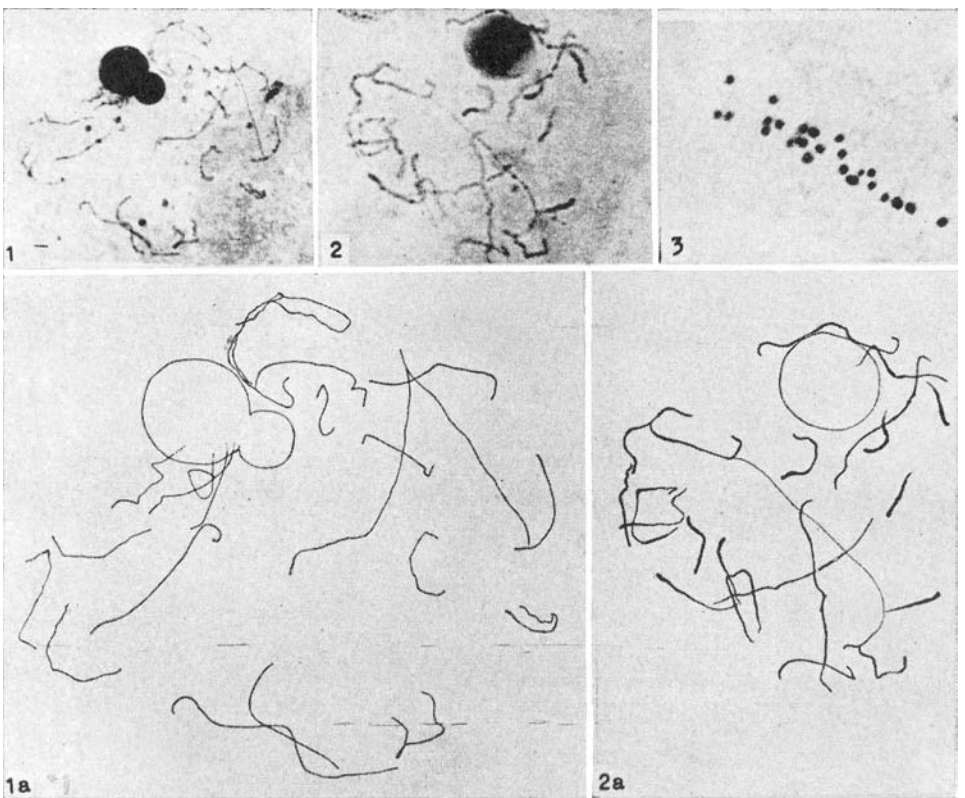

Fig. 3.1–3. Pachytene studies on *Oryza sativa* × *officinalis* hybrid.
1 – Pachynema showing 24 univalent chromosomes; 1a – Camera lucida drawing of the same; 2 – Late pachynema showing 24 unpaired chromosomes; 2a – Camera lucida drawing of the same; 3 – First metaphase showing $1^{II} + 22^{I}$.

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A preliminary study of an F_1 between *sativa* (variety Taichung 65 of the *japonica* type) and *officinalis* (line W 012 of the National Institute of Genetics, Japan) led us to the following findings: from a number of well spread and easily analyzed PMCs at pachynema and early diplonema, with the exception of a partly paired bivalent in one PMC at diplonema, 24 unpaired chromosomes were invariably observed (Fig. 3.1, 1a and 3.2, 2a). This unpaired condition persisted till metaphase I (Fig. 3.3).

Lack of homology between the chromosomes of the two species caused the nearly complete univalent formation in the hybrid. Our findings support the earlier observations of NANDI (1936, 1938), RAMANUJAM (1937), MORINAGA and KURIYAMA (1960) and NEZU *et al.* (1960) that *sativa* and *officinalis* have two distinct genomes, A and C, respectively.

DISCUSSION IN SESSION ON GENETIC AND CYTOGENETIC EVIDENCE FOR SPECIES RELATIONSHIPS

Discussion of hybrid breakdown in certain interspecific crosses suggested the possibility that, in addition to genic systems, cytoplasmic factors could also affect the viability of hybrids. The ensuing discussion concerning the interrelationship between *Oryza perennis, sativa* f. *spontanea* and *sativa* revealed that the group was divided into two schools of thought. One school envisaged the *spontanea* forms as derivatives from hybrids between *perennis* and *sativa.* This observation also applied to the so-called *formosana.* The other school regarded both *spontanea* and *sativa* as derivatives of *perennis.* However, no critical evidence is yet available to support or refute either one of the postulates. The participants also discussed the need for defining the *perennis* type.

Discussion followed on the conflicting reports concerning meiotic behavior in *sativa* × *glaberrima* hybrids. The influence of environment upon pairing behavior, the genic control of chromosome pairing and genetic diversity in plant material were mentioned as possible explanations for the differences in research results. Certain workers in this area proposed to exchange experimental materials for comparative study.

A question referring to the paper by S. V. S. SHASTRY was raised on the mode of origin of the "frying pan-type trivalents" in diploid hybrids. The author suggested that such trivalents presumably arose through segmental reduplication. On the question of "differential segments" in relation to species relationships, the author maintained that isolating mechanisms and chromosome differentiation should be treated as separate topics in describing species in a purely genetic or taxonomic sense. The next question concerned the relative amounts of heterochromatin in chromosomes in relation to the putative primitiveness of species. Various possibilities were suggested, such as deletion, conversion, condensation, and partial alocycly. Past studies on interspecific cross involving *australiensis* involved only one single strain. This again demonstrated the need for more diverse material in genome analysis to sample variation within various species.

On a question on the hybridization of *sativa* with *ridleyi,* the group agreed that genome analysis for those species within the section *Sativa* Roschev. is nearly complete, whereas for other sections, additional study is needed.

With reference to his report, H. W. LI pointed out that coconut milk was extensively used in the artificial culture of young caryopses of inter-specific hybrids. The author also clarified that he studied prophase pairing of the *sativa* × *australiensis* hybrid at diplotene, which suited his material better than pachytene.

On the subject of pachytene analysis, H. KIHARA commented that ERNST'S (1939) investigations showed that pachytene pairing cannot be accepted as the sole criterion for chromosome homology. As MCCLINTOCK (1933) suggested, the pachytene stage may be interpreted as the time when no chromosome remains single due to some form of attraction. KIHARA recounted an instance in a wheat hybrid in which perfect pairing was observed at pachytene and no pairing was noted at metaphas I, showing that at pachytene even partially homologous chromosomes appeared to conjugate in a regular manner. M. T. HENDERSON emphasized the need to differentiate genes controlling pairing in different genomes and the genomes themselves.

The participants urged standardization of genome symbols in *Oryza species* and elected a committee composed of H. KIHARA (chairman), H. W. LI, T. MORINAGA, R. H. RICHHARIA and T.-T. CHANG (secretary) to study the problem and to report their recommendations to the Symposium on the last day of the conference.

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SESSION V

NATURE OF INTERVARIETAL HYBRID STERILITY IN *ORYZA SATIVA*

CYTOGENETIC STUDIES AT THE LOUISIANA AGRICULTURAL EXPERIMENT STATION ON THE NATURE OF INTERVARIETAL HYBRID STERILITY IN *ORYZA SATIVA**

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Despite the regular occurrence of partial or complete sterility in interspecific and intergeneric hybrids, investigators differ in opinion concerning all of the causes of this phenomenon. Essentially the same is true with the cause of partial sterility in intervarietal hybrids within a species. Although several examples of intervarietal hybrid sterility within wild species of plants have been discovered and investigated, virtually in all cases there still remain differences of opinion as to the cause of the sterility. Two general classes of explanations account for the cases of sterility in intervarietal hybrids within wild species. According to one type of explanation, the hybrid sterility is caused in some manner by the action of genes. The second explanation is that sterility is due to structural differentiation in the chromosomes of the parental varieties.

Although sterility in hybrids, both interspecific and intervarietal, in species other than cultivated rice has been the subject of considerable research, many scientists still disagree as to its causes. Although intervarietal hybrid sterility comparable to that in *Oryza sativa* appears to be extremely rare in other cultivated species, it is probable that the cause of the sterility in rice is basically similar to that causing sterility in several wild species. As in the sterility of interspecific hybrids, it is not entirely caused by failure of the chromosomes to pair. Consequently, it is not surprising that the cause of sterility in certain intervarietal hybrids of cultivated rice should be disputed.

The research was of two general types. In one type, cytological studies were made of chromosomes and chromosome behavior during meiosis in F_1 plants, some of which were partially sterile and others fertile, representing several intervarietal rice hybrids. The second type of research involved a genetic study of the behavior of sterility in hybrid populations,

CYTOLOGICAL STUDIES OF CHROMOSOME BEHAVIOR DURING MEIOSIS

The cytological experiments have been published (YAO, HENDERSON AND JODON,

* Dr. BIRDIE P. YEH, SEW YING YAO, and Dr. H. R. CAFFEY, former graduate students in the Department of Agronomy at Louisiana State University, conducted the research reported here. The research was financed in part by a grant from The Rockefeller Foundation. Mr. N. E. JODON provided much of the materials used in the studies. Some phases of the research have been published (YAO, HENDERSON, AND JODON, 1958), while portions have not (CAFFEY, 1959).

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1958; HENDERSON, YEH AND EXNER, 1959). One study consisted primarily of a detailed examination of chromosome pairing during the pachytene stage of meiosis for evidence of inversions in a group of seven intervarietal hybrids — one fertile and six partially sterile. Structures which appeared to represent loops characteristic of chromosomes heterozygous for inversions were found at a low frequency in five of the seven hybrids. The experiment showed structural differentiation of the chromosomes in the form of inversions. The partial sterility may have resulted primarily from cryptic structural hybridity arising from included inversions. At the same time, ordinary inversions of the type that cause loops at pachytene would not account for any appreciable fraction of the sterility occurring in the hybrids, a fact overlooked in several previous publications.

Somewhat later, the anaphase stage of meiosis in six fertile and six partially sterile hybrids was investigated. A minimum of 428 PMCs per hybrid was examined, and in 10 of the hybrids, the number of PMCs exceeded 1000. Only examples representing unquestionable cases of bridges accompanied by chromatin fragments were recorded as such. Cells at anaphase with clear cases of bridges and acentric fragments were found in 9 of the 12 hybrids. Cells with bridges and fragments had low frequency in all hybrids. Four of the hybrids with bridges and fragments were fertile. No examples of cells with bridges and fragments were discovered in three varieties or in a hybrid between two closely related varieties.

The discovery of bridges accompanied by acentric fragments at anaphase in 9 of 12 hybrids probably signifies the presence of paracentric inversions in most hybrids between distantly related varieties of rice. Although the presence of ordinary inversions would not account for any measurable degree of sterility, it shows that a high degree of structural differentiation exists in the chromosomes of rice and supports the probability that sterility in certain hybrids may be caused by more complex included inversions which could not be detected. The presence of included inversions capable of causing hybrid sterility is entirely circumstantial and the cytological results did not reveal any evidence contradictory to the hypothesis that the sterility is genic in cause.

GENETIC STUDIES OF HYBRID STERILITY

The genetic studies were conducted in F_1, F_2 and F_3 generations of an intervarietal hybrid which showed sterility typical of that found frequently in *indica-japonica* crosses (YAO *et al.,* 1958; HENDERSON *et al.,* 1959; CAFFEY, 1959). One parent was designated CI 6008 and the other, selected by a rice farmer in Louisiana from a field of the Zenith variety, was known as Dischler's Selection. CI 6008 belongs to the indica group while the classification of the other parent is unknown. Degree of fertility of individual plants in the hybrid populations was expressed both as percentage of florets setting seed and percentage of pollen grains stainable in acetocarmine. The study involved 866 F_2 plants and 91 F_3 lines.

For 100 F_2 plants chosen at random, a highly significant positive correlation coefficient of 0.68 was found between percentage of florets setting seed and percentage of stainable pollen. Among the means of the 91 F_3 lines, an even higher correlation coef-

ficient of 0.77 occurred between seed set and stainable pollen. Though the agreement was not perfect in all cases, it was apparent that the sterility had basically the same effect on seed setting and development of functional pollen. Consequently, the genetic studies presented in this paper are restricted to reduction in stainable pollen as an expression of sterility because it is subject to less environmental variation than is seed setting.

Individual plants of the parent varieties ranged in stainable pollen from approximately 85 to 95%, with means of 92 and 94%. Thus, variation in environmental conditions slightly reduced fertility and caused variation of about 10% among individual plants. These data show that any hybrid plant with percentage of stainable pollen of 85 or above in the experiment probably had normal fertility. Those hybrid plants with less than 85% stainable pollen were probably partially sterile.

Pollen stainability of the F_1 was only 4.3%. These data were obtained from an F_1 plant grown in the greenhouse during the winter however, and undoubtedly were lower than when grown in the field. When grown in the field the preceding summer the F_1 had 41.5% of florets setting seed indicating that the F_1 generation had a high degree of sterility, although probably not as high as shown by the pollen stainability data.

Among the 866 F_2 plants evaluated, there was a continuous range from 1 to 98% in stainable pollen. For convenience in handling and presenting the results, the F_2 plants were placed in 10 classes, with a 10 percent range within classes. The results, in the form of frequency distributions, for the parents and 866 F_2 plants are presented in Table 1.

TABLE 1

PREQUENCY DISTRIBUTIONS FOR PERCENTAGE OF STAINABLE POLLEN IN THE PARENTS AND 866 F_2 PLANTS

	Number of plants in each stainable pollen range										
Population	1–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	Mean
CI 6008									3	7	92.0
Dis. Sel.									4	15	93.7
F_2	100	54	58	71	135	128	69	62	121	68	52.7

Table 1 shows that the F_2 data were trimodal, although a large number of plants occurred in each fertility class. One mode occurred in the most sterile range of 1 to 10% stainable pollen. The highest mode occurred in the range of 40 to 60%, with 30% of the F_2 population falling within this partially sterile range. A third mode occurred in the 81 to 90% range. Most of the F_2 plants in this and the 91 to 100% ranges undoubtedly were completely fertile.

Apparently, several inherently different classes of plants occurred in the F_2 population, although the exact number could not be determined because of the continuous distribution. Obviously, these F_2 data do not fit into any distinct ratio characteristic of a qualitative trait. Neither was the F_2 behavior typical of a quantitative character.

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In order to determine the breeding behavior through progeny performance of the various classes of F_2 plants, 10 F_2 plants from each of the range classes in Table 1 were selected, and F_3 lines were grown from them. Nine of the 100 F_3 lines, having fewer than 10 plants which headed, were discarded. Each plant of the remaining 91 F_3 lines was evaluated for pollen stainability. Unfortunately, space does not permit presentation of the complete data for the 91 F_3 lines.

The means of the F_3 lines ranged continuously from 21.6 to 92.1 in percentage of stainable pollen, a behavior similar to the F_2. A correlation coefficient of 0.71 was calculated between mean pollen stainability of the F_3 lines and the percentage stainable pollen for the F_2 plants from which the lines were derived. The regression coefficient of means of F_3 lines on the F_2 plant values was 0.50, indicating heritability of 50 percent for fertility. The moderately high level of heritability for fertility confirms the conclusion drawn from the F_2 data that a large number of inherently different plants occurred in the F_2 population.

Eleven F_3 lines appeared to breed true for complete fertility. These lines were derived from F_2 plants which were highly fertile. All of the remaining F_3 lines contained some plants which definitely were partially sterile. In all of these lines, except one, however, some completely fertile plants were found. This also was observed in lines with mean fertility as low as 22%, This phenomenon of completely fertile plants occurring in lines showing partial sterility was so distinctive and consistent that it must be considered when interpreting results. As stated previously, one F_3 line did not contain any completely fertile plants, However, this line contained only 13 plants, with some having as high as 50% of stainable pollen. It seems probable that, had a larger number of plants been grown in this line, completely fertile ones would have been obtained. In any event, this exceptional F_3 line did not breed true for a particular level of sterility, since the plants ranged from less than 5% to 50% in stainable pollen.

To illustrate that a large number of the F_3 lines represented inherently different fertility classes, Table 2 shows the means and frequency distributions obtained for a selected group of seven lines. The 91 F_3 lines showed a wide and continuous range in mean fertility; hence, the actual number of inherently different lines could not be determined,

TABLE 2

FREQUENCY DISTRIBUTIONS AND MEANS OF 7 F_3 LINES SELECTED TO INDICATE THE INHERENT DIFFERENCES AMONG 91 F_3 LINES TESTED

	Number plants in following % stainable pollen ranges										
Line No.	1–10	11–20	21–30	31–40	41–30	51–60	61–70	71–70	81–90	91–100	Mean
19									3	12	91.9
38							2	1	4	7	86.7
14	1			1		1			5	4	75.2
61	1			1	1	2	3	2	3	1	65.0
80	1	1	1	2	1		1	1	1	1	49.4
73	5	2			4	3	1	1		1	38.6
85	10	1			2	1				1	21.9

or even estimated. The ones presented in Table 2 were so distinct in behavior that there is no doubt of their inherent difference in fertility.

Line 19 obviously was completely fertile. Line 38 had a few partially sterile plants but differed only slightly from line 19. In line 14, 9 of the 12 plants appeared to be completely fertile but the other three plants showed the complete range of partial sterility, Conversely, in lines 61 and 80 a majority of plants were partially sterile. In lines 73 and 85 a large percentage of the plants were highly sterile, yet one completely fertile plant occurred in each line.

A purely objective analysis of the genetic studies with F_2 and F_3 populations suggests that the results do not prove the cause of sterility. However, any genic explanation would seemingly have to be highly contrived, complex and possible, but improbable. The extremely large number of inherently different classes in F_2 and F_3 and the strong tendency of all partially sterile F_3 lines to contain completely fertile plants do not support a logical genic explanation. On the other hand, all of the behavior observed in F_2 and F_3 would be expected if the parent strains differed in respect to several included inversions involving varying chromosome lengths. For these reasons, the writer feels that the cytological and genetic data together indicate stronger evidence that the sterility was due to structural differences in the chromosomes, probably of the included inversion type.

ADDENDUM

During and immediately following the Symposium, attention was called to the possibility that many of the conferees may not be familiar with the concept of included inversions and their possible role in causing intervarietal hybrid sterility in *Oryza sativa.* Consequently, the following information is added to explain more completely included inversions which may account for this sterility,

An inversion is a gross structural rearrangement within a chromosome in which a chromosome segment becomes inverted, or reversed from its normal position. Assume, for example, that a chromosome possesses the following genes in alphabetical order, A B C D E F G H I, but the segment C through G becomes reversed in its position within the chromosome. An inversion will be formed, with the new gene order A B G F E D C H I. Inversions have been found in a wide variety of species, both plant and animal. In some plant genera, such as *Tradescantia* and *Paris,* essentially all individual plants contain them.

Two kinds of inversions are recognized based on the relationship of the inverted region to the centromere. In one class, called paracentric, the centromere is not included in the inverted segment, with the inversion restricted to one arm of the chromosome. In the second type, called pericentric, the centromere is included in the inverted region. The paracentric type is by far the more common and for this and other reasons the discussion presented herein will be limited to that form.

If a pair of homologous chromosomes differ in an inversion, the segment involved will interfere with pairing and chiasma formation. Should the inverted region be long, pairing can be achieved by development of a loop configuration at zygotene and

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pachytene stages of meiosis. Chiasma formation in the loop will result in one chromatid with two centromeres, which upon separation of the pair of chromosomes at anaphase will produce a chromatid bridge, and a fragment without a centromere (acentric). The acentric fragment is lost. The process causes the development of some gametes with a chromosome deficiency and others with a duplicated region. Both abnormal types of gametes usually are unable to function and partial sterility results.

When the inverted segment is short, as will be the case for essentially all inversions in rice due to smallness of the chromosomes, the loop figure will rarely occur. Instead, when the homologous chromosomes pair, there will be a strong tendency for the inverted regions to lie side-by-side in an association which is frequently termed "non-homologous pairing". In such cases, chiasma formation is inhibited in the non-homologously associated region, the dicentric bridge and fragment do not occur at anaphase and sterility is not produced. In cells both with and without the loop at pachytene, chiasma formation in chromosome regions outside the area of the inversion does not produce a bridge and fragment or sterility. As a consequence of these relationships, single inversions usually cause no measurable degree of sterility in species with short chromosomes and are tolerated in natural populations of many plants.

Paracentric inversions can be detected by: (1) a change in linkage relationships, (2) the occurrence of the loop configuration at pachytene and (3) the presence of cells with a bridge and acentric fragment at anaphase. For various reasons, the first two of these criteria cannot be utilized for most species and the low frequency of cells with bridge and fragment at anaphase in a short chromosome species, such as rice, makes detection difficult.

The net effect of the facts summarized above concerning single or simple inversions in a plant such as rice is that hybrids may differ in a large number of inversions without this being detected, unless several hundred pollen mother cells are examined at anaphase for the occurrence of bridges accompanied by fragments. Furthermore, single inversions, even if numerous, will not account for the high degree of sterility that is characteristic of *indica-japonica* hybrids.

If paracentric inversions are as numerous in rice as cytological research indicates, there will occasionally be cases involving two inversions within the same chromosome arm, producing adjacent, overlapping and included inversions, depending on the locations of the two inverted regions in relation to one another.

The included type of inversion is of special interest here. The following scheme illustrates how it can arise:

(I) A B C D E F
(II) A E D C B F
(III) A E C D B F

In the preceding scheme it is assumed that the original chromosome identified as (I), has the gene sequence A B C D E F and that an inversion occurs involving the segment B through E to form chromosome (II). It is then assumed that a second inversion develops later in chromosome (II) involving the segment D through C, forming chromosome (III) with the gene sequence A E C D B F. The reinversion

forms what is termed an included inversion because the inverted segment is entirely within the region of the first change.

If both inversions are paracentric, a hybrid which brings into association the original chromosome (I) and inverted chromosome (II) will merely be heterozygous for a simple inversion that will probably be detectable only through the occurrence of an occasional cell at meiotic anaphase with a bridge and fragment and there will probably be little if any measurable reduction in fertility. The same relationship will be true for a hybrid having the two inverted chromosomes, (II) and (III).

However, the meiotic behavior in the hybrid which contains original chromosome I and reinverted chromosome (III) will differ in several important aspects. For one thing, the typical inversion loop will not occur at anaphase, and, consequently, there will be no anaphase cells with bridges and fragments. In short chromosomes, it is probable that "non-homologous pairing" will occur in the displaced B and E segments. This means that likely the presence of inversions in the hybrid cannot be detected by the usual cytological methods. On the other hand, the regions with C D in the two chromosomes will be brought into homologous association through pairing, despite the fact that this region is involved in the inversions. This can be illustrated more clearly by arranging the two chromosomes side-by-side in the following manner:

(I) A B C D E F
(III) A E C D B F

Chiasma formation in the C D regions will form deficient and duplicated chromatids, resulting in sterility. The degree of sterility should be much higher than in plants heterozygous for a simple inversion because sterility is not dependent on loop formation. In fact, the degree of sterility caused by the included inversion should be one-half of the frequency with which chiasmata are formed in the C D regions.

Thus, heterozygosity for an included inversion may be virtually undetectable in many species but can cause much higher sterility than simple inversions. The condition described is one form of what has been termed "cryptic structural hybridity" by STEBBINS, who defined it as "chromosomal sterility due to heterozygosity for structural differences so small as not to materially influence chromosome pairing at meiosis."

It also has been pointed out that a system of translocation and retranslocation between chromosomes can also lead to cryptic structural hybridity capable of causing an even higher degree of sterility than included inversions. In order for cryptic structural differences to arise through retranslocation, however, it is necessary to have a high rate of single translocations, which can be detected readily by the presence in almost all pollen mother cells of multivalents at diakinesis and metaphase of the first meiotic division. Several cytological studies have shown that metaphase is normal, with 12 bivalents, in rice hybrids of the *indica-japonica* type. The absence of evidence for translocations in these hybrids combined with the cytological proof that inversions are common has led the author to the opinion that sterility in intervarietal hybrids of cultivated rice may be a result of cryptic structural hybridity arising from included inversions.

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IS STERILITY GENIC IN *JAPONICA - INDICA* RICE HYBRIDS?

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Pioneering investigations of KATO and his collaborators (1928, 1930) led to the classification of the cultivated rice, *Oryza sativa* L., into two subspecies, *japonica* and *indica*, based upon hybrid sterility as one of the important criteria. Since then, several workers, including TERAO AND MIZUSHIMA (1939), MORINAGA AND KURIYAMA (1955, 1958), OKA (1953b, 1956b, 1957a), KUANG (1951), MELLO-SAMPAYO (1952), SAMPATH AND MOHANTY (1954), VENKATASWAMY (1957), SAMPATH (1959), YAO, HENDERSON AND JODON (1958), HENDERSON, YEH AND EXNER (1959), have concentrated on the nature of sterility in these hybrids and the validity of this classification. The major controversy that held the attention of these workers was whether the nature of hybrid sterility is genic or chromosomal. Evidence in favor of either of these hypotheses was limited until YAO *et al.* (1958) and then SHASTRY AND MISRA (1961a, b) introduced pachytene analysis.

Previous workers (TERAO AND MIZUSHIMA, 1939; JONES AND LONGLEY, 1941; KUANG, 1951) supported the view that, although the chromosome pairing in these hybrids was apparently normal at metaphase I, the sterility of hybrids might be due to small chromosomal changes not detectable at this stage (cryptic structural hybridity). In support of this view, CUA (1952) obtained a partial evidence for preferential pairing in allotetraploids. The next step was an inquiry into the nature of structural differences. MELLO-SAMPAYO (1952) described the occurrence of anaphase bridges and suggested inversion heterozygosity as a cause of sterility. SAMPATH AND MOHANTY (1954) supported this view with the reservation that the extent of occurrence of anaphase bridges did not cause all the sterility in these hybrids. HSIEH AND OKA (1958) disputed the role of inversions, since, they observed bridges occurring in both the pure-line parents and the hybrids. HENDERSON *et al.* (1959), likewise, reported that bridges with fragments were infrequent (0.08%) and that bridges without fragments were more frequent, although the latter did not exclusively account for all of the sterility. They suggested that either the inversions were pericentric or the typical loops in pairing were not formed at pachytene.

T. VENKATASWAMY (1957), a collaborator of S. SAMPATH, first indicated that translocations brought about chromosomal differentiation between the subspecies, but I am not convinced. SAMPATH (1959), supporting the view of VENKATASWAMY (1957) indicates that the origin of duplication-deficiencies in the gametes might cause the

sterility in these hybrids. While the conclusions of both workers are significant, critical cytological evidence was not forthcoming.

During the period 1953–1957, OKA, largely observing the apparent normal pairing at metaphase I and the disputable evidence in favor of inversion heterozygosity, postulated that purely genic models account for sterility. The erratic nature of segregation for sterility (discussed below) made it necessary to postulate a series of genic systems such as the "Gametic-development genes", "duplicate-fertility genes", and certation to account for the various facets of the segregation pattern for sterility. These genic systems also were postulated to account for segregation distortion and other phenomena associated with hybrid sterility in these crosses. These postulates led HU (1960b) to conclude that even between different species, *sativa* and *glaberrima*, the predominating means of genetic differentiation might be by gene mutation. He further suggested that the sterility in *Oryza sativa* × *glaberrima* hybrids is probably due to recombination between G.D. genes as postulated by OKA (1953b).

Before presenting the evidence of the nature of sterility in *japonica-indica* hybrids, it is necessary to examine the segregation for sterility in these hybrids. KUANG (1951) reports that fertility improved in selfed generations of the hybrids. K. RAMIAH and his collaborators at Coimbatore, India, made similar observations. The unpublished data I obtained from the Central Rice Research Institute, Cuttack, India, are summarized as follows:

(1) There was no consistency with regard to the degree of sterility and the choice of parents. No single *japonica* or *indica* variety in combination with all others tested could give highly fertile or highly sterile F_1 hybrids.

(2) While reciprocals did not, in general, differ in sterility, in a limited number of cross-combinations, the differences were as large as 20–30%; in one cross (MO.2 × Taihoku 6) the reciprocal hybrids exhibited 28 and 98% sterility. The role of cytoplasm, although limited in evidence, was positive at least in some cases. This view was confirmed by the differences in sterility which persist even in the F_2 generation.

(3) Irrespective of the extent of sterility in the F_1 generation, the F_2 generation exhibited a wide variation in sterility so that the sterility characteristic of individual F_1 hybrids was lost in later generations. This observation leads to the conclusion that sterility is "recombinational".

(4) It is possible to obtain "true breeding steriles", "true breeding fertiles" and widely segregating cultures by appropriate selection. This was confirmed up to the F_5 in a limited number of crosses. Marked segregation for sterility was also noted from F_3 to F_5 in some crosses.

(5) Although not critically tested by statistical procedures, a general correlation between height and fertility, pubescence and sterility, and a tendency to lose *japonica* type plants was indicated.

(6) In several F_2-F_5 populations, albino, xantha and "non-flowering" plants were observed. These may have resulted from disharmonious recombinations in the hybrids. VENKATASWAMY (1957) reports "narrow leaf", "sterile lemma" and "dwarf sterile" types in some progenies. The problem involved, therefore, is to suggest a possible

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explanation for genetic differentiation between the subspecies and to account for anomalous segregation for sterility and the appearance of abnormal plants in the progenies.

SHASTRY AND MISRA (1961b) employed four *japonica-indica* F_1 hybrids for a detailed analysis of prophase stages. Sterility in these hybrids ranged from 43–76 percent. Pairing at metaphase I in all cases was normal and no anaphase bridges were recorded. Previous workers, not having examined the stages prior to diakinesis, failed to find quadrivalents which were recorded in two out of four hybrids studied by the above authors. This observation was confirmed in seven other hybrids investigated later (MISRA, unpublished). These observations, which indicate heterozygosity for translocations, are highly significant, as STEBBINS (1958) suggests that the G.D. genes of OKA (1953b) might well be small translocated segments. Some proponents of the genic theory of sterility discard the view because cytological evidence in favor of translocations is inadequate. The genetic evidence also supports the role of translocation in the differentiation of the subspecies. MIZUSHIMA AND KONDO (1960) conclude that the *Sp* (*A*) locus might be located in different linkage groups in *japonica* and *indica* rices. The discrepancy between our results and those of other workers seems to result from the choice of the stage analyzed. The low frequency of quadrivalents at diplotene and the regular bivalent formation at later stages only supports the conclusion that the segments involved are small and that they might be located in terminal or subterminal regions. Unlike inversions which will influence fertility subsequent to recombination within the inverted segments, translocations, by the independent assortment of chromosomes, can result in sterility. Consequently, although the frequency of quadrivalents may be low, translocations may be regarded as a factor in producing sterility.

Pachytene pairing in the *japonica-indica* hybrids studied by SHASTRY AND MISRA (1961b) was exceedingly abnormal. In a hybrid which exhibited 76.44% pollen sterility 10 out of 12 bivalents exhibited one or other types of structural abnormality, such as reversed repeats, terminal heteromorphicity, loose pairing and pairing-failure in the form of differential segments. The total length of differential segments was 31.04% of the total chromatin length. This value indicates the extent of structural hybridity that could not be detected during apparently normal pairings at post-diplotene stages, and it serves to caution against the excessive reliance cytologists have placed upon metaphase pairing as a criterion in deciding the nature of sterility. STEBBINS (1950) says that the so-called cryptic structural hybridity remains cryptic mainly because in many plant genera the pachytene stage is not amenable for analysis.

The occurrence of differential segments was the predominant irregularity indicating structural hybridity in these hybrids, SHASTRY AND MISRA (1961b) interpret these segments to be most probably due to translocations. The main reasons for this conclusion are: (1) in diploid species it is the most preponderant chromosomal alteration (Darlington, 1932), and (2) inversion heterozygosity would entail no genetic unbalances due to differential segments and cannot account for the sterility of hybrid plants unless accompanied by chromosomal bridges which were infrequent. The percentage of segments showing structural changes to the total chromosomal length (termed by us

as the "differential index") is closely related to sterility (Table 1). The results of observations of several other crosses confirmed the earlier conclusions (MISRA, unpublished). In as many as eight species of *Oryza,* twenty varieties of *sativa* and three strains of *sativa* var. *fatua, perennis* and *officinalis* studied by pachytene analysis, no abnormalities in pairing were recorded. This constitutes the strongest support for the validity of the pairing data for *japonica-indica* hybrids. It is well known (PROPACH, 1940; KOOPMANS, 1951; HUTCHINSON, 1940; STEBBINS, 1950) that the progeny of cryptic structural hybrids may be unthrifty in growth because of genetic unbalances resulting from recombinations. In view of the extensive structural hybridity in *japonica-indica* hybrids (YAO *et al.,* 1958; SHASTRY AND MISRA, 1961), the chlorophyll-deficient and other abnormal plants may best be interpreted as the results of haplo-viable deletions. Consequently, the hypothesis of VENKATASWAMY (1957) that *indica-japonica* hybrids are highly unstable genetically can be more precisely defined as a result of deletions in gametes. The resulting hybrid progeny might owe their survival to the probable secondary polyploid origin of *sativa* (SAKAI, 1935b; NANDI, 1936).

TABLE 1

DIFFERENTIAL INDICES AND POLLEN STERILITY

Hybrids	Total chromatin length involved in differential segments (in μ)	Total chromatin length of complement (in μ)	Differential index	Pollen sterility (%)
T. 21 × A. 18	83.5	269.0	31.04	66.44
N.P. 130 × 7473	43.5	237.0	18.31	46.01
Norin 6 × N. 32	53.5	362.0	11.90	43.00

The chromosomal differentiation between *japonica* and *indica* rices, as reflected in pairing at pachytene, strongly suggest that these two subspecies should be treated separately in linkage studies, as pointed out by SHASTRY AND MISRA (1961b).

SUMMARY

Normal chromosomal pairing at metaphase I and the infrequent occurrence of anaphase bridges in *japonica-indica* hybrids led to the hypothesis that there are no restrictions to homology between the chromosomes of *japonica* and *indica* rices and that the differentiation between these subspecies is largely due to gene mutations. To account for the sterility in these intersubspecific hybrids, OKA (1953b, 1956b, 1957a) offers genic explanations. This view requires considerable revision in view of the extensive pairing abnormalities recorded at pachytene by SHASTRY AND MISRA (1961). The differential segments, which are most common in these hybrids and which are interpreted as caused by translocations, may account for sterility, non-recovery of some recombinants and the occurrence of abnormal plants in hybrid progenies. Genic explanations of sterility are therefore redundant since the basic premise of normal homologous pairing was not satisfied at pachytene.

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CONSIDERATIONS ON THE GENETIC BASIS OF INTERVARIETAL STERILITY IN *ORYZA SATIVA*

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Since KATO (1930) classified varieties of *Oryza sativa* into the *indica* and *japonica* types, the sterility of intervarietal F_1 hybrids has been regarded as a criterion for estimating their phylogenetic relationships. Rice breeders became interested in *indica-japonica* hybridization. However, various problems concerning the intrinsic nature of this sterility remain unsolved. These are reviewed in this paper, mainly in the light of my experimental results.

CLASSIFICATION OF HYBRID STERILITY PHENOMENA BY UNDERLYING MECHANISMS

Although the sterility of interspecific hybrids has long been observed in both plants and animals, its genetic bases have been only partly analyzed. DOBZHANSKY (1937, 1951) distinguishes between genic and chromosomal sterility, according to the type of underlying genetic disharmony. Genic sterility is due to a disharmonious interaction of parental genes in F_1 individuals. It usually results in degeneration of sexual organs in animals. Chromosomal sterility is due to the failure of chromosomes in F_1 hybrids to pair normally because of structural differences between the parents. On the other hand, STEBBINS (1958a, pp. 165–168) distinguishes between haplontic and diplontic sterilities as proposed by RENNER (1929) and MUNTZING (1930). This was more "operational" for experimental workers than DOBZHANSKY'S classification. In STEBBINS' words, "haplontic sterility acts on gametes or gametophytes, while diplontic sterility affects diploid tissues either in the F_1, before or at meiosis, or in certain zygotes and early embryos of the segregating F_2 generation".

The writer prefers STEBBINS' classification for these reasons: (1) it is difficult to distinguish between genic and chromosomal causes of sterility, (2) adopting haplontic vs. diplontic classification, the terms can indicate the mode of inheritance of sterility, the former being subject to gametic selection while the latter undergoes zygotic selection.

We find both types of sterility in intervarietal hybrids of rice. In both cases, meiosis is normal and sterility results from deterioration of microspores and megaspores beginning at the first division stage of the haploid nucleus (OKA AND DOIDA, 1962), The major difference between them is that in haplontic sterility, which appears in F_1 hybrids, the deterioration of gametes can be attributed to a genotypic unbalance of

gametes, while in diplontic sterility, which appears in F_2 and later generations, the deterioration of gametes seems to be due to an effect of the genotype of the plants which produce the gametes. Thus, haplontic or F_1 sterility occurs only in the heterozygotes for sterility factors and is not fixable, while diplontic or F_2 sterility occurring in homozygotes can be fixed, as will be mentioned later. These two types of sterility may be called "gametophytic" and "sporophytic" sterilities, respectively.

WHETHER OR NOT *Oryza sativa* VARIETIES ARE DIFFERENTIATED IN CHROMOSOME STRUCTURE

The cultivated species *sativa* and *glaberrima,* as well as their corresponding wild relatives, *perennis* and *breviligulata,* have the same genome, A; their F_1 hybrids show normal pairing of chromosomes (MORINAGA AND KURIYAMA, 1957b; NEZU *et al.* 1960). However, on the basis of the occurrence of a low frequency of univalents, rod-shaped chromosomes and anaphasic bridges in some F_1 hybrids between these species, YEH AND HENDERSON (1961) argue that the genome A could be subdivided into A_1 (A; *Oryza sativa* and Asian forms of *perennis*), A_2 (A^{cu} and A^b; American and African forms of *perennis*) and E (A^g; *glaberrima* and *breviligulata*). In intervarietal F_1 hybrids of *Oryza sativa,* previous observers consistently recognize that the pairing of chromosomes in diakinesis through first metaphase is normal. Anaphasic bridges, with or without fragments, are found at a frequency of about one percent. Several workers are inclined to consider these to be due to inversions (MELLO-SAMPAYO, 1952; SAMPATH AND MOHANTY, 1954; YAO *et al.,* 1958; HENDERSON *et al.,* 1959). However, HSIEH AND OKA (1958) show that such anaphasic bridges occur not only in F_1 hybrids but also in parental pure-lines, having nothing to do with F_1 sterility. Hu (1962.) also reaches the same conclusion using pure-lines obtained from haploid plants. This does not absolutely contradict the possibility of inversions existing between rice varieties, but indicates that the anaphasic bridges usually found are due to causes other than inversions, possibly to a reunion at the ends of breakage of sister chromatids at diplotene, as often found in irradiated plants (cf. SCHWARTZ AND MURRAY, 1957).

Similarly, rod-shaped chromosomes, which were considered an indication of structural differentiation by YAO *et al.* (1958), have been found in pure-lines (Hu, unpubl.). Repicrocal translocations bringing about rings of four in heterozygotes are frequently found in the progeny of irradiated plants. But for an unknown reason, they are scarcely found in hybrids between cultivated varieties.

SHASTRY AND MISRA (1961) found that in an *indica-japonica* F_1 hybrid, the chromosomes at pachytene remained unpaired along about 30 percent of the total length. They consider this to be indicative of the amount of structural differences (differential segments) present between the parental strains. However, the senior author (SHASTRY *et al.,* 1960) reports that in an F_1 hybrid between *sativa* and *officinalis,* the parents being far more distantly related than between *indica* and *japonica* types of *sativa,* the pachytene chromosomes had formed 12 normal bivalents which were at later stages resolved into univalents. According to MAGUIRE (1962), pachytene paring-failure in maize

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seems to be distributed in chromosomes at random, its frequency being related in a certain manner to the total chromosome length. Recently, WU *et al.* (1964) found that the failure of pachytene pairing, as pointed out by SHASTRY, might be due to a repulsion between chromosomes which begins to work in the early diplotene stage.

On the other hand, the number of nucleoli formed in somatic telophasic cells varies between two and four among varieties of *Oryza sativa* (SELIM, 1930; SAKAI, 1938). According to OKA AND KAO (1956), however, this variation is continuous if measured in terms of the relative nucleolus-forming ability of certain chromosomes.

Thus, it appears that cytological evidence is not sufficiently strong to conclude that *sativa* varieties are differentiated in chromosome structure; we have no particular reason for assuming differentiation of *sativa* varieties to be structural. However, the pairing of chromosomes may be controlled by certain genes, as found in *Triticum* species (OKAMOTO, 1957; RILEY, 1960). If rice had genes promoting chromosome pairing, the chromosomes would pair regardless of a certain amount of structural differences. Cases of sterile interspecific hybrids showing normal meiosis are known in various plant genera, viz., *Primula* (VALENTINE, 1953), *Agropyron* (STEBBINS AND PUN, 1953), *Bromus* (BARNETT, 1957), etc. In these cases the causes of sterility have generally been assumed to be "cryptic" structural differences. A case closely resembling that in *Oryza* might be found in intraspecific hybrids of *Galeopsis tetrahit* (MÜNTZING, 1930, 1932). In this case, haplontic factors causing F_1 sterility were considered duplications and deficiencies brought about by translocations. Thus, in higher plants, speciation is in most cases believed accompanied by structural differentiation. Therefore, we cannot reject the possibility that a certain amount of structural differentiation is involved in the phylogenetic differentiation of *sativa* varieties.

Confronted with this problem, an efficient method of estimating the amount of structural differences present is to examine the hybrid between tetraploid strains of two given varieties to determine whether the chromosomes tend to pair preferentially between those derived from the same parent. CUA (1951), OKA (1954), OKA *et al.* (1954), and MASIMA AND UCHIYAMADA (1955) have attempted this experiment in *sativa.* The results indicate that the F_1 hybrids between distantly related tetraploid strains generally show a higher fertility and a smaller number of quadrivalent chromosomes (by about two) than the parental autotetraploid. However, OKA (1955b) points out that the high fertility of tetraploid hybrids might be largely due to heterosis, because it could not be fixed in later generations in spite of continued selection for higher fertility, and the decrease in quadrivalent chromosomes might also be of the same nature. He shows further that the F_2 ratios for glutinous endosperm, apiculus coloration, phenol reaction and other characters fitted well those expected values under the assumption of random chromosome or chromatid pairing. Therefore, in those hybrids between tetraploid strains of *sativa,* it seems difficult to confirm preferential pairing of homogenetic chromosomes,

These considerations suggest that structural differences between *sativa* varieties, if present, might be minor, and that differentiation is mainly genic. Dealing with this problem, STEBBINS (1958a, pp. 176–178) states, "the strongest evidence for the existence

of haplontic sterility controlled by gene interaction exists in *Oryza*... Small chromosomal rearrangements could be expected to segregate in the same way as genes, and to show linkage relationships. This detailed study of a particular example serves to emphasize the difficulty of distinguishing between the genic and the chromosomal bases of hybrid sterility, and to indicate further that both the disharmonious effects of gene recombination as well as the results of deficiencies and duplications for chromosomal segments may be operating to produce any particular series of examples of hybrid sterility which, as in *Oryza*, are obviously conditioned by many genetic factors."

At present, we have no good method of looking into the fine structure of genetic substance in higher plants. It seems rather useless to discuss whether the nature of varietal differentiation in rice is chromosomal or genic, while a more important problem may be the origin of sterility factors.

GENIC ANALYSIS OF INTERVARIETAL F_1 STERILITY IN *Oryza sativa*

The intervarietal F_1 sterility in *Oryza sativa* is characterized by the following features:
(1) the F_1 plants grow normally and show no degeneration of the ovary, the anther or the other sexual organs;
(2) there is no disturbance in chromosome pairing, even in highly sterile hybrids;
(3) after normal meiosis, microspores and megaspores begin to deteriorate at the first division stage of the haploid nucleus;
(4) the percentage of normal gametes during flowering depends on the cross-combination. In almost all crosses investigated by TERAO AND MIZUSHIMA (1939), the percentage of good pollen was approximately the same as that of normal embryo sacs. The degree of sterility measured by the percentage of good pollen is not much affected by environmental conditions:
(5) in most cases, reciprocal crosses show no significant difference in the percentage of good pollen in F_1 hybrids.

These facts suggest that the partial sterility of F_1 hybrids might be due to deterioration of gametes carrying a recombination of parental chromosomes or genes. Generally speaking, in the cross of $A_1A_1B_1B_1 \times A_2A_2B_2B_2$, gametes carrying A_1B_2 or A_2B_1, may deteriorate. To look into the genetic basis, the writer attempted crossing experiments of the following design (cf. OKA, 1953a and b, 1957a and c). Two relatively closely related strains were chosen which showed a difference in F_1 pollen fertility when each was hybridized with a third distantly related strain; in one such experiment, two Philippine strains, 219 and 221, and a Japanese strain, 563, were used. The former two were completely interfertile, while 563 × 219 gave a partly sterile hybrid (about 65% in pollen fertility) and 563 × 221 a highly fertile F_1 hybrid. Strains 221 and 563 were glutinous (denoted by *gl* in this paper), while 219 was non-glutinous (+). The F_1 and F_2 plants of 219 × 221 were each crossed with 563. Then the plants obtained from these crosses segregated into four classes, i.e., (a) partly sterile and heterozygous for glutinous, (b) partly sterile and homozygous, (c) fertile and heterozygous, and (d) fertile and homozygous (Table 1).

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TABLE 1

SEGREGATION OF FERTILE AND PARTLY STERILE PLANTS IN 563 × F_1 (219 × 221) AND 563 × F_2 (219 × 221)

Gametic genotype	When hybridized with 263		× F_1 experiment			× F_2 experiment		
	Fertility	*gl* gene	Freq	Exp. no.	Obs. no.	Freq.	Exp. no.	Obs. no.
x_1 – + X_2	Part. sterile	Heteroz.	½(1–*p*)	10.0	11	¼(2–3*p*+2p^2)	107.6	114
x_1 – *gl* X_2	Part. sterile	Homoz.	½ *p*	6.0	6	¼(3*p*–2p^2)	39.4	41
X_1 – + X_2	Fertile	Heteroz.	½ *p*	6.0	6	¼(3*p*–2p^2)	39.4	38
X_1 – *gl* X_2	Fertile	Homoz.	½(1–*p*)	10.0	9	¼(2–3*p*+2p^2)	107.6	101
			x^2 = 0.20, P>0.8			x^2 = 0.902, P>0.5		

The pattern of segregation shown in Table 1 can be explained by assuming that a genetic factor X which works in the gametes as a development maintainer is duplicated, the loci being independent, and microspores and megaspores carrying the double-recessive combination (x_1x_2) deteriorate. Those putative duplicate haplontic lethals are called "gametic-development genes" (or abridged as G.D. genes) in the writer's papers.

Following this hypothesis, the genotypes of the three strains used in the above experiment may be 563: X_1 -*gl* x_2; 219 : x_1 - + X_2; and 221: X_1 -*gl* X_2 (- shows linkage), respectively. Then the F_1 of 219 × 221 will produce four kinds of gametes which pro–duce the four plant classes, respectively, shown in Table 1. Letting the recombination fraction between X_1 and *gl*, or x_1 and + be *p*, the F_1 data gave p = 0.375, and the F_2 data p = 0.208, giving a good fit of observed numbers to expected ones. The value of p obtained from the F_2 data might be, in view of a larger number of plants observed, more reliable than that from the F_1 data.

Further, in each line derived from the cross of F_2 plants of (219 × 221) with 563, the numbers of the four kinds of plants were compared with the numbers to be expected if the given line carries each of the 10 genotypes into which the F_2 will segregate. Then, the probability that the observed numbers would result from each genotype was calculated for each line, and was transformed into the probability that the given line carries each genotype, by using BAYES' theorem. From the above computations, the probabilities found in respective lines were summed up regarding each genotype, and were regarded as an estimate of the actual segregation ratio of the F_2 genotypes. A maximum likelihood estimation of the recombination fraction obtained from this genotypic ratio, p = 0.210, was consistent with the former value, 0.208, and the estimated genotypic ratio gave a good fit to the expected one (OKA, 1953a). It may be concluded that the difference between 219 and 221 in F_1 pollen fertility with 563 can be satisfactorily explained by the hypothesis of duplicate haplontic lethals, and in this case, a G.D. genepair is linked with the glutinous gene with a recombination value of 21 percent.

The results of similar experiments using other strains could also be accounted for in the same manner as above. In one of them, in which the F_1 and F_2 plants between two Taiwanese strains 108 and 143 were crossed with 563, a difference was found between

the reciprocal crosses, (108 × 143) × 563 and 563 × (108 × 143). The latter produced more partly sterile plants than the former, or more than expected from the hypothesis, This was considered to be due to a certation resulting from a low fertilizing ability of pollen grains with double-dominant combinations of G.D. genes (OKA, 1953b). This may also be due to a second set, linked with the G.D. genes in the form of repulsion, affecting only the development of pollen grains.

It was deduced that several sets of G.D. genes might be concerned in usual varietal crosses. In the above experiments, if one set was assumed, partly sterile plants must have theoretically a 75% pollen fertility owing to the deterioration of x_1x_2 gametes. Actually, those plants showed a range in fertility, for instance, 65% to 35% in the first experiment. This suggests that a second set of G.D. genes, Y_1/y_1 and y_2/Y_2, might be involved, in addition to the first set, X_1/x_1, and x_2/X_2, and Y_1 is linked with X_1 while Y_2 is independent of X_2. In other words, we may assume that a chromosome, if recombined with either one of two other chromosomes, brings about partial sterility. Further, in repeating experiments of the same design with different strains, some of them showed several frequency peaks in the variation of pollen fertility. For instance, crossing the F_1 of two Taiwanese strains 160 and 143 with 563 gave a wide range of pollen fertility values with five frequency peaks, which could be accounted for by assuming at least five sets of G.D. genes distributed in seven pairs of chromosomes (OKA, 1956b). These facts suggest that the G.D. gene-sets may be regarded as polygenes. It seems that the sterility relationships in sativa as a whole involve many sets of G.D. genes. The fertility of a plant heterozygous for *k* sets is expected to be 0.75^k.

MODIFIED SEGREGATION RATIOS AND RESTRICTION ON RECOMBINATION DUE TO GAMETIC SELECTION

The gametic output of a plant heterozygous for a set of gametic-development genes will be $1/3X_1X_2 : 1/3X_1x_2 : 1/3x_1X_2$, which will give rise to a 2 : 1 ratio of $X_1 : x_1$ or $X_2 : x_2$. Therefore, when a gene A/a is linked with a pair of G.D. genes with a recombination fraction *p*, the F_1 gametic ratio of A : a will be $1 + p : 2 - p$ (repulsion)

TABLE 2

THE MODIFIED F_2 RATIO OF AA : Aa: aa BY THE EFFECT OF A SET OF GAMETIC-DEVELOPMENT GENES

Repulsion :	AA	Aa	aa
Coupling:	aa	Aa	AA
Fertile	$(1+3p^2)$ $-sp(1+p)$	$(2+6p-6p^2)$ $-s(1+2p-2p^2)$	$(4-6p+3p2)$ $-s(1-p)(2-p)$
Part. sterile	$2p(1-p)$	$2(1-2p+2p^2)$	$2p(1-p)$
Total	$(1+p)^2$ $-sp(1+p)$	$2(1+p)(2-p)$ $-s(1+2p-2p^2)$	$(2-p)^2$ $-s(1-p)(2-p)$

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or $2-p : 1+p$ (coupling). Letting the fertilizing capacity of double-dominant pollen be $1-s$, the F_2 will segregate in a manner as shown in Table 2. The table shows that the ratio of AA : Aa : aa may vary from 1 : 2 : 1 to 1 : 4 : 4 (or 4 : 4 : 1), while the heterozygous class for A/a contains more partly sterile plants than the homozygous class. It will then be expected that phenotypically dominant plants have a lower mean fertility and a larger variance than recessive ones. The same tendency will also be expected in back-crosses.

The writer has investigated segregation ratios and their relation to fertility variation in several crosses between distantly related strains. In most cases, the results gave a good fit to the above expectation (OKA, 1953c). In a recent experiment, two strains, 414 (*indica*, non-glutinous) and 563 (already referred to, *japonica*, glutinous), were used. Distributions of pollen fertility in back-crosses of their reciprocal F_1 hybrids with 563 are given in Table 3. The data in the table show that there was no significant difference between reciprocal crosses, and in the pooled data, the ratio of homozygotes to heterozygotes for the glutinous gene, 55 : 32, significantly deviated from the 1 : 1 ratio. The table also shows that the heterozygous class contained more sterile plants than the homozygous class. Assuming a pair of G.D. genes to be linked with the glutinous gene, the recombination fraction was estimated to be 0.065 from the segregation ratio. Having computed the distribution of pollen fertility under the assumption that fertility variation due to causes other than the G.D. genes results in a normal distribution, the observed distributions fitted well the expected ones, as shown in Table 3.

TABLE 3

DISTRIBUTIONS OF NORMAL POLLEN PERCENTAGE IN HYBRIDS BETWEEN TWO DISTANTLY RELATED RICE STRAINS, 414 AND 563

Class	% of good pollen									No. of plants	Mean (%)	Remarks
	100	90	80	70	60	50	40	30	20			
(a) Comparison between reciprocal crosses:												
414 × 563 F_2	13	12	10	6	4	3	2	3	1	54	77.4	χ^2 = 2.98
563 × 414 F_2	6	11	10	4	2	4	6	2	1	46	72.0	P>0.50
(414 × 563) × 563	4	9	4	12	10	9	2	4		54	67.0	χ^2 = 5.00
(563 × 414) × 563	4	3	5	15	10	3	1	1	1	33	68.5	P>0.50
(b) Comparison between + *gl* and *gl gl* classes in back-crosses:												
+ *gl* Obs.	4	7	6	11	12	9	2	4		55.0	66.4	χ^2 = 3.60
+ *gl* Exp.	3.37	5.84	9.72	11.93	11.04	7.57	3.78	1.73		55.0	63.6	P>0.50
gl gl Obs.	4	5	3	6	8	3	1	1	1	32	69.6	χ^2 = 6.79
gl gl Exp.	3.29	5.23	7.50	7.50	5.01	2.44	0.79	0.21	0.03	32.0	74.2	P>0.20
(c) Comparison between + +, + *gl* and *gl gl* classes in F_2:												
++	7	5	5	2	2	3	1	2		21	76.2	
+ *gl*	8	14	13	7	4	4	5	3	2	60	72.7	
gl gl	4	4	2	1			2			13	82.3	

This may serve as an example showing that modified segregation ratios in partly sterile hybrids can be explained by the hypothesis of gametic-development genes. Also in F_2, as shown in Table 3, there were more non-glutinous homozygotes than glutinous homozygotes, and the heterozygous class contained more sterile plants than the homozygous class. The fertilizing capacity of pollens carrying the double-dominants was estimated from the data by the formulas given in Table 2; it was found that $s = 0.836$. However, as will be mentioned later, the fertility variation in F_2 may be due not only to G.D. genes but also to sterility factors having a diplontic effect.

We may then consider a case in which two independent genes A/a and B/b, controlling certain characters, are linked respectively with two pairs of G.D. genes, X_1/x_1, and x_2/X_2, which belong to the same set. For instance, the F_1 genotype may be $\frac{A- X_1}{a - X_1}\frac{b-X_2}{B - x_2}$. Since both the pollen and embryo sacs carrying x_1x_2 will deteriorate and the pollen carrying X_1X_2 will have a low fertilizing capacity, gametes with ab and AB will decrease to a certain extent, resulting in a relative increase of gametes with parental gene combinations, Ab and aB. Accordingly, the independent genes A/a and B/b may appear as if they were linked. This restriction on recombination may be called pseudo-linkage due to gametic selection.

Having looked for cases falling under this expectation, a few examples were presented (OKA, 1956d). In one of them in which the apiculus-pigmentation gene C/c and the phenol-reaction gene *Ph/ph* are concerned, recombination fractions between those genes and two pairs of G.D. genes were estimated, respectively.

In addition to the writer's work mentioned above, modified F_2 ratios for glutinous endosperm and apiculus pigmentation were observed by MIZUSHIMA AND KONDO (1961, 1962). The cross combinations they observed could be grouped into several different types of modification of F_2 ratios for the glutinous gene, which had no apparent correlation with the degree of F_1 sterility. This may be because the genes causing F_1 sterility are not always linked with the glutinous gene. They have assumed, dealing with modified ratios for glutinous endosperm, a factor which eliminates one half of the glutinous pollen, and a genic system which is essentially the same as the writer's gametic-development genes for apiculus pigmentation,

SPOROPHYTIC STERILITY AND HYBRID BREAKDOWN

As demonstrated in the preceding sections, the F_1 sterility between *Oryza* sativa varieties is apparently haplontic or gametophytic. It is expected that haplontic sterility occurs only in heterozygotes for the sterility factors and therefore it is not fixable. According to the hypothesis of gametic-development genes, a fertile plant should produce only fertile plants in its selfed progeny, and a partly sterile plant should produce fertile plants and partly sterile ones whose fertility is not lower than that of the parent. Actually, partly sterile plants are often found in the F_2 and later generations derived from a highly fertile F_1 hybrid, and lines true-breeding for partial sterility

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appear in the progeny of such partly sterile segregants. This suggests that rice has another sterility mechanism which differs from that for F_1 sterility.

With regard to this type of sterility, OKA AND DOIDA (1962) obtained the following experimental results. First, having compared in many crosses the mean seed fertility of F_2 plants with the F_1 fertility, they found no correlation between the two generations as shown in Fig. 1. Secondly, in crosses which had a high F_1 fertility, the F_2's

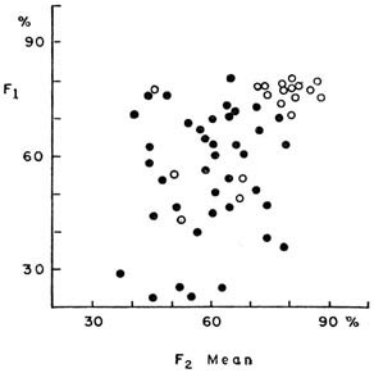

Fig. 1. Comparison between fertility of F_1 hybrid and mean fertility of F_2 population ● = *indica* × *japonica* ○ = *indica* × *indica* or *japonica* × *japonica* (from OKA AND DOIDA, 1962.)

showed variation in fertility due to segregation, with different modes of distribution according to the particular cross combination concerned. Thirdly, in two crosses, 1 × 719 *(indica* × *indica)* and 563 × 104 *(japonica* × *indica),* some progeny lines derived from partly sterile F_2 plants appeared to breed true for partial sterility. Crossed with the parental strains, the F_1 showed a partial sterility, and the fertility variation in the F_2 could be explained by a 1 (fertile) : 2 (partly sterile) : 1 (highly sterile), or 1 (fertile) : 3 (partly sterile) ratio, In those partly sterile plants, deterioration of microspores and megaspores was found to begin at the stage of first nuclear division, in the same manner as in F_1 sterility. These facts indicate that this sterility is diplontic or sporophytic and might possibly be due to a physiological effect of the diploid genotype which blocks the development of gametes. As the parental strains are completely fertile, the sterility factors must be duplicated or of a complementary nature. The abovementioned inheritance of this sterility can be well explained by assuming that the parental strains are of the genotype $A_1A_1a_2a_2$ and $a_1a_1A_2A_2$, respectively, and the true-breeding partly sterile lines are $a_1a_1a_2a_2$. Since the F_1 between parental strains ($A_1a_1A_2a_2$) was fertile and the first backcrosses ($A_1a_1a_2a_2$ or $a_1a_1A_2a_2$) were partly sterile, it may be that at least two dominant A's (A_1A_1, A_2A_2 or A_1A_2) present in the diploid tissue are needed to bring about normal fertility. In one of these crossing experiments, it was found that a_1 or a_2 was linked with the apiculus-pigmentation gene, *C,* with a 9.5% recombination value. These sterility factors of sporophytic effect were called "duplicate-fertility genes".

The same genic system as above also was postulated for the occurrence of weak plants in F_2 (OKA, 1957c). In a cross between two distantly related strains, 451 (*indica*)

and 521 (*japonica*), the F_1 plants grew normally, while the F_2 segregated into normal and weak plants in a ratio approximately 11 : 5. The weak plants ceased to grow after developing four to five leaves and became yellowish. By back-crossing the F_1 to parental strains, a 3 normal : 1 weak ratio was found. Taking another strain, 647, which showed F_2 weakness neither with 521 nor with 451, the F_1 of 647 × 521 was crossed with 451. In the second generation of this cross, four out of nine lines showed a 11 normal : 5 weak segregation. From these experimental results, it was concluded that the genotypes of strains 451, 521 and 647 might be $A_1A_1a_2a_2$, $a_1a_1A_2A_2$ and $A_1A_1A_2A_2$, respectively, while that of the weak segregants might be $a_1a_1a_2a_2$, $A_1a_1a_2a_2$, or $a_1a_1A_2a_2$. These duplicate genes, called complementary recessive lethals, display the same behavior as the duplicate-fertility genes.

The occurrence of sterile or weak plants in F_2 and later generations, which could be largely explained by double-recessive combinations of duplicate genes, may be regarded as a tendency of hybrids to break down. These phenomena will be frequently encountered in hybrids between distantly related *sativa* varieties, and may be used as an index of phylogenetic relationships in the same manner as F_1 sterility. However, an important difference between the F_1 sterility and the present phenomenon is that the former occurs in heterozygotes and causes gametic selection, while the latter occurs in homozygotes and results in zygotic elimination. According to the writer's computation (OKA, 1955a, 1957b, partly unpublished), gametophytic sterility disappears rapidly in segregating generations, practically by F_3, but sporophytic sterile plants will increase after F_3 and will continue to appear for many generations. For breeders who hybridize distantly related varieties, the latter would be a more serious obstacle than the former.

It may be noteworthy that both the gametophytic and sporophytic sterilities between *sativa* varieties could be explained by duplicate or complementary genes; duplicate genes whose double-recessive combinations have a deteriorating effect may be called complementary lethals. It seems that duplication of genes is a characteristic feature in rice.

CYTOPLASMIC STERILITY AND OTHER GENETIC UNBALANCES FOUND IN HYBRIDS

Reciprocal crosses between *Oryza sativa* varieties usually show no significant difference in F_1 pollen or seed fertility. KATSUO AND MIZUSHIMA (1958) found, however, a reciprocal difference in F_1 seed fertility between wild-rice strains of the *spontanea* type (*O. perennis*) and Japanese varieties; those with *O. sativa* as the maternal parents gave higher fertility than the reciprocals. HINATA AND OKA (1962a) found the same phenomenon in one of three cross combinations between an Indian *spontanea* and three *sativa* strains for which variance analysis was made. They did not find reciprocal difference in pollen fertility or in other characters. It seems that cytoplasmic differentiation which brings about a reciprocal difference in seed fertility occurs sporadically in this plant group.

On the other hand, KITAMURA (1962) backcrossed a Philippine variety "Tadukan'

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and a Japanese variety "Norin 8," using the latter as the recurrent pollen parent, and selected two strains from the BC_3 generation. Crossing the two strains with various Japanese varieties, he found that when the Japanese varieties were used as the maternal parent, all the F_1 hybrids were fertile, but some reciprocal F_1's with "Rikuu 132" and several other varieties as the pollen parent were partly sterile. The sterility was found to be attributable to a difficulty in the dehiscence of anthers; the sterile plants had normal pollen and embryo sacs. It seems that there is a disharmonious interaction between the cytoplasm of Tadukan and certain genes of the Japanese varieties.

The writer (OKA, unpubl.) also backcrossed two strains, 414 (*indica*) and 504 (*japonica*), using the latter as the recurrent pollen parent. The results showed (Table 4) that repetition of backcrosses until BC_6 did not bring about a remarkable improvement in fertility, but if the partly sterile plants were selfed, the progeny was almost completely fertile. Since a backcross of the reciprocal combination with the recurrent parent as the maternal plant did not restore fertility, the sterility appearing in this experiment cannot be attributed to cytoplasmic effect. It also was found that pollen and seed fertilities were parallel among those plants, and the dehiscence of the anthers was normal. It is rather difficult to account for this sterility. A possible explanation might be that a pair of genes A/A_s is concerned and if A is present in the tissue of maternal plants, gametes with A, deteriorate. Then, letting the genotypes of parental strains 414 and 504 be AA and A_sA_s, respectively, the F_1, AA_s, will be expected to have a 50% fertility, and to produce gametes carrying only A.

In various plant groups, cases are known in which sterility results from disharmonious interactions between genotype and cytoplasm, embryo and endosperm, or between embryo and the maternal tissue (cf. STEBBINS, 1958,). However, I know of no previous report of a disharmonious interaction between gametes and the maternal tissue. If the above hypothesis holds true, it may be inferred that at the locus of A/A_s there might be many other multiple alleles which do not bring about sterility, and A_s might have evolved from such allelomorphs.

TABLE 4

FERTILITY VARIATIONS OBSERVED IN A BACK CROSS EXPERIMENT, (414 × 504) × 504 × 504

Generation	% of seed setting									Number of plants
	90	80	70	60	50	40	30	20	10	
F_1							3	8	1	12
B_1			1		1	5	6	6	9	28
B_3 (8 lines)				2	14	32	35	12	6	101
B_5 (5 lines)				1	1	16	12		1	31
B_6 (6 lines)			4	3	14	6	10			37
504 × B_5 (6 lines)		2	3	3	12	5	1			26
B_5F_2	19	18	11	4			1			53
B_1(504 × 414) × 504			1		3	6	5	4	4	23

This hypothesis can explain the occurrence of intervarietal F_1 sterility and modified F_2 ratios due to gametic selection, but it fails to account for the tendency to restrict the recombination of independent genes. On the other hand, the gametic-development gene hypothesis cannot account for the above experimental results. This suggests that the causes of intervarietal sterility in *Oryza sativa* are complex. It might be possible that some of the gametic-development genes can interact with the genotype of the maternal tissue, through some biochemical reaction, like exchange of nutrients.

In addition to the various types of sterility referred to, the weakness of F_1 plants is found among intervarietal crosses, although it is not common. In two out of about 900 cross combinations observed, the F_1 plants ceased to grow after developing several leaves. This weakness was found to be due to a set of complementary dominant lethals (OKA, 1957c). The various phenomena due to a genetic unbalance of hybrids so far found in intervarietal crosses in *sativa* may then be summarized as follows:

PHENOMENON	INCIDENCE	GENETIC BASIS
F_1 weakness	rare	Complementary dominant lethals
F_1 sterility	common	(a) Gametic-development genes (= complementary haplontic lethals) (b) Disharmonious interaction between gametic and maternal genotypes (c) Non-dehiscence of anthers due to cytoplasmic effect
Hybrid breakdown,		
(a) weakness	common (?)	Complementary recessive lethals
(b) sterility	common	Duplicate-fertility genes

RELATION BETWEEN F_1 STERILITY AND DIFFERENTIATION IN CHARACTERS

In many plants and animals, interspecific sterility is found to be correlated with differences in other characters. Sterility is then considered as an isolating mechanism which promotes differentiation. Cases are also found however, in which sterility barriers do not develop so much between species in spite of morphological, ecological or distributional differences. On the contrary, highly developed sterility barriers may be found between varieties of a species which do not differ much in other characters. Thus, as worked out by STEBBINS (1950), the mode of correlations between sterility and differentiation in other characters varies among groups of plants. A species may be heterogeneous for sterility mechanisms (DOBZHANSKY, 1951, pp. 199–202).

Since KATO classified *Oryza sativa* varieties into the *indica* and *japonica* types, it has been generally believed that F_1 sterility and differences in characters simultaneously occur between the two types. However, TERAO AND MIZUSHIMA (1939) show that the sterility relationships are too complicated to allow the classification of varieties into two groups. RICHHARIA *et al.* (1962) argue further that the intervarietal sterility might be of no use for estimating phylogenetic relationships, because some *indica-japonica* hybrids were highly fertile while other crosses within the same types gave sterile F_1 hybrids. My work on this problem (OKA, 1958b) led to these conclusions: based on the

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pattern of character associations, *sativa* varieties from Asian countries can be largely divided into two major groups corresponding to the *indica* and *japonica* types (called Continental and Insular groups, respectively), though an array of intergrades appears between them. Those varieties could also be classified into the same two groups if their F_1 pollen fertility relationships with a certain set of tester strains were arranged according to similarity. It was found further that by treating the data with the technique of principal component analysis, the first component axis extracted from the variation was indicative of the tendency of those varieties to be differentiated into the *indica* and *japonica* types (HINATA AND OKA, 1962a). Thus, considering the variations as a whole, the *indica-japonica* differentiation may be recognized in both character associations and sterility relationships.

These investigations pointed out that by the pattern of character associations the *indica-japonica* differentiation could be projected more clearly than by sterility relationships. For instance, having computed two discriminant formulas both maximizing the differences between the *indica* and *japonica* types, one combining the measurements of characters and the other combining F_1 pollen fertilities with a set of test-strains, the former gave a discontinuous distribution of scores by which the strains could be divided into two groups, while the latter gave a continuous distribution (Fig. 2). This suggests that in the evolution of cultivated rice, differentiation in characters might go ahead of that in sterility relationship. The F_1 sterility might be rather an outcome of differentiation than an effective isolating mechanism.

From F_7 and F_8 populations of *indica-japonica* hybrids which had been propagated in bulk or in pedigrees without artificial selection, I took many strains at random, and investigated various characters and F_1 pollen fertilities with a set of test-strains (OKA, 1956d, 1957d and e). The results showed that (1) among certain characters determined by a single gene (glutinous endosperm, phenol reaction, red pericarp, etc.), the combinations representing parental strains were more frequent than expected under the assumption of random combination, (2) characters controlled by many genes (potassium

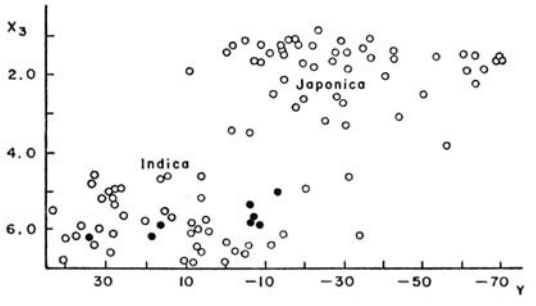

Fig. 2. Scatter diagram showing the relation between two discriminant scores for classifying *indica* and *japonica* types, one (abscissa) combining F_1 pollen fertilities with five test-strains, and the other (ordinate) measurements of four characters. ● = wild ○ = cultivated
(From OKA AND CHANG, 1962.)

chlorate resistance, low temperature resistance, drought resistance, degree of grain shedding, etc.) tended to be correlated in the same way, though with lower correlation coefficients, as among varieties of Asian countries, and (3) strains having the character association of *indica* or *japonica* type tended to show a high F_1 fertility with the test-strains of *indica* or *japonica* type, respectively. Examples showing these tendencies are in Table 5.

TABLE5

CHARACTER COMBINATIONS AND F_1 STERILITY RELATIONSHIPS FOUND AMONG F_7 LINES DERIVED FROM A HYBRID POPULATION, 414 × 563

Test-strain	Group of F_1 sterility reaction						
	A	B	C	D	E	F	Number of lines
108 *(indica)*	+ + –	+ + –	+ + –	+ + –	—	– – –	
414 *(indica)*	+ – +	+ – +	+ – +	+ – +	—	– – –	
563 *(japonica)*	– – –	– – –	+ + +	+ + +	—	– + +	
647 *(japonica)*	– – –	+ + +	— — —	+ + +	—	+ – +	
Obs. no.	9 417	15	2151	5	6	13 4 8	99
Exp. no.*	30 25.3	32 36.1	1 5.5	5 8.1	8.1	25 15.9	c^2 = 12.0 P<0.02
Group of character-combination**							
I and II	17	11		1		2	31
III	7	13	1	1		7	31
IV	3	4		1	2	10	20
V and VI	3	4		2		26	17
Phenol reaction							
Positive	21	18	1	3	3	8	57
Negative	9	11		2	3	17	41

+ and – show F_1 pollen fertility being higher or lower than 87.5%.

* Expected under the assumption of random association.

** Combination of phenol reaction, $KClO_3$ resistance, weight for grain shedding, awn length, and endosperm destruction in alkali-test. I: Similar to 414 (*indica* type); VI: Similar to 563 *(japonica* type): II and III: Different from I in one and two characters, respectively; IV and V: Different from VI in one and two characters, respectively.

NAGAMATSU AND OMURA (1960) made a similar experiment. Most strains taken from the hybrid population showed sterility relationships similar to those of Japanese varieties, possibly because plants with characters of the *japonica* type had been unconsciously selected.

These experimental results indicate that the *indica-japonica* differentiation present between parental strains is not completely disrupted by hybridization, or in other words,

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the genotype of hybrid populations is able to restore the differentiation to a certain extent. This may be explained by gametic and zygotic selections caused by many sets of duplicate genes. As their double-recessive combinations are eliminated, there might arise a tendency for gametes or plants carrying recombinations of parental genes to be selected. This trend is a hindrance to obtain desired recombinants from hybrids. It seems that more detailed studies in this respect are needed.

MECHANISMS OF DIFFERENTIATION IN *Oryza sativa* VARIETIES

As already mentioned, both the haplontic and diplontic sterilities, as well as the occurrence of weak F_2 plants found in intervarietal hybrids of *sativa,* can be interpreted on the hypothesis of duplicate genes. Recently, MIZUSHIMA AND KONDO (1960) found that in some F_2 hybrids between distantly related varieties, the apiculus-pigmentation gene, *C,* of divergent parents, could be located on different chromosomes. They found that in the F_2 between strains with a pigmented apiculus, a few colorless plants occurred. In addition to these, the presence of duplicate genes controlling empty-glume length, pigmentation of certain organs and other characters have early been known on the basis of 15 : 1 F_2 ratios (CHAO, 1928; MITRA AND GANGULI, 1932; etc.). Thus, it seems that the germplasm of *sativa* might be duplicated to some extent.

Duplication of gene loci results either from doubling of chromosomes or from translocations. As mentioned, there has been much discussion whether *sativa* varieties are structurally differentiated. Although a large number of cytological workers are inclined to assume the presence of cryptic structural differences, the evidence is not sufficient. If there were many minute structural differences, there should also be differences large enough to produce visible disturbance of meiosis.

The hypothesis that *Oryza sativa* is a secondary balanced polyploid was set forth first by SAKAI (1935b) and NANDI (1936) on the basis of pronounced secondary associations of meiotic chromosomes. It has then become a matter of dispute whether secondary association is due to a latent homology between chromosomes. According to HU (1962), the observed facts in rice do not fit the view that secondary association takes place between heterochromatic parts of chromosomes at random (THOMAS AND REVELLE, 1946; HIRAYOSHI, 1957), or that it is an artifact of microtechniques (BROWN, 1950). Different workers (SAKAI, 1935b; NANDI, 1936; PARTHASARATHY, 1938; OKUNO, 1944; cf. HAGA, 1940), using different techniques, have consistently recognized the same pattern of associations, the maximum being written as 2(3) + 3(2). HU (1962) found this association pattern also in *Oryza* species other than *sativa,* namely, *perennis, glaberrima, breviligulata, officinalis, australiensis, brachyantha,* etc. Therefore, if, as STEBBINS (1950, pp. 360–362) suggests, secondary association was due to the presence of homologous segments inserted in non-homologous chromosomes by translocations, it must be assumed that such translocations would have taken place in the ancestral plants of the genus, but have not contributed to speciation. Further, HU (1957, 1960b) has observed intragenome pairings in haploid plants of *sativa* and *glaberrima,* concluding that the pattern of pairing, which did not differ between the two species, could

be explained on the same basis as for secondary association in diploids. These facts strongly suggest that species of the genus *Oryza* might have been derived from a species involving a doubling of certain chromosomes.

In the light of this hypothesis, the occurrence of many duplicated loci may be considered to be mainly due to the doubling of chromosomes in the ancestral plants. *Oryza sativa* might have arisen from the Asian *perennis* or *balunga* type of wild rice belonging to *perennis* (MORISHIMA *et al.*, 1961; OKA AND CHANG, 1962). It was found that the Asian forms of *perennis* tended to show a high F_1 fertility with various *sativa* varieties, which gave partly sterile F_1 hybrids (HINATA AND OKA, 1962a). The rice varieties collected from the Jeypore Tract of Orissa, India, including an array of intergrades between wild and cultivated forms, appeared to be gradually differentiated into the *indica* and *japonica* types approaching cultivated forms. Those intermediate wild-cultivated plants mostly showed high F_1 fertilities with different *sativa* strains in the same manner as the Asian forms of *perennis,* but a part of them approaching cultivated forms gave partly sterile F_1 hybrids with certain *sativa* strains. These facts support the hypothesis of duplicate genes for intervarietal sterility. The wild forms might have double-dominant genotypes for those duplicate genes, and deficiencies and mutations at one or the other of the loci might give rise to a differentiation into partly intersterile varieties.

When genes are duplicated (OKA, 1957a), a mutation at one locus may be concealed by the gene with a similar effect at the other locus, so that genic changes which are otherwise fatal may be retained in the population. The species will then tolerate a high genetic load and will enjoy a large potentiality of variation. Further as already mentioned, differences in duplicate genes will result in a restriction on free recombination of other genes, and will promote differentiation.

The *indica* and *japonica* types differ in temperature response, fertilizer response and in other physiological responses to outer conditions (cf. OKA, 1956c, 1959; OKA AND RU, 1957). We have assumed that in the course of evolutionary change toward cultivated forms, the differentiation in certain characters might proceed ahead of that in sterility relationships. If duplicate genes controlling intervarietal sterility are linked with other gene complexes determining adaptability to different environments, it will be possible that plants selected for different ecological requirements are gradually differentiated into partly intersterile groups. As the *indica-japonica* differentiation seems to have advanced in parallel with that of wild plants to cultivated forms, its initiating factor might be a differential response to a certain new environmental condition arising in a cultivated habitat.

SUMMARY

Different interpretations of the genetic basis of intervarietal sterility in *Oryza sativa* were discussed. Emphasis was laid on the following points:

(1) We have no particular reason for assuming that *sativa* varieties are differentiated in chromosome structure. Differentiation might be mainly due to genic changes.

(2) The intervarietal F_1 sterility is haplontic or gametophytic, and can be accounted

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for by assuming sets of duplicate genes which work in the gametes as development maintainers. A number of sets of such duplicate genes may be concerned with sterility between distantly related varieties.

(3) The F_1 sterility is accompanied by gametic selection, which results in modification of segregation ratios. It also brings about a restriction on recombination of ndependent genes.

(4) In the F_2 and later generations of intervarietal hybrids, partly sterile or weak segregants are found, and from the partly sterile plants, true-breeding partly sterile lines can be obtained. This sterility is diplontic or sporophytic and is not correlated with F_1 sterility. This penomenon, which may be considered as a partial breakdown of hybrids, can also be explained by duplicate genes of sporophytic effect.

(5) Sterilities due to disharmonious interactions between genotype and cytoplasm and between gametic and maternal genotypes have been found in intervarietal hybrids. The varieties of sativa thus seem to be differentiated by a complex of mechanisms.

(6) Although the intervarietal F_1 sterility is correlated on the whole with differences in characters, the latter more clearly differentiate varieties. In the progeny of hybrids between *indica* and *japonica* types, character combinations and sterility relationships tend to be associated in the same manner as it was observed among varieties of the *indica* and *japonica* types.

(7) The Asian forms of *perennis,* from which *sativa* might have arisen, generally showed high F_1 fertilities with *sativa* varieties of different types. These wild plants are not differentiated into *indica* and *japonica* types; the differentiation appears to advance as the plants approach cultivated forms. It may then be inferred that the wild forms have dominant alleles at many duplicated loci, and mutations or deficiencies at one or the other of them might result in the differentiation of *sativa* varieties into partly intersterile groups.

(8) The origin of duplicated loci may be accounted for by assuming secondary polyploidy.

THE SIGNIFICANCE OF HYBRID STERILITY IN RICE

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PART I

During the period 1953–55, I collaborated on a project to evolve new varieties of rice from hybrids between *japonica* and *indica* varieties. My part was to trace the cause of hybrid sterility and to estimate its effects on the breeding program. I obtained no definite answers in that period. Subsequently, the experimental results from other institutions on varietal differentiation in *Oryza sativa* were studied, and the mode of inheritance of semisterility was re-examined at the Central Rice Research Institute, Cuttack. An interpretation of the hybrid sterility was thought out, based on information on the origin of cultivated rices. This interpretation is presented in this paper. It is highly probable that genetic differentiation in *sativa* has resulted in continuous variation but a discontinuity can be found between particular groups, namely, *indica* and *japonica.* This discontinuity will be the main subject discussed.

REVIEW OF LITERATURE

Results and interpretations of other workers have been used in the study. The monograph by NAGAI (1958) on *japonica* rice is a standard reference. This text includes a list of Japanese workers who contributed to the study of ecological adaptation in different groups of rice, pointing out the relationship of soil and climatic factors with the choice of varieties being grown. NAGAI also has discussed in detail the genecology of rice in relation to regional distribution of variety-groups. MATSUO (1952) did basic work on ecological differentiation. He analyzed variations in morphological and agronomical characters of a large varietal collection, and demonstrated the association of characters giving rise to the three main groups, A, B and C, which may be regarded as subspecies. OKA conducted similar work on a larger scale. He contributed a series of papers on "Phylogenetic differentiation of rice". His studies show that in the wide range of variation present in *sativa,* a pattern with three variety-groups, called the Temperate-Insular, Tropical-Insular and Continental, is found. For classifying varieties, OKA (1958b) gives certain diagnostic characteristics tested with discriminant functions and hybrid sterility. OKA AND CHANG (1959, 1960) also discuss the role of introgressive hybridization in causing variation. OKA (1953, *et seq.*) also has contributed

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to the study of hybrid sterility, but since he is including a review article on this subject in this symposium, past work is not reviewed here.

MORINAGA AND KURIYAMA (1954a, 1955, 1958), in three important articles on hybrid sterility in rice, provide data on the extent of spikelet sterility in a large number of F_1 combinations of rice varieties from different groups and countries. These data are indispensable for the present study, and some of the findings are cited.

(1) F_1 fertility of even closely related varieties rarely approached 90 percent and usually reached only 75 percent (under the conditions in Japan).

(2) In every group from a country there was considerable variation in fertility when representatives were crossed with testers.

(3) Even in intra-*indica* crosses, hybrid sterility could be high.

(4) The "tjereh" (*indica*) and "bulu" (*javanica*) types of Indonesia were distinguished by their hybrid sterility when crossed to each other or to *indica* and *japonica* tester strains.

(5) In contrast, the "aus" and "aman" varieties of Bengal were largely cross-fertile, but differed in amount of sterility when crossed with *japonica* varieties.

(6) Short-day treatment often affected fertility, but there were wide differences. In Burmese × Japanese varietal combinations, 8-h days increased fertility over 11-h treatments. In Javanese × Japanese combinations and Javanese × Chinese combinations, natural day length gave increased fertility over 8-h treatments. In "tjereh" × japonica there was a similar trend and increase in fertility under natural day length was high.

In an analysis of the relationships among "aus" of Bengal, "bulu" of Indonesia and *japonica* varieties, MORINAGA AND KURIYAMA found that some "aus" varieties and some "bulu" varieties gave moderately fertile hybrids with *japonicas.* However, in "aus" × "bulu" combinations, they recorded a large variation among three cross combinations. In two combinations, where "bulu" was the female parent, the mean fertility was 4.5 percent. In one combination where "aus" was the female parent, the fertility was 91.6 percent. They also found that the first two sterile combinations gained about 30 percent in fertility following vegetative propagation for one year. The authors do not discuss this feature, but it is possible that cytoplasmic differences are present between some of the "aus" and the "bulu" varieties.

MORINAGA AND KURIYAMA (1954b) conducted a series of experiments on photoperiodism in six varieties and constructed photoperiod response curves. These six included the *japonica* varieties, Zuiho and Fukoku. SAMPATH AND SESHU (1961) used their results to show that the genetics of photoperiod response in *japonica* × *indica* hybrids give a clue to differentiation of the two groups.

Japanese workers had believed that structural differentiation of chromosomes played a part in the evolution of *japonica* from the tropical groups. CUA (1952) and MASHIMA AND UCHIYAMADA (1955) utilized tetraploids derived from *japonica* × *indica* hybrids to support this hypothesis. YAO *et al.* (1958) were the first to use pachytene analysis in F_1 hybrids to obtain direct evidence for chromosomal differences. Further evidence was obtained by HENDERSON *et al.* (1959) by pachytene analysis. SHASTRY AND MISRA

(1961), also by pachytene analysis of four hybrid combinations, demonstrated the presence of "differential segments". They emphasized that the differentiation observed would account for the semisterility occurring in such hybrids.

Studies on hybrid sterility have been made at the Central Rice Research Institute since 1951, during the course of a hybridization project. Data were collected cooperatively, and salient features of incidence of sterility, in a large number of *japonica* × indica hybrids and in some hybrid progenies, were given in the reports to the International Rice Commission. A report to the International Rice Commission (Sixth Meeting, Penang, 1955) contained a brief consideration of the factors affecting semisterility, environmental, cytoplasmic, genic and chromosomal. The relevant portions are repeated in this paper.

BUTANY *et al.* (1961), from the results obtained in this project, reported on the general features in *japonica* × *indica* hybrid sterility at the Rice Research Workers' Conference, Cuttack, 1959. They included observations which confirm those given in the present paper as follows: spikelet sterility exceeds pollen sterility in most instances by 10 to 50 percent. In 16 *japonica* × *indica* combinations, the reciprocal crosses differed in spikelet sterility by more than 10 percent. The incidence of spikelet sterility varied with season, and was higher in March-April than in September-October. In three selected hybrid combinations, the F_2 sterility dispersion was similar even though the F_1 sterilities were different.

VENKATASWAMY (1957) completed a study on sterility in *japonica* × *indica* hybrids and its inheritance. The results are recorded in an unpublished thesis. Since this work was done at Cuttack, with my collaboration, the findings and inferences are given with later acknowledgement.

Among the unpublished work conducted at Cuttack are studies on *spontanea* rices, generally classified as *Oryza sativa* var. *spontanea.* SAMPATH AND GOVINDASWAMY (1958) pointed out that in Orissa, conditions favor natural crossing between *sativa* and *perennis* and field evidence suggests that such natural crossing is the source of *spontanea* rices. MADHAVAN NAYAR (1958), GOPALAKRISHNAN (1959), and HAKIM (1962) obtained clear evidence of the hybrid origin of *spontanea* varieties. Their unpublished work showed that the hybrids between *sativa* or *glaberrima* and *perennis* showed semisterility and there was considerable sterility present in their progenies.

The incidence and inheritance of semisterility in *spontanea* is of interest and its causes are yet to be traced. In some cultures of the Asian wild rice collection, 50 percent or more semisterility is present, and their progeny are also semisterile. It is possible that the causes of semisterility in these wild rices are the same as those operating in *Oryza sativa* hybrids.

General experience with *spontanea* varieties from different countries led SAMPATH AND SEETHARAMAN (1962) to suggest that introgressive hybridization of *sativa* by geographical varieties of *perennis* could have contributed to the origin of different groups of rice. Their hypothesis is based on the known differences in the forms of *perennis* from different countries and on the close affinity of the perennial wild rice of Taiwan with the *japonicas.* RICHHARIA *et al.* (1962) cite examples of a wide range of

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sterility observed in rice hybrids and they do not consider hybrid sterility to be a criterion of relationship. They also point to the role of introgressive hybridization as a source of variation in rice.

EXPERIMENTAL

The following abbreviations are used in this text:

J = *japonica*
I = *indica*
spontanea = *Oryza sativa* var. *spontanea* or *fatua*
FAO = work done at the Central Rice Research Institute under a project sponsored by the Food and Agriculture Organization.

The figures given as percentages refer to sterility and never to fertility.

Under the FAO and a related Indian project, selected *japonica* varieties were crossed with a few *indica* varieties from participating countries. In the extended program of these projects, the *japonica* varieties Zuiho and Fukoku were crossed with a larger number of selected *indicas.* The F_1 plants of those *japonica* × *indica* crosses were grown at Cuttack, and seeds for the F_2 were sent to different countries. The total number of J × I combinations was about 360. In most crosses the J was the female parent. The F_1 hybrids were grown in the July-November season, although the crossed seeds were often sown in February, and the F_1 plants were vegetatively propagated in July.

(*a*) *Pollen Sterility.* This was scored from iodine mounts. Since pollen sterility was generally lower than the spikelet sterility, the possibility of error in scoring was examined. The pollen from J × I hybrids varied in size and also in staining reaction with iodine (brown to black). A pollination study showed that both undersized and brown-staining pollen are viable and both, therefore, were scored as fertile.

(*b*) *Spikelet Sterility.* This was measured from samples of five panicles. The variation in spikelet fertility in any one combination was often large. For example, in Fukoku × DJ. China, the F_1 mean sterility was 81 percent and the standard deviation (S.D.) was 6.5 percent. Therefore, sterility readings are given to the nearest integer and a S.D. of 5 percent may be generally applicable. Spikelet sterility approximately equals embryo sac sterility since there are always enough fertile pollen grains to fertilize viable egg cells. Zygotic sterility, as shown by partly developed ovules, varies from 2 to 7 percent and may be ignored. Another type of zygotic sterility, namely fully formed but non-germinating seeds, occurs but is rare. The J × I spikelet sterility in the F_1 plants of about 300 combinations was used to prepare a histogram, adopting class intervals of 10 percent. The distribution was nearly normal with a mode of 50–60 percent sterility. Since the parents were about 10 percent sterile, this value was deducted from counts of spikelet sterility.

The pollen sterility was considerably lower than spikelet sterility. In a sample of 60 cross combinations, the mode was 20 to 30 percent, and none was more than 80 percent

TABLE 1A

COMPARATIVE HYBRID STERILITY OF *indica* WITH TWO *japonica* VARIETIES

indica parent (female)	Spikelet sterility as percentages *japonica* varieties (male)	
	Fukoku	Zuiho
C 32–1 (Burma)	76	87
C 401 (Burma)	72	58 (?)
Katak Tara (Aus)	29	36
Dular (Aus)	11	15
Habiganj 8 (Aus)	26	48
Hastikalmi (Aus)	27	43
Habiganj 7 (Aus)	18	52
Peta (Tjereh)	62	73
Untung (Tjereh)	35	82
Salak (Tjereh)	65	67
Ve Vang (Viet Nam)	92	96
Tai chet cu (Viet Nam)	90	91
Ramia	78	89
Lupang Puti (Viet Nam)	35	95
Wag Wag (Philippines)	62	9s
Pa (Thailand)	46	64
Khao Unpai (Thailand)	62	63
Khao Tao (Thailand)	76	81
Leyang Yei (Thailand)	73	81

sterile (FAO). The counts given in Table 1B of pollen and spikelet sterility in many J × I combinations suggest that this difference is common. This difference between pollen and spikelet sterilities persists even in the F_2, and the data from the F_2 of Bhadas × Fukoku are given in Table 2 (VENKATASWAMY, 1957). From the F_2 of Taichung 65 (J) × Guinangang (I) 47 plants were scored for pollen and spikelet sterility. Only in five instances did the first exceed the second. The averages were: pollen sterility 42 percent, spikelet sterility 68 percent (FAO).

(*c*) *Environmental Effect.* Sterility is markedly affected by environmental conditions. The same F_1 combinations were more sterile in south Malaya than in north Malaya. Similar differences were between North Sumatra and Java (FAO). The hot dry conditions of March and April increased sterility at Cuttack. This feature was used to compare the behavior of Zuiho and Fukoku in cross combinations involving the same *indica* varieties. During October to November, 17 Zuiho × *indica* combinations had a mean sterility of 75 percent while the Fukoku × indica mean was 60 percent. In April to May, the corresponding figures were 79 and 91 percent respectively. Thus, sterility increased during the hot months, but to a larger extent in the Fukoku hybrids (FAO).

(*d*) *Segregation for Sterility.* It was generally found that there is wide segregation for

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TABLE 1B

COMPARATIVE HYBRID STERILITY IN J × I COMBINATIONS

indica parent (male)	*japonica* parent (female)	Pollen sterility percent	Spikelet sterility percent
(Burma)			
C 24 – 02	Norin 8	28	53
C 24 – 02	Gimbozu	9 (?)	50
D 17 – 88	Norin 20	32	86
D 17 – 88	Norin 18	56	77
D 17 – 88	Taichung 65	70	89
D 17 – 88	Gimbozu	50	90
C 28 – 16	Norin 20	54	92
C 28 – 16	Norin 6	72	75
C 28 – 16	Rikuu 132	36	55
(India)			
T 1145	Norin 6	26	68
T 1145	Norin 18	34	70
T 1145	Norin 20	23	62
T 1145	Gimbozu	9 (?)	77
T 812	Norin 6	41	79
T 812	Norin 20	61	77
T 812	Gimbozu	32	82
Nigersail	Asahi	31	66
Nigersail	Norin 20	6 (?)	70
Nigersail	Taichung 65	22	59
(Philippines)			
Elon Elon	Norin 8	13	72
Elon Elon	Norin 20	10 (?)	75
Apostol	Norin 18	43	74
Apostol	Gimbozu	46	65
(Indonesia)			
Mas	Norin 8	24	70
Mas	Gimbozu	26	83
Mas	Asahi	17	72
(Malaya)			
Serandah Kuning	Gimbozu	48	87
Serandah Kuning	Norin 18	42	89
Nachin 57	Gimbozu	39	69
(Thailand)			
Nang mol	Norin 6	37	68
Nang mol	Norin 18	14	72
Nang mol	Asahi	24	72
Puang Nagern	Norin 6	12	64
Puang Nagern	Gimbozu	13	51

TABLE 2

CORRELATION BETWEEN POLLEN STERILITY AND SPIKELET STERILITY IN F_2 OF BHADAS (I) × FUKOKU (J), NUMBER OF PLANTS IN SPIKELET STERILITY CLASSES (10 PERCENT CLASS RANGE)

Number of plants in pollen sterility classes	Number of plants in spikelet sterility classes									
	95	85	75	65	55	45	35	25	15	Total
95	3	–	–	–	–	–	–	–	–	3
85	3	2	2	–	–	–	–	–	–	7
75	2	4	1	4	1	–	–	–	–	12
65	2	4	1	4	1	1	–	–	–	12
55	3	2	2	3	1	1	–	–	–	12
45	1	2	2	1	6	3	1	–	–	16
35	1	–	4	–	3	1	4	–	–	17
2s	1	1	–	2	5	2	3	5	–	19
15	–	3	1	1	–	4	9	2	2	22
Total	16	18	13	19	17	11	17	7	2	120

sterility in all the F_2 and later generations of J × I. Plants more sterile than the F_1 parent were present in most F_2 populations. In the F_1 of Zuiho × Dular, the sterility was only 15 percent. Dular is an "aus" variety of Bengal and is equally fertile with Fukoku. But F_2 plants with more than 70 percent sterility are found (FAO). The segregation pattern for sterility observed in the F_2 of six J × I combinations is given in Table 3 (VENKATASWAMY, 1957). From the F_2 onwards, selection for fertility is effective but plants more sterile than the parent occur in some F_3-F_5 families (FAO and VENKATASWAMY). It is believed that there is no uniform and rapid increase in fertility on selfing.

(*e*) *Homozygous Semisteriles.* Selection for homozygosity in plant characters and in semisterility was started from a random sample of J × I progenies. One culture, J × I (148), became uniform in the F_6 and this was confirmed in the F_7 and F_8. The

TABLE 3

INCIDENCE OF SPIKELET STERILITY IN F_2 (APRIL SEASON). NUMBER OF PLANTS IN 10 PERCENT CLASS RANGE

J × I Combination	Sterility in F_1	95	85	75	65	55	45	35	25	15	Total	Mean	S.D.
Fukoku × Bhadas	33	6	8	17	15	19	22	13	12	8	120	53	22
Bhadas × Fukoku	68	16	18	13	19	17	11	17	7	2	120	63	23
Gaisenmochi × Bhadas	27	19	15	9	16	11	11	17	9	14	121	55	27
Fukoku × B 405	69	14	23	18	6	12	4	9	1	4	91	69	21
Fukoku × Milagrosa	17	2	7	5	12	15	22	24	12	11	110	46	20
Fukoku × Concejala	55	3	2	2	3	3	1	–	5	5	24	50	29

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sterility varied from 70 to 80 percent. There was a number of abnormalities at meiosis in this culture. In 18 out of 200 cells (in all stages of division) one to two quadrivalents were observed (VENKATASWAMY, 1957). By colchicine treatment and chromosome doubling in a haploid obtained from the F_3 of Rikuu 132 × Meyong Ebos, homozygosity could be obtained directly and quickly. The diploid culture showed semisterility which varied from 27 to 52 percent depending on environment and season (VENKATASWAMY). This culture was extraordinarily sensitive to environment, and sterility varied from 10 to 80 percent. Another J × I semisterile culture reached uniformity in the $F_{10}$and is being maintained. In this culture (C. 20), the pollen sterility is 35–40 percent and spikelet sterility 90 percent. One quadrivalent was found in 18 out of 23 cells in diakinesis, and in another ten cells two more bivalents were close together, as if in contact. From a hybrid between Fukoku and a local variety of *perennis* (termed *balunga*) homozygous semisteriles were found in F_5 and uniformity was confirmed in F_6 and F_7. Pollen sterility was 5–10 and spikelet sterility was 60–70 percent in this culture.

(*f*) *Cytoplasmic Factor*. In the J × I combinations of Rikuu 132 with Adt. 18 and Gimbozu with T. 1145, the reciprocal crosses differed significantly in pollen sterility. Similarly, in the combination of Taihoku 6 with MO. 1, J × I was 97 percent sterile while I × J was 25 percent sterile. The means for the F_2 were 66 and 53 percent respectively (FAO). In the Fukoku × Bhadas combination the reciprocals differed in pollen sterility by 13 percent and in spikelet sterility by 35 percent. This difference in pollen sterility was not statistically significant. The F_2 Mendelian ratios for husk color and awning were noted in the reciprocals of this combination. Numerical differences observed in the small populations scored were not statistically significant (VENKATASWAMY, 1957).

DISCUSSION

The experimental work reported here is restricted to J × I combinations. These alone are not adequate to trace the factors controlling hybrid sterility in rice. The extensive studies by Japanese workers, particularly MORINAGA AND KURIYAMA (1954a, 1955, 1958) and OKA (1957a and 1958b) are pertinent.

By what genetic process did the sterility barriers arise and what was the effect of selection preserving them in the different regions? To solve these problems it is necessary to trace the factors causing semisterility. If semisterility were solely due to recessive mutations in duplicate loci essential for gamete development, then an explanation would involve drift but not selectional effects. The following objections can be raised against such a simple hypothesis:

(1) Gametic lethals would not be so sensitive to environment.

(2) Homozygotes should not show semisterility, but this is not supported by observation.

(3) There would be a rapid elimination of gametic lethals and correspondingly a rapid uniform recovery of fertility.

(4) It is improbable that a progeny could be more sterile than the parent.
(5) The wide difference between pollen sterility and spikelet sterility in F_1 hybrids and in hybrid progenies is not explained.

OKA (1957b), in discussing the role of recessive genes in hybrid sterility, postulates a system, wherein "duplicate fertility genes" are inferred to operate in addition to gametic lethals. An alternative interpretation of factors inducing semisterility is offered here, along with reasons.

The analysis of genic components of semisterility is at present difficult, but critical experiments can be designed from the data now available. For example, the Zuiho × *indica* combinations are more sterile than the Fukoku × *indica* combinations. A critical cytogenetical study of Zuiho × Fukoku would reveal whether a genic or chromosomal difference is important in this instance. One possible explanation has been overlooked previously. Genes having some other clear phenotypic effects could have a pleiotropic modifying effect on sterility. Fukoku is known to differ from Zuiho in four dominant loci: a complementary gene for black hull, at least one gene for awning (FAO) and duplicate factors for earliness (SAMPATH AND SESHU, 1961). The first two are primitive characters and may help reduce sterility. The latter duplicate genes may be responsible for the increase of sterility in April-May season. An intensive study of the FAO data and of MORINAGA AND KURIYAMA'S data for both agreement and disagreement might suggest further decisive genetical experiments.

The published data on hybrid sterility lead to the inference that segmental interchanges may be present within regional groups like *indicas.*

The existence of cytoplasmic differentiation, which contributes to hybrid sterility, is probable. The significance of this factor is discussed in Part II. The relative importance of genic and chromosomal factors in causing semisterility has to be considered in relation to the ecological differentiation of rice.

It is believed that the *indica* and *japonica* varieties are differentiated in chromosomal organization and that this would contribute to hybrid sterility. An extreme view, suggested by SHASTRY AND MISRA (1961), is that the main cause of sterility is in chromosome structural differences which, on segregation, would cause death of a proportion of gametes (haplontic sterility). Such an interpretation does not agree with (a) sensitivity to environment, (b) increase in sterility of segregants, (c) occurrence of homozygous semisteriles, and (d) varying differences between Zuiho and Fukoku when used as testers. In addition, it may be stressed that segmental interchanges in rice chromosomes do not produce clear phenotypic effects; hence, the selective value of such changes is difficult to estimate. The problem of ecological adaptation remains unexplained.

It is evident that the gametic lethals would be rapidly eliminated, and the persistent sterility in the F_2 and later generations would be of complex origin. The consistent and large difference between pollen and spikelet sterility in each hybrid indicates that cellular physiology restricts full gamete development (diplontic sterility). Such a restriction could be selective or non-selective, depending on the nature of segregation at meiosis. This restriction is called "squeeze". "Squeeze" can also operate in pure

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strains. For instance, SAKAI (1959) conducted periodical sowings of three pure strains and recorded that the varieties differed in their maximum sterility.

The pattern of inheritance of semisterility in rice hybrids is visualized as follows. In the F_2 and later generations, gametic lethals (genic or chromosomal) become less important. By recombination of loci or gene blocks concerned with cellular physiology and with homeostasis, efficiency of gamete formation is affected and "squeeze" begins to operate. If the loci concerned become homozygous, the culture breeds true for semisterility. In terms of differential segments of J as compared with I, it implies survival of the deficiency-duplicates in a haploid condition. This inference requires evidence for acceptance. The occurrence of highly sterile progeny from moderately sterile parents and of homozygous semisteriles suggests that this explanation may be correct. The occurrence of quadrivalents in homozygous semisteriles also supports this. An independent check also is possible, and X-ray irradiated cultures are being screened for homozygous semisteriles. BORA AND RAO (1958) used X-rays and thermal neutrons to induce mutations in rice varieties. They suggest that deficiency-duplicates for small segments of chromosomes are viable in *sativa.* Results of other workers in this field, e.g. CARPENA AND RAMIREZ (1960), possibly support this view.

This interpretation is important in tracing the origin of cultivated rice, but it is not essential to explain the genetic discontinuity between J and I varieties. The discontinuity observed could have arisen by introgressive hybridization of *indica* varieties from China with the wild rice of Taiwan (SAMPATH AND SEETHARAMAN, 1962). This hypothesis does not explain the origin of chromosomal differences between the ancestral species and *sativa* and the distinctive features of Taiwan's wild rice. The differentiation of Taiwan's wild rice could have occurred in the following manner. Translocation heterozygotes would survive in natural populations since a large seed production is not essential in perennial plants. Their progenies would carry a continuous range of deficiency-duplicates and also mutants. If any of them showed adaptation to a changing environment or a new ecological situation, they would survive. Mutations in these, being random, could lead to increased fitness as well as reduced sterility. Since this species has been in existence for a geological period, the time required for such evolution was available.

It is deduced that there are two different problems for further study. One relates to reorganization of genetic material or "repatterning of chromosomes". The other relates to changes in gene function, that is mutation. The possibility of survival of deficiency-duplicates for small segments of chromosomes permits a particular mode of evolution. Mutations, recombinations, and selection are deemed the main causes of evolution, and these could operate on repatterned chromosomes as effectively as on unchanged genomes. One kind of change in chromosome organization as represented by "included inversions" are probably present in *sativa* (HENDERSON, 1964). If the other process suggested above is also operating in the evolution of wild rices, a working hypothesis on the genetic differentiation of *sativa*, from the ancestral species, can be constructed. Studies conducted on semisterility in "synthetic" and natural *spontanea* varieties suggest that chromosomal repatterning has accompanied the evolution of

Oryza sativa, and the causes of semisterility in *spontanea* varieties are similar to those in *japonica* × *indica* hybrids. The report by SHASTRY (1964, this Symposium p. 112) that in a *spontanea* from Madhya Pradesh, India, differential segments could be observed in pachytene stage, suggests that chromosome structural differences between ancestral species and *Oryza sativa* can be experimentally ascertained.

The conclusion is that evolution and differentiation of *sativa* is parallel to and interconnected with its origin from *perennis.*

PART II

It is probable that some *japonica* strains have a cytoplasmic factor that contributes to hybrid sterility. The decisive evidence for such a factor is obtained by successive backcrossing to affect genome substitution in an alien cytoplasm. Important researches on cytoplasmic sterility have been made in other crops, and the well known work of KIHARA in *Triticum* and allied genera is cited as an example. As regards rice varieties, in addition to the evidence cited previously, an important contribution was made by OKA (1958a). He records that successive backcrossing by J in an I × J hybrid gave semisterile cultures, and that selfing eliminates this sterility. KITAMURA (cf. CHANG, 1963) from test crosses with rice varieties from different regions, infers that regional differences in cytoplasmic types could be detected through suitable testers from a *japonica* group. Oka's work as well as the FAO findings suggest that the cytoplasmic factor is genically controlled and new techniques have to be devised to establish their presence. In order to explain the work in progress, a "model" is presented.

MODEL

The *"hoja blanca"* or white leaf virus is endemic to South and Central America. *Oryza latifolia* and possibly other grasses are resistant to this virus and constitute a source of resistance. The wild rices of this region will be subjected to severe selection and the survivors will carry resistance through cytoplasmic organelles, the resistance being indirectly genetically controlled. The genetical features of this model are: the virus differs between continents and large islands, and wild plants will be resistant to the endemic virus; the cytoplasm of the resistant types carries factors which induce sterility in appropriate hybrids.

Evidence: JENNINGS (1961) found that in Colombia the old local varieties of rice included cultures resistant to *"hoja blanca"*. This supports the model in that genes for resistance are present in *Oryza sativa* and selection had taken place. ATKINS AND ADAIR (1958), however, recorded that out of 867 varieties from Japan, Taiwan and China, 362 were found to be resistant to *"hoja blanca",* and that almost all of the resistant types were *japonica* varieties. This finding could disprove the model, since *"hoja blanca"* does not occur in Japan. There is the possibility that a virus disease, "yellow dwarf" or "stripe disease" (*Oryza* virus 2), present in Japan, is serologically similar to *"hoja blanca"* and that selection for one is effective for the other.

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Experimental: The *japonica* varieties likely carrying the cytoplasmic factor are Rikuu 132, Taichung 65, Taihoku 6, Asahi, Aikoku and Norin 36 (FAO). The "bulu" varieties Sukanandi and Genja Beton likely carry the same factor (inferred from MORINAGA AND KURIYAMA, 1958). The Chinese *spontanea* varieties (found near Canton) probably carry a cytoplasmic factor causing sterility (inferred from SAMPATH AND RAO, 1951). This factor may be different from that found in *japonica* varieties (KATSUO AND MIZOSHIMA, 1958).

The African group of *Oryza glaberrima, stapfii* and *breviligulata* give highly sterile hybrids with *sativa.* These African species are being tested for the cytoplasmic factor at Cuttack, and backcrossing has been started with *sativa* × *glaberrima* combinations.

In Afrasian combinations, the semisterility found could be due to a combination of genic and cytoplasmic factors. Because of this complexity, experimental modification of F_1 hybrid sterility is being attempted. One experiment gave negative results: irradiation of stem buds of *sativa* × *glaberrima* F_1 by g-rays to a dosage of 2000 r did not reduce the sterility. Soaking the F_1 stem buds for 24 h in 0.2 percent caffeine solution reduced sterility in one out of three combinations treated. Experiments to reduce sterility by chemicals are being continued.

The seed setting in Afrasian combinations is poor. Tetraploidy has been induced in the following four combinations: *sativa* (*indica*) × *glaberrima, sativa* (*indica*) × *glaberrima* (as *stapfii*), *sativa* (*japonica*) × *breviligulata* and *breviligulata* × wild rice from Taiwan. These hybrid tetraploids are being used for studies in semisterility.

American × Asian wild rice combinations have been obtained. One collection of wild rice from Amazonas, Brazil, belonging to Dr. T. MORINAGA, has been used as one parent. This culture is tentatively identified as a *spontanea.* The F_1 combinations of this culture with a Cuban *spontanea,* with a Cambodian *spontanea* and with *breviligulata* from Mali, West Africa, were highly sterile. The combination with Cambodian *spontanea* is being selfed, caffeine-treated, as well as colchicine-treated for induction of tetraploidy.

There is a possibility that other regions may contain a new cytoplasmic factor. For this purpose, crosses are being made using New Guinea and Australian wild rices.

CONCLUSION

This part is included to draw attention to an important problem in hybrid sterility. It is deduced that cytoplasmic barriers to hybrid fertility have arisen in different regions, concomitant with the development of resistance to local pathogens. If the interpretation offered were valid, an important line of research could be started, the results of which would be of great interest to other biologists.

A PRELIMINARY NOTE ON THE PACHYTENE ANALYSIS OF *JAPONICA* × *INDICA* HYBRIDS

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SHASTRY AND MISRA (1961) report that the pairing of chromosomes at pachynema in *indica* × *japonica* hybrids was quite abnormal, lending evidence for structural hybridity. The varieties used in their studies were N. P. 130, N-32 and T. 21 of the *indica* type; Norin 4, A-18, 7942 and 7374 of the *japonica* type.

We also have studied pachytene figures in the following *japonica* × indica hybrids : Taichung 65 × Bir-mei-fen, Taichung 150 × Wu-kuan-chin-yu, and Kaohsiung 64 × PTB 25, and in the *indica* × *japonica* hybrid, Bir-mei-fen × Nakamura. Examination of hundreds of slides of PMCs of these parents and hybrids, which contained a large number of analyzable configurations at pachytene, showed that the pairing in the hybrids was quite normal as in the parents. There was no clear-cut evidence of structural hybridity. We found one chromosome with a "reversed repeat", as described by SHASTRY AND MISRA, only in the Bir-mei-fen × Nakamura hybrid (Fig. 3).

Among the PMCs studied, chiasmata became evident at early diplonema as the paired homologous chromosomes opened out to form loops of varying sizes. Fig. 2 shows two chromosomes found in a PMC at this stage in Kaohsiung 64 × PTB 25 beginning to open. Such configurations are not considered as evidence of structural hybridity as they were frequently observed in the parents (Fig. 6). At late diplonema, the paired chromosomes open up further forming more loops (Fig. 7), similar to those described by SHASTRY AND MISRA as gross structural hybridity. This phenomenon was found in both the parents and hybrids.

It may be concluded that the chromosome behavior at pachytene in the parents and in the hybrids between them is identical. No apparent indication of gross structural changes at the proper pachytene stage in the *japonica* × *indica* hybrids was detected. The details of this investigation will be published elsewhere.

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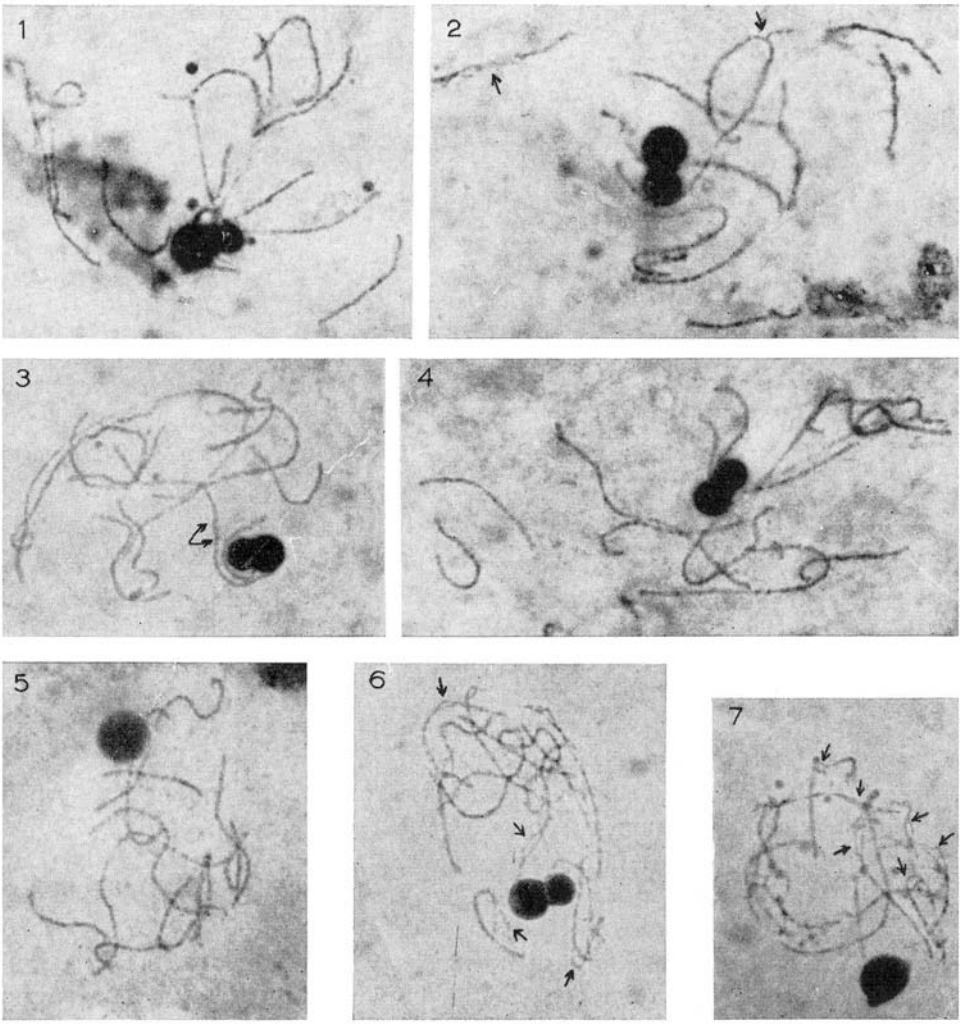

FIGS. 1–12

1 – Kaohsiung 64 × PTR 25, 12 bivalents well paired at pachytene stage in meiosis; 2 – idem, the paired homologous chromosomes open out to form loops in one chromosome and simply open out at the end of another; 3 – Bir-mei-fen × Nakamura, the so-called "reverse repeats" found on one chromosome denoted by arrows: 4 – idem, showing well paired 12 bivalents; 5 – Bir-mei-fen, one of the parents used, showing 12 bivalents well paired at pachytene stage in meiosis; 6 – idem, early diplotene stage, loops and open ends can be seen in some chromosomes; 7 – idem, later diplotene stage in meiosis, loops can be seen almost in each chromosome.

DISCUSSION IN SESSION ON NATURE OF INTERVARIETAL HYBRID STERILITY IN *ORYZA SATIVA*

One fact emphasized in the discussion was that the sterility is not limited to hybrids between varieties of the *indica* and *japonica* races. Sterility is most common in these wide crosses but may also generally occur in intervarietal hybrids within each of these races. Furthermore, some hybrids of the *indica-japonica* type are completely fertile.

One question was whether Completely fertile F_2 plants derived from partially sterile hybrids tend to resemble the parents in morphological characters. This indicates difficulty in obtaining desirable recombination types when rigid selection is practiced in F_2 for complete fertility. The answer was that in the genetic studies reported here no association was found in F_2 and F_3 between degree of fertility and any morphological characters in which the parents differed. However, a need for more specific investigation of the possible influence from selection for high fertility in the early segregating generations on gene recombination was indicated.

A general tendency among F_2 and F_3 plants for the percentage of stainable pollen to be higher than the percentage of florets setting seed was revealed. Its cause is not known but several possible explanations were offered.

A question was asked regarding whether all F_3 lines with partially sterile plants invariably also contain some completely fertile plants. It was pointed out that this was almost always true in the genetic studies so far reported, providing strong evidence that the basic mechanism causing the sterility characteristically results in the occurrence of completely fertile plants in semisterile F_3 lines. A few cases have been reported of lines in which no completely fertile plants were found, but their rarity suggests that they may not be related to the marked sterility of F_1 and F_2 populations.

A large portion of the discussion revolved around the effect of the absence of evidence for translocations at pachytene and metaphase I on validity of the proposal that the sterility is due to cryptic structural hybridity arising from translocations. It appeared that no entirely satisfactory answer was as yet available concerning this question.

Another subject related to the appropriate use of the terms haplontic and diplontic in reference to various expressions of sterility is intervarietal rice hybrids. Participants agreed that all forms of sterility reported to date should be described as haplontic, since in all examples of intervarietal hybrid sterility in rice which have been investigated, deterioration occurs in the microspores and megaspores after meiosis has been completed in an apparently normal manner. Further discussion revealed that the problem with terminology resulted from the occurrence of partially sterile plants in F_2 and F_3 of a few crosses in which the F_1 generation was completely fertile. The suggestion was made that this type of sterility be described as “sporophytically controlled.”

SESSION VI

INHERITANCE STUDIES, GENE MARKERS AND LINKAGE GROUPS

GENETIC SEGREGATION AND LINKAGE, IMPORTANT PHASES OF RICE RESEARCH

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INTRODUCTION

With many significant, recent advances in genetics, it is well to begin with a discussion of "formal" or "classical" genetics. Developments in cytogenetics, bacterial genetics, evolutionary genetics, and population genetics have recently overshadowed inheritance studies of the Mendelian type. However, quoting from a review of a new book on genetic linkage[1], "There is bound to be, in the near future, a recrudescense of interest in the more formal aspects of Mendelian inheritance".

How amenable is rice to genic and linkage analysis? Renewed study of inheritance of discrete characters and their linkage relationship seems essential in the development of the scientific appreciation of such essential a food crop as rice. Rice deserves investigation by the ecologist, morphologist, taxonomist, physiologist, entomologist, pathologist, agronomist, economist, and even the sociologist, among others, but the resulting picture would be incomplete without an adequate knowledge of the genetic behavior.

Furthermore, it is from an understanding based on the fundamentals of genetic principles that the crop can be bred to its utmost usefulness. Studies of gene segregation and linkage relationships surely will continue to be valuable training for future rice breeders. Although in many plant breeding studies in which the individual assortment of the genes cannot be followed, we can advance on the basis of the particulate nature of character inheritance.

The enormous value of rice is incentive enough for conducting studies to enhance our knowledge of the crop, including intensive investigations of rice genetics. Dr. K. RAMIAH has often emphasized the wide variability of rice, and studies on the segregation of a wide spectrum of characters have been published. R. H. RICHHARIA (1961) estimates that some 300 genes affecting some 50 characters have been identified. Thus, it is appropriate that we consider the inheritance and linkage of discrete characters,

REVIEWS

Because of the scattered publication and language diversity, summaries and reviews

[1] Review by R. C. Lewontin of NORMAN T. J. BAILEY, *Introduction to the Mathematical Theory of Genetic Linkage,* Oxford University Press, 1961, in American Scientist, 1962, Vol. 50: 320A – 322A.

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are an important part of the literature on rice genetics. The historical development of rice genetics can be traced by means of the reviews.

Apparently, segregation of bran colors, reported by J. E. VAN DER STOK in 1908 was the first record of Mendelian inheritance. S. KATO'S extensive early work in Japan was summarized by H. ANDO in 1916.

YAMAGUCHI (1927) presented a comprehensive discussion of much of the work in rice genetics up to that time, with emphasis on Japanese contributions. Mendelian segregation had been reported for awns, long glumes, dwarfs, waxy endosperm, red and purple kernel colors, and resistance to *Piricularia oryzae.* Complementary factor ratios were found for the segregation of the color characters and pleiotropic effects had been noted.

IKENO (1927) in his monograph on genetic studies of the rice plant, mentions pubescence; in a cross between long- and short-haired varieties, the segregation was non-Mendelian, but glabrous types were unknown to him. He holds resistance to *Piricularia oryzae* to be dominant and monofactorial in spite of wide fluctuations in the F_2. Easy threshing was recessive to difficult threshing. He credits KATO with a 3:1 ratio for liguleless. It was KATO who predicted the probability of duplicate gene ratios for this character, reported only recently (GHOSE, BUTANY AND SEETHARAMAN, 1957). IKENO says that HOSHINO in 1902 discovered xenia in seed produced on waxy plants pollinated with normal pollen.

IKENO discusses an unstable large grain strain which he attempts to explain as a gene mutation which back-mutated. Normals which occurred again gave rise to abnormal plants, indicating chromosomal irregularities instead of gene action as he thought.

MATSUURA (1933) summarized genetic results reported up to 1929 under the following headings :

(1) Colors such as red, purple, gold, and furrow colors in glumes, awns, and kernel.
(2) Color in vegetative parts.
(3) Morphologic characters, such as glume length, awns, sinuous neck, a spindly type of plant, and dwarfs.
(4) Waxy endosperm.
(5) Time of heading.

The discussion of color characters takes up a large part of MATSUURA'S review. Reported characters were listed as controlled by 1, 2, 3, 4, or multiple genes. He listed 111 ratios altogether, of which 72 had to do with color. Workers had found that colors in various combinations of parts were influenced by the same genes, but the term "pleiotropy" had not come into use. Apparently, pleiotropic effects and the linkage concept were confused.

SAKAI (1935a) reviewed publications dated between 1930 and 1935, mostly the work of K. RAMIAH, Y. YAMAGUCHI, J. W. JONES and K. NAKAYAMA. Color inheritance continued to make up a large part of the studies; vegetative and flowering parts were still placed in separate categories. Chlorophyll deficiences were studied in India and Japan. In the United States, J. W. JONES reported a 3:1 ratio for non-shattering *vs.*

shattering. Laxness of panicle, brittleness, and ligulelessness were studied and additional work on awns, dwarfing, and heading time was reported. RAMIAH found tallness usually dominant, although the reverse was encountered.

The 1936 USDA Yearbook contains an article by J. W. JONES, briefly summarizing genetic research with rice. His tabulation of F_2 segregations and ratios follows MATSUURA'S closely and was supplemented by the work of RAMIAH, KADAM, and his own studies in California.

KADAM AND RAMIAH'S (1943) tabulation of F_2 ratios serves as a review although this was not the authors' purpose in preparing their "Symbolization of Genes in Rice". Segregation ratios for about 180 contrasted characters were listed. The linkage summary by JODON (1948) included a tabulation of additional reports on the segregation of 15 characters as a supplement to Appendix III of KADAM AND RAMIAH.

Some of the characters, which were new in the literature at the time, included chlorophyll deficiencies, *Cercospora oryzae* resistance, certain dwarfs, fragrant flower, lazy and other types of growth habit, spikelet length, round spikelet, some types of sterility and stunted plants. More than 200 citations were included in the bibliography, of which about 80 were dated 1935 or later.

NAGAO (1951) in a report on genetic studies in Japan, describes some new mutants: open spikelet, claw-shaped spikelet, double awns (awns on both lemma and palea), triangular hull, neckleaf and glossy leaf.

JONES (1952) describes several mutations found in Caloro; two "blackleaf" physiologic diseases, hullspot, Sathi type and neckleaf, all controlled by single genes. He also reports two aberrant plant types induced by X-ray irradiation; compact-sterile and short-small seed.

I contributed a few mutations to the stock of marker genes: a virescent, a semidominant long-glume, fragrant flower, whitehull, Cercospora resistance, blackleaf, long-grain dwarf, and blackspot from X-ray irradiation (JODON, 1957, 1958).

The monograph by RAMIAH AND RAO (1953) covers the findings of Indian workers up to about 1950, and NAGAJ'S *"Japonica Rice"* (1958) does the same for Japanese work up to about 1957.

The expanded IRC report (ANON., 1963), published by the USDA, includes lists of character pairs and their segregations, tabulated after the manner of KADAM AND RAMIAH, thus giving a rundown of genetic studies through 1960.

The reported Mendelian characters were grouped for convenience in the IRC report under the following categories or types:

A = Anthocyanin colors	E = Modified structures
B = Other colors	F = Modified composition
C = Presence of structures	G = Modified growth habit
D = Size and shape	H = Physiologic and disease

The following table gives a very rough estimate of the extent of inheritance studies and the volume of accumulated information.

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TABLE 1

ACCUMMULATION OF INFORMATION ON MENDELIAN INHERITANCE IN RICE AS INDICATED BY LITERATURE REVIEWS

Period	Number of reports on segregation due to the number of genes indicated				Number of symbols	Number of publications	Reviews by
	1	2	3	4			
Up to 1927	–	–	–	–	40	?	YAMAGUCHI (1927)
to 1930	76	37	7	2	None	60	MATSUURA (1933)
to 1942	174	70	21	4	118	200	KADAM AND RAMIAH (1943)
Increase, 1930 to 1942	98	33	14	2		140	
Up to 1960	280	130	63	34	97	320	ANONYMOUS (1963)
Increase, 1942 to 1960	106	60	42	30		120	

Many of the possible patterns of Mendelian inheritance have been demonstrated in rice as indicated in the literature. The following are examples :

3 : 1	Straw *vs.* goldhull (*Gh* : *gh*)
1 : 2 : 1	JONES' Sathi type mutation
13 : 3	Inhibitors such as purple leaf (*I–Pl*)
15 : 1; 63 : 1	Resistance to *Cercospora* races, duplicate genes (*Ce* : *ce*)
4 : 8 : 3 : 1	Epistasis (*Gm* and *g*)
9 : 7; 27 : 37 ; 81 : 175	Apiculus color; complementary genes (*C, A, P*)
C and *Rc*	Basic genes for anthocyanin and bran colors
C, C^{B}, etc. H^{m}, H^{i}, etc.	Allelic series
C^{B}, *A, P*	Pleiotropic effects : color in apiculus, outer glumes, stigma, internode and sheath.

GENES SUITABLE FOR LINKAGE STUDY

Besides those already found, do other spontaneous mutations exist? Workers in tomato genetics, although theirs may be a newer field, reported 175 new mutants between 1955 and 1960 (CLAYBERG *et al.,* 1960). The varieties of rice have been extensively inventoried and classified, but some heritable freak characters doubtless remain undiscovered. The brittle character, for example, probably was found only once. This illustrates the rarity of radical mutations which are fairly viable and useable as marker genes. In relation to this gene, modifying genes restricting the drastic effect of the brittle character perhaps may be found, but so far none has been recognized. Possibly, mutations are found more frequently in hybrid lines because of disruption of genic influence on the mutation rate.

Sometimes I ask rice breeders visiting the Rice Experiment Station in Louisiana if they know of mutations paralleling various ones of those found in other cereals which might occur in rice. Probably it had never occurred to any of them that a mutation such as the free-threshing or naked kernel character might appear in rice. Possibly, someone will discover a rice with yellow endosperm or with solid straw, both of which might have distinct economic as well as genetic value. A simply inherited character, glabrous, has made life much more pleasant for combine and drier operators in our mechanized system in the United States.

Hidden genes may be manifested in segregating populations when freed from epistatic and inhibitor genes, Some of the genes in the "twilight zone" between discontinuous and blending inheritance, the major genes involved in bimodal inheritance, may be found segregating free of modifying genes in advanced generation lines. For example, we have isolated strains segregating distinctly for 30-day differences in maturity. In the F_2 of a natural cross of red rice on one of our long-grain varieties, typical long-grain segregates amounted to 21.5% of the population. This indicates that a major gene controls kernel width.

A virtual reservoir of genes important for linkage studies probably exists in the resistance reactions to disease organisms, especially to particular races of fungus. RYKER (1943) identified several physiologic races of *Cercospora oryzae* and demonstrated that resistance to each was controlled by single or duplicate genes. His results made possible subsequent tests for linkage with genes for several morphologic characters.

Piriculuria oryzae also is comprised of numerous physiologic races. Genes for resistance to *Piriculuria,* with help of plant pathologists, could be readily tested for linkage, In such tests, the segregation of other characters would be classified in the F_2 and the disease reaction on the basis of inoculated F_3 progenies.

Naturally, we will turn to induced mutations to augment our collection of contrasted genes, thus extending to the maximum the number of genes that can be placed on the linkage map, According to JONES (1936 USDA Yearbook) mutations obtained by means of X-ray, ultraviolet light, and temperature were reported in Japan in 1934. JONES (1952) later reported X-ray induced mutations from Caloro. I obtained one good marker from a limited number of irradiated plants (JODON, 1958).

LINKAGE

The linkage relationships of many of the genes for which segregations have been reported have been tested in a limited number of combinations, if at all. The previously reported ratios would be verified in the course of testing for linkage.

With 12 chromosomes the chances of finding, in any one cross, any two pairs to be members of the same linkage group is somewhat less than 1 in 12. There is somewhat less possibility in any rice cross of finding linkage than in barley or corn with 7 and 10 chromosomes, respectively. Thus it is desirable to make crosses involving as many contrasted characters as possible. Only four definite instances of crossing over out of 63 combinations of ratios in 10 crosses were found in a study I made of 19 characters

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(JODON, 1957). NAGAO AND TAKAHASHI (1960) used 217 combinations between 23 gene pairs as the basis of their postulated 12 groups. Only 13 definite linkages occurred, or 1 in 17. In lima beans (11 chromosomes), 10 linked combinations were found out of 90 paired combinations tested (ALLARD AND CLEMENT, 1959). Progress is being made with linkage studies, even in organisms with as many as 20 chromosomes, such as mice.

It is, of course, highly desirable in linkage studies to have marker genes which permit fairly normal development of the plants. Some mutant characters were so unfavorable that deficient phenotypic classes result, complicating the calculation of crossing over. Liguleless is one example; although liguleless plants seem quite normal, deficiency ratios were found in F_2. From 100 F_3 seed of each type 89 normal but only 81 liguleless seedlings emerged. It would be convenient to have tester lines carrying multiple marker genes so that a new mutation could be tested simultaneously in one or a few crosses for linkage with several groups. However, viability and also crossability limit the extent to which this can be realized.

Genes which are especially suitable for use as markers are limited in number. My choices would include the following:

	MARKER GENES	
LINKAGE GROW	FIRST CHOICE	SECOND CHOICE
1	*wx, C, Cl, v*	—
2	*Pr*	*lg, d_2, Pl*
3	*A, Rd*	—
4	*g, Rc*	d_o
6	*gh*	d_1
8	*la*	—
9	*nl*	—
10	*bl*	—
11	*bc*	—
12	*gl*	—

F. R. PARNELL (cf. ANONYMOUS, 1963 ; JODON, 1948) is credited with the first instance of crossing over found in rice. This was between color in glumes and internode lines. YAMAGUCHI (1927) reported the *C-wx* linkage in 1921. In his 1927 review he also lists liguleless and glume color.

MATSUURA listed 5 linkage groups :

(1) Apiculus color and waxy, to which a maturity gene had been added.
(2) Red kernel and another maturity gene.
(3) Hull color and purple internode lines.
(4) Color in ligule and glumes.
(5) Color in stigma and leaf sheath.

Apparently, very little attention was given to linkage relationships in genetic studies conducted during the 1930's.

All available results of segregations showing crossing over were assembled in my mimeographed 1948 "Summary of Rice Linkage Data" (JODON, 1957). The data were divided into eight provisional groups, which seemed to be the minimum number that could be assumed. Linkages between characters, for which pleiotropic genes possibly were involved and with roughly similar crossing over, were assumed to be the same. A tabulation of characters between which independent inheritance had been shown was also included; 30 or possibly more genes had been tested in combinations which showed no linkage.

In 1953, I prepared a review of rice genetics for the American Society of Agronomy, taking note of the four groups described by NAGAO in his 1951 paper, which gave results of previously unavailable Japanese work. It was eventually published in the Journal of the Agricultural Association of China, with revisions drawn from the monograph of RAMIAH AND RAO (1953). However, the linkage group affinities of many of the color genes were left in question and the suggested groupings were tentative.

NAGAO AND TAKAHASHI (1960) recently published results of their linkage findings in Japanese varieties, and were the first to postulate 12 groups. Five of the groups were based on linkage of only two loci. The linear order of three loci in each of four groups and of six loci in one group was determined. Two additional loci, tested for linkage and found to be independent of each other and of each of the above 10 groups, were proposed as markers for the two remaining groups. The 12 groups proposed by NAGAO AND TAKAHASHI (1940) were used as the basis of the linkage section of the IRC report (ANONYMOUS, 1963) and other available data were collated with theirs as far as possible.

The F_2 segregation of a cross between the variety Toro and red rice mentioned above is presented (Table 2) as an example of an inheritance study which afforded data on linkage relationships. The segregation of six character pairs was studied. The observed numbers were in reasonable agreement with the expected ratio on the basis of single gene segregation for five of the characters and for a duplicate gene segregation for the remaining character. The 15 possible combinations of the 6 characters were tested for linkage. The chi-square test indicated that for three combinations inheritance was not independent. Shattering and blackhull were linked with 27.5% crossing over. Neither was linked with any other character, and as the linkage apparently has not been reported previously, further tests would be necessary to place the controlling genes in one of the established groups.

Glabrous and awned were linked with 28.5% crossing over. Linkage between glabrous and a gene for awns has been designated as Group 12 (ANONYMOUS, 1963). Red bran also appeared to be linked with glabrous, and 37.5% crossing over was calculated, since independent inheritance between red bran and awned was indicated by the Chi-square test and the calculated linkage was 44%. The gene order appeared to be *Rc-gl-An.* However, the basic gene for red bran *(Rc)* has been reported to be in Group 4 (ANONYMOUS, 1963), and the results obtained from the Toro × red rice cross may have been due to chance.

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TABLE 2

SEGREGATION AND Chi-SQUARE TEST FOR INDEPENDENT INHERITANCE OF SIX CHARACTERS IN THE F_2 OF THE CROSS TORO × BLACKHULL RED RICE*

Segregating characters	Gene pairs	Observed ratios (numbers)**	Chi-square (Independence)				
			Sh : sh	*Bh : bh*	*Rc : rc*	*Gl : gl*	*Lk : lk*
Shattering *vs.* non-shattering	*Sh* : *sh*	125 : 33					
Blackhull *vs.* strawhull	*Bh* : *bh*	112 : 46	20.00				
Red *vs.* non-red bran	*Rc* : *rc*	115 : 43	0.20	3.11			
Pubescent *vs.* glabrous	*GI* : *gl*	120 : 38	0.24	1.83	5.60		
Wide *vs.* slender-grain	*Lk* : *lk*	124 : 34	3.45	0.15	0.58	1.96	
Awned *vs.* awnless	*An* : *an*	146 : 12	0.14	0.11	0.25	8.36	1.44

* Unpublished data, Rice Experiment Station, Crowley, Louisiana. J. S. KARYIANNIS classified the F_2 population.
** Expected numbers (3 : 1 ratio) 118.5 : 39.5 and (15 : 1 ratio) 148.1 : 9.9.

Results from a number of crosses, in which nine characters were tested in certain combinations for linkage with marker genes for five groups, plus a few miscellaneous genes, are summarized briefly (Table 3). Purpleleaf and whitehull were known to be in Group 2, but the other characters were "unplaced". Only 26 combinations of the 45 possible between 9 characters and 5 linkage groups were obtained, Eight of the nine characters were shown to be independent of the Group 12 marker. Blackspot was found to be independent of all markers in all five groups, but the tests for the other characters were much less complete. No instance of linkage was discovered among the combinations tested.

Various instances of crossing over have been reported which require further investigation for placement in the respective linkage groups (ANONYMOUS, 1963). The

TABLE 3

INDEPENDENT INHERITANCE OF NINE CHARACTERS TESTED IN CERTAIN OF THE POSSIBLE COMBINATIONS WITH MARKER GENES IN FIVE LINKAGE GROUPS AND WITH VARIOUS OTHER GENES*

Characters tested	Linkage groups and marker genes								Other genes independently inherited**
	1			4		6	9	12	
	wx	*c*	*Cl*	*g*	*Pr*	*gh*	*nl*	*gl*	
Purple pericarp (*Prp*)			×	×		×		×	*An, Rk, Psh, Ef*
Purple leaf (*Pl*)		×	×			×		×	*bl*
Purple-internode (*Pin*)		×	×			×		×	*Rk*
Whitchull (*Wh*)	×							×	*An,* d_{22}
Round kernel (*Rk*)	×	×	×	×				×	
Dwarf (d_{22})				×				×	*rc*
Blackleaf (*bl*)		×	×			×		×	*Pr, An*
Blackspot (*bs*)	×	×	×	×	×	×	×	×	*rc*
Dwarf (d_{21})				×					*Psh*

* Unpublished data, Rice Experiment Station, Crowley, Louisiana.
** See IRC list of symbols (ANONYMOUS, 1963).

first recorded linkage in rice, that between a hull color and purple internode lines, reported by PARNELL remains to be placed in the appropriate linkage group. Purple pericarp has been reported to be linked with purple collar, purple sheath, and scented kernel, and unpublished data also indicate linkage with a dwarf and with a blackleaf character. Other reported linkages not yet placed include those of Kuang's between awns and a dwarf, and awns and blackhull (cf. ANONYMOUS, 1963).

RAMIAH AND RAO (1953) described two patterns of pigmentation in morphologic structures, one independent of Group 1 and the other independent of the first and also of Group I. These color patterns behaved as heritable units, except that rare unexpected types appeared, possibly the result of crossing over.

Some of the reported linkages between purple characters involving different plant parts may or may not involve the same genes, and further substantiation may be needed for some. Purple colors vary with light and nutrition and are subject to fading; consequently, errors in classification may occur. Such errors, together with those due to natural crossing and mixtures, could lead to indications of actually non-existent linkage.

WORK IN PROGRESS

Genic segregation and linkage in rice remain fields of interest for many workers. In the United States continued support is needed for cytogenetic studies at Louisiana State University. Graduate students at Louisiana State University desiring problems in plant breeding probably will continue to have the opportunity to work on phases of problems of economic importance involving both quantitative and qualitative characters. Usually, material is available at Crowley for this type of study and several students have conducted their research there. Similar informal arrangements are made on occasion between the Beaumont Station and Texas A & M College geneticists.

The program at Crowley, as at other rice stations over the world, is primarily variety improvement with genetics something of a stepchild. A number of crosses for genetic studies are made each year, and more F_2 populations grown than it has been possible to classify. At present there are on hand sheaves representing many crosses from which it is hoped that considerable interesting data may be obtained. Dr. C. N. BOLLICH is taking up an extended investigation of lodging. Some of the ramifications of this problem probably will lead to inheritance studies.

It is anticipated that studies on the inheritance and linkage relationships of genes for resistance to disease organisms, especially *Piricularia,* will be initiated in Louisiana and Texas. Pathologists in the United States Department of Agriculture have established methods for testing progenies of crosses for resistance to straighthead and the hoja blanca virus, as well as *Piricularia.*

SUGGESTIONS FOR FUTURE WORK

At the 1962 Rice Technical Committee meeting the following recommendations were made regarding research in genetics and cytogenetics:

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Studies should be conducted "on the mode of inheritance of many characteristics, such as (a) reaction to disease, insects, pesticides, temperature and to levels of soil fertility and other soil and water characteristics such as alkalinity and concentration of salts; (b) resistance to lodging; and (c) properties associated with cooking and processing characteristics. Genetic and cytogenetic research also should include the synthesis of strains with a normal complement of chromosomes that have marker genes for use in linkage studies. A search also should be made for the 12 trisomics in rice as these could be used in linkage studies."

I have already pointed out some possibilities for inheritance studies: look for spontaneous and induced mutations, watch segregating populations for hidden characters, and exploit genes for resistance to diseases. The discovery of new characters is worth recording in print, together with illustrations and the demonstration of their mode of inheritance. Up to this point at least, Mendelian studies might be classed as amateur sport. The study of newly discovered genes should include determining the loci in the respective linkage groups. A great deal of work remains to be done to locate already known genes, as was pointed out. A set of tester varieties with wide adaptation is needed. RICHHARIA (1961) has suggested parallel studies of characters in *Oryza sativa* L. and *Oryza glaberrima* Steud. Association of quantitative characters with linkage groups is possible by means of chi-square tests. Qualitative characters may be profitably studied along with quantitative or economic characters in varietal improvement studies. The economic importance of characters such as awns calls for study. Another possible approach would be the comparison of crossingover of linked genes in different cross combinations. The identity of genes from various sources should be investigated as should also the behavior of genes in different genetic backgrounds. A method study, comparing efficiency of backcross and F_2 data, might be worthwhile. Environmental factors which influence crossing over also might be investigated.

Perhaps close linkages should be restudied in the light of the new concepts of the gene. Rice genetics investigations would be fostered by improved publication outlets. Publication in journals of limited distribution is not adequate.

SUGGESTIONS REGARDING METHODS OF STUDY

Every precaution should be taken to overcome differential survival. Fungicidal seed treatments might help save some of the weaker segregates which may not emerge under conditions of direct seeding as used in the United States. "Aldrin" (an insecticide used as a seed treatment) might help small or weak seedlings survive submergence at the initial flood as it seems to invigorate seedlings. Systemics such as "Thimet" could be used to overcome borers and other insects. Ordinary transplanting methods would appear to be very unfavorable for the survival of weak segregates, but I have seen no comment on these.

Working space must be provided between the rows. It is convenient to plant two rows fairly close together and leave an alley of at least 18 inches between pairs of rows. Proper spacing must be used for individual plant identification.

Recording of data and classification of phenotypes can be simplified. Small bits of paper attached to the tips of panicles with stapling pliers may be used to record heading dates. A bit of masking tape serves the dual purpose of providing a surface to record data and of holding the culms of a plant up and together. Thus the plants can be collected in bundles, making envelopes necessary only if there is shattering. If classification of all characters can be made on mature plants, only one selected culm per plant need be retained. Classification can then be accomplished simply by separating successively the members of each pair of characters, and eliminating counts until all the phenotypic classes have been sorted out.

The minimum size of population adequate to determine segregation and crossing over is important because of the space and time involved. Usually 300 to 600 or more F_2 plants from single F_1 plants, have been grown in Louisiana, considerably more than necessary for most purposes. Mathematically determined information seems to indicate that 225 plants adequately distinguish between most segregation ratios and to determine linkage.

Contamination of segregating populations by natural crosses occurring in the F_1 should not be overlooked. Semisterile F_1's grown in the field probably are vulnerable, but obvious off-crosses in F_2 populations are very few.

Most workers feel that because so few seed are obtained from cross-pollinations backcrossing is not feasible. Perhaps an even greater impediment to the use of backcrosses for linkage studies is the necessity of verifying each plant as actually the progeny of the intended combination. Results of backcross studies have not been published. However, various techniques for crossing now in use might be employed in ways that would make mass pollinations possible in the field as well as in the greenhouse. High percentages of seed set were obtained in the greenhouse at Beaumont by removing anthers by suction, after clipping the glumes, and then dusting on pollen by jarring panicles in which florets had opened without bursting of the anthers. WELLS AND CAFFEY (1956) reported successful use of scissor emasculation and mass pollination of cereal grains in the field. In Greece, pollen is collected in the field on slips of paper. In India, anthers are flushed out by enclosing panicles in bamboo chambers, a technique which should be adaptable to getting the volume of pollen needed to fertilize florets emasculated by hot-water treatment.

CONCLUSIONS

Some points of interest may be inferred regarding current genetic investigations from recent Mendelian and linkage studies.

(1) About 20 papers reporting results of inheritance studies have been published since 1959,

(2) The matter of symbolization remains chaotic, but *C* and *A* seem to be coming into use for apiculus colors.

(3) Relatively very few "new" characters have been described: supercluster, leaf hair genes, a long-glume modifier, twisted long-grain, length of second leaf, and breadth of second and flag leaves.

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(4) The patterns of genic interaction described by NAGAO and TAKAHASHI (1956) were not altered in crosses between some of their lines and those from other areas.

(5) Duplicate or complementary gene action appears to be involved in the inheritance of certain characters for which only single-gene segregations were previously known.

(6) Linkage relationships which are not yet adequately investigated in relation to the 12 groups have been reported.

(7) Rice genetics is far from being "mined out". Much more complete genetic information will be needed before the so-called "fine structure" of the genes can be investigated.

In the preparation of this paper the question recurred : Has rice genetics any unique features compared with the findings in other plants? Dr. K. RAMIAH has suggested in some publications that the mutant types in rice include an uncommonly large proportion of dominants. However, the International Rice Commission list of symbols indicates about 50–50 dominants and recessives. It is Dr. M. T. HENDERSON's opinion that major genes play an unusually prominent part in quantitative inheritance in rice. Possibly an exceptional number of characters are controlled by duplicate (polymeric) genes. Also as indicated in this paper, fewer instances of crossing over are encountered than might be expected.

Be this as it may, there is no crop which can be studied under a wider diversity of geographic and ecological conditions.

CERTAIN CONSIDERATIONS ON GENIC ANALYSIS AND LINKAGE GROUPS IN RICE

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INTRODUCTION

Genic analysis in rice has been underway in India for about five decades. Earlier studies were directed towards confirming the application of Mendelian principles to character inheritance in rice and were confined to indica varieties. Though contributions also came from U.S.A., the two main lines of investigations in India and Japan took place independently. Although in a majority of these investigations identical or similar characters have been genically analyzed, it has not been possible to arrive at uniformity in the identification of gene loci concerned or in the mode of gene action and interaction. RICHHARIA (1945) summarized available genetical data in rice and in other crops. RAMIAH AND RAO (1953) brought together in their monograph the genetical data obtained in India through 1948. Recently, JODON *et al.* (ANON., 1963) summarized the information on genic analysis and linkage relationship of genes available from India, Japan and U.S.A. The abstract bibliography of rice from the College of Agriculture, University of the Philippines is a useful contribution. The present paper attempts to draw attention to certain aspects of genic analysis, based on the findings reported since 1960 and the work now in progress at different rice research institutions in India and elsewhere.

PART I

EXPERIMENTAL

(1) *Inheritance of 'fuzzy' character of hull*

Earlier workers have reported monogenic or digenic segregation for the hairiness *vs.* glabrousness of hulls (RAMIAH AND RAO, 1953; SAMPATH AND SESHU, 1958; NAIR, 1958, Central Rice Research Institute, unpublished records). The hairy phenotypes were represented by varieties with hairs of a 0.3 to 0.5 mm length distributed over the surface of the lemma and palea and most densely in the apiculus region. In the new phenotype 'fuzzy', the hairs are comparatively long, smooth and densely distributed, giving the grain a fuzzy appearance. In crosses where the inheritance of the 'fuzzy' character was studied, the conclusion was that a new gene designated as *Fg* (Fuzzy glumes) is par-

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tially dominant over the normal hairy gene *Gl,* although they are not allelic. *Fg* exerts its influence in the presence of *Gl.* Fuzzy glumed phenotypes in the segregating population exhibited a certain range of variation in the density of the long hairs; this might be due to the influence of modifiers.

(2) *Superclustering*

Clustering refers to the arrangements of spikelets in the panicle. When varieties with spikelets arranged in "3's" were crossed to normals, the inheritance of clustering was monogenic, with higher clustering exhibiting incomplete dominance (RAMIAH AND RAO, 1953; NAIR, 1958; C.R.R.I. Annual Report, 1957–58). RAMIAH AND RAO (1953) observe that the degree of clustering varies from two to seven spikelets per cluster. KADAM AND D'CRUZ (1960a) also report that the intensity of clustering is considerably influenced by modifying genes. These authors found no normal plants in the F_2 population of some crosses. The F_2 consisted of plants with two to eight spikelets per cluster, while both parents had spikelets arranged in clusters.

The newly found phenotype, superclustering, may be considered to involve a high degree of condensation of the primary and secondary branches of the panicle with the result that each bunch consists of 10 to 30 spikelets, occasionally as many as 40 (BUTANY AND SEETHARAMAN, 1960). The superclustered type of *indica* rice (Ac. 3776) was crossed to a normally clustered variety. The panicles of the F_1 plants had spikelets arranged in "2's" at the tips of various branches. The spikelets were densely arranged along the entire length of the spikes. In the F_2 population, in addition to plants exhibiting varying degrees of clustering, normal plants were also recovered, suggesting that the gene for this character designated as *Scl* was non-allelic to *Cl.* The presence of modifier genes influencing clustering could be inferred from the spectrum of clustering exhibited by the F_2 and F_3 plants; these data suggested that the number of major genes might not be more than two,

(3) *Ligulelessness*

Ligulelessness in an *indica* variety Shala was reported first by PAWAR *et al.* in India. Earlier investigators in Japan (cited by NAGAI, 1958) found this character controlled by a single recessive gene *lg.* GHOSE *et al.* (1957) concludes from their studies on *indica* varieties that four duplicate genes govern the presence or absence of auricle, ligule and junctura. The absence or presence of these parts was inherited as a unit. In subsequent investigations (C.R.R.I., unpublished records), monogenic, digenic, and trigenic ratios were obtained. Further, liguleless strains of the *japonica* type, when intercrossed, always produced F_1 plants without auricle, ligule and junctura, showing that the genes in both parents are situated at the same locus. The present investigation has confirmed this observation, Some of the liguleless *indica* strains were then crossed with *japonica* liguleless ones; again the F_1 plants had no auricle, ligule and junctura. This suggests that the *indica* and *japonica* types have the same gene controlling ligulelessness. To account for more complicated segregation ratios reported by other workers, however, cytological interpretation would be necessary. It is possible that

in liguleless strains, a portion of the chromosome containing dominant genes for ligule formation is deleted, the extent of deletion being different in different strains. Or, the segment of the chromosome containing those genes might be transmitted as a single block in *japonica* varieties while in *indica* the genes act as separable units. Further work in in progress,

(4) *Awnedness*

The development of awns has been studied either as a qualitative or as a quantitative character. The awned character is generally dominant and two or three gene loci have been identified (SETHI *et al.,* 1937; RAMIAH AND RAO, 1953; C.R.R.I. Annual Report, 1956–57). Complementary genes have also been recorded. Duplicate genes for awning has been reported by ALAM (1931, cited by GHOSE *et al.,* 1960), At the C.R.R,I., different ratios were reported. It seems that five genes probably are involved in the expression of awning (GHOSE *et al.,* 1960).

The phenotypic expression of awning is known to be variable, however. The phenotype in any segregating population shows variation both in the degree of awning and in the length of awns, It also is known that when a tetraploid is induced from an awnless type, its spikelets tend to develop awns. These observations led MISRO *et al.* (1961) to conclude that awn development is controlled by a polygenic system. In addition, a dominant gene inhibiting the expression of awning also has been found (MISRO AND MISRO, 1954).

The occurrence of palea-awn (double awning) has been observed. The double awning has been reported to be controlled by a single recessive gene *da* (TAKAHASHI, 1950). The uncommon triple awning has been considered only as a teratological variation (SEETHARAMAN, 1962b), It is known that in *Oryza australiensis* and, to a lesser extent, in *breviligulata,* short palea-awn is present. Long double awns were also observed in the sativa × *breviligulata* amphidiploids. Even the original diploid plants of this cross showed double awning. This suggests that double awning is not exclusively governed by a recessive gene.

(5) *Anthocyanin pigmentation*

Anthocyanin coloration in different plant parts is a striking feature. The genetical behavior of this character has received considerable attention since the initiation of genetical research on rice in India. A review of the published papers reveals that little critical evidence is available, however, to reconcile the different results in a comprehensive general scheme. Such a plan has been postulated by NAGAO (1951) for the *japonica* varieties. Investigations at the Central Rice Research Institute show that an extension of this plan to explain the results obtained in the *indica* varieties is premature. It is not applicable to all cases.

Available genetical data enable us to formulate a few broad conclusions as follows :

(i) The occurrence of pigment in any plant part depends on the complementary action of two basic genes, *C* and *A* (KADAM AND RAMIAH, 1943), corresponding to the *C* and *Sp* (now designated as *A*) of NAGAO (1951).

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(ii) Genes for pigmentation are usually dominant.

(iii) Recent studies with different purple-leaved varieties in the *indica* group strongly indicate that at least three genes (and probably more in a few cases) interact to produce anthocyanin coloration in this plant part. The interrelationship of these genes needs further study.

(iv) An inhibitor present in most colorless varieties prevents the development of purple color in the leaf blade (KADAM, 1936; RAMIAH AND RAO, 1953; etc.).

(v) A varying number of genes (two to six or more) is involved in the expression of color in various plant parts. (RAMIAH, 1935; Annual Report of Central Rice Research Institute, 1957–58; GHOSE *et al.,* 1963; NAIR, 1958, etc.). Some of them act as 'localizers'.

(vi) Evidence (C.R.R.I. unpublished records,) indicates that differences exist in the effect of an inhibitor gene or genes in their ability to suppress the expression of color in one or more organs at a time. A biochemical explanation may be that inhibition is determined by the nature of anthocyanidin present in the plant parts.

(vii) Localization genes play an important role in the expression of color in various plant parts. Pleiotropic effect of those genes needs reinvestigation, with particular attention to a few exceptional cases.

(viii) The presence of genes for apiculus coloration may not always be required for the occurrence of pigmentation in other parts.

(ix) The presence and effect of anti-inhibitory genes must be investigated.

NAGAI (1958) classifies genes controlling the development and distribution of anthocyanin pigment into four categories, i.e., (a) gene complex controlling the production of chromogenic substance or its precursors including primary and secondary converters, (b) complementary genes, (c) distributors, and (d) inhibitors. The distributing genes are probably synonymous with localization genes.

In most investigations on anthocyanin coloration only two classes, colored *vs.* non-colored, have been distinguished. No attempt to differentiate the grades has been made, though these are present. NAGAO (1951) explains exceptional variation in the apiculus coloration on the basis of a multiple allelic series at the *C* and *Sp* (A) loci. Similar studies need to be undertaken in the *indica* group.

Genes controlling the pH value of cell sap or the degree of oxidation of anthocyanidin are known in plants other than rice. It has been established in different plants that a more oxidized state of anthocyanidin is dominant over a less oxidized state (LAWRENCE *et al.,* 1939), and a low pH value over a high pH value (BUXTON, 1932). Differences in the intensity of coloration in rice could be due to such differences.

(6) *Role of pigment-localization genes*

NAGAO (1951) in his studies on the interrelationships of genes controlling anthocyanin pigmentation in *japonica* varieties emphasizes pleiotropic effects of single genes in several cases (*Pl, Pla,* etc.). In contrast, KADAM AND RAMIAH (1943) conclude that the localization genes play a considerable role in the pigmentation of various plant parts.

Evidence for the latter conclusion is as follows:

(i) A variation in intensity of pigment has been observed in segregating populations, even when pigment generally occurs in plant parts such as leaf sheath, septum, and internode, the intensity is not always the same. This variation suggests that independent genes control the colorations in different parts.

(ii) RAMIAH (1945) and MISRO *et al.* (1960) observe that in a large varietal collection, the patterns of pigment distribution were never uniform and showed independence for different parts.

(iii) In segregating progenies, the ratios of colored to non-colored plants differ according to the different plant parts (NAIR, 1958; IYER, 1959).

These observations suggest the significant role of localization genes in the *indica* group; however, in the *japonica* group, a gene can, in a particular combination with one or more localization genes, produce coloration in more than one part. It is probable that anthocyanin pigmentation is governed by different gene systems in the two subspecies.

(7) *Gene at Pr locus*

A critical observation of the genetic stock collection reveals that varieties differ even in the extent the apiculus color spreads down to the floral glumes (lemma and palea), and that these differences are inherited and are therefore under genic control. In test crosses between two parents differing in extent of the apiculus color spreading, the greater spreading nature dominated over the less spreading behavior. Also, in a few cases, a monogenic ratio has been obtained (unpublished records). These findings strongly suggest the existence of a multiple allelic series at this locus.

NAGAO (1951) asserts that the gene *Pr* (former *Rp*) is responsible for the distribution of color over the entire surface of the floral glumes. No multiple allelic series has been suggested for the *japonica* group. The absence of variation in the *japonica* varieties for this character, as a whole, as contrasted to *indica* varieties, where different phenotypic expressions have been observed, might explain the failure to identify multiple allelic series at this locus in the *japonica* varieties.

(8) *Nature of endosperm*

In rice, the glutinous endosperm is controlled by a single recessive gene (*gl* or *wx*). The non-glutinous endosperm shows complete dominance and the phenomenon of 'xenia' has been observed when the glutinous variety is used as the female parent (PARNELL *et al.*, 1922; RAMIAH AND RAO, 1953).

A new approach to the genetics of this character is now possible. Starch in rice, as in other cereals, consists of two fractions, i.e., amylose and amylopectin. The starch-iodine blue test (HALICK AND KENEASTER, 1956; BEACHELL, 1957), shows the partial dominance of low iodine value (high amylose content). SEETHARAMAN (1959) concludes that the difference in the ratio of amylose to amylopectin in the varieties studied is controlled by one pair of major genes and several modifiers. In these investigations the amylose content was expressed in terms of 'Iodine transmission value', The intensity

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of blue coloration being dependent on amylose only, this test is quantitative and is preferable to the normal qualitative tests. The glutinous starch gives a characteristic reddish coloration. The new technique thus shows that the chemical reactions leading to a differentiation of starchy and non-starchy endosperm are probably controlled by more than one pair of genes. By using such biochemical tests, genic analysis of the nature of endosperm starch can advance. It has been reported that even in glutinous varieties starch found in the cells of leaf and other vegetative parts is of non-glutinous nature.

The presence or absence of abdominal white is associated with the endosperm. RAMIAH AND RAO (1953) reports variation in the extent to which the chalky region progresses towards the center of the grain. The Central Rice Research Institute also studied this variation. Recently, UEDA AND OTA (1961) classified abdominal white into five types. Although this character is reported to be controlled by one dominant gene (NAGAI, 1958), it is more likely that the number of genes involved may be two or more.

There is another group of varieties whose endosperm is chalky in appearance but gives a blue color with iodine solution. N. E. JODON first showed this phenotype to me. We have observed it in our Institute's collection. The endosperm is soft and crumbly. This character may be considered as an extreme form of abdominal white.

Besides these, quality factors such as translucency and hardness are commercially important. Genic analysis of these characters may be best accomplished with the cooperation of biochemists.

(9) *Duration to flowering*

The duration to flowering or the number of days from seed germination to panicle emergence is an important physiological character. This trait has been investigated by several geneticists, but there is little unanimity in their opinions. Different conclusions have been given regarding the nature of dominance relationship. RAMIAH AND RAO (1953) summarize available information from India. The mode of inheritance has been simple in some cases, while in others interaction of two or more genes has been suggested.

CHANDRARATNA (1952) indicates that estimation in terms of sensitivity, optimum photoperiod and minimum duration, all being varietal characteristics, would facilitate a correct interpretation of the data on flowering time, According to CHANDRARATNA (1952), photoperiod response closely relates to and influences 'duration to flower' and is more an end result of a series of reactions of differential nature than an isolated phenomenon. In one of his experiments (1955), sensitivity has been found to be controlled by one pair of genes located in chromosome-I. In such a case, a series of multiple alleles or a series of modifiers has to be postulated to account for the wide range in photosensitivity found among rice varieties. Minimum duration has been found to be polygenic (CHANDRARATNA, 1952).

The duration to flowering of a variety depends upon the reaction of its genotype to the given environment. But the importance of recording the date of sowing, the place

of experimentation and other related matters seems to have been overlooked in past publications. Information on these respects may provide clues to explain some of the discordant results.

The influence of temperature also requires attention. SAMPATH AND SESHU (1961) infer from their studies that temperature and/or some other environmental factors affect the dominance relationship of photoperiodic response.

MORINAGA AND KURIYAMA (1954d) summarized results of investigations on photoperiodic response of rice varieties in Japan. Six pairs of genes have been reported to control photoperiodic response, of which two pairs appeared to exert an indirect influence by controlling the response to temperature. Interaction of genes also has been reported.

(10) *Dwarfs*

RAMIAH (1953) summarized available information on the genetics of dwarfness. Dwarfness is usually controlled by one or two recessive genes. Dominant dwarfs have never been reported in India.

Dwarf mutations controlled by different genes can result in considerable reductions in plant height. It is important, therefore, to distinguish between different genes by their effects. KADAM (1937) suggests that five recessive genes (d_1 to d_5) control dwarfness.

NAGAO (1951, cited by NAGAI, 1958) concludes from investigations on dwarfs in Japan that at least eight recessive genes (d_1 to d_8) are present. HSIEH (1962) recently reports another recessive gene, d_9.

BUTANY *et al.* (1959) conclude that the dwarf character they studied resulted from an interaction of three recessive genes (symbolized as d_a, d_b and d_c); the presence of any two of these genes would result in a dwarf.

SHASTRY AND PATNAIK (1962) observe that the mode of inheritance of their dwarfs fell into two distinct classes, one showing distinct segregation and the other showing a continuous variation in plant height.

The problem, then, would be how to distinguish the effect of different dwarf genes. A study of different dwarf types, as done in Japan, may provide valuable information.

(11) *Observations on interspecific hybrids*

Interspecific hybrids can be utilized to provide evidence for dominance relationships of genes as well as to detect parallel variation. In the study of F_1 hybrids between members belonging to the two cultivated species, viz., *Oryza sativa* and *glaberrima*, the long acuminate ligule, long glume (in certain cases), awning, branched habit and perennating nature exhibit dominance. Similar dominance relationships also exist in intra-*sativa* hybrids and in certain cases in intra-*glaberrima* hybrids. The mode of inheritance of genes responsible for the expression of anthocyanin coloration is similar in both groups in several cases. When the members of the two species are separately crossed to *Oryza perennis*, the nature of dominance of several genes in one is similar to the other (SEETHARAMAN, 1962a).

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However, in the lax nature of the panicle, in the shattering habit and also in the presence of awns in certain cases, the F_1 plants of interspecific hybrids between the two cultivated species show a 'reversion' to the wild ancestral type (SEETHARAMAN, 1962).

This evidence supports the hypothesis that the two cultivated species *sativa* and *glaberrima* are derived from a common ancestral type by parallel evolution. For certain characters, mutations have occurred at the same loci, while for the expression of a few others, mutations have occurred at different loci, resulting in identical phenotypes (SEETHARAMAN, 1962). Complementary genes are inferred to control the expression of certain characters in hybrids between these two cultivated species. Available evidence also suggests that these two species show differences in the linkage relationship of genes influencing anthocyanin coloration (RICHHARIA AND SEETHARAMAN, 1962).

(12) *Inheritance of other characters*

KADAM AND D'CRUZ (1960a) conclude that:

(i) The black color of the lemma is conditioned by four complementary genes (Hb_a, Hb_b, Hb_c, Hb_d)

(ii) Four complementary genes (Kr_a, Kr_b, Kr_c, KT_d) control the roundish shape of spikelet ; and

(iii) Short (normal) glumes are due to two duplicate genes (G_1 and G_2).

PART I I

This review of genetical investigations in rice during the past five decades shows that in several cases, interaction of duplicate genes, complementary genes and genes with multiple alleles controls expression of characters in *indica* varieties. Even in the inheritance of morphological characters with high expressivity, a complicated interrelationship of genic effects has been suggested. Three reasons may account for the complexity and lack of consistency in results :

(i) The polyploid origin of the genus *Oryza*, and consequently, of the species *sativa*, would account for the presence of duplicate and triplicate genes as a result of duplication of chromosomes. Under such conditions, mutation or loss of function of a few genes would not be deleterious and, consequently, the mode of inheritance would depend upon the degree of such changes that had occurred in the strains.

(ii) Phenotype is the end result of a series of biochemical reactions. A block in the reaction series at any particular stage will change the end product. Therefore, a number of gene loci would each have a different effect on the character studied, and in the process, naturally, one, two or more genes would be identified.

(iii) Polygenic systems also would contribute to the establishment of adaptability and evolution. Such systems also may control physiological characters like duration to flowering, plant height, grain size, and other metric characters, They may, in

fact, be responsible for the complexity encountered in analyzing these characters in a qualitative manner.

Whatever the cause in a particular instance, it generally is experienced that in the *japonica* subspecies more clear-cut modes of inheritance are often observed. Thus, inheritance and the nature of gene action may not be identical in the two subspecies, indica and japonica.

Additional evidence in support of the existence of differences in genic constitution between the two subspecies can be listed as follows:

(i) Different crossover values for the same set of genes have been obtained (NAIR, 1958; RICHHARIA *et al.,* 1960).

(ii) A new linkage between genes for the coloration of apiculus and stigma has been reported (D'CRUZ, 1960).

(iii) Recent investigations by MISRO AND SHASTRY (1962) have shown that genes for anthocyanin coloration are present in chromosomes other than those reported by NAGAO AND TAKAHASHI (1959).

(iv) The causes for the sterility in inter-subspecific crosses have been attributed to structural hybridity resulting from chromosomal differentiation (YAO *et al.,* 1958; HENDERSON *et al.,* 1959; SHASTRY AND MISRA, 1961). If this interpretation is accepted, the genes present in the differential segments would have a different linkage relationship. On the other hand, if the gametic lethal system suggested by OKA (1953b) is operative, a selective elimination of the gametes has to be assumed to explain the ratios.

RICHHARIA AND SEETHARAMAN (1962) suggest that the linkage relationship of genes in the two subspecies is not identical. They further point out that differences in linkage relationship are also detectable in the two cultivated species, *sativa* and *glaberrima.* Additional evidence supporting the former contention recently obtained

	Crossover value in	
	Present data	*Japanese data*[1]
Between gene for apiculus coloration and gene for glutinous endosperm	36.0	22.8
Between gene for apiculus coloration and gene for cluster habit	38.5	40.3
Between gene for cluster habit and gene for glutinous endosperm	linkage not detectable	linked
Betweengenefor liguleless condition[3] and inhibitor-gene for purple leaf blade color	14.0–19.0	29.5[2]

[1]Data taken from NAGAO AND TAKAHASHI (1960).
[2]Between gene governing liguleless condition and gene for purple leaf blade.
[3]Identical with *lg* of NAGAO AND TAKAHASHI.

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is given below. This relates to crossover values calculated between genes influencing certain easily identifiable and well-defined characters. The study is confined to *indica* varieties and, for a comparative study, the values reported by Japanese workers also are cited.

The present data show that while the arrangement of the three gene loci (*gl-C-Cl*) is similar in the two groups, the relative distances between the genes are not identical in the two subspecies, Differences in crossover values also exist between certain genes in group 2.

These differences in crossover values and those in linkage relationship emphasize a need for comparative studies concerning gene loci in the indica and japonica subspecies. GHOSE *et al.* (1960), JODON (1948, 1955, 1956), NAGAO AND TAKAHASHI (1960) and JODON *et al.* (ANON., 1963) summarized information on linkage relationship. Further critical studies can be conducted based on the available data.

For identifying complementary and duplicate genes, a good method of approach would be by 'process of elimination'. Another line of approach may be made by identifying the interactions of a given gene with some other genes with a distinct effect, as has been demonstrated in Japan (*C* or *Sp* loci and *Rc* or *Rd* loci).

Progress in the genetic investigation of the *indica* subspecies in parallel with that of the *japonica* subspecies would depend upon the availability of tester stocks. The tester stocks used by Japanese investigators belong to the *japonica* subspecies and their value for analyzing linkage relationships in the *indica* varieties is limited. The needed establishment of tester stocks in the *indica* group may be achieved either by selection of exceptional types with novel phenotypes from genetic stock collections or by a process of isolation of homozygous recessives with different marker genes. It is desirable that investigators maintain their tester stocks or other valuable material so as to promote exchange of materials for the common purpose of obtaining critical evidence.

LINKAGE GROUPS AND GENE SCHEMES OF SOME STRIKING MORPHOLOGICAL CHARACTERS IN JAPANESE RICE

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During the past several years that S. NAGAO and I have studied genic analysis in Japanese rice varieties, strains segregating simultaneously for two or more monogenically inherited characters became available and some multiple gene-markers were built up. These segregations, supplemented with hybrids made specifically to study linkage, resulted in indications of 12 linkage groups corresponding to the haploid number of chromosomes. This paper summarizes these results and presents a linkage map of Japanese rice on a trial basis as the first step toward systematic study of the linkage relationships of rice.

To one without adequate knowledge or information on gene systems of characters concerned, experimental results on linkage would be difficult to understand and would not furnish enough data for mapping linkage groups. For example, the expression of anthocyanin color in the apiculus of Japanese varieties depends on the complementary effect of three genes, *C* (chromogen), *A* (activator) and *P* (spreading the chromogen of *C* throughout the apiculus). Therefore, if the available information merely indicates that a certain character is linked with apiculus coloration, it is impossible to determine with which apiculus coloration gene, *C, A, P,* or possibly another gene, the causal gene of the said character is linked.

Thus, a brief resumé of character expression of genes worth mentioning in this paper precedes the presentation of linkage data.

Linkage intensities were calculated using Immer's tables, except when indicated otherwise. In the present paper, I shall use the gene symbols recommended by the IRC. To avoid confusion, I have inserted in parentheses previously used symbols in the descriptive notes of the genes referred to, whenever applicable.

CHARACTER EXPRESSION OF GENES INCLUDED IN LINKAGE STUDIES

(1) *Genes for anthocyanin and its related color expression, the so-called 'tawny'.*

Coloration due to the presence of anthocyanin and its related pigments occurs quite commonly in several parts of the rice plant. The anthocyanin color shows a wide scope of variation, from pink to purplish black, and its related color expression, the so-called 'tawny', ranges from light to dark brown (TAKAHASHI, 1957). Among causal

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genes of these colorations, the following seven were examined for their loci in linkage groups :

C ..... Chromogen for anthocyanin color
A ..... Anthocyanin activator *(Sp)*
P ..... Completely colored apiculus *(A)*
Ps ..... Purple stigma
Pr ..... Purple hull *(Rp)*
Pl ..... Purple leaf
Pn ..... Purple node

According to NAGAO AND TAKAHASHI'S genic scheme, the occurrence of the anthocyanin color depends on the complementary action of genes *C* and *A* ; *C* is the basic gene for the production of chromogen, and *A* exerts its activating effect on *C* and turns the chromogen into anthocyanin.

C and *A* both comprise multiple allelic series of genes: six alleles have been found at the *C* locus and four at the *A* locus (NAGAO, 1951 ; TAKAHASHI, 1957; NAGAO, TAKAHASHI AND KINOSHITA, 1962). They are arranged in the rank of dominancy as $C^{B} > C^{Bp} > C^{Bt} > C^{Br} > C^{Bm} > C^{+}$ and $A^{E} > A > A^{d} > A^{+}$.

The expression of anthocyanin color of the apiculus is generally attributed to the complementary effect of *C* and *A,* which are essentially considered to be color-producing genes. However, with these genes alone, coloration is so limited and appears so thinly scattered at the very tip of the apiculus that plant of this genotype usually is considered to be colorless in ordinary outdoor observation. For distinct coloration in the apiculus, it is necessary, in the presence of *C* and *A,* for another gene *P* to be present. *P* is concerned with the spreading of chromogenic substances over the entirety of the apiculus. The majority of the Japanese varieties or strains I examined possess *P* in common, and it follows that the principal color types of the apiculus studied so far mainly resulted from combinations of any alleles of the *C* and *A* loci (NAGAO AND TAKAHASHI, 1956; TARAHASHI, 1957).

The rank of dominancy of each allele at the *C* and *A* loci is in direct proportion to the potency of chromogen production and also to the assimilative ability of chromogenic substances in the production of anthocyanin pigment, respectively.

In the absence of *C* or *A,* the anthocyanin color does not appear and the plant is uncolored at the time of flowering. Upon ripening and when *C* is present alone or with A^{d}, that is when it is without A^{E} or A, *C* makes the apiculus brown or 'tawny' in several intensities of color shade, depending on which allele of *C* is present. In this phenomenon, it is assumed that, when *A* is absent, the chromogenic substances produced by *C* change to brown pigment. A^{d} is less potent than A^{E} and A, that is, only a fraction of the chromogenic substances produced can be utilized in the formation of anthocyanin pigment. Therefore, when *C* coexists with A^{d} the remaining quantity of unchanged chromogenic substances is turned into brown pigment. This is why certain plants with the genotype $C^{B} A^{d}$ and $C^{Bp} A^{d}$ show a particular mode of coloration in which the anthocyanin and the tawny colors overlap.

In these genic schemes of the anthocyanin color, there is no need to propose a modifying gene or genes which convert color hue or shade to explain several color types in rice plant.

With regard to the action of genes, *Ps, Pr, Pl* and *Pn* are considered functional in distributing the anthocyanin color into respective parts, and thus coloration occurs in these parts when these genes coexist with two basic genes at the *C* and *A* loci. The hue and shade of the colored parts is connected with the combination of *C* and *A.*

In most Japanese varieties, stigma coloration occurs by the dual effect of *P;* but in addition to this, there is another gene, *Ps,* which localizes the pigment in the stigma (TAKAHASHI, 1958). In a case where an allele at the *C* locus is less potent than C^{Bt}, no color develops in the stigma in spite of the presence of *P* and/or *Ps* together with *C* and *A.*

Pr is a gene which distributes the color over the entire surface of floral glumes, viz., lemma and palea, and in some cases the rachilla. This also holds true in the tawny color of these parts.

Pl, the purple leaf gene, is comprised of three alleles, *Pl, Pl^W* and *Pl^+.* The distribution effects of *PI* and *Pl^W* are similar to each other, showing coloration in the entire surface of the leaf blade, leaf sheath, collar, auricle, ligule, node, and internode. However, they are principally different in the following points : (i) color by *Pl^W* almost fades out in the later part of the growing period, whereas color by *Pl* does not show any noticeable change until maturity, (ii) *Pl^W* causes purple pericarp regardless of its exposure to direct sunlight during its development, while pericarp color by *Pl* is expressed only when it is exposed to the direct sunlight, and (iii) as to internode coloration a striking expression is observed as the pleiotropic effect of *Pl^W*, but as to node or collar coloration *Pl* is more effective than *Pl^W* (NAGAO, TAKAHASHI AND KINOSHITA 1962). There remains, however, a possibility that *Pl* and *Pl^W* are pseudoallelic.

Pn, the so-called purple node gene, is connected with the distribution of color in leaf apex, leaf margin and the entire surface of stem node, collar, auricle and ligule. The part most strikingly colored by *Pn* is the stem node.

(2) *Genes for other colors.*

Among genes for coloration other than the anthocyanin and its related colors, the following were included in our linkage studies :

Rc ..... Brown pericarp
Rd ..... Red pericarp
gh ..... gold hull (*rg*)
I-Bf ..... Inhibitor for dark (or brown) furrows in lemma and palea (*I-F* or *df*)

Rc is responsible for the production of the pigment in the so-called brown rice, which has dark-brown irregular speckles on a reddish brown background. *Rd,* when *Rc* co-exists, is responsible for spreading the color of *Rc,* giving a dark red pericarp and seed coat, or red rice. *Rd* itself does not produce any pigment. Thus, the genic

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constitution of red rice is assumed to be *Rc Rd*, that for brown rice *Rc* Rd^+, and that for white rice either Rc^+ Rd^+ or Rc^+ *Rd* (NAGAO, 1951).

In connection with the red pericarp character, besides the effects of *Rc* and *Rd*, reddish brown color is expressed by a pleiotropic effect of Pl^w (as mentioned before, Pl^w is a gene for purple leaf) when it co-exists with *C* in the absence of *A*.

Some varieties have yellow pigment in the cell wall of the floral glumes and internode. There are two types of this coloration; one is self colored and is called 'ripening gold or gold hull', and the other 'dark or brown furrows', in which only the interveins of the floral glumes are colored. The 'gold hull' is a single recessive (gene symbol *gh*) to normal straw color; the 'dark furrows' is due to a dominant gene *Bf;* and the coexistence of *Bf* and its inhibitor *I-Bf* or the deficiency of *Bf* cause the normal straw color. According to this scheme the genic formulae are given as:

gold ..... gh *Bf I-Bf, gh* + *I-Bf, gh Bf* +, *gh* + +
furrows ..... + *Bf* +
normal ..... + *Bf I-Bf,* + + *I-Bf,* + + +

(3) *Genes for presence of floral structures*

Among several morphological traits, awning, pubescence of floral glumes and long empty glumes will be briefly mentioned.

An ..... Awned
gl ..... glabrous glume and leaf
g ..... recessive long glume (*lng*)

The only distinct criterion in the grouping of awnedness is awned *vs.* awnless, and in this respect 3:1, 15:l and 63:l are the representative segregation ratios in Japanese varieties. However, and although the scaling of the awnedness was conducted in a simplified form, five types of this character are roughly explained on the basis of three pairs of multiple genes. The medium-awned, the short-awned and the tip-awned are characterized by the action of the genes An_1, An_2 and An_3 respectively. The fully awned can be produced by the mutual effect of An_1 and An_2, An_3 is considered to be the weakest in its action, no remarkable effect being seen upon the awnedness of An_1 or An_2, even if An_3 coexists with these genes. Accordingly, the five types can be denoted as follows (NAGAO AND TAKAHASHI, 1942):

Full awn ..... An_1 An_2 An_3 or An_1 An_2 +
Medium awn ..... An_1 + An_3 or An_1 + +
Short awn ..... + An_2 An_3 or + An_2 +
Tip-awn ..... + + An_3
Awnless ..... + + +

Four kinds of major genes, Hl_a, Hl_b, *Hg* and *gl* exist, all of which are responsible for an expression and development of pubescence of floral glume and/or leaf. A gene *gl* is responsible for glabrous leaf and floral glumes, and Hl_a and Hl_b are complementary in producing long pubescence on leaves, but when they co-exist with gl the hairs are remarkably shortened. The last one, *Hg,* is a gene for long pubescence on floral glumes and *Hg* exerts its pleiotropic effect on pubescence of the leaf margin, auricle and

panicle branch. As a result of these schemes, the following hair types have been accounted for (NAGAO, TAKAHASHI AND KINOSHITA, 1960):

Floral Glumes	*Leaf*	*Genes concerned*
pubescent	pubescent	Hl_a Hl_b *Hg*
pubescent	glabrous	*Hg gl*
glabrous	pubescent	Hl_a Hl_b *gl*
glabrous	glabrous	*gl*

In some varieties, empty glumes are as long as, or longer than, the lemma and palea. It is known that these two types are generally governed by the respective single genes, *g* and *Gm,* except in the few cases where duplicate genes are considered to exist, giving a F_2 ratio of 15 normal (short) *vs.* 1 long.

These are the prevailing types of long empty glumes. However, there is still another type of such glumes, in which the length of the empty glumes is uneven, giving a long empty glume on the palea side and a normal short empty glume in the lemma side. It was revealed that this type is given when a suppressor, *Su-g* coexists with *g.* Thus the relations between phenotypes and their genotypes are: normal short (+ +, *su-g* +) uneven long *(Su-g g)* and even long *(+ g)* (NAGAO, TAKAHASHI AND KINOSHITA, 1960).

(4) *Genes for modified structures*

The following morphologically modified characters will be discussed.

Cl ..... Clustered spikelets *(Scl)*
d_7 ..... 'cleistogamous' dwarf
Dn ..... Dense (*vs.* normal) panicle
Ur ..... Undulate rachis
ri ..... verticillate arrangement of rachis
nl ..... neck leaf, bract leaf at the basal node of panicle *(hk, nk)*
lg ..... liguleless
la ..... lazy

Clustered spikelets are usually controlled by a simple pair of allelomorphs *(Cl),* "clustered" being incompletely dominant.

A cleistogamous mutant found by NAGAO AND TAKAHASHI (1954b) has a singular form, with compact panicles, small spikelets and somewhat short plant height. Morphologically, this plant has abnormal glumes in which the lower parts can not be differentiated and the lemma and palea are united. This mutant behaves as single recessive to the normal, and is designated as d_7 in its gene symbol.

The writer found a variant with a singular panicle type which is characterized by a Japanese barnyard grass-like panicle. A single gene, *Dn,* is responsible for this character, showing that dense is incompletely dominant over normal.

In connection with the panicle density, there is another type of dense panicle of

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which the causal gene is designated as *Ur* (NAGAO, TAKAHASHI AND KINOSHITA, 1958). The *Ur* is incompletely dominant over the recessive allelomorph. This panicle type has well-branching rachises, and it is characterized by undulating rachises, showing an open panicle. The cause of undulation seems to be attributable to the growing of branching rachises in the flag leaf sheath, which produces this irregularity by mechanical suppression.

Verticillate or whorled arrangement or rachis is the name given to the particular arrangement of branches on the panicle stem, when five or even more rachises are borne around the basal node of the panicle. In the writer's examination, this behaves as a simple recessive (gene symbol *ri*) to the normal. The letters 'ri' are an abbreviation of 'rinshi', which in Japanese means whorled branches.

Neckleaf is a bract arising at the basal node of panicle. Usually the large part of the panicle is enclosed in the bract leaf. When this is longer than the panicle, no spikelets appear from the sheath, while the panicle is already mature. This character is a single recessive (*nl*) to the normal.

It is known that in a liguleless leaf, the auricle and collar are also absent. In every instance, liguleless behaves as a simple recessive (*lg*) to the normal, the liguled form.

Normally the stem of rice grows upright from the surface of the soil; however, one type of growth habit which is called lazy, spreading or prostrate has been reported by many workers. This type of growth is characterized by the stem growing obliquely so that the young plant has an extreme spreading form. This is a simple recessive (*la*) to the normal.

(5) *Genes for dwarfness*

Dwarf forms of Japanese rice plants are roughly classified into two main types, one is the 'Daikoku' type which is more common, and the other is the 'Bonsai' type. In the former, all plants are similar in plant height, nearly two-thirds to one-third of the original forms. The leaves are upright, short and rigid, and have a deep green color. The panicle is short and compact, and in some forms it is erect even when ripened. The grains are small and roundish. The latter, 'Bonsai' type, is characterized by many tillers with narrow and slender leaves, and with small panicles with not so round or small floral glumes.

The following genes for the 'Daikoku' type dwarf characters were examined as to their loci in linkage groups:

d_1 ..... 'Daikoku' dwarf
d_2 ..... 'Ebisu' dwarf
d_7 ..... 'cleistogamous' dwarf
d_8 ..... 'Norin 28' dwarf

The 'Daikoku' and 'Ebisu' dwarfs are simple recessives to the normal, their causal genes being d_1 and d_2 respectively. Character expression by d_1 is the typical 'Daikoku' type with short and stout stems, short, sinuate but broad leaves, erect and compact panicles, small and round floral glumes.

The appearance of d_2 is similar to that of d_1. However, the d_2 plant is relatively taller

than the d_1 and the floral glumes are not so reduced in size when they are compared with the original normal form.

As mentioned in the preceding paragraph, the 'cleistogamous' dwarf is characterized by its closed glumes, but at the same time it shows the 'Daikoku' type of dwarf stature, somewhat short and broad leaves, erect and compact panicles, and small and round floral glumes.

The 'Norin 28' dwarf was considered to be a simple recessive (d_8) to the normal. The character expressions by d_1 and d_8 are almost the same, but they definitely belong to different loci.

Here are dwarf forms of the 'Bonsai' type. Among them is the so-called 'tillering' dwarf of NAGAO, which I studied with respect to its causal genes and their linkage relationships.

d_3 ..... One of the multiple genes for 'tillering' dwarf
d_4 ..... do
d_5 ..... do

In crosses between the 'tillering' dwarf and the normal forms, three Segregation ratios, 3:1, 15:1 and 63:1, were found, indicating that three multiple genes d_3, d_4 and d_5 are responsible for this character (NAGAO, 1951).

Besides the 'Daikoku' and 'Bonsai' types, another 'lop-leaved' dwarf characterized by sinuous panicle neck and leaves with lopped blades and shortened sheaths were examined, The causal gene is designated as d_6.

(6) *Genes for modified composition*

The following three genes and their character expressions will be mentioned :

wx ..... waxy (glutinous) endosperm (*m* or *gl*)
sh ..... shattering or easy threshing, recessive to difficult or intermediate threshing
bc ..... brittle culm

Though certain varieties are called waxy or glutinous, they contain neither waxy nor real gluten substances. The recessive gene which governs the waxy endosperm is designated as *wx*. Many workers have found a deficiency in the number of waxy segregates in F_2 from crosses of waxy × non-waxy. Various hypotheses have been suggested to explain this.

The worst form of shattering is exhibited by the wild rices, but, even in some varieties of cultivated rice, some degree of shattering is inevitable. It is known that in crosses between wild and cultivated forms more than one gene are involved and shattering behaves as a single or double dominant gene over non-shattering, In crosses among cultivated varieties showing varying degrees of shattering, there are three modes of segregation, viz., dominant, intermediate or recessive. I found a case where non-shattering behaves as a monogenically dominant gene over shattering. The causal gene of shattering is designated as *sh*.

The character 'brittle culm' behaves as a simple recessive to the normal type without exception and is designated as *bc*. The brittle culm mutant has a comparatively lower content of a-cellulose in its cell wall.

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(7) *Genes for chlorophyll deficiency*

The linkage relationships of the following genes have been examined:

fs ..... fine stripes in leaf margin of young plants
gw ..... green-and-white-striped
v ..... virescent

The 'fine striped' type is characterized by the appearance of minute white flecks or fine white stripes at the tip and margin of the leaf blade in young plants. This is a simple recessive to the normal green. The expression of the causal gene (*fs*) to a large extent depends upon the environmental conditions, showing no phenotypic difference between plants with + and fs when they are grown under relatively high temperature conditions (TAKAHASHI, 1950).

The green-and-white-striped types mentioned above are some of the most common striped patterns in rice. This is a simple recessive character and is designated as *gw* in its gene symbol (TAKAHASHI, 1950).

Besides these two types of variegations, the virescent type and its causal gene, *v*, which Mr. JODON sent me, is being studied.

(8) *Genes for other characters*

Three genes, *Ph, bl* and *Wh,* are the objects of the description in the present paragraph.

Ph ..... Phenol staining
bl ..... Physiological disease showing dark brown or blackish mottled discoloration of leaves (*mt* or *mg*)
Wh ..... White hull (*Hw*)

It has been known that a single dominant gene, designated as *Ph,* is connected with the presence of the substance by which the pericarps and hulls, when treated with an aqueous solution of phenol, are stained brownish purple.

Dark-brown mottled discoloration is a singular color type in which brown spots begin to develop shortly after panicle emergence. The discoloration of the chlorophyll appears first on the leaves as brown spots resembling fungus lesions. It spreads and by maturity extends even into panicles, giving the entire plant a dirty brown appearance. Many crosses indicate that a single recessive gene *bl* (NAGAO AND TAKAHASHI, 1954a) causes this color type. Crossing JONES' (1952) and JODON'S (1957) brown or black spot mutants with my mottled type revealed that these were governed by different genes.

White hull, in contrast to straw color, appears dull chalky white. JODON (1957) first analyzed this and reported that the dull chalky white color was caused by a single dominant gene, *Wh.* According to him, *Wh* is not allelic to the gold hull gene, *gh.* I verified this observation.

LINKAGE RELATIONS

(1) *Linkage data involving the above-mentioned genes*

Both linked inheritance data and independent inheritance data are essential to

demarcate each linkage group. In this paper, emphasis is given to linkage relationships within each group. For experimental results on independence between groups, only summarized recombination values will be given.

Linked genes and their segregation modes examined by NAGAO and the writer are shown in Table 1. The summarized data of recombination values are listed in Table 2.

TABLE 1

LINKED GENES AND THEIR SEGREGATION MODES IN JAPANENSE RICE

Gene pair	Linkage phase and number of crosses	Segregation mode					Re-combination value	c^{2*}	n	P
		AB	Ab	an	ab	Total				
Group I (*'wx'*-group)										
d_4–wx (3:l) (3:l) (21.5%)	r 2	309 (285.5)	122 (133.1)	122 (133.1)	5 (6.5)	558	21.5 ± 2.70	2.94	3	0.5–0.3
wx–C (3:l) (3:l) (22.8%)	c 2	1148 (1124.1)	167 (174.9)	175 (174.9)	242 (258.1)	1732	22.8 ± 0.79	1.87	3	0.7–0.5
d_4-C (3:1) (3:l) (38.2%)	r 2	295 (298.8)	127 (118.9)	114 (118.9)	21 (20.3)	557	38.2 ± 2.41	0.82	3	0.9–0.8
Pla–wx (3:l) (3:l) (44.7%)	c 1	385 (388.5)	123 (117.0)	113 (117.0)	53 (51.5)	674	44.7 ± 1.83	0.52	3	0.95–0.90
Cl–c (3:l) (3:l) (40.3%)	c 2	360 (360.5)	114 (98.5)	84 (98.5)	54 (54.5)	612	40.3 ± 1.81	4.58	3	0.3–0.2
Cl–wx (3:l) (3:l) (41.0%)	c 1	77 (73.9)	14 (20.6)	26 (20.6)	9 (10.9)	126	41.0 ± 4.00	3.99	3	0.3–0.2
Group II ('PI'-group)										
d_2-d_3 (3:l) (3:1) (25.4%)	r 1	207 (194.6)	86 (88.2)	79 (88.2)	5 (6.1)	377	25.4 ± 3.21	1.99	3	0.7–0.5
d_3–Pl (15:l) (3:l) (35.2%)	r 1	354 (349.7)	125 (121.9)	21 (27.5)	3 (3.9)	503	35.2 ± 5.53	1.89	2	0.5–0.3
Pl–lg (3:l) (3:1) (30.9%)	c 7	1325 (1344.4)	289 (271.0)	267 (271.0)	272 (260.8)	2153	30.9 ± 0.84	2.02	3	0.7–0.5
lg–Ph (3:l) (3:l) (7.4%)	c 1	218 (229.3)	13 (11.5)	11 (11.5)	79 (68.8)	321	7.4 ± 1.03	2.30	3	0.7–0.5

* c^2 for goodness of fit, under respective linkage intensities.

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Table 1 *(continued)*

Gene pair	Linkage phase and number of crosses	Segregation mode				Total	Re-combination value	c^{2*}	n	P
		AB	Ab	aB	ab					
Ph–Pr (3:1) (3:1)	c 2	333	55	64	86	538	24.2 ± 1.47			
(24.2%)		(346.3)	(57.2)	(57.2)	(77.3)			2.38	3	0.5-0.3
d_2–*Pl* (3:1) (9:7)	c 2	232	157	51	59	499	38.1 ± 2.80			
(38.1%)		(223.8)	(152.0)	(58.1 j	(65.1 j			1.91	3	0.7-0.5
d_3-*lg* (15:1) (3:1)	c 2	146	44	13	7	210	39.7 ± 7.13			
(39.7%)		(149.1)	(47.7)	(8.4)	(4.8)			3.99	3	0.3-0.2
lg–Pr (3:1) (3:1)	c 5	486	93	103	109	791	28.2 ± 1.31			
(28.2%)		(497.4)	(95.8)	(95.8)	(101.9)			1.37	3	0.8-0.7
Pl–Pr (3:1) (3:1)	c 2	341	99	114	30	584	48.6 ± 2.06			
(48.6%)		(325.1)	(112.9)	(112.9)	(33.1)			2.79	3	0.5-0.3
d_2–*lg* (3:1) (3:1)	c 5	920	297	243	96	1556	47.2 ± 1.24			
(47.2%)		(886.5)	(280.6)	(280.6)	(108.5)			8.69	3	0.05-0.02
d_2–*Ph* (3:1) (3:1)	c 1	165	65	48	17	295	51.5 ± 2.99			
(50.0%)		(165.9)	(55.3)	(55.3)	(18.4)			2.78	3	0.5-0.3
d_2–*Pr* (3:1) (3:1)	c 2	341	121	90	26	578	52.8 ± 2.16			
(50.0%)		(325.1)	(108.4)	(108.4)	(36.1)			8.19	3	0.05-0.02
Pl–P (3:1) (3:1)	c 2	580	4	13	147	744	2.1 ± 0.34			0.005
(2.1%)		(550.3)	(7.7)	(7.7)	(178.3)			1.85	2	-0.001
Group III (*A*-group)										
A–Rd (3:1) (3:1)	c 9	2298	4	10	797	3109	0.3 ± 0.07			
(0.3%)		(2326.7)	(5.0)	(5.0)	(772.2)			5.9s	2	0.1-0.05
Rd–Pn (3:1) (3:1)	r 2	241	132	137	13	523	26.8 ± 2.70			
(26.8%)		(270.9)	(121.4)	(121.4)	(9.4)			7.63	3	0.1-0.05
A–Pn (3:1) (3:1)	r 2	333	173	162	19	687	29.9 ± 2.31			
(29.9%)		(358.9)	(155.4)	(156.4)	(15.4)			4.69	3	0.2-0.1
Group IV (*g*-group)										
d_6–*g* (3:1) (3:1)	r 3	383	178	156	3	720	14.0 ± 2.45			
(14.0%)		(363.5)	(176.5)	(176.5)	(3.5)			3.42	2	0.2-0.1

* c^2 for goodness of fit, under respective linkage intensities.

Table 1 (continued)

Gene pair	Linkage phase and number of crosses	Segregation mode AB	Ab	aB	ab	Total	Re-combination value	c^{2*}	n	P
g–Rc	c	947	194	181	192	1514	28.9			
(3:l) (3:l)	4						± 0.97			
(28.9%)		(947.8)	(188.0)	(188.0)	(190.2)			0.47	3	0.95-0.90
d_6–*Rc*	c	1557	319	330	321	2521	29.9			
(3:l) (3:l)	3						± 0.76			
(29.9%)		1573.9)	(321.3)	(321.3)	(310.4)			0.79	3	0.9-0.8
d_6–d_7	r	192	85	87	18	382	39.4			
(3:l) (3:1)	1						± 2.62			
(39.4%)		(206.3)	(80.2)	(80.2)	(15.3)			2.33	3	0.1-0.5
Group V (*I-Bf*-group)										
I–Bf–Ps	c	114	35	33	18	200	42.1			
(3:l) (3:1)	2						± 3.25			
(42.1 %)		(116.8)	(33.2)	(33.2)	(16.8)			0.25	3	0.98-0.95
Group VI (d_1 -group)										
gw–d_1	r	745	304	323	9	1381	17.8			
(3:l) (3:l)	4						± 1.75			
(17.8%)		(701.4)	(334.3)	(334.3)	(10.9)			6.12	3	0.2-0.1
d_1–*gh*	r	3 50	138	148	11	647	27.8			
(3:l) (3:l)	2						± 2.42			
(27.8%)		(336.0)	(149.3)	(149.3)	(12.5)			1.62	3	0.7-0.5
gw–gh	r	282	101	106	20	509	40.9			
(3:l) (3:l)	2						± 2.43			
(40.9%)		(275.9)	(105.9)	(105.9)	(21.4)			0.45	3	0.95-0.90
gh–An_3	c	265	70	77	34	446	42.9			
(3:1)(3:1)	2						± 2.20			
(42.9%)		(259.4)	(75.2)	(75.2)	(36.4)			0.67	3	0.9-0.8
Group VII (*fs* -group)										
Ur–fs	c	414	90	80	103	687	27.5			
(3:l) (3:l)	2						± 1.39			
(27.5%)		(433.8)	(81.5)	(81.5)	(90.3)			3.61	3	0.5-0.3
fs–Dn	*c*	511	147	160	88	906	41.1			
(3:1) (3:l)	2						± 1.51			
(41.1 %)		(531.6)	(147.9)	(147.9)	(78.6)			2.92	3	0.5-0.3
Since the gene *Dn* is epistatic to the gene *Ur*, the recombination value in *Ur-Dn* was calculated by Fisher's maximum likelihood method as follows:										
Ur–Dn	r	246	27	44	20	337	46.2			
(1:2:1) (3:l)	1						± 4.99			
(46.2%)		(252.8)	(24.4)	(41.9)	(17.9)			0.79	3	0.9-0.8

* c^2 for goodness of fit, under respective linkage intensities.

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Table 1 *(continued)*

Gene pair	Linkage phase and number of crosses	Segregation mode AB	Ab	aB	ab	Total	Re-combination Value	c^{2*}	n	P
Group VIII (*la*-group)										
la-sh		215	98	74	15	402	38.7 ± 2.82			
(3:l) (3:l)										
(38.7%)		(216.1)	(85.5)	(85.5)	(15.1)			3.38	3	0.5-0.3
Group JX (*nl*-group)										
nl–ri	r	853	404	391	51	1699	32.4 ± 0.67			
(3:1) (3:1)	4									
(32.4%)		(897.5)	(377.4)	(377.4)	(46.7)			4.97	3	0.2-0.1
Group X (*bl*-group)										
bl–d_5	c	215	6	50	6	277	25.1 ± 2.09			
(3:l) (15:l)	1									
(25.1%)		(200.2)	(7.6)	(59.5)	(9.7)			4.84	3	0.3-0.2
Group XI (*bc*-group)										
bc-d_8	r	178	51	50	9	288	43.5 ± 3.19			
(3:1) (3:1)	1									
(43.5%)		(157.6)	(58.4)	(58.4)	(13.6)			6.34	3	0.1-0.05
d_8–*An*$_1$	c	840	23	39	247	1149	5.4 ± 0.50			
(3:l) (3:l)	3									
(5.4%)		(831.6)	(30.2)	(30.2)	(257.1)			4.76	3	0.2-0.1
bc–An$_1$	r	170	46	58	14	288	48.4 ± 3.00			
(3:l) (3:1)	1									
(48.4%)		(166.0)	(54.0)	(54.0)	(14.0)			2.77	3	0.5-0.3
Group XII (*gl*-soup)										
gl–An$_2$	c	272	68	76	41	457	39.5 ± 2.08			
(3:1) (3:1)	1									
(39.4%)		(271.0)	(71.7)	(71.7)	(42.5)			0.51	3	0.95-0.90
gl-Hg	c	269	70	67	40	446	38.7 ± 2.08			
(3:l) (3:l)	1									
(38.7%)		(264.9)	(69.6)	(69.6)	(41.9)			0.25	3	0.98-0.95

(2) *Trial construction of linkage maps estimated from the above data*

Based on the data mentioned above, twelve linkage groups are postulated and the genes of each group are arranged in the order presented in Fig. 1. Here, each group is provisionally designated with a roman numeral, but at the same time, it is temporarily named with the symbol of one of the representative genes.

I would like to mention the process of estimating the location of genes in each linkage group.

(*a*) *'wx' Linkage group* (*Group I*). – This group corresponds to Group I of JODON'S system. The best-established case of linkage in this group is that between the apiculus color gene and the waxy or glutinous endosperm gene. Of the three genes, *C, A* and *P* which are responsible for apiculus coloration, *C* is linked with *wx* for waxy endosperm. The recombination value between *C* and *wx* is about 23%, although there is considerable variation from this value in several crosses. Based on this linkage relation it is suggested that *C* is identical with genes described under different names by many workers; for example, *Ap* for purple apiculus (JODON, 1948 and others), *S* for reddish apiculus (YAMAGUCHI, 1926), Ap_4 for colored apiculus (CHAO, 1928), *Ty* for tawny color of ripened apiculus (CHAO, 1928), and others.

Besides the *C,* two genes, d_4 for 'tillering' dwarf and *Cl* for clustered spikelets are inserted in this group. The percentage of recombination value between d_4 and *wx,* and between d_4 and *C,* are 22% and 38% respectively. Fluctuations of these values in individual crosses make precise mapping difficult, However, these data may indicate that d_4 is located in this group in the order of $d_4 - wx - C$.

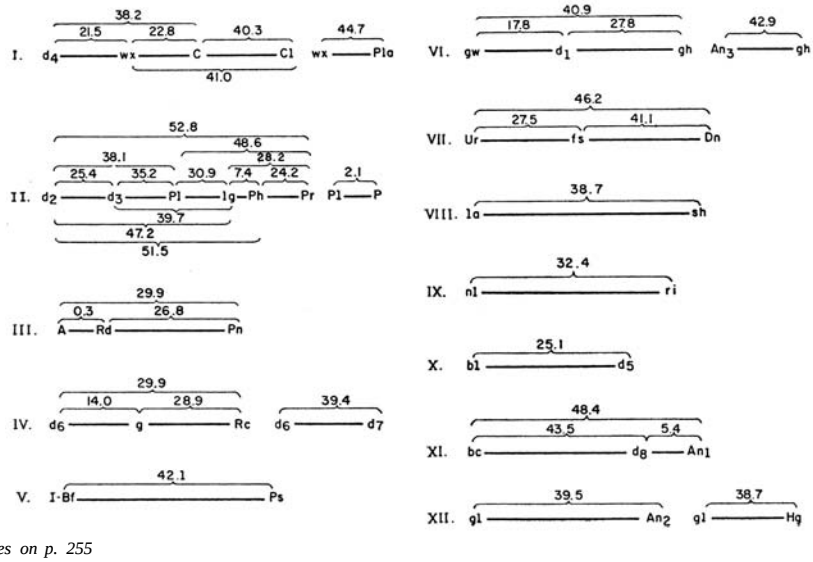
In addition to those mentioned above, linkages between *C* and *Cl,* and between *wx* and *Cl,* first reported by JODON (1940), were reexamined, giving recombination values of 40% and 41% respectively. Further work would be necessary to determine whether *Cl* is located on the same side of *wx,* putting *C* in the center, or not. However, available data suggest the relative gene sequence of $wx - C - Cl$.

On the whole, therefore, the gene order of this group may be arranged as follows:

$$d_4 \text{ ——— } wx \text{ ——— } C \text{ ——— } Cl$$

Fig. 1

Trial construction of linkage maps in Japanese rice.



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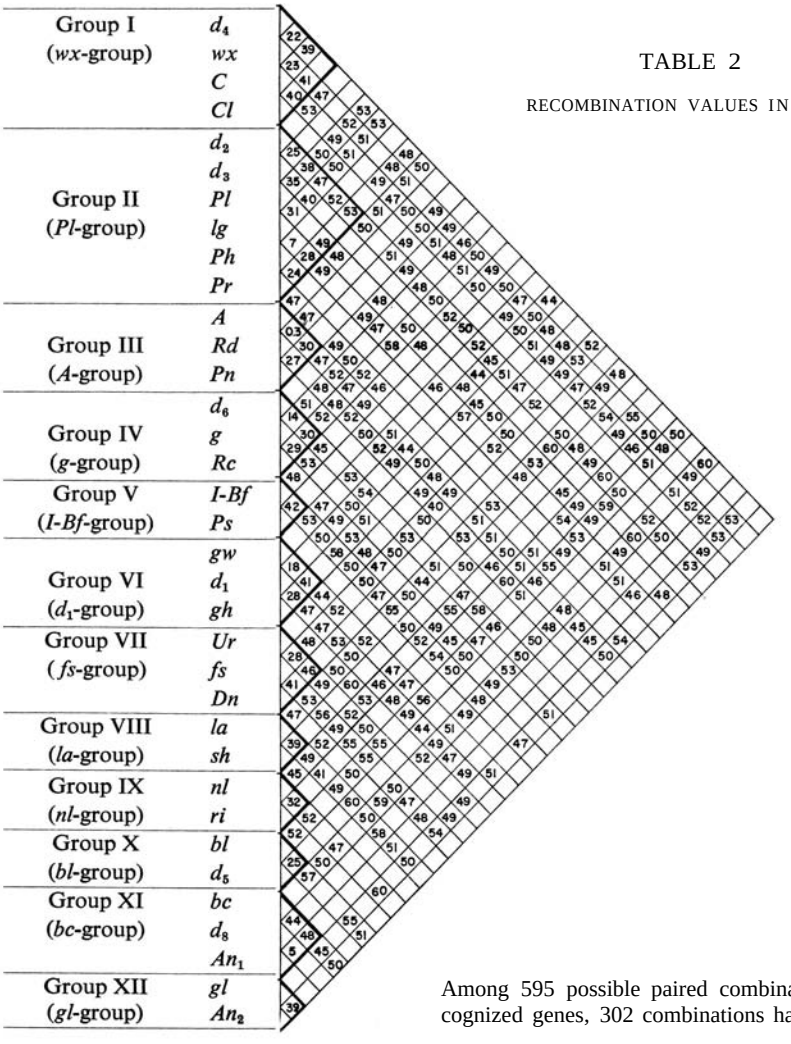
(b) *'Pl' Linkage group (Group II).* – This group is based upon linkages among *Pl* (purple leaf), *lg* (liguleless) and *Ph* (phenol staining). MORINAGA *et al.* (1942, 1943) first reported linkages between *Pl* and *lg*, and between *Pl* and *Ph*, giving recombination values of 21% and 7% respectively.

NAGAO and I confirmed and further added four genes to this group. These are d_2 ('ebisu' dwarf), d_3 ('tillering' dwarf), *Pr* (purple hull) and *P* (completely colored apiculus). The average recombination value between *Pl* and *lg* is 31%. However, it varies from 24% to 32% in the coupling phase. Linkage intensity between d_2 and *Pl* amounts to about 38%, through two crosses, in which leaf color segregated 9 colored *vs.* 7 colorless, indicating that besides *Pl*, an activator gene *A* is concerned with the character expression of leaf color. As will be mentioned in the next paragraph, *A* belongs to

TABLE 2

RECOMBINATION VALUES IN JAPANESE RICE



Among 595 possible paired combinations between recognized genes, 302 combinations have been tested.

another linkage group, indicating a linkage between d_2 and *Pl.* An examination of linkage intensity between *lg* and d_2 yields linear order of three genes, d_2, *Pl* and *lg.* In five crosses an average recombination value of 47% was calculated, indicating that the probable order is d_2 – *Pl* – *lg.*

Linkages between *Ph* and *lg,* between *Ph* and *Pr,* and between *Pr* and *lg,* are given as approximately 7%, 24%, and 28% respectively, suggesting that the sequence of these genes is *lg* – *Ph* – *Pr.*

Thus, two sets of gene maps, d_2 –*Pl*– *lg* and *lg* – *Ph* – *Pr* are provided. In connecting these two groups, linkage between d_2 and *Pr* should be examined, since *lg* is considered to be the base point in the two maps. In this respect two crosses were made, and a 53% recombination value was obtained, indicating that d_2 and *Pr* are located apart. Based on these results, sequences of five genes would be d_2 – *PI* – *lg* – *Ph* – *Pr.*

To further verify this assumption, such linkages as between d_2 and *Ph,* or between *Pl* and *Pr* were examined. Testing of three cross combinations resulted in recombination values of 52% and 49%, respectively. As mentioned above, in linkage intensities be–tween d_2 and *lg,* and between *lg* and *Ph* are 47% and 7% respectively, the gene sequence of d_2 – *lg* – *Ph* may be expected. The value of 49% between *Pl* and *Pr* may also prove or show the linear order of genes, *Pl* – *lg* – *Pr.*

With regards to a linkage between d_2 and d_3, a cross of two genotypic plants, + d_3 d_4 d_5 (‘tillering’ dwarf form) and d_2 + d_4 d_5 (‘ebisu’ dwarf form) was made. In this cross the genes d_4 and d_5 do not affect the mode of segregation ratio, and therefore two dwarf forms should be monogenically inherited, The observed result agrees with that expected based on an approximate 25% recombination value in repulsion phase. It follows that d_3 might be assigned to the present linkage group,

In this connection, a linkage between d_3 and *Pl* was ascertained, the recornbination value between them being 35%. As the cross over between d_2 and *Pl* was 38%, d_3 might be located between d_2 and *Pl.* Examination of another linkage, viz,. between d_3 and *lg,* of which linkage intensity was approximately 40%, and varied from 31% to 47%, proved this supposition correct. The probable order of d_2, $d_3$and *lg* is d_2 – d_3 – *lg.*

On the whole, therefore, the order and distance of the above mentioned six genes, d_2, d_3, *Pl, lg, Ph* and *Pr* might be diagrammatically indicated as follows:

$$d_2 \text{———} d_3 \text{———} Pl \text{———} lg \text{——} Ph \text{———} Pr$$

P is another gene which might possibly be assigned to this group. Two crosses of *CAP* + × *CA* + *Pl* give segregation that undoubtedly shows close linkage between the genes *P* and *Pl,* with a recombination value of 2%.

(c) *‘A’ Linkage group (Group III).* – The members of this group are A (anthocyanin activator), *Rd* (red pericarp) and *Pn* (purple node). Before going further, we should recall some considerations on gene interaction between *A* and *Pn.* The action of *Pn* is expressed only in the coexistence of *CA,* and in this connection such crosses as *C* ++ × *CA Pn* and *CA* + × *C* + *Pn* are inappropriate in examining linkage intensity between *A* and *Pn,* as there is no way of discriminating two genotypes of + *Pn* and ++, both of them being colorless in their stem nodes. Linkage between *A* and *Pn* is

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observable through the cross combination in which both the parents are different from each other in A-locus in such a way as *A vs.* A^d, but not in a way as *A vs.* +,

Crosses were made between two genotypic plants of C^B *A Pn* and C^B A^d +, and a linkage was found in the coupling phase.

In addition, other linkages of this group, between *A* and *Rd* and between *Rd* and *Pn*, were examined by studying a cross Combination of C^{Bp} *A+Rc Rd* (purple apiculus with green node and red pericarp) × C^BA^dPn++ (red apiculus with red node and white pericarp). Three recombination values regarding the linkages of *A-Pn, A-Rd* and *Rd-Pn* are given as 32%, 0.2% and 30% respectively. This result indicates a probable order of these genes as: *A – Rd – Pn.*

To further verify the close linkage between *A* and *Rd*, additional crosses were made. In all of F_2 segregations with approximately 0.3% recombination value, about the same magnitude as those shown above was recognized. By adding some data on these linkages a diagrammatic expression of the gene order and the magnitude of gene distance may be as :

A ——— *Rd* ——— *Pn*

(d) *'g' Linkage group (Group IV)*

This group consists of linkages among d_6 ('lop-leaved' dwarf), g (recessive long empty glumes), *Rc* (brown pericarp) and possibly d_7 ('cleistogamous' dwarf). The gene d_6 gave a 14% recombination value with *g, g* gave a 29% recombination value with *Rc,* while *Rc* gave a 30% recombination value with d_6, suggesting that these genes are linked in the order of $d_6 - g - Rc$.

An additional gene, d_7, possibly is assigned to this group, by adapting a linkage between d_7 and d_6, with a 39% recombination value. Due to the lack of sufficient data of linkages between d_7 and other genes, the location of d_7 is not determined yet.

d_6 ——— *g* ——— *Rc* d_6 ——— d_7

(e) *'I-Bf' Linkage group (Group V)*

This group is based upon a single weak linkage with a 42% recombination value between *I-Bf,* an inhibitor for dark brown furrows in lemma and palea, and *Ps,* a gene for purple stigma color.

I-Bf ——————— *Ps*

(f) *'d_1' Linkage group (Group VI)*

This group includes three genes, namely, d_1 for "Daikoku" dwarf, *gh* for gold hull, and *gw* for green and white striped leaves and panicles. The recombination values are calculated as: 18% for d_1 and *gw,* and 41% for *gw* and *gh,* and 28% for d_1 and *gh,* suggesting that the probable order of the concerned genes would be $gw - d_1 - gh$. To set up accurate magnitude of gene distances of the above groups, however, further studies should be made, because of estimating the above linkages certain correction values were applied under the repulsion phases of gene combination concerned.

It is possible that one of the genes for awnedness may be inserted in this group. This possibility is based upon a linkage between gold hull and awnedness with an intensity of 43% recombination value.

$$gw \text{ ———— } d_1 \text{ ———— } gh \quad gh \text{ ———— } An_3$$

(*g*) *'fs' Linkage group (Group VII)*

This is a group of which postulated members are *fs* (fine stripes of young leaf), *Ur* (undurate rachis) and *Dn* (dense panicle). The gene fs shows linkages with *Ur* and *Dn,* in the intensity of recombination values of 28% and 41% respectively.

Linkage also would be expected between *Ur* and *Dn.* In this connection it should be mentioned that besides an action of *Dn,* a dense panicle also results as a pleiotropic effect of *Ur.* The former behaves as epistatic over the latter in their character expression, thus the discrimination between two genotypes, *Dn Ur* and *Dn* + is difficult. In the present examination of combined segregation of *Ur* and Dn, F_2 plants were assorted into four classes as shown below, where 46% of a recombination value between *Ur* and *Dn* was calculated by Fisher's maximum likelihood method.

1	2	3	2
Dn Ur	+ *Ur* (*Ur* homo)	+ *Ur* (*Ur* hetero)	+ +
Dn +			

Based on these results, gene sequences would be as follows.

$$Ur \text{ ———————— } fs \text{ ———————— } Dn$$

(*h*) *'la' Linkage group (Group VIII)*

Prostrate or lazy growth habit governed by *la* and shattering of grains caused by *sh* are found linked with each other with an intensity of 39% recombination value. This constitutes the linkage group *la.*

$$la \text{ ———— } sh$$

(*i*) *'nl' Linkage group (Group IX)*

Based on a linkage between *nl* (neck leaf) and *ri* (whorled arrangement of rachises) and with results that these two genes are independent of many other genes of known linkage groups, the *'nl'* linkage group has been postulated. The linkage intensity between *nl* and *ri* is approximately 30%. Though this value was obtained in the repulsion phase, considerably small variation of recombination values, 28% to 34%, was observed in various crosses examined.

$$nl \text{ ———————— } ri$$

(*j*) *'bl' Linkage group (Group X)*

This group comprises two genes, *bl* (brown mottled discoloration of leaves and panicles) and d_5 ('tillering' dwarf). The *bl* is linked with d_5 with a 25% recombination value. The numerical value of this linkage intensity itself is not so trustworthy in that (*i*) segregation of normal *vs.* dwarf is 15:1 and (ii) only a single cross combination was available in estimating this linkage. Therefore, to obtain a more precise value, further

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examination which involves such a cross as $+\ d_3\ d_4+\ \times\ bl\ d_3\ d_4\ d_5$ should be made.
bl —————— d_5

(k) *'bc' Linkage group (Group XI)*

Insofar as NAGAO and the writer have examined, the gene *bc* for brittle culm shows no linkage with any of the genes belonging to the above-mentioned linkage groups. As regards possible genes of this group, d_8 for 'Norin-28' dwarf and An_1, one of multiple genes for awnedness, may be worthy of note.

The genes d_8 and An_1 show a striking linkage in which the recombination value is approximately 5%, though some variation, from 3% to 10% ,was noted in crosses examined. As to linkage intensities of *bc* with d_8 and with An_1, recombination values of 44% and 48% were given respectively.

These results may indicate the gene sequence, as shown below.

bc —————— d_8 ———— An_1

(l) *'gl' Linkage group (Group XII)*

The *gl* gene responsible for glabrous floral glumes and leaves shows no linkage with genes located in the linkage groups mentioned above. A single dominant gene for awnedness, An_2, and a gene *Hg* for pubescence in floral glumes seem linked with *gl*, Thus these three genes appear to be members of linkage group XII.

Recombination value between *gl* and An_2, approximately 40%, is similar to the value reported by BREAUX NORRIS (1940) in crosses of U.S. varieties. Between *gl* and *Hg*, a recombination value of 39% was given. Here, it must be mentioned that *Hg* opposes *gl* in expressing pubescence in the floral glumes. Therefore, the classification of four genotypes *+Hg*, ++, *gl Hg* and *gl+* by glume hair types is difficult. The *gl* exerts its effect not only on the floral glumes but also on the leaves. In contrast, the effect of *Hg* is limited to the floral glumes. Thus, the discrimination of the above four genotypes should be made by the following mode of character expression.

GENOTYPE	PHENOTYPE	
	glume hair	*leaf hair*
+ Hg	++	+
++	+	+
gl Hg	+	—
gl +	—	—

As to the expected linkage between An_2 and *Hg*, as yet there is no trustworthy numerical value of recombination.

gl ————— An_2 *gl*————— *Hg*

OTHER RICE LINKAGES REPORTED IN JAPAN AND TAIWAN

According to OKA (1953b), his postulated genes X_1 and Y_1, which are said to be re-

sponsible for gametic development, are assumed to be linked with *wx* in group I, in the sequence of $X_1 - Y_1 - wx$. To this group NAGAMATSU AND OMURA (1961) add two genes, *dp* for depressed palea, and *v* for virescent seedling. The linear order of four genes concerned in their experiment was concluded as *wx – dp – C – v.*

To the group II, two additional genes, *Xa,* a gene for resistance to *Xanthomonas oryzae,* and *lop,* a gene for lopped leaf, may be inserted. These results are based on two sets of linkages obtained by NISHIMURA (1960) and NAGAMATSU AND OMURA (1961); these are shown as *Xa – lg – Ph* and *Pl – lop.* HSIEH (1961a) in Taiwan reported additional genes in this group. They are *Ps,* for stigma color, *d* for dwarfness, and *Pi* for *Piricularia* resistance. Linkage relations of these genes are *lg – Pr, Ps – Pr, Ps – Ph, d – Pl* and *Pi – lg.*

HSIEH (1960) adds three genes to group III, viz. *lgt, d* and *ts,* responsible for the expression of such characters as long twisted grain, a type of dwarf plant, and twisted stem, respectively. The arrangement of these genes is assumed to be *lgt – d – ts – A.* He also reports a possibility of linkage between *A* and $I\text{-}Ps_2$, one of the inhibitors for stigma coloration, although this possibility should be confirmed by further tests.

NAGAMATSU AND OMURA'S (1961) finding that the so-called 'long stemmed Daikoku' (a Daikoku type dwarf similar to JODON'S intermediate dwarf) is linked with *la* (lazy) indicates the existence of a dwarf gene in group VIII.

LINKAGE GROUPS SUMMARIZED BY JODON

JODON (1948) summarized linkage data and tentatively proposed eight linkage groups, They are mainly based on work in the United States and India. RAMIAH AND RAO (1953) placed genes for anthocyanin coloration in different plant parts, along with some other genes, into three linkage groups. JODON (1955, 1956) modified his earlier paper of 1948 and suggested seven linkage groups (Table 3).

Following JODON'S I956 report, additional experimental findings as mentioned below have been made.

According to JODON'S later report (1957), a gene for white hull (*Wh*) is linked with a gene for liguleless (*lg*) and it also appears to be linked with a gene for apiculus coloration (Ap_b). By adding some other data of his experiments, he concluded the following gene sequence of his group II: $gh\text{-}Ap_b\text{-}lg\text{-}Wh$. RICHHARIA, MISRO, BUTANY AND SEETHARAMAN (1960) reported two sets of linked genes, in which the concerned genes are *Lsp* (colored sheath, *Psh*), *Lxp* (colored leaf axil, *Px*), *Ntp* (colored internode, *Pin*), and *Ap* (colored apiculus). These are assigned a position in the combined (II and Ill) group of JODON in the order of *Lsp-Lxp-Ntp-Ap.*

G, one of the duplicate genes for short glumes, and *Kra,* one of the complementary genes for round shape of spikelets are reported to be linked with the gene *A* presumed to be an activator of anthocyanin coloration (KADAM AND D'CRUZ, 1960a). These three genes are arranged in the sequence of *A – G – Kra,* and are considered to belong to JODON'S group IV, instead of group I or II.

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TABLE 3

LINKAGE GROUPS OF RICE
(after JODON, 1955, 1956)

Group	Gene	Character expression
I	*C* (*Ap*)	Colored apiculus (chromogen)
	**Lmp* (*Pla*)	Colored leaf margin
	**d*$_3$	dwarf
	Hf	Colored hull-furrows (dark furrows)
	**Anr*	Red apiculus (modifier)
	wx	waxy
	Fl$_1$	Maturity
	v	virescent
	Cl	Clustered spikelets
	fm (*sf*)	sterile?
	**Fl*$_3$	Maturity
	**Lp*	Colored leaf blade
II & III	*A* (*Apb*)	Colored apiculus (activator)
	Pr (*Rd*)	Red pericarp
	Sp	Purple stigma
	Np (*Pn*)	Purple node
	Pa	Purple auricle?
	Lax	Colored leaf axil
	Lsp	Purple sheath
	hg (*gh*)	gold hull
	Ntp	Colored internode
	Gp	Purple empty glumes
	Lgp	Purple collar (ligule)
	Lmp	Purple leaf margin
	Hp	Purple hull (caryopsis and lemma)
	Hw (*Wh*)	White hull
	lg	liguleless
	Ph	Phenol staining
	Lbp (*Pl*)	Purple leaf blade
	**d*$_2$	Dwarf
	**sk*	semi-sterile?
IV	*Pbr* (*Rc*)	Brown pericarp (bran)
	g	long empty glumes
	**d*$_6$	dwarf
	Ntp (*Pin*)	Colored internode (same as Pn?)
	Fl	Maturity
V	*Jp*	Purple junctura or collar
	Prp	Purple pericarp
	Lsp	Purple sheath
	O	Scent or aroma in grain
VI	*Hb*	Colored hull (black)
	Ntv	Purple internode
	An	Awn
	d	dwarf
VII	*Lh* (*gl*)	Pubescence
	An	Awn
VIII	*Ce*	*Cercospora* resistance
	d	dwarf

* Location estimated by Japanese data alone.

In addition to the above, two sets of linkages between color genes involving the stigma color gene are reported. They are *Psh* (purple sheath) – *Ap* – *Ps* (purple stigma) (SHAFI AND AZIZ, 1959) and a close linkage between a glume color gene and a stigma color gene (D'CRUZ, 1960).

CONSIDERATIONS

(1) *Identification of linkage groups*

In this section the writer will critically identify two series of linkage groups, viz., the groups summarized by JODON and the groups proposed by NAGAO and the writer.

As can be seen through Fig. 1 and Table 3, these two series of linkage groups do not coincide with respect to the loci of some genes, and thus can not be brought together under one general series of rice linkage groups. Especially, the differences are seen in the location of *A, P, Pl, Pn, Pr* and *gh.* In Japanese rice, these genes belong to three different linkage groups, viz., *(P–Pl–Pr), (A–Pn)* and *(gh),* while in the other varieties genes which are probably identical with those genes in Japanese rice have been assigned positions in the linkage group termed as combined (II–III) group of JODON.

This discrepancy cannot be satisfactorily explained at present, because little information has been accumulated in the identification of gene systems in distantly related rice varieties. However, the following presumptions may be considered as probable causes of such differences.

(a) It may be due partly to the existence of minor structural differences of chromosomes between different varieties. In this connection, MIZUSHIMA AND KONDO (1959, 1960, 1961, 1962) crossed Japanese and Indian varieties and observed an anomalous mode of segregation on characters governed by *wx, C, A, Rc* and *Rd.* They proposed a hypothesis attributing this mode of character segregation to minor structural differences of chromosomes between parental varieties.

(b) JODON (1955) suggests that anthocyanin color characters may be controlled by different gene systems in different varieties. This is also worthy of consideration and should further be studied.

(c) Other difficulties are in the proper identification of characters and of the causal genes involved. It is highly probable that in some of the reported data, where linkage involves color character, different workers have found the same linkage relationships although their classification is based on different plant parts. On the other hand, recombination data in some respects account for the correlation of two characters only and, without further analysis on their gene systems, postulated linkage. In this respect, it should be recalled that, as already mentioned in the introduction part of the present paper, if the reported data merely indicate that a certain character is associated with leaf color, for example, it is actually impossible to determine which gene, chromogen *(C),* activator *(A),* localization *(Pl)* or inhibition *(I-Pl),* is linked with the causal gene of the said character.

Linkage studies in rice have not been subjected to systematic work, and only a fraction

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of the possible paired combinations between recognizable characters has been tested. In linkage studies it is desirable to plan hybridization systematically so that complete genic analysis of characters is obtainable, especially when dealing with those affecting coloration. Inadequate genic information on complex characters complicates linkage work.

(2) *Future problems*

As the first steps in comprehensive and systematic research, the following proposals, some of which suggest cooperative work, are discussed.

(a) *Exchange of gene stocks between workers or some kind of a pooling system along the blood bank line of thought.* – This would facilitate identification of genes and the comparison of genic systems and linkage groups.
Consolidation and summarization of available information (including pictures) on characters and their causal genes would be desirable too.

(b) *Building up multiple markers and induced mutants.* – The former would be of much benefit in the assignment of new or untested genes to their respective linkage groups. Viable, easily identified mutants would be valuable for use in linkage studies. Information on effective irradiation methods should be exchanged, together with the actual products of mutation.

(c) *Utilization of reciprocal translocations.* – By using known genetic stocks, a complete set of reciprocal translocation homozygotes should ne prepared. According to N_1-SHIMURA's finding (1961), the chromosomes VI and XI, which he designated, bear two series of genes belonging to the Japanese linkage groups I and II, respectively.

(d) *Acceleration of further research on sterility of intervarietal hybrids.* – Inadequate knowledge of this important phase of rice genetics would hamper introduction of genes from distantly related varieties.

(e) *Finding of correlation between marker-genes and agronomic characters.* – Though this may not be an urgent problem at present, we have to bear this in mind constantly. TORIYAMA'S results (1962), in which he reported that one of the effective factors (a polygene) for cold tolerance in Japanese varieties is located in Group II, are worthy of mention. This may well be the first step in this area of Japanese rice breeding.

INHERITANCE OF RESISTANCE IN RICE TO THREE RACES OF THE BLAST FUNGUS

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Inheritance of resistance to U.S. races 6, 8, and 16 of the rice blast fungus *(Piricularia oryzae* Cav.) was studied in F_1, F_2 and F_3 generations of a cross between two unnamed strains, designated '1709' and 'CI 9418'. Strain 1709 was resistant, while CI 9418 was susceptible to all three races. In testing for reaction, seedlings in the 2 or 3 leaf stage were inoculated with a spore suspension of the individual races under greenhouse conditions.

Most of the research involved reaction to race 6. Resistance to this race appeared completely dominant in F_1. The F_2 results showed a good fit to the ratio of 3 resistant: 1 susceptible, suggesting that the parents differed by only one pair of alleles. For 135 F_3 lines tested, there was a ratio of 1 homozygous resistant: 2 segregating: I homozygous susceptible, confirming the assumption that resistance to race 6 in strain 1709 is due to a single gene. This gene was provisionally designated Pi_1. Several lines of evidence indicated that one or more modifier genes producing minor effects on degrees of resistance and susceptibility were also segregating in the cross.

A group of F_3 lines of known reaction to race 6 also were tested for resistance to races 8 and 16. Basically similar reaction of the lines was found for the three races, suggesting that one major gene pair governed reaction to races 6, 8 and 16 in this cross. It appeared, however, that reaction to each race was influenced by a different set of modifiers.

In addition to the value of the study in genetic analysis, the results showed that simple, inexpensive greenhouse tests could advantageously be used to detect resistant lines during the early segregating generations of a breeding program.

DISCUSSION IN SESSION ON INHERITANCE STUDIES, GENE MARKERS, AND LINKAGE GROUPS

Discussions on the need for more complete linkage maps emphasized the usefulness of developing translocation stocks, trisomics, and monosomics in supplementing the conventional method of estimating recombination values. Some of the technical difficulties in developing and maintaining trisomics were discussed. A method of obtaining primary and tertiary trisomics from interchange heterozygotes also was mentioned. The restriction that environment places upon the seed production of tester stocks of different ecotypes necessitates the separate development of more complete linkage maps of the so-called *japonica* and *indica* types at different centers. The next logical step would be a detailed analysis of the relative location and linkage relationships of apparently identical genes in the two variety-groups. Preliminary studies have indicated that such differences in linkage relationship might exist. The workers in this area also proposed to exchange testers more extensively in the future.

T. T. CHANG reported on the manner in which The International Rice Research Institute handles its world collection of cultivated varieties. Notes are being taken on some 42 plant and grain characteristics for each of the 8000 varieties in the collection at the rate of 2000 entries a year. Eventually, the recorded notes will be transferred to IBM cards and cataloged. Meanwhile, seed stocks are maintained in cold storage to supply rice breeders with the desired germ plasm over a long period of years. Similarly, a stock of over l000 entries in genetic testers, mutants and strains of *Oryza* species is being maintained.

SESSION VII

CONCLUDING SURVEY

THE PRESENT STATUS OF TAXONOMICAL, GENETICAL, AND CYTOGENETICAL INVESTIGATIONS IN RICE

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The task set before me of summarizing this first international 'Symposium on Rice Genetics and Cytogenetics' is truly imposing. What contributions that can be made at this juncture are largely anti-climactic. At best, one can only recapitulate what has already been said and thoughtfully discussed.

History has been made here. The excellent summaries and reports which characterized the entire week's proceedings laid the groundwork for long-lasting cooperative effort to advance rice improvement.

This symposium has emphasized that, as in any science, the state of hereditary knowledge of the rice plant has advanced through a series of more or less sequential steps, each being prerequisite to later advances, and each contributing to the synthesis that has emerged here.

In the area of classical genetics, documentation of the mode of pollination has been followed, in turn, by the establishment of a large number of simply inherited characters, the elucidation of gene interactions in multifactor inheritance, the association of factors into inheritance groups and finally the emergence of intra-group inheritance patterns based on the simple supposition that relative strengths of factor association are functions of distance on a chromosome map. Studies such as these can be pursued independently, of course, and require only that mechanical control of pollination is maintained, that adequate classification techniques are available, that appropriate statistical techniques be used, and, most important, that good, basic, fundamental reasoning be employed.

Ideally, classical genetic studies can best be carried out with variants of a single variety, in a single environment, with each established variant remaining available to help identify and characterize subsequent variants. Under such conditions an orderly and relatively uncomplicated development may be maintained. When two or more laboratories are engaged in similar studies, orderly development depends upon intercommunication and collaborative effort, and requires free exchange of material. Even under ideal collaborative arrangements, complications resulting from differential expression of characters in different environments, differential expression of characters in different genetic backgrounds, and, in rice, even differential adaptation of varieties in which genetic tester stocks have been established must be considered.

Perhaps the most important barrier to orderly development of any science results

from inadequate intercommunication. It is particularly fitting that Dr. KIHARA should open this symposium with his excellent presentation of the need for standardization of genetic symbols and nomenclature in rice. It is gratifying that great progress in this direction has been made through the efforts of The International Rice Commission of the FAO.

This symposium can perhaps be most easily summarized by commenting on the sessions in the order in which they occurred. Such comments, cannot be entirely free of personal bias, but this would be true regardless of who might be entrusted with this task,

SESSION I

In Session I, the present status of the taxonomy of the genus *Oryza* was capably reviewed. The Linnean system of taxonomic classification, as generally employed, implies, essentially, a static concept. The system attempts to establish relationships which depend on relatively gross anatomical and morphological features and from which a filing system for plant forms emerges. It is inevitable that differences in concept regarding the degree of refinement in establishing category limits and the degree of permissible overlapping of categories will arise.

In recent years, new techniques and new tools have been used on the species concept. The breeding test has been evoked as a taxonomic tool. Cytology has come to be regarded as a taxonomic aid to establish numerical relationships of chromosomes, to compare karyotypes, and even to elucidate pairing behavior. Ecological and geographical considerations have been introduced. Statistical methods have been developed to differentiate population swarms. Biochemical differences have been proposed as further refinements. Concurrent with the introduction of these tools a concept of population dynamics has evolved, resulting, perhaps naturally, in suggestions for taxonomic revisions. Without Commenting on the relative merits of such suggested revisions it would appear that it might be necessary to attempt agreement (1) on the appropriate tools to be employed, and (2) on the criteria to be used in establishing category limits. It is significant that of taxonomists who responded to the questionnaire on which Dr. CHANG reported, greatest agreement on criteria centered around the use of morphology of vegetative and floral organs.

SESSION II

Session II was concerned with gene symbolism and nomenclature. Important progress has been made. The committee on gene symbolization has been active. Broad generic designations of certain types of genes have been established. There is now agreement developing in which subscripts and superscripts will be assigned. Tests of allelism have been arranged. The need for an exchange of tester stocks has been recognized and the establishment of a gene bank considered. These denote increased cooperation among rice research workers.

It is perhaps pertinent to comment briefly on the role of a central agency in liaison

and improved communication. It has been deemed desirable that once a year a central agency should solicit, from all rice workers, short statements of results and research in progress. Such statements would be largely preliminary. Results of allele tests might be reported, the discovery of new genes might be indicated, detection of new linkages would be informative, and certainly new research techniques should be called to the attention of others. It is visualized that such statements would not be vehicles for proposing hypotheses nor of promulgating abstract ideas lest the volume of material become too unwieldy for easy dissemination and reference. It is heartening that IRRI is willing to serve as an information gathering and compiling center and that IRC is willing to serve as a dissemination center.

Another function of a central agency could well be to maintain and distribute tester material as needed. It could likewise receive, maintain, and distribute publications, serving as an information clearing house. It should certainly maintain a roster of research personnel to facilitate direct contact between workers with similar interests.

It would be clearly understood that such a central agency would in no way supplant individual prerogatives either in research or in publication; it should serve only to facilitate and encourage collaboration among individuals. Provided that there remains adherence to this concept, and that the rice newsletter will become established, the opportunity for continued evolution toward uniformity and more complete understanding appears great.

SESSION III

The third session on chromosome morphology brought a second independent discipline into focus. Cytology, like classical genetics, has progressed through more or less sequential steps coincident with improvements in technique. Chromosome numerical relations were first studied, these studies being followed by morphological comparisons, and finally by establishment of homology relationships.

The recent progress in the use of haploids to establish confidence limits in differentiating chromosomes of a genome is remarkable. Too, the number of cytologists who find meiotic analysis feasible is constantly growing. Despite a lack of diagnostic features on some chromosomes of certain types, this method is likely to become an increasingly important tool both in the study of pairing relationships and in assigning linkage groups to specific morphologically recognizable chromosomes. In addition, its potential use in karyotype analysis was strongly indicated.

SESSION IV

Here we begin to effect a synthesis of what might be termed the primary disciplines, i.e., taxonomy, genetics, and cytology. This permits greater comprehension in biological description. Synthesis of such primary disciplines provides the basis for both a broader and deeper insight into species relationships. This session brought out a differentiation

between studies of species as morphological entities, and studies of species relationships as a function of dynamic evolutionary forces.

Many disciplines and criteria may be brought to bear on this particular problem. Among those mentioned in the various presentations were such factors as:

(1) Morphological, physiological and biochemical differences
(2) Ecology and geographical distribution
(3) Natural hybridization and introgression
(4) Crossability and reproductive isolation
(5) Weakness or sterility in artificial hybrids
(6) Genome and karyotype analysis
(7) Gene arrangement differences
(8) Statistical quantification of population swarms

For purposes of clarifying interspecific relationships, it is necessary to use every technique and refinement available to elucidate and to characterize the ebb, flow, and homeostasis of heredity material. It is the essence of population dynamics. Each of the speakers in session IV considered one or more of the above factors. They generally agreed that a state of flux has existed and still exists. The predominant mechanism for change, if indeed a predominant mechanism exists, will continue to be a suitable topic for discussion and resolution for some time to come.

The clear demonstration of differential timing of meiotic events in different chromosome genomes in the same cell of a *sativa* × *australiensis* cross is intriguing and, to the speaker's knowledge, not documented elsewhere. This demonstration adds critical evidence for the concept that, such as biochemical processes, each sequential step in the meiotic process is under genetic control. Other instances may be mentioned, such as the existence of such genes which condition asynapsis, desynapsis, polymitotis, divergent spindle, and polysomaty.

SESSION V

This session was devoted to possible explanations for reproductive isolation in taxonomically closely related forms. It is difficult to obtain critical evidence which differentiates a genic from a chromosomal mechanism as the primary explanation for the reproductive barrier.

The clear cut demonstration of heritability of degree of sterility certainly might be used as evidence for genetic control. The isolation of true-breeding partially sterile lines with different degrees of sterility provides cogent evidence. On the other hand, the fact that F_1 sterility can be accounted for by sets of duplicate factors does not necessarily establish genetic proof; nor does the demonstration of linkage of sterility with other factors.

From the purely cytological studies, the weight of the chromosomal evidence, based as it is on an analysis of length of unpaired segments in relation to total chromatin length, is partially offset by the inverse relation between the 'differential segment'

length and total chromatin length. In other words, the shorter the total chromatin length, the longer the relative length of unpaired segments. This relationship would seem to indicate that the results were confounded with the meiotic stage observed. This confounding tends to inflate the ratio of differential segment length to total length. Despite some inflation, the direct relationship of this ratio to degree of sterility as determined from pollen analysis cannot be overlooked.

The clear demonstration, in parental material, that there are pairing lapses which are interpreted to reflect the later pachytene stages does not necessarily preclude acceptance of the relation of relative differential segment length to pollen sterility.

In summary, it appears that the primary mechanism for the partial reproductive barrier that exists between *japonica* and *indica* types has not been clearly established. The question of whether a genic or a chromosomal hypothesis should be invoked remains to be resolved. There has been little or no evidence which would suggest that the alternate concepts are mutually exclusive.

From a breeding standpoint it might be interesting to compare the co-distributions of quantitative traits and sterility in succeeding generations to assess the magnitude of the recombination barrier occasioned by the reproductive barrier. Such a study would be most interesting in populations in which total variability has been maintained.

SESSION VI

The excellent presentations on inheritance studies, gene markers, and linkage groups hardly require comment. The painstaking analysis of factor interaction of genes involved in pigment production is truly remarkable. The magnitude of the coordinated studies leading to the establishment of 12 linkage groups is impressive. The natural consequences and logical future opportunities lie in a coordinated cytological and genetic approach.

The next step is the assigning of specific linkage groups to specific morphologically identifiable chromosomes. A variety of materials could prove useful for this purpose. A set of chromosomal interchange testers would be valuable. So, too, would a set of primary trisomic stocks.

When the linkage groups are assigned to chromosomes, the further problem of orienting the linkage groups with respect to chromosome ends will remain. In other crops various techniques have been used. In some cases linkages between established interchange break points and marker genes have been helpful. Tertiary trisomic stocks derived from unequal disjunction in heterozygous translocation stocks could be used. Inversions, if available, would be helpful. The necessity of using F_2 rather than test cross populations would complicate such studies. However, it is important to be able to take advantage of chromosomal anomalies whenever they occur in attacking special problems.

After linkage groups have been oriented, the genetic placement of the centromere will still remain a challenge.

PRESENT STATUS AND OPPORTUNITIES

One final conclusion is the fact that the present state of hereditary knowledge in rice compares favorably to that in most other crop plants.

In maize, the 10 linkage groups have been assigned to the 10 chromosomes; the linkage maps have been oriented, and for at least a few chromosomes, the genetic locus of the centromere has been fairly precisely determined.

In wheat, linkage groups have been assigned to chromosomes, but no orientation of linkage maps has been accomplished.

In barley, linkage groups have been assigned but in only a few chromosomes has the map been oriented.

Trisomic analysis in *Datura* has become classical and has been completed in *Lycopersicon.* Monosomic analysis in *Nicotiana* and *Triticum* has been largely completed and could be undertaken in rice amphidiploids.

It will readily be understood that, as the linkage maps become more complete, it will become more and more difficult to place a gene in its proper position in the map. In many cases, gene order determination will have to depend on a single recombination event marked by genes on either side of the two between which recombination occurs. Experience has shown, however, that if studies of mutational events, or recombination of subunits within the functional gene locus are desired, it is essential that closely linked marker genes be available.

One of the ultimate aims in inheritance studies is the eventual complete characterization of the hereditary material, e.g., the structure of a gene, the manner of its reduplication, the mechanism of segregation, and the way in which its effect on the organism is mediated. Recent years have seen geneticists who work on lower forms and microorganisms take the lead in the solution of some of these problems. The seriation of vast numbers of loci separable by recombination within a single functional locus in bacteriophage has given a much clearer concept of the complexity of gene structure. The fact that alleles of the waxy locus in maize, which have resulted from different mutational events, can be separated by recombination and arranged in a linear order adds validity to the concept of sub-units within a single functional unit.

Rice researchers should remain alert to opportunities uniquely provided by the rice plant to make important contributions to fundamental aspects of biology and heredity. The extraordinarily wide geographic distribution provides a rare opportunity to study evolution and speciation. The tremendous variability in grain quality characteristics in relation to its importance as a food crop provides a unique opportunity to study the biosynthesis of starch and related compounds. A few examples illustrate this point. The classical work of Dr. BARBARA MCCLINTOCK on controlling elements in maize, the contributions by Dr. R. A. BRINK on paramutation at the *R* locus in maize, and the many studies dealing with the amylose — amylopectin ratio represent important advances which could not have been made except with higher organisms. In addition, the possibility of direct or indirect application of such fundamental studies to the

improvement of the crop is sufficient justification for achieving greater biological understanding of the rice plant.

I am grateful to The International Rice Research Institute for providing this opportunity to spend this week with you. I owe a debt of gratitude to all of you as participants for having broadened my own horizons. I am confident that you, too, have achieved new breadth of understanding by this international endeavor.

AREAS OF RESEARCH MERITING STRENGTHENED OR NEW RESEARCH

HITOSHI KIHARA AND STERLING WORTMAN, presiding

In a discussion of the many interesting and important problems facing the world's rice geneticists and cytogeneticists, the following areas were agreed upon as fields for increased attention:

(1) Extensive genetic analysis of economic traits, especially of physiological and quantitative characters of importance to plant breeders.

(2) Development of more complete linkage maps, both in *indica* and *japonica.*

(3) Completion of a set of trisomics and a set of translocations within a single *japonica* variety, within a single *indica* variety, and within a single 'ponlai' variety.

(4) Clarification of the nature of intervarietal sterility, using both cytogenetic and genetic approaches, with special emphasis on studies at leptotene, pachytene, and diplotene.

(5) Extension of studies of genome analysis to species in sections other than *Sativa,* which is relatively complete, and the utilization of amphidiploids.

(6) Clarification of species relationships still in dispute. Further collections of African material are necessary and are now being planned for 1963–64.

(7) Studies on the inheritance of resistance to *Piricularia oryzae,* to stem borers and to other important diseases and pests.

(8) A continuing and thorough search of the world collection of rice varieties for characters of interest to taxonomists, geneticists, and plant breeders.

(9) Preservation of genetic material used in important genetic studies, particularly those stocks used as analyzers. These might be deposited with IRRI for later use by scientists wishing to verify earlier results or to make comparative genetic studies.

(10) Genetic studies on hybrid populations and on the interspecific transfer of desirable traits.

RESOLUTIONS

HERBERT H. KRAMER, Chairman

The chairman of the Committee on Taxonomy, M. T. HENDERSON, submitted to the conferees a report of the Committee's attempt to formulate a standard classification and nomenclature of the genus *Oryza.* This report outlined both areas of agreement and disagreement. Following a discussion of the classification and nomenclature for the wild forms in the *perennis–fatua* complex, the conferees approved the committee's report and voted for a temporary adoption of classification No. 1, in which the names *Oryza sativa* var. *fatua, Oryza perennis* subsp. *cubensis, Oryza perennis* subsp. *balunga* and *Oryza perennis* subsp. *barthii* were proposed for various forms of the complex. For full text of the report see Appendix I.

The conferees also designated the group as a 'Standing Committee' to continue efforts in developing a classification plan that will be useful and acceptable to all rice workers and which will incorporate information from cytogenetic investigations and other studies.

The chairman of the Committee on Genome Symbols, H. KIHARA, presented a report in which agreement was reached on the rules for symbolization and the recommended genome symbols for reasonably well studied *Oryza* species. The conferees adopted the report as presented. The text of the report is in Appendix II.

The Director and the Associate Director of the IRRI briefly outlined the international cooperative program of the Institute. The international activities include resident training of research scholars, research fellowships, technical conferences, germplasm bank of cultivated varieties and genetic stocks, and support of research activities elsewhere. It was emphasized that IRRI's effort will be mainly to strengthen existing research of international and economic importance.

H. KIHARA also reported on the discussion among interested parties about a cooperative plan of collecting experimental material in Africa. Two teams will be sent: one to north-central Africa mainly to study cultivated and wild growing populations, and the other to Madagascar and east Africa to collect wild species. The above plan will be a cooperative venture of the National Institute of Genetics of Japan, Academia Sinica of China, the Central Rice Research Institute of India and The International Rice Research Institute. Participants in these discussions also agreed to deposit at

the IRRI a duplicate set of plant and seed samples collected for use by other workers. On the subject of gene symbolization, the IRRI agreed to monitor new gene symbols. The IRC Newsletter will carry reports of this nature. In an effort to achieve uniformity, the conferees also urged the Institute to compile and publish a booklet to describe, with illustrations, the various parts of a rice plant and a number of mutant traits.

In closing, the conferees unanimously agreed that the symposium was highly useful in promoting the exchange of ideas and information and that similar meetings should be held in the future. The conferees also expressed appreciation to the Ford Foundation, The Rockefeller Foundation, the Government of the Philippines, the International Rice Commission, and The International Rice Research Institute for making the symposium possible.

The associate director of the Institute thanked the conferees for their cooperation and active participation.

APPENDIX 1

REPORT OF COMMITTEE APPOINTED TO ATTEMPT A STANDARD CLASSIFICATION AND NOMENCLATURE OF THE GENUS *ORYZA*

The Committee appointed on February 4, 1963 by the conferees of the Symposium on Rice Genetics and Cytogenetics held at The International Rice Research Institute is composed of S. SAMPATH, T. TATEOKA and M. T. HENDERSON. Members of the Committee unanimously agreed that a standard classification and nomenclature should be adopted and used uniformly by all rice research personnel. Such standardized classification should serve the needs and interests of all groups conducting research on rice but should be designed especially for those engaged in research in the fields of taxonomy, cytogenetics, genetics, and breeding.

It is the opinion of the Committee that, for a number of reasons, the standardized classification and nomenclature should be based primarily on the previous classifications of Roschevicz, Chevalier, Chatterjee, and others which used only the conventional morphological characters of taxonomy. However, consideration also should be given to the needs of other workers. In fact, it should be kept in mind that any classification adopted will be used principally by cytogeneticists, geneticists and rice breeders rather than by specialists in taxonomy. For this reason, utilitarian nature of the classification for these groups is fully as vital as observance of the rules of conventional taxonomy.

The Committee agrees that in the light of presently available evidence it should recognize the following forms as distinct, valid species of the genus *Oryza.*

sativa L.
glaberrima Steud.
breviligulata A. Chev. et Roehr.
australiensis Domin
schlechteri Pilger
coarctata Roxb.
officinalis Wall. ex Watt
minuta J. S. Presl ex C. B. Presl
eichingeri A. Peter
punctata Kotschy ex Steud.
latifolia Desv.
ridleyi Hook. f.
alta Swallen
brachyantha A. Chev. et Roehr.
angustifolia C. E. Hubbard
perrieri A. Camus
tisseranti A. Chev.
longiglumis Sansen
meyeriana (Zoll. et Mor. ex Steud.) Baill.

The Committee agrees further that the form commonly designated *Oryza subulata* Nees should be excluded from *Oryza* and recognized as *Rhynchoryza subulata* (Nees) Baill.

The Committee thinks that there are still uncertainties concerning the following aspects of taxonomy in *Oryza:*

(1) Relationships and nomenclature among the taxa commonly designated *Oryza sativa* var. *fatua* (or f. *spontanea*) and *Oryza perennis* (Asiatic, American and African subspecies or varieties).

(2) Relationship of the form usually designated *Oryza stapfii* Rosch. to *Oryza glaberrima* and *breviligulata.*

(3) Relationship between *Oryza granulata* Nees et Arn. ex Watt and *meyeriana.*

(4) Relationship between *Oryza alta* and *grandiglumis* (Doell) Prod.

(5) The status of the taxa previously designated *Oryza ubanghensis* Chev. and *malampuzhaensis* Krish. et Chand.

Additional information concerning these aspects is needed before a reliable conclusion can be reached. It is recommended that participants of the Symposium appoint a Standing Committee to accumulate further evidence on these questionable relationships and to formulate recommendations at a later date by means of correspondence.

The appropriate classification and nomenclature for the wild forms called *sativa* var. *fatua* (or f. *spontanea*) and Asiatic, American and African types of *perennis* appear to constitute the most difficult problem remaining in the taxonomy of *Oryza.* The Committee, despite lengthy discussion, could not reach agreement on a proposed classification of these forms. Consequently, three alternative treatments are presented for consideration by all the members of the Symposium. Each of these possible classifications are presented below.

The Committee agrees that the form commonly designated *Oryza stapfii* does not warrant species rank and should be combined with one of the other African annual species. However, they could not decide on whether to consider this form as a variety of *Oryza glaberrima* or of *breviligulata.*

Genus *Oryza*	Proposed alternative classification*		
Previous Classification	1	2	3
sativa var, *fatua* or f. *spontanea*	*sativa* var. *fatua*	*sativa* var. *fatua*	*rufipogon* subsp. *rufipogon*
perennis	*perennis*		
American form	subsp. *cubensis*	*perennis*	*rufipogon* subsp. *glumaepatula*
Asian form	subsp. *balunga*	*balunga*	*rufipogon* subsp. *rufipogon*
African form	subsp. *barthii*	*barthii*	*barthii*

* Proposed classification number 1 in the above scheme was adopted by the Symposium on February 8 as a tentative or temporary classification for the group.

APPENDIX 2

RECOMMENDATION OF THE COMMITTEE ON GENOME SYMBOLS FOR *ORYZA* SPECIES

The Committee nominated on February 5, 1963 by the conferees of the Symposium on Rice Genetics and Cytogenetics at The International Rice Research Institute, Philippines, is composed of H. KIHARA (chairman), T.T. CHANG (secretary), H. W. LI, T. MORINAGA and R. H. RICHHARIA. This Committee met February 6 and agreed on a number of principles for assigning and using genome symbols. The recommended rules for symbolization enumerated below were adopted by the conferees of the Symposium on February 8.

(1) In designating individual genomes, alphabetical letters (capitalized), after the MORINAGA scheme, should be used.
Example: A for the genome of *Oryza sativa.*

(2) The genome of diploid species should be indicated by two letters, the tetraploid species by four letters, and so forth.
Examples: AA for *Oryza sativa;*
BBCC for *Oryza minuta.*

(3) To distinguish between species with basically similar genomes but detectable differences in meiotic behavior, fertility relationships and other criteria in their interspecific hybrids, literal superscripts (in small letter) should be used to indicate subgroups in these species. Initial letter or letters of representative species should be used.
Examples: AA for *Oryza sativa* and *perennis* subsp. *balunga* (Asiatic form)
A^bA^b for *Oryza perennis* subsp. *barthii* (African form)
$A^{cu}A^{cu}$ for *Oryza perennis* subsp. *cubensis* (American form)
A^gA^g for Oryza *glaberrima* and *breviligulata*

(4) A summary of recommended genome symbols for reasonably well studied *Oryza* species is given as follows: *(see the table on page 254).*

(5) Workers, proposing the use of new symbols in the future, should communicate with the geneticist of The International Rice Research Institute. The geneticist will in turn correspond with other members of the Committee for clearance. If accepted, the above recommended rules and symbols and those to be added later will be announced in the issues of the International Rice Commission Newsletter and other scientific journals.

Genomes	*Oryza* species	Distribution
AA	***sativa**, *sativa*** var. ***fatua*** (or f. ***spontanea***),	
	perennis subsp. ***balunga***	Asia
A^bA^b	***perennis*** subsp. ***barthii***	Africa
$A^{cu}A^{cu}$	***perennis*** subsp. ***cubensis***	America
A^gA^g	***glaberrima*, breviligulata, stapfii***	Africa
CC	***officinalis***	Asia
BBCC	***minuta****,	Asia
	eichingeri	Africa, Asia
CCDD	***latifolia,* alta, grandiglumis, paraguaiensis***	America
EE	***australiensis***	Australia
FF	***brachyantha***	Africa

* Representative species of the subgroup.

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SUBJECT INDEX