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CYTOGENETICAL STUDIES IN ORYZEAE AND PHALARIDEAE

I. CYTOGENETICS OF SOME X-RAY DERIVATIVES IN RICE (*ORYZA SATIVA* L.)[1]

BY N. PARTHASARATHY

Botany Department, King's College, London

(With Plate I and Forty-four Text-figures)

CONTENTS

1. INTRODUCTION

EVER since the importance of X-rays as a fruitful source of inducing germinal changes in living organisms came to be recognized from the work of Muller (1927) on *Drosophila*, extensive experiments on the application of this radiation to plants have been initiated by numerous investigators, as Goodspeed (1929*a*), Goodspeed & Avery (1930) in *Nicotiana*, Horlacher & Killough (1933) in cotton, Katayama (1935)

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in wheat, Navashin (1931) in *Crepis*, De Mol (1930) in *Hyacinthus* and *Tulips*, Levitsky & Araratian (1932) in *Secale*, *Vicia* and *Crepis*, Catcheside (1935) in *Oenothera*, and others.

The genetical and cytological changes involved were in the main identified to be gene mutations, translocations, inversions, duplications and deficiencies following chromosome breakage. Muller (1927) obtained genetical evidence in his X-ray experiments on *Drosophila*, of numerous changes of varied kinds in the gene alignment, including translocations. Some of these changes were checked cytologically (Muller, 1928; Painter & Muller, 1929). From the study of pachytene stages McClintock (1931*a*) was able to demonstrate deficiencies, translocation and an inversion in *Zea Mays*. Stone (1933) and Mather (1934) found a number of metaphases and anaphases which showed a great deal of fragmentation. They think that this fragmentation leads to inversions and translocations by the reattachment of broken parts. Catcheside (1935) found that the only structural changes in chromosomes surviving the division after their origin were segmental interchanges and perhaps small deficiencies.

Most of the mutations so induced, from the point of view of the plant breeder, are useless, inviable and lethal, but the great advantage lies in the wide range of material offered due to the possibilities not only of the alteration of the gene, but also of the reshuffling of the gene arrangements, both in the chromosome and between chromosomes. The survival value of these changes, however, depends upon the nature of the changes. Some of them are eliminated even after a few somatic mitoses if the vegetative tissues or root tips are irradiated. Mather & Stone (1933) showed that after irradiation of root tips of *Crocus* and *Tulipa*, the fragments lacking the spindle attachments and chromosomes with two attachments are not perpetuated. The former fail to divide at anaphase and lose the power of movement, and the latter, due to two centromeres which act independently, are broken in half the number of cases and thus are eliminated in half the number of cells at each division. Cells possessing such mechanically unsound chromosomes are eliminated and so make no important contribution to the progeny of irradiated organisms. Changes which may survive somatic divisions may not all of them escape the sexual generation. Gates (1930) has suggested the application of X-rays to heterozygous fruit plants, where the naturally occurring mutations are rare. There is the additional advantage of being able to propagate vegetatively the somatic mutations induced which are really economic. Goodspeed (1932) and Goodspeed & Avery (1930) were able to get new stable forms in *Nicotiana* quite different from the control, as a

result of chromosome fragmentation. They conclude that such changes as fragmentation and fusion are probably productive of initial and secondary alteration in the number and morphology of the chromosomes. These, together with other changes in reorganization of the chromatin, may produce complex unstable structural types, from which, however, stable derivatives distinct from the control may be isolated.

In addition to structural changes consequent on the rearrangement of chromatin in the chromosome, the production of haploid and polyploid forms has been claimed by the use of Rontgen rays. Katayama (1935) obtained haploid plants from X-rayed spikes of *Triticum monococcum*. Webber (1933) described three haploid plants of *Nicotiana glutinosa*, two from X-rayed seed and one apparently of spontaneous origin. However, opinion seems to differ regarding X-rays as a cause of inducing haploidy and polyploidy. Stadler (1931), after X-ray treatment of the pollen of maize, found no indication of induced polyploidy, and the haploids which were observed might equally well have occurred under natural conditions. A few rice haploids were isolated in Coimbatore Rice Research Station (unpublished records) from X-rayed progenies of rice, but the rate of occurrence was so low, as noted in actual progenies, that it could not be claimed that X-rays were the causative agent in inducing haploidy.

A lot of work has been done on the relation between the rate of mutations and radiation dosage. Stadler (1931) found that the loss of dominant endosperm characters from maize pollen was directly proportional to dosage. Catcheside's (1935) observations on X-rayed *Oenothera* are in conformity with other workers (Stubbe, 1933) in that an increased dosage produces an increased effect.

More recently, X-ray treatment has been utilized to determine the time of division of the chromosomes with reference to particular stages of cell division. When the chromosomes are broken or interchanged under X-rays before they have divided, the changes produced will be exactly paired in the chromatids at metaphase and are described as due to chromosome breaks. When the cells are treated after the chromosomes have divided, the changes they undergo are more likely to be independent in each of the chromatids and are recognized at metaphase as due to chromatid breaks. The results of the various investigators are not unanimous. Huskins & Hunter (1935) found chromatid breaks which they consider proved the second meiotic anaphase chromosomes to be double. Marshak (1935), White (1935), and Nebel (1936) found configurations to show that chromosomes are at least double before synapsis.

Riley (1936) and Mather (1937) conclude that chromosomes divide during the post-meiotic resting stage. The *Drosophila* experiments of Demerec (1935) show that X-rays delete a number of longitudinally adjacent salivary gland chromosome bands. The chromonemata are for the most part closely adpressed even though multiplicity of strands can be most clearly demonstrated. A number of these behave as single units for X-rays—so Huskins (1937) holds that while chromatid breaks are almost decisive evidence of doubleness at the time of irradiation, chromosome breaks are no evidence of singleness. Gates (1938) points out that the X-ray evidence is conflicting and no valid inference can be drawn from it alone.

As regards the mode of action of X-rays, it is suggested that the changes induced are due to the direct bombardment of the highly energized electron (Catcheside, 1936). This may alter the gene or break the chromosome at any locus. White (1937) found in three species of Orthoptera that the effect of X-rays was to cause chromosomal disintegration, and the fact that the chromosomes were not affected during the prophase stages when they were surrounded by the nuclear membrane leads him to suggest that irradiation acts indirectly on the chromosomes through the production of probably enzymatic substance or substances in the cytoplasm which act on the chromosomes when the nuclear membrane disappears.

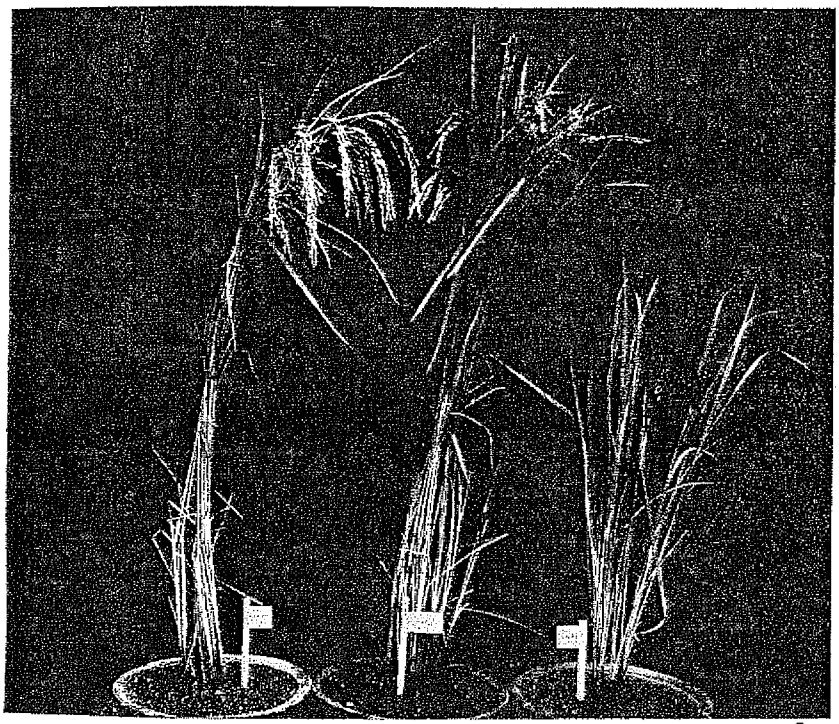
The scope of X-radiation in cytogenetics is unlimited though the results cannot be controlled, as the changes are induced at random in a highly organized system. In the hands of a plant breeder it is a quick but speculative method of not only inducing new variations, but also of breaking up certain undesirable combinations of characters due to gene linkage.

The present paper deals with the cytogenetics of three mutants derived from the X_3 generation.

2. Origin of the material

Investigations were started at the Rice Research Station, Coimbatore, during 1933–4, when the seeds of a pure line of rice were exposed to X-rays. The seeds were treated under three different conditions: (1) dry state, (2) wet state (soaked in water for 24 hr.), (3) germinated state. The treatment consisted of unscreened exposure under a water-cooled Coolidge tube with copper anti-cathode operated at 53 kV. and a tube current of 10–11 mA. at a target distance of 17 cm. The lots were exposed for 1, 2 and 3 hr. The percentage of surviving seedlings in the different

cases varied with the condition and duration of treatments (Ramiah *et al.* 1934). The wet and germinated seeds were badly affected, while in the dry seeds the lethal effect increased with the dose. Amongst the surviving plants several types of mutations affecting the stature of the plant, size of grain, size of leaf and chlorophyll contents of leaves were noticed. These types were only very few, but pollen sterility of varying



Normal Semi-sterile Dwarf

Text-fig. 1.

degrees was noticed in most of the plants. Seeds from each of the plants were collected separately and X_2 generation was raised. A number of mutations, mostly in chlorophyll content—several of which are quite new and not found as spontaneous mutations in rice—and those affecting plant stature and grain size, sterility, etc., were isolated, and the genetical behaviour in further generations was studied (Ramiah & Parthasarathy, 1938).

In the progeny of a semi-sterile mutant obtained from the line (X-rayed

dry seed—1 hr. exposure) in X_2, a number of dwarf mutants was found to occur, besides semi-sterile and normal plants (Text-fig. 1). The semi-sterile plants were just like normal plants in general features of growth and could be identified only after the emergence of the earhead, by the examination of pollen and later by seed setting. The dwarf mutants could not be recognized in the seedling stages, but after two months' growth they could easily be isolated from the nature of their short stature. The earheads emerged a fortnight later than the normal and semi-sterile plants; the spikelets were small and some of them were malformed. The pollen was well filled and normal though the flowers did not set seed.

Two other mutants were isolated from the X_2 generation; one was termed "stumpy" from the nature of the thick culms or tillers. The number of tillers produced was smaller than in normal plants and the height slightly shorter. The other mutant was the "beaked sterile", which was also characteristically different from the normal in having the flowering glumes beaked or curved, with sterility up to 50 %. These were also slightly shorter than the normal plants. These two mutants were taken up for cytological study, as they did not breed true, and pure types could not be isolated in further generations.

3. Breeding behaviour of the mutants

(*a*) The semi-sterile mutant was isolated in X_2, and gave rise to the following progeny: semi-sterile 51; dwarfs 16; normals 37. The dwarfs, as mentioned before, did not set seed and a few plants in each of the other two groups were carried forward for the next generation. The normal plants bred pure, while the semi-sterile gave rise to the three groups again in the following proportions (Table I):

(*b*) The "stumpy" was derived from the same X_1 line which gave rise to the above semi-sterile mutant. Only one such plant was observed in X_2 generation and seeds of this plant when grown gave in X_3: normal 69; stumpy 25; and a new type with long narrow grain 7. The stumpy, as mentioned before, were slightly shorter in stature and, besides having thick and fewer culms than the normal, were characteristic in possessing panicles or earheads in which the spikelets were arranged rather closely, and a certain amount of sterility up to 25 % was noticeable. Table II gives the behaviour in the next generation.

(*c*) The beaked sterile was isolated in another line which was characteristic in having beaked spikelets, nearly 50 % of the flowers failing to set seed. This mutant also did not breed true and appeared only in smaller

TABLE I

Showing segregation of the "semi-sterile" mutants

Generation	No. of plants chosen	Character of selection	Behaviour		
			Normals	Dwarfs	Semi-sterile
X_2	1	Semi-sterile	37	16	51
X_3	10	Normal		All normals	
	19	Semi-sterile	33	9	58
			25	2	36
			28	23	46
			29	13	57
			30	15	54
			25	11	48
			41	16	41
			38	12	73
			25	20	60
			12	7	22
			38	19	56
			59	18	71
			25	9	34
			34	21	44
			55	18	51
			8	3	14
			42	9	65
			41	17	62
			42	15	59
		Total	667	273	1002
		Expected 1 : 1 : 2	485·5	485·5	971
		Deviation	181·5	212·5	31

$\chi^2 = 154{\cdot}89$ ($P = 0{\cdot}01$, $\chi^2 = 9{\cdot}21$).

TABLE II

Showing the segregation of the "stumpy" mutant

Generation	No. of plants chosen	Character of selection	Behaviour		
			Normals	Stumpy	Long and narrow grain
X_4	3	Normal		All normals	
	3	Stumpy	75	36	2
			86	32	5
			85	34	1
		Total	246	102*	8
	1	Long narrow grain	84	—	41

* Percentage of stumpy plants 29.

proportions in the next generation. Table III gives the breeding behaviour and shows that only two types of plant appeared, while in the "stumpy" a new type of plant appeared which also did not breed pure. The probable clue for this is explained later from the difference of these mutants in their cytological behaviour. Seeds of the "semi-sterile", "stumpy", "beaked sterile", along with the normal, were sown at the Courtauld Genetical Laboratory, Regent's Park, London, during the summer of 1937 for cytological study.

TABLE III

Showing segregation of the "beaked sterile" mutant

Generation	No. of plants chosen	Character of selection	Behaviour	
			Normals	Beaked sterile
X_3	3	Beaked sterile	70	13
			66	18
			64	18
X_4	8	Normals	All normals	
	10	Beaked sterile	75	13
			98	15
			32	2
			9	6
			96	18
			57	13
			117	13
			68	31
			53	12
			87	16
		Total	892	188

Percentage of beaked sterile 17·4

4. Cytological technique

As the mutants do not breed true, fixation was possible only after their character could be recognized. The dwarfs from the "semi-sterile" mutant could be identified after 2 months' growth, and root tips for this were fixed early enough. Root tips were fixed in La Cour's 2BE for 24 hr., washed and dehydrated by the chloroform method. Sections were cut 12μ thick, bleached for 24–48 hr. in a mixture of 3 parts of alcohol and 1 part of 20 vol. hydrogen peroxide and stained by Newton's iodine gentian violet technique. The root tips for other mutants were fixed late, after the flowers were collected, and unfortunately good plants were not secured for study. Attempts to raise roots again from the stubbles failed. For the meiotic stages, the young spikelets were clipped at the top, exposing the anthers, dipped in Semmens' (1937) modification of Carnoy (3 parts of abs. alcohol, 1 of acetic acid and 1 of chloroform)

for a few seconds and then transferred to Navashin's fluid for 24 hr. The fixed material was straight away taken to 50 % alcohol and then carried on to wax in the same way as the root tips. Sections were cut 14–16μ thick, and all the slides were mordanted in 1 % chromic acid solution for about 15–20 min. before staining for better differentiation of the chromosomes from the cytoplasm. Aceto-carmine smears were made, to determine the right stages for fixation.

5. CYTOLOGICAL BEHAVIOUR

(i) *Semi-sterile mutant*

Stages earlier than diakinesis were not studied, as the chromosome threads were too delicate for proper fixation. Instead of the twelve bivalents usually observed in normal rice plants (Text-fig. 1), associations of four chromosomes and ten bivalents were noted. Out of four semi-sterile plants examined, one plant showed the presence of one or two fragments in addition to the full complement, and as the genetical behaviour of this plant was the same as that of the plant with fragments, the descriptions and drawings refer mostly to this plant.

(*a*) *Association of four chromosomes.*

Occasional quadrivalent formation in rice has been reported by Nandi (1936). This would correspond to the occasional bivalents that are formed in the haploid rice (Morinaga & Fukushima, 1934) and the rare case of sexivalent formation in the auto-triploid rice (Ramanujam, 1937). Multivalents are met with in diploids which have their related chromosomes in the haploid complement. Sakai (1935) and Nandi (1936), by studying the secondary pairing in rice, have concluded that rice is a secondarily balanced allotetraploid derived by hybridization of two five-chromosomed species with the duplication of two chromosomes due to meiotic irregularities in the hybrid, followed by the doubling of chromosomes; thus giving $n=12$ as the fundamental number of the genus *Oryza*. In the normal variety from which this mutant was isolated, I have observed the maximum secondary association of two groups of three and three groups of two bivalents similar to the observations of the above investigators as well as of Ramanujam (1938) in *O. sativa* and *O. Barthii* (Parthasarathy, unpublished). With such a constitution a maximum of two sexivalents in the triploid, two quadrivalents in the diploid and two bivalents in the haploid is possible, though the probability of their occurrence is in the inverse order, the chance being greater in the haploid, due perhaps to the absence of competition in pairing

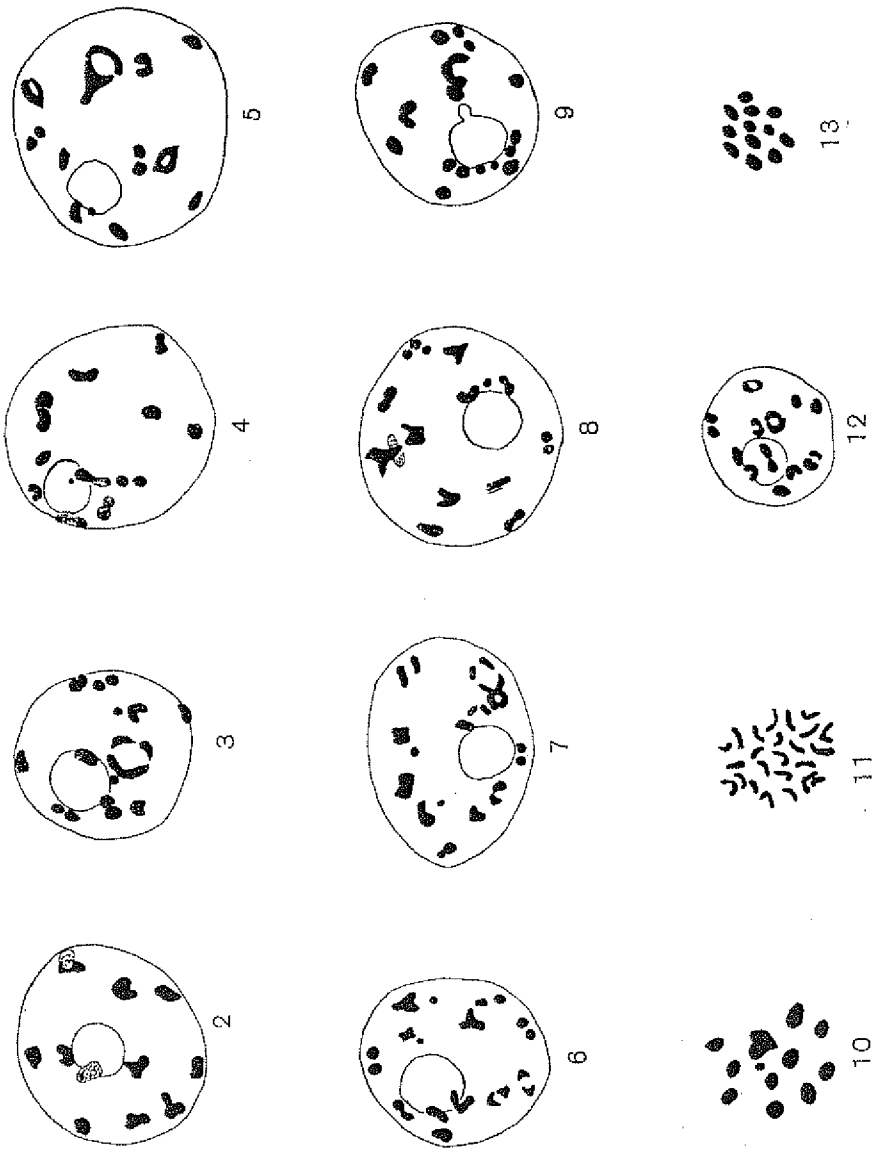
between the undifferentiated duplications still present in the ancestral homologues.

The association of four chromosomes found in the semi-sterile plant is not rare, but is found in almost all the pollen mother cells examined. The configurations are of different types depending on chiasma formation, and are described later. This sort of association in a diploid which normally forms only bivalents was due to interchange of segments between non-homologous chromosomes, as first suggested by Belling (1925) to explain the partial sterility previously recorded by him (Belling, 1914) in *Stizolobium*. Belling and Blakeslee (1926) inferred the occurrence of an interchange of segments between non-homologous chromosomes in the trisomic forms of *Datura*. This hypothesis has been proved to be adequate in interpreting chromosome ring formation in plants arising from hybridization or irradiation and also in naturally occurring forms. If two non-homologous chromosomes *AB* and *CD* (each chromosome regarded as being composed of two segments) exchange segments *B* and *D*, the resulting chromosomes will be *AC* and *BD*. The heterozygote derived by this interchange will have the constitution *AB*, *BC*, *CD*, *DA*, while the original plant will have the constitution *AB*, *AB*, *CD* *CD*. The latter will form two bivalents and the former will give a ring of four on the accepted promise that homologous portions of chromosomes pair. In *Datura* (Blakeslee, 1928, 1929), *Pisum* (Hakansson, 1929, 1931, 1934; Sansome, 1929; Pellew & Sansome, 1931) and in *Polemonium* (Clausen, 1931), hybrids with a ring of four have been obtained by crossing different homozygous races or species. Hybrids with ring of six or two rings of four involving two interchanges have been produced either by crossing two parents which differed in two interchanges, as in *Datura*, or by crossing two single interchange races which have one gametic type in common, as in *Oenothera* (Gates & Catcheside, 1931), in *Pisum* (Sansome, 1932) and in *Campanula* (Gairdner & Darlington, 1931).

Chromosome ring formation was first observed in *Oenothera* by Gates (1908). The existence of naturally occurring forms which show rings at meiosis was later observed in *Campanula*, *Rhoeo*, *Humulus*, *Tradescantia*, *Anthoxanthum*, etc., and the hypothesis of Belling has given a clue to the behaviour of chromosomes, though the exact mode of origin of these forms in nature is still a matter of conjecture. The interchange could have given rise to the heterozygote at one step, or the heterozygote might have given rise to homozygous races from the original types and interchange forms, the hybridization of which could have produced the heterozygote again, but the fact that the homozygous combinations are

lethal in *Oenothera* favours the direct interchange hypothesis (Darlington, 1937). In X-rayed stock reciprocal interchanges between non-homologous chromosomes have been recorded by previous investigators: Muller (1930) in *Drosophila*, McClintock (1931*b*) in *Zea Mays*, Katayama (1935) and Thompson & Thompson (1937) in *Triticum*, Catcheside (1935) in *Oenothera*, and Ramiah *et al.* (1934) in rice.

In the X_1 generation of X-rayed rice seed, a semi-sterile plant was found to show a ring or chain of four chromosomes in diakinesis. Random orientation of this ring or chain gave half the number of inviable gametes. For *AB*, *BC*, *CD*, *DA* may disjoin giving rise to *AB*, *CD* and *BC*, *DA* (viable gametes) or *AB*, *BC* and *CD*, *DA* (inviable due to deficiency of *D* and *B* segments). Further observations in this plant or its progeny were not made. In the present material, observations at diakinesis showed that out of 100 cells examined 16 cells showed rings or chains (Text-figs. 3, 4 and Pl. I A, B). The next were of different configurations showing interstitial chiasmata between either or both the adjacent chromosomes forming the association of four. The most common configuration is the one with an interstitial chiasma in one of the pairs (Text-fig. 5 and Pl. IC). Sansome (1933) calls this the median chiasma. The failure at one of the ends where the second pair is attached to the pair with the interstitial chiasma gives rise to the configuration like a necktie (Text-fig. 6). The presence of terminal chiasmata at free ends of the pair with interstitial chiasma gives a "figure-of-eight" configuration (Text-fig. 7), quite different from the "figure-of-eight" described by Sansome (1932), which is composed of six chromosomes. Text-fig. 8 represents interstitial chiasma formation in both the pairs. The position of the attachment constriction could not be identified because of the small size of rice chromosomes, but from the metaphase configuration it could be said that the interstitial chiasma is towards the side of the association with the second pair, as otherwise only a ring configuration could result at metaphase (Pl. ID). (For convenience the chromosomes forming interstitial chiasmata are considered as pairs, but strictly they are not homologous for the whole lengths.) Since the ends of the first pair associated with the ends of the second pair, cannot be homologous by virtue of their association with ends of different chromosomes, the chiasmata could have been formed only in the homologous regions, as the occurrence of chiasma implies previous pairing.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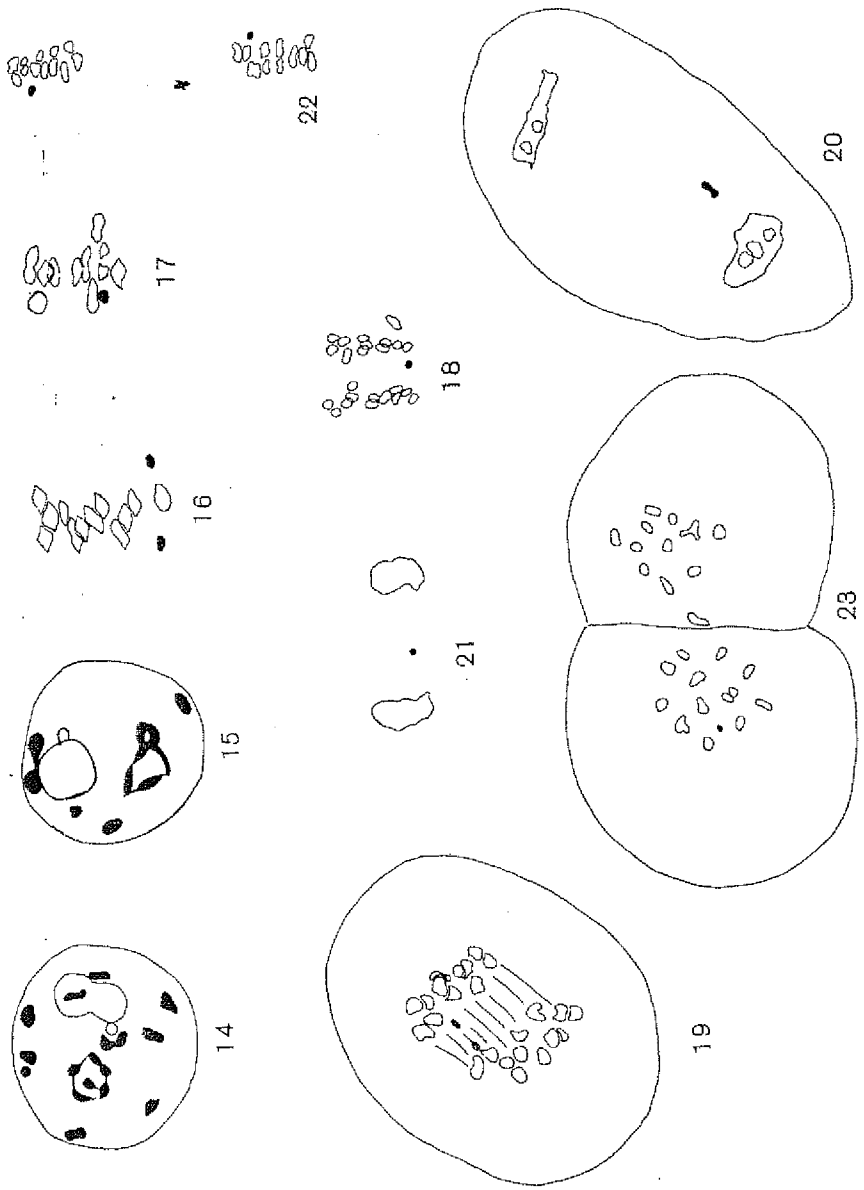
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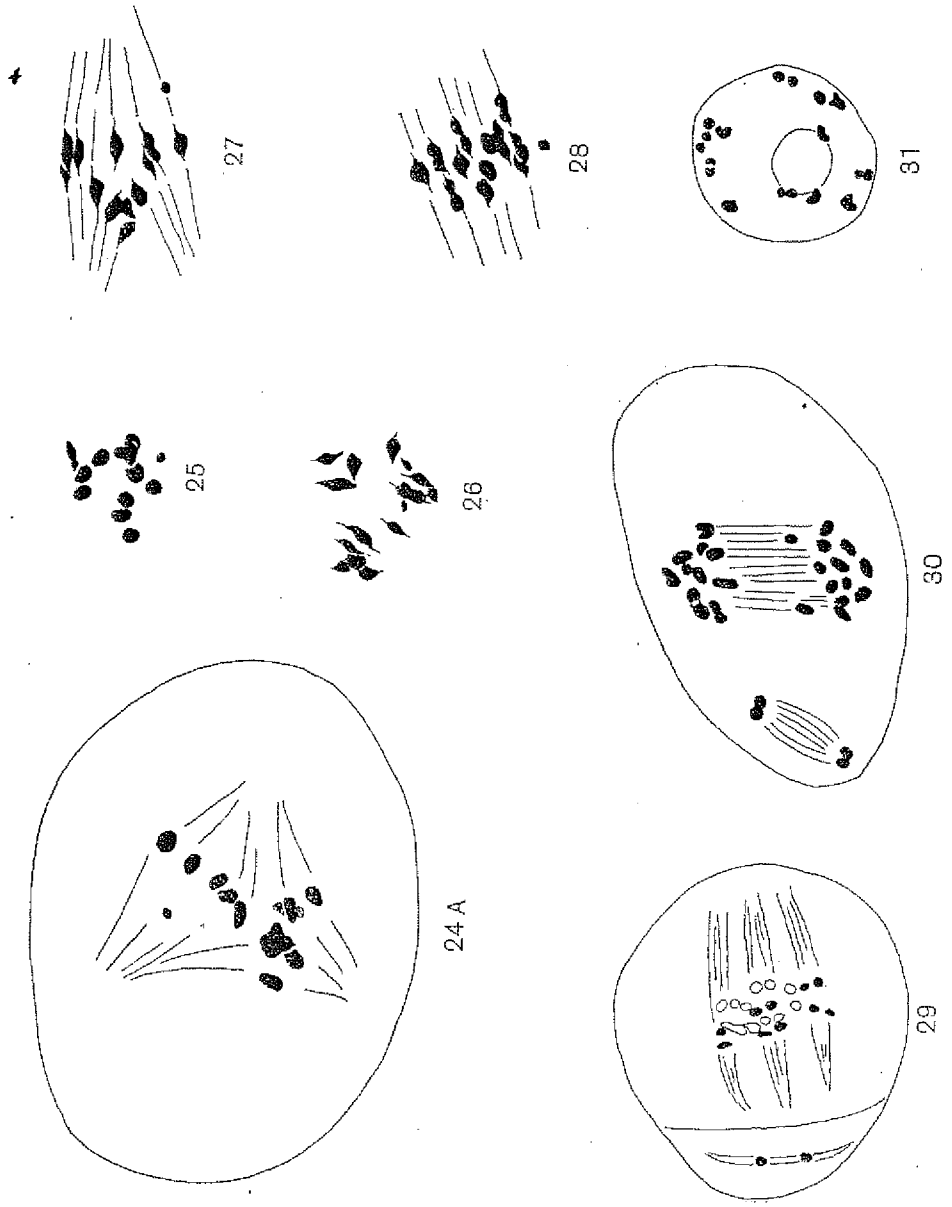
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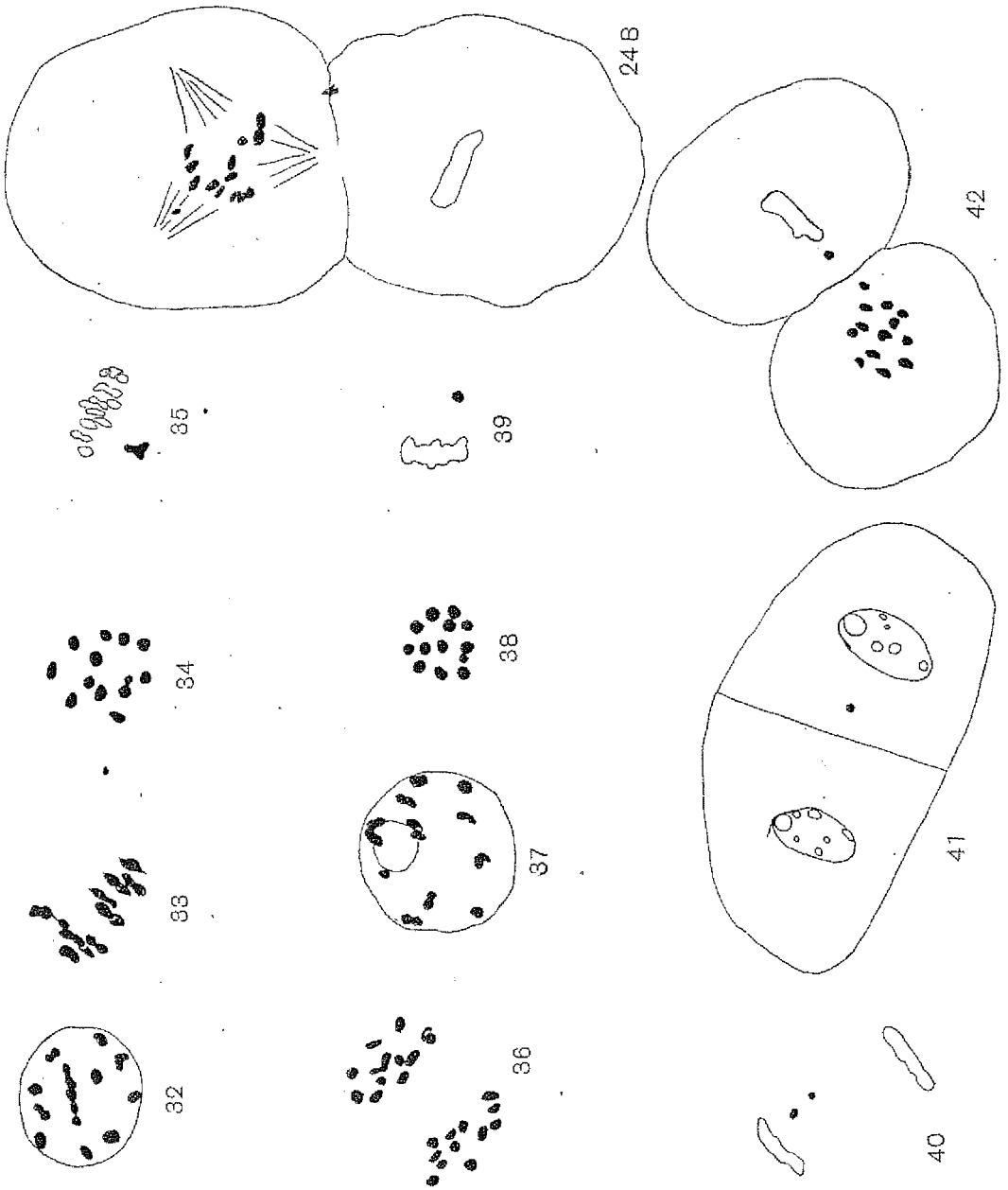
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(*b*) *Disjunction.*

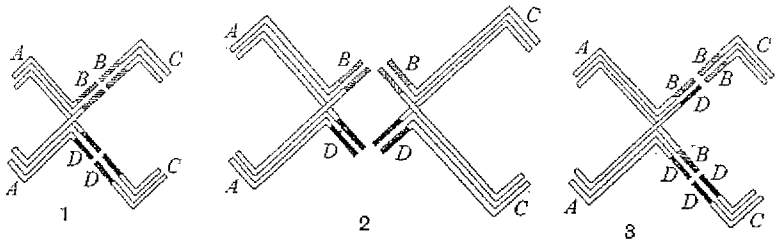
The regular disjunction of the four chromosomes in a ring in most of the artificially produced heterozygotes, as in *Datura* and *Zea*, only takes place by alternate chromosomes going to the same pole and is associated with zigzag arrangement of the chromosomes. This is the only configuration in the ring or chain which will give rise to viable gametes. The gametes resulting from such a configuration will be of the parental type and if only such occurred, the offspring of the ring-formation hybrid would consist only of the parental types and the hybrid type, but the chances of non-disjunction involving the deficiency of a segment due to adjacent chromosomes passing to the same pole are equal, and hence 50 % of the gametes are aborted. Thus the orientation of the ring of four on the spindle in these hybrids is random, but in the semi-sterile mutant described here, though the orientation on the spindle is random, the non-disjunction is brought about in a different way due to crossing-over of chromatids at the interstitial chiasma in the proximal portions of the interchanged segments. The result of this in the first metaphase gives in half the cases chromatid non-disjunction (see Text-fig. 43, diagram 1).

Janssens (1909, 1924) was the first to attempt to link up genetical crossing-over with chromosome behaviour at meiosis. He believed that changes of partner amongst chromatids, to which he gave the name chiasmata, observable in diplotene to metaphase, were intimately connected with genetical crossing-over. His idea of partial chiasmatypy has since been adopted by Belling and others as the explanation of crossing-over. This hypothesis assumes changes of partner amongst chromatids at diplotene as a result of previous crossing-over, the chromatids of parental or somatic chromosomes tending to remain in association on either side of the chiasma. The classical theory elaborated by Sax (1930) assumed that the chromosomes separate out at diplotene without breaking, the diplotene loops being due to alternate equational and reductional splits and that the breaking of chiasmata between diplotene and metaphase causes the crossing-over.

In the present case, the evidence on the assumption that homologous portions of chromosomes pair shows that a chiasma is only the result of crossing-over and not the cause of it. In Text-fig. 43, diagram 3, it will be seen on the basis of classical theory, that of the two chromatids of *A*, one end *B* is homologous with the *B* end of *BC* chromosome and the same end *B* of the second chromatid of *A* is homologous with the *D* end of the *DC* chromosome, which is contrary to the assumption of homology and

hence proves that crossing-over has occurred before the formation of chiasma.

The result of this crossing-over at the chiasma referred to in the association of four chromosomes brings about chromatid non-disjunction in 50 % of the cases, causing semi-sterility. This is similar to the case of *Pisum* (Sutton, 1935). This sort of non-disjunction was first observed by



Text-fig. 43. Diagrams 1 and 2 illustrating chromatid non-disjunction (i) metaphase configuration showing the interstitial chiasma in the homologous region proximal to the interchanged segments in the chromosomes *A B* and *A D*.

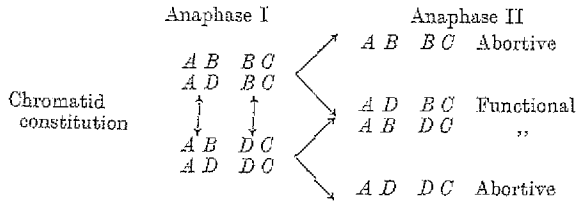


Diagram 2. Metaphase configuration showing the interstitial chiasma in both the pairs of chromosomes (*AB*, *AD*) and *BC*, *DC*).

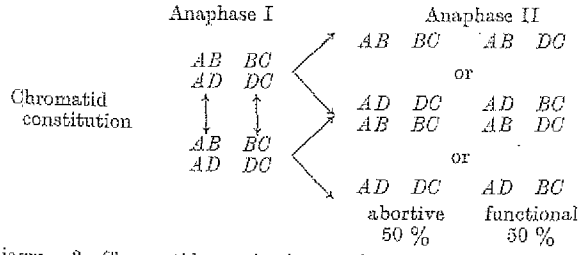


Diagram 3. Chromatid constitution on the basis of crossing-over according to classical theory (see text).

Sansome (1933), and occurred as a result of a chiasma in the X-segment of the figure-of-eight configuration observed by her in the association of six chromosomes in *Pisum*.

If a chiasma is formed in a similar region in the other pair (Text-figs. 10 and 43, diagram 2), the consequences will be similar in the production of 50 % non-disjunctional gametes. So the three main different types of configuration observed in the semi-sterile plant are similar in effect in

giving rise to 50 % non-viable gametes. This is similar to the artificially produced rings of four either by hybridization or by X-rays in *Zea*, *Pisum* and *Datura*, where 50 % of gametes are sterile. In the case of *Oenothera* (Catchesides, 1935) and in *Triticum* (Thompson and Thompson, 1937), the arrangement of the chromosomes forming the association is zigzag, with the result that alternate chromosomes pass to the same pole. In *Triticum* this sort of disjunction results in fertile progeny and in a single case in *Datura*, may be inferred from the result (Blakeslee & Cleland, 1930). In *Oenothera*, on the other hand, semi-sterility is due to homozygous interchanges being lethal to spores carrying them. Thompson & Thompson conclude that the same mechanism which operates in giving regular orientation of chromosomes in *Oenothera* is present in the genus *Triticum*.

The reciprocal interchanges giving rise to 50 % sterility are equal or nearly equal and involve large segments (Cooper & Brink, 1931). Burnham (1932) found in maize a reciprocal interchange involving a large segment and a very small terminal portion of the satellite giving rise to a chain-of-four configuration, due to failure of synapsis at the end having the small homologues. In this case only 25 % sterility resulted, due to one of the chromosomes having only a very small deficiency and therefore being functional. He presumes that all the interchanges may be reciprocal and the so-called simple translocations giving rise to chains of four (Burnham, 1930) may on careful cytological examination reveal minute reciprocal interchange. This is important with reference to the idea of non-fusibility of the whole ends of chromosomes (Stadler, 1932).

In addition to the types of non-disjunction involving chromosome or chromatid segments, there is yet a possibility of numerical non-disjunction involving whole chromosomes—occasionally three chromosomes may pass to one pole and one to the other. This type of non-disjunction was first observed by Gates (1908) in *Oenothera* and was later suggested as the cause of the origin of the trisomic mutants (Gates & Thomas, 1914). Hakansson (1929) suggested the occurrence of trisomics in the progeny of semi-sterile *Pisum* plants as a result of his observation of second metaphase plates. Trisomics were recorded in the progeny of semi-sterile plants in maize (Burnham, 1930) and in *Pisum* (Sansome, 1933). It is quite probable that the examination of a sufficient number of first anaphase or second metaphase plates may show this type of irregularity, though it is rare in the present material.

(c) *Genetical consequences.*

The semi-sterile plant produces, as a result of the cytological behaviour described, two types of functional gametes in equal proportions (*AB*, *CD*) (*BC*, *DA*). On selfing, the progeny should give rise to three types of plants genotypically; (1) normal plants with the original complement (*ABAB CDCD*), (2) heterozygous plants (*ABBC CDDA*), (3) plants homozygous for the interchange (*BCBC DADA*), in the proportions of 1 : 2 : 1 respectively. In the interchanges studied in maize, Anderson (1935) reports that plants homozygous for interchange could not be phenotypically distinguished from the normal plants. Only breeding tests by hybridization between the plants could isolate the two types.

Associated with the interchange is the unexpected linkage of factors which are known to be inherited independently as they are carried by different chromosomes. In *Pisum*, Hammerlund (1923) found unexpected linkage between factors which were ordinarily independent. Hakansson (1929) examined this plant cytologically and found a ring of four chromosomes, proving that this was due to segmental interchange.

In the progeny of the original semi-sterile plant, a certain proportion of dwarf mutants appeared, the actual number being much less than 25 % of the population. The semi-sterile plants like the parent plant formed nearly half and the normal plants were much more than one-fourth of the total population. The pollen formation of the dwarfs was regular although the plants did not set seed. These dwarfs were examined cytologically and were found to possess 24 chromosomes (Text-fig. 11), the same as the normal rice plants, the pollen meiosis was normal, showing twelve bivalents (Text-figs. 12 and 13) which segregated regularly giving rise to the normal tetrad of spores. The breeding data for two generations indicate that the semi-sterile plants alone give rise to dwarfs while the normal plants breed true. The ratio of the normal semi-sterile and dwarf plants in the progeny of the heterozygous plant (semi-sterile) is roughly in the proportion of 1 : 2 : 1 respectively, although there is a very significant reduction in the number of dwarfs, and a significant excess in the number of normal plants. From the data one is led to presume that the dwarfs represent plants homozygous for the interchange. Hybridizing these with normal plants should yield the heterozygous semi-sterile plant. Attempts will be made to confirm this.

Seed germination tests, which were the same for both normals and semi-sterile, indicate that the significant reduction in dwarfs is not caused by lower viability of the zygote, but is due to the fact that the pollen carrying the interchanges may not compete equally well with the pollen

carrying uninterchanged chromosomes. This is clearly indicated by Table IV. This lower functional activity of pollen may be due to very slight gene deficiency associated with the interchange.

TABLE IV

Indicating expected proportions of phenotypes from the semi-sterile plant on the assumption of pollen competition

		Functional pollen	
		Normal (*AB.CD*)	Dwarf (*BC.DA*)
Assuming normal pollen functions better than pollen with interchanged segments in fertilization		$\frac{1}{2}+x$	$\frac{1}{2}-x$
Egg		$\frac{1}{2}$	$\frac{1}{2}$
Zygote	Normal plant *AB AB CD CD*	Semi-sterile *AB BC CD DA*	Dwarf *BC BC DA DA*
	$\frac{1}{4}+x/2$	$\frac{1}{2}$	$\frac{1}{4}-x/2$
	Assumed $X=\frac{1}{5}$ (20 %)		
	$\frac{1}{4}+\frac{1}{10}$	$\frac{1}{2}$	$\frac{1}{4}-\frac{1}{10}$
Expected proportions in the total population of 1942 plants	679·7	971	291·3
Actual	667	1002	273
Deviation	12·7	31	18·3

$\chi^2=2{\cdot}173$. P between 0·50 and 0·30.

If there be only simple interchange without any other change in the chromosome material, the plant with homozygous interchange should have the full complement like the normal plants and so should not differ phenotypically, as in maize, *Datura* and *Pisum*, where the plants homozygous for the interchange are not recognizable except by breeding tests.

(*d*) *Origin of dwarfs and position effect.*

There are thus two possibilities regarding the origin of dwarfs:

(1) A deficiency at the point of interchange: as the pollen formation is normal this possibility has to be excluded, since deficiency in chromosome segments leads to imperfect pollen development. Marquardt (1937) however describes in *Oenothera* reciprocal deletion in the interstitial segments near the translocation regions. These deletions do not affect the viability of the plant as they involve only the heterochromatic regions which he considers to be inert and devoid of genes. This is quite contrary to the view of Darlington (1936*b*), who considers the interstitial segments as intimately linked with the potential complex and any deficiency in these would be detrimental to the complex.

(2) The second possibility is gene mutation at or near the point of

interchange, which is indistinguishable from "position effect". It is found in *Drosophila* that translocations are usually accompanied by mutations. A translocation which occurred spontaneously (Burkart, 1931) was found to be associated with a mutation to light body colour. In the X-ray induced chromosomal aberrations, the coincidence of the loci of mutations with those of chromosome breakages strongly suggests that these two phenomena are interrelated. Oliver's (1932) data justifies the conclusion that the rearrangement of chromosomal materials tends to be accompanied by mutations, and various hypotheses were suggested to explain this fact. The whole problem is adequately reviewed by Dobzhansky (1936). The best explanation for the phenomenon is that afforded by the position effect hypothesis. In the words of Muller & Altenburg (1930) "The alteration in intermolecular surroundings of the genes directly adjacent to the points of breakage and re-attachment, in other words, the alteration in intergenic contiguities, has in itself brought about a change in the quantity or quality of the physico-chemical action of these genes upon the protoplasm, so as to make them somewhat different genes, as though gene mutation has taken place in the genes on either side of the breakage and attachment points". To put it in a simpler way, the shifting of a portion of a chromosome, or a group of genes from one place to another, in the same or in different chromosomes, tends to influence the action of adjacent genes, producing in effect the result of gene alteration or mutation. This position effect hypothesis was arrived at by Sturtevant (1925) on the basis of his studies of the unequal crossing-over at the bar locus in *Drosophila melanogaster*. Normally the homologous chromosomes are broken at the same level between the loci of the same two genes, with the result that the exchange of segments does not involve the loss or gain of a single gene to either chromosome. It was found in the bar locus in *Drosophila* that mutations occurred which were always accompanied by crossing-over in the immediate vicinity of that locus in the chromosome. The chromosomes carrying the bar may be broken one to the right and the other to the left of the bar locus, and thus by crossing-over, one of the resultant chromosomes will have two bar genes and in the other there will be no bar gene. The phenotypic effect of these changes showed that two bar genes located adjacently in the same chromosome produce a stronger effect on the eye size than when each of these are situated in separate chromosomes. That bar eye is itself a duplication of certain discs in the salivary chromosomes has been demonstrated by Bridges (1936). This is one of the many illustrations of position effect given by various investigators.

Most of these cases may still be explained without assuming position effect. The phenotypic change may be due to gene alteration or mutation at the point of break. The crucial test of the position effect hypothesis would be the reversibility of the change with the restoration of the original phenotype. That is, if a gene changes its behaviour due to the disruption of its original association, will it revive its normal expression if its original associations are restored? If it does, then it means that no permanent alteration has taken place in the gene material, and the phenotypic effect of this change would really be due to position effect. Such a proof was obtained by Grüneberg (1937). In a stock homozygous for a long inversion of the *X*-chromosome, and inseparably associated with a gene for very rough eye surface, animals appeared in which the "rough" gene has reverted to the normal. Associated with this back mutation was a reinversion. It was found that the breakage points leading to reinversion were identical with those of the original inversion, the normal order of genes being restored precisely. This has also been confirmed cytologically by Emmens (1937) by a study of the salivary chromosomes in the same material. Since the phenotypic effect appeared simultaneously with the inversion and disappeared with reinversion, this case furnished crucial evidence of position effect.

Muller *el al.* (1935) have shown that most of the apparent mutations produced by X-rays are due to gross and minute intergenic rearrangements (position effects) so that the latter being of such a nature, a genetic discrimination between them and true intragenic change (gene mutation) would be difficult or impossible. Recently, Goldschmidt (1937, 1938) has doubted the very existence of "genes", and according to him all mutations are the result of chromatin rearrangements. The problem of the "gene" is more complex and uncertain than ever, but the fact remains that a "gene mutation" is the phenotypic expression of a change in the chromosome thread and this change, whatever it is, denotes a "difference" which has arisen at certain loci in the thread and is perpetuated. This view was put forward by Gates (1915, 1933), who considered genes as differentiations of many kinds and sizes which have arisen in the core of the chromosomes during their evolution, making them a nest of catalytic substances.

It remains to be considered whether the dwarf mutants occurring in the semi-sterile progeny are the result of position effect due to segmental interchange. On a comparison with homozygous interchange races derived from experimentally evolved segmental interchange heterozygotes, as in *Datura*, maize and *Pisum*, where not a single case of position

effect has been reported, it may not be different in rice. Further, there is practically no difference in appearance between the normal plants and the heterozygote, in which there is one set of interchanged chromosomes, as in *Drosophila*, where the mutations accompanying breaks in the chromosomes occurred in heterozygous state as most of the homozygous translocations were lethal, and so the probability of position effect is very doubtful. The origin of the dwarfs may therefore be due to minute deficiency or gene mutation at or near the point of interchange of segments in one of the chromosomes.

(*e*) *Interlocking of a bivalent in the ring of four in the semi-sterile mutant.*

In two cells at diakinesis configurations, composed of six chromosomes, were observed in semi-steriles (Text-figs. 14 and 15). Evidently this condition represents interlocking of a bivalent in the ring or association of four. This interlocking does not persist at metaphase, as a number of the plates examined did not show this phenomenon. The occurrence of interlocking is due to the distribution of threads at zygotene. When two chromosomes pair, a third chromosome of another pair may remain between the pairing threads, and the formation of chiasma on either side in both the chromosome pairs will bring about interlocking of bivalents observed at diakinesis and metaphase. Interlocking has been observed to take place in zygotene in *Dendrocoelum* (Gelei, 1921), *Viviparus* (Belar, 1928) and in *Allium* (Levan, 1933). Interlocking between bivalents has not been so far reported in normal diploid rice and its occurrence in segmental interchange hybrids is significant, which perhaps indicates that the chances of a chromosome intervening between the four pairing arms of the association of four are greater than in the simple straight pairing bivalent threads.

(*f*) *Behaviour of fragments.*

As mentioned already, one semi-sterile plant out of four examined has fragments of chromosomes in addition to the full complement. These had no visible effect on the phenotype. In addition to the ten bivalents and the association of four chromosomes, fragments, the number of which varied from one to three, even in the same plant, were observed to be present from diakinesis. One was more frequent than two, and three was rare. They were usually found free or associated with one another or with individual bivalents. At first division, most of the plates showed only one fragment. Two cells showed two in each (Text-figs. 16 and 30), in one of which both were towards one pole, while in the other they were opposite one on each side of the equatorial plate, having evidently

separated before the other bivalents. The single fragment seen in most of the cells behaved irregularly. The inclusion of the fragment in the daughter cells seemed to depend upon its position during metaphase I. If it is nearer to one pole it may be included in one of the daughter cells and divide at division II. Second division plates showed the fragment mostly only in one of the daughter cells (Text-fig. 23), or it may divide late on the spindle and only one of the halves is then included in the telophase nucleus, the other half being left out in the cytoplasm (Text-fig. 21). When the fragment is situated in the equatorial plate, it divides always later than the bivalents (Text-figs. 18, 19). If its division is during early anaphase, each of the halves is included in the daughter cells (Text-fig. 22), while if it is during late anaphase, after the chromosomes have moved to the poles, the chances of their inclusion in the daughter cells are remote (Text-fig. 20). Second division plates also show the same type of irregularity, so the chances of the elimination of the fragment are great, which is quite obvious from the fact that only one plant out of four contained these fragments, although all the plants were derived from the same ancestor. The fragments must have originated as a result of X-radiation, and their persistence for two generations indicates that they have autonomous movements which is possible only if they have the spindle attachment region, Navashin (1932). Mather & Stone (1933) have demonstrated that fragments without attachment region (acentric fragments) are passive and are lost during cell division. Such fragments arising out of crossing-over in relatively inverted segments in inversion heterozygotes are similarly not perpetuated, owing to the absence of spindle attachment.

Gates & Thomas (1914) found that one of the fifteen chromosomes of *Oenothera lata* may sometimes fragment and degenerate during meiosis, but occasionally male gametes may be formed with one or more fragments in addition to the haploid complement. This condition was surmised to be the cause of the origin of plants with an extra fragment from the progeny of *Oenothera lata* crossed with *O. Lamarkiana* (Lutz, 1916). Fragments have been reported in other genera and their behaviour was irregular, resulting in their elimination in the progeny. Goodspeed (1929*b*) reports the occurrence of fragments in five plants in X_1 generation of irradiated *Nicotiana*. In one of the plants the presence of an extra fragment had no effect on the external morphology of the plant, as it resembled the control, and this instance is similar to the case reported here in rice. Probably these fragments represent inert material situated near the spindle attachment.

Cases of persistent fragments noted to occur in X_2 and X_3 progenies are mentioned by Goodspeed & Avery (1930). Their appearance at metaphase I is ascribed to either primary or secondary origin, but in either case they have not suffered somatic elimination. In rice the fact that several pollen mother cells show only one fragment suggests that the other fragment or fragments get eliminated in the premeiotic divisions, so the chances of survival of such fragments are reduced in every generation and they tend to be completely eliminated in the course of a few generations.

(*g*) *Spindle.*

The disappearance of the nuclear membrane, terminating diakinesis, (Text-fig. 25) leads to the grouping together of bivalents due to the loss of surface charges, the presence of which was inferred by Gates (1909) in diakinesis from the mutual repulsion exhibited by chromosomes at that time. A later stage shows the chromosomes spread out and orientated in various directions (Text-fig. 26). Some of them are found to have developed thin tapering fibres at both the attachment regions, indicating that each chromosome is surrounded by its own spindle. Text-figs. 24*a*, *b*, represent tripolar spindles in both first and second divisions similar to the observations of Kuwada (1910) and Selim (1930) in rice. Successive stages from tripolar spindle in first division to the normal bipolar spindle are represented in Text-figs. 27, 28.

In this connexion it would be worth mentioning the occurrence of a compound spindle in the homotypic division in haploid rice (Text-fig. 29). Marked secondary association was prevalent, and it was found that some of the secondarily associated chromosomes had spindles with distinctly separate poles. Another cell in anaphase I in the semi-sterile rice showed a bivalent, which was off the plate, having a separate spindle (Text-fig. 30). These observations support Gates' (1932) opinion that the spindle is a compound structure.

Evidence of Hughes-Schrader (1924), F. Schrader (1932), and Davie (1933), tends to show that the central spindle is intranuclear in origin. The regular bipolar orientation of the spindle indicates the operation of an external agent or agents situated on either side of the equator. The existence of this external agent is also inferred from the formation of cell walls in portions of cells devoid of chromosomes, as in certain grass hybrids and haploids (Kagawa & Chizaki, 1934; Chizaki, 1934). The occurrence of unattached spindles (Darlington & Thomas, 1937), in *Lolium-Festuca* hybrid, where the spindle passes completely round the

chromosomes as though avoiding them, is further evidence for the inference of an outside influence. The compound spindle observed in rice is found to occur in various stages in the above grass hybrid, from the compact bipolar spindle down to irregularities where each chromosome has a spindle of its own. The authors attribute the cause of this abnormality to the lack of co-ordination in the timing relationships between the pole-directing or determining agents (external agents) and the centromere spindles. According to them the multipolar condition in higher plants must probably be due to the fact that the external pole-determining agents exist as diffused particles instead of congregating or uniting as they perhaps normally do when the regular bipolar spindle is formed. All these observations point to the conclusion that some agent outside the nucleus is responsible for organizing the spindles. It is quite probable that these agents perform the function of centrosomes, though at times they fail to co-ordinate, resulting in the conditions outlined above.

(ii) *The stumpy and beaked sterile mutants*

(*a*) *Association of five chromosomes.*

The examination of diakinesis and metaphase in the above two mutant plants showed that they are trisomics. Their cytological behaviour, however, is different. In the stumpy, the extra chromosome is associated in a chain of three (Text-fig. 31) or five (Text-fig. 32) or may occur as an unpaired free univalent. Sometimes the chain of five may break up, giving a chain of four and a univalent. The different associations are as follows:

	Chromosome association			
	Five	Four and one	Three and 1 bivalent	Free univalent and 2 bivalents
No. of cells	10	3	9	4

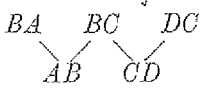
The multivalents are always in the form of chains and are never found united at the ends to give a ring configuration. If the additional chromosome were only a simple extra duplication, not more than an association of three could be possible, except in very rare cases where associations of five could be formed similar to the rare occurrence of a quadrivalent in diploid and sexivalent in a triploid, reported in rice and mentioned in the early part of this paper. The regular behaviour giving rise to associations of five indicates structural change in the extra chromosome. If *AB*, *CD*, *EF*, etc., represent the haploid complement of twelve chromosomes, each chromosome being represented as composed of two segments, the extra chromosome in "stumpy" is represented as *BC*.

On the basis of pairing of homologous segments, the maximum of five chromosomes could be formed as *BA*, *AB*, *BC*, *CD*, *DC*. Smaller associations might result from competition in pairing, where more than two homologous sets are involved. I could not make out the associations of five and four in metaphase, though diakinesis showed them very clearly. Associations of three could easily be recognized at metaphase and these were either in the form of a chain (Text-fig. 33) or a *Y*. The *Y* trivalent was characteristic in not being in the equatorial plane along with other bivalents (Text-fig. 35). The lack of proper orientation of the trivalent is perhaps due to its asymmetrical nature, having the centromeres not in a line with the axis of the spindle (Darlington, 1936*a*).

The homologues of the trivalent separate at anaphase without any apparent irregularity, two going to one pole and one to the other (Text-fig. 36). The behaviour of the univalent, however, is rather varied. When it happens to be at or near the equator, it may split simultaneously with the bivalents (cf. Text-fig. 42) or lag behind until all the bivalents have completely disjoined, after which it may divide, with the probability that the split halves will not be included in the daughter nuclei. If the univalent is nearer one pole it may be included in the telophase nucleus and divide in the next division, or it may divide at late anaphase and one of the halves only be included in the daughter nucleus (Text-figs. 40, 41). So the theoretical chance of half the spores containing the extra chromosome is not fulfilled. From the breeding results, it is seen that the extra chromosome is not functional on both pollen and ovule side, as otherwise we should expect tetrasomics ($2n+2$), which are viable in rice (Ramanujam, 1937). Most probably ($n+1$) pollen is not functional. Reciprocal crosses between diploids and trisomics will indicate the extent to which pollen and ovules are responsible for the transmission of the extra chromosome.

(*b*) *Disjunction.*

From the multivalent associations it is probable that the extra chromosome in the various gametes may not be the same. In a chain of five the probable functional form of disjunction will be



so that the extra chromosome will be the interchange chromosome, while in the association of three it may be either

$$(1)\ \frac{BA\ BC}{AB} \quad \text{or} \quad (2)\ \frac{BA\ AB}{BC}.$$

In the latter case one of the gametes will be deficient and the other will get an extra chromosome similar to one of the chromosomes of the haploid complement. It is thus possible to get in the progeny a type of trisomic different from the parental form. A new type of mutant, "long and narrow grain", has actually been found. It occurred in a small proportion in the progeny of the stumpy mutant (vide Table II). This plant has not yet been examined cytologically, but from the breeding behaviour it also resembles a trisomic.

Cytological examination of the beaked sterile showed that it was different from stumpy in not having higher associations than three chromosomes. Out of 50 cells examined at diakinesis and metaphase, in 45 cells the extra chromosome was unpaired and free (Text-figs. 37, 38). The univalent was at the equator in 15 cells and in the rest of the cells nearer one pole or the other (Text-fig. 39). Univalent behaviour as regards division and inclusion in the daughter nuclei was similar to that described for "Stumpy", but due to the greater proportion of cells having the free univalent the chances of the inclusion of the extra chromosomes are much less, which accounts for the smaller proportion of trisomics in the progeny. This trisomic is evidently one of the primary types, as associations of more than three were not met with. The origin of trisomics in general is due to non-disjunction. The failure of the chromosomes to disjoin may rarely occur in the diploids during meiosis, giving rise to daughter cells, one with $(n+1)$ and the other with $(n-1)$ chromosomes. In *Datura*, Belling & Blakeslee (1924) observed eight cases in 1137 sporocytes. Numerous other cases have been reported by Goodspeed (1923), Ruttle (1927), etc. Non-disjunction may occur in somatic divisions as well. Hedayetullah (1932) inferred pre-meiotic non-disjunction in *Oenothera* from his observations of groups of trisomic cells in diakinesis.

Non-disjunction is more likely in polyvalent associations than in purely bivalent associations. Trisomics arise regularly in the progeny of triploids. In rice they have been found to occur in the progeny of an auto-triploid rice (Ramanujam, 1937). The occurrence of trisomics in the progeny of structural heterozygotes as a result of chromosome non-disjunction has been recorded in maize in the progeny of semi-steriles (Burnham, 1930). These were the tertiary trisomics in which the extra chromosome was composed of parts of two different members of the haploid set, and these form chains of five chromosomes as in stumpy mutant in rice: the fact that the stumpy mutant was isolated from the same line as the semi-sterile may be a confirmation of the nature of the extra chromosome. Hakansson (1929) suggested the possibility that

trisomics may occur in *Pisum*, as a result of his observations of second metaphase plates. Sansome (1933) found two trisomic plants in the offspring of a plant with associations of six chromosomes in *Pisum*.

The number of trisomics involving different chromosomes will correspond to the number of chromosomes in the haploid complement in a simple diploid. The isolation of these different types of trisomics is important from a general point of view, to determine the characters associated with particular chromosomes. In maize, the occurrence of a triploid plant in 1925 was the starting point for the determination of linkage groups (McClintock, 1929; Randolph & McClintock, 1926). In *Oenothera*, the number of trisomics is much more than the number of chromosomes in the haploid complement, due to different types of non-disjunction peculiar to ring formation and combinations with the two different complexes (Catcheside, 1936*b*; Ford, 1936). Another peculiar feature in *Oenothera* is that some of these trisomics are true breeding. Gates & Nandi (1935) report a case of a true breeding trisomic. The reasons for their extraordinary behaviour are given by Catcheside (1933, 1936*b*).

6. DISCUSSION

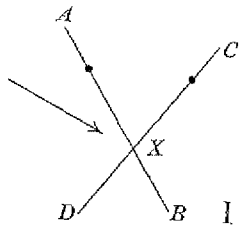
Segmental interchange has been inferred as a result of hybridization in geographical races and species, as in *Datura* and *Pisum*. In maize, *Nicotiana*, wheat and rice it arose out of X-radiation. Navashin & Gerassimova (1936) have found that exactly similar changes to those produced by irradiation are characteristic of seeds germinated after a long period of dormancy. In the naturally occurring genus *Oenothera*, this interchange is maintained in a heterozygous state by special lethal mechanisms associated with homozygosity, while in some ring forming plants, such as *Rhoeo*, *Campanula*, etc., it is evident that some such mechanisms are in the process of evolution, as evidenced by the persistence of the ring forms. From all these it can be surmised that among the many structural changes that are characteristic of species evolution, segmental interchange occupies a prominent position, as it has not only a survival value, but under certain conditions it has been specially favoured by nature.

The existence of this structural change in nature leads one to infer the special mechanisms involved in favouring this change. Segmental interchange between homologous chromosomes is obviously the result of the pairing of homologous threads accompanied by breaks in the identical loci, and reunion of the parental threads, known as "crossing-

over". Here special conditions of pairing between homologous chromosomes during meiosis bring about the inferred change. No such pre-existing conditions are known to account for segmental interchange between non-homologous chromosomes, which we find to be one of the factors determining species evolution. Irradiation experiments have shown that the primary change induced by X-rays is fragmentation. Fragmentation followed by reunion of the fragments with broken ends of other chromosomes brings about translocation. The evidence so far indicates that fragments are never known to unite with whole ends of chromosomes. Stadler (1932) has pointed out that simple translocations and terminal inversions have never been shown to follow irradiation. Supposed simple translocations (Burnham, 1930) have been later shown to be reciprocal translocations where one of the segments was very small. There thus seems to be a fundamental difference between whole and broken ends. Peto (1935) found in heat-treated barley that the broken ends of chromatids have an unsatisfied attraction which makes them unite at random with any other broken end located in the vicinity.

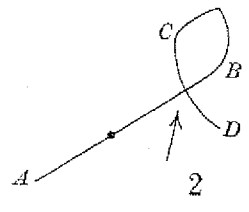
Stadler (1932) considers that the mechanism of reciprocal translocation involves random breakage and the subsequent reattachment of broken ends with an unlimited time interval between the two events, extending even to numerous cell generations, but as the acentric fragments are known to be lost at mitosis, a delay before reunion could take place is improbable. The most probable view is that of Catcheside (1935), who considers that when breaks occur at the overlap of two chromosome threads, the broken ends immediately rejoin at random. In this manner, Text-fig. 44 explains how an inversion, segmental interchange and deletion, etc., could take place as a result of the impingement of the ray at the crossing points. The type of rearrangements from such breaks and reunions will depend upon which of the two broken ends unite. For instance, the end in *A* uniting with the end in *C* will give an acentric fragment and a dicentric thread which are not mechanically sound and are eliminated, so the efficient combinations are the original combination and the new ones giving reciprocal translocations. It can be seen from the diagram how two interchanges can take place to give a ring of six by the overlap of three chromosomes in two different ways, (3) or (4). The chances of three threads passing at the same point are more remote than the probability 3, so it is clear how rings involving more than four chromosomes can occur directly as a result of the bombardment of X-rays in *Oenothera* (Catcheside, 1935), and in maize (Anderson, 1935). This hypothesis of breakage and direct reunion seems to be a more

probable explanation of reciprocal translocations found in nature, as well as under the influence of X-rays. These changes may occur at any stage in the life cycle of the plant organism. Seeds when treated to X-rays show the result of interchange in the meiotic divisions of X_1 generation. Similarly when pollen is treated and pollinated as in *Oenothera*,



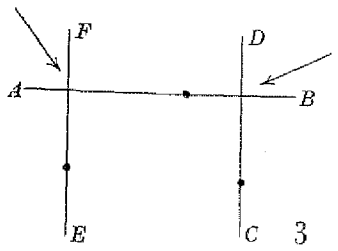
Different kinds of reattachment

(1) *AB CD* (original)
(2) *AC DB*
Dicentric acentric
(3) *AD BC* (reciprocal interchange)
(4) *AXD*, and *CX* (deletion of *XB* segment)



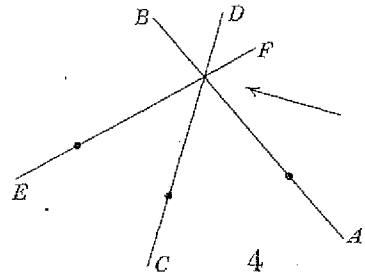
A B C D (Original)
A C B D (Inversion)

A B C } Deletion of
A C B } *D* segment



Interchanged functional chromosomes
AB CD EF (original)
AE BF CD (one interchange)
AD BC EF (one interchange)

AE BC DF (two interchanges)



AB CD EF
AD BC EF
AB DE CF
CD BE AF
BE CF AD
DE BC AF

Text-fig. 44. Illustrating the recombinations after breakage at the overlap of the chromosome threads.
The arrow indicates the point of breakage of chromosomes.
Black dot indicates the position of the centromere.

the F_1 plants may show rings at meiosis. Such changes are also known to occur spontaneously in the pre-meiotic divisions, as in *Secale cereale* (Darlington, 1933) where a group of eight cells showed evidence of interchange.

Darlington (1929*b*) considers another possibility of the occurrence of segmental interchange between non-homologous chromosomes. In an

organism subject to structural changes in the process of evolution, there is the probability of a translocation of an interstitial segment from one chromosome to a corresponding part of one not homologous with it. The steps leading to this involve a series of interchanges. Thus if a chromosome is considered to be differentiated into three segments as *A X B*, and if *A* and *B* get interchanged with different segments from two different chromosomes, we can have a chromosome of the make-up *C X E* leading to the reduplication of *X* segments, and the pairing of these two different chromosomes in the *X* segment will be the basis for a new interchange—*BC* and *AE* chromosomes. This is an indirect way, but may represent an infrequent method by which such changes are brought about. In *Pisum*, in a ring of six chromosomes (Sansome, 1932), crossing-over in the homologous interstitial segment in an otherwise non-homologous chromosome as illustrated above, was the cause of a new segmental interchange. Darlington (1931) has stressed the importance of this phenomenon and has explained the origin of the half-mutants in *Oenothera* on the basis of such crossing-over (Sweet, 1938).

Morgan *et al.* (1925) have suggested that translocations may be caused by the interlocking of non-homologous chromosomes during the process of synapsis as described by Gelei (1921), Sax (1930) and Catcheside (1932), consider that this phenomenon may be responsible for segmental interchange. If the interlocking between bivalents is not released at later stages, any strain may involve breaks of the chromatids at the point of contact, so that a section of one chromosome might become attached to another non-homologous chromosome. They consider the prevalence of interlocking observed in *Oenothera* (Cleland, 1922) as significant in this connexion.

Though segmental interchange between non-homologous chromosomes occurs in nature in plants like *Pisum* and *Datura*, and chromosome rings arise as a result of hybridization between the different races involving the interchange, the heterozygous state is not advantageous to the plants as it produces sterility. This is due, as we have seen, to the random orientation of the ring on the spindle. Artificially produced interchange heterozygotes in maize, rice and *Nicotiana*, give the same result, so here we find no further evidence of the evolutionary stages which lead to the highly evolved complex heterozygote in *Oenothera*.

In the case of *Triticum* and a solitary case in *Datura* (Blakeslee & Cleland, 1930), fertile hybrids are produced due to the zigzag arrangement of the ring on the spindle. Segregation leads again to the production

of homozygous and heterozygous types. This condition may represent the possible initial stages of the ring forms in nature. *Rhoeo*, *Campanula*, *Anthoxanthum* and *Aucuba* are still further advanced as they show the incipient stage of evolution due to the persistence of ring forms in nature.

The elimination of homozygotes occurs to some extent in *Campanula* (Gairdner & Darlington, 1931), and this condition is the beginning of the perfect mechanisms involved in the elimination of homozygotes in *Oenothera*.

7. SUMMARY

1. The genetical and cytological behaviour of three mutants derived from X_3 generations of irradiated rice seed are described.

2. The semi-sterile mutant was heterozygous and gave rise to plants like the parent, besides normal fertile plants and dwarfs roughly in the proportions 2 : 1 : 1. Semi-sterility was found to be due to the association of four chromosomes during meiosis. The commonest configuration involved a chiasma in the homologous regions proximal to the interchange in a pair of chromosomes. This indicates crossing-over of the interchanged segments resulting in chromatid non-disjunction and 50 % gametophytic abortion.

3. One of the semi-sterile plants exhibited the presence of two fragments which had no visible effect on the phenotype. The behaviour of the fragments indicated that they suffer rapid elimination during the formation of gametes.

4. The dwarf plants arose only in the progeny of semi-sterile plants while the normal fertile plants bred true. Cytological examination showed that the dwarfs contained the normal complement of chromosomes and the meiosis was regular, resulting in the formation of fertile pollen, although the plant did not set seed. The origin of this mutant is discussed; it is probably due either to a small deficiency or a gene mutation at or near the point of interchange.

5. The two other mutants, "stumpy" and "beaked sterile", did not breed true to type, and were found to be $(2n+1)$ types. In the case of "stumpy" a chain of five chromosomes was of frequent occurrence and indicated that the extra chromosome was composed of segments derived from the two non-homologous chromosomes of the haploid set. The other trisomic was of the primary type, the extra chromosome representing the duplication of one of the chromosomes of the haploid set.

6. The cytological basis of reciprocal translocations and its importance in the evolution of species in plants is discussed.

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LEGENDS TO TEXT-FIGS. 2–42.

Text-fig. 2. Diakinesis in normal rice showing 12 bivalents.

Text-figs. 3–8. Diakinesis in semi-sterile mutant showing association of four chromosomes and ten bivalents and fragments.

Text-fig. 3. A ring of four and two free fragments.

Text-fig. 4. A chain of four and a single free fragment.

Text-fig. 5. Association of four with a chiasma in one pair—one free fragment.

Text-fig. 6. Association of four giving a necktie configuration. Two fragments, one associated with a bivalent.

Text-fig. 7. "Figure of eight"—two free fragments.

Text-fig. 8. Association of four with a chiasma in each of the pairs. Two fragments associated with different bivalents.

Text-fig. 9. Twelve bivalents with two fragments paired.

Text-fig. 10. "Semi-sterile mutants" polar view of metaphase I.

Text-fig. 11. "Dwarf mutant" somatic metaphase ($2n \times 24$).

Text-fig. 12. "Dwarf mutant" diakinesis.

Text-fig. 13. "Dwarf mutant". Polar view of metaphase I.

Text-figs. 14 and 15. "Semi-sterile." Interlocking of a bivalent in the ring of four.

Text-fig. 16. Semi-sterile. Side view of metaphase I showing fragments disjoined before the bivalent.

Text-fig. 17. "Semi-sterile" side view of metaphase I. Single fragment associated with a bivalent.

Text-fig. 18. "Semi-sterile" anaphase I. The fragment is undivided at the equator.

Text-fig. 19. "Semi-sterile." The fragments dividing later than the bivalents.

Text-fig. 20. "Semi-sterile." Fragment dividing at very late anaphase I.

Text-fig. 21. "Semi-sterile." Fragment not included in a daughter nucleus.

Text-fig. 22. "Semi-sterile." Side view of metaphase II with a fragment in each of the daughter cells.

Text-fig. 23. "Semi-sterile." Polar view of metaphase II, a fragment in one of the daughter cells.

Text-fig. 24*a*. "Semi-sterile mutant." Tripolar spindle, division I.

Text-fig. 24*b*. "Semi-sterile mutant." Tripolar spindle, division II.

Text-fig. 25. "Semi-sterile mutant." Prometaphase I.

Text-fig. 26. "Semi-sterile mutant." A slightly later stage.

Text-fig. 27. "Semi-sterile mutant." Convergence of tripolar spindle.

Text-fig. 28. "Semi-sterile mutant." Convergence of bipolar spindle.

Text-fig. 29. "Haploid mutant" anaphase II. Showing the compound spindle.

Text-fig. 30. "Semi-sterile mutant" compound spindle. Two fragments towards one pole.

Text-fig. 31. Stumpy mutant ($2n+1$). Diakinesis. A chain of three chromosomes.

Text-fig. 32. Stumpy mutant ($2n+1$). Diakinesis. A chain of five chromosomes.

Text-fig. 33. Stumpy mutant ($2n+1$). Side view of metaphase I chain trivalent.

Text-fig. 34. Stumpy mutant ($2n+1$). Polar view of metaphase I.

Text-fig. 35. Stumpy mutant ($2n+1$). Side view of metaphase I. Y trivalent not oriented at equator.

Text-fig. 36. Stumpy mutant. Anaphase I showing 13 and 12 chromosomes on either side of the equator.

Text-fig. 37. "Beaked sterile." Diakinesis showing two bivalents and the unpaired univalent on the nucleolus.

Text-fig. 38. "Beaked sterile." Polar view of metaphase I univalent not in the equatorial plane.

Text-fig. 39. "Beaked sterile." Side view of metaphase I showing the univalent off the plate.

Text-fig. 40. "Beaked sterile." Divided univalent near one pole at late anaphase.

Text-fig. 41. "Beaked sterile" one-half of the divided univalents not included in the daughter nucleus.

Text-fig. 42. "Beaked sterile." Polar view of metaphase II, each daughter cell having one-half of the divided univalent.

EXPLANATION OF PLATE I

All drawings were made at bench level with the aid of a camera lucida. An achromatic objective N.A. 1·3 was used in conjunction with Zeiss eyepiece K. 25, giving approximate magnifications of 4000 diameters. These were reduced to half size in reproduction.

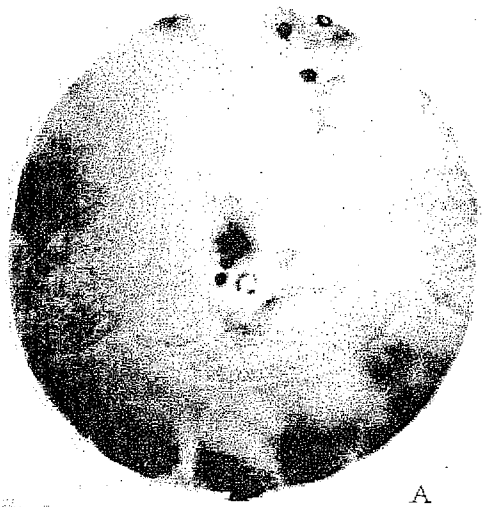
A. "Semi-sterile mutant." A ring of four chromosomes at diakinesis (cf. Text-fig. 3). ($\times 2000$.)

B. "Semi-sterile mutant." A chain of four chromosomes at diakinesis (cf. Text-fig. 4). ($\times 2000$.)

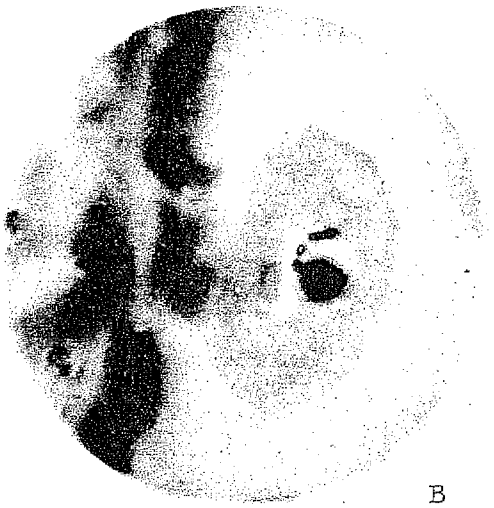
C. "Semi-sterile mutant." Association of four chromosomes with a chiasma in one of the pairs at diakinesis (cf. Text-fig. 5). ($\times 3000$.)

D. "Semi-sterile mutant." Side view of metaphase I, showing the configuration of four chromosomes and a free fragment. ($\times 3000$.)

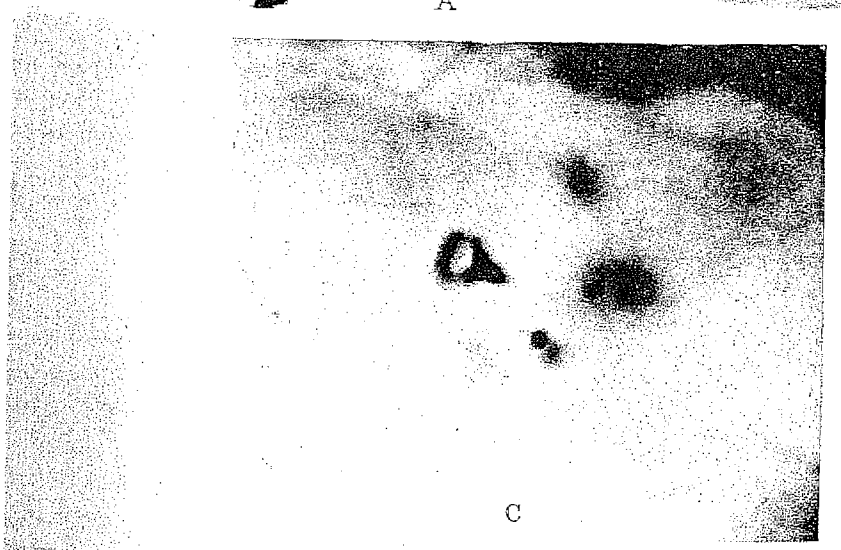
E "Stumpy mutant" ($2n+1$). A chain of five chromosomes at diakinesis. The fifth chromosome is out of focus (cf. Text-fig. 32). ($\times 3000$.)



A



B



C

D E