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CHROMOSOME PAIRING IN AUTO-POLYPLOIDS

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The paper comprises a review of chromosome pairing in auto-polyploids, with emphasis in auto-tetraploids, at pachytene and discusses its bearing on the origin of different kinds of multivalent types of diakinesis and metaphase I of meiosis, utilising the data already available in maize and *Brassica* to a large extent, and in *Sorghum, Tripsacum* and tomato to a lesser extent.

Certain generalisations on the chromosome pairing at pachytene in polyploids have been made. Pachytene pairing is usually between homologous parts only at any point. Four homologues may form two separate pairs or one or more exchanges of partner may take place in an association. The exchanges show a lack of intereference. The frequency of exchanges in a chromosome arm is proportional to its length. The number of effective pairing blocks increases with the chromosome length up to a certain limit beyond which it decreases and the mean length of the pairing block increases with the length of the particular chromosome of the complement. Association of all four homologous centromeres in an auto-tetraploid occurs frequently and these are to be taken as points of exchange of partner to satisfactorily account for the frequency of multivalents at diakinesis and metaphase I. The positions of exchanges suggest that the initial points of pairing are generally distributed at random along the length of the chromosome, irrespective of the fact whether they are differentiated or undifferentiated chromosomes and that the initial points of pairing may be asociated particularly with ends of arms, centromere and knob regions.

I feel grateful for being asked to deliver the Bashambar Nath Chopra Memorial Lecture endowed to commemorate the memory of Dr. B. N. Chopra by his family members and I take this opportunity to pay my humble homage to his memory.

As is well known, the sexually reproducing higher organisms, plants and animals, are characterized by the phenomenon of alternation of generations between the gametophytic and sporophytic generations, the former constituted by cells with haploid chromosome number and the latter by diploid chromosome number. The proportion of the life-cycle occupied by these two generations is varied according to the group of organisms they belong to. The diplod state is brought about by fusion of male and female gametes, the process being known as fertilization. As the chromosome number in the nuclei is constant for the species concerned, the doubled chromosome number in the zygote and sporophyte is to be reduced to half through a special kind of cell division called meiosis which has been defined by its superficial phenomena "as the occurrence of two divisions of a nucleus accompanied by one division of its chromosomes and results in the production of four nuclei, each of which has

one chromosome of each set in the mother nucleus, provided that their distribution has been regular". The first of these two division steps is known as Meiosis I and the second as Meiosis II. Meiosis I is characterised by pairing of two homologous chromosomes, one from each of the haploid set. Pairing is the phenomenon of chromosomes coming together in pairs and the paired chromosomes become self duplicated and exchange portions at places along the length of the chromosome. When the exchange involves non-sister chromatids, it leads to the formation of the chiasmata and the phenomenon is known as cross-over and recombination. As is well known this phenomenon forms the corner stone of the edifice of variation, heredity and evolution.

Meiosis I may be subdivided into Prophase I, Metaphase I, Anaphase I and Telophase I stages and similarly Meiosis II into Prophase II, Metaphase II, Anaphase II and Telophase II. Prophase I is a prolonged process and is further distinguished into leptotene, zygotene, pachytene, diplotene and diakinesis stages and it is during this phase that important differences arise between chromosome pairing in diploids and auto-polyploids resulting in Metaphase I configurations of complexity in the latter. This lecture is devoted to explain how these different configurations arise in relation to pairing behaviour of chromosomes during prophase I.

For our purposes polyploids may be recognized into two broad catogories, namely, (i) Auto-polyploids, and (ii) Allo-polyploids. The latter are those with more than two sets of chromosomes and arise from diploids in which the haploid sets are different from each other such as in hybrids, while auto-polyploids are those which have more than two sets of similar haploid sets, three, four or more chromosomes of each kind in the set. The auto-polyploids with three sets are known as auto-triploids, with four as auto-tetraploids, with five sets as auto-pentaploids and so on. In considering the phenomenon of pairing, I would like to restrict myself to that occurring in auto-tetraploids, a few of which have been investigated by my co-workers and myself.

The prophase I chromosomes are in their most attenuated condition at the leptotene stage and through the process of condensation they become thicker and shorter as prophase I progresses. Pairing is not evident at leptotene at which stage the chromosomes lie scattered within the nucleus. At this stage and subsequent stages up to pachytene, the chromosome presents an appearance of a string of unequal beads placed at unequal distances from each other. The beads are called chromomeres. They are constant in number, size and position for each chromosome and at the pachytene stage serve as landmarks of identification of particular chromosomes in the haploid set. In fact Lima-de-Faria (1952) constructed chromosome maps for the different chromosomes in the haploid complement of rye. Later Reddi (1958) and Ehrlich (1960) also identified chromosomes on the same basis in *Sorghum purpureo-sericeum* and *Saintpaulia ioantha* respectively. Gottschalk and his associates (Gottschalk 1951, 1954; Gottschalk and Peters 1956) used the number, size and position of the macrochromomeres in the heterochromatic sections of the pachytene chromosomes in the identification of different chromosome types in Solanaceae.

For purposes of quantitative study of pairing in chromosomes at the pachytene stage the criteria of (i) relative lengths, (ii) arm ratio (as determined by the centromere position), (iii) knobs, if present, and (iv) chromomere pattern have been

successfully employed in identifying individually each chromosome of the haploid set. Indeed this has been done in our laboratories in maize, *Sorghum, Pennisetum, Brassica,* Solanums and others (Venkateswarlu 1950, 1963; Venkateswarlu and Reddi 1956; Reddi 1958; Venkateswarlu and Pantulu 1968*a*, 1968*b;* Venkateswarlu and Kamala 1971; Venkateswarlu and Bhiravamurthy 1969) and similarly by others elsewhere in tomato, rye, rice and a few other plants. Pachytene chromosomes are also broadly distinguishable into (i) differentiated chromosomes, and (ii) undifferentiated chromosomes. Differentiated chromosomes show regions of deep stainability the extent and distribution of which have been good aids for identifying individual chromosomes of the haploid set, in addition to the other criteria. These occur in tomato, some solanaceae, eu-Sorghums, *Plantago, Pennisetum* and *Tripsacum,* etc. The undifferentiated chromosomes stain uniformly all along the chromosome and occur in maize, teosinte, rye, barley, rice and others.

Pachytene Pairing in Auto-Tetraploids

In auto-tetraploids there are four chromosomes for each of the kind of chromosomes in the complement while in diploids there are only two of each kind. All the four chromosomes of any particular chromosome of the complement, present in an auto-tetraploid are described as homologues as pairing affinities among them are equal but the pairing can be only between any two of the four homologues at a given region. If such a 2/2 pairing involves the whole length of the chromosome, it would result in the formation of two pairs or bivalents. Thus from any such set of four homologues there result two bivalents at diakinesis and metaphase I, ofcourse if chiasma formation occurs between the chromatids of the pairing chromosomes. The formation of two pairs from out of the four homologues does not always take place. In a proportion of cases the pairing may be between two of the chromosomes for some length, and along the length of the chromosome different pairs may be involved in a group of four homologues. Where two different such pairs meet having one chromosome in common between them, they exchange partners and the number of such exchanges may vary in the same plant from cell to cell. There are 0–2 exchanges in maize, 0–3 in *Tripsacum* and *Sorghum,* 0–4 in *Brassica* and 0–6 in triploid and pentaploid tulips and aneuploid *Hyacinthus* (Venkateswarlu 1950, 1962; Tantravahi 1968; Reddi 1970; Venkateswarlu and Kamala 1974; Newton and Darlington 1929; Darlington 1937).

From the earlier works on prophase pairing (Newton and Darlington 1929; Darlington 1929) it was suggested that there are two phases in the process of pairing, an earlier phase of generalized attraction among all four homologues of the same chromosome in an association of four and a second phase of attraction between any two of them only at any particular point along the length of the chromosome.

As the exchanges are fewer than initial points of pairing between any two of the homologues, it is clear that particles neighbouring to those which come to pair first are largely influenced in their choice of partner by the pairing of the adjoining particles and thereby the chromosomes come to pair in blocks. This is one of the conclusions reached by Newton and Darlington (1929) and Darlington (1929) from their qualitative observations on pairing of prophase chromosomes of aneuploid *Hyacinthus* and triploid tulips. The position of paired chromosomes lying between two exchange

points or an exchange point and the end of the arm concerned is recognised as a pairing block. It should be inferred that there are two pairing blocks if there is one exchange and three when there are two exchange points in an association and so on. Thus the number of pairing blocks would be one more than the number of exchange points and if there is no exchange, the four homologues form two bivalents and the pairing block would be one.

As there are quantitative data on the pachytene pairing only in a few auto-tetraploids namely maize, tomato, *Sorghum* and *Brassica*, further consideration in this lecture is given only to these to make some generalizations as follows :

(*i*) There have been observed in all the above auto-tetraploids certain cases where all the homologous centromeres are stuck together. However, it is only in maize that the quantitative data on diakinesis and metaphase I are also readily available (Venkateswarlu 1950). The question whether associations of the four homologous centromeres represent cases of exchange of partners or merely cases of interlocking could be examined in maize alone. Table I shows the data in maize on centromere associations.

On the assumption that the centromere associations do not represent cases of exchange of partners, the 35 cases of homologous centromere association with no exchange elsewhere should form 70 bivalents at diakinesis and metaphase I. Including these cases in a total of one hundred observed associations with no exchange, 200 bivalents should be expected at metaphase I. From the metaphase I observations, about 43 per cent of the bivalents in the tetraploid cells are ring bivalents. If the centromere associations represent true interlocks, it would be expected that 43 per cent of the 70 or about 30 bivalents, formed from associations showing homologous centromere associations would show interlocking at metaphase I. Amongst 60 metaphase nuclei, only one case of true interlocking between two bivalents was actually encountered. Further in an organism like maize with an unpolarised meiotic nucleus, pairing commences at random and it is therefore not possible that interlocking would always be at the site of the centromere. On the other hand the assumption that the centromere associations represent cases of exchange of partners during prophase is supported by the following considerations. An analysis of 60 metaphase I nuclei showed that 74 per cent of the possible trivalents and quadrivalents are formed. However, only 48.7 per cent of the observed pachytene associations showed one or two exchange of partners which could lead to the formation of multivalents. Of the pachytene associations, about 18 per cent showed no exchange of partners but showed only homologous association of all the four centromeres. If these are also taken as representing an exchange of partner at the site of the centromere, the percentage of multivalents expected would be near to the observed (48.7 per cent actually observed + 18 per cent of centromere associations = 66.7 per cent) namely 74 per cent. Therefore, the centromere associations seem to represent exchange of partners during prophase I. This conclusion derives support by the circumstance that the structure of the centromeres also shows a linear arrangement of chromomeres as in the rest of the parts of the chromosome. It may be relavant to state here that Gottschalk (1955) found in tomato single exchanges located mostly in insertion (centromere) regions in chromosome 11. Further, reduction in the percentage of multivalents as resulting from exchanges of partner at sites other than centromere is not due to lowered

TABLE I

Homologous association of all four centromeres			No homologous association of centromeres but with exchange elsewhere	Total associations examined
With no exchange elsewhere	With exchange elsewhere	Total		
35	45	80	115	195

TABLE II

Frequency of different numbers of exchanges of partner in 4n maize
(after Venkateswarlu 1950)

Chromosome		Number of exchanges (*X*)					Mean no of exchanges for group of four	X^2	*P*
		0	1	2	3 or more	Total			
1–10 taken together	Obs:	100	74	21	0	195	0.60	2.3655	0.5–0.3
	Cal:	107.2	63.96	19.04	4.38	195			

chiasma frequency as actually it is observed in 4n maize (colchiploid) the chaisma frequency turned out to be significantly more than twice that in the corresponding diploid meiotic nuclei in the same plant.

(*ii*) *Frequency of different numbers of exchanges*— Table II summarises the data for associations of four chromosomes at pachytene with none, one, two, three or more exchanges in auto-tetraploid maize. These data have been compared with the expectation that exchanges occur at random without mutual interference and in all the cases the poisson expectation* of independent occurrence of exchanges along the length is very good. Thus there does not seem to be any interference between the occurrence of one exchange with a neighbouring one.

Similar analysis has also been made recently for 4n *Brassica campestris* var. *oleifera* (Venkateswarlu and Kamala 1974) and in this species also the occurrence of exchanges along the length are according to poisson distribution.

(*iii*) *Distribution of exchanges between the two arms of a chromosome*—A chromosome has two arms demarcated by the centromere. Its position in various chromosomes of the haploid set varies from median, submedian to subterminal positions thus permitting the grouping of chromosomes into symmetrical and asymmetrical ones. In the last mentioned category one arm will be markedly shorter than the other. In an auto-tetraploid where pachytene associations have been studied, it is possible when adequate number of observations have been made, to study the

*For this purpose the formula $n.\ \frac{e^{-m}\ m^x}{\lfloor x}$ has been used; where m = observed number of exchanges for group of four, n = total number of groups of four observed, and x = the number of exchanges.

distribution of exchanges between the arms and to say that the number of exchanges occurring in any arm is proportional to its length or not. Studies on this aspect were first made by me in colchicine induced auto-tetraploid maize and subsequently by others in *Tripsacum laxum* (Tantravahi 1968), *Sorghum miliaceum* and *S. panicoides* (Reddi 1970) and *Brassica campestris* (Venkateswarlu and Kamala 1974). From all these data it seems clear that the occurrence of one or more exchanges is proportional to the length of the arm. Far few auto-tetraploids could be studied with reference to the pachytene associations to say whether any other factors are also involved.

(*iv*) *Position of exchanges*—The actual position of an exchange is related to the position of initial points of pairing. When initial points of the same two of the four homologues are involved all along the length of the chromosome, they form two bivalents, if chiasma formation takes place between them. However, where initial points involve two different chromosomes, with one of them being common, the pairing regions spread and where they meet each other, there occurs an exchange of partners and the number of exchanges varies according to the number of combinations of pairing along the length of the chromosome. From the data available, the initial points of pairing seem to be situated in a random fashion along the length of the chromosome. From the work on auto-tetraploids with differentiated chromosomes (Reddi 1970; Venkateswarlu and Kamala 1974) the exchanges occur also in the heterochromatic as well as euchromatic regions. Peters (1954) recorded a higher frequency of exchanges in euchromatic regions in *Solanum tuberosum* and *S. andigenum* while as already stated, Gottschalk (1955) observed in autotetraploid tomato single exchanges located mostly in centromere regions (88.9 per cent) in chromosome 11. One more point emerges from the study of adequate samples of pachytene associations in chromosomes 6 and 9 in induced auto-tetraploid maize (Venkateswarlu 1950). In these chromosomes, the exchanges are clustered between the centromere and the ends of the arms and also to some extent between a knob on an arm and its end. This suggests that the centromeres, the ends of the arms, and the knobs where present, seem to be more favoured points of initial attraction between any two of the four homologues.

(*v*) *Number and length of pairing blocks*—Pachytene stage represents the stable pairing between chromosomes which is two by two pairing in any region. Of the three, four or more chromosomes in auto-polyploids, the whole chromosome does not act as a unit but only part of the length of a chromosome forms a unit of pairing, such a unit of pairing being designated as a "pairing block" (Stone and Mather 1932). Some inferences have been drawn by Darlington and Mather (1932) and Stone and Mather (1932), on the basis of the study of diakinesis and metaphase I configurations in triploid tulips and diploid and triploid *Hyacinthus* respectively. A verification of these inferences has become possible now owing to the availability of quantitative data in auto-tetraploids of maize, *Tripsacum* and *Brassica* (Venkateswarlu 1950, 1962; Tantravahi 1968; Venkateswarlu and Kamala 1974), based on direct observations and measurements of number and length of pairing blocks in particular chromosomes of their chromosome complements at the pachytene stage. These observations show that the mean length of an effective pairing block increases with the increase in length of the chromosome. It is also noticed that

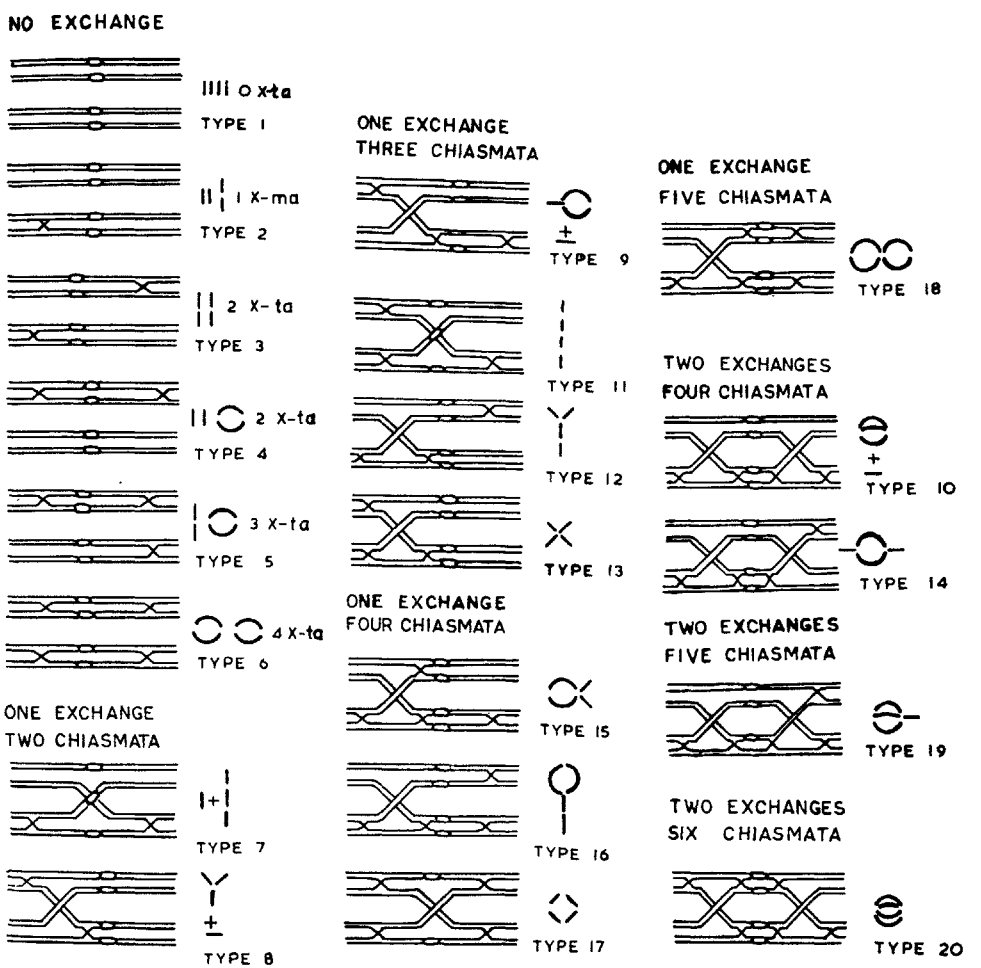

FIG 1. Diagram showing the various types of pachytene configurations with the minimum number of exchanges of partners and their distribution and the minimum number of chiasmata and their distribution at appropriate places along the length of the chromosome required for the formation of various configurations ranging from univalents to quadrivalents at diakinesis (*Linnert's diagram* 1948, *slightly modified*). The diakinesis configurations with type numbers assigned to them by Darlington (1937) are also given side by side.

there is a decrease in the mean number of effective pairing blocks beyond a certain limit in the length of the chromosome. The inferences made by Stone and Mather (1932) on the basis of a study on diakinesis and metaphase I stages in triploid *Hyacinthus* are in accord with the observations made at the prophase I chromosome associations of a few auto-tetraploids mentioned above.

(*vi*) *Relation between pachytene configurations and diakinesis and metaphase I configurations*—During the prophase I, the chromosomes undergo a gradual condensation as meiosis progresses in time, and the condensation process is maximum at diakinesis and metaphase I stages, while chiasmata formed involving two portions of non-sister chromatids in the regions paired become evident at the diplotene

stage. At this stage, in some organisms the intercalary chiasmata shift their position to the ends of the arms concerned due to (*i*) further condensation of the chromosomes, and (*ii*) repulsion forces between pairs of chromosomes. This process is known as terminalization. In such organisms where terminalization is complete certain characteristic configurations ensue. In auto-tetraploids these have been given numbers from 1 to 20 (see Fig. 1) which represent all possible configurations in an auto-tetraploid. However, all these kinds have not so far been observed to occur together in the same species. For the formation of configurations representing trivalents and quadrivalents two conditions are required to be fulfilled, namely, (*i*) formation of minimum number of exchanges followed by (*ii*) formation of minimum number of chiasmata, both distributed at appropriate places in one or both arms (as the case may be) along the length of the chromosome during prophase I pairing. Total failure of chiasma formation in the paired regions will lead to the formation of four univalents in auto-tetraploids and three univalents in auto-triploids irrespective of the presence of exchanges. If no exchanges are formed during prophase it will lead to the formation of two bivalents in tetraploids and a bivalent and a univalent in triploids provided the necessary chiasmata at appropriate places are formed. If one exchange and two chiasmata are formed at appropriate places, trivalent types 7 and 8 arise; if one exchange and three chiasmata are formed trivalent type 9 and quadrivalent types 11 to 13 result; if one exchange and four chiasmata are formed quadrivalent types 15 to 17 occur; if one exchange and 5 chiasmata are formed quadrivalent type 18 would follow. If two partner exchanges are formed during prophase I pairing accompanied by four chiasmata at appropriate places, trivalent type 10 and quadrivalent type 14 ensue; if two exchanges with five chiasmata are formed it would result in quadrivalent type 19 and with six chiasmata in type 20. However, there are auto-polyploid organisms with more than two partner exchanges, these represent more than minimum conditions necessary and do not make any difference as to the type of configuration provided, however, terminalization of chiasmata is complete. The 20 kinds of configurations (recognised as Types 1-20 by Darlington 1937) and the relation to pachytene configurations and chiasmata formation are shown in diagram drawn after Linnert (1948).

These are the processes involved in the pairing of chromosomes in auto-polyploids and two stages may be broadly recognised as pachytene pairing and metaphase I pairing.

Ultimately the metaphase multivalents orient in a number of ways and at the end of meiosis I, each daughter nucleus contains only half the number of chromosomes, which is maintained in the daughter nuclei formed after meiosis II.

The above account represents the processes involved in the pairing of auto-polyploids and not many studies are available because of the time-taking nature of the work and tireless patience involved as also choice of material amenable for such studies both at pachytene and later at diakinesis and metaphase I. Such studies go a long way in providing fundamental information about our understanding of the genetic systems in polyploid crop plants and because of its possible use in polyploid breeding work.

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