The Segregation of Heteromorphic Homologous Chromosomes in Pollen Mother-Cells of *Triticum vulgare*

By

C. Leonard Huskins and Jane D. Spier,
McGill University, Montreal

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Introduction

On account of their significance for an understanding of meiosis and of the relationship of chromosome behaviour to genetic phenomena, heteromorphic chromosome pairs have been the subject of many studies, but very diverse conclusions have been reached by different workers.

Robertson (1915) showed that the unequal bivalents which he studied in *Tettigidea parvipennis* and *Acridium granulatus* invariably underwent pre-reduction, i.e. they segregated in the first meiotic division and split equationally in the second. At the time he held lack of equational first divisions to be direct evidence against the chiasma-type theory of genetic crossing-over. Later (1931) he mentions that some figures of his earlier (1916) paper indicate that crossing-over may have taken place at some previous time.

Wenrich (1916) found in two individuals of *Phrynotettix magnus* a heteromorphic bivalent that divided reductionally at the first division in one half of the observed cases and equationally in the other half. He considered that "In the cases in which [bivalent] C divides reductionally in the first division, the spindle-fibre attachment is necessarily shifted to the distal ends", and held that the shape or position of the tetrad when it is first brought under the influence of the spindle determines the mode of division. McClung (1928) considered this a possibility for a heteromorphic bivalent of *Mecostethus gracilis*, but stated that sufficient work had not been done on critical stages to make it certain.

Carothers (1926, 1931) describes segregation of six pairs of unequal homologues in *Trimerotropis citrina*, *Mecostethus gracilis*, and *Amphitornis bicolor*, three species of the Acrididae. The ratio of pre- to

post-reduction varies for these unequal pairs. For one bivalent segregation takes place at the first division in only one per cent of the cells, for another in 95 per cent. Carothers remarks that the cause of such "differential behaviour" is a question of interest, but does not examine it in relation to the chiasmotype or any other theory of crossing-over, though later, in relation to synapsis, she notes that the "phenomenon of crossing-over occurs during the process, either by means of chiasmatype or otherwise".

Belling (1931 b) has shown that the behaviour of a heteromorphic bivalent in *Aloe purpurescens* conforms to expectation on the partial chiasmotype theory of crossing-over, and the observations of Robertson, Wenrich, and Carothers can be interpreted in accordance with this hypothesis. Direct evidence from the comparative behaviour of different heteromorphic chromosomes of one species has, however, previously been lacking and three possible explanations of "differential behaviour" are still held by various workers. These are: (1) that equational first divisions are due to reciprocal segmental interchange, i.e. crossing-over, between non-sister chromatids, sister chromatids always being associated at the spindle attachment; (2) that either sister or non-sister chromatids may be associated at the attachment; (3) that the position of the spindle attachment may change during meiosis, owing to the position occupied by the bivalent at the time of spindle formation.

During the course of other studies, a number of wheat plants with heteromorphic bivalents were discovered. These differed in degree of inequality and therefore promised to provide evidence significant for the general problem. Three of them were selected for this study.

Material and Methods

One heteromorphic bivalent was found in each of the following plants of *Triticum*:

- (a) A heterozygous speltoid 43-chromosome wheat, grown from Nilsson-Ehle's "C-series strain 26-1295".
- (b) A 42-chromosome plant from a sub-compactum form provided by Dr. Å. Åkerman, Svalöf, Sweden.
- (c) A 42-chromosome plant from a heterozygous speltoid, series C, which was received from Professor Jur. Philiptchenko, of the Institute of Genetics, University of Leningrad.

Spikelets of (a) with the ends of the glumes clipped off were fixed by Kihara's method (1924)—Carnoy 6:3:1, followed by medium Flemming. The material was paraffin embedded and cut at 14 microns. Newton's iodine gentian-violet staining method was used. Metaphase plates and anaphase stages of pollen mother-cells were examined.

Anthers of (b) and (c) were dissected and aceto-carmin smears made of the pollen mother-cells. These were made permanent by the McClintock (1929) method and the behaviour of the heteromorphic homologous chromosomes was followed through metaphase and anaphase.

Observations

Heteromorphic Bivalent of (a): The presence of this heteromorphic pair was noted earlier (Huskins 1928) as a "cytological abnormality due to the loss of approximately half a chromosome". The spindle-fibre attachment on the larger chromosome is median; that on the smaller, terminal (Text figs. 1-3; Plate 9, photomicrograph 1).

Figs. 1-4. $\times 1900$. 1. Chromosome complement of plant (a). 20 bivalents, one unequal, and 1 trivalent. 2, 3. Reductional division of unequal pair of plant (a). 4. Equational separation of lagging univalents of plant (a).

Following pairing, the heteromorphic chromosomes appear invariably to undergo a reductional first division. Typical metaphase figures

are shown in Text figs. 1 and 3, and an anaphase separation is illustrated in Text fig. 2. Such reductional separations were found in 56 nuclei at metaphase and 4 at anaphase.

In 34 nuclei the unequal homologous chromosomes were found to be unpaired. In this case, as univalents, they evidently behave independently of each other and move by chance to the same or to opposite poles. Occasionally, at late anaphase, lagging unpaired univalents undergo an equational division on the plate (Text fig. 4).

Heteromorphic Bivalent of (b): Unlike the bivalent of (a), both members of this pair have a submedian attachment constriction (Text fig. 8; Plate 9, photomicrographs 2 and 4). Moreover, they are always paired at metaphase.

In 35 of the 39 cells in which the heteromorphic pair could be distinguished clearly, the first maturation division was reductional (Text figs. 5, 8).

Figs. 5-8. $\times 1300$. 5, Chromosome complement of plant (b). 21 bivalents, one unequal. 6, 7, Chromosome complement of plant (b), showing heteromorphic bivalent as a ring chromosome with unequal arms extending from the ring. 8, Reductional first divisions of heteromorphic bivalents of plant (b). Fig. 9. Equational separations of unequal bivalent of plant (b). $\times 2660$.

In three cells, however, a ring bivalent with unequal arms extending from the ring was found at metaphase (Text figs. 6, 7, 9). Obviously, an equational separation will result from such a figure. One other cell showed an equational separation of the heteromorphic pair at a slightly later stage (Text fig. 9).

Heteromorphic Bivalent of (c): In the third unequal pair, the attachment constrictions are also both sub-median. There is a slightly greater discrepancy in size between the homologues than in (b), and at metaphase the members may be paired or unpaired.

This heteromorphic pair was studied in 48 metaphase nuclei. In 31 it was found to be undergoing reductional separation and in 11 it was dividing equationally (Text fig. 10; Plate 9, photomicrograph 6). In 6 nuclei, the unequal homologues were unpaired (Text fig. 11; Plate 9, photomicrograph 5). In two cases, comprising one reductional and one equational separation, the heteromorphic pair was associated with another pair of chromosomes in a quadrivalent configuration. This observation, though very significant

Fig. 10. Equational first division of unequal homologous chromosomes of plant (c). $\times 2660$.

Fig. 11. Chromosome complement of plant (c), showing unpaired, unequal homologous chromosomes. $\times 1300$.

for the speltoid problem, is germane to the present study only in that the equational separation within the quadrivalent shows, on the chiasma theory of metaphase pairing, that the longer of the unequal chromosome arms is capable of forming at least two chiasmata.

Discussion

a. *Origin of Unequal Bivalents:* Carothers (1931) holds that in three of the unequal chromosome pairs which she studied in the Acrididae the inequality is due to an increase of chromatin material and "is brought about by the transformation of terminal granules into chromosome vesicles which become densely chromatic and are maintained"

Robertson (1915) and Wenrich (1916) speak of chromosome deficiencies as the cause of inequality in this same group of Orthoptera. It is clear that the chromosome inequality in the Nilsson-Ehle (a) material is due rather to a loss than to an increase in chromatin material, since the chromosome complements of normal plants fail to show bivalents comparable in size to the smaller chromosome of the unequal pair and none of them have terminal attachments. The short chromosome has evidently been broken at the attachment constriction, and the half lacking the point of spindle-fibre attachment has been lost.

b. *Unequal Bivalents in Relation to "Pre- and Post-Reduction" and Crossing-over*: The work of Robertson, Wenrich and Carothers shows very clearly that pre- or post-reduction is a function of each bivalent and not of the chromosome complement as a whole. This cytological evidence is paralleled by the genetic observations of Allen (1926) on *Sphaerocarpus*, of Dodge (1931) on *Neurospora*, and of Goldschmidt and Katsuki on gynandromorphs and mosaics of the silkworm moth (Goldschmidt, 1932).

The decisive results of Stern (1931) on *Drosophila melanogaster*, and of Creighton and McClintock (1931) on *Zea Mays* have established conclusively the correlation between genetic and cytological crossing-over. As a corollary of their work, it is now clear that in these two organisms a given division may be reductional for one *part* of a chromosome and equational for the rest; in short, that the type of separation is a function not of whole chromosomes but of their constituent parts.

This was first postulated in the partial chiasmatype theory of Janssens (1909). His theory was first based upon observations on *Batrachoseps* and later substantiated (1924) by his observations on the Orthopteran genera, *Chorthippus* (*Stenobothrus*) and *Stethophyma* (*Mecostethus*).

The observations of Stern and of Creighton and McClintock do not bear upon the problem of whether chiasmata cause crossing-over, as Sax (1930) maintains, or are themselves caused by it, as current formulations of Janssens' partial chiasmatype hypothesis hold (see Belling, 1931 a & b, Darlington, 1932, and others). The present observations being upon metaphase and later stages likewise lack significance in this respect. They do indicate that equational first divisions of heteromorphic bivalents are due to crossing-over, however it may have occurred. This hypothesis, the first of the three possible explanations suggested, fits all the observations.

The heteromorphic chromosomes of (a), that is, those with median and terminal attachment constrictions, showed only reductional first divisions. As seen in text figure 12 (A, B), that is the result to be expected on the first explanation. Crossing-over in this bivalent can occur only between equal arms and therefore would not result in a visibly equational separation. If non-sister chromatids may be associated at the spindle-fibre attachment, as the second explanation demands, equational separations might result, as shown in text figure 12 (C). These were not observed.

Chromosomes of (b) and (c), on the other hand, showed both equational and reductional first divisions. Equational separations are to be expected if sister chromatids are associated at the spindle-fibre attachment, and if an exchange between non-sister chromatids of the unequal arms takes place in some of the divisions (Text fig. 13, B).

Figs. 12 and 13. Explanation in the text.

The second explanation, that equational separations result from association of non-sister chromatids at the spindle-fibre attachment, would fit the behaviour of the chromosomes of (b) and (c) (Text fig. 13, C). Whether sister chromatids are always associated at the attachment constriction is not definitely known. The absence of equational separations of the unequal chromosomes of (a) is significant in this respect. If non-sister chromatids can associate at the spindle attachment, equational separations of these chromosomes of (a) might be expected to occur as frequently as those of (b) and (c). The number of cells of (a) recorded is, of course, not very large, but many more nuclei of it than of (b) or (c) have been examined and, further, if equational first division separations of the chromosomes of (a) occurred, they would be very easy to detect, while those of (b) and (c) are more difficult to distinguish with certainty. The difference of behaviour between the

unequal chromosomes of (a) and of (b) or (c) is therefore even greater than the numbers indicate.

The third possible explanation, that a change in position of spindle-fibre attachment occurs, is rendered improbable by the observations of many workers that the attachment constriction is a characteristic "fixed" feature of chromosome structure. Where changes do occur, they have been found to be transmitted from parent to offspring (Carothers, 1921). In view of this constancy, a change from one end to the other of a chromosome in 50 per cent of cases, as would be necessary to explain Wenrich's observations, is most improbable. Further, this change would involve association of non-sister strands at the attachment constriction also. In these *Triticum* heteromorphic bivalents, the spindle attachment has clearly remained in the same position, whichever mode of segregation has occurred.

The frequent lack of pairing of the chromosomes of (a) is in accord with the chiasma theory of metaphase pairing.

Summary

A heteromorphic bivalent, having one terminally and one medianly attached chromosome, always segregates reductionally in the first meiotic division.

Two heteromorphic bivalents, with sub-median attachments on each chromosome sometimes separate equationally in the first division.

The spindle attachment is shown to remain unchanged, and the possibility that non-sister chromatids may be associated at the spindle attachment is not in accord with these observations. The hypothesis that sister chromatids are associated at the spindle attachment and that equational first divisions are due to crossing over is in full accord with the observations.

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Explanation of Plate

Plate 9

- Photomicrograph 1. Unequal homologous chromosomes of plant (a).
- Photomicrograph 2. Unequal homologous chromosomes of plant (b).
- Photomicrograph 3. Chromosome complement of plant (c). See Text fig. 5.
- Photomicrograph 4. Reductional separation of heteromorphic bivalent of (b).
- Photomicrograph 5. Unpaired unequal homologous chromosomes of (c). See Text fig. 10.
- Photomicrograph 6. Equational separation of unequal homologous chromosomes of (c). See Text fig. 11.