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Chiasmata in spermatocytes of *Drosophila melanogaster*

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1. INTRODUCTION

The present paper deals with the nature of the chiasmata which can be seen at meiosis in the autosomes of *Drosophila melanogaster* males.

Much confusion has accumulated around the problem of recombination. Morgan in 1915 defined recombination genetically in terms of crossing-over as a result of exchange of homologous parts between homologous chromosomes. Janssens & Darlington (1930) gave a cytological definition according to which 'a chiasma is an exchange of partners in a system of paired chromatids'.

While the genetical definition is unquestionable—the cytological definition in its original form quoted above led to some divergence of opinion. Kaufmann (1934) described chiasma-like configurations in mitosis of neurocytes in *Drosophila melanogaster* male and female larvae; Cooper (1949) observed similar configurations in meiosis of *Drosophila* males. Both these papers argue against the interpretation of every chiasma as a visible proof for the occurrence of genetical crossing-over.

According to Kaufmann the separation of associated and duplicated homologous chromosomes during mitosis begins with the opening of the threads in two planes at right angles to each other, and if separation takes place at about equal rates, both between homologous threads and sister strands, distinct chiasmata results. Such chiasmata cannot be correlated with those observed at meiosis, but it would seem that caution is necessary in concluding that the presence of a chiasma is a sufficient proof that crossing-over has occurred.

Cooper reported that in *Drosophila melanogaster* males the frequency of autosomal bivalents having chiasmata at meiosis lies between 1 and 7·6%, giving an adjusted chiasma frequency of 3·8% per autosomal arm. This finding, together with the well-known fact that as a rule there is no genetically detectable crossing-over in *Drosphila* males, led Cooper to the conclusion that chiasmata may arise without antecedent crossing-over. He emphasized the uncertain genetical significance of the presence or absence of chiasmata in forms for which parallel genetic data are not available.

Observations of Kaufmann and Cooper seemed to undermine the whole principle of chiasma as morphological counterpart of crossing-over. This led Darlington (1932) to suggest that in *Drosophila* males two reciprocal chiasmata (i.e. two chiasmata, such that one cancels out the genetical effects of the other) are formed in the

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proximal inert heterochromatic regions. Although this suggestion can never be experimentally proved or disproved, there is some circumstantial evidence against it. The occurrence of two successive chiasmata requires a certain minimum length of the chromosome; while this length is perhaps available in the long heterochromatic segments of the sex chromosomes, the autosomal proximal heterochromatic regions are very short and cannot accommodate two chiasmata. Detailed crossing-over studies by Green (1962) showed that in flies homozygous for a duplication and containing in addition autosomal inversions, double crossovers occurring in less than 10 map units are recovered. In normal chromosomes and without the enhancement of inversions two reciprocal chiasmata cannot possibly occur in short proximal regions of the autosomes.

At the present time and by generally accepted definition a chiasma is a place in a bivalent at late prophase of meiosis where (1) the pairs of homologous chromatids remain in contact although they are separated in other parts and (2) an exchange of homologous parts between the paternal and maternal chromosomes has taken place.

The phenomena described in both parts of this definition are usually considered as two faces of the same thing, they are understood to be inseparable. In the present paper some evidence is given suggesting that, the place of contact, does not necessarily mean 'exchange of parts'.

2. MATERIAL AND METHODS

Three types of male were used: Ore-K as a representative of wild-type; T143/+, a stock of translocation heterozygotes; and T99/99, a double translocation stock. The translocation stocks were obtained from Dr H. Slizynska, who analysed them cytologically.

The T143/+ male contains a translocation of the following cytological constitution (given in terms of C. B. Bridges' reference system for salivary gland chromosomes):

21 ... 40—centromere of II—41 ... 56/94 ... 100
61 ... 80—centromere of III—81 ... 94/56 ... 60

Here both autosomes have each one arm unchanged, and this permits homologous pairing. Pairing of the translocated segments leads to combination of both bivalents into a huge structure.

The stock T99/99 contains two simultaneously produced autosomal translocations between the second and third chromosomes. The rearranged chromosomes have the following cytological constitution:

(1) 21 ... 40—centromers of II—41/91 ... 100
61 ... 80—centromere of III—81 ... 91/40 ... 21
(2) 21 ... 40—centromere of II—41/96 ... 100
61 ... 80—centromere of III—81 ... 96/40 ... 21

In these two translocations both second and third chromosomes have become uneven-armed and can be identified in mitotic and meiotic metaphase. When

chiasmata *sensu stricto* are produced in a bivalent with unequal arms an asymetrical configuration is formed, and such a configuration has indeed been found in meiosis in females of the stock T99/99.

Males two to three days old were dissected in acetocarmine and their testes were left in the stain for about 15 minutes. Afterwards the material was transferred in a drop of acetocarmine onto an albuminized slide and covered with a siliconized cover slip. The spreading of the drop between the cover slip and the slide produces some slight squashing of the testes. No pressure was applied. After 5–10 minutes the preparation was placed in 70% alcohol, in which the cover slip fell off. The preparation was then stained in basic fuchsin or in Feulgen strain and mounted in Euparal.

3. OBSERVATIONS

Figure 1–4 (Plate I) show meiotic stages in wild-type males. The first two photographs show the end of metaphase in side view. The ends of the homologous arms of each pair of autosomes are connected together so that the two homologues form one large ring. The area included between the contacting arms contains much stainable, Feulgen-positive material.

Figure 3 shows the beginning of anaphase separation. Homologous autosomes have become separated. The two arms of each individual chromosome show non-homologous association, so that each chromosome has assumed a ring or heart-shaped configuration. There is still much stainable material inside the ring.

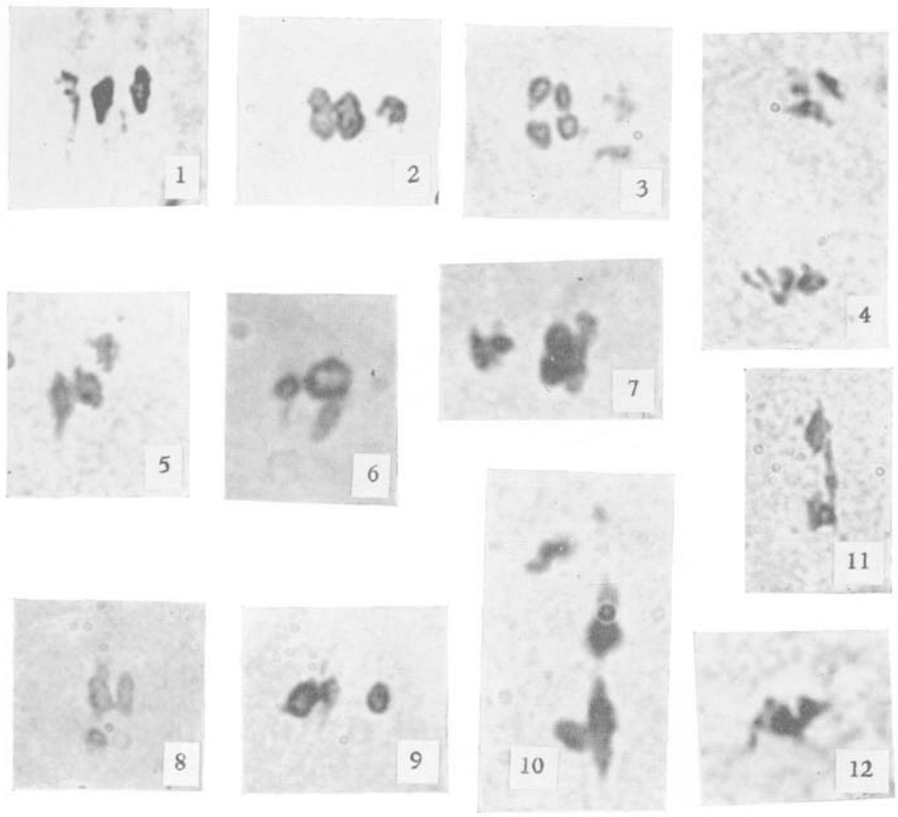
Figure 4 shows telophase. The arms have lost their contacts with each other, so that the distal ends of all chromosomes are free and the chromosomal elements assume the typical V-shaped figures. There is no stainable material around them.

Figure 5 shows a side view of meiotic metaphase of a T143/+ male. The autosomal bivalents behave as if there were no translocation between them.

Figures 6–11 show metaphase of meiosis in males of stock T99/99. As in the wild type metaphases, the autosomal bivalents have no loose free ends. The bivalents formed from the associated chromosomes invariably assume the shape of a ring or of an oval in metaphase. This is clearly different from the asymmetrical configurations observed in the oöcytes, which are typical for chiasma formation in submediocentric chromosomes (Fig. 12).

The size relations of these bivalents in males suggest that association may occur between homologous or non-homologous chromosomes. Rings of similar sizes indicate non-homologous association, while rings of unequal sizes results from homologous association. From the pictures of incipient anaphase one may suspect that associated non-homologous chromosomes may move together to the same pole without final separation.

The behaviour of the sex chromosomes in regard to pairing, chiasma formation (interstitially in X and proximally in Y), and separation differs from that of the autosomes. The present observations on the sex chromosomes fully confirm most of the facts recorded by Cooper.



EXPLANATION OF PLATE

Photographs represent chromosomes in meiosis of *Drosphilia melanogaster* males; Nos. 1–4 of Ore-K, No. 5 of T143/+ and Nos. 6–11 of T99/99 and No. 12 of female from T99/99 strain. Magnification about 900 ×.

Fig. 1. End of meiotic metaphase, the two autosomes differ slightly in size, are connected by chiasma-like structures and contain much stainable material.

Fig. 2. Another view of meiotic metaphase showing different staining of the autosomes.

Fig. 3. Anaphase: autosomes have separated but the arms of individual chromosomes show terminal associations assuming ring or heart shaped configurations; there is much stainable material between them.

Fig. 4. Telophase: chromosomes have assumed typical V-shaped figures and there is no stainable material about them.

Fig. 5. Side view of meiotic metaphase: presence of translocated segment in one arm of each autosome does not prevent its association with non-homologous partner.

Figs. 6 and 9. The autosomes are of unequal size and differ in intensity of staining, evidently the translocated segment acquired the character of the chromosome to which it has been attached.

Figs. 7 and 8. The two autosomes (also showing inequality of staining) are of similar size.

Figs. 10 and 11. Chiasmata and separation of sex chromosomes, the autosomes appear to move to the same pole without disjunction.

Fig. 12. Side view of metaphase plate in an oöcyte of T99/99 shows asymmetrical configuration typical for chiasma formation in sub-metacentric chromosomes.

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4. DISCUSSION

From the above observations the following tentative conclusions can be drawn: In the first meiotic metaphase of *Drosophila melanogaster* males the autosomes are connected by their free ends. This tendency to be connected is so strong that after separation of the homologous ends the association is transferred to the nearest chromosome end. Evidently the stainable substance is the main factor in this association and its amount on the chromosomes is so large that even non-homologous ends are kept together. It follows that this surface association between the ends of the arms of the same chromosome (that is between non-homologous ends) is of the same nature as the preceding association of homologous ends, which imitated chiasmata.

Thus, association of autosomal bivalents in males of *Drosophila melanogaster* is not of chiasmatic nature. It is a surface association only. It is therefore not related to any exchange of parts between the associated chromosomes and does not result in genetic recombination.

The autosomes are not synchronous in reaching the maximum charge of stainable substance and normally these time differences ensure the association of homologues and their orderly segregation. In the case of translocations, the translocated segment becomes synchronous with the main body of the host chromosome and this may result in non-homologous association. Generally the change of homology in the case of translocation heterozygotes might be expected to result either in disengagement of the chromosome arms or in arrest of the terminalization of chiasmata—neither has been observed here and the terminalization of 'chiasmata' proceeds as if there were no translocation but perfect homology.

Chromosome pairing is presumed to serve two main purposes: control of segregation and opportunity for genetic crossing-over. The observed surface associations of non-chiasmatic nature and the absence of genetic crossing-over in *Drosophila melanogaster* males suggest that only one of these functions has been preserved.

A similar separation of the functions of chromosome pairing is seen in the first meiotic division in the mouse, and probably in all mammalian oöcytes (Slizynski, 1960). After the pachytene stage, there is a rapid disappearance of stainable materials from the chromosomes, which thus become diffuse. In the following stage, the dictyotene, the bivalents are not visually separable. During this stage however, the chiasmata formed at the end of pachytene undergo terminalization. When the chromosomes appear again in prometaphase, most chiasmata are terminalized and there is rapid reformation of stainable materials around the chromosomes.

Thus in mouse oöcytes the stainable material on the chromosomes appears to be connected with pairing and with repulsion, both of which are movements of the whole chromosomes, but certainly is not necessary for the movements and terminalization of chiasmata, which evidently can proceed without any stainable material on the chromosomes.

The similarity between meiosis in *Drosophila* males and mouse females lies in the fact that in both cases the first anaphase separation is mechanically independent of chiasma formation.

Summing up it can be concluded that the two parts of the definition of a chiasma (quoted in the introduction) are not necessarily two faces of the same thing—there can be places of contact which do not result from the exchange of parts, but are due to the fusion of the stainable materials aggregated on the chromosomes.

SUMMARY

On the basis of chromosome behaviour in three strains of *Drosophila melanogaster* males—a wild-type strain, a translocation heterozygote strain and a double translocation strain—it is concluded that the cytologically detected apparent chiasmata in the autosomes are only surface associations of homologous chromosomes and do not involve exchange of chromosome material. Such chiasma-like associations occur also between non-homologous chromosomes. The stainable, Feulgen-positive material of the chromosomes is thought to be responsible for the surface associations.

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