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The Pattern of Polytene Chromosome Conjugation and Crossing-over in Interspecific Hybrids of *Drosophila*

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Summary. The pairing of polytene chromosomes was investigated in the hybrids between three closely related species of *Drosophila* belonging to the *virilis* species group. It was found that within the same hybrid different chromosome bands lost the ability to pair by differing degrees. Furthermore, the same chromosome sections paired with different frequencies depending on the hybrid involved. This study revealed that poor polytene chromosome pairing in the hybrids is not due to specific genetic interaction in the hybrids, but depends solely on the properties of the homologous loci themselves. It was also of interest to find whether the pattern of polytene chromosome somatic pairing resembled in some way the picture of chromosome synapsis during meiosis. To obtain evidence for this, crossing-over in the hybrid 5th chromosome was analyzed both genetically and cytologically (from salivary gland chromosome observations). It was found that the sections of the fifth chromosome which were characterized by a high frequency of conjugation in the salivary glands of hybrids also exhibited a high frequency of crossing-over in hybrid females. It may be concluded that sections of the polytene chromosome characterized by a low frequency of conjugation behave in the same manner in meiosis, and thus rarely take part in genetic recombination.

Introduction

For many years, biologists have been challenged by problems of chromosome pairing. The most interesting studies in this field have been made using the distant plant hybrids characterized by the abnormal formation of bivalents in meiotic prophase. However, the discovery of polytene chromosomes (Painter, 1933) in salivary gland nuclei of *Drosophila* gave a new subject for the study of chromosome pairing (the so called "somatic synapsis"). As in the case of plant hybrids, the conjugation of polytene chromosomes in interracial or interspecific *Drosophila* hybrids was found to be incomplete in comparison with "pure" species whose salivary gland chromosomes were intimately paired throughout their whole length (Pätau, 1935; Kerkis, 1936; Dobzhansky and Tan, 1936; Horton, 1939; Cordeiro, 1968). In summarizing the contradictory hypotheses concerning the causes of incomplete polytene chromosome conjugation in distant hybrids, Dobzhansky and Tan (1936) postulated three possible causes: very minute heterozygous rearrangements may prevent pairing because of a "conflict of the attractive forces"; specific interactions of genes may determine chromosome synapsis in the hybrid genotype; finally, point mutations disturbing the identity of homologous loci may lead to their "estrangement" (Kerkis, 1936) and the subsequent weakening of attractive forces between them.

Because of the scarcity of information about the nature of polytene chromosome conjugation it was decided to restrict these studies to salivary gland chromosomes of *Drosophila* interspecific hybrids. From the genetic point of view, the most interesting and puzzling problem seemed to be the incomplete pairing of polytene chromosomes in those regions which exhibit a quite symmetrical disc order and a similar number of discs. In the genetic literature the pattern of "somatic synapsis" in polytene chromosomes was often taken as a model of what takes place at meiosis. But such an extrapolation without experimental proof is untenable. The question now arises: Is there any correspondence between the pattern of "somatic synapsis" and the picture of homologous chromosome pairing in meiosis?

Unfortunately, it is not possible to compare directly the two types of pairing. But it is possible to attack the problem by studying the frequency of crossing-over in those chromosome regions exhibiting relatively intimate synapsis in the polytene chromosomes of interspecific *Drosophila* hybrids and in the regions characterized by constant lack of conjugation. If there is any correspondence between the schemes and forces involved in the two types of synapsis, we should expect a significant decrease in crossing-over frequencies in the asynapsed regions when compared with those chromosome sections exhibiting a high frequency of conjugation in the salivary gland nuclei of hybrids.

Materials and Methods

Three species of the *Drosophila virilis* group — *D. virilis*, *D. texana* and *D. littoralis* (Patterson and Stone, 1952) were used in the present studies. *D. littoralis*, found and described in the USSR by Sokolov (1959), does not correspond to the species with the same name in the Patterson and Stone classification. All three species can be hybridized under experimental conditions and produce viable and fertile hybrids. The interspecific hybrids were obtained in the following crosses:

I ♀ *virilis* × ♂ *texana*
II ♀ *virilis* × ♂ *littoralis*
III ♀ *texana* × ♂ *littoralis*

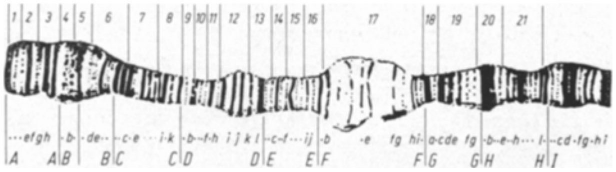

Fig. 1. The distal end of the fifth chromosome of *D. virilis* (Hsu, 1952), subdivided into sections

Aceto-orcein smear preparations of the salivary glands of third instar hybrid larvae were made. The quantitative analysis of the fifth chromosome conjugation in the salivary gland nuclei of the hybrids was performed. As an aid to analysis, the distal end of the fifth chromosome was subdivided into 21 arbitrary sections. The limits of these sections are indicated in Fig. 1 on the cytological map (Hsu, 1952) of the *D. virilis* fifth chromosome.

To compare the conjugation of the fifth chromosomes in the hybrids, 500 chromosomes of hybrid (I), 390 in hybrid (II) and 310 in hybrid (III) were examined.

To obtain data on the role of the genome in incomplete polytene chromosome conjugation in the interspecific hybrid, the stock of *D. virilis* carrying recessive marker genes scarlet (*st*) and eosinoid (*es*) eye colours in the fifth chromosome and a wild-type stock of *D. texana* were used. By means of eleven backcrosses one *D. texana* fifth chromosome in the heterozygous condition was transferred into an otherwise intact *D. virilis* genotype. Furthermore, a relatively small section of a *D. texana* fifth chromosome, including the wild type alleles *st* and *es* incorporated into the *D. virilis* fifth chromosome by spontaneous crossing-over, was also transferred by backcrossing into the *D. virilis* genotype. A comparative analysis was made of the mode of pairing in the fifth chromosomes of F_1 *virilis* × *texana* hybrids, and in the genotype of *D. virilis* into which a *texana* fifth chromosome had been substituted (FB_{11}). (Three hundred 5th salivary gland chromosomes were examined in each case.)

Crossover studies

The frequency of crossing-over in the fifth chromosome between the two markers *st* and *es* was measured in three different genotypes: in the pure *D. virilis* species (I); in F_1 *virilis* × *texana* hybrids (II); and in the case where the fifth chromosome of *D. texana* in heterozygous condition had been transferred by eleven back-crosses into the *D. virilis* genotype (III). Preliminary cytological experiments showed that in the last case in *FB*II all other *D. texana* chromosomes except the fifth were replaced by *D. virilis* chromosomes.

All test crosses were carried out following a standard procedure in order to minimize variations in recombination frequency due to maternal age and temperature. Females heterozygous for the fifth chromosome to be tested were collected as virgins and individually mated with males of *D. virilis* homozygous for the recessive markers.

The Cytological Studies of Crossing-Over

The hybrid females from *virilis* by *texana* crosses were backcrossed to *D. virilis* males (*FB*I) to investigate the crossing-over cytologically.

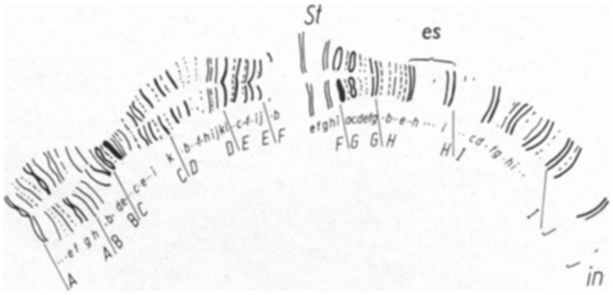

Fig. 2. The typical picture of fifth chromosome conjugation in *D. virilis* × *D. texana* F_1 hybrids (The position of the gene markers is indicated)

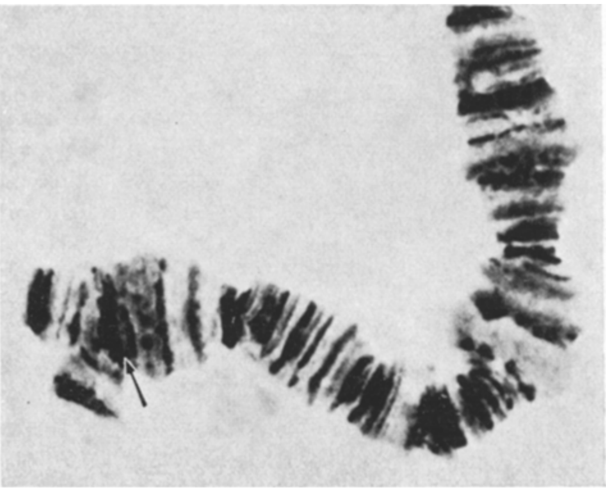

Fig. 3. The cytologically observed crossover in FB_1 (The arrow indicates the place of exchange)

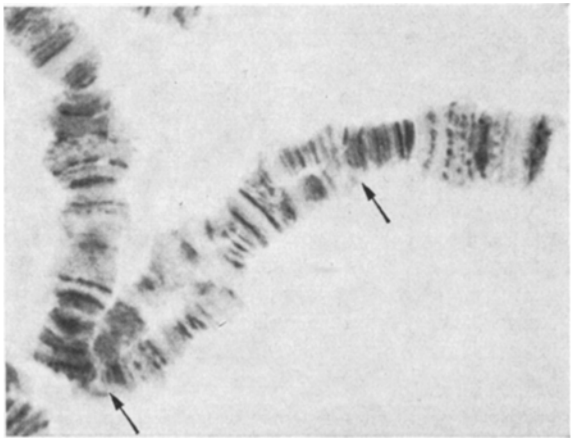

Fig. 4. A cytologically seen crossover in FB_1 resulting from a double exchange

The crossing-over in the distal end of the fifth chromosome was easily observed by the change in the typical polytene chromosome conjugation scheme when studying salivary gland chromosomes in *FB*I (fig. 2, 3, 4). To prove the genetic validity of the cytologically observed crossovers, flies, phenotypically identified as crossovers between *st* and *es* in FB_1 progeny were collected and individually crossed to *D. virilis* flies (FB_2). In this series of experiments the cytological positions of the genetic exchange were identified in the polytene chromosomes of FB_2 larvae.

Results and Discussion

Polytene chromosome conjugation in interspecific hybrids and the role of the hybrid genome.

The results of the quantitative study of polytene chromosome conjugation in three interspecific hybrids are shown in Fig. 5. It is noteworthy that the disc pattern in the distal ends of the fifth chromosomes is quite similar in all three species crossed. Thus the interspecific hybrids studied differ from the hybrids between two subspecies of *Chironomus* described by Keyl (1965) in which, within unpaired chromosome segments, the bands appear to correspond in their linear arrangement but vary in their size and relative DNA content.

It is evident from Fig. 5 that different loci in the same hybrid lose the ability to conjugate by differing degrees. Some chromosome sections paired quite frequently while others only rarely paired with their homologues. Furthermore, as illustrated in figure 5, the disturbances in pairing differed significantly in the three hybrids: *virilis* × *texana* hybrids (I) were characterized by the poorest conjugation; *texana* × *littoralis* hybrids (III) showed more complete pairing; and *virilis* × *littoralis* hybrids occupied an intermediate position. As it was evident that hybrids between closely related species exhibited different modes of polytene chromosome pairing, it became important to know whether the poor conjugation was due to the specific action of imbalanced genotypes in hybrids or whether the ability to conjugate depends solely on the properties of the homologous loci themselves. To do this quantitatively, conjugation of the distal ends of *D. texana* and *D. virilis* fifth chromosomes was studied in F_1 *virilis* × *texana* hybrids (I), and in the *D. virilis* genotype where the whole *D. texana* fifth chromosome (II) or its region (cut by crossing-over) (III) have been transferred by backcrossing.

The results of this study are presented in Fig. 6. It is obvious that there is no significant difference in the frequency of conjugation in all three cases.

It should be emphasized that the *D. virilis* and *D. texana* fifth chromosomes exhibited quite similar modes of conjugation in the F_1 hybrid between these species and in the *D. virilis* genome. Furthermore, a rather small section of the *D. texana* fifth chromosome also conjugates with a homologous region of the *D. virilis* fifth chromosome in the genotype of *D. virilis* just as in F_1 hybrids.

These results permit the conclusion that incomplete polytene chromosome pairing in interspecies hybrids depends on the properties of the chromosome loci.

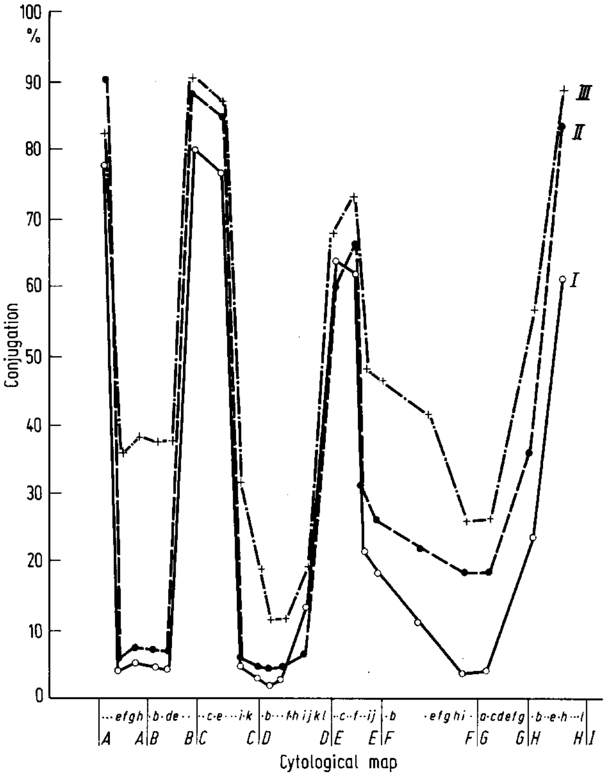

Fig. 5. The conjugation of the fifth chromosome in the interspecific hybrids:

♀ *D virilis* × ♂ *D. texana* I
♀ *D. virilis* × ♂ *D. littoralis* II .
♀ *D. texana* × ♂ *D. littoralis* III

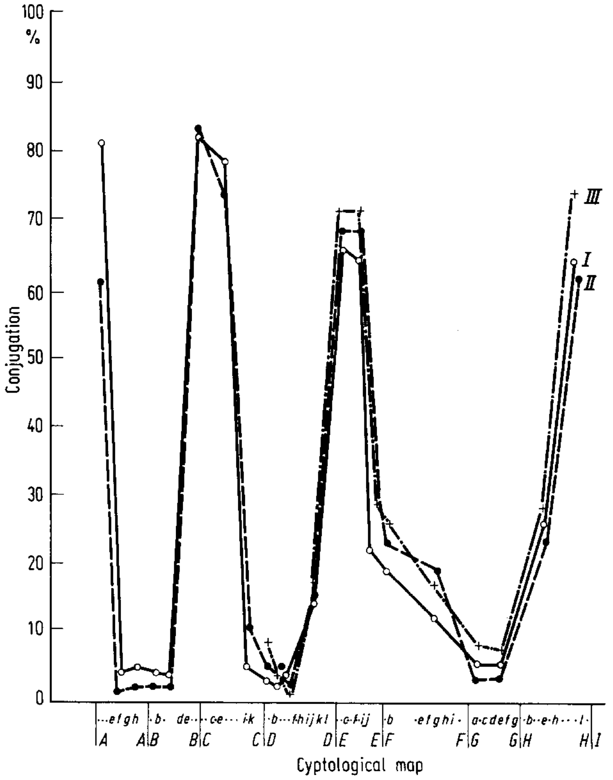

Fig. 6. The conjugation of the distal ends of *D. virilis* and *D. texana* 5th chromosomes in different genotypes: I = in *D. virilis* × *D. texana* F_1 hybrids; II = in the genotype of *D. virilis* (FB_{11}); III = The conjugation of the section of *D. texana* fifth chromosome in the genotype of *D. virilis*

These observations are also in good agreement with the modern concept of chromomeric organization of the chromosome. According to this hypothesis, the chromomere is regarded as a molecular unit of replication and transcription and is relatively independent of other chromomeres in its activity (Plaut and Nash, 1964; Beermann, 1966; Pelling, 1966).

(According to the polytene hypothesis, Koltzoff, 1934, bands of polytene chromosomes result from the specific association of thousands of identical chromomeres at the same level.) Based on the studies reported here, the ability to conjugate must now be added to such autonomous properties of the chromomere as replication and transcription. There is good reason to believe that units of conjugation within the polytene chromosomes are no larger than one or several discs. Mutation, by differentially altering the nucleotide sequences in the homologous loci of chromosomes of related species in the process of divergent evolution, might account for the poor pairing of chromosomes in interspecific hybrids. The investigation by Laird and McCarty (1968), who found relatively profound differences in the base composition and nucleotide sequences when comparing the DNA of closely related *Drosophila* species (e.g. *D. melanogaster* and *D. simulans*), provides experimental support for this speculation. The extent to which the attraction between homologous loci is decreased in the interspecific hybrids probably depends on quantitative molecular changes which have occurred in the chromosomes of related species. Thus in hybrids between closely related species, the degree of polytene chromosome pairing abnormalities may be used as an important feature in estimating the phylogenetic position of the species concerned. Based on the study of polytene chromosome conjugation in the hybrids, the three species constitute the following series: *D. virilis* — *D. littoralis* — *D. texana*. This seriation corresponds to Sokolov's suggestion (1959) on the relationship of these species within the *virilis* group.

Studies on the correspondence between polytene chromosome synapsis and pattern of crossing-over in interspecific virilis texana hybrids.

So far, the data have been concerned with comparative studies of conjugation in interspecific hybrids. When it became evident that incomplete pairing in the hybrids did not depend on a specific interaction of genes determining synapsis of chromosomes, but rather that the homologous conjugation may be regarded as an autonomous property of chromosome sections, it became important to learn whether the scheme of salivary gland chromosome conjugation resembles in some way the pattern of chromosome synapsis in meiosis. This question may be approached through the study of crossing-over between markers (*st* and *es*) localized by Fujii (1942) in the region characterized by incomplete pairing of the polytene chromosomes in *virilis* × *texana* hybrids (fig. 3).

These experiments were based on the expectation that if the correspondence between the two types of pairing really exists the frequency of crossing-over between the two markers should be much lower in hybrids than in pure species. Table 1 summarizes the crossing-over date between *st* and *es* in three different genotypes.

Table 1. *The frequency of crossing-over between st and es in three different genotypes*

Experiment	Total number of flies	Number of cross-overs	Crossing-over (% ± m)
1. Standard *D. virilis* species	3216	497	15,4±0,63
2. *Virilis* × *texana* F_1 hybrids	2348	470	20,0±0,82
3. The hybrid fifth chromosome in *D. virilis* genotype (after eleven back-crosses)	1113	25	2,2±0,43

These data seem rather contradictory. The expected decrease in crossing-over frequency in F_1 hybrids was not found. Instead, a significant increase in the crossing-over frequency between the markers was observed in the hybrids in comparison with the unchanged *D. virilis* standard. However this contradiction can be easily explained. Recent experiments (Evgenev, 1970) showed that the unexpected high-frequency of crossing-over in the fifth chromosome of *virilis* × *texana* F_1 hybrids results from the interchromosomal effect of heterozygous inversions contained in the hybrid genotype. After these inversions were eliminated by backcrossing a dramatic decrease in crossing-over frequency in the hybrid fifth chromosome was observed ($F\ B_{11}$).

Thus, the very low frequency of crossing-over observed in experiment 3 (Table 1) favours the hypothesis of the correlation between the extent of "somatic synapsis" in *Drosophila* salivary gland nuclei and the meiotic pairing of chromosomes necessary for genetic recombination.

To obtain further experimental proof of our hypothesis, crossing over was studied cytologically among the progeny of hybrid females. The frequency of exchanges in synapsed and asynapsed regions was determined (cf. Materials and Methods). While carrying out this experiment we were asking whether these cytologically observed crossovers would correspond to genetic ones. To answer this question we

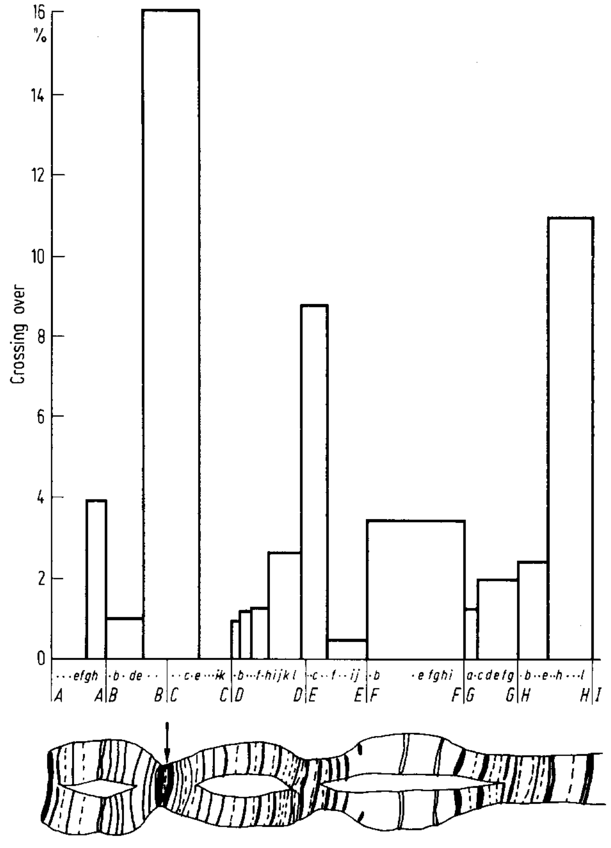

Fig. 7. The distribution of crossing-over in the distal ends of the fifth chromosomes in *D. virilis* × *D. texana* hybrids (F_1)

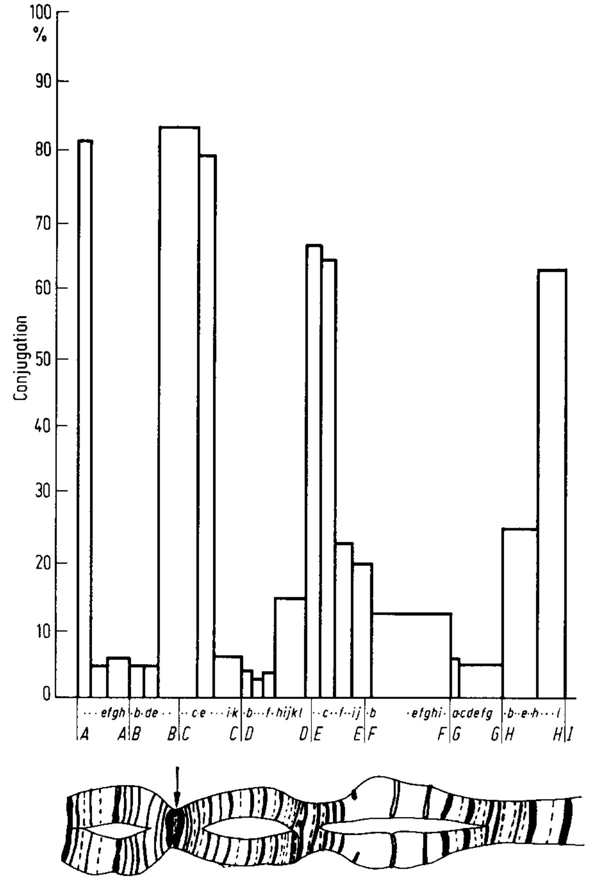

Fig. 8. The frequency of conjugation in the distal ends of the fifth chromosomes in *D. virilis* × *D. texana* hybrids (F_1)

cytologically studied the polytene chromosomes of the progeny of phenotypically observed crossovers $\left(\frac{st\ +}{st\ es}\right.$ and $\left.\frac{+\ es}{st\ es}\right)$ (see Materials and Methods). The analysis indicated that all cytologically observed places of crossing-over were situated in the region between *st* and *es* markers. These experiments proved that cytologically seen changes in polytene chromosome conjugation in the first backcross ($F\ B_1$) really result from crossing-over in ***virilis*** × ***texana*** hybrids females.

The results of the cytological studies of crossing-over in the fifth chromosome of the hybrids are shown in Figure 7. From a comparison of Fig. 7 and Fig. 8 (which represents the data of polytene chromosome conjugation studies in *virilis* × *texana* F_1 hybrids) it is obvious that there is a positive correlation between the pattern of salivary gland fifth chromosome conjugation in hybrids and the distribution of crossing-over in this chromosome in hybrid females.

In other words, the sections of the chromosome characterized by a high frequency of conjugation in the salivary glands of hybrids also exhibited a high frequency of crossing-over in hybrid females. Thus this set of experiments further supports the validity of the supposed correspondence between the patterns of "somatic synapsis" in the salivary gland nuclei and of meiotic synapsis necessary for genetic exchange.

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