

# Proximal Chiasma Localization within an Interstitial Chromosome Segment, a Likely Correlate of Adjacent-2 Segregation of Translocation Causing Multivalents in the Mouse

by P. de Boer\*

Two T70H/+ translocation-carrying male mice were used in an investigation into the relation between the segregation pattern of the translocation caused multivalent at anaphase I and the position of the only chiasma in a long interstitial segment. Moreover, the relation between meiotic stage (from early diakinesis to metaphase I) and chiasma movement was assessed. It appeared that pronounced movement of a chiasma within the multivalent was linked with chiasma terminalization in an adjacent segment, either on the same side of the translocation breakpoint or on the other side.

On summing the CIV and CIII+I configurations (making up 97.9% of all configurations found) and focusing on the multivalents from late meiotic cells, 41.3% had a proximal chiasma in the long interstitial segment of the multivalent. In total, a percentage of 33.5% of all secondary spermatocytes were characteristic of adjacent-2 segregation (i.e., homologous centromeres move to the same pole at anaphase I). Results obtained with T70H translocation trisomics, which are briefly discussed in this paper, confirm the tendency of proximal chiasma frequencies in late meiotic cells to coincide with the frequency of absence of homologous centromere separation within the T70H translocation multivalent. The importance of this phenomenon for normal bivalent behavior during anaphase I is considered.

## Introduction

It is well known that during the first meiotic division of an organism, heterozygous for a reciprocal translocation, homologous centromeres can move to the same pole. This type of segregation of the translocation caused multivalent is called adjacent-2 segregation (1). Searle et al. (2) gave evidence of this phenomenon in males and females heterozygous for reciprocal translocations between acrocentric mouse chromosomes. When the translocation involves the exchange of segments of strikingly unequal length, a chiasma in the nontranslocated (interstitial) pairing segment, produces two homolo-

gous chromosomes with chromatids of unequal length. These marker chromosomes enable one to cytologically quantify the amount of adjacent-2 segregational events. A mouse translocation fulfilling these two prerequisites is T(1;13)70H. Heterozygote males were studied cytologically by de Boer (3). An average adjacent-2 frequency of 25.2% was obtained, which seems high when compared to other mouse translocations. The present paper seeks to explain the reason(s) for the very significant amount of adjacent-2 segregations within this translocation. It specially tries to uncover why the consistent presence of a chiasma in a long interstitial segment (i.e. between the centromere and the point of exchange) does not prevent missegregation of these segments, which always would be the case when bivalents in the normal male mouse are involved (4).

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## Materials and Methods

The T(1;13)70H male mice used were among the material described by de Boer (3). Preparation of meiotic cells, preferential staining of centromeric heterochromatin, notation of diakinesis and first meiotic metaphase stages and notation of translocation configurations, pairing segments and second metaphase chromosomes follow those described and illustrated by de Boer (3). A pachytene pairing-cross with symbols is given in Figure 1. Besides these parameters, assessments were made of the number of chiasmata in the long translocated segment of chromosome 3 1 ( $1_t$ ). Chiasma-positions were subjectively scored in this segment and in segment  $13_t$  (the nontranslocated (interstitial) part of chromosome 13, containing th3 centromere). Therefore, these segments were subdivided in three parts: proximal (*p*) intermediate (*p/d*) and distal (*d*). Care was taken to sharply define the proximal and distal chiasmata, thus automatically producing the third (*p/d*) class. A proximal chiasma in segment  $13_t$  caused the two centric heterochromatin masses originating in chromosome 13 to be closely apposed. A distal chiasma in segment  $1_t$  causes an end-to-end association of chromosome 1 and chromosome  $13_t$ .

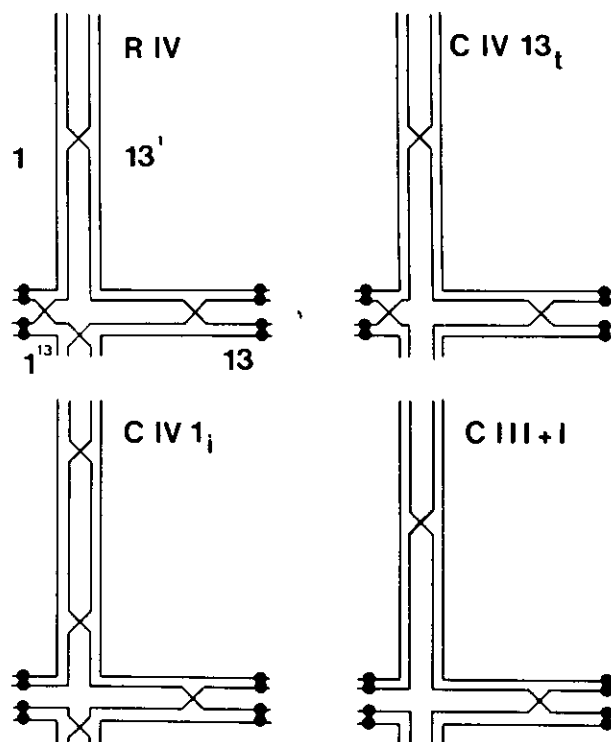
## Results

From two males a total of 838 primary spermatocytes was used, of which 97.9% possessed a CIV $1_t$  (no chiasma in  $1_t$ , see Fig. 1) or a CIII+I ( $1_t+13_t$ ) configuration (Fig. 1). Altogether, 796 cells with the latter two configurations allowed scoring of chiasma positions and were used in the following computations. When diakinesis proceeds to metaphase I, the chiasma in segment  $13_t$  has a tendency to terminalize. This causes an increase in the fraction of CIII+I configurations in the spiralization classes 2 (mid-diakinesis) and 3 (late diakinesis and metaphase I), confirming results published earlier (3, 5). The data presented here are given in Table 1.

Table 2 gives the distribution of chiasma positions within segment  $13_t$  for CIV $1_t$  and CIII+I ( $1_t+13_t$ )

**Table 1. Relation between the two main T70H derived configurations during diakinesis, metaphase I, and spiralization class indicative of meiotic stage. Class 3 cells contain the most contracted bivalents.**

Configuration	Spiralization class		
	1	2	3
CIV $1_t$	101	327	87
CIII+I( $1_t+13_t$ )	16	144	121
Ratio CIV $1_t$ /CIII+I( $1_t+13_t$ )	6.3	2.3	0.7



**FIGURE 1.** Pachytene cross depicting T70H length ratios (based on G-banding) and the majority of configurations found at diakinesis-metaphase I.

configurations. Within CIV, the position of this chiasma does not change when meiosis proceeds ( $\chi^2 = 4.91$ , n.s.). For CIII+I, this rule does not hold ( $\chi^2 = 10.00$ ,  $0.025 < p < 0.05$ ), mainly due to a relative lack of proximal chiasmata in mid diakinesis and an excess in the later stages. Combining all meiotic stages, a comparison can be made between the chiasma position within segment  $13_t$  of CIV and CIII+I configurations (table 2), and then a clear difference is observed ( $\chi^2 = 66.82$   $p < 0.005$ ).

Combining the spiralization classes, most of the CIII+I configurations were seen to originate from the CIV configurations through chiasma terminalization, and second, the chiasma position of  $13_t$  is a constant for CIV configurations through the meiotic stages observed, so the position of the chiasma changes when the transition CIV→CIII+I occurs. Table 2 indicates that a more distal chiasma position occurs.

When only one chiasma is present in segment  $1_t$  (see Fig. 1) there is no change of position observable with regard to the *p*, *p/d* and *d* segments, when spiralization proceeds. This holds true for both the CIV and CIII+I configurations. As found earlier (5), the fraction of the cells containing two chiasmata in segment  $1_t$  drops when meiosis proceeds. When the

**Table 2. Spectrum of chiasma positions for chromosome segment 13<sub>1</sub>.**

Configuration	Chiasma position		
	<i>p</i>	<i>p/d</i>	<i>d</i>
CIV <sub>1</sub>	257 49.9%	229 44.5%	29 5.6%
CIII+I(1 <sub>1</sub> +13 <sub>1</sub> )	63 22.4%	172 61.2%	46 16.4%

chiasma positions in this segment are compared between cells with one or two chiasma(ta) (Table 3), a difference is apparent. When the distal chiasma in segment 1<sub>1</sub> terminalizes, the proximal chiasma moves into the intermediate (*p/d*) region.

To correlate the chiasma position of segment 13<sub>1</sub> with the behavior of the T70H-multivalent at anaphase I, we have measured the number of secondary spermatocytes which are produced after non-disjunction of the two chromosome 13-centromeres of the multivalent. The almost consistent presence of one chiasma in segment 13<sub>1</sub> (99.4%) enables one to score secondary spermatocytes for this type of non-disjunctional event by either the presence of two 13;13<sup>1</sup> chromosomes or its absence. Chromosome 13;13<sup>1</sup> can be recognized by the difference in length of the two chromatids and by the fact that the 13<sup>1</sup> chromatid is the longest of the complement (3) (see Fig. 2). The bulk of nondisjunctional events for the two 13-centromeres is due to adjacent-2 segregation, but numerical nondisjunction may be involved as well. The frequency obtained for the present material is 33.5% (*n*=200). It is interesting to compare this frequency with the frequency of proximal chiasmata in segment 13<sub>1</sub> of multivalents of spiralization class 3, 41.3% (*n*=208). These two frequencies are not significantly different ( $\chi^2 = 2.68$ ). When the figure of 41.3% proximal chiasmata is broken down for CIV<sub>1</sub> and CIII+I(1<sub>1</sub>+13<sub>1</sub>) configurations, it is 56.3% (*n*=87) for the former and 30.6% (*n*=121) for the latter. There is no evidence for preferential production of secondary spermatocytes by primary spermatocytes containing either of these two.

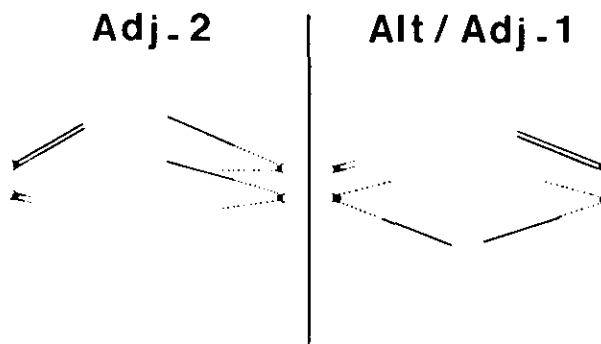
**Table 3. Relation between the number of chiasmata in segment 1<sub>translocated</sub> and chiasma position.**

<i>n</i> <sub>chiasma 1<sub>1</sub></sub>	Chiasma position		
	<i>p</i>	<i>p/d</i>	<i>d</i>
1	11 1.7%	349 52.4%	306 45.9%
2	102 39.2%	28 10.8%	130 50.0%

## Discussion

The foregoing results indicate the existence of chiasma movement during early diakinesis-metaphase I within the translocation-multivalent. However, both in segments 1<sub>1</sub> and 13<sub>1</sub>, movement noticeable with the method of subjectively assessing positions, occurs when an adjacent chiasma terminalizes. For 13<sub>1</sub>, this leads on average to a less proximal position of the interstitial chiasma.

In both configurations (CIV and CIII+I), the (remaining) chiasma in 1<sub>1</sub> has never shown signs of precocious terminalization in spiralization class 3 cells. The mechanism for adjacent-2 segregation we propose uses this fact, and the fact that in 41.3% of all late cells, the two 13-centromeres are positioned closely together through a proximal chiasma. This will make them function like two centers of motion moving to the same pole instead of to opposite poles. The chiasma in 1<sub>1</sub> causes the two 13-centromeres and



**FIGURE 2.** Illustration of adjacent-2 and alternate/adjacent-1 segregation of the T70H/+ multivalent. The 13;13<sup>1</sup> marker chromosome with chromatids of unequal length is clearly recognizable. Chromosome 13 portions are dotted.

the centromere of chromosome 1 to be at different sides of the equator.

We have searched for experimental designs to test the mechanism proposed. One way of doing this is to increase the proportion of CIII configurations which should result in a decrease of the adjacent-2 frequency (see the results). In order to obtain such a situation, we have bred translocation trisomic mice by crossing Ts(1<sup>13</sup>)70H tertiary trisomic male mice with T70H/T70H (homozygous) females. Up to 50% of the offspring receives the small translocation chromosome 1<sup>13</sup> from the father and chromosomes 1<sup>13</sup> and 13<sup>1</sup> from the mother. The two 1<sup>13</sup> chromosomes form a bivalent in 47.6% of all primary spermatocytes (CIII+II), CIV<sub>1</sub> make up 14.4% and CIV<sub>1</sub>+I, 34.5% (*n*=1200, de Boer et al., in preparation). It could be shown that ≈15% of all secondary spermatocytes were produced by CIV<sub>1</sub>+I config-

urations whereas the formed two configurations yielded the balance. Provisional results indicate that the adjacent-2 frequency among CIV<sub>1</sub>+I cells is much higher ( $\approx 45\%$ ,  $n=82$ ) than among CIII+II plus CV cells ( $7.7\%$ ,  $n=470$ ). Of the latter two configurations, 13% of the late diakinesis-metaphase I spermatocytes possessed a proximal chiasma in segment 13<sub>1</sub>. For CIV<sub>1</sub>+I, this figure was 24%, thus lower than expected on the basis the adjacent-2 frequency. The incomplete transmission from the first to the second meiotic division of this configuration probably affects segregation as well. These results strengthen the proposed model of a relation between proximal chiasmata in an interstitial segment at the time of orientation within the spindle and subsequent adjacent-2 segregation of the multivalent.

Pertinent is whether these results have a bearing upon the behavior of structurally normal bivalents during the first meiotic division. Faulty terminalization of proximal and/or terminal chiasmata in our view increases the risk of first-anaphase nondisjunction. Maguire, in a recent study (6) describing a true desynaptic mutant in maize, stresses the role of biochemical mechanisms in sister-chromatid alignment and chiasma terminalization. The process described here, proximal localization of a chiasma in

a too advanced stage of the first meiotic division and the increased risk of nondisjunction involved, could well be termed opposite of desynapsis, both conditions being linked with chromosome missegregation.

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