Heredity **66** (1991) 453–458

Received 17 September 1990

Positional control of chiasma distribution in the house mouse. Chiasma distribution in mice homozygous and heterozygous for an inversion in chromosome 1

IVAN P. GORLOV, TATYANA YU. LADYGINA, OLEG L. SEROV & PAVEL M. BORODIN
Institute of Cytology and Genetics, Academy of Sciences of the USSR, Siberian Department, Novosibirsk 630090, USSR

An examination of the chiasma distribution in chromosome 1 of male mice homozygous and heterozygous for a distal inversion In(1) 12Rk and in normal males was carried out. No differences in chiasma distribution were found between homozygotes for the inversion and homozygotes for normal chromosome 1. A significant decrease in the frequency of bivalents bearing chiasmata in the pretelomeric region was found in heterozygotes. This, in its turn, produced a redistribution of chiasmata in the proximal non-inverted part of bivalent 1. These results could be interpreted as evidence for positional control of the chiasma distribution pattern: the distance of certain parts of the chromosome from the telomere and chiasmata interference are more important for determination of the chiasma frequency in a given region than its genetic content.

Keywords: chiasma, chromosome 1 inversion, house mouse.

Introduction

The distribution of chiasmata within bivalents is, with rare exceptions (Strickland, 1958; Olson *et al.*, 1978), never random (Jones, 1986). If it were random, chiasmata would occur with equal probability in all parts of the bivalent and the position of two chiasmata in the same bivalent would be independent of each other. Jones (1987) mentions two principal identifiable limitations of chiasma distribution within bivalents: localization and interference.

There is an obvious tendency for chiasmata to arise preferentially or exclusively in certain regions of the bivalent (Darlington, 1931; Mather, 1937, 1938; Henderson, 1963; Fox, 1973). Extreme subcentrometric and/or subtelomeric localization of chiasmata has been found in some species (Callan & Perry, 1977). However, many species demonstrate not a qualitative, but a quantitative localization of chiasmata in the sense that they are more frequent in some regions than in others. For example, long bivalents (1–3) of the house mouse display a tendency to subcentromeric and subtelomeric localization, while the remaining bivalents show predominantly subtelomeric localization. Relatively few chiasmata arise in the interstitial part of the bivalents (Maudlin & Evans, 1980; Gorlov *et al.*, 1987). The latter observation could be considered as a reflection of the second limitation of chiasma distribution within a bivalent, namely, chiasma interference. This can be inferred from comparisons of chiasma distributions in bivalents with one or two chiasmata. These distributions differ considerably, so that single chiasmata tend to occupy rather central positions in bivalents while double chiasmata occupy relatively more proximal and distal locations.

Studies of the molecular mechanisms of crossing-over have revealed some hot spots of recombination determined by the primary nucleotide sequence in DNA (Steinmetz *et al.*, 1987). A case was found where a single base substitution in yeast led to a 10-fold increase in recombination frequency (Ronticelli *et al.*, 1988). It has also been suggested that the localization of minisatellite DNA, which is homologous to the Chi-sites of prokaryotes, plays an important role in the control of chiasma distribution within bivalents (Jarman & Wells, 1989). *In situ* hybridization on human meiotic metaphase I chromosomes, using the labelled minisatellite core sequence, showed clusterings of autoradiographic grains principally at or around chiasmata (Chandley & Mitchell, 1988).

The aim of this study was to investigate the factor(s) controlling chiasma localization in mice. More impor-

tantly does the genetic content of a definite region (i.e. localization of certain DNA sequence) or the position of this region with respect to centromere and telomere determine its chiasma frequency?

Materials and methods

Male and female mice homozygous for a paracentric inversion in chromosome 1 (In(1) 12Rk–In12) were gifts of Dr T. H. Roderick from the Jackson Laboratory, Bar Harbor, Maine, USA.

High-resolution G-band staining (Ikeushi, 1984) was used for the precise localization of the breakpoint of this inversion on the cytological map of mitotic chromosome 1. Surface-spread preparations of synaptonemal complexes (SC) from the spermatocytes of males heterozygous for In12 were made according to the method advanced by Solari (1980), stained with $AgNO_3$ (Howell & Black, 1980) examined and photographed with an electron microscope JEM (Jeol, Japan) at 80 kV. The locations of the endpoints of synapsis inside and outside the inversion loops were presented as proportions of the distance between the centromere and the corresponding point to the mean length of the two lateral elements of the given SC. These data were used to localize the breakpoints of the inversion on the map of the SC.

The F_1 heterozygotes In12Rk/+ were produced by crossing In12Rk homozygotes with a CBA/Lac strain. The latter possessed chromosome 1 marked by a small block of subcentromeric C-heterochromatin (Forejt, 1973), which was used to identify the bivalent 1 in diplotene–diakinesis spreads. Homozygotes and heterozygotes for In12, as well as normal homozygotes, were obtained from F_2 crosses among F_1 progeny. Karyotypes of the individuals were identified by a biochemical marker, peptidase 3. The gene for peptidase 3 (Pep-3) has been localized inside the inverted segment of chromosome 1 (Roderick *et al.*, 1981). In12 contained allele Pep-3^a (slow variant). Normal chromosome 1 derived from CBA/Lac strain carried allele Pep-3^b (fast variant). Electrophoretic separation and histochemical staining were performed as described previously (Rubtsov *et al.*, 1982). F_2 homozygotes for the inversions, selected for the analysis of chiasma distribution, also possessed a small C-positive block. They were crossovers between the centromere and the inversion.

Three, 3-month-old males of each genotype were used to study chiasma distribution. Diplotene–diakinesis chromosome spreads were prepared after hypotonic treatment and methanol:acetic acid (3:1) fixation by a routine air drying technique (Evans *et al.*, 1964). Slides were processed by the C-band staining technique to identify subcentromeric C-heterochromatin in chromosome 1 (Sumner, 1972).

Well-spread diplotene and diakinesis nuclei (Fig. 1) were traced using a drawing apparatus. Each bivalent of chromosome 1, marked by a small precentromeric C-band, was subdivided into 10 equal parts. The frequency of bivalents with chiasmata in each part was calculated. Differences between individual males within genotypes in chiasma distribution were tested according to the methods used by Laurie & Jones (1981). The distribution patterns of chiasmata between genotypes were compared statistically by Chi-square method for two empirical distributions with 9 d.f. (Kendall & Stuart, 1973). Differences in chiasma frequency (f) between certain regions of the bivalent were tested using F-criterium after ρ-transformation, with $\rho = 2 \arcsin \sqrt{f}$ (Fisher & Yates, 1938; Auscombe, 1948).

Results

Cytological mapping of the inversion

So far inversion In12 has been localized only on the genetic map (Roderick, 1981). The proximal breakpoint was found to be situated in the middle of the map (45 cM from the centromere), and the distal breakpoint is located near the telomere. According to the pattern of high-resolution banding (Fig. 2), we localized the proximal breakpoint between subbands E1 and E3 and the distal breakpoint at band H6. The distances of

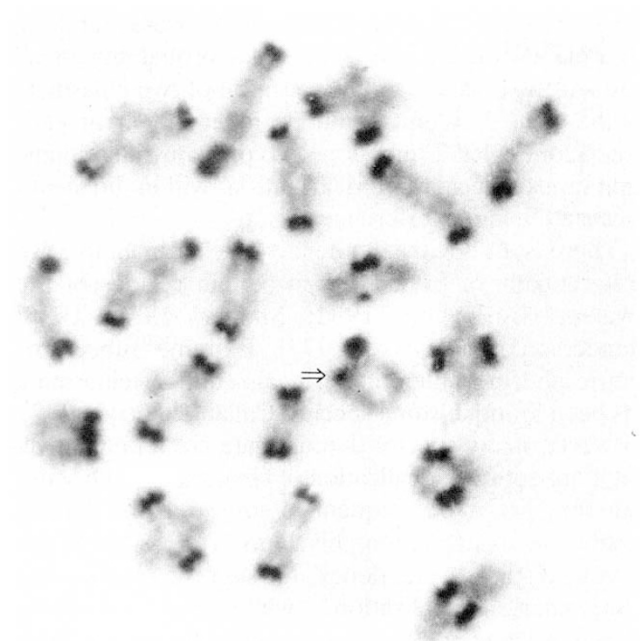

Fig. 1 Diakinesis in a male mouse heterozygous for the small C-positive block in chromosome 1 (arrowed).

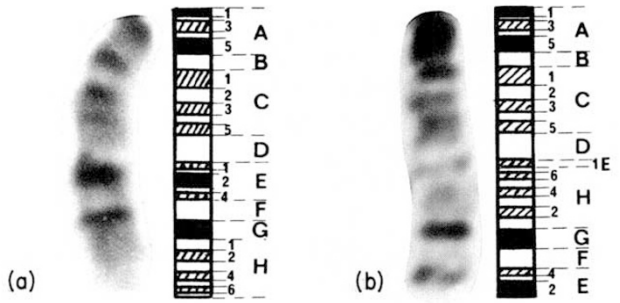

Fig. 2 Microphotograph and schematic presentation of mitotic metaphase chromosome 1. G-band staining. Arrows show breakpoints. (a) Normal chromosome, (b) In(1) 12Rk.

these points from the centromere were 0.57 and 0.98, respectively.

Synaptonemal complexes (SCs) were studied in a total of 149 silver-stained surface-spread pachytene nuclei with full paring of all non-inverted chromosomes from three heterozygous males. Approximately one-half of the nulei showed all straight-paired bivalents. Bivalent 1 in these nuclei was heterologously paired in the inverted segment. The remaining nuclei showed three types of heteromorphic synaptic configuration. Incomplete inversion loops with fully paired ends of axes (Fig. 3a) were found in a small fraction (2.01 ± 1.10 per cent) of the nuclei. A more frequent configuration (34.89 ± 3.89 per cent) was an incomplete inversion loop with unpaired distal segments of the axes and with the asynaptic zone in the proximal part of the loop (Fig. 3b). The third configuration found in 8.05 ± 2.21 per cent of the nuclei is shown at Fig. 3c. It had no inversion loop but its telomeric ends were unpaired. The relative length of SC from the centromere to the proximal point of asynapsis in configurations *a* and *b* was 0.52 ± 0.02, and from the centromere to the distal point of asynapsis in configuration *a* was 0.95 ± 0.02. This agrees with the positions of the breakpoints of the inversion on the mitotic map of chromosome 1. They correspond to segments 6 and 10 respectively of the diplotene–diakinesis bivalent.

More details on the chromosome pairing and synaptic adjustment in single and double heterozygotes for inversions in chromosome 1 are published elsewhere (Borodin *et al.*, 1990). The main purpose of this study of SC was to compare the synaptic pattern of

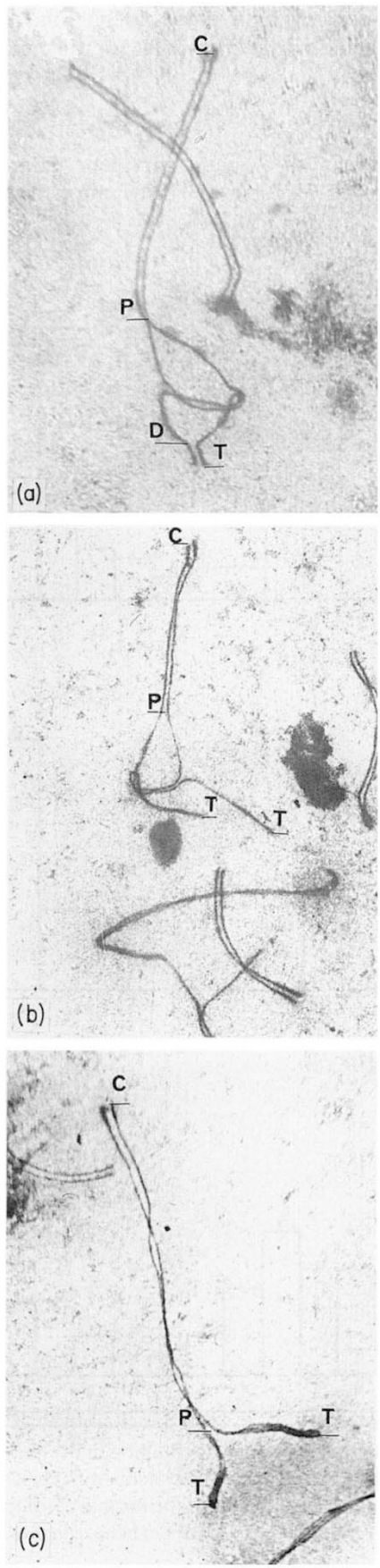

Fig. 3 Different synaptic configurations of the chromosome 1 in male mice heterozygous for In(1) 12Rk. Electron micrograph. Silver staining. C = centromere, P = proximal endpoint of straight synapsis, D = distal endpoint of straight synapsis, T = telomere.

chromosome 1 with the pattern of chiasma distribution along bivalent 1 in heterozygotes for the inversion.

Chiasma distribution

The numbers of C-band diplotene and diakinesis nuclei scored for chiasma distribution from normal, heterozygous and homozygous for In12 males were 249, 210 and 305, respectively. There were no differences between individual males within genotypes in chiasma distribution (chi squares for normal, heterozygous and homozygous were 26.7, 24.7, 20.0, respectively, $P > 0.05$), thus the data within the genotypes were pooled.

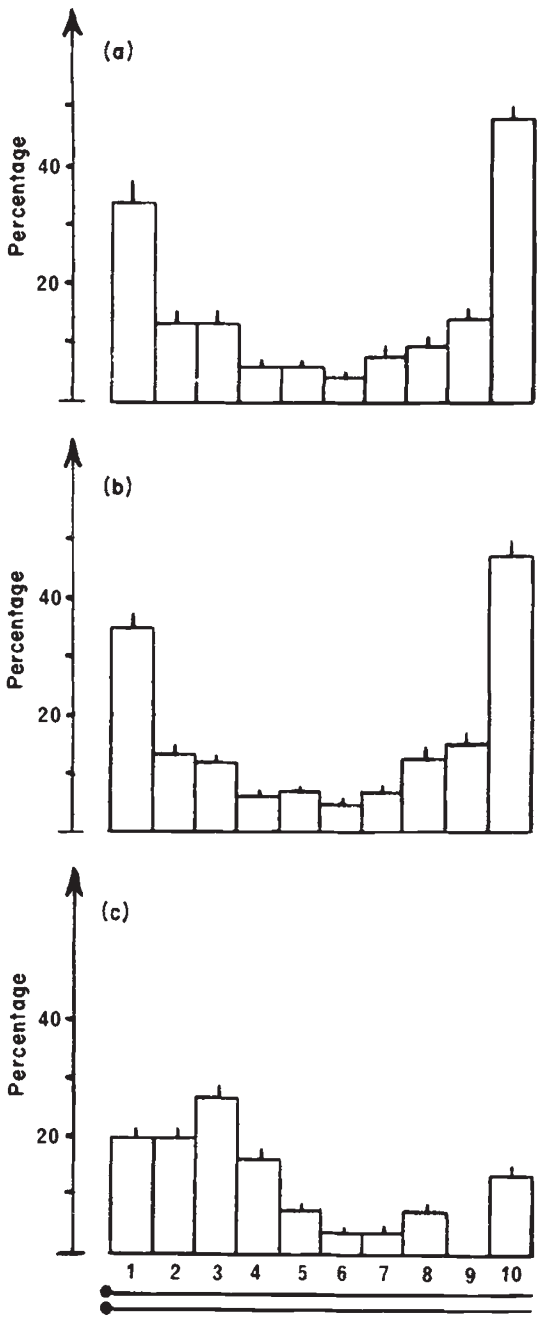

Fig. 4 Chiasma frequency distribution in the ten segments of the bivalent 1 (percentages of bivalents with chiasma in the segment). (a) Homozygotes for the normal chromosome 1, (b) homozygotes for In(1) 12Rk, (c) heterozygotes for In(1) 12Rk.

Figure 4 shows the distributions of chiasmata along bivalent 1 in the three genotypes.

The chiasma distribution in normal and homozygous for In12 males (Fig. 4a,b) was identical (chi square = 3.6, $P < 0.5$), i.e. inversion of the chromosomal material did not produce any inversion of the pattern of chiasma distribution. Homozygotes did not differ from normal males in the total chiasma number (1.62 ± 0.03 and 1.67 ± 0.03, respectively, $t = 1.18$, $P > 0.05$).

As the breakpoints of the inversion were localized inside segments 6 and 10, it would be incorrect to compare the chiasma frequency within these regions between the normal and inversion homozygotes. Chiasma frequency in region 7 in normal mice was significantly lower than in region 9 ($F = 4.2$, $P < 0.05$). On being transferred to the pretelomeric region 9 in the inversion homozygote, however, it displayed as high a frequency as region 9 in the normal homozygotes and vice versa ($F = 0.96$, $P > 0.01$).

Chiasma distribution in heterozygotes (Fig. 4c) differed drastically from those in normal and inversion homozygotes (Chi square = 94.7, $P < 0.01$). Chiasmata occurred in the telomeric region in heterozygotes three times less frequently than in the same region in homozygotes. This is not surprising because, as we have shown, this region was unpaired in about 40 per cent of pachytene nuclei. Weak and unstable synapsis inside the inversion loop and a high incidence of heterosynapsis led to a decrease in chiasma frequency in the inverted region. As a result, the total chiasma number per bivalent was much smaller in heterozygotes (1.21 ± 0.03) than in homozygotes and normal males ($t = 10.37$, $P < 0.01$). A crossover suppression in the distal part of the bivalent produced chiasma redistribution in the proximal non-inverted part. The subcentromeric peak characteristic of homozygotes was smoothed out in heterozygous males.

Discussion

The aim of this study was to investigate which of the factors that determine the probability of chiasma occurrence in a definite region of the chromosome are the more important, the genetic content of the region (i.e. localization of certain DNA sequence) or its position with respect to the centromere and telomere?

Inversion homozygotes represent a convenient model for the examination of this problem. Different parts of the region that are confined by the breakpoints significantly differ from each other in chiasma frequency. Chiasmata rarely occur near the proximal breakpoint (region 7) and occur more often in the subtelomeric part (region 9) of the normal bivalent. If the chiasma localization in any part of any chromosome is determined predominantly by the genetic content of this part, the pattern of chiasma distribution in the bivalent 1 in homozygotes for the inversion is inverted. We have not found, however, any differences in chiasma distribution patterns between normal and inversion homozygotes. On the other hand, we have found an altered pattern of the chiasma distribution in the proximal non-inverted part of bivalents in heterozygotes, while the order of genes in this region in homozygotes and in heterozygotes was the same.

Similar results were found in a study of chiasma distribution along bivalent 1 of two chromosomally differentiated taxa of the grasshopper *Caledia captiva* (Shaw & Wilkinson, 1980). These taxa were supposed to differ by a pericentric inversion in chromosome 1, transforming an acrocentric chromosome of 'Torresian' variety into a metacentric chromosome of 'Moreton' variety. Nevertheless, chiasma distributions in both homozygotes were almost identical to a proximal/distal pattern of chiasma localization. The distribution pattern of chiasma in the non-inverted part of chromosome 1 in heterozygotes was very different from that observed in either parent.

These results can be interpreted as evidence of the positional control of the chiasma distribution pattern. The distance from any part of the chromosome to the telomere and the action of chiasma interference are more important for the determination of chiasma frequency in the given region than the genetic content of this region.

This is not to deny the existence of a local site-specific control mechanism. Thousands of potential sites of recombination exist as evidenced by the data obtained in molecular studies (Stern & Hotta, 1987). They are dispersed evenly throughout the genome and vary in their recombinogenic activity. They are responsible for the fine control of recombination, however, the positional control of chiasma distribution eclipses these microvariations. Chiasma determination appears to be a characteristic not of a particular site but of the position of the segment on the chromosome. The subtelomeric segments of any site composition are characterized by higher chiasma frequencies than any other segments of the chromosome.

In general, our data fit Mather's (1937, 1938) sequential model, in which chiasma determination follows a linear and temporal sequence from the fixed point in a bivalent. He takes the centromere as the starting point. A number of data on chiasma distribution in different species have been interpreted in terms of this model. The only differences in this interpretation concern the consideration of telomeres rather than centromeres as the starting point (see Jones, 1987, for a review).

It has been suggested that chiasma distribution can be inferred from pairing characteristics such as the position of the initiation point and the rate of synaptic progression (Moens, 1969; Sybenga, 1975). A close correlation between pairing frequencies of definite segments and the recombination frequencies in respective regions has been demonstrated (Maguire, 1966, 1977; Parker *et al.*, 1982; Parker, 1987).

In our experiment a correlation between the pairing failure in the telomeric region of chromosome 1 in inversion heterozygotes and a substantial decrease in the frequency of chiasmata in the pretelomeric segment was also shown. The leading role of the mammalian telomere as the starting point of pairing has been demonstrated in different studies (Speed, 1989). This may be the reason why the segment of the chromosome located near the starting point has the highest chiasma frequency (Maudlin & Evans, 1980; Gorlov *et al.*, 1987). Recombination frequency in the distal part of the bivalent decreases in proportion to the distance between the telomere and the segment. We have shown that this is true regardless of its genetic content.

From this point of view it is interesting to reconsider the evolutionary role of inversions. In addition to the classical interpretation of inversions as tools of conservation of co-adaptive gene complexes (Dobzhansky, 1970) and as factors that affect gene expression through the position effect, we would like to emphasize their 'recombinational position effect'. Homozygotes for an inversion may differ from normal homozygotes with respect to recombination between certain genes of the chromosome. It can affect the rate of the combinative variation for certain traits and, as a result, determine the probability of fixation of 'normal' or inverted chromosome.

Acknowledgements

We are extremely grateful to Dr L. V. Vysotskaya and Dr B. F. Chadov for their helpful comments on the manuscript, and to Mrs Marina I. Rodionova for technical assistance.

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