A TANDEM DUPLICATION THAT LOWERS RECOMBINATION THROUGHOUT A CHROMOSOME ARM OF *DROSOPHILA MELANOGASTER*[1]

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THE use of polytene chromosomes to analyze chromosomal aberrations reveals structural details that are inconspicuous or invisible in condensed meiotic or mitotic chromosomes. One type of aberration difficult to analyze in organisms lacking somatically paired polytene chromosomes is the tandem duplication. In Drosophila, the extent of a duplication can be accurately determined in polytene chromosomes and its effect on recombination studied in meiotic chromosomes. The tandem duplications hitherto investigated in *Drosophila melanogaster* have either not affected or have increased meiotic recombination in the vicinity of the duplication, depending on whether the female studied was hetero- or homozygous for the duplication. As a result of these studies, GREEN (1962) has concluded that tandem duplications increase crossing over by increasing effective pairing.

The present communication explores the behavior of a tandem duplication that *lowers* recombination throughout a chromosome arm. Crossover reductions associated with the addition of chromosomal segments are difficult to reconcile with PRITCHARD's (1955) effective pairing hypothesis which states that pairing at the time of meiotic recombination is limited to a few short segments of homologous chromosomes. The present data are in better agreement with the concept that homologous chromosomes are usually at least roughly aligned with homologous regions brought into proximity before meiotic recombination takes place.

MATERIALS AND METHODS

The tandem duplication described here was recovered in a search for crossover suppressors from irradiated Canton-S females (THOMAS and ROBERTS 1966). Details of the screening procedure used to recover this aberration, Dp(2;2)619, can be found in the above reference. Briefly, the method used permits simultaneous screening of the major chromosomes of an irradiated genome for crossover suppressors by using markers spaced approximately 50 crossover units apart. Stocks were made of the crossover suppressors, and salivary chromosomes were examined.

Information on recombination in proximal and distal regions of chromosome arm 2L was obtained by crossing males heterozygous for the duplication and a balancer chromosome marked by *Cy* (curly wings) to "all" females. These females carried the following markers in chromosome arm 2L: aristaless (*al*, 2–0.0, aristae reduced), dumpy (*dp*, 2–13.3, wings truncated), black (*b*, 2–48.5, dark cuticle), and purple (*pr*, 2–54.0, eye color). Non-*Cy* F_1 females were then crossed to "all" males, and the progeny scored for recombination. Recombinant chromosomes

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TABLE 1

Meiotic recombination in the left arm of chromosome 2 with Dp(2;2)619 absent, heterozygous, and homozygous

Genotype of P females	Avg. no. of progeny per female	Noncrossover progeny	Phenotype and no. crossovers/interval and (% crossing over/interval) 1 *al-dp*	2 *dp-b*	3 *b-pr*	Double crossovers 1,2	1,3	2,3	Total progeny	Total % crossing over
al dp b pr / + + + +	299	*al dp b pr* 811 + 902	*al* 207 *dp b pr* 125 (12.2)	*al dp* 345 *b pr* 393 (26.0)	*al dp b* 80 *pr* 78 (6.0)	24	7	15	2987	44.2
Dp(2;2)619 / *al dp b pr*	257	*al dp b pr* 1227 + 1636	*al* 18 *dp b pr* 5 (0.88)	*al dp* 69 *b pr* 48 (3.9)	*al dp b* 41 *pr* 37 (2.5)	4	0	0	3085	7.3
al dp Dp(2;2)619 / Dp(2;2)619 *b pr*	9	*al dp* 237 *b pr* 276	*dp* 3 *al b pr* 15 (3.1)	*al dp b pr* 29 + 36 (10.7)	*al dp pr* 1 *b* 17 (3.2)	0	1	1	616	17.0

bearing the duplication were then used to measure recombination in duplication homozygotes: *al dp* Dp(2;2)619/SM1, *Cy* males were crossed to Dp(2;2)619 *b pr*/SM1, *Cy* females, and the non-*Cy* female progeny were crossed to "all" males.

RESULTS AND DISCUSSION

In the course of screening the chromosomes of the female that yielded Dp(2;2) 619, it was noted that recombination was normal in the other arm of chromosome 2 and in chromosomes 1 and 3; it is therefore unlikely that a gene mutation is responsible for the observed crossover reduction. Figure 1A shows the entire length of chromosome arm 2L in a female heterozygous for the crossover suppressor. It is apparent that there is no inversion or translocation present, but the greater thickness of the chromosome just proximal to the "shoebuckle" landmark indicates the presence of a long duplication extending from 26A to 28E. Figure 1B shows the extent of the duplication more clearly; the tandem nature of the duplication and the bands that comprise it can be seen in Figure 1E.

Table 1 compares recombination in three regions of the left arm of chromosome 2 in controls, duplication heterozygotes and duplication homozygotes. That the number of offspring recovered from duplication heterozygotes is comparable to that recovered from control females is an indication that elimination of crossover progeny is not responsible for the observed crossover reductions in 2L. Although equal numbers of the reciprocal classes were not obtained in region 1 of duplication heterozygotes, recombination in this region would not be greatly increased relative to the control value even if a correction were made for possible elimination of *dp b pr* recombinants. Recombination in the duplication heterozygote is greatly reduced in all three regions of 2L, but the reduction is more extreme in the distal regions.

Females homozygous for the duplication, bearing four doses of 26A to 28E, lay fewer eggs than controls and duplication heterozygotes and most of these eggs fail to hatch. This pronounced difference in fertility of heterozygotes and homozygotes provides a convenient means of assuring the presence of the duplication in the homozygous condition: any presumed homozygotes that were actually heterozygotes would have produced many more offspring than the mean. (In addition, the presence of the homozygous duplication in these infertile females was confirmed by cytological examination of the salivary chromosomes of several progeny of one such female.) None of the pair matings of the 70 homozygous females yielded progeny in numbers greatly exceeding the mean of nine per female, nor were there appreciable differences in recombination frequency between broods of different homozygous females. The frequency of reversion resulting from exchange within the duplication in Dp(2;2)619/SM1, *Cy* females is, therefore, less than 1% and, judging from the stability of stocks used to produce marked homozygous females, is probably rare. There is, however, no striking phenotypic change associated with heterozygosity or homozygosity for the duplication that would readily allow precise estimation of reversion frequency.

Evaluation of the amount of crossover reduction in duplication homozygotes is complicated by the low fertility of these females and the failure to recover equal

numbers of reciprocal crossover classes in regions 1 and 3. If it is assumed that selective elimination of *al dp pr* recombinants is responsible for the inequality of the two crossover classes in region 3, doubling of the number of *b* recombinants would bring recombination in this region to the control value. The *dp* recombinant class is deficient in number in region 1, but an assumption of selective elimination of *dp* recombinants and an adjustment similar to the one considered for region 3 still leaves recombination in region 1 at less than half the control value. It should be emphasized that in the region that includes the duplication, region 2, reciprocal recombinant classes are recovered in approximately equal numbers. After the possibility of selective elimination of recombinants among the progeny of these infertile duplication homozygotes has been taken into consideration, it appears that although recombination throughout 2L is greater in duplication homozygotes than in heterozygotes, recombination in at least the two distal regions is much lower than in the controls.

The behavior of tandem duplications studied previously might lead one to predict crossover increases in the presence of Dp(2;2)619 at least proportionate to the amount of genetic material added to chromosome arm 2L. GREEN (1962) has shown that several tandem duplications on the X chromosome increase recombination locally in excess of the length of genetic material added and has proposed that tandem duplications increase crossing over by increasing effective pairing.

The effective pairing hypothesis was advanced by PRITCHARD (1955) to account for localized negative interference in Aspergillus. According to this hypothesis, chromosome pairing prior to recombination is limited to a few short chromosomal segments with a high probability of exchange within such effectively paired segments. Additional support for the hypothesis was claimed from crossover increases observed in a triplicated chromosomal region of Aspergillus; however, recombination in two adjacent regions was reduced (PRITCHARD 1960).

The effects of chromosomal duplication (equivalent to triplication referred to above) on recombination have been extensively studied in *D. melanogaster*. Duplication of chromosomal segments ordinarily leads to crossover reductions in and near the region of homology (DOBZHANSKY 1934; E. H. GRELL 1964). As pointed out by R. F. GRELL (1965) such crossover reductions are not in accord with the effective pairing hypothesis. Prior to the discovery of Dp(2;2)619, the crossover increases observed in the presence of three different homozygous tandem duplications appeared to represent a set of data consistent with the predictions of the effective pairing hypothesis (GREEN 1962). The behavior of Dp(2;2)619 indicates that tandem position of duplicated material does not lead invariably to crossover increases. Dp(2;2)619 is considerably longer than the tandem duplications of the X chromosome studied by GREEN, but recombination is not increased even in females homozygous for the duplication. Instead, a profound depression of recombination extends to all regions of the chromosome arm in duplication heterozygotes and to at least the distal regions of homozygotes.

If the chromosome pairing preceding recombination were, in fact, limited to short, effectively paired segments, one would expect to observe crossover increases

in a duplicated region (PRITCHARD 1960). The crossover decreases observed throughout chromosome arm 2L in the presence of Dp(2;2)619 suggest that exchange pairing in Drosophila involves a chromosome configuration with properties more complex than the configuration proposed by the effective pairing hypothesis. The present data can be accounted for by postulating that homologous chromosomes are ordinarily aligned so that homologous regions are brought into proximity throughout a chromosome arm before recombination occurs. A close

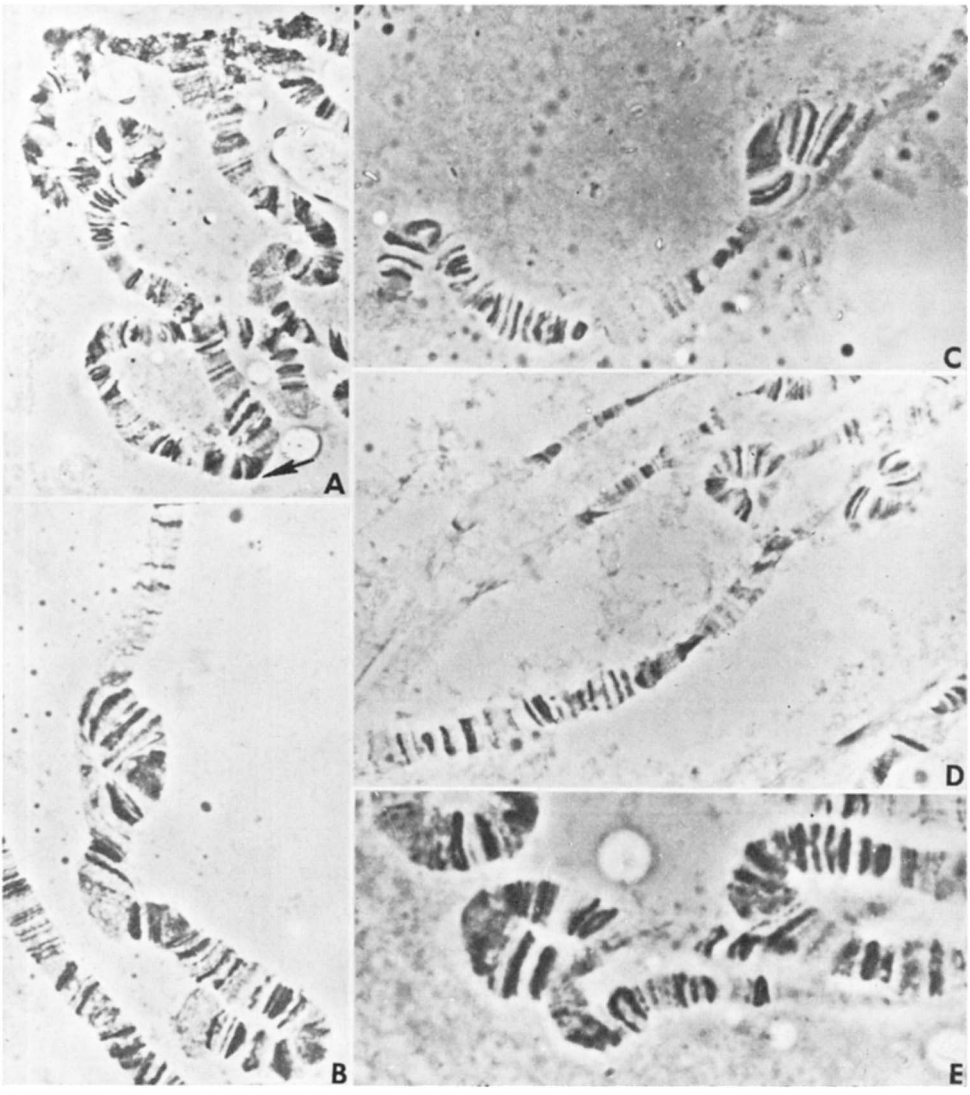

FIGURE 1.—Polytene configurations of Dp(2;)619. (A) Heterozygote showing entire 2L which is normal except for duplication (arrow). (B) Heterozygote in greater detail. (C) Homozygote. (D) Homozygote with duplicated regions of homologous chromosomes unpaired. (E) Heterozygote (unpaired) with tandem nature of duplication clearly visible (see Figure 2E).

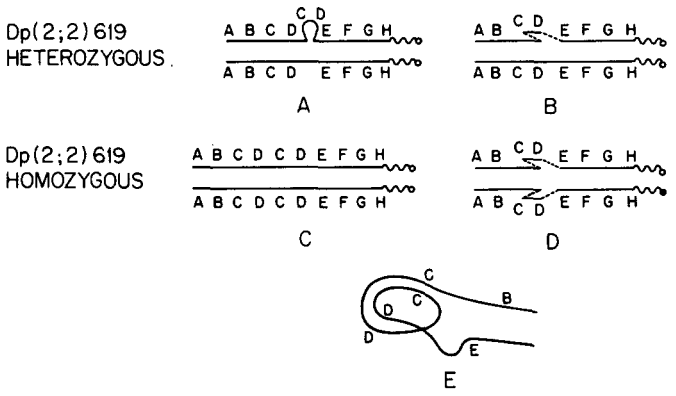

FIGURE 2.—(A–D) Diagrams of possible pairing configurations in left arm of chromosome 2 in the presence of Dp(2;2)619 (see DISCUSSION for explanation). (E) Diagram of the duplicated region of the unpaired chromosome seen in Figure 1E.

examination of the behavior of Dp(2;2)619 and other crossover suppressors may help elucidate the properties of this pairing configuration.

Figure 2 is a diagram of alternative pairing configurations in duplication heterozygotes and homozygotes. In Figures 2A and 2C the pairing of homologous chromosomes is maximal. The configuration that leads to crossover increases in meiotic cells in the presence of certain tandem duplications is not known because Drosophila oocytes do not provide good cytological material. One would predict, however, that the configuration diagrammed in Figure 2A would produce little change in recombination throughout the chromosome arm whereas the one diagrammed in Figure 2C would increase recombination at least to a degree equivalent to the extra chromosomal material present.

The extent to which somatic pairing of polytene chromosomes resembles meiotic pairing is also conjectural, but there is little doubt that there is some correspondence. Complex aberrations, when heterozygous, reduce or prevent meiotic recombination between homologues; flies heterozygous for complex rearrangements have asynapsed chromosomes in their salivary gland nuclei more frequently than aberration-free flies or flies carrying simple two-break rearrangements. (A photograph of the salivary gland chromosomes of a fly heterozygous for a complex rearrangement, SM5, may be seen in THOMPSON (1962).)

NEWTON and DARLINGTON (1930) have shown that when three or more homologous chromosomes are present in meiotic cells of plants, only two are synapsed at any particular point. Where chromosomes can be seen during meiosis in insects, in tetraploid spermatocytes of the long-horned grasshopper, for example, more than two chromosomes are never associated at the same level (WHITE 1948). Evidence that this is also true for Drosophila oocytes is indirect, owing to the difficulty of observing chromosomes in oocytes: DOBZHANSKY (1934) found that recombination between normal homologues is reduced in the presence of free duplications and duplications attached to the base of the X chromosome. The greatest crossover reductions were observed in regions homologous to the dupli-

cation and in adjacent regions, and the degree of crossover reduction was positively correlated with the length of the duplication. DOBZHANSKY interpreted these crossover reductions as evidence of competitive pairing, that is, as an indication that the duplication competes with either of the homologues for a pairing partner. Competitive pairing is consistent with the hypothesis that pairing in meiotic cells of hyperploid Drosophila is, as in other organisms, by twos. However, the three polytene homologues in salivary nuclei of Drosophila triploids pair along their length, as readily as do the polytene chromosomes of diploids (PAINTER 1934). COOPER (1938) suggested that polyteny may impose radial symmetry with respect to the available pairing surface while meiotic chromosomes have a single pairing surface. The greater length of polytene chromosomes, as well as the presumably greater time interval available for pairing in somatic nuclei, may also facilitate pairing in salivary nuclei compared with somatic nuclei (SWANSON 1957).

With these differences between somatic and meiotic pairing in mind, it is now profitable to consider the pairing of the duplication in salivary gland nuclei. Figures 1A and 1B, duplication heterozygotes, show pairing configurations like that of Figure 2B. The configurations illustrated here are observed consistently in salivary nuclei of duplication heterozygotes. Failure of the chromosome carrying the duplication to pair with its homologue (as in Figure 1E) is rare. Figures 1C and 1D, duplication homozygotes, show pairing configurations like that of the diagram in Figure 2D. Again, it is uncommon to see homologous chromosomes unpaired in the region of the duplication except in an occasional nucleus where the chromosomes have been subjected to considerable tension in the process of squashing (Figure 1D and E).

The crossover reductions observed when recombination in duplication hetero- and homozygotes is compared with controls (Table 1) are difficult to explain if one assumes that pairing relations in meiotic cells are as in Figure 2A and C respectively. Crossover reductions are explicable, however, if the pairing observed in somatic cells (Figure 1 and diagrammed in Figure 2B and D) corresponds to the pairing that occurs in meiotic cells.

Pairing of hyperploid regions in salivary nuclei is apparently facilitated by the polyteny of somatic chromosomes. If, as COOPER (1938) has suggested, meiotic chromosomes have a single "surface" available for pairing, then that aspect of chromosome pairing observed in salivary nuclei which probably corresponds to pairing in meiotic nuclei is the strong tendency for pairing of the duplication with the homologous region on the *same* chromosome rather than with the corresponding region of the homologous chromosome. The near-perfect pairing of the whole complex (the two homologues and their duplicated regions) observed in salivary nuclei is probably not typical of the pairing relations of the two homologous chromosomes in meiotic nuclei, judging from the crossover suppression caused by presence of the duplication (Table 1).

The pairing configuration in meiotic cells under consideration here may correspond to the synaptinemal complex seen by electron microscopists in meiotic cells (MOSES and COLEMAN 1964). MEYER (1964) has reported that the synap-

tinemal complex is absent in Drosophila males (where meiotic recombination is absent) and is formed by the paired sections of chromosomes of triploid females but not by unpaired chromosomes. The central linear element of the synaptinemal complex, presumed to be at the junction of synapsed meiotic chromosomes (MOSES and COLEMAN 1964), may form the single available pairing surface proposed by COOPER as an explanation of chromosome pairing by twos in meiotic cells of hyperploid organisms. However, it is preferable at this time to distinguish between the pairing properties of meiotic chromosomes inferred from genetic data and light microscopy and the pairing inferred from structures seen in electron microscopy of meiotic cells. Consequently, the pairing configuration of homologous chromosomes in meiotic cells which is presumed to precede meiotic recombination will be referred to here as the "recomplex." (This term has the advantages of brevity, and of suggesting a possible identity with the synaptinemal complex.)

The tandem duplication under consideration here has approximately twice the effect on recombination of the longest duplication studied by DOBZHANSKY. Duplication 105, attached to the base of the X chromosome (DOBZHANSKY 1934), included loci extending from *y* to *cv*—equivalent to the first four divisions of the salivary map—and lowered recombination in the X chromosome to ½ the control value. Dp(2;2)619, when heterozygous, although shorter than Dp 105, reduces recombination in the left arm of chromosome 2 to ⅙ the normal value. The lack of proportionality of the degree of crossover reduction to the size of the duplication in this case suggests that competitive pairing alone is not adequate to account fully for the crossover suppression caused by Dp(2;2)619.

Evidence from the distribution of translocation breakpoints associated with crossover suppression (ROBERTS 1965) suggests that the location of Dp(2;2)619 may contribute to its effect on recombination. In Drosophila females heterozygous for certain reciprocal translocations, recombination within a translocated arm may be reduced to a fraction of the control value (comparable to crossover reductions caused by inversion heterozygosity) by a single, distally located breakpoint. (A detailed account of the crossover suppressing translocations recovered from irradiated sperm is in preparation, but one such translocation, recovered from an irradiated oocyte, has been described in THOMAS and ROBERTS 1966.) Perhaps the simplest explanation for the effectiveness of certain distal translocation breakpoints in lowering recombination throughout a chromosome arm is that if pairing is ordinarily initiated in distal regions, a diminution of the ability of this region to pair resulting from structural heterozygosity may reduce or prevent pairing throughout the chromosome arm (ROBERTS 1965). One other point suggested by the translocation data may be relevant to this consideration of the behavior of Dp(2;2)619: certain chromosome arms (2L in particular) appear to be more sensitive to crossover suppression by translocation than others.

If the distal location and size (see below) of Dp(2;2)619 are the major factors responsible for its effectiveness in reducing pairing and subsequent recombination, it may be profitable to speculate on formation of the recomplex. If, when attractive forces between homologous regions are first manifested, homologous

chromosomes are usually outside the limits of the forces of attraction, Dp(2;2)619 will then be likely to form a synapsed segment with the adjacent homologous region on the same chromosome (as in Figure 1E) completing pairing by twos in this region. The probability of this distal region pairing with its homologue in meiotic cells is thereby reduced and the likelihood of recomplex formation is consequently diminished; one can thereby account for the lowering of recombination in the *b-pr* region, far from the duplication, to half the control value (Table 1). This hypothesis would lead one to predict crossover reductions in females homozygous for Dp(2;2)619, and this is borne out by the data. However, recombination in duplication homozygotes, although much lower than in controls, is greater than in the heterozygotes. This suggests that pairing in the homozygote is more likely to be successful. The reason for this is not apparent, but equality of arm length is one factor not considered in the above model that many facilitate pairing in the homozygote. In contrast with meiotic cells, the development of polyteny in somatic cells permits pairing in the duplicated region and throughout the chromosome arm.

Dp(2;2)619 is a small aberration for the amount of crossover reduction it causes. but a long duplication compared with the tandem duplications whose effects on recombination have been investigated previously. The tandem duplications studied by GREEN increased rather than decreased recombination, but they differ from Dp(2;2)619 in location as well as in size: one duplication is near the tip of the X chromosome and the other two are near the base. Nevertheless, one would predict, from the present data and those of DOBZHANSKY, that consistent pairing of a tandem duplication with the adjacent region on the same chromosome (as in Figures 2B and 2D) would lead to at least local crossover reductions in any euchromatic region. There is little information available on the pairing relations of these tandem duplications in salivary gland nuclei, but the Bar duplication usually fails to pair with the adjacent homologous region on the same chromosome even in tandem triplications, as can be seen by the diagrams of BRIDGES (1936) found in most genetics textbooks. The longest of the crossover-stimulating tandem duplications studied by GREEN (1962) was Bx^{r49k}, a duplication of bands in 17 A, B and part of C. It is possible that a minimum length is required for frequent looping back of a tandem duplication, a configuration that should reduce recombination in meiotic cells, and that Bx^{r49k} is shorter than this minimum length. The failure of short tandem duplications to reduce recombination can be most simply explained if the minimum length required for recognition or attraction of tandem duplications to adjacent homologous regions is between the length of Bx^{r49k} and Dp(2,2)619.

The smallest free duplication studied by DOBZHANSKY (1934) may pair competitively if a slight (but statistically insignificant) crossover reduction observed in the region of homology is due to the presence of the duplication. E. H. GRELL (1964) has shown that a duplication attached close to its region of homology produces greater crossover reductions (presumably pairs more competitively) than one that is free or attached to a site remote from the region of homology. Tandem duplications should have a greater opportunity to pair with their regions

of homology than free duplications of the same length but tandem duplications equal to and shorter than Bx^{r49k} apparently do not do so. If the minimum length required for a free duplication to pair competitively is, in fact, less than that required for a tandem duplication to pair with the adjacent homologous region, this may reflect one property of the recomplex (structural rigidity over short distances, for example).

Finally, it is possible to reconcile the inhibiting effect of a long tandem duplication with the stimulating effects of short tandem duplications on recombination by postulating that the long duplication interferes with recomplex formation but the short duplications do not, although they may modify pairing within the formed recomplex. Studies of asymmetrical pairing of tandem duplications have provided evidence that when short regions within a chromosome are considered, pairing is discontinuous (GREEN 1962; ROBERTS 1965; JUDD 1965). If, within the recomplex, the regions of precise pairing (presumably involving complementary DNA bases) that lead to crossovers are discontinuous and short, as the available evidence suggests, this is consistent with the hypothesis that pairing throughout most of the recomplex is approximate.

SUMMARY

Dp(2;2)619 is a long tandem duplication that lowers recombination throughout the left arm of chromosome 2, behavior that is inconsistent with a prediction of the effective-pairing hypothesis. The crossover reductions caused by this duplication are attributed to its length and distal location: the great length of the duplication permits pairing of the duplication with the adjacent homologous region, which, in turn, interferes with recomplex formation.

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