Genetica (1962) **33**: 154-164

THE EFFECTS OF TANDEM DUPLICATIONS ON CROSSING OVER IN *DROSOPHILA MELANOGASTER*

M. M. GREEN

Department of Genetics, University of California Davis, California

(*Received October 15, 1962*)

Each of three tandem duplications, *Bar*, *Beadex*^{r49k} and *Dp*(*1*)*z-w*, when homozygous increases crossing over in their environs in excess of the genetic length of the duplication.

Detailed crossing over studies with *Dp*(*1*)*z-w* showed that in the duplication homozygotes interference is reduced and when combined with heterologous autosomal inversions, double crossovers occurring in less than 10 map units are readily recovered.

These results are interpreted in terms of the concept of effective pairing and suggest that tandem duplications increase crossing over by increasing effective pairing.

Among the features of crossing over, pairing between homologous chromosomes at some meiotic stage appears essential. Whether the crossover event involves breakage and reunion of wholly replicated chromatids, or localized copy-choice concurrent with chromatid replication, some type of intimate association between homologues presumably takes place. It has been suggested, especially to explain the occurrence of localized negative interference in *Aspergillus*, that this necessary condition for crossing over is effective pairing (PRITCHARD, 1960a, b). Effective pairing is understood to represent the localized, intimate pre-pachytene chromosomal association occurring in a fraction of cells undergoing meiosis and is considered to be distinct from pachytene synapsis prerequisite to regular meiotic disjunction.

This study of the possible influence of short tandem duplications on the frequency of crossing over within and without the duplicated segment was undertaken on the presumption that it could provide useful information bearing on the question of pairing and crossing over. Two alternative effects of such duplications may be anticipated. Crossing over would be increased in the duplication homozygote equivalent to the genetic length of the duplicated segment. This is expected if pairing in the duplication homozygote is primarily symmetrical and equivalent to that between normal, unduplicated chromo-

somes. Alternatively, crossing over may be significantly greater than expected from the genetic length of the duplicated segment. This may be expected where both symmetrical and asymmetrical pairing occurs between the homologous duplications, such that the overall pairing frequency relative to that between normal chromosomes is appreciably increased. Frequencies of crossing over greater than the standard values for specific genetic intervals need not be considered exceptional since both genetical and environmental conditions have been described which bring about an increase. It is highly probable that for most genetic intervals, the maximal potential crossover frequency is usually not realized.

Experimental

The duplication selected for detailed study is one located distal to the centromere near the tip of the X chromosome in *Drosophila melanogaster* in which the chromosome segment between the *zeste* eye (*z*) and *white* eye (*w*) loci is duplicated in tandem. Cytologically, this segment is associated with salivary gland chromosome bands 3A4–3C1, inclusive. The origin of the duplication, designated *Dp z-w*, has been described in detail elsewhere (GREEN, 1961).

Crossing over between the loci *y-z-w-spl* (*y* = *yellow* body, *spl* = = *split* bristles) was studied, employing the mutants y^2, *z*, w^{a2} and *spl*. For these intervals, the standard crossover values are: *y-w* ca. 1% and *w-spl* ca. 0.8%. Among 336 observed crossovers in the *y-z-w* interval, 115 or 0.33 occurred between *y* and *z* and the remaining 221 or 0.67 between *z* and *w*. Thus, the genetic length of the added segment is ca. 0.67 crossover units. Parenthetically, it is of interest to point out that as measured by salivary gland chromosome length, the *y-z* section is at least four times longer than the *z-w* section yet the majority of described loci are distributed between *y* and *z* with only one localized within *z-w*.

Throughout, an attempt was made to keep the normal and duplication chromosomes as nearly genetically alike as possible. Heterozygous females were collected within eight hours of eclosion and mated immediately. Three females per mating were allowed to oviposit together for three consecutive intervals totalling ten days. Care was taken to provide uniform media both for raising heterozygous females and their progeny at a temperature controlled to 22–23°C.

Results

In the first experiments, the crossing over frequency within the interval y^2-z-w^{a2} was determined for homozygous standard chromosomes, homozygous *Dp z-w* and their heterozygote. The results tabulated in Table 1, 1)–3) show that in the homozygous duplication a striking increase in crossing over was realized, in excess of that expected from the genetic length of the duplication alone. Furthermore, not only was crossing over increased within the duplicated segment, but in the unduplicated distal segment as well. A second series of experiments was set up to include the interval w^{a2}-*spl* and to exploit the effects of the heterozygous autosomal inversions *Cy* (*Curly* wings) and *Ubx* (*Ultrabithorax-130*) in increasing crossing over in the X chromosome. The tabulation of these data in Table 1, 4)–6) confirms the results of the first series and shows, in addition, that crossing over in the unduplicated w^{a2}-*spl* interval was also increased. While a three-fold increase in crossing over within the *y-spl* interval

TABLE 1

CROSSING OVER IN *Dp z-w* HOMOZYGOTES AND HETEROZYGOTES
(Intervals: 1 = y^2-z; 2 = z-w^{a2}; 3 = w^{a2}-*spl*)

* Genotype P ♀♀	No. crossovers/interval, and (% crossing over/interval)						Total Progeny	Total % crossing over
	1	2	3	1,2	1,3	2,3		
1) y^2w^{a2}/z	9 (0.27)	20 (0.60)					3329	0.87
2) $y^2Dp\ w^{a2}/z$	5 (0.23)	15 (0.69)					2166	0.92
3) $y^2Dp\ w^{a2}/z\ Dp$	36 (1.06)	95 (2.81)					3383	3.87
4) $y^2w^{a2}spl/z$	11 (0.51)	14 (0.66)	18 (0.84)				2130	2.01
5) $y^2Dp\ w^{a2}spl/z\ Dp$	105 (1.35)	209 (2.67)	152 (1.96)		1	1	7854	4.98
6) $y^2Dp\ w^{a2}/z\ Dp\ spl$; $Cy/+$; $Ubx/+$	467 (4.41)	479 (4.50)	301 (2.84)	5	4	2	10799	11.75

* Duplication *z-w* abbreviated as *Dp*.

was found in the duplication homozygotes, a six-fold increase was achieved by supplementing the duplication homozygotes with heterozygous autosomal inversions. Both increases are well in excess of expectation based on the genetic length of the duplicated chromosome segment.

Aside from the increase in crossing over, a most noteworthy event was the recovery of double crossovers within the genetically marked intervals. While only two double crossovers were found in the duplication homozygote without heterozygous autosomal inversion, they are of significance because they occurred within a genetic interval of 5 crossover units. In *Drosophila* interference is expected to be complete in so short an interval (MORGAN, BRIDGES & STURTEVANT, 1925). Even more striking is the recovery in appreciable numbers of all possible double crossovers among the progeny of those females heterozygous for the autosomal inversions. The double crossing over frequencies are sufficiently increased to permit calculation of coefficients of coincidence. For the three intervals, designated 1, 2 and 3 in Table 1, coincidence values as follows were found: intervals 1; 2 = 0.24, intervals 1; 3 = 0.31, and intervals 2; 3 = 0.15. Thus, the combination of duplication homozygosity and heterologous inversions has effected a significant reduction in interference. While double crossovers in the standard *y-w-spl* interval effected by heterozygous autosomal inversions have been recovered (REDFIELD, 1955, 1957; GREEN, 1960), they are comparatively rare and have not, until now, been obtained in so high a frequency.

Of particular importance is the observation that concurrent with the increased frequency of crossing over, asymmetrical pairing and crossing over ("unequal" crossing over) between the homologous duplication chromosomes occurred such that both triplication and unduplicated (normal) chromosomes were recovered. An estimate of the frequency of "unequal" crossing over was obtained by progeny testing single crossover males of the phenotype $y^2z^+w^+spl$ from cross 6, Table 1, to determine whether they did or did not possess the duplication. (The progeny test simply involved crossing to homozygous *zeste* ♀♀. Those F_1 ♀♀ heterozygous for *z* and the duplication have a variegated eye color, while those carrying a normal chromosome are wildtype.) Among 85 males tested, three or ca. 3.5% proved to possess normal, unduplicated chromosomes which presumably arose

by unequal crossing over. These males represent only one-fourth the possible unequal crossovers, the reciprocal triplication could not be quantitatively found nor was the alternative unequal crossover class scored. Assuming this is an accurate estimate, the unequal crossovers could make up as many as 14% of all crossovers within the duplication region.

Among the double crossovers found, one male of the genotype $y^2z\ w^{a2}$ merits special mention because his X chromosome proved to be normal, i.e. no longer carrying the duplication. This male's X chromosome was, therefore, the product of concurrent asymmetrical pairing and double crossing over in a short genetic interval. The significance of this event will be discussed below.

To complement the crossing over data of *Dp z-w*, a comparable series of experiments was carried out employing a short tandem duplication proximal to the centromere at the right end of the X chromosome. For this purpose the tandem duplication of the *Beadex-wing* (*Bx*) locus, Bx^{r49k}, herein designated $(Bx^+)_2$, was selected. Earlier, genetic analysis established that neither the *small eye* (*sy*) locus 0.2 crossover units to the left of *Bx* nor the *fused* wing (*fu*) locus, 0.1 unit to the right are duplicated in $(Bx^+)_2$ (GREEN, 1953). Therefore, the total genetic length of $(Bx^+)_2$ is something less than 0.6 crossover units. Since unequal crossing over is known to occur frequently in $(Bx^+)_2$ homozygotes, a tandem triplication, $(Bx^+)_3$ was available for study as well. From homozygotes of the latter, tandem quadruplications, $(Bx^+)_4$, have been found and one was included here. For each replication homozygote, the total crossing over was de-go termined for the genetic interval *forked* bristles (*f*) to *carnation* eye

TABLE 2

CROSSING OVER IN THE *f-car* INTERVAL AS INFLUENCED BY TANDEM REPLICATIONS OF THE Bx^+ LOCUS

Genotype P ♀♀	Frequency crossovers in *f-car* interval	Total progeny scored	% crossing over
$f\ Bx^1\ car/+$	326	7572	4.30
$f\ (Bx^+)_2\ car/(Bx^+)_2$	361	5567	6.48
$f\ (Bx^+)_3/(Bx^+)_3\ car$	381	4531	8.41
$f\ (Bx^+)_4/(Bx^+)_4\ car$	381	4358	8.74

color (*car*) localized respectively to the left and right of the *Bx* locus. In the control, crossing over in the same interval was measured in females *Bx*/+. The results listed in Table 2 show that in the duplication homozygotes, a 50% increase in crossing over was realized as compared to the control. In the triplication and quadruplication homozygotes, the crossing over frequencies were doubled. In each case the increased crossing over was in excess of the overall genetic length of the repeated segment.

Finally, one crossover series was carried out to test whether so small a duplication as *Bar* eye (*B*) can effectively increase crossing over. Within the interval *f-B* crossing over was compared in heterozygous ♀♀ *f B*/++ and homozygous ♀♀ *f B*/+ B^i (B^i = *infrabar* whose duplication is identical to that of *B*). Among 3,366 progeny of the former, 5 crossovers were found equal to a frequency of 0.15%. Among 4,122 progeny of the latter, 14 crossovers (including 3 unequal crossovers) were recovered equal to a frequency of 0.34%. Thus, a duplication homozygote as short as *B*, effectively increases crossing over in its genetic environs.

Discussion

Among the genetic properties shared by the three tandem duplications studied here, the most significant appear to be the following: (1) All when homozygous undergo asymmetrical pairing and crossing over, i.e., "unequal" crossing over. (2) The frequency of crossing over increased in each duplication homozygote in excess of the genetic length of the duplicated segment. The increase is not limited to the duplication and includes adjacent unduplicated marked genetic intervals. (3) In the case of *Dp z-w* homozygotes, double crossovers occur within the marked genetic intervals where complete interference is expected. Furthermore, the combination of duplication homozygosity and heterologous autosomal inversions effected a striking reduction in interference.

What bearing do these facts have on the mechanism of crossing over? And what mechanism of crossing over best fits these data?

As noted at the outset and discussed in detail by PRITCHARD (1960a), crossing over apparently requires the interplay of two independent events occurring in concert. Thus, effective pairing, or

pairing which brings segments of homologous chromosomes (or nonsister chromatids) into an intimate association occurs followed by exchange between the homologous genetic segments at the point of contact. From this, it follows that the probability of a crossover occurring within a specified genetic interval depends on the combined probability of effective pairing and exchange. Presumably, by increasing or decreasing the occurrence of one or the other or both events, the frequency of crossing over can be maximized or minimized.

The very fact of unequal crossing over suggests that effective pairing between the homologous segments is increased in homozygous tandem duplications. It may be safely assumed that in duplication homozygotes the frequency of symmetrical pairing is in the least equivalent to the pairing frequency between those identical, unduplicated segments of homologous normal chromosomes. If, as is generally accepted, chromosome pairing is a property of the individual allelic loci, it is quite possible that because of the added loci, symmetrical pairing is increased in homozygous duplications. The recovery of unequal crossovers means that in duplication homozygotes, in addition to symmetrical pairing, asymmetrical pairing and exchange occurs. Thus, the total frequency of effective pairing is best represented as the sum of both symmetrical and asymmetrical associations.

On this basis alone, it is not difficult to see why crossing over between the homozygous duplicated segment is increased in excess of the genetic length of the duplication. Between normal chromosomes effective pairing for any given interval is primarily, if not exclusively, symmetrical. (The extreme rarity with which the products of asymmetrical pairing and crossing over, duplication and deficiency, are recovered attests to this conclusion.) It may be reasonably assumed that in a tandem duplication, the frequency of symmetrical associations and exchanges for each segment is equivalent to its frequency in a normal chromosome. Thus, exclusively symmetrical pairing should lead at a minimum to a crossing over frequency twice that of the unduplicated interval. When the "unequal" crossovers are added, the total frequency of crossing over exceeds that expected from the genetic length of the duplicated region.

The increased frequency of crossing over found in genetic intervals adjacent to either end of the duplications is in part similarly explicable in terms of increased effedtive pairing. By this is meant that the

effects on pairing imposed by tandem duplications are not delimited to the length of the duplications themselves but extend to adjacent regions. This implies that by increasing the probability of pairing at one defined genetic interval, the probability of pairing in immediately adjacent intervals is similarly increased. Thus, an increase in the crossing over frequency in these adjacent regions is effected.

Some comment on the length of the effective pairing segment is in order at this point. The occurrence of "unequal" crossing over argues in support of the idea that pairing between wholly replicated chromatids is neither prerequisite to nor necessary for the actual genetic exchange. Since "unequal" crossovers are, in part, the result of asymmetrical synapsis between halves of the duplications, it follows that the effective point of contact need be only very short. Indeed for any duplication arbitrarily designated A–B–a–b, asymmetrical pairing requires effective association between A–B of one chromatid and a–b of the nonsister chromatid. Thus, the maximum length of the effective pairing segment need only be equal to the genetic length of the duplicated segment, the interval A–B. "Unequal" crossing over has been described for duplications of varying cytogenetical lengths. Thus, the *Bar* duplication represents a repeat of eight bands (BRIDGES, 1936; MULLER *et al.*, 1936), the *Star* duplication of four bands (LEWIS, 1941), *Abruptex* of the band 3C7 (SCHULTZ in MORGAN *et al.*, 1941) and a duplication of the *w* locus involving only one band (GREEN, unpublished), of the band 3C3. Since the latter one band duplications readily undergo "unequal" crossing over, it follows that their required effective pairing segment must be exceedingly short, involving at most the distance equivalent to one salivary gland chromosome band. Even in the longer duplications, the effective pairing segment may be quite short. Thus, in the case of *Bar*, duplication of the 16A1–2 doublet has been associated with the phenotypic change (SUTTON, 1943) and asymmetrical pairing over the length of this doublet alone is sufficient to yield the exceptional crossovers. While it is hazardous to extrapolate salivary gland chromosome dimensions to meiotic chromosome lengths, it is nonetheless clear from these considerations that effective pairing requires association of only fractions of the total meiotic chromosome length.

The occurrence of crossing over between asymmetrically synapsed duplications or between asymmetrically paired homologous loci, e.g.,

between the *w* and *z* loci (GREEN, 1961; JUDD, 1961), represents added information in support of the notion that pairing along their entire lengths of wholly replicated chromatids is neither prerequisite to nor necessary for the exchange event. Furthermore, the positive correlation of asymmetrical pairing of duplications with an increased crossing over frequency can be accommodated best by a concept of crossing over where either chromatids pair in a touch-and-go fashion and exchange is sequential or where exchange takes place concurrent with chromatid replication of the copy-choice type. By touch-and-go pairing is meant the intimate association of different regions of homologous chromosomes at distinctive time intervals. This implies that at any given time pairing occurs at one site along the chromosome before or after that at an adjacent site. Thus, any unpairing of nonsister chromatids imposed by neighbouring asymmetrical associations would be transitory and the unpaired regions could become paired following exchange or unpairing at the point of asymmetrical synapsis. A copy-choice hypothesis implies a similar, if not identical, phenomenon. Here crossing over occurs in conjunction with DNA replication and replication is not simulatenous along the chromosome. The one recovered double crossover cited earlier in which one exchange involved asymmetrical pairing within *Dp z-w* and the second involved symmetrical pairing outside the duplication can be better accommodated by the notion of sequential crossing over than by one of simultaneous crossing over. Two simultaneous crossovers means simultaneous pairing between homologues at two close but separate regions. Such an event is difficult to envisage, especially when pairing at one of the sites is in all likelihood asymmetrical. The latter would very likely impose certain physical stresses which militate against concurrent pairing in adjacent regions. With sequential pairing and exchanges, such stresses should not occur for adjacent regions pair at different times.

Probably the most interesting observation reported here is the recovery from females homozygous *Dp z-w* of occasional double crossovers within the y^2-w^{a2}-*spl* interval and the regular recovery of all possible double crossovers when these females were made heterozygous for autosomal inversions. As a general rule in *Drosophila*, interference is complete over intervals less than ten crossover units in length. However as pointed out earlier, rare double crossovers have

been uncovered in the *y-w-spl* interval when, under the influence of heterologous autosomal inversions, total crossing over has been increased to approximately 7–10 crossover units. As indicated, the combined effects of homozygous *Dp z-w* and heterozygous autosomal inversions facilitated a measurable decrease in interference over intervals of equivalent genetic length. Since at least two double crossovers were found among the progeny of homozygous *Dp z-w* females devoid of autosomal inversions, it follows that the aforementioned decreased interference was, in part, effected by the increased pairing impaired by the homozygous duplications. The influence of the added autosomal inversions, known to increase multiple crossovers (see SCHULTZ & REDFIELD, 1951 for a detailed discussion), may be attributed to one or the other of the following effects. On the one hand, the autosomal inversions may effect an increase in effective pairing between the X chromosomes which, when added to the influence of the homozygous tandem duplications on effective pairing, facilitates a marked increase in crossing over. Implicit in this suggestion is the notion that the exchange frequency for any specified genetic interval is more or less constant and increased crossing over can be accommodated primarily through increased pairing. On the other hand, the autosomal inversions could conceivably increase crossing over by increasing the exchange frequency. This, when combined with the increased effective pairing of the duplications, could effect the observed increase in crossing over. There is at the moment, no critical information which recommends the choice of either one or the other interpretation, or perhaps a combination of the two.

REFERENCES

BRIDGES, C. B. (1936). The *Bar* "gene" a duplication. *Science* **83**: 210–211.

GREEN, M. M. (1953). The *Beadex* locus in *Drosophila melanogaster:* Genetic analysis of the mutant Bx^{r49k}. *Zeit. f. Vererbungsl.* **85**: 435–449.

GREEN, M. M. (1960). Apparent double crossing over in a short genetic interval in *Drosophila melanogaster*. *Nature* **186**: 990–991.

GREEN, M. M. (1961). Further data on non-homologous pairing and crossing over in *Drosophila melanogaster*. *Genetics* **46**: 1555–1560.

JUDD, B. H. (1961). Analysis of products from regularly occurring asymmetrical exchange in *Drosophila melanogaster*. *Genetics* **46**: 1687–1698.

LEWIS, E. B. (1941). Another case of unequal crossing over in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci.* **27**: 31–34.

MORGAN, T. H., C. B. BRIDGES & A. H. STURTEVANT (1925). The genetics of *Drosophila*. *Bibliogr. Genetica* **2**: 1–262.

MORGAN, T. H., J. SCHULTZ & V. CURRY (1941). Investigations on the constitution of the germinal material in relation to heredity. *Carnegie Inst. Wash. Ybk.* **40**: 282–287.

MULLER, H. J., A. A. PROKOFYEVA-BELGOVSKAYA & K. V. KOSSIKOV (1936). Unequal crossing over in the *Bar* mutant as a result of duplication of a minute chromosome section. *C. R. Acad. Sci. U.S.S.R.* **1**: 87–88.

PRITCHARD, R. H. (1960a). The bearing of recombination analysis at high resolution on genetic fine structure in *Aspergillus nidulaus* and the mechanism of recombination in higher organisms. *Tenth Symp. Soc. Genl. Microb.*: 155–180.

PRITCHARD, R. H. (1960b). Localized negative interference and its bearing on models of gene recombination. *Genet. Res.* **1**: 1–24.

REDFIELD, HELEN (1955). Recombination increase due to heterologous inversions and the relation to cytological length. *Proc. Nat. Acad. Sci.* **41**: 1084–1091.

REDFIELD, HELEN (1957). Egg mortality and interchromosomal effects on recombination. *Genetics* **42**: 712–728.

SCHULTZ, J. & HELEN REDFIELD (1951). Interchromosomal effects on crossing over in *Drosophila*. *Cold Spring Harbor Symp. Quant. Biol.* **16**: 175–195.

SUTTON, EILEEN (1943). *Bar* eye in *Drosophila melanogaster*: A cytological analysis of some mutations and reverse mutations. *Genetics* **28**: 97–107.