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Title: THE EFFECTS OF TANDEM AND NON CONTIGUOUS DUPLICATIONS ON FERTILITY, VIABILITY AND CROSSING OVER IN *Drosophila melanogaster*

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X-ray or EMS (ethylmethanesulfonate) induced suppressors of the Minute loci M(2)z and $M(3)w^{124}$ were recovered in the 2L and 3R arms chromosome arms of *Drosophila melanogaster*. A total of thirty three suppressors of Minute were recovered: twenty were suppressors of M(2)z, and thirteen were suppressors of $M(3)w^{124}$. Cytologically, all were duplications of one or more polytene bands.

Frequencies of crossing over in flies heterozygous for duplications of seven or more polytene bands were reduced with only one exception, and the degree of reduction of crossover frequencies increased with increasing length of the duplication. Many of the duplications were strong reducers of crossover frequencies. Some reduced the frequency of crossing over throughout a chromosome arm to ten percent or less of the control value for the corresponding chromosome arm. Within an arm the position of the duplica-

tion influenced the regional pattern of crossing over. A short (10 polytene band) homozygous 2L duplication increased the frequency of crossing over in the vicinity of the duplication in excess of the duplication's genetic length.

When examined cytologically, all duplications isolated in this study showed evidence of intrachromosomal pairing back of the duplicated portions of the genome. Single band tandem duplications showed pairing to the presumptive parent band as evidenced by connecting strands of chromatin to the presumptive parent band or apposition to the presumptive parent band in either synapsed or desynapsed polytene strands. All direct tandem duplications isolated of seven or more polytene bands were always seen to form intrachromosomal loops in analysable polytene figures.

The Minute loci used in this study to select for duplications were effectively localized by using the overlapping series of duplications generated by the selection technique. M(2)z was localized to the 25A1-2 doublet or the surrounding interband regions, and $M(3)w^{124}$ was localized to the 95A1-2 doublet or the surrounding interband regions. The use of overlapping duplications isolated as suppressors of the Minute loci should be useful in localizing the positions of other Minute loci as well.

Cyogentic and genetic data were obtained which indicated that the dumpy locus (dp) is located in the 25A3-4

doublet or the 25A2-3 interband region.

A mechanism is considered by which newly induced small direct tandem duplications could be stabilized in a population, and thus serve as a potential source of new genes in a population. The mechanism is based on the observations that small heterozygous tandem duplications can reduce crossing over in the environs of the duplications, and that small direct tandem duplications have little or no detectable effect on the fertility or viability of the duplication bearing flies.

The Effects of Tandem and Noncontiguous Duplications on Fertility, Viability and Crossing Over in *Drosophila melanogaster*

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THE EFFECTS OF TANDEM AND NON-CONTIGUOUS DUPLICATIONS ON FERTILITY, VIABILITY AND CROSSING OVER IN *Drosophila melanogaster*

I. INTRODUCTION

The factors controlling the behavior of chromosomes in meiotic cells are still in need of study. In particular the sequence of events leading to synapsis of homologues is still not well defined.

It is difficult to study meiotic cells in *Drosophila* because the chromosomes are small. However, by using polytene salivary gland cells it is possible to determine the breakpoints of aberrations and to define their location accurately in the genome. By analysing the pairing configurations of the aberrations it is sometimes possible to gain some insight into meiotic pairing configurations.

With the use of multiply marked stocks the number and position of exchanges can be easily determined. This information is useful in analysing the meiotic behavior of aberrations. Studies using chromosome aberrations to disrupt pairing have given some insight into the functional differentiation of chromosome arms with respect to synapsis. For example, translocation studies of Roberts (1970, 1972) have delineated distal-medial regions of autosome arms which are particularly sensitive to disruption by the presence of a translocation breakpoint.

Rhoades (1938) was the first to study the effects of

a duplication on crossover frequencies. Using a translocation fragment of chromosome 2 inserted into the Y chromosome, he observed a reduction in crossover frequencies both in the region of the duplication and outside the region of the duplication.

Dobzhansky (1934) and Grell (1969) used free duplications of the X chromosome to study recombination. They found that the greater the length of the duplication the more crossover frequencies were reduced.

Studies by Roberts (1966) and Kalish (1975) showed that long tandem duplications can act as powerful crossover suppressors. In contrast, very short X chromosome tandem duplications when either heterozygous or homozygous increased frequencies of crossing over (Green, 1962).

Systematic study of the behavior of tandem duplications had been hampered by the lack of a suitable selective technique. This deficiency was remedied with the development of the anti-Minute technique by Grell (1969). The Minutes are a class of dominant bristle mutants which are homozygous lethal. This technique is based on an observation by Schultz (1929) that one dose of the Minute allele is recessive to two doses of the wild type allele. Duplications are selected as suppressors of the Minute phenotype. There are some forty Minute loci scattered throughout the genome of Drosophila melangaster. Since this technique gives duplications of different lengths one can

select for a series of duplications of different lengths in different portions of the genome.

The purpose of this study was to investigate in detail the cytology and genetic behavior of a series of duplications of different lengths in different parts of the Drosophila melanogaster genome. Viability and fecundity studies were also conducted in order to provide, in concert with the cytological and genetic data, estimates of the evolutionary potential of newly induced tandem duplications.

II. MATERIALS AND METHODS

Production of Duplications and Culture of Flies

Lewis' Standard Drosophila Medium (Lewis, 1960) was used for all experiments. The flies were grown in ½ pint or ¼ pint glass milk bottles or 30 ml shell vials. Cultures were maintained at 25°C±1°C for all experiments.

Containers to restrain the flies during irradiation were made from pieces of tygon tubing seven centimeters long and five millimeters internal diameter with tiny air holes one centimeter apart. Corks were used as closures. About 200 flies were placed in each container. The flies were not left in these containers more than one hour, and they were usually left less than half an hour, because the humidity increased with time and the flies would stick together. Immediately after irradiation the flies were transferred to shell vials with media.

Female flies were irradiated with X-rays from a GE 300 KVP machine with a 0.25 mm Cu filter at an average dose rate of 10.9 rads/sec (range 3.1-27.8) and average total dose of 4,000 R (range 3960-4042). The height of the machine head from the containers ranged from 3.1 cm to 22.5 cm.

One stable suppressor, SuM2z10, was induced by EMS (ethylmethanesulfonate) as an incidental product of an independent line of research by Laurie Mac Phail.

Induction of SuM Duplications

$\frac{r}{r}$ females * X $\frac{Bal}{M}$ males **

↓

Genotype	$\frac{Bal}{r}$	$\frac{M}{r}$	$\frac{M}{r\ SuM}$
Phenotype	Balancer	Minute	wild type (rare)

Back cross of potential SuM Duplications

$\frac{M}{r\ SuM}$ X $\frac{Bal}{M}$

↓

Genotype	$\frac{Bal}{r\ SuM}$	$\frac{M}{r\ SuM}$	$\frac{Bal}{M}$	$\frac{M}{M}$
Phenotype	Balancer	wildtype if SuM is stable	Balancer Minute	dies
	Used for stocks	Used for stocks		

Figure 1 Diagram of anti-Minute technique

* Females were homozygous for recessive (r) mutant(s) so non-virginity can be detected. They were X-rayed to induce chromosome breakage. Treated maternal chromosomes are indicated by dotted lines.
SuM = Suppressor of Minute

** The Balancer (Bal) stocks carried crossover suppressors and dominant markers.

Selection for Duplications

The method used to select for duplications was the "anti-Minute" technique developed by Grell (1969) which selects for duplications on the basis of the duplication's ability to suppress the phenotype of Minute mutants (Figure 1). Minutes at two different loci were used to select for Suppressors of Minute (SuM). One Minute, M(2)z, was on the second chromosome near the tip of 2L. The other Minute, $M(3)w^{124}$, was on the third chromosome near the tip of 3R.

Irradiated females were mass mated to the appropriate Minute males (Balancer/Minute)(Figure 1). Optimum culture conditions were achieved with 40-50 irradiated females and 40-50 Minute males mated in each 1/2 pint milk bottle with transferral of flies to fresh media every two days for eight days. Three classes of progeny were expected in the F_1 progeny with dominantly marked balancer or Minute phenotypes predominating (Figure 1). Only the rare phenotypically wild type bristled flies, potential suppressors of Minute, were saved. The F_1 wild type Suppressors of Minute/Minute (SuM/M) flies were backcrossed to the corresponding balanced Minute stock (Balancer/Minute) to check the stability of the suppressor, and to establish balanced stocks of the stable suppressors of Minute. Several sublines (3-7) were established for each stock.

The potential suppressors of Minute when backcrossed fell into four categories. The first category consisted of those which were either misclassified or somehow transiently modified as none of their progeny showed suppression of Minute. These flies were discarded. The second category consisted of phenotypically mosaic flies (those possessing only some wild type bristles); these did not transmit the ability to suppress Minute to their progeny. Flies in this category were also discarded. The third category consisted of flies, which while phenotypically wild type, gave both progeny carrying a suppressor and progeny not carrying a suppressor. These flies were presumably gonadal mosaics. Four of five flies in this category eventually gave stable suppressors and stocks were established. The fourth category consisted of flies whose progeny all carried Suppressors of Minute; stocks were established from these flies.

From the F_2 progeny of the F_1 wild type SuM/M X Bal/M (Balancer/Minute) cross, two types of stock were established: Bal/SuM females X Bal/SuM males and M/SuM females X M/SuM males (Figure 1). The Bal/SuM stock was a balanced stock of the suppressor if the Bal/SuM flies were viable and the homozygous suppressor flies were lethal. If, however, the homozygous suppressor flies were viable, sublines would frequently lose the balancer and homozygous sublines would become established.

SuM/M balanced sublines were practical because the Minute gene is homozygous lethal, and the SuM is usually a crossover suppressor. They were started for three reasons: The first reason was to have a balanced subline available if the Bal/SuM stock subline proved inviable, i.e. in cases when the suppressor was inviable when heterozygous for the balancer chromosome and homozygous lethal as well. The second reason was to have a stock for cytology that did not require outcrossing, and the third reason was to have a stock in which limited crossing over away from the M-SuM region might occur. Crossing over could then lead to elimination of lethals induced at loci different from the suppressors, but on the same chromosome. Elimination of lethals was possible if the SuM were a weak crossover suppressor since the chromosome containing the Minute gene contained no crossover suppressors.

A third method was occasionally necessary to maintain a suppressor if both the Bal/SuM stock were inviable and the M/SuM stock also had poor viability. In such cases usually involving the longest duplications, for each generation, M/SuM males were picked from the cross Bal/M females X M/SuM males and mated to Bal/M females again.

When the F_2 progeny of the F_1 wild type fly gave both wild type and M progeny (usually because the fly was a gonadal mosaic) the F_2 wild type flies were mated to the

Bal/M stock and the F_3 progeny used to establish the Bal/SuM or SuM stocks. This was necessary because one could not reliably establish the Bal/SuM stock with the F_2 flies since some of the F_2 flies with the balancer chromosome would contain the duplication while some would not.

Suppressors were induced in females homozygous for recessive mutants so any progeny from non-virgin females could be detected in the F_1 and discarded. In the first experiment to collect SuMs, cn bw; e females were used to select for both second and third chromosome suppressors, but because ebony interfered with the scoring of the F_2 crossover progeny for the third chromosome suppressors, this stock was not used again. In subsequent experiments cn bw females were used to select for second chromosome suppressors and p^p females were used to select for third chromosome suppressors.

Cytology

Larvae to be used for cytology were grown at 18°C in 1/2 pint milk bottles with Fleishmanns dry yeast added to the medium. To prepare polytene chromosomes for observation, standard salivary gland staining and squashing techniques were used (Lefevre, 1976).

For cytological analysis of the aberrations we used chromosomes that were well stretched, stained and desynapsed for precise localization of the breakpoints. To

accomplish this for all the duplications, cytology was done using a variety of stocks. The choice of stocks depended upon the length of the duplication. If the duplication were moderately large to large (100 bands or more) enough desynapsed figures were observed using the SuM stock or outcrossed stocks (SuM/Bal X wild type). If the duplication were short, however, desynapsis of the homologues occurred infrequently when over wild type or Minute, so Bal/SuM stocks were used. The presence of the multiply inverted balancer chromosome increases the frequency of desynapsis with its homologue by interfering with somatic paring.

The number of bands for each duplication was determined from Bridges' revised maps (Bridges, 1941, Bridges, 1942). In cases where the breakpoints are given just to a lettered subdivision, e.g. 24E, the number of bands was determined from the beginning of the lettered subdivision.

Recombination

Three types of genetic marker stocks were used for the recombination studies with heterozygous duplications. Two were used to determine recombination throughout an entire arm or most of an arm: "all" was used for the 2L arm, and "streroca" was used for the 3R arm (see Tables 1 and 2 for a description of these stocks). Of the seven mutants in the "all" stock only five, those lying in

regions likely to be affected by the rearrangement, were scored. Four of these, al, dp, b, pr, were in 2L and one, c, was in 2R. All the mutants of the "streroca" stock were scored. The third stock, ed dp cl, was used to study recombination in a short interval around the small duplications of the second chromosome. A corresponding stock for the small duplications of the third chromosome does not exist because of a lack of markers in the appropriate region.

For the F_1 crosses several marker stock females were crossed in shell vials to several males carrying the duplication. F_1 heterozygous females (marker/duplication) were individually testcrossed to 3-5 males of the marker stock. Females were individually testcrossed so non-virgin or contaminant females could be detected.

Crossing over in homozygous small duplications was studied in duplication bearing stocks made from the F_2 crossover flies of the ed dp cl/ duplication cross described above. F_2 crossover flies (e.g. ed or dp cl) were mated to the Bal/M flies, and if the F_2 crossover fly contained the duplication a balanced stock was started. For the crossover study two of these duplication bearing stocks with the appropriate markers were crossed to obtain F_1 females homozygous for the duplication and heterozygous for the markers. These F_1 females were testcrossed to homozygous ed dp cl males. Crossover values from

TABLE 1. LIST AND DESCRIPTION OF MUTANTS USED.

For more complete description see Lindsley & Grell (1968).

SYMBOL	NAME	LOCATION	DESCRIPTION
al	aristaless	2-0.0	aristae reduced
b	black	2-48.5	body, legs, & veins darkened
bw	brown	2-104.5	eye color brown
c	curved	2-75.5	wings curved
ca	claret	3-100.7	eye color ruby
cl	clot	2-16.5	eye color dark maroon
cn	cinnabar	2-57.5	eye color bright red
dp	dumpy	2-13.0	truncated wings
e	ebony	3-70.7	body color black
ed	echinoid	2-11.0	eyes large & rough
lt	light	2-55.0	eye color pink
M(2)z	Minute	2-12.9	reduced bristles
M(2)zB	Minute	2-12.9	deficiency of Bridges
$M(3)w^{124}$	Minute	3-79.7	reduced bristles
p^{p}	pink-peach	3-48.0	eye color orange
pr	purple	2-54.5	eye color ruby
ro	rough	3-91.1	eyes rough
sp	speck	2-107.0	wing axils speckled
sr	stripe	3-62.0	stripe on thorax
st	scarlet	3-44.0	eye color vermilion
Ore-R	Oregon-R		wild type; Roseburg, OR

TABLE 2. LIST OF STOCKS USED

For more complete description see Lindsley & Grell (1968).

cn bw

cn bw; e

p^p

al dp b pr c pl sp = "all"

st sr e ro ca = "streroca"

Ore-R

M(2)z/In(2LR)SM5, al^2 Cy lt^v cn^2 sp^2

$M(3)w^{124}$/In(3LR)TM3, y^+ ri p^p sep bx^{34e} e^s

$In(3LR)Ubx^{130}ca$/T(3:4);83B

Ore-R/ed dp cl females were used as control values.

Whenever the F_1 crosses were started for a recombination experiment, "phenotype checks" were also started at the same time with males from the same subline that was used to start the recombination crosses. The purpose of this cross was simply to determine if the subline still contained the suppressor rather than wild type flies resulting from contamination or breakdown of the duplication. The phenotype check cross consisted of Bal/M females X either Bal/SuM or M/Sum males. Wild-type progeny indicated that the suppressor was still present. Crossover studies were conducted for all duplications recovered. Selected ones were repeated to check the reproducibility of the results.

Egg Hatch and Viability Studies

In order to determine egg hatch and viability, a duplication was made heterozygous over Ore-R. The Ore-R wild type was chosen, rather than the stock in which duplications were induced, so duplications on 2L and 3R could be compared in a similar, although admittedly not identical, background. Ore-R flies were used as controls. The usual cross made for the studies was F_1 duplication/ Ore-R females X Ore-R males. The reciprocal cross, Ore-R females X F_1 duplication/Ore-R males was made for selected duplications. The crosses generating heterozygotes and

the controls were raised in shell vials on media from the same batch of food to eliminate any possible batch differences in the food. The F_1 females were aged 5-7 days so egg production would be near maximum. The females were then individually mated to 3-4 males in 1/4 pint bottles with standard Lewis' food dyed dark blue with Schilling Blue Food Color (McCormak and Co.) with 10 ml/5 liters of media added to improve egg visibility. One half hour before the flies were transferred, three to four drops of a yeast + sucrose + water mixture were added to each bottle with a Pasteur pipette. The mixture was made by adding a pinch of Fleishmanns Bakers Yeast and a pinch of sucrose to five ml of water. After the percent hatch was determined, a piece of Saran Wrap was used to cover the top of the bottle to reduce moisture loss.

Females were placed on the food at time 0 and transferred to fresh food at 24 hours. The number of eggs laid was counted shortly after the transfer. The percent of eggs hatched was determined at 48 hours. The number of pupae and adults was determined at 14-16 days.

The total number of eggs counted at 24 hours and 48 hours should be the same. Occasionally, however, some eggs (usually less than 0.5%) were obscured by bacterial growth at 48 hours and could not be scored accurately with respect to hatching. The percent hatched (equivalent to percent making it to the larval stage, or %L), was based

on the number that could be scored at 48 hours. Since some of the obscured eggs did in fact hatch, there being as many adults as there were eggs laid in some of these cultures, the 24 hour count was more desirable as a basis for calculating percent pupae (i.e., percent surviving to the pupal stage) and the percent adults (i.e., the percent surviving to the adult stage). By using the 24 hour count, pupal and adult percentages were never greater than 100 percent. The percent larvae lost during the larval stages (%LL) was calculated as %LL = %pupae - % hatched, and the percent lost during the pupal stage (%PL) was calculated as %PL = % adults - % pupae.

If none of the eggs hatched from a female, the data from that female were excluded since it was not determined whether the cause for the lack of hatch was sterility or lack of insemination.

RESULTS

Thirty-three stable suppressors of Minutes were isolated from X-irradiated females; 19 were suppressors of M(2)z and 13 were suppressors of $M(3)w^{124}$. One stable suppressor of M(2)z was induced by the chemical mutagen EMS (ethylmethanesulfonate). Cytological analysis showed that all suppressors were visible duplications of one band or more. One stock which contained Suppressor of Minute (3)w number 6 (SuM3w6) also contained a long duplication (Dp(3:3)DJB 1) near the base of 3L (Figure 2A). This raised the total number of duplications recovered to 34 (Tables 3 and 4). The frequency of recovery for the M(2)z suppressors was 0.03 percent, and the frequency of recovery for the M(3)w suppressors was 0.05 percent.

The duplications isolated were of a wide range of lengths varying from one to 502 bands (Bridges' notation). Furthermore, there was a significant difference between the lengths of the duplications recovered in the two chromosome arms. The longest 3R duplication (502 bands) was more than twice as long as the longest 2L duplication (227 bands). The mean number of bands for the 3R duplications (200 bands), was more than twice as great as the mean number of bands for the 2L duplications (92 bands). In spite of a large difference of the variances, these differences in mean band length were significantly differ-

Figure 2. PHOTOGRAPHS OF POLYTENE CHROMOSOMES SHOWING PAIRING CONFIGURATIONS OF THE DUPLICATIONS.

2A Dp(3:3)DJB 1

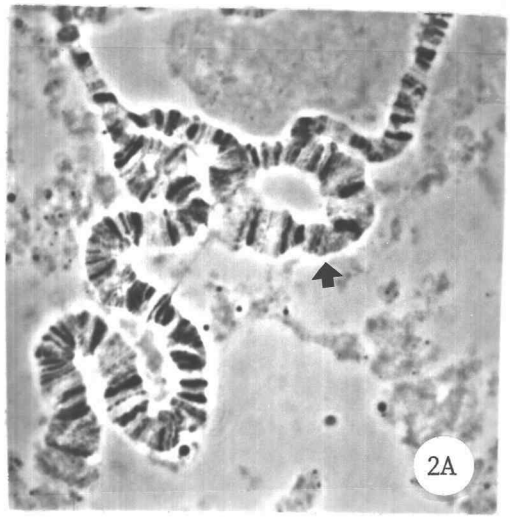
2B SuM2z1

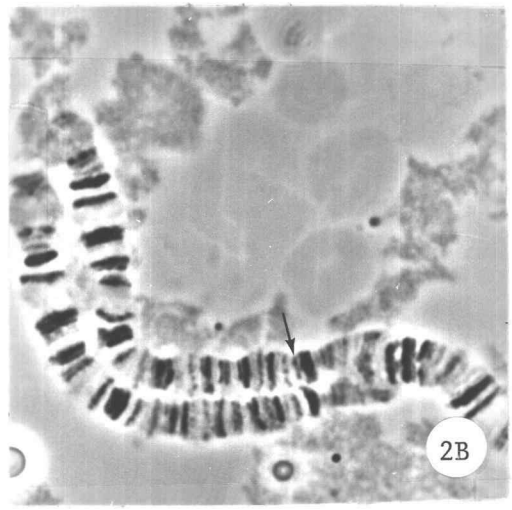
2C SuM2z11

2D SuM3w5

2E SuM2z5

2F SuM2z5


2A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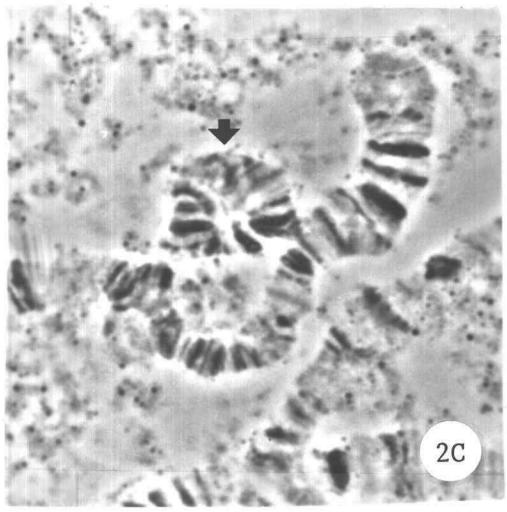
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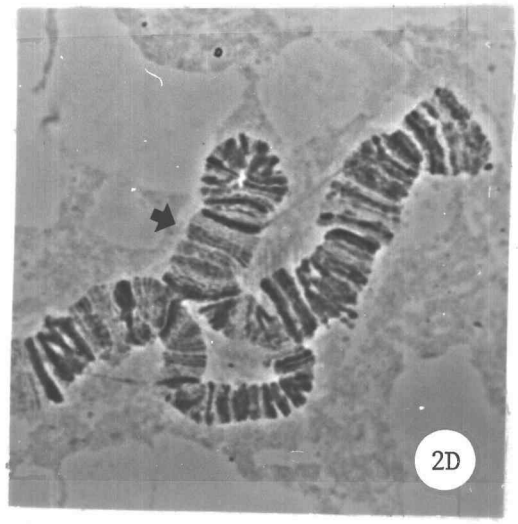
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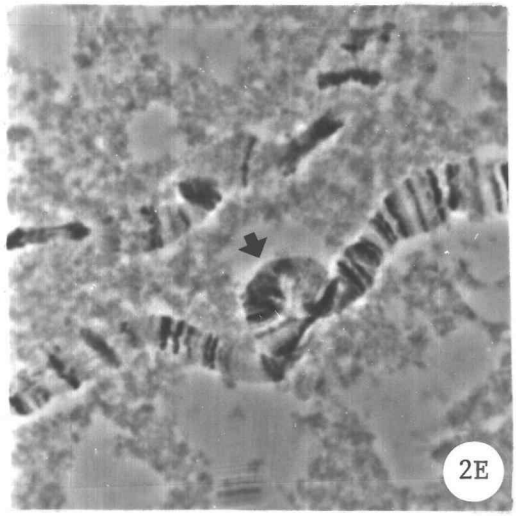
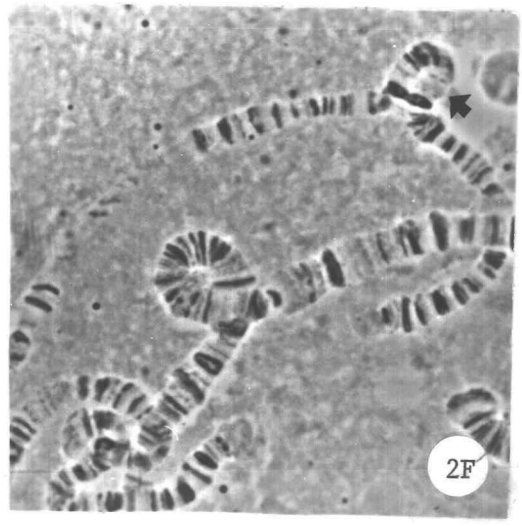

2E


2F

ent by a t-test at the 5 percent level of significance. The longest 3R duplication represented 43 percent of the length of the 3R polytene arm, and the longest 2L duplication represented 28 percent of the length of the polytene arm. It appears, therefore, (assuming that breaks are induced randomly in both arms) that aneuploidy is more tolerated in the segment of 3R studied than in 2L.

Twenty-nine of the duplications were contiguous direct repeats (tandem repeats). To illustrate, the gene order for a tandem repeat might be --ABAB--. Two were contiguous but, since they were single band duplications, the order could not be determined cytologically. Three of the duplications, SuM2z5, SuM3w5 and SuM3w13, were non-contiguous reversed repeats (e.g. with a gene order --ABCDBA--) in which the duplicated segment was inserted in the same arm from which it originated.

The second chromosome with the one band duplication, SuM2z1 (Figure 2B), when heterozygous uncovers several members of the dp pseudoallelic series. Furthermore, heterozygotes for SuM2z1 with dp alleles dp^{o2}, dp^{v2}, or dp^{ov} give more extreme dp phenotypes than their respective homozygotes. A deficiency has not been detected cytologically, but since this is the way the dp alleles behave in the presence of known deletions, a submicroscopic deletion is probably present.

TABLE 3. ABREVIATIONS AND DESCRIPTIONS OF SUPPRESSORS OF MINUTE(2)z.

ABREVIATIONS	NUMBER BANDS	TYPE*	REARRANGEMENT	CYTOLOGY
SuM2z1	1	T	Dp(2;2)SuM(2)z1	Dp(2;2)24F8-25A1;25A2-3
SuM2z2	7	T	Dp(2;2)SuM(2)z2	Dp(2;2)24F6;25A4
SuM2z3	10	T	Dp(2;2)SuM(2)z3	Dp(2;2)24F1(2);25A2-3
SuM2z4	12	T	Dp(2;2)SuM(2)z4	Dp(2;2)24F1(2);25A4
SuM2z5	12	NR	Dp(2;2)SuM(2)z5	Dp(2;2)24F1;25A4:26C
SuM2z6	25	T	Dp(2;2)SuM(2)z6	Dp(2;2)24D1(2);25A4
SuM2z7	40	T	Dp(2;2)SuM(2)z7	Dp(2;2)24A1(2);25A2-3
SuM2z8	42	T	Dp(2;2)SuM(2)z8	Dp(2;2)24A1(2);25A3(4)
SuM2z9	55	T	Dp(2;2)SuM(2)z9	Dp(2;2)24E1(2);25E1(2)
SuM2z10	69	T	Dp(2;2)SuM(2)z10	Dp(2;2)24F8-25A1;26C1(2)
SuM2z11	87	T	Dp(2;2)SuM(2)z11	Dp(2;2)22D6;25A2-3
SuM2z12	100	T	Dp(2;2)SuM(2)z12	Dp(2;2)24E1(2);26F
SuM2z13	122	T	Dp(2;2)SuM(2)z13	Dp(2;2)22F;25E2
SuM2z14	135	T	Dp(2;2)SuM(2)z14	Dp(2;2)21B7;25A4
SuM2z15	143	T	Dp(2;2)SuM(2)z15	Dp(2;2)23D1;26F
SuM2z16	152	T	Dp(2;2)SuM(2)z16	Dp(2;2)23E-F;27D-F1
SuM2z17	156	T	Dp(2;2)SuM(2)z17	Dp(2;2)22A3;25F2
SuM2z18	195	T	Dp(2;2)SuM(2)z18	Dp(2;2)23D1(2);27B4
SuM2z19	206	T	Dp(2;2)SuM(2)z19	Dp(2;2)22A1;27C2
SuM2z20	227	T	Dp(2;2)SuM(2)z20	Dp(2;2)23F;29B

*T = Tandem duplication and NR = Noncontiguous reversed repeat.

TABLE 4. ABREVIATIONS AND DESCRIPTIONS OF SUPPRESSORS OF MINUTE(3)w.

ABREVIATIONS	NUMBER BANDS	TYPE*	REARRANGEMENT	CYTOLOGY
SuM3w1	1	T	Dp(3;3)SuM3w1	Dp(3;3)95A1(2)
SuM3w2	23	T	Dp(3;3)SuM3w2	Dp(3;3)94E6(7);95B
SuM3w3	48	T	Dp(3;3)SuM3w3	Dp(3;3)94E4;95D1
SuM3w4	69	T	Dp(3;3)SuM3w4	Dp(3;3)94A1(2);95A
SuM3w5	69	NR	Dp(3;3)SuM3w5	Dp(3;3)95A1;96B1(2);97E
SuM3w6	175	T	Dp(3;3)SuM3w6	Dp(3;3)93F;96B
SuM3w7	206	T	Dp(3;3)SuM3w7	Dp(3;3)93D;95D
SuM3w8	226	T	Dp(3;3)SuM3w8	Dp(3;3)92E1(2);96A1(2)
SuM3w9	232	T	Dp(3;3)SuM3w9	Dp(3;3)95A1(2);98B
SuM3w10	291	T	Dp(3;3)SuM3w10	Dp(3;3)91F;95A2
SuM3w11	373	T	Dp(3;3)SuM3w11	Dp(3;3)92E1(2);98A
SuM3w12	382	T	Dp(3;3)SuM3w12	Dp(3;3)93D5;99B1(2)
SuM3w13	502	NR	Dp(3;3)SuM3w13	Dp(3;3)90E;98B
———	191	T	Dp(3;3)DJB1**	Dp(3;3)70F;76A

*T = Tandem duplication and NR = Noncontiguous reversed repeat.

**Not a SuM3w; one of two duplications recovered in the SuM3w6 stock.

Cytology

All suppressors of Minutes recovered were cytologically visible duplications that indicated their homology by looping or pairing back with adjacent band(s). In the single band duplications, SuM2z1 and SuM3w1, pairing back was evidenced by the presence of strands of chromatin extending between the duplicated band and its presumptive "parent" band in attenuated preparations, and by curving of the band toward and apposition of the band to the presumptive parent band in less attenuated preparations.

Tandem duplications of seven bands or more clearly showed the presence of an intrachromosomal loop caused by pairing back of the duplicated chromatin upon itself in both synapsed and desynapsed homologues (Figures 2A and 2B). These intrachromosomal loops were always seen in analysable figures. The appearance of synapsed strands of tandem duplications from seven to about fifty bands in length, was a highly localized "fuzziness" of the bands resulting from stretching and distortion of the bands involved in pairing back. This fuzziness was difficult to distinguish from fuzziness caused by other distortions resulting from the preparation of polytene chromosomes. Fortunately, fairly accurate localization of the Minute loci by the technique of overlapping duplications (see below) permitted the appropriate region to be carefully examined. However, to confirm the presence of a small

duplication, desynapsed figures, where the duplicated strand can be examined apart from its normal homologue, were essential.

A loop is most clearly and regularly seen in either synapsed or desynapsed strands in the larger duplications (about 90 bands or more)(Figures 2A and 2C). In the larger duplications, presumably because of mechanical difficulties, a portion of the basal part of the duplication loop was usually desynapsed (Figures 2A and 2C). Large polytene bands usually delimited the ends of these desynapsed portions of the basal part of the loop. The size of this desynapsed portion differed from duplication to duplication. For a given duplication it was usually quite constant although occasional unstretched figures would show pairing of the usually desynapsed region, and conversely, occasional figures would show desynapsis of the normally paired delimiting large polytene bands. The loop was never entirely desynapsed with either the small or large duplications.

The three noncontiguous reversed repeats also showed intrachromosomal pairing in the polytene chromosomes. The type of pairing configuration seen with the noncontiguous reversed repeat is illustrated by the duplication SuM3w5 (Figure 2D). The small noncontiguous reversed repeat, SuM2z5, when synapsed with its homologue, mimicked the appearance of a tandem duplication (Figure 2E), but when

desynapsed its true nature was apparent (Figure 2F).

Cytological Localization of the Minute Loci

The usual method of localization of genes to specific salivary chromosome regions or bands has been by the use of overlapping deletions. The use of overlapping duplications to localize genes became practical with the development of the "anti-Minute" technique, a technique of selecting for duplications as suppressors of Minutes. The duplications recovered in this study using the "anti-Minute" technique covered a wide range of lengths and positions, and proved to be surprisingly effective in localizing the Minutes.

Localization of M(2)z

M(2)z is probably located in the 25A1-2 doublet or the immediately adjacent interbands. The evidence for this comes from a set of overlapping duplications recovered as suppressors of M(2)z. Duplications SuM2z3 and SuM2z7 have their rightmost breakpoints between 25A2 and 25A3. SuM2z10 has its leftmost breakpoint to the left of 25A1 but after 24F8, the last band in the 24F section (Table 3). Since only 25A1-2 is common to these duplications and all suppress M(2)z, it appears that M(2)z is located in 25A1-2 or the surrounding euchromatin. Corroboration for this conclusion comes from SuM2z1 (Figure 2b). That this

apparent one band duplication frequently shows pairing to the 25A1-2 doublet in heterozygotes, is independent evidence that it is the 25A1-2 band which is duplicated. Both lines of evidence support the assignment of M(2)z to the 25A1-2 band.

Localization of $M(3)w^{124}$

$M(3)w^{124}$ is probably located in the 95A1-2 doublet. The evidence for this comes from a set of overlapping duplications recovered as suppressors of $M(3)w^{124}$. Duplication SuM310 has its rightmost breakpoint just to the right of 95A1-2, while SuM3w9 has its leftmost breakpoint just to the left of 95A1-2 (Table 4). Since only the band(s) 95A1-2 is common, and both are suppressors of $M(3)w^{124}$, it appears that $M(3)w^{124}$ is located in 95A1-2 or the adjacent interband regions. Corroboration for this conclusion comes from the duplication SuM3w1. This one band duplication frequently shows pairing to the 95A1-2 doublet in desynapsed strands. Consequently, it should be a duplication for the 95A1-2 band. This is in agreement with the assignment of $M(3)w^{124}$ to the 95A1-2 band from the set of longer overlapping duplications.

Localization of dp and the Genetic Order of M(2)z and dp

Previous attempts to localize dp have narrowed the

location to the 24D-25A2-3 region. In the course of localizing M(2)z, we have accumulated evidence that dp is probably located in the 25A3-4 doublet. The evidence for this is both cytological and genetic. As mentioned above, the rightmost breakpoint of duplication SuM2z3 (which does not include dp) is between 25A2 and 25A3. The rightmost breakpoint of the duplication SuM2z4 (which does include dp) is to the right of 25A4, but before the next detectable band, 25A5; 25A3-4 is the only band that differs at the rightmost end of these duplications. This evidence combined with the genetic data given below localized dp.

F_2 crossover males, in particular the dp cl flies, from the cross SuM2z duplication/ed dp cl females X ed dp cl/ed dp cl males were individually mated to M(2)z/SM5 Cy females to determine whether they contained the Minute suppressor. If the dp locus is included in the duplication, then F_2 dp cl males should not contain the duplication. In contrast, if the dp locus is not included in the duplication then some dp cl males should contain the duplication and some should not (Figure 3). Duplication SuM2z3 females yielded dp cl males, half of which contained the duplication, and half of which did not contain the duplication (Table 5). We interpret this as evidence that the dp locus is not duplicated in SuM2z3.

Conversely, eight dp cl flies involved in the cross with SuM2z4 tested did not contain the SuM2z4 duplication.

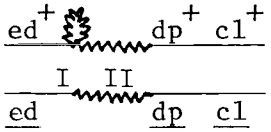
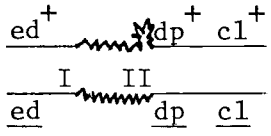
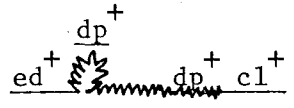
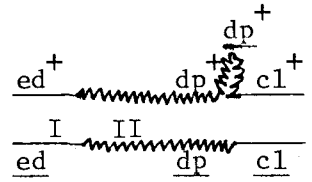
FORMAL PAIRING CONFIGURATION	SCO CROSSOVER IN REGION	PHENOTYPE (ONLY dp cl CLASS)	SUMMARY
Where duplication does not include dp locus			
	I	dp cl	
	II	Dup dp cl	dp cl flies with and without the Duplication
	I or II	Dup dp cl	
Where duplication does include dp locus			
	I	dp cl	dp cl flies always lack Duplication
	I or II	dp cl	

Figure 3 Consequences of inclusion of the dp locus in the duplication: presence or absence of the duplication in the dp cl class of F_2 crossover flies for the cross

$$\frac{\text{Duplication}}{\text{ed dp cl}} \text{ females} \times \frac{\text{ed dp cl}}{\text{ed dp cl}} \text{ males}$$

Dup = Duplication, SCO = Single Crossover

Table 5. Presence or absence of duplications in F_2 cross-over males from the cross duplication/ed dp cl ♀ X ed dp cl/ed dp cl ♂♂

	ed		dp cl		ed dp		cl	
Duplication	w	w/o	w	w/o	w	w/o	w	w/o
SuM2z3	2	2	3	3	0	11	14	0
SuM2z4	3	4	0	8	0	2	4	0
SuM2z2	2	2	0	6	0	0	6	0

Number of flies scored

w = with or containing the duplication
w/o = without or not containing the duplication

This implies that the dp locus is included in the SuM2z4 duplication. Corroborative evidence comes from SuM2z2, which also includes 25A3-4. None of the dp cl SuM2z2 F_2 crossover males tested contained duplications. This suggests that the dp locus is included in SuM2z2. Since SuM2z4 and SuM2z2 include 25A3-4 and include the dp locus, while SuM2z3 does not include 25A3-4 and does not include the dp locus, the dp locus is probably in the 25A3-4 band or the adjacent interbands. Genetic evidence from the gene order of M(2)z and dp is consistent with the localization of M(2)z and dp described above. The gene order of M(2)z and dp was determined from the cross described in Table 6. An inversion, In(3LR)Ubx ca was introduced into the genome in this cross to increase the frequency of crossing over by means of interchromosomal effects. Since flies of this cross which represent a single crossover between dp and M(2)z are ed and dp cl M, dp must be to the right of M(2)z. No ed dp M or cl flies were found as would be expected if dp were to the left of M(2)z. Consequently the gene order is ed M(2)z dp cl.

A rough estimate of the map distance between dp and M(2)z can be obtained by correcting for the interchromosomal stimulation of crossover frequencies due to the presence of M(3LR)Ubx ca. The average of all the controls in the crossover studies with ed dp cl (see crossover section) was used as a base for the crossover values for the ed-dp and dp-cl regions. Table 7 shows the stimulation

TABLE 6. DETERMINATION OF THE GENETIC ORDER OF M(2)z and dp.

The cross used was as follows:

$$\frac{\text{M(2)z}}{\underline{\text{I}}\quad\underline{\text{II}}\quad\underline{\text{III}}}{\underline{\text{ed}}\qquad\underline{\text{dp}}\quad\underline{\text{cl}}} \;;\; \frac{\text{In(3LR)Ubx ca}}{+}\,♀♀ \;\text{X}\; \frac{\underline{\text{ed}}\qquad\underline{\text{dp}}\qquad\underline{\text{cl}}}{\underline{\text{ed}}\qquad\underline{\text{dp}}\qquad\underline{\text{cl}}}\,♂♂$$

NCO		SCO						DCO		
		I		II*		III		I & III		
M	ed dp cl	ed M	dp cl	ed	M dp cl	ed dp	M cl	ed M cl	dp	TOTAL
1246	1712	81	31	5	1	94	38	3	1	3285

Number of flies scored is given.

*Critical classes for ordering M(2)z and dp.

TABLE 7. MAP DISTANCE FOR THE REGION M(2)z-dp

	MAP UNITS M(2)z-dp	MAP UNITS ed-dp	MAP UNITS dp-cl	RATIO OF INVERSION/ CONTROL ed-dp	RATIO OF INVERSION/ CONTROL dp-cl	AVERAGE RATIO OF INVERSION/ CONTROL	CORRECTED MAP UNITS
INVERSION PRESENT	0.17	3.81	4.27	2.35	1.06	1.7	$0.1 = \frac{0.17}{1.7}$
INVERSION ABSENT (Control)	—	1.62	3.93				

of the two regions (1.7 times control). Since the inversion stimulated map distance between M(2)z and dp is 0.17 map units, the unstimulated map distance is in the order of 0.10 map units (0.17/1.7). dp and M(2)z are closely linked as expected. It is interesting to note that the genetic distance between M(2)z and dp (0.1 map units) is about the same internal genetic distance as the dp locus itself.

Other workers place dp at a slightly different location. According to Morgan et al. (1938), Df(2)M(2)c is a "loss of the section of bands from just to the right of the faint bands in 24D to half through the shoebuckle set of four bands in 25A." It uncovers the dp locus. Df(2)M(2)z^{B}, another deficiency, has breakpoints 24E-25A1-2 (Lindsley and Grell, 1968). It too uncovers the dp locus. This suggests that the dp locus is located in or to the left of 25A1-2. Our studies localize dp to 25A3-4, thus there is an apparent contradiction between our localization and that of Bridges. However, the shoebuckle consists of two heavy polytene bands with a very short interband region between them and precise localization of a breakpoint in this region is difficult. It is possible that the breakpoint of the two deficiencies Df(2)M(2)c and Df(2)M(2)z^{B} described above as being between 25A2-3 might lie in the very beginning of the 25A3-4 bands. If only a portion of the dp locus need be missing to eliminate function of the dp locus, then the discrepancy is eliminated. Alternat-

ively, the dp locus could be located in the 25A2-3 interband region. The discrepancy would be eliminated in this case as well if Df(2)M(2)c, Df(2)M(2)z^{B} and the SuM2z's described above all had a breakpoint in the 25A2-3 interband region.

Crossover Studies

Previous studies (Roberts 1972) delineated distal-medial regions in autosome arms where the presence of a translocation breakpoint was most apt to reduce crossover values. For both the 2L and the 3R arms a series of duplications was deliberately generated with breakpoints near these sensitive regions. Crossing over was studied both within and without the area covered by a duplication, and crossover frequencies were calculated for individual genetic regions as well as for the whole arm. Crossover frequencies for individual regions as well as for the whole arm were expressed as a percent of the control value. The "whole arm" crossover values are calculated by summing the crossover values for each genetic region in that arm.

2L Duplications

To study crossing over throughout 2L, the "all" stock with a series of recessive visible gene markers, was used (see Tables 1 and 2 for a description of the stock and the markers). It divides 2L into three genetic regions, al-dp,

dp-b, b-pr, which account for approximately 24 percent, 65 percent, and 11 percent respectively of the total crossing over in 2L. Collectively these markers include more than 99 percent of the genetic length of 2L.

All of the 2L duplications recovered were selected as Suppressors of M(2)z which is located in the 25A1-2 doublet. Thus, all the duplications isolated have at least part of their duplicated chromatin covering the 25A1-2 region of the genome (Figure 8). Some of the 2L duplications isolated in the distal 1/4-1/3 of the 2L chromosome arm were very effective in reducing whole arm crossover frequencies. SuM(2)z15 for example, reduces the frequency of crossing over within 2L to 8.8 percent of the control value (Table 8, Figure 4).

For all the 2L duplications isolated, whole arm crossover suppression is primarily a function of the length of the duplication (Figure 4)(Table 8). For duplications over 55 bands, as the length of a duplication increases, its effectiveness in reducing crossover values rapidly increases. For duplications 120 or more bands in length crossover values are usually reduced to 20 percent or less of the control value. The longest duplication, SuM(2)z20, reduced crossing over within the arm to 8 percent of the control value.

The general pattern of crossover frequency reduction with increasing length holds for the individual regions as well (Figures 5-7) with some minor differences. All the

TABLE 8. SUMMARY OF WHOLE ARM CROSSOVER DATA FOR HETEROZYGOUS 2L DUPLICATIONS.

	MAP UNITS	% OF CONTROL	NO. OF BANDS
Control*	45.6	100.0	
SuM2z2	44.3	97.2	7
SuM2z4*	48.0	105.3	12
SuM2z5	41.5	91.3	12
SuM2z6	41.8	91.7	25
SuM2z7	44.9	98.5	40
SuM2z8	44.6	97.8	42
SuM2z9*	44.8	98.3	55
SuM2z10	20.3	44.5	69
SuM2z11*	26.4	57.9	87
SuM2z12	26.5	58.1	100
SuM2z13	9.5	20.8	122
SuM2z14*	39.2	86.0	135
SuM2z15	4.0	8.8	143
SuM2z16	3.0	6.6	152
SuM2z17	9.3	20.4	156
SuM2z18*	4.9	10.8	195
SuM2z19*	14.4	31.6	206
SuM2z20	3.7	8.1	227

*Mean value for those experiments which were repeated.

This table summarizes data given in Table 17 in the Appendix.

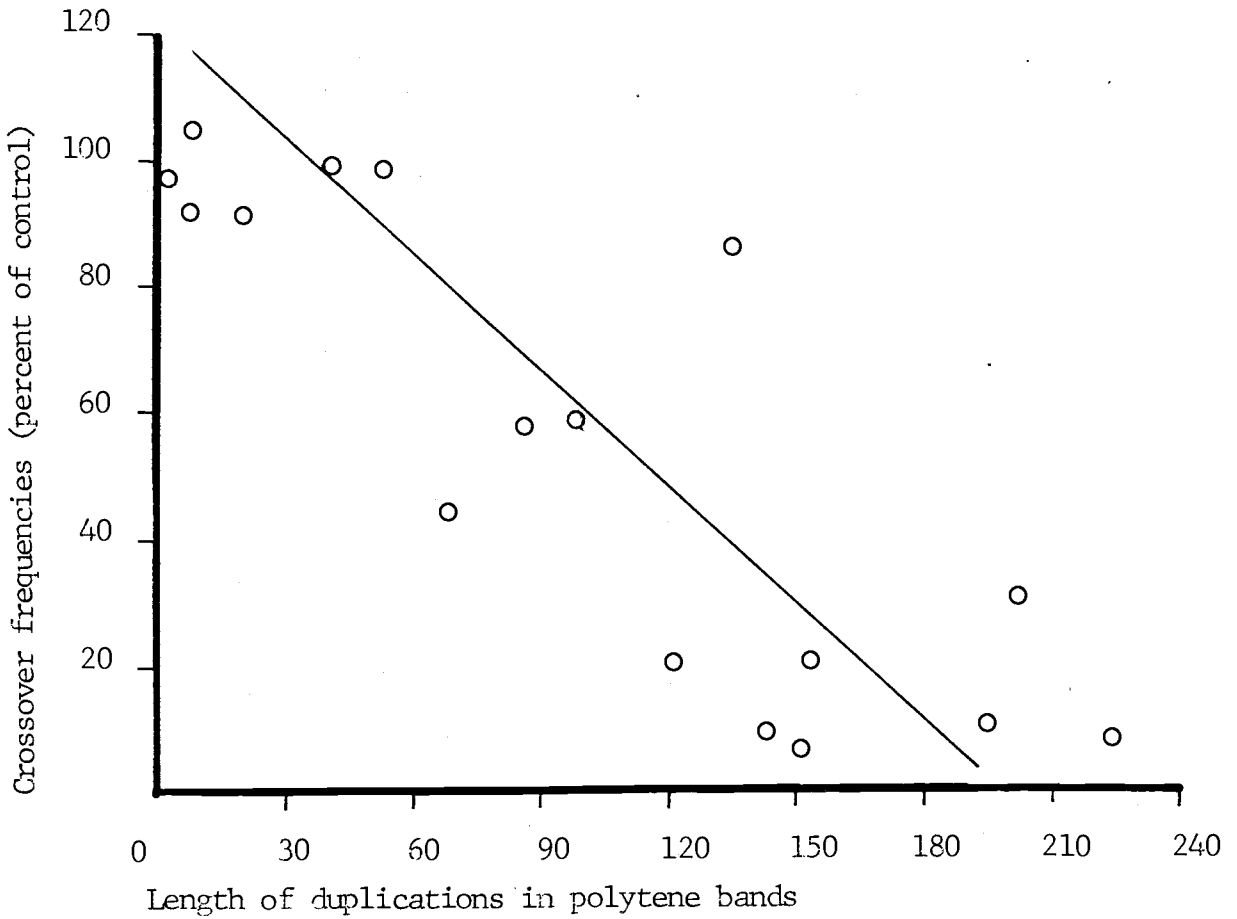


Figure 4 Crossover frequencies versus duplication length for SuM2z/al dp b pr ; whole arm values

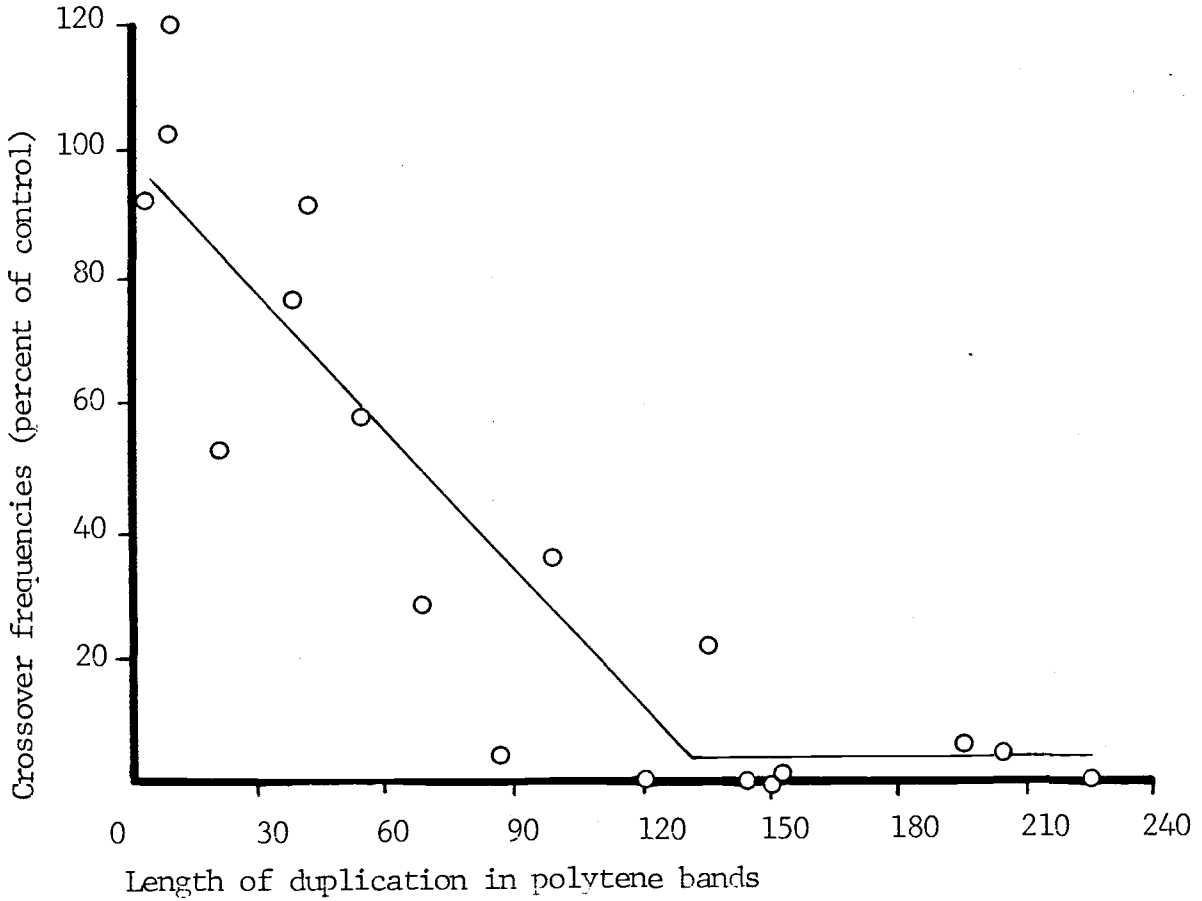


Figure 5 Crossover frequencies versus duplication length for SuM2z/al dp b pr ; al-dp region

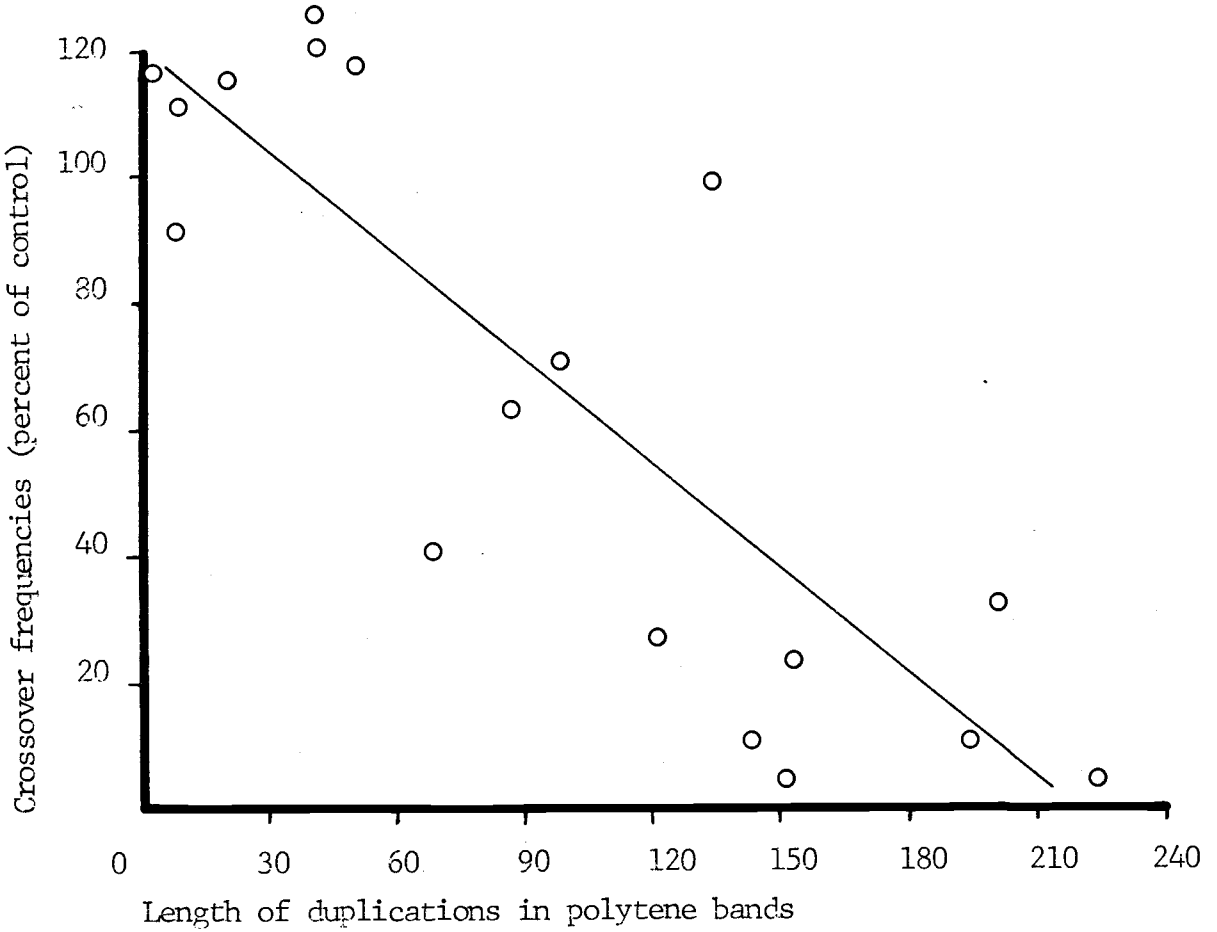


Figure 6 Crossover frequencies versus duplication length for SuM2z/al dp b pr ; dp-b region

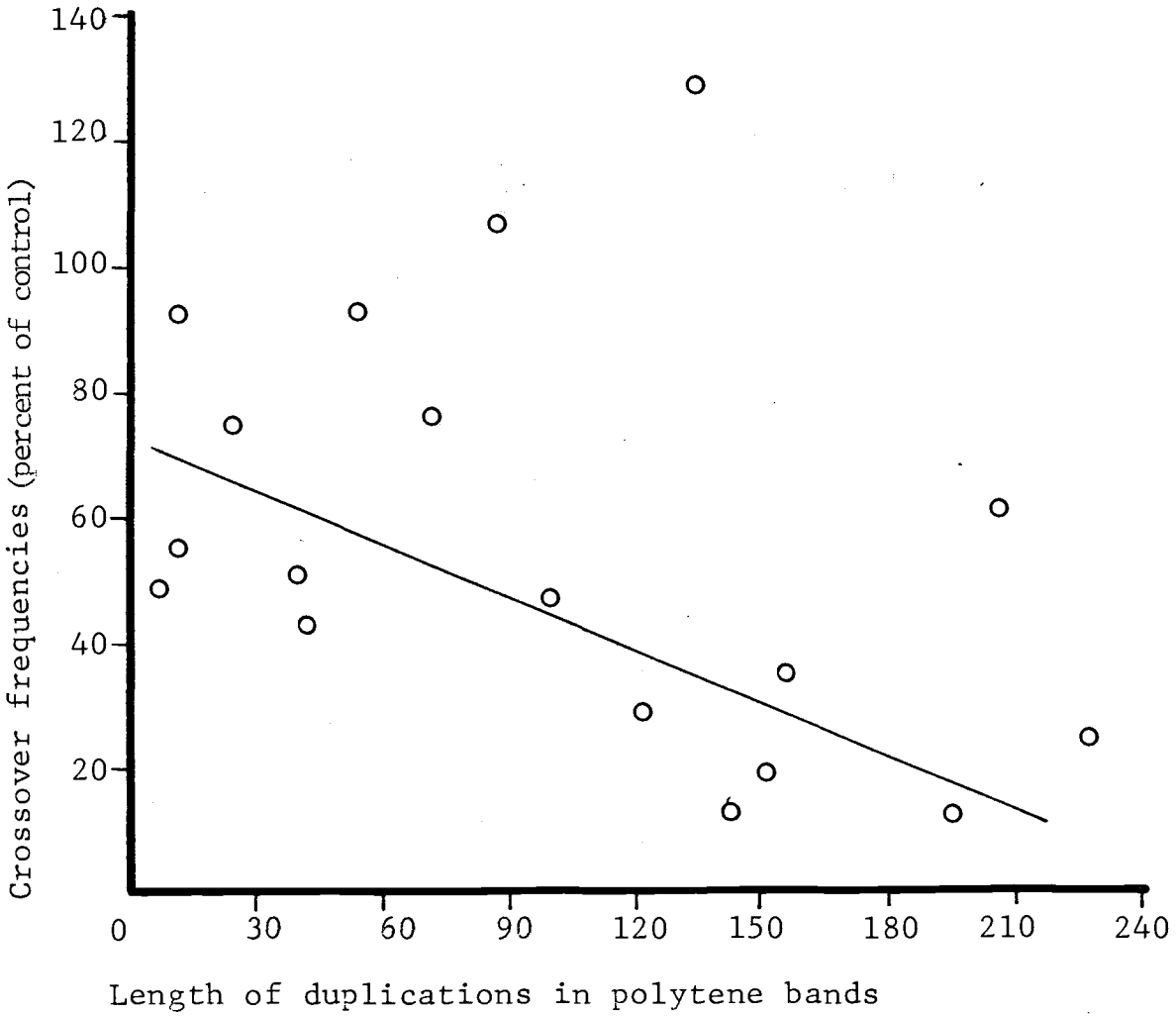


Figure 7 Crossover frequencies compared to duplication length in the b-pr region

FIGURE 8. LOCATION AND LENGTH OF SuM2z DUPLICATIONS.

Rectangles represent duplicated portions of 2L. Location of marker genes and M(2)z is given. Distance between marker symbols is proportional to the genetic length of the regions between them.

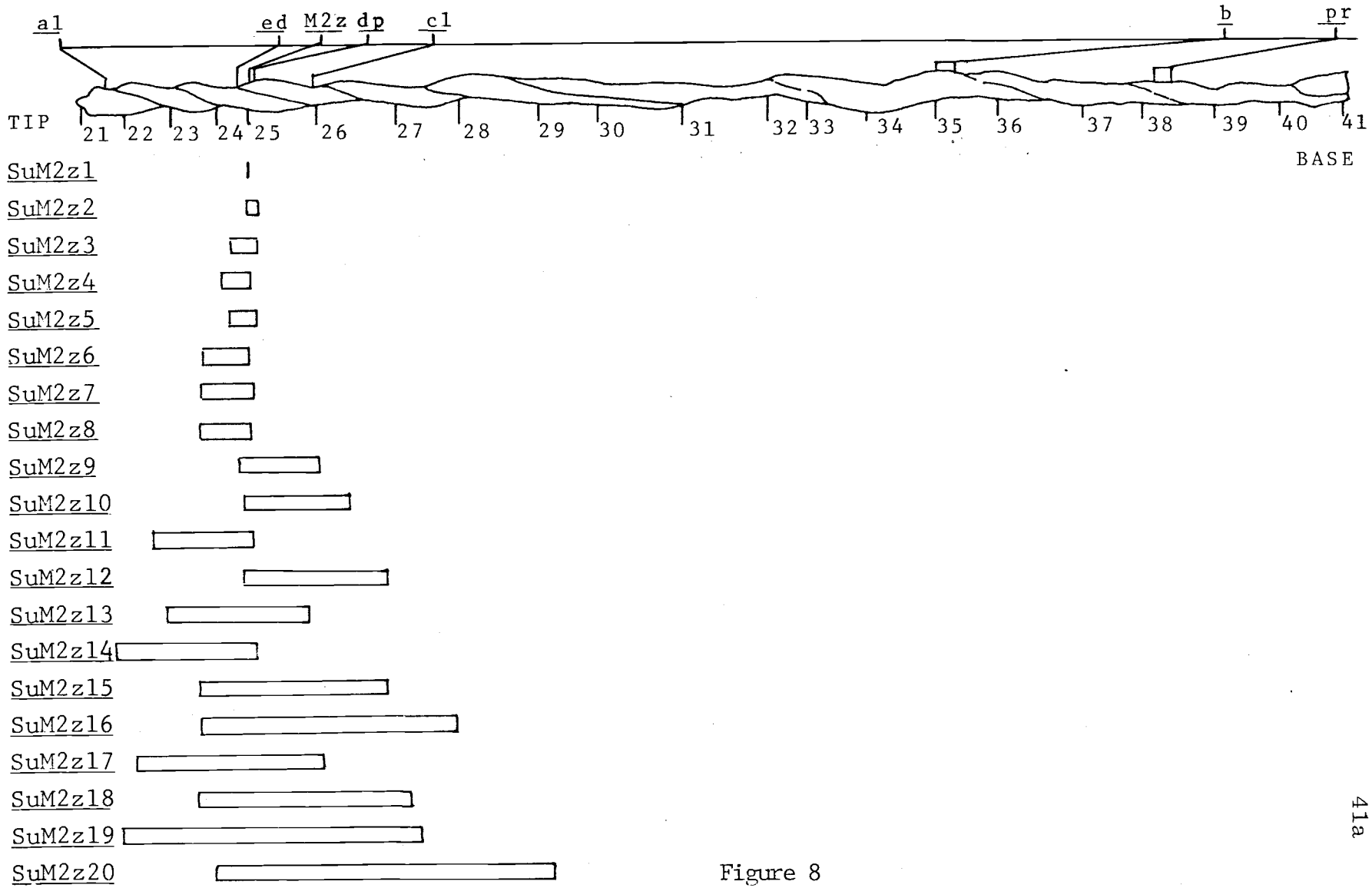

al
ed
M2z
dp
cl
b
pr
TIP
21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41
BASE
SuM2z1
SuM2z2
SuM2z3
SuM2z4
SuM2z5
SuM2z6
SuM2z7
SuM2z8
SuM2z9
SuM2z10
SuM2z11
SuM2z12
SuM2z13
SuM2z14
SuM2z15
SuM2z16
SuM2z17
SuM2z18
SuM2z19
SuM2z20

Figure 8

TABLE 9. SUMMARY OF CROSSOVER VALUES AND PERCENT OF CONTROL FOR THE CROSSES.
SuM2z/al dp b pr c x al dp b pr c/al dp b pr c

NAME	NUMBER OF BANDS	al-dp	% OF CONTROL	dp-b	% OF CONTROL	b-pr	% OF CONTROL	pr-c	NUMBER OF FLIES COUNTED
SuM2z2	7	10.4	92.9	29.4	114.0	4.5	51.7	15.8	728
SuM2z4	12	11.6	103.0	27.9	108.0	8.5	97.7	20.1	2132
SuM2z5	12	13.6	121.4	23.0	89.1	5.0	57.5	21.2	1038
SuM2z6	25	6.0	53.6	29.0	112.4	6.8	78.2	23.0	383
SuM2z7	40	8.7	77.7	31.5	122.0	4.7	54.0	----	739
SuM2z8	42	10.4	92.9	30.3	117.0	3.9	44.8	20.0	595
SuM2z9	55	6.6	58.9	29.7	115.0	9.5	97.7	25.2	1302
SuM2z10	69	3.3	29.5	10.4	40.3	6.6	75.9	24.4	817
SuM2z11	87	0.6	5.4	16.1	62.4	9.7	111.5	18.5	1431
SuM2z12	100	4.1	36.6	18.1	70.2	4.3	49.4	18.6	1135
SuM2z13	122	0.1	0.9	6.8	26.4	2.6	29.9	----	970
SuM2z14	135	2.6	23.2	24.9	96.5	11.8	135.6	25.9	1037
SuM2z15	143	0.1	0.9	2.7	10.5	1.9	21.8	----	933
SuM2z16	152	0.0	0.0	1.2	4.7	1.8	20.7	----	827
SuM2z17	156	0.1	0.9	6.0	23.3	3.2	36.8	----	772
SuM2Z18	195	0.7	6.3	3.1	12.0	1.1	12.6	15.7	1323
SuM2z19	206	1.0	8.9	8.2	31.4	5.5	63.2	20.9	1877
SuM2z20	227	0.1	0.9	1.4	5.4	2.2	25.3	----	1019
Control	---	11.2	100.0	25.8	100.0	8.7	100.0	19.6	6767

This table summarizes data given in Table 17 in the Appendix.

duplications were selected as suppressors of M(2)z and consequently have at least part of the duplication in the al-dp region. As can be seen in Figures 4 and 5, when cross-over frequencies for the al-dp region are compared to cross-over frequencies for the whole arm, a given length of duplication shows a greater reduction in crossover frequencies for the al-dp region than for the whole arm. Generally the frequency of crossing over is detectably reduced in the al-dp region for duplications of 25 or more bands. Although duplications of less than 25 polytene bands do not detectably reduce crossing over in the relatively long al-dp region, they do reduce crossing over in the ed-dp region, the region in the immediate vicinity of the duplication (see below).

The b-pr region is the most proximal region and the region farthest removed from the duplications. None of the duplications isolated extended into this region (Figure 8). The correlation of size and crossover reduction is weak in this region, but Table 9 and Figure 7 show that if a duplication is 120 or more bands, crossing over in the b-pr region is usually reduced to 30 percent or less of the control. This demonstrates that with some of the larger duplications crossover frequency reductions can occur well beyond the limits of their breakpoints. If a duplication is sufficiently large (e.g. SuM(2)13, 122 bands) the duplication need only extend to the 25 E subdivision in order to reduce crossover frequencies to low levels in the b-pr region. If shorter

(e.g. SuM(2)z12, 100 bands) a duplication must extend farther toward the base (into the 26F subdivision) before significant crossover frequency reduction occurs (Tables 3 and 9).

Apparently, in some cases, position of the duplication in the genome is important as to the degree of crossover reduction seen. For example, duplication SuM2z14 (135 bands) located in the region distal to the shoebuckle region (25A1-4), does not reduce crossing over in the b-pr region, even though it is long and covers almost the entire region from the tip of 2L to the shoebuckle (Figure 8 and Table 9). On the other hand, a shorter duplication, SuM2z12, (100 bands) which has most of its duplicated portion proximal to the shoebuckle, does reduce crossing over in the b-pr region. In this case the effect of position seems to override the effect of length on crossover frequency reduction.

It is interesting to note that SuM2z14 and SuM2z11 are two duplications confined to the region distal to the shoebuckle region and SuM2z14, the longer of the two, is a less effective crossover reducer in the 2L chromosome arm than is SuM2z11, the shorter duplication. Neither reduced the frequency of crossing over in the b-pr region. There may even be a slight stimulation of crossing over in this region. However, comparing SuM2z14 and SuM2z11 (Table 9), it can be seen that SuM2z14 reduces the frequency

of crossing over little or not at all in the dp-b region, the region adjacent to the duplicated region. The SuM2z11 duplication does reduce crossing over in this region. These results were reproducible in two experiments (Table 17 in Appendix). SuM2z14 may also be a less effective crossover reducer in the al-dp region too, although the results were not reproducible for this region (Table 17 in Appendix).

The reason for the relative ineffectiveness of the longer duplication, SuM2z14, as a crossover reducer in 2L compared to SuM2z11 is unknown. Perhaps the duplication of a locus in SuM2z14 but not in SuM2z11 (in the region 21B7-21D6) may partly compensate for the crossover suppressive effects of this duplication by some physiological mechanism.

The genetic marker curved (c) a mutant gene in 2R (cytogenetic location unknown) was scored for some of the duplications to determine if there were any interbrachial effects on crossing over. No significant increases or decreases were seen (Table 9).

Studies of Short Heterozygous Duplications:
ed dp cl Studies

With widely spaced genetic markers such as are found in the "all" stock, slight effects on crossing over can be easily missed. With the "all" stock the shorter duplica-

tions (with lengths up to 55 bands) showed no detectable crossover frequency reduction throughout the 2L arm and little or no crossover frequency reduction in the region in which they were located. To determine if there were any effects on crossing over in the immediate vicinity of these small duplications, the ed dp cl stock was used (see Table 2 for a description of the mutants). The cytogenetic location and the relative positions of these mutants and the short duplications studied are shown in Figure 8. Crossing over was studied in nine short duplications: eight were tandem repeats and one was a noncontiguous reverse repeat (Tables 10 and 10a).

ed-dp Region

Crossing over relative to the eight short tandem repeats will be first considered in the ep-dp region. The single band tandem duplication, SuM2z1 (Table 10a), will be discussed later. The remaining seven tandem duplications (Table 10) ranged in size from seven to fifty bands. With six of these seven duplications, crossing over was reduced relative to the controls in the ed-dp region; with the seventh, SuM2z3, crossing over was not reduced.

Of the three duplications of twelve bands or less, two, SuM2z2 (7 bands) and SuM2z4 (12 bands) reduced crossing over slightly and repeatedly but not significantly; the third,

TABLE 10. CROSSOVER STUDIES WITH SHORT 2L HETEROZYGOUS DUPLICATIONS.
SuM2z/ed dp cl♀ x ed dp cl/ed dp cl ♂♂

Duplication	Tandem repeat or non-contiguous repeat	Number of bands	Region: ed-dp Map units	ed-dp % of Control	ed-dp Change*	Region: dp-cl Map units	dp-cl % of control	dp-cl Change	N	Locus covered by duplication** ed	dp	cl	Number of repeat experiments
Control			1.62			3.93			4452				
SuM2z2	T	7	0.73	45	-r	4.21	107	+r	2710	-	+	-	2
SuM2z3	T	10	1.59	99	0	4.13	405	+	3937	-	-	-	3
SuM2z4	T	12	0.81	50	-r	4.30	109	+r	3327	-	+	-	2
SuM2z5	NR	12	0.9	56	-a	4.0	102	+a	2367	-	+	-	2
SuM2z6	T	25	0.24	15	-ars	2.44	62.1	-r	2518	+	+	-	2
SuM2z7	T	40	0.39	24	-ars	2.68	68.2	-4	2528	+	-	-	2
SuM2z8	T	42	0.32	20	-s	3.03	77.1	-	1054	+	+	-	1
SuM2z9	T	55	0.29	18	-s	1.61	41.0	-s	2297	?	+	?	1

a = averages for the experiments which were repeated, r = reproducible,
s = significant at the 5% level of significance

* + = Increase, 0 = No change, - = decrease

** + = Duplication included, - = duplication not included,
? = uncertain whether duplication included

This table summarizes data given in Table 18 in Appendix.

TABLE 10a. CROSSOVER STUDIES WITH THE SHORT HETEROZYGOUS DUPLICATION SuM2z1
SuM2z1/ed dp cl ♀ x ed dp cl/ed dp cl ♂♂

Name	Type of Duplication	Number bands	Region ed-cl	Number counted
Control	———	——	4.89	4452
SuM2z1	Tandem repeat	1	5.65	372

SuM2z3 (10 bands) showed no crossover suppression. All the duplications of 23 bands or more significantly suppressed crossing over in the ed-dp region to 24 percent or less of the control value.

It is interesting to note that neither SuM2z3 (10 bands nor SuM2z7 (40 bands) includes the dp locus but both have relatively more crossing over than the other duplications of about the same size which do include the dp locus. If this difference is real, it implies that the presence of the dp locus in the duplication decreases the amount of crossing over (see also homozygous duplication studies).

The results for SuM2z1, a one band duplication are shown in Table 10a. Crossover values are given from ed-cl rather than from ed-dp and dp-cl because SuM2z1 is probably deficient for the dp locus. As a consequence, all the F_2 crossover flies are phenotypically dp so crossing over cannot be determined for the individual regions. Although the sample size is small, the results show a small but not significant increase in crossover frequencies.

SuM2z2, SuM2z3, SuM2z4 and possibly SuM2z9 do not include the ed locus so the proportion of the observed crossover effects which are the result of intra-duplication or extra-duplication effects cannot be determined. SuM2z6 and SuM2z8 definitely do include the ed locus, and the observed crossover suppression seen with these duplications

in the ed-dp region is the result of crossover suppression within the duplication.

dp-cl Region

In the dp-cl region the three duplications of twelve bands or less show a small but not significant increase in crossing over. The four duplications of 23 bands or more show a decrease in crossing over from between 77 percent to 41 percent of the control values. Only the longest of these duplications, SuM2z9, which extended most of its length into the dp-cl region gave significant crossover reduction in the dp-cl region. The other three showed a consistent but not significant pattern of crossover frequency reduction. Since these three extend minimally or not at all into the dp-cl region, this indicates that crossover frequency reduction extends beyond the limits of the breakpoints of even these small duplications.

Crossing over in the Noncontiguous Reversed Duplication, SuM2z5

Cytologically, SuM2z5 is a short 10 band noncontiguous reversed repeat inserted into the 26C polytene subdivision (Table 3). The breakpoints of this duplication are 24F1-25A4. Of the three genetic markers ed, dp, cl, cytologically only the dp locus is included in the duplication. The data for the testcross of the heterozygote

SuM2z5/ed dp cl females are shown in Table 11. These results are consistent with a model of the duplication in which genetically just the dp locus is included in the duplication (Table 12).

According to this model the ed-dp class is the only unequivocally detectable double crossover class (DCO). About six map units separate ed from the reversed duplicated region inserted at 26C. Chromosome interference is usually complete for map distances of 10 map units or less in Drosophila, thus few or no DCO flies are expected to be recovered. No ed dp flies were found among 2357 flies scored. The remaining DCO classes were not unequivocally identifiable because they were phenotypically like either a noncrossover (NCO) class or a single crossover (SCO) class. Thus it is possible that some DCO flies were present but went unrecognized.

Among the SCO classes only one of the two reciprocal crossover classes is unequivocally identifiable as a SCO class for a given crossover region. The phenotype of the corresponding reciprocal class is the same as that of at least one other crossover class. For example, although the dp cl class can be the result of a SCO only in region I (Table 12), its reciprocal class, the ed class, can arise from a SCO in either region I or II.

If we assume there are few or no DCO flies among the flies scored we can get an estimate of the amount of cross-

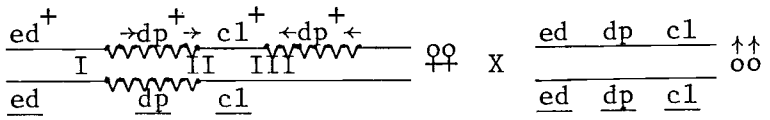
TABLE 11. CROSSOVER DATA FOR THE CROSS.
SuM2z5/ed dp cl ♀ x ed dp cl/ed dp cl ♂♂
Number of Flies Scored

NCO		SCO				DCO	
+	ed dp cl	ed	dp cl	cl	ed cl	ed dp	N
1161	1052	62	11	47	34	0	2367

See text and Table 12 for details

NCO = Noncrossover, SCO = Single crossover, DCO = Double crossover

Table 12 A model for the SuM2z5 duplication: expected phenotypic classes from the cross



CLASSES			DUPLICATION	PHENOTYPE
NCO	1)	$\underline{ed}^{+}\underline{dp}^{+}\underline{cl}^{+}\underline{dp}^{+}$	+	wild type
	2)	ed dp cl	-	ed dp cl
SCO I	3)	$\underline{ed}\ \underline{dp}^{+}\underline{cl}^{+}\underline{dp}^{+}$	+	ed
	4)	$\underline{ed}^{+}\underline{dp}\ \underline{cl}$	-	dp cl
SCO II	5)	$\underline{ed}^{+}\underline{dp}^{+}\underline{cl}$	-	cl
	6)	$\underline{ed}\ \underline{dp}\ \underline{cl}^{+}\underline{dp}^{+}$	+	ed
SCO III	7)	$\underline{ed}^{+}\underline{dp}^{+}\underline{cl}^{+}$	-	wild type
	8)	$\underline{ed}\ \underline{dp}\ \underline{cl}\ \underline{dp}^{+}$	+	ed cl
DCO I-II	9)	$\underline{ed}^{+}\underline{dp}\ \underline{cl}^{+}\underline{dp}^{+}$	+	wild type
	10)	ed dp cl	-	ed cl
DCO I-II	11)	$\underline{ed}^{+}\underline{dp}\ \underline{cl}\ \underline{dp}^{+}$	+	cl
	12)	$\underline{ed}\ \underline{dp}^{+}\underline{cl}^{+}$	-	ed
DCO II-III	13)	$\underline{ed}^{+}\underline{dp}^{+}\underline{cl}\ \underline{dp}^{+}$	+	cl
	14)	$\underline{ed}\ \underline{dp}\ \underline{cl}^{+}$	-	ed dp

NCO = noncrossover, SCO = single crossover, DCO = double crossover

+ = duplication present, - = duplication absent

→ or ← indicates orientation of duplicated segment

ing over in the SuM2z5 heterozygote. For the ed-dp region, region I, we must also assume that the frequency of the ed class will be the same as that for the dp cl class, since the ed class is not unequivocally identifiable. Making these assumptions, the map distance calculated for the ed-dp region is 0.9 map units. The control value for the ed-dp region is 1.6 map units (Table 10), thus, crossover frequencies appear to be reduced slightly in the ed-dp region, the region in which the duplication is located. Similar calculations for the dp-cl region show little or no change in the crossover frequency for the dp-cl region (4.0 map units for the SuM2z5 heterozygote v.s. 3.9 map units for the control (Table 10). The behavior of this small reversed noncontiguous duplication is similar to the behavior of a small tandem direct duplication, SuM2z4, with the same or very similar breakpoints (Tables 3 and 10).

In summary, studies with the short heterozygous 2L tandem or noncontiguous duplications studied with the ed dp cl stock showed that crossover frequencies were usually reduced in the ed-dp region, the region in which the duplications were located. For duplications of 12 bands or less, there may be a small increase in crossover frequencies in the dp-cl region, the region immediately adjacent to the duplications. For duplications of more than 12 bands, crossover frequencies were reduced in the dp-cl region.

Crossover Studies with Short Homozygous 2L Duplications

Crossover increases have been reported in the presence of short homozygous tandem duplications of the X chromosome (Green 1962). We endeavored to determine if short autosomal duplications behave similarly. Most of the duplications recovered in the present study could not be obtained as homozygotes for one or more of the following reasons: lethality of homozygotes (e.g. SuM2z9), inability to obtain marker homozygotes because of strong crossover suppression in the heterozygotes (e.g. SuM2z7), or inclusion of one or more markers within the duplication so that no duplication bearing F_2 crossover flies with the included loci could be recovered.

Two duplications, SuM2z3 and SuM2z4 were suitable for study as homozygotes (Table 13). SuM2z3, a short 10 band duplication had its left breakpoint at 24F1-2 and its right breakpoint between 25A2 and 25A3. An increase in crossing over from a control value of 5.55 map units to 8.05 map units is seen with the SuM2z3 duplication over the ed-cl region. Since SuM2z3 does not cover any of the loci ed, dp, or cl used in this study, crossing over can be determined for the ed-dp and dp-cl regions. Consideration of increases in the ed-dp and dp-cl regions show that most of the increase seen comes in the ed-dp region, the region where the duplication is located. The estimated maximum

TABLE 13. CROSSOVER DATA BY REGION FOR SHORT HOMOZYGOUS 2L DUPLICATIONS; SuM2z/ed dp cl. DATA FOR REGIONS GIVEN IN PERCENT CROSSOVER (map units).

Name	Number Counted	Map Units ed-dp	Ratio SuM Control	Map Units dp-cl	Ratio SuM Control	Map Units ed-d	Ratio SuM Control
Control	7682	1.62		3.93		5.55	
SuM2z4	5284	----		----		5.91	1.06
SuM2z3	1304	3.30	2.04	4.75	1.25	8.05	1.45

genetic length of this duplication is about 0.6 map units. SuM2z3 increases crossover frequencies in the ed-dp region from a control value of 1.62 map units to 3.30 map units or an increase of 1.68 map units. This represents a 5.5 fold increase in map units compared to the length of the duplication (1.68/0.6). This increase is probably significant and thus a homozygous autosomal 2L duplication appears to increase crossing over in excess of its genetic length as does the homozygous X chromosome duplication, Dp(1;1)z-w, studied by Green (1962). No markers were located within the SuM2z3 duplication so whether the increases occur within or outside the limits of the duplication cannot be determined.

There is a small but not significant increase in crossing over in the dp-cl region as well. If this increase is real it would imply that crossing over is stimulated beyond the limits of the duplications breakpoints.

SuM2z4 is a short (12 band) duplication. Like SuM2z3 its left breakpoint is at 24F1-2. Its right breakpoint extends two bands beyond SuM2z3's right breakpoint to 24A3-4. SuM2z4 includes the dp locus, hence crossing over could only be measured from ed-cl rather than from ed-dp and dp-cl as was possible for the SuM2z4 duplication. From Table 13 it appears that in the ed-cl region there is little or no increase in crossing over when compared to the average of the controls. When compared to the

control value scored at the same time as the SuM2z4 duplication (4.7 map units, (not shown)) there appears to be a small but not significant increase in crossing over. Thus we might tentatively conclude that there is an increase with this homozygous duplication as well, but these results obviously need to be confirmed.

Interestingly, SuM2z3 which does not include the dp locus shows a larger increase in crossing over than does SuM2z4 which does include the dp locus. This increase of crossing over with SuM2z3 compared to SuM2z4 was seen in studies with short heterozygous duplications as well.

Crossover Studies with the 3R Duplications

All of the 3R duplications isolated were selected as suppressors of M(3)w which has been localized in the 95A1-2 doublet. Thus all the 3R duplications have at least part of their duplicated chromatin covering the 95A1-2 region of the genome (Figure 10).

The stock used to study crossing over throughout 3R was the "streroca" stock, which contained, in order, the five recessive visible mutants st, sr, e, ro, and ca (See Table 1 for a description of the mutants). Since M(3)w is located in the ro-ca region, all of the SuM's have part of their duplicated chromatin in this region (Figure 10). Four of the five mutants (sr, e, ro, ca)

are in 3R the fifth mutant, st, is located about two map units on the other side of the centromere in 3L. The four mutants in 3R are located in the distal half of 3R: the proximal half of 3R is unmarked. Since st was close to the centromere, it was assumed initially that no significant error would be introduced if crossing over in the st-sr region were equated with crossing over in the proximal half of 3R. This assumption had to be modified in the light of more detailed studies (see below).

We expected that the 3R duplications would behave like the 2L duplications and crossing over would be either unaffected or reduced in the proximal half (st-sr region) of 3R. Consideration of the data in Table 14a indicates that with one exception, SuM3w9, crossover frequencies in SuM bearing flies are increased relative to the average of the controls in the st-sr region. However, no correlation can be detected between the amount of increases and the length of the duplication.

The data for an experiment in which recombination was studied for the SuM3w4 and SuM3w5 duplications are shown in Table 14b. The SuM3w4 duplication showed an increase in crossover frequencies for the st-sr region compared to the control for that experiment. The SuM3w5 duplication showed no marked change in crossover frequencies. The data for this experiment were not combined with the rest of the data and compared to the average of the

TABLE 14. SUMMARY OF CROSSOVER VALUES BY REGION FOR THE CROSS SuM3w/st sr e ro ca ♀ X st sr e ro ca/st sr e ro ca ♂♂

Name	Number of bands	st-sr	T of Control	sr-e	% of Control	e-ro	% of Control	ro-ca	% of Control
-A-									
Control (average)		20.45	100	8.5	100	22.7	100	11.3	100
SuM3W1	1	30.8	150	9.2	108	24.4	107.0	11.3	100
SuM3w2	23	28.6	140	11.0	129	18.1	79.7	12.9	114
SuM3w3	48	38.3	187	7.1	83.5	11.5	50.7	9.4	83.2
SuM3w5	69	30.7	150	11.2	132	8.1	35.7	5.7	139
SuM3w6	175	28.9	141	3.2	37.7	0.92	4.1	2.3	20.4
SuM3w8	226	33.4	163	1.6	18.8	2.0	8.8	8.4	74.3
SuM3w9	232	5.7	28	0.42	4.9	0.35	1.5	0.25	2.5
SuM3w11	373	32.7	160	3.2	37.6	1.7	7.5	5.5	48.7
SuM3w12	282	56	274	1.9	22.4	0.97	4.3	0.97	8.6
				sr-ro*					
SuM3w7	206	29.9	146	3.5	11.2			4.5	39.8
SuM3w10	291	25.5	125	4.9	15.7			7.4	65.5
-B-									
Control*		30.4		32.5				15.5	
SuM3w4	69	38.7	127	19.7	60.6			12.1	78.1
SuM3w5	69	29.7	98	16.5	50.8			3.7	23.9

*Recombination could be determined only for the sr-ro region for the duplications listed below since an ebony allele was present on the duplication bearing chromosome arm.

**Control only for SuM3w4 and SuM3w5 below.

These tables summarize data given in Tables 19 and 20 in the Appendix.

controls from the other experiments. They were kept separate and compared to their own control due to a large difference in the st-sr region between the control for this experiment and the controls for the other experiments. The reason for this abnormally high value is most probably misclassification of the sr phenotype for this experiment, the first in which "steroca" was used. The sr phenotype is variable in expression and practice was needed to score it accurately; misclassification was not a problem in later experiments.

The reason for the crossover frequency increase in SuM3w bearing flies relative to the controls as seen in the st-sr region, cannot be definitely determined from the data available. The increase in crossing over does not seem to be related to the length of the duplication because one duplication, SuM3w9, showed a decrease and it is intermediate in length relative to the other SuM3w duplications. However, there is a possiblity that unselected or "floating" rearrangements may increase crossing over in this region. This region includes the centromere which is known to be sensitive to interbrachial and interchromosomal effects.

Chromosome rearrangements in one arm of a chromosome can increase the frequency of crossing over in another arm of a chromosome (interbrachial effects) or in non-

homologous chromosomes (interchromosomal effects)(reviewed in Luccessi 1976, Luccessi and Suzuki 1968). Interbrachial or interchromosomal effects commonly result in a marked increase in the frequency of crossing over in the centromeric region. If interbrachial or interchromosomal effects were to be a factor in causing the increase in frequency of crossing over with the SuM3w flies, one would expect to find unselected rearrangements in all the SuM3w flies except SuM3w9, the only one which showed a decrease. A limited search of the SuM3w stocks uncovered an unselected inversion in 3L in the SuM3w11 stock, a stock showing a crossover frequency increase, and no unselected rearrangements in SuM3w9. This follows the expected pattern, but since no unselected rearrangements were found in the other SuM3w stocks, all of which showed increases in crossover frequencies, no definite conclusion can be drawn. Obviously a more thorough search of the stocks is necessary.

If these increases are the result of interbrachial or interchromosomal effects, the effects of the duplications in the proximal half of 3R may be masked. Because of this uncertainty, a whole arm crossover value was not calculated; instead, a half arm crossover value for the distal sr-ca region was calculated. The crossover values were summed only over the regions from sr-ca. Crossover

frequencies were expressed as a percent of the average of all the controls except the control for the SuM34 and SuM35 duplications (Table 14b). The percent of control values so calculated versus the length of the duplication is shown in Figure 9 and Table 15. Examination of the half arm crossover values shows that the short duplications SuM3w1 (one band) and SuM3w2 (23 bands) gave no marked crossover frequency reduction, while the intermediate length duplications had crossover frequencies between 28 and 35 percent of the control value, thus showing a definite reduction in crossover frequencies. Duplications of 160 or more bands gave crossover frequencies that were 26 percent or less of the control value. For the one duplication, SuM3w9, which suppressed crossing over throughout the entire 3R arm, the frequency of crossing over was reduced to about 14% of the corresponding control value (data for this calculation in Table 19 in Appendix).

The crossover frequency data from SuM3w4 and SuMw5 (Table 14b) are plotted in Figure 9 as a percent of their own control, as their own control showed a relatively high value for the st-sr region, presumably because of the misclassification of sr. The crossover frequencies for the other region involving sr, sr-ro, should be considered suspect as well, and thus comparisons were made to their own control.

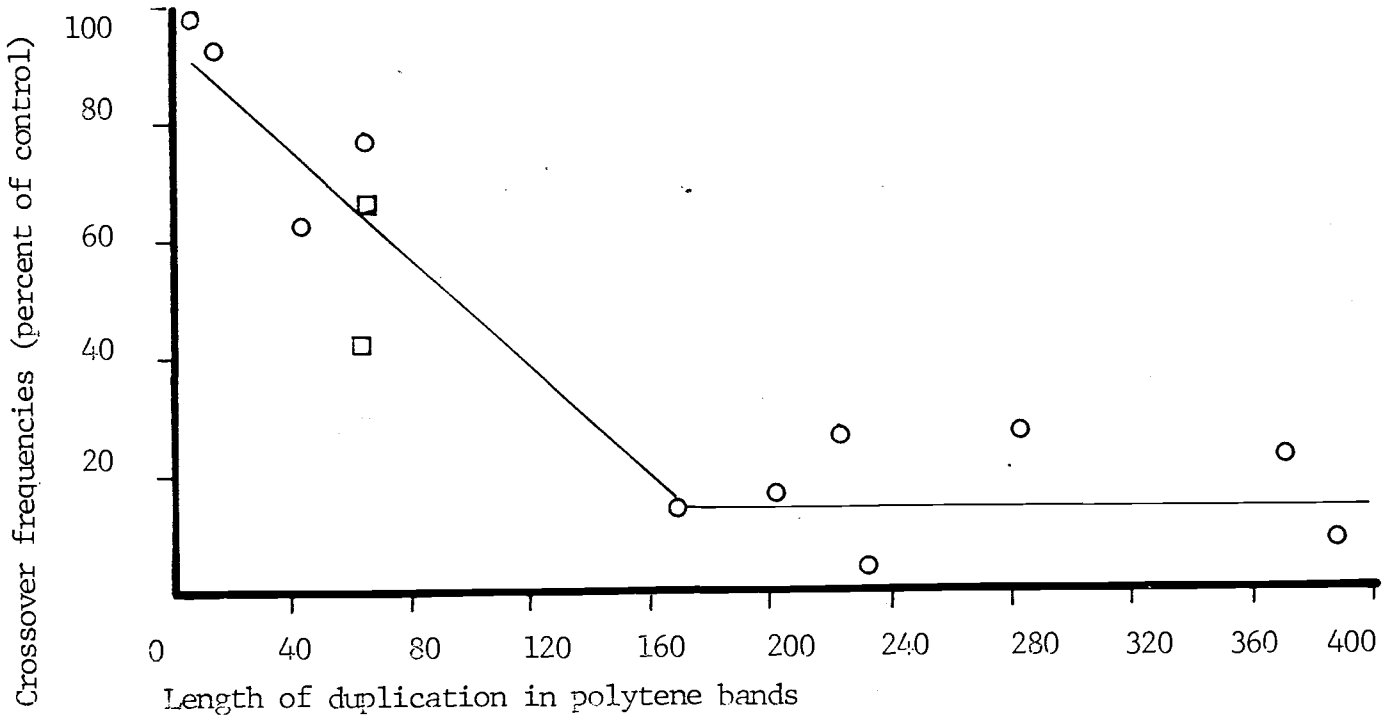


Figure 9 Crossover frequencies versus duplication length for SuM3w/st sr e ro ca ; half arm values

□ represents values from the duplications SuM3w4 and SuM3w5 which were compared to their own controls (see text).

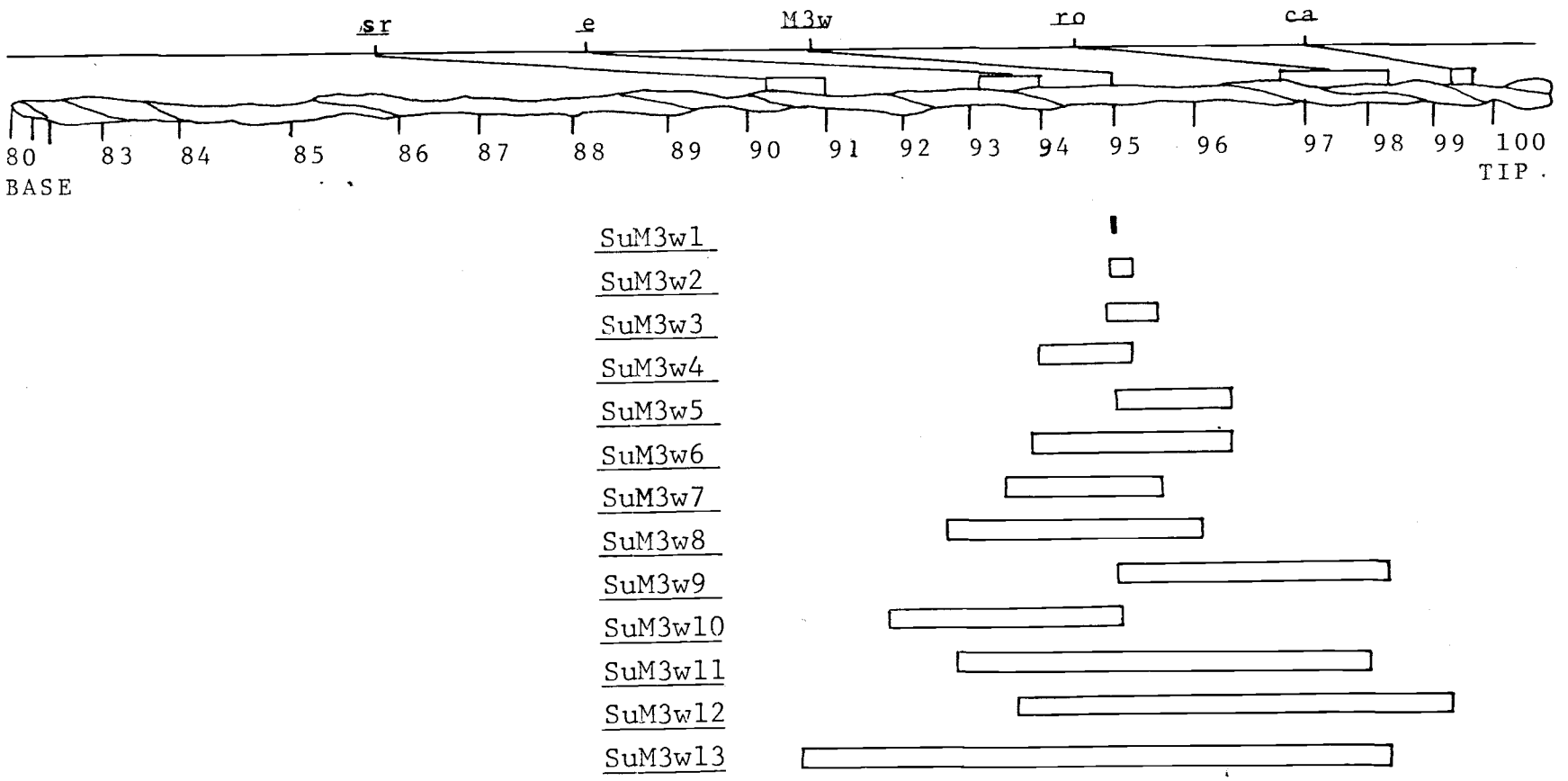


Figure 10 Location and length of SuM3w duplications in 3R

Rectangles represent duplicated portions of 3R. Location of marker genes and $M(3)w^{124}$ is given. Distance between marker symbols is proportional to the genetic length of the regions between them.

TABLE 15. HALF ARM CROSSOVER DATA FOR SuMw/st sr e ro ca, females. DATA FOR sr-ca REGION GIVEN IN PERCENT CROSSOVER (map units)

Name	Number bands	Map Units sr-ca	Percent of Control
Average of Controls		45.2	100.0
SuM3w1		44.9	106
SuM3w2	23	42.0	92.9
SuM3w3	48	28.0	62.0
SuM3w5	69	35.0	77.4
SuM3w6	175	6.4	14.2
SuM3w7	206	8.0	17.7
SuM3w8	226	12.0	26.6
SuM3w9	232	1.8	4.0
SuM3w10	291	12.3	27.2
SuM3w11	373	10.4	23.0
SuM3w12	382	3.8	8.4
Control*		48.0	100.0
SuM3w4	69	31.8	66.3
SuM3w5	69	20.2	42.1

*SuM3w4 and SuM3w5 below are compared to this control (see text for details).

This table summarizes data given in Tables 19 and 20 in the Appendix.

Consideration of crossing over in individual regions from sr to ca indicates that if the duplication is sufficiently long, crossover frequency reductions can occur beyond the limits of a duplication's breakpoints with the 3R duplications, such as was already shown for the 2L duplications. For example, both SuM3w3 and SuM3w4 (Table 14a) show a slight reduction of crossing over in the ro-ca region, while SuM3w3 shows a small reduction in crossover frequency in the sr-e region as well. Both of these regions are beyond the limits of the breakpoints of these duplications. It is not known whether SuM3w4 would also show a reduction in crossover frequency in the sr-e region, because SuM3w4 could not be scored for the sr-e region as the e allele is present in SuM3w4. SuM3w5 shows a pronounced reduction in crossover frequency in the ro-ca region (Tables 14a and 14b). This is not surprising since SuM3w5 is a noncontiguous reversed repeat inserted in the ro-ca region.

Subtle regional shifts in crossover patterns with a slight shift in the positions of the duplication (as in the 2L duplications) could not be detected with the third chromosome duplications due to technical problems. Duplications about the same length and type but with slightly different positions were not recovered, or if recovered, had an e allele in the stock which interfered with the

scoring of the markers. The e allele is present in some stocks because in the initial X-ray experiment, the duplications were induced in a cn bw; e stock. An e allele was therefore present in those stocks (SuM3w4, SuM3w5, SuM3w7, and SuM3w10). Since e was also in the "streroca" stock, it did not segregate in these experiments; thus, crossing over could be determined only for the longer sr-ro interval, rather than for the two shorter intervals sr-e and e-ro. Attempts were made to eliminate the e allele in these stocks by outcrossing. Removal of e was successful only for SuM3w5 and the crossover results are in Table 14a.

In summary, the SuM3w duplications were tested with the "streroca" stock to determine whether or not they reduced crossover frequencies as the SuM2z duplications did. With some qualifications, it appears that the SuM2z and SuM3w duplications do behave similarly. As measured by half arm crossover values, crossover frequencies in SuM3w heterozygotes decreased with increasing length of the duplication for the sr-ca region, a region which includes most of the distal half of the 3R chromosome arm. Only one long duplication, SuM3w9, reduced crossing over in both the sr-ca region and the st-sr region. The st-sr region includes the centromeric region as well as the proximal half of the 3R chromosome arm. The remaining

SuM3w duplications generally increased recombination in the st-sr region. The increases seen in the st-sr region may have been due to the presence of unselected chromosomal rearrangements in the SuM3w stocks or to interbrachial effects. Since decreases in frequencies of crossing over throughout the 3R arm were seen with one long SuM3w duplication but, not with any others, it is possible that decreases in crossover frequencies in more proximal regions could have been masked by interchromosomal or interbrachial effects. If this were the case, then the SuM3w duplications behave similarly to the SuM2z duplications.

Egg Hatch and Viability of Newly Induced Tandem Duplications

Table 16 shows egg hatch and viability studies for four 2L duplications. Two separate experiments were run; SuM2z14 was tested in both experiments to check the reproducibility between experiments. The results indicate that duplications up to 135 bands (SuM2z14) have no marked effects on any of the parameters measured (percent egg hatch, percent eggs or larvae developing to pupae or adults). The long duplication, SuM2z19, decreases egg hatch, percent pupae, and percent eggs reaching adulthood about 15 percent relative to the control. A reciprocal cross was made with the SuM2z19 to check for any detect-

able maternal effects. None were found.

Table 16 also summarizes comparable studies carried out for the 3R duplications. The 3R duplications ranged in length from one to 175 bands. The data show that compared to the control, duplications up to 48 bands in length have little or no effect on the parameters measured (percent egg hatch, percent pupae, or percent adults). The duplication SuM3w4, which is 69 bands long does not reduce egg hatch compared to the control, but does reduce the percent pupae and percent adults. The decrease seen for the percent of fertilized eggs reaching the adult stage (26.9 percent) is primarily accounted for by larval losses (%LL=19.8). The long SuM3w duplication reduces all parameters measured. Most of the losses (%LL=64%) occurred during the larval stages.

TABLE 16. EGG HATCH AND VIABILITY DATA FOR TANDEM DUPLICATIONS OF DIFFERENT LENGTHS.

	Egg Hatch %	% Pupae	% Adults Emerging	No. Eggs Laid	No. Bands
		2L Duplications - Exp. 1			
Control	91.3	81.3	80.3	380	---
SuM2z1	92.1	86.2	85.8	247	7
SuM2z12	90.2	84.2	81.5	487	100
SuM2z14	92.0	87.6	83.9	242	135
		Exp. 2			
Control	97.3	96.2	96.0	511	---
SuM2z14	88.5	91.6	89.7	204	135
SuM2z19*	84.9	88.5	84.0	151	206
SuM2z19**	87.4	88.1	87.3	252	206
		3R Duplications			
Control	97.6	87.3	85.7	677	---
SuM3w1	97.6	88.9	87.4	405	1
SuM3w3	97.4	94.0	91.0	232	48
SuM3w4	95.2	75.4	73.3	232	69
SuM3w5	86.0	22.1	17.7	217	175

* ♀♀ were heterozygous for SuM2z19: the cross was SuM2z19/+ ♀ X +/+ ♂♂

** ♂♂ were heterozygous for SuM2z19: the cross was +/+ ♀ X SuM2z19/+ ♂♂

IV. DISCUSSION

Effects of Duplications on Crossover Frequencies; Implications for the Mechanism of Meiotic Pairing and Comparison of the Relative Effectiveness of Duplications and Translocations

It is well known that inversions can be very effective in reducing crossover frequencies, but several other types of chromosome aberrations can be effective crossover frequency reducers as well (reviewed by Roberts, 1976). For example, translocation studies by Roberts (1972) delineated disto-medial regions of autosomes which were particularly sensitive to disruption by the presence of a single translocation breakpoint. Translocations with breakpoints in sensitive regions in some cases reduced frequency of crossing over to 10 percent or less of the control value. Studies by Roberts (1966) and Kalish (1975) have shown that some disto-medially located long duplications can also be very effective as crossover reducers. Our studies confirm and extend these observations and show that several distally located 2L duplications can reduce crossover frequencies throughout the entire 2L arm. In one case, SuM3w9, a long distally located 3R duplication, suppressed crossover frequencies throughout the 3R arm.

In the case of the best known and most studied crossover suppressors, inversions, there are well known meiotic mechanisms to account for part of the reduction in cross-

over frequencies seen with heterozygotes. Paracentric inversions reduce crossing over in part by elimination of crossover products as dicentric and acentric chromatids by oriented meiotic divisions (Sturtevant and Beadle 1936). Pericentric inversions can reduce crossing over in part by elimination of recombinant aneuploid segregants as a result of their lethal effects in zygotes (Roberts 1967). For reversed repeats, crossovers can theoretically be eliminated from exhanges between reversed repeats and their homologues by mechanisms similar to those for paracentric inversions (see below). There are no comparable mechanisms of crossover chromatid elimination to account for the crossover reduction in duplication heterozygotes found with the long direct tandem duplications. The explanation probably involves disruption of meiotic pairing by the presence of the duplications.

For recombination to occur, the preconditions for exchange (e.g. pairing) must be satisfied, and the exchange process itself must occur (reviewed by Baker and Hall 1976). The decreases in crossover frequencies seen with chromosome aberrations such as the large duplications examined in this study and those studied by Roberts (1966), Nix (1973), and Kalish (1975) are probably the result of interference with the pairing process. This interference could possible be the result of mechanical disruption of

the chromosome in the region of the aberration or perhaps be due to disruption of pairing at a site necessary for the initiation of pairing or the maintenance of pairing.

Mechanical disruption might result from the presence of an intrachromosomal loop in the region of the duplication (see below). A loop might produce at least localized regions of asynapsis. Asynapsis resulting from mechanical disruption in heterozygotes might be expected to reduce pairing in the immediate vicinity of the duplication, and thus reduce recombination in the immediate vicinity of the duplication. However, it would seem unlikely that disruption would occur much beyond the limits of the duplication. Long duplications reduce crossover frequencies well beyond their breakpoints (Tables 9 and 14). Thus, mechanical disruption does not explain all of the effects of long duplications on crossover frequencies.

The effectiveness of these duplications (and other aberrations as well) as reducers of crossover frequencies is better explained if they are considered to act by interrupting synapsis of the meiotic chromosomes during meiotic prophase at a major site or sites involved in the initiation or maintenance of pairing. Crossover decreases then might occur either through failure to regularly initiate pairing or through a destablilization of pairing once initiated. In the case of long tandem duplications which are very effective crossover suppressors throughout

an entire chromosome arm, the formation of an intra-chromosomal loop could disrupt pairing if it were to form prior to the time of the initiation of the pairing of the homologues (Roberts 1966). If the attractive forces between homologous regions are first manifested when the homologous chromosomes are sufficiently far away from each other, then the probability of intrachromosomal loop formation may be higher than interchromosomal pairing. Meiotic chromosomes in Drosophila, like chromosomes for at least some other organisms, probably pair at most two at a time for any given region of a chromosome (Dobzhansky 1934). If the intrachromosomal loops form perferentially with the adjacent homologous region on the same chromosome, thus completing the pairing by twos for the region, the probability of pairing with the homologue is reduced since the pairing sites are saturated. If the loop is in a region important for the initiation or maintenance of pairing throughout the chromosome arm, pairing at sites well removed from the site of the aberration (duplication) might be reduced or absent, and thus crossover frequencies could be profoundly affected at sites far removed from the duplicated region.

The site for initiation or maintenance is probably not near the tip of a chromosome. If meiotic synaptic paring proceeds in a zipperlike fashion (i.e. pairing is polarized) from a point near the tip of a chromosome

toward the centromeric region (base) of a chromosome, then interruption of pairing at or near the initiation point could be expected to disrupt pairing proximal to the initiation point. This could account for the reduction in crossover frequencies in regions well beyond the limits of the breakpoints of some of the distally located duplications (e.g. crossover reductions in the b-pr region with some of the 2L duplications, Table 9). However, the translocation studies of Roberts (1970, 1972) show that translocations with breakpoints near the tips of the autosomes reduce crossing over little or not at all. These translocation studies make location of a initiation site on or near the chromosome tip unlikely.

Another possibility is that pairing might begin in the centromeric region and proceed toward the tip of the chromosome. If this were the case, it would be expected that crossover frequencies would be reduced only in the vicinity of the duplications and in regions distal to the duplications. This was not observed (Tables 9 and 14). The translocation studies of Roberts (1970, 1972) also show that the region near the base of the chromosome are relatively insensitive to disruption by translocations with breakpoints in that region. These observations make a centromere to tip pairing model unlikely.

Comparing similarly located translocations and dupli-

cations reveals similarities and differences in the effect of the two types of aberrations on crossover frequencies. Most of the duplications isolated do not include the most sensitive region to crossover disruption by rearrangement (from polytene subdivisions 29-34 of Bridges map of 2L (1942) as defined by Roberts' translocation studies. Nonetheless much of the 2L arm is very sensitive to disruption, and translocation breakpoints in the region in which many of these duplications were located reduced crossing over to 20 percent or less of the control values. Since several of these duplications also reduce crossing over to 20 percent or less, the present duplication studies confirm the sensitivity of the 2L arm to disruption by any aberrations.

The third chromosome (3R) duplications with breakpoints similar to or identical with those of a translocation which was very effective in reducing crossover frequencies, do not reduce crossing over frequencies nearly as effectively as does the translocation. For example, the duplication SuM324 with its leftmost breakpoint at the beginning of the 94A subdivision of the polytene chromosome reduces frequency of crossing over to 66 percent of the control (Table 15), while the translocation, T(3;4)94A (Roberts, 1972), with its breakpoint also in the beginning of the 94A subdivision, reduces the frequency of crossing over to 10 percent of the control.

Also, SuM3w2 with its left breakpoint in the 94E subdivision reduces crossing over to 93 percent of the control, while T(3;4)94C;101 with a breakpoint in the 94C subdivision, a short distance away from 94E, reduces crossing over to 18 percent of the control. Since the translocation data represent whole arm crossover values while the duplication data represent half arm crossover values, it is possible the differences are even more marked. Since different types of rearrangements with similar breakpoints show different degrees of effectiveness in reducing crossover frequencies, it appears the breakpoints *per se* are not the only factor in reducing crossover frequencies.

The reason for the differences between the duplications and translocations as reducers of crossover frequencies is unknown. However, with translocations, nonhomologous chromatin would be in the vicinity of the apparent site of the initiation or maintenance of paring. Perhaps this nonhomologous chromatin could play a role in increasing the effectiveness of translocations as reducers of crossover frequencies. Alternatively, in the case of Roberts (1972) study, the differences could be due to the presence of chromosome 4. All the translocations studied by Roberts (1972) involved chromosome 4 and one of the other autosomes. If chromosome 4 is usually in the

chromocenter during meiotic prophase, as recent studies suggest (Nokkala and Puro 1976), pairing may be inhibited by mechanical tensions.

Short Heterozygous Autosomal Tandem Duplications: Comparison with Short X Chromosome Duplications and Consideration of Pairing Configurations Relative to Crossover Frequencies

Crossover studies with long heterozygous tandem duplications have shown that the frequency of crossing over is decreased both within and outside the limits of these duplications (Roberts 1966, Nix 1972, Kalisch 1975). This is in contrast to studies on three very short X chromosome duplications made by Green (1962). The duplications Green studied varied in length from eight to 18 polytene bands. One was located in the distal 1/4 of the X chromosome and two were located proximally relative to the centromere. Green observed an increase in crossover frequencies both within and outside the limits of the duplication with these short heterozygous duplications relative to their controls. These observations have been repeated by other workers (reviewed by Kalisch 1975). Kalisch (1975) studied a short (34 bands) distally located X chromosome tandem duplication and found the frequency of crossing over was reduced in heterozygotes. All the distally located 2L duplications from seven to 55 bands in length studied in our laboratory showed reduced fre-

quency of crossing over as heterozygotes in the region of the duplication with only one exception. Thus, while the very short heterozygous X chromosome duplications increase crossing over within the duplication, a longer heterozygous X chromosome duplication and very short and longer heterozygous autosomal 2L duplications decrease crossing over in the region of the duplication.

Extrapolation from polytene pairing configurations to meiotic chromosome behavior should be made with caution (see Roberts 1966 and Kalisch 1975 for a discussion of this point). Nevertheless, the polytene pairing configurations of all the short 2L duplications showed intrachromosomal loops. The decrease in recombination frequencies within the region of the duplications in heterozygotes seen with the very short and longer autosomal, and with the longer X chromosome duplications could be the result of meiotic intrachromosomal loop formation which could result in either mechanical disruption of paring, or, as was suggested above for the very long duplications, interference with the initiation or maintenance of pairing.

Alternatively if the meiotic chromosome were a semi-rigid structure over the distances encompassed by the short duplications, and intrachromosomal loop formation were unlikely because of the difficulty in pairing back,

then simple mechanical interference from the presence of the extra chromatin might reduce the probability of pairing in the region of the duplication. If this were to interfere with the formation of the synaptonemal complex in this region, recombination could be reduced. Factors which might contribute to the semirigidity could be the synaptonemal complex itself or coiling properties of the chromosome. Cytological observations on meiotic chromosomes of Drosophila are needed to determine the type of pairing configurations found in duplication heterozygotes to distinguish between the mechanical interference model and the iniation site model discussed previously. Such data are not at present available because of the extreme difficulty of working with Drosophila oocyte material, although recent advances (Nokkala and Puro, 1976) promise to resolve this question.

The models just presented can account for the decreases in crossover frequencies seen in the region of the duplication in heterozygotes with the autosomal duplications and with the longer X chromosome duplications. However, short (one-20 band) heterozygous X chromosome duplications can apparently increase crossing over within the region of the duplication (Green 1962, reviewed in Kalisch 1975). Pritchard (1955, 1960) has proposed an effective pairing model to account for localized negative

interference and increases seen with duplications studied in Aspergillus. According to this model, chromosome pairing prior to recombination is limited to a few short chromosomal segments with a high probability of exchange. Effective pairing would be discontinuous and occur as a precondition to pairing along the entire chromosome. Recent evidence from studies on the synaptonemal complex (reviewed by Moses 1968 and Westergaard and vonWettstein 1972) indicate that there is a rough alignment along a whole chromosome prior to recombination which would make Pritchard's model untenable. However, if something akin to localized effective pairing is occurring within the synaptonemal complex (i.e. there may be regions within the synaptonemal complex in which pairing is enhanced and there is a high probability of exchange) this might explain the increases seen in the X chromosome duplication heterozygotes. Why should localized effective pairing be the case for only the short X chromosome heterozygous duplications? Perhaps differences in rigidity of the chromosome or synaptonemal complex may be responsible. If the X chromosome were relatively more rigid than the autosomal chromosomes over the distances involved for the short X chromosome duplications, then the formation of intrachromosomal loop (which could lead to decreases in crossover frequencies) would be less likely. The pairing sites

within the duplicated regions would tend to remain available for pairing with the homologue, and thus regions with a high probability of exhcnage could pair interchromosomally. This might account for the increases seen in crossover frequencies within the limits of the duplication with the short X chromosome heterozygotes.

Decreases in crossover frequencies are seen beyond the limits of the short (more than 12 bands) heterozygous 2L autosomal duplications (Table 10). These crossover frequency decreases could be accounted for by either a mechanical disruption of pairing (perhaps associated with the presence of an intrachromosomal loop) which extends a short distance beyond the limits of the short duplication, or by interference with a site necessary for the initiation or maintenance of pairing as perviously discussed.

However, difficulties arise when we consider that increases in crossover frequencies can occur outside the limits of the very short heterozygous X chromosome duplications and possibly also beyond the limits of even the short (7-12 band) heterozygous autosomal duplications (which decrease recombination in the region in which the duplication is located). In the case of the short autosomal duplications increases in crossover frequencies are not significant, but they are consistently seen in all the

short duplications studied (Table 10). If the crossover frequency increases beyond the limits of the small duplications are real, the models considered above are inadequate to account for the crossover frequency increases since the regions in question are not duplicated. An adequate explantion is not available at the present time to account for these crossover frequency increases. Perhaps future work will lead to suggestions in terms of unique mechanical or physiological models which might account for this phenomena.

Short Homozygous Tandem Duplications: Effects on Recombination and Meiotic Pairing Models and Comparison to Short Heterozygous Tandem Duplications

When homozygous, the short X chromosome tandem duplications studied by Green (1962) increased the frequency of crossing over in the environs of the duplications. In the distally located X crhomosome duplication studied by Green, Dp(1:1)z-w, genetic markers were situated both within and outside the region of the duplication. One might reasonably expect that the addition of duplicated genetic material in the homozygote would increase recombination equal to an amount equivalent to the genetic length of the material added, but this was not the case. The frequency of crossing over was increased both within and outside the region of the duplication, but the in-

creases seen within the duplication were in excess of the genetic length of the duplication. At least one short 2L autosomal duplication, SuM2z3, probably also increases crossover frequencies in excess of its genetic length as well (Table 13).

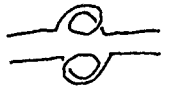
Localized effective pairing sites within the synaptonemal complex (see above) might explain the increases seen in the small homozygote duplications. One might then expect that localized effective pairing would result in increases in any duplication when homozygous. However, Roberts (1966) has shown that in a long homozygous duplication, crossover frequencies are profoundly reduced. This finding is inconsistent with the localized effective pairing model. These conflicting observations may be resolved if the relative rigidity of the chromosome or synaptonemal complex in the region encompassed by the short duplications is an important factor. Semirigidity of the synaptonemal complex or chromosome over short distances may increase the likelihood of localized effective pairing by reducing the probability of intrachromosomal loop formation. An extended loop (in contrast to the intrachromosomal loop) may then be formed and pairing opportunities increased (Figure 11). Rigidity of the synaptonemal complex or chromosome may not be as adequate over longer distances to prevent intrachromosomal loop

INTRACHROMOSOMAL LOOP

Heterozygote

Interchromosomal pairing absent--asynapsis and recombination reduced.

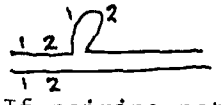
Homozygote



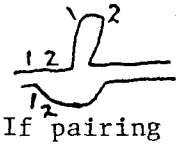
Interchromosomal pairing absent--asynapsis and recombination reduced.

EXTENDED LOOP

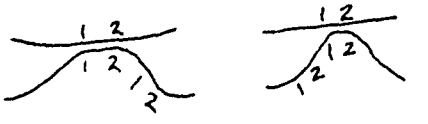
Heterozygotes



If pairing not distorted, then little or no effect on recombination.

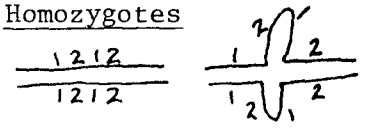


If pairing distorted, then ansynapsis and recombination reduced.

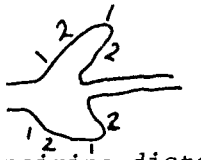


If localized effective pairing, then recombination increased.

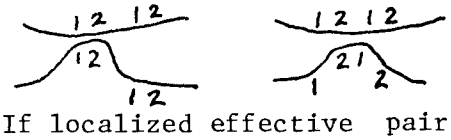
Homozygotes



If pairing not distorted, then little or no effect on recombination.



If pairing distorted, then asynapsis and recombination reduced.



If localized effective pairing, then recombination increased.

Figure 11 Diagram of some possible pairing configurations in duplication heterozygotes and homozygotes. Not all possible configurations are shown.

formation.

The increase in crossing over seen within the duplication with the short heterozygous X chromosome duplication (about 1.5 fold increase, Green 1962) is relatively less than the increase seen with the homozygous X or homozygous autosomal duplications (4-5 fold increase). The difference might be accounted for by the increased opportunity for pairing in homozygotes compared to heterozygotes since there are four copies of the duplicated portion of the genome in homozygotes compared to three copies in heterozygotes.

A closer look at the models which have just been presented to account for the crossover behavior of the small heterozygous and homozygous duplications shows that there are inconsistencies with respect to the properties required of the synaptonemal complex or of the chromosome in the region of a duplication. Intrachromosomal loop formation was invoked to account for the decreases in recombination seen with the short heterozygous autosomal duplications. This would imply that in the region encompassed by the duplication, the autosomal chromosome would be sufficiently flexible that intrachromosomal loops could form relatively frequently. However, to account for the increases seen in the homozygotes it was presumed that extended loops, the result of a semirigid structure, were

formed relatively frequently. This leads to a paradox since, for example, the short 2L autosomal duplication in the heterozygotes but extended loops in the homoqygotes to account respectively for the decreases and increases in recombination seen with these duplications.

Perhaps intrachromosomal loop formation is also unlikely with the very short heterozygous autosomal duplications as well as with the short homozygous autosomal duplications because of semirigidity of the synaptonemal complex or chromosome. The decreases in recombination seen with the very short heterozygous autosomal duplications could then be explained if, for example, an extended loop were to form and the extended loop were to cause distortion and buckling (Figure 11) of the synaptonemal complex in the vicinity of the duplication. Desynapsis might be likely and recombination decreased in the heterozygote. However, if the pairing configurations in the X chromosome and autosome were similar, this model would not account for the increases in crossing over seen with the short heterozygous X chromosome duplications. No one simple pairing model appears adequate to explain all of the observations for both short heterozygous and homozygous duplications.

Another difficulty with the models proposed above is that they cannot explain crossover increases seen

outside the limits of the short homozygous duplications. Increases were seen outside the limits of the breakpoints of the small X chromosome duplication studied by Green (1962), and SuM2z3 (Table 13), a short autosomal duplication, may also have increased the frequency of recombination outside the limits of its breakpoints. There appears to be no adequate explanation at the present time which resolves these difficulties. Perhaps we are forced to conclude that simple mechanical models may not be adequate to account for all of the observations. Other factors (more complex mechanical factors or physiological factors), the nature of which are unknown at present, may be responsible for part of the changes seen in recombination frequencies seen with these small duplications.

EVOLUTIONARY POTENTIAL OF SMALL DIRECT TANDEM DUPLICATIONS

That duplications have played an important role in the evolution of new genomes is well documented (reviewed by Ohno 1970, Smith 1970, and Watts and Watts 1969). Duplication of the entire genome has probably played an important role in the evolution of plants (Stebbins 1951) and possibly some vertebrates (e.g. cypriaid and salmonid fishes, reviewed in Ohno 1970). Total duplication of the genome of animals is generally lethal. Sectional duplications, i.e. duplications of only part of a genome (e.g. tandem or noncontiguous duplications) may have played a more important role in the evolution of new genes in animals.

A general model by which a sectional duplication might evolve into a new gene was considered by Lewis (1951). In this model, once a duplication of part of the genome has arisen new mutations could accumulate in one copy of the duplication while the other copy retained its essential function "shielding" the duplication from elimination through stabilizing selection. The copy in which mutations accumulate could then eventually differentiate and acquire a new function.

The spontaneous rate of duplication formation in Drosophila is probably quite low on a per locus basis in

laboratory populations. In our laboratory no spontaneous duplications were found at the M(2)z locus among 5000 flies counted in one control experiment, and in an X-irradiation experiment, 13,000 flies were scored and no duplications were recovered as SuM's. Thus it appears that the rate of formation of duplications may be 1/13,000 or less. The rate of formation in natural populations may be in the same order of mangitude.

In spite of the probable low rate of formation of duplications in natural pouolations, there is evidence that duplications, in particular tandem duplications, are present in natural populations. For example, in higher vertebrates the β and δ hemoglobin chains, which have similar amino acid seauences and are closely linked, may be recently evolved tandem duplications. Also, the a^2 haptoglobin gene, a gene for one of the serum proteins involved in Fe transport, is probably the result of a partial direct tandem duplication (reviewed in Harris 1975). Observations such as these have led to the assumption that tandem duplication of genetic material has played a prominent role in the evolution of new genes.

To serve as a source of new genes, the newly induced duplications must persist in a population long enough to evolve to a new function. Newly formed duplications probably arise at a low rate, and because of random drift

or accidents, many would be lost. It seems likely that duplications which have persisted and spread throughout a population have done so because of a selective advantage associated with the duplication (Spofford 1972). Spofford (1972) has considered a mechanism whereby organisms with duplications could be at a selective advantage, and thus the duplications could spread throughout a pouplation. In her model, two alleles coding for two different dimers of a dimeric enzyme interact and confer a selective advantage to heterozygotes carrying both alleles (heterotic advantage). The maximum frequency of heterozygotes for these alleles in a population would be 50 percent because of segragation in the heterozygotes for these alleles. A duplication could confer a permanent heterotic advantage if the duplicated portion of the genome were to include the two alleles which are advantageious in the heterozygote. The fraction of the population carrying both alleles could then increase to more than 50 percent. The frequency of the duplication in the population could obviously then increase and perhaps spread throughout a population.

The types of sectional duplications which have been considered most likely to give rise to new genes are reversed tandem or noncontiguous direct or reversed duplications (Lewis 1945, Spofford 1972), because meiotic

mechanisms exist which can stabilize reversed and non-contiguous direct duplications. For example, interchromosomal crossover exchanges involving the duplicated regions of reversed duplications, give dicentric bridges which could result in the elimination of these crossover products. The noncrossover chromosome would still contain the duplication, and thus, the reversed duplication bearing chromosome could persist in a population. For non-contiguous direct repeats sufficiently separated in the genome, intrachromosomal or interchromosomal exchanges could lead to large duplications or deficiencies which would reduce the viability of the zygotes carrying the aberrations. Similar mechanisms are not available for stabilizing direct tandem duplications.

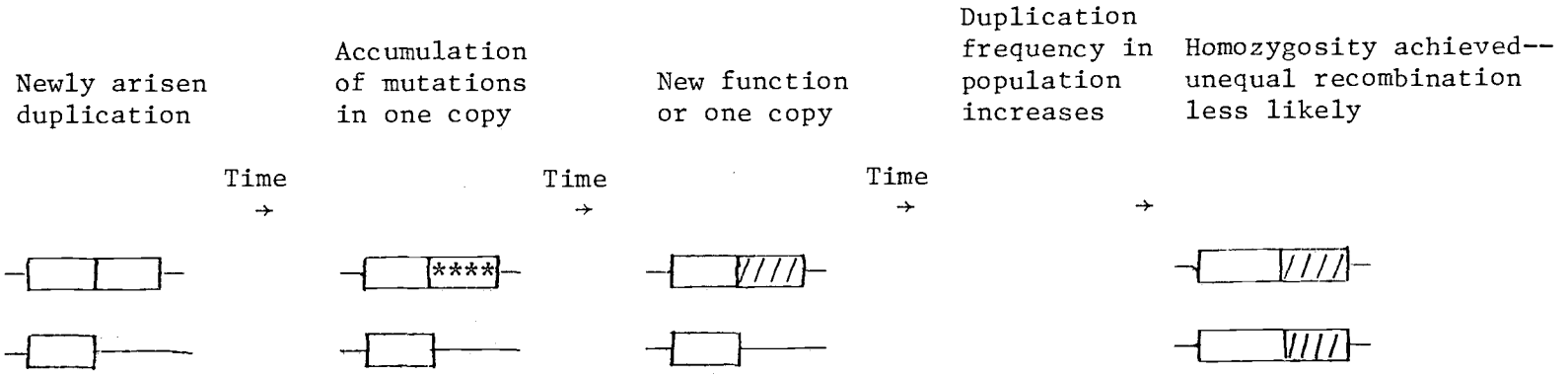
Direct tandem duplications when homozygous can be lost by unequal homologous crossing over (Sturtevant 1925 and reviewed in Roberts 1976). This has led Spofford (1972) to suggest that "tandem duplications must be regarded as inherently unstable as long as the genetic length of the duplicated region permits crossing over at rates significantly above the per generation mutation rates." Flies carrying newly induced autosomal direct tandem duplications will most likely be heterozygous and not homozygous for the duplication. Our crossover studies with small heterozygous 2L duplications demonstrate that

small direct tandem duplications can reduce the frequency of crossing over within the limits of the duplications. This suggests that small autosomal direct tandem duplications in heterozygotes may be stabilized to some extent by reduced crossing over within the duplicated portion.

If recombination were sufficiently reduced within the duplication in heterozygotes, the slowly accumulating mutations would be less likely to be exposed to the forces of Natural Selection because the duplication would be less likely to be broken apart by recombination (Figure 12). Therefore, mutations could continue to accumulate in one of the duplicated portions of the direct tandem duplication leading eventually to a potentially new gene evolving in one copy of the duplication.

One other aspect of the intital stages of the evolution of new genes deserves closer examination. If newly induced duplications were to lower fertility and viability, flies bearing the duplications would be at a distinct selective disadvantage. Duplications which reduced fertility and viability would probably be quickly eliminated from a population. Fertility and viability studies with the duplications isolated in this study show that the larger heterozygous duplications reduce fertility and viability very strongly (Table 16). In contrast, flies carrying small direct tandem duplications reduce fertility

WITHOUT RECOMBINATION



WITH RECOMBINATION

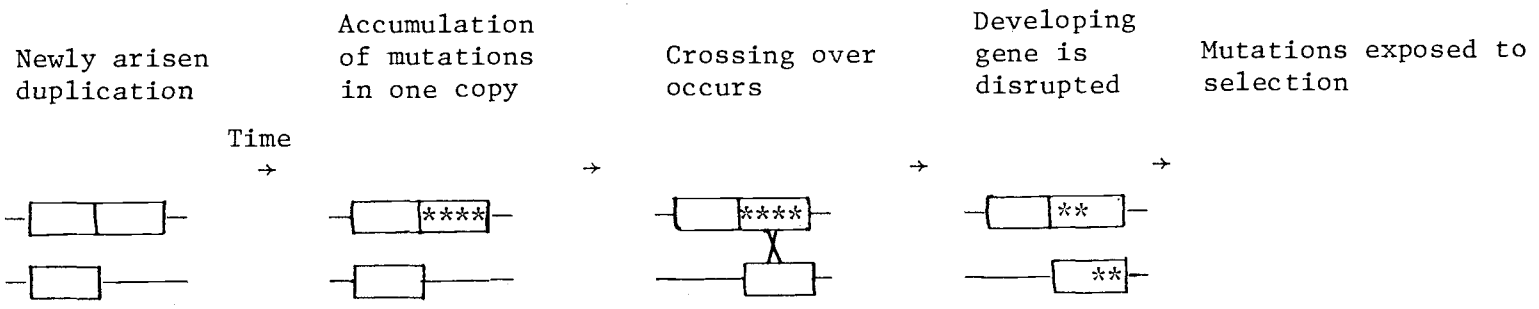


Figure 12 Consquences of recombination in heterozygous direct tandem duplications

[ ] = original unmutated copy [****] = copy accumulating mutations

[////] = copy with new function

and viability little or not at all. This suggests that flies heterozygous for small direct tandem duplications may not be at a pronounced selective disadvantage.

Spofford (1972) has suggested that duplications with a heterotic advantage can become established in a population. If one or a few loci are duplicated in addition to the locus with the heteretic advantage then, by being coupled to the loci conferring heterotic selective advantage, these duplicated loci could spread throughout the population as well. The loci involved with the heterotic advantage would not be expected to diverge because the heterotic advantage would be lost, but the "extra" duplicated loci might not have this same restriction. If the duplication conferring the slective advantage reduces recombination and has little or no effect on fertility or viability then one copy of a coupled extra locus (loci) could accumulate mutations and eventually evolve with subsequent functional divergence as suggested by Lewis (1951).

The X-ray induced duplications recovered in this study showed an excess of direct tandem versus noncontiguous repeats (31 direct tandem/3 noncontiguous). This is expected on the basis of the number of chromosome breaks necessary to give the two types of duplications. Direct tandem duplications can arise from a minimum of two breaks, one in each of two chromatids. Noncontiguous duplications,

however, require at least three breaks (e.g. two in one chromatid and one in the other chromatid, Figure 13). Assuming the induction of each break is an independent event, direct tandem duplications would then be expected to be induced more frequently than noncontiguous repeats. Since we recovered no reversed tandem duplications, perhaps reversed tandem repeats also arise by three hit events as well. If a similar ratio of tendem to noncontiguous repeats is induced in natural populations, it would suggest that a much larger pool of new direct tandem repeats would be available for natural selection to act upon than would be the case for noncontiguous repeats. If on the average, selection pressures on direct tandem duplications are similar to those for noncontiguous repeats, and if the advantage obtained by the reduction in crossover frequencies in short heterozygous direct tandem duplications is comparable to the advantage obtained by the meiotic mechanisms which eliminate recombinants in the noncontiguous duplications, then it might be very likely that direct tandem duplications could be a source of new genes in evolution.

Potential problems which could make this model for direct tandem duplications less tenable are relatively high frequencies of intrachromosomal recombination, and interchromosomal recombination within the short tandem

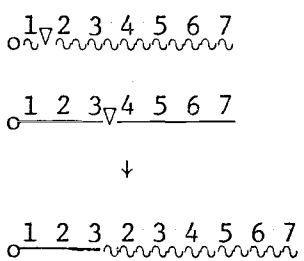
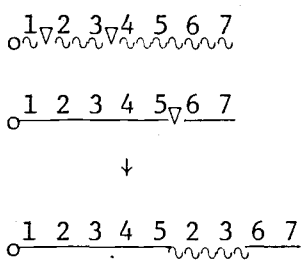
Number of chromosome breaks	Direct tandem	Number of chromosome breaks	Noncontiguous (Direct illustrated)
2		3	

Figure 13 Number of chromosome breaks needed to give direct tandem or noncontiguous duplications

∇ = breakpoint

duplications. As Peterson and Laughnin (1963) have shown, intrachromosomal recombination can occur in small direct tandem duplications. Intrachromosomal recombination can result in the loss of tandem duplications, and thus could act to break up newly deveoping genes. The frequency of intrachromosomal recombination for the short X chromosome duplication studied by Peterson and Laughlin was low (about 1/18,000), perhaps in the order of the forward mutation rate for new duplications. Thus, elimination of newly developing genes by intrachromosomal recombination may not be too troublesome for the model, at least not with the short X chromosome duplication studied.

The short 2L duplications, however, may be another matter. The short 2L duplications decrease frequencies of interchromosomal recombination while short X duplications increase frequencies of interchromosomal recombination. If this difference is due to more frequent formation of intrachromosomal loops in the 2L duplications than in the X duplications, and intrachromosomal loop formation increases the probability of intrachromosomal recombination, then you would expect a higher frequency of intrachromosomal recombination in the 2L duplications than in the X duplications. If intrachromosomal recombination is frequent, this could mean that the formation of intrachromosomal loops, which supposedly protect duplications

from interchromosomal recombination and increase their evolutionary stability, would make them more vulnerable to intrachromosomal recombination and thus decrease their evolutionary stability. Intrachromosomal recombination was not studied with the short 2L duplications isolated in this study. Data are obviously needed on this point to determine if intrachromosomal recombination is sufficeiently low so that premature disruption of the tandem duplication is unlikely.

The other point to be considered is whether interchromosomal recombination is reduced sufficiently to make the model plausible. Data in Table 10 suggest that for duplications of 40 or more bands (in which recombination could be measured within the duplication e.g. SuM2z8) the frequency of recombination is relatively high (0.32 percent). It is still possible that the frequency of recombination within the smaller duplications (7-25 bands) may be reduced sufficiently to hold the duplication tightly linked to the master copy until positive selection pressure develops for the duplication. Unfortunately, genetic markers were not present within the small duplications so the amount of recombination within the small duplications could not be ascertained. Obviously, data on this point are needed.

To summarize, the rather strong suppression of cross-

ing over in the neighborhood of the most frequently induced duplication (the direct tandem duplication) when heterozygous suggests that a duplication and its master copy may be held together long enough perhaps by intrastrand pairing and concomitant crossover suppression to permit independent evolution of new function. In other words, the direct tandem duplication would seem to be preadapted both by reason of its frequency of occurrence as well as by its predisposition to intrastrand pairing to be the most common source of new genes.

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VI. APPENDIX

TABLE 17 CROSSOVER DATA BY REGION FOR SuM2z /al dp b pr c ♀♀ ++

Data for regions given in %crossover (map units)*

NAME	CONTROL NUMBER**	NUMBER COUNTED	al-dp	dp-b	b-pr	pr-c
Control 1		680	13.5±2.5	26.9±3.4	8.8±2.1	20.9±3.1
Control 2		1465	8.5±1.5	24.3±2.2	7.0±1.6	19.4±2.0
Control 3		692	11.3±2.4	26.6±3.3	9.4±2.2	18.2±2.9
Control 4		1179	13.8±2.0	19.8±2.3	11.4±1.8	16.2±2.1
Control 5		345	10.7±3.2	29.6±4.8	7.5±2.8	18.0±4.0
Control 6		200	13.0±4.7	22.0±5.7	14.0±4.8	23.5±5.9
Control 7		1043	8.4±1.6	26.8±2.6	5.7±1.4	20.8±2.4
Control 8		1163	10.7±1.8	30.2±2.6	6.2±1.4	———
SuM2z2	5	728	10.4±2.2	29.4±3.3	4.5±1.5	15.8±2.7
SuM2z4	2	1205	12.3±1.9	27.6±2.6	8.9±1.6	19.1±3.7
SuM2z4	5	927	10.9±2.0	28.2±2.9	8.1±1.8	21.0±2.6
SuM2z5	5	1038	13.6±2.1	23.0±2.5	5.0±3.1	21.2±2.5
SuM2z6	5	383	6.0±2.4	29.0±4.6	6.8±2.5	23.0±4.2
SuM2z7	8	739	8.7±2.1	31.5±3.3	4.7±1.5	———
SuM2z8	5	594	10.4±2.4	30.3±3.3	3.9±1.6	20.0±3.2
SuM2z9	2	1302	8.6±1.4	25.7±2.5	10.2±1.7	20.7±2.2
SuM2z9	6	546	5.7±2.0	30.6±3.9	7.3±2.2	26.4±3.7
SuM2z9	7	876	5.5±1.5	32.7±3.1	8.0±1.8	28.5±3.0
SuM2z10	5	817	3.3±1.2	10.4±2.1	6.6±1.7	24.4±3.0
SuM2z11	2	1425	0.8±0.4	14.8±1.8	9.4±1.5	18.5±2.0
SuM2z11	average***	602	0.3±0.5	17.4±3.0	10.0±2.4	———
SuM2z12	3	1135	4.1±1.7	18.1±2.3	4.3±1.2	18.6±2.3
SuM2z13	8	970	0.1±0.2	6.8±1.6	2.6±1.0	———
SuM2z14	2	429	0.2±0.5	27.5±4.2	7.0±2.4	19.1±3.7
SuM2z14	4	608	4.9±1.7	22.2±3.3	16.6±3.0	32.6±3.7
SuM2z15	8	933	0.1±0.2	2.7±1.0	1.9±0.9	———
SuM2z16	8	827	0.0±0.0	1.2±0.7	1.8±0.9	———
SuM2z17	8	772	0.1±0.3	6.0±1.7	3.2±1.2	———
SuM2z18	6	246	1.2±1.3	3.2±2.2	1.2±1.4	15.4±4.5
SuM2z18	7	1077	0.2±0.2	2.9±1.0	1.0±0.6	16.0±2.2
SuM2z19	1	539	0.0±0.0	9.8±2.5	7.6±2.2	27.5±3.8
SuM2z19	2	439	1.1±1.0	6.2±2.3	3.9±1.8	10.7±2.9
SuM2z19	5	899	0.9±0.6	8.6±1.9	5.1±1.4	24.4±2.8
SuM2z20	8	1019	0.1±0.2	1.4±0.7	2.2±0.9	———
			al-b			
SuM2z1	5	683	44.7±3.7		7.0±1.9	———

* 95 percent binomial confidence limits are given.

** The control number indicates which control values are appropriate for each SuM2z duplication.

*** SuM2z11 was compared to the average of controls 1-8.

TABLE 18 CROSSOVER DATA BY REGION FOR SuM2z/ed dp cl ♀♀ : SHORT HETEROZYGOUS DIRECT TANDEM DUPLICATIONS

Data for regions are given in map units with 95 percent binomial confidence limits indicated. The control number indicates which control values are appropriate for each SuM2z duplication.

NAME	CONTROL NUMBER	NUMBER COUNTED	ed-dp	dp-cl
Control 1		1738	1.61±0.59	3.28±0.84
Control 2		1243	1.53±0.68	4.42±1.14
Control 3		1755	1.65±0.60	4.16±0.93
Control 4		959	1.67±0.81	3.86±1.22
SuM2z2	1	883	0.68±0.54	4.53±0.37
SuM2z2	4	1827	0.77±0.40	3.89±0.89
SuM2z3	1	1019	1.28±0.69	4.02±1.21
SuM2z3	2	1451	1.45±0.62	3.72±0.97
SuM2z3	4	1467	2.04±0.72	4.64±1.08
SuM2z4	1	1219	0.90±0.53	4.18±1.12
SuM2z4	3	2108	0.71±0.36	4.41±0.80
SuM2z6	1	1109	0.27±0.31	1.89±0.80
SuM2z6	4	1409	0.21±0.24	2.98±0.89
SuM2z7	3	1901	0.37±0.27	3.21±0.79
SuM2z7	average*	744	0.40±0.45	2.15±1.04
SuM2z8	1	627	0.32±0.44	3.03±1.34
SuM2z9	1	1054	0.29±0.32	1.61±0.76

* SuM2z7 was compared to the averages of controls 1-4.

TABLE 19 CROSSOVER DATA BY REGION FOR SuM3w/st sr e ro ca ♀♀

SuM3w stocks are without an e allele on the duplication bearing chromosome. Data for regions are given in map units with 95 percent binomial confidence limits indicated. The control number indicates which control values are appropriate for each SuM3w duplication.

NAME	CONTROL NUMBER	NUMBER COUNTED	st-sr	sr-e	e-ro	ro-ca
Control 1		1492	21.8±2.1	10.1±1.5	22.4±2.1	11.9±1.6
Control 2		567	19.2±3.3	7.4±2.2	20.3±3.3	10.9±2.6
Control 3		1115	22.2±2.4	8.6±1.7	22.7±2.5	12.6±1.9
Control 4		655	18.6±3.0	7.9±2.1	25.2±3.3	9.8±2.2
SuM3w1	average*	480	30.8±4.1	9.2±2.6	24.4±3.8	11.3±2.8
SuM3w2	4	1177	35.1±2.8	8.7±1.6	19.5±2.3	15.8±2.1
SuM3w3	3	686	35.1±3.6	7.6±2.0	13.3±2.5	9.6±1.9
SuM3w3	4	272	41.5±3.8	6.6±1.9	9.6±2.2	9.2±2.2
SuM3w5	1	420	30.7±4.4	11.2±3.0	8.1±2.6	5.7±2.2
SuM3w6	2	218	29.9±6.4	3.2±2.4	0.9±1.3	2.3±2.0
SuM3w8	3	503	33.4±4.1	1.6±1.1	2.0±1.2	8.4±2.4
SuM3w9	1	145	7.3±4.2	0.8±1.5	0.7±1.3	0.3±0.8
SuM3w9	1	727	4.1±1.5	0.0±0.0	0.0±0.0	**
SuM3w11	3	343	32.7±5.0	3.2±1.9	1.7±1.4	5.5±2.4
SuM3w13	average*	78	25.6±9.7	3.8±4.2	6.4±5.4	5.1±4.9

* SuM3w1 and SuM3w13 were compared to the averages of controls 1-4.

** These flies could not be scored because a ca allele or a deficiency for ca was present.

TABLE 20 CROSSOVER DATA BY REGION FOR SuM3w/st sr e ro ca ♀♀

SuM3w stocks carry an e allele on the duplication bearing chromosome. Data for regions are given in map units with 95 percent binomial confidence limits indicated. The control number indicates which control values are appropriate for each SuM3w duplication.

NAME	CONTROL NUMBER	NUMBER COUNTED	st-sr	sr-ro	ro-ca
Control 1		444	30.4±4.3	32.5±4.4	15.5±6.8
Control 2		310	21.9±4.6	41.8±5.5	14.5±3.9
SuM3w4	1	354	38.7±5.1	19.7±4.1	12.1±3.4
SuM3w5	1	478	29.7±4.1	16.5±3.3	3.7±1.7
SuM3w7	2	822	29.9±3.1	3.5±1.3	4.5±1.4
SuM3w10	2	889	25.5±2.9	4.9±1.5	7.4±1.7