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Tandem duplications in *Drosophila melanogaster*

III. Intrachromosomal exchange of a heterozygous tandem duplication

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SUMMARY

During meiosis the two parts of a tandem duplication are able to pair in a double loop instead of pairing with the corresponding region of the homologous chromosome. The frequency of intrachromosomal exchange within this double loop was measured in heterozygous females of tandem duplication *Dp(1; 1)Gr* by the phenotypes of the exceptional F_1 males. The intrachromosomal exchange frequency is increased significantly by both the 'interchromosomal effect' of heterozygous inversions in the autosomes and by a double inversion in the homologous *X* chromosome. Distribution of the exchange events depends on the pairing situation and its frequency within the double loop. The analysis of clusters of intrachromosomal recombinants observed favours the assumption that this exchange type is exclusively a meiotic event.

1. INTRODUCTION

Investigations in *C(1)RM* females have shown (Anderson, 1925) that the intrachromosomal exchange process and interchromosomal crossing-over take place, in principle, according to the same mechanism. On the other hand, deviations from the exchange frequencies expected occurred after double loop formation between both parts of paracentric tandem duplications (Peterson & Laughnan, 1963*a*; Green, 1968; Laughnan & Gabay, 1970). Finally, data on tandem duplications with duplicated regions of varied size support the assumption that such deviations originate from the meiotic pairing situations of the duplicated regions, along with other factors (Kalisch, 1975). In the following paper it will be shown to what extent the intrachromosomal exchange frequency of a tandem duplication can be altered by changes in the meiotic pairing situation and by the 'interchromosomal effect' of heterozygous inversions.

Recombinants whose origin can be explained by an intrachromosomal exchange process have sometimes been found as clusters among the progeny of single cultures (Kalisch & Becker, 1970). Since the strains used in these experiments carried 'mutable genes', it could not be excluded that clustering was influenced or even caused by these genes. Therefore the present paper will examine to what extent intrachromosomal exchange processes can occur premeiotically without the influence of 'mutable genes'.

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2. MATERIAL AND METHODS

Table 1 shows the synopsis of gene symbols used in the experiments. A more detailed description of these factors and those which will also be mentioned in this paper has already been given elsewhere (Lindsley & Grell, 1967). The *Dp(1; 1)Gr* tandem duplication chromosome, which causes lethality in hemizygous males, has the order: *Dp(1; 1)3A2-3; 8B4-C1*. It was marked by $y^2(w^- spl\ sn^3)(w^c\ sn^3)$ in all the experiments. Other details of *Dp(1; 1)Gr*, as well as culturing and testing methods used, have already been given by Kalisch (1973, 1975).

Table 1. *Synopsis of gene symbols and chromosome mutations used in text**

Symbol	Location or constitution	Phenotype or Properties
y^2	*1·0·0*	Body colour yellow; hairs and bristles black
w^-	*1·1·5*	White eyes, def. in the *white* locus; viable in males
w^{11E4}	*w*-allele	White eyes
w^c	*w*-allele	Red eye colour; distinguishable from *wild* type
spl	*1·3·0*	Split bristles; rough eyes
sn^3	*1·21·0*	Bristles twisted and shortened
FM1	$In(1)sc^8 + dl-49,\ y^{31d}\ sc^8\ w^a\ lz^S\ B$	Bar eyes; male viability reduced
SM1	$In(2LR)SM1,\ al^2\ Cy\ cn^2\ sp^2$	Curly wings
TM2	$In(3LR)Ubx^{130},\ Ubx^{130}\ e^s$	Halteres about twice normal size

* For more details see Lindsley & Grell (1967). Further gene symbols used in text have already been described elsewhere (Kalisch, 1973, 1975; Green, 1969).

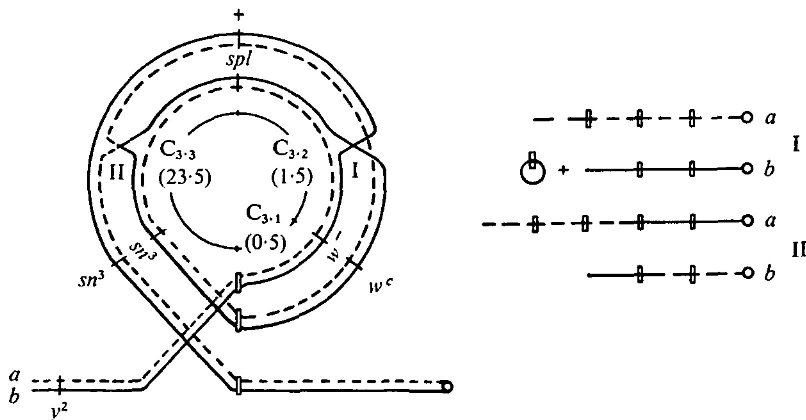

Fig. 1. Intrachromosomal exchange possibilities after double loop formation in the *X* chromosome of tandem duplication *Dp(1; 1)Gr* (modified after Kalisch, 1975). Open bars symbolize the limits of the duplicated sections. The innermost circle indicates the regions ($C_{3\cdot1}$–$C_{3\cdot3}$), in which the intrachromosomal exchange is demonstrable by the phenotype of the F_1 males on the basis of the marker genes used. Position of the marker genes is strongly schematized; the distances between these markers in the *wild*-type chromosome are given in parentheses. I = isochromatid exchange; II = unequal sister chromatid exchange. Both exchange types can occur in the regions $C_{3\cdot1}$–$C_{3\cdot3}$. The figure's right half shows results in regard to both sister chromatids (*a* and *b*).

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Intrachromosomal exchange and its recognition by the F_1-male phenotypes. Fig. 1 shows a configuration of complete meiotic pairing (double loop) of the two chromosome sections in a tandem duplication. An intrachromosomal exchange in this configuration is called an *isochromatid exchange* if it occurs between two homologous regions within the same chromatid. This type of exchange leads to a normally structured chromatid (without the duplication) and an acentric ring, as well as to a sister chromatid which has not been involved in this exchange event (type I; Fig. 1). If, instead, the intrachromosomal exchange within the double loop takes place between sister chromatids, it is called an *unequal sister chromatid exchange* (type II; Fig. 1). This type of exchange leads to a triplication chromatid and a chromatid without the duplication (Peterson & Laughnan, 1963*a*). In both types of intrachromosomal exchange only one of the exceptional chromatids (I*b* and II*b*; Fig. 1) can be phenotypically identified among the male progeny of *Dp(1; 1)Gr* females. Chromatid types I a and II a cause lethality in the hemizygous *Dp(1; 1)Gr* males. Intrachromosomal double exchange could also not be identified because of the lethality of the males. Among the female progeny single and double exchange recombinants cannot be identified by the marker genes used (Kalisch, 1975).

3. RESULTS

(i) *Influence of heterozygous inversions on the intrachromosomal exchange frequency*

Control experiments have revealed that the sum of all measurable intrachromosomal exchange processes within the heterozygous *Dp(1; 1)Gr* tandem duplication equals 0·24% (Kalisch, 1975). The 'interchromosomal effect' of heterozygous inversions (Sturtevant, 1919) and the effect of a double inversion in the homologous *X* chromosome on intrachromosomal exchange processes in *Dp(1; 1)Gr* were investigated. For this purpose, the effects of *FM1*, *SM1* and *TM2* (Table 1) were tested in the experiments listed in Table 2. A significant increase in the exchange frequency is to be observed due to the combined effect of autosomal inversions and *FM1* (no. 4; Table 2). Furthermore, a much stronger effect was found in the case of *FM1* (no. 3) than in the combined effect of *SM1* and *TM2* (no. 2; Table 2). A significant increase of patroclinous F_1 males is to be observed in connection with the presence of the heterozygous autosomal inversions.

Analysis of the experimental data listed in Table 2 required consideration of the following technical problems. All experiments are based on single cultures. This was necessary in order to be sure that all the females of the P generation contained the *Dp(1; 1)Gr* chromosome (intrachromosomal exchange processes in those *Dp(1; 1)Gr* females, which were used to produce the females of the P generation in Table 2 could not be phenotypically recognized by the marker genes of the heterozygous females of the P generation). The following difficulties were encountered in the F_1 analysis of experiments nos. 3 and 4 of Table 2, as well as no. 2 of Table 3. Apart from the C_3 exceptions, viable F_1 males in these experiments carry *FM1*, which severely reduces viability. F_1 females carrying *FM1* may

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Table 2. *Effect of heterozygous inversions on the intrachromosomal exchange frequencies in heterozygous* Dp(1; 1)Gr *females*

No.	Genotype of parental females*	No. of single cultures	Total F_1 male progeny	Patroclinous ♂♂ among the F_1		C_3 male exceptions among the F_1 progeny†	
						N	%
1	*Dp(1;1)Gr/(+); +/+; +/+* ‡	365	15269	11	(1/1388)	37	0·24
2	*Dp(1;1)Gr/(+); SM1/+; TM2/+* ‡	118	1487	(5)§	(1/255)§	29	1·95
3	*Dp(1;1)Gr/FM1; +/+; +/+*	83	2680‖	18	(1/1489)	180	6·72
4	*Dp(1;1)Gr/FM1; SM1/+; TM2/+*	18	372‖	11	(1/34)	37	9·94

* In each single culture one of these females was crossed with three *sc* w^{11E4} *spl* sn^3/Y males.

† C_3 = intrachromosomal exchange recombinants type I*b* and II*b* of Fig. 1.

‡ The gene markers of the normally structured female *X* chromosome in different experiments of no. 1 were +, w^{bf} *cv*, *spl cv f*, *sc ec cv* and in the case of no. 2 +, *spl cv f* (Kalisch, 1975).

§ Based on 106 single cultures with a total F_1 male progeny of 1277 in a separated experiment.

‖ Because of the limited viability of the *FM1* males, data were calculated from the heterozygous *Dp(1; 1)Gr* females. For details see text, p. 277.

also have reduced viability, and the expected number of viable males was therefore calculated as 100/75 × the number of *Dp(1; 1)Gr* females, to allow for the fact that the hatching frequency of the latter is 25 % below that of flies with wild-type *X* chromosomes (Kalisch, 1975).

(ii) *Distribution of intrachromosomal exchanges in the double loop*

The distribution of intrachromosomal exchanges was measured in three different regions ($C_{3.1}$–$C_{3.3}$ in Fig. 1) on the basis of the marker genes used. Assuming a complete meiotic double loop pairing, one must expect exchange frequencies in

Table 3. *Distribution of the intrachromosomal exchanges in heterozygous* Dp(1; 1)Gr *females*

(Exchange types according to Fig. 1. Experiment no. 1 equals no. 1 in Table 2, whereas the data of no. 3 in Table 2 and of no. 2 in this Table are based on different experiments.)

No.	Genotype of parental females*	Total F_1 male progeny	Male exceptions			Total C_3 recombinants	
			$C_{3\cdot1}$	$C_{3\cdot2}$	$C_{3\cdot3}$	N	%
1	*Dp(1;1)Gr/(+)*†	15269	0 (0·74)§	0 (2·22)§	37 (34·04)§	37	0·24
2	*Dp(1;1)Gr/FM1*	4378‡	2 (6·58)§	3 (19·74)§	324 (302·68)§	329	7·51

* Genotype of the parental males in both experiments: *sc* w^{11E4} *spl* sn^3/Y.

† For the gene markers of the *X* chromosomes see footnote ‡ in Table 2.

‡ Estimated from numbers of F_1 heterozygous *Dp(1; 1)Gr* females, as explained in the text.

§ Numbers in parentheses show the expected distribution according to the distribution in normally structured *X* chromosomes.

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Table 4. *Distribution of the intrachromosomal recombinants in single cultures of heterozygous* Dp(1; 1)Gr *females in comparison with the Poisson distribution*

No.	Genotypes of parental females*	No. of single cultures	Total number of C_3 male exceptions	Frequencies†	Single cultures with the following amount of C_3 male exceptions								
					0	1	2	3	4	5	6	7	8
1	*Dp(1; 1)Gr/(+); +/+; +/+*	365	37	(A)	331	31	3	0	—	—	—	—	—
				(B)	330·26	33·36	1·68	0·05	—	—	—	—	—
					$\chi^2[3] = 1{\cdot}25;\ P > 0{\cdot}7$								
2	*Dp(1; 1)Gr/(+); SM1/+; TM2/+*	118	29	(A)	92	21	5	0	—	—	—	—	—
				(B)	91·90	22·97	2·87	0·24	—	—	—	—	—
					$\chi^2[3] = 1{\cdot}99;\ P > 0{\cdot}5$								
3	*Dp(1; 1)Gr/FM1; +/+; +/+*	83	180	(A)	12	22	18	17	6	5	2	1	0
				(B)	9·43	20·46	22·21	16·06	8·71	3·78	1·37	0·42	0·11
					$\chi^2[8] = 4{\cdot}11;\ P > 0{\cdot}8$								

* For details see Table 2.
† (A) Actual, (B) expected.

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relation to the gene distances in normally structured *X* chromosomes. Table 3, none the less, shows that exchanges in the regions $C_{3\cdot1}$ and $C_{3\cdot2}$ appear more seldom, not only in *Dp(1; 1)Gr*/(+) females but also in *Dp(1; 1)Gr/FM1* females, than would otherwise be expected in the light of the above mentioned assumption.

(iii) *Clustering of intrachromosomal recombinants*

From the progeny of the single cultures in experiments nos. 1–3 of Table 2 the frequency of exceptional C_3 flies among the males of the F_1 generation per single culture was determined. The distribution of these empirically determined values was compared with the theoretical values of the Poisson distribution (Table 4). The data of Table 4 show that the C_3 clusters found are based on a random distribution of independent exchange events. In all three experiments the distribution of intrachromosomal recombinants fits the Poisson distribution. A comparable investigation in the females of the genotype *Dp(1; 1)Gr/FM1; SM1/+; TM2/+* was not carried out, owing to the difficulties of evaluation of the F_1-progeny as mentioned on page 277.

4. DISCUSSION

The data of experiment no. 3 in Table 2 show a significant increase of intrachromosomal exchanges in heterozygous *Dp(1; 1)Gr* flies, due to the presence of the double inversion *FM1* in the homologous *X* chromosome. For this behaviour the following possible explanation is offered. Because of the strong structural heterozygosity of the *X* chromosomes, the normal meiotic pairing behaviour between the corresponding chromomere sections of both chromosomes is often disturbed (Kalisch, 1975). As a result a frequent pairing of both duplication sections in a double loop may occur. It may therefore be assumed that the increased exchange frequency in relation to the control experiment (6·72 % versus 0·24 %) is caused by the altered pairing situation during meiosis. This assumption is also confirmed by experiments nos. 2 and 4 of Table 2. These data show that the intrachromosomal exchange is even further increased when autosomal inversions and *FM1* are present in the same genome. The difficulties of chromosome pairing and segregation between the *X* chromosomes occurring during meiosis, due to structural heterozygosity (Cooper, Zimmering & Krivshenko, 1955), are mirrored in the increase of the number of patroclinous males among the F_1 progeny.

The distribution of C_3 recombinants in Table 3 shows that the exchange processes in the double loop are not distributed in correspondence with those in normally structured *X* chromosomes. This becomes particularly apparent from the progeny of *Dp(1; 1)Gr/FM1* females. For both halves of the tandem duplication, the data show a significant exchange suppression in the distal region, as opposed to the middle and the proximal ones. A similar exchange suppression may be assumed for the proximal end according to recent experiments (Kalisch, unpublished data); but in the present experiments this could not be established for *Dp(1; 1)Gr* on the basis of the gene markers used. These data lead to the

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assumption that the meiotic pairing figure of the double loop can, just as all structurally heterozygous configurations, lead to pairing difficulties in those regions in which the normally paired sections undergo transition to structurally heterozygous regions. The uneven distribution of exchange processes in the double loop thus may be attributed to a frequent non-pairing of these transitional chromosome sections during meiosis.

In previous publications, intrachromosomal exchange processes in duplications have been described which occurred as clusters among the F_1 progeny, and it has been concluded that these clusters arise from premeiotic processes (Kalisch, 1970; Kalisch & Becker, 1970; Laughnan, Gabay & Montgomery, 1971; Peterson & Laughnan, 1963*b*). However, in the exchange processes described by Kalisch (1970) and Kalisch & Becker (1970) apparent cluster formation was shown to be due solely to the high mutability of one or more genes within the chromosome sections concerned. This mutability does not stand in direct relation to the intrachromosomal exchange processes, but rather works in a manner similar to other 'mutable genes' and 'mutator genes' described in *Drosophila* (Green, 1973). Since such a high mutability does not exist in the *Dp(1; 1)Gr* strain, it must be assumed that the normal case of a tandem duplication is represented by *Dp(1; 1)Gr* and that the intrachromosomal exchanges in heterozygous *Dp(1; 1)Gr* females, as well as all other tandem duplications (Kalisch, 1975), are exclusively the result of meiotic processes.

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