

THE MECHANISM OF NON-DISJUNCTION IN THE *CLB* STOCK OF *DROSOPHILA MELANOGASTER*.

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(With One Text-figure.)

I. INTRODUCTION.

MISCELLANEOUS observations during several years of work with the so-called "*CLB*" stock¹ of *D. melanogaster* have shown that *XXY-CLB* females produce an exceedingly high percentage of exceptional offspring. Such flies are heterozygous for the *CLB* chromosome, which contains a large inverted section preventing nearly all crossing-over between the *X*'s. It was thought that an investigation of non-disjunction in this stock might possibly lead to some new findings bearing on the problem of interdependence of crossing-over and chromosome disjunction, and experiments were therefore undertaken with this idea.

II. SECONDARY NON-DISJUNCTION IN THE *CLB* STOCK.

In order to study secondary non-disjunction in the *CLB* stock exceptional females heterozygous for this chromosome, and moreover carrying a *Y*-chromosome, were mated to different males containing non-inverted *X*-chromosomes. The regular female offspring of such flies were studied in respect of non-disjunction by crossing with *bobbed* or *bobbed-lethal* males. These offspring consisted of four groups of females. One group received from the mother a *CLB X*-chromosome together with a *Y*-chromosome; such females served for a more precise study of the influence of the *CLB* chromosome on secondary non-disjunction. Another group differed from the first by absence of a *Y*-chromosome; this gave the possibility of studying the effect of the *CLB* chromosome on primary non-disjunction. A third group consisted of females which received from their mother a non-inverted *X* and a *Y*-chromosome: these served to show whether the autosomes of the *CLB* stock had any influence on

¹ This stock, obtained by Muller (1923), has been extensively used since then in genetical work with *Drosophila*. The *CLB* females are heterozygous for an *X*-chromosome carrying the genes *scute*, *tan*, *vermilion*, *small-wing* and *Bar* and having a recessive lethal effect. Moreover, this chromosome contains an inversion, the left break of which lies in the vicinity of *cross-veinless* (Muller and Stone, 1930) and the right break between *Bar* and *fused* (Painter, 1933).

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secondary non-disjunction. Lastly, the fourth group of females carried two non-inverted *X*-chromosomes and no *Y*-chromosomes.

The presence or absence of a *Y*-chromosome in all these types of regular F_1 females was determined by their breeding behaviour. In order to distinguish between the *XX* and *XXY* females with the necessary precision, only such crosses as gave more than a hundred flies were taken into consideration.

Table I shows the offspring obtained from the first group of females, *i.e.* from *XXY* females heterozygous for the *CLB* chromosome.

The percentage of female exceptions in the offspring of such *XXY-CLB* flies is calculated directly from the experimental data. In estimating the percentage of male exceptions as well as the mean percentage of both

TABLE I.

Series	Regular ♀♀	Exceptional ♀♀	Total ♀♀	Regular ♂♂	Exceptional ♂♂	Total ♂♂	Total flies	Percentage of exceptional ♀♀	Percentage of exceptional ♂♂ (corrected)	Percentage of exceptional flies (corrected)
♀ $\frac{CLB}{sc\ v} \times \delta\ f\ bb$	867	439	1306	383	458	841	2147	33.6	37.4	35.5
♀ $\frac{CLB}{sc\ w} \times \delta\ w^e\ bb^l$	1093	707	1800	571	636	1207	3007	39.3	35.8	37.5
♀ $\frac{CLB}{sc\ w^e\ ec} \times \delta\ w^e\ bb^l$	1351	579	1930	595	583	1178	3108	30.0	32.9	31.4
♀ $\frac{CLB}{y\ cl\ f\ +\ cr} \times \delta\ y\ v\ bb$	920	587	1507	426	560	986	2493	39.0	39.7	39.3
Total	4231	2312	6543	1975	2237	4212	10755	35.3	36.2	35.7

sexes together, a correction is introduced to allow for the inviability of half of the regular males, *viz.* those that receive the *CLB* chromosome.

The analysis of Table I shows that females heterozygous for the chromosomes of the *CLB* stock show a very high percentage of secondary exceptions, and that the percentage of such exceptions among the female and male offspring is approximately equal. A second series of experiments answers the question whether this high percentage of secondary exceptions is due to the presence of the *CLB* *X*-chromosome or to the influence of the autosomes from the *CLB* stock. This series consists of crosses with *bobbed* and *bobbed-lethal* males of regular *XXY* females, likewise derived from exceptional *CLB* mothers, but carrying two non-inverted *X*-chromosomes. The results of this series are summarised in Table II, and show that the *CLB* autosomes exert no appreciable influence on the process of secondary non-disjunction (at any rate in a heterozygous condition). Consequently, the high percentage of secondary exceptions

in the first series must be evidently attributed solely to the presence of the *CLB X*-chromosome.

From a theoretical point of view, it is natural to suppose that the increase of secondary non-disjunction in females heterozygous for the *CLB X*-chromosome is due to a weakening of the synaptic affinity between the *X*'s, caused by the existence of an inversion in one of them. The experimental data of Dobzhansky (1933) concerning the rôle of inversions during the meiotic processes in flies carrying translocations also tells strongly in favour of such an interpretation. Nevertheless, an alternative explanation is possible. It might be assumed that the decrease of the synaptic affinity between the *X*-chromosomes in the case studied is caused, not by a chromosome rearrangement, but by the presence in the inverted *X*-chromosome of some specific genes influencing its behaviour during synapsis. The following experiment was undertaken

TABLE II.

Series	Regular ♀♂	Exceptional ♀♂	Total ♀♂	Regular ♂♂	Exceptional ♂♂	Total ♂♂	Total flies	Percentage of exceptional ♀♂	Percentage of exceptional ♂♂	Percentage of exceptional flies
♀ $\frac{sc}{sc v} \times \delta f bb$	2904	113	3117	2664	151	2815	5932	3.6	5.4	4.5
♀ $\frac{sc w}{sc w^e ec} \times \delta w^e bb^t$	1365	44	1409	1294	34	1328	2737	3.1	2.6	2.8
Total	4269	157	4526	3958	185	4143	8669	3.5	4.5	3.9

in order to decide between these two possibilities. *XXY* females, carrying a normal (non-inverted) *X*-chromosome and a reinverted *CLB* chromosome, containing the genes *scute*, *tan*, *vermillion*, *small-wing* and *Bar*, were studied in respect of non-disjunction. The reinverted chromosome in question arose through a spontaneous complete reinversion of the *CLB* chromosome, in consequence of which it regained its normal gene arrangement and lost its lethal effect. This latter peculiarity can be explained either by a supposition that the lethal effect of the *CLB* chromosome is due to a position effect, or by assuming that this chromosome had lost the lethal factor it carried by means of double crossing-over in the region between the gene *scute* and the point of the left break of the inversion. Excluding this region, it is evident that in respect of the genes it carries this reinverted *X*-chromosome is identical with the *CLB* chromosome.

Crosses of *XXY* females carrying a normal *X*-chromosome and a

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reverted *ClB* chromosome with *forked bobbed* males gave the following results (Table III):

The low percentage of exceptions obtained in these crosses shows clearly that the increase of secondary non-disjunction in flies heterozygous for the *ClB* chromosome cannot be ascribed to the action of any specific genes located to the right of the left break of the inversion. Experiments with *ClB* chromosomes which had lost their left parts (including the locus of *white*) through rare cases of single crossing-over, makes it evident that no such genes are present likewise in this region. These facts strongly support the supposition that the increase of

TABLE III.

Series	Regular ♀♀	Exceptional ♀♀	Total ♀♀	Regular ♂♂	Exceptional ♂♂	Total ♂♂	Total flies	Percentage of exceptional ♀♀	Percentage of exceptional ♂♂	Percentage of exceptional flies
$\frac{+sc\ t\ v\ sm\ B}{sc} \times \delta\ fbb$	1410	38	1448	1021	24	1045	2493	2.6	2.3	2.5

secondary non-disjunction in females heterozygous for the *ClB* chromosome is due to the weakening of the synaptic affinity between the *X*'s caused by the presence of an inversion in one of them.

The percentage of exceptions in the offspring of different *XXY-ClB* females shows a considerable variability, ranging from about 15 to about 55 per cent. Nevertheless, there evidently exists no essential difference between these females in respect of the process studied. This can be seen from Fig. 1, which shows the distribution of all the crosses

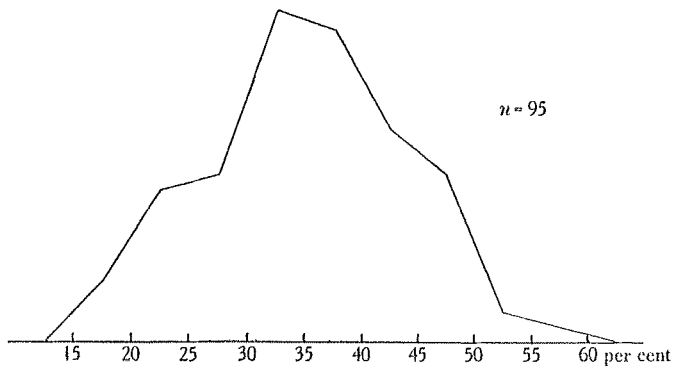


Fig. 1.

included into Table I according to the percentage of secondary exceptions in their offspring. The character of the frequency curve speaks for the accidental nature of the variations observed.

III. THE MECHANISM OF SECONDARY NON-DISJUNCTION IN THE *CLB* STOCK.

Bridges (1916) in his classic work on non-disjunction had arrived at the conclusion that in *XXY* females the sex-chromosomes do not synapse in a triad, but two synapse, leaving the third unsynapsed; at the reduction division the two synapsed chromosomes disjoin and pass to opposite poles; and the free chromosome goes with one or the other at random. On the basis of this conception, Bridges had theoretically estimated the highest possible percentage of secondary non-disjunction as equal to 33.3 per cent. This maximal value can be established only when no homosynapsis takes place, *i.e.* when the synaptic partners are always represented by one of the *X*'s and the *Y*-chromosome, the other *X* remaining free.

In a later work, Anderson (1929) suggested that crossing-over is independent of the presence of a *Y*-chromosome, and prior to the processes which determine the distribution of the *X* and *Y*-chromosomes; and that the type of distribution where the two *X*-chromosomes go to the same pole occurs most freely in those cases where little or no crossing-over takes place near the right (spindle fibre) end of the *X*-chromosomes. This hypothesis is not contrary to the experimental data obtained by Bridges; but since it is based on a case of high non-disjunction caused evidently by some chromosomal aberration, the nature of which is not sufficiently clear, the question remains open whether this hypothesis can be applied to all cases of secondary non-disjunction in *Drosophila*, or whether it must be restricted only to the special case studied by Anderson. The analysis of the mechanism of secondary non-disjunction in the *CLB* stock, with its high percentage of exceptions approaching the maximal possible value given by Bridges, seemed therefore to be justified.

According to the hypotheses both of Bridges and Anderson, the *Y*-chromosome should go at random with one or the other *X*-chromosome, when the regular *XY* gametes are formed. Consequently, the ratio between the *XX* and *XXY* regular females ought to be 1 : 1. Only if such a ratio could be actually found would it be worth while to test the applicability of these hypotheses to the case studied.

Table IV shows the ratio between the *XX* and the *XXY* regular females in the offspring of exceptional *CLB* mothers.

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The fact that in both the *Bar* and non-*Bar* groups of these females the ratio between the *XX* and *XXY* types is very near to 1 : 1, indicates that in *XXY-CLB* flies the *Y*-chromosome goes with either one of the *X*-chromosomes at random.

If the older conception of Bridges were true, the fact that *CLB-XXY* females give in their offspring about 35 per cent. of exceptions would mean that in such females one of the *X*'s always synapses with the *Y*-chromosome, while the other *X* is left unsynapsed. Such a possibility can be easily tested. If no homosynapsis takes place in these females and one of the *X*-chromosomes is always left as an univalent, evidently no crossing-over between the *X*'s can be possible. Though the *CLB* inversion greatly inhibits crossing-over between the *X*-chromosomes, it yet does

TABLE IV.

Type of <i>X</i> -chromosome received from the mother	Regular <i>XXY</i>	Regular <i>XX</i>	Total
<i>CLB</i>	♀♀ 47	♀♀ 49	♀♀ 96
Normal (non-inverted)	68	63	131
Total	115	112	227

TABLE V.

		Regular offspring			Exceptional offspring		Total
♀♀	♂♂	Percentage of single crossing-over in the left non-inverted region	Percentage of double crossing-over in the inverted region	Percentage of single crossing-over in the right non-inverted region	♀♀	♂♂	
1739	843	0.77 ± 0.11	0.15 ± 0.08	0.08 ± 0.06	3	9	2594

not prevent it completely, and a study of crossing-over is therefore possible even in *CLB* flies. In order to obtain a control illustrating the process of crossing-over in *XX-CLB* females, such females, carrying the *CLB* chromosome and a non-inverted *X*-chromosome with the genes *yellow*, *cut*, *forked* and *carnation* were mated to *yellow vermilion bobbed* males. Table V gives the results obtained from these crosses. It can be easily seen that the crossing-over values are very low throughout the chromosome. Crossing-over takes place most frequently in the left non-inverted section, *i.e.* in the region between *sc* and the left break of the inversion. The frequency of crossing-over is equal here to 0.8 per cent. Crossing-over between *cr* and the right break of the inversion and double crossing-over in the inverted section are much more rare, taking place in about 0.1 per cent. each.

XXY-CLB females, heterozygous for the *y ct f cr* chromosome, were

mated to *y v bb* males in order to decide whether crossing-over can occur in them. The results of these series are summarised in Table VI. This table shows that crossing-over not only does take place between the *X*-chromosomes, but seems at first sight to be here even more frequent than in *XX-ClB* females. It seems therefore evident that Bridges' conception of the behaviour of the sex chromosomes during synapsis in *XXY* females does not hold true for the case studied.

On the contrary, the hypothesis suggested by Anderson accords well with the experimental data stated above. On the basis of this hypothesis it is to be expected that crossing-over between the *X*'s will not be influenced by the presence of a *Y*-chromosome, and consequently the percentage of cross-over chromosomes among the gametes of *XX* and *XXY* females will be equal. The percentage of cross-over chromosomes in the gametes of *XX-ClB* females can be estimated directly from their offspring (Table V). The percentage of cross-over chromosomes in the

TABLE VI.

Regular offspring					Exceptional offspring			
♀♀	♂♂	Percentage of single crossing-over in the left non-inverted region	Percentage of double crossing-over in the inverted region	Percentage of single crossing-over in the right non-inverted region	♀♀	Percentage of single crossing-over in the left non-inverted region (equationals)	♂♂	Total
920	426	4.56 ± 0.68	0.33 ± 0.11	—	587	0.50 ± 0.09	560	2493

regular gametes of the *XXY-ClB* females can be likewise computed directly from the experimental data. 0.2 per cent. of the exceptional gametes of these females were cross-overs of the "equational" type resulting from crossing-over in the left (non-inverted) region. In order to determine the number of cross-overs of the "reciprocal" type, 417 exceptional daughters obtained from *XXY-ClB* females were tested by means of mating them to *y v bb* males. None of them proved to be carrying cross-over chromosomes. The percentage of crossing-over in exceptional gametes can be therefore regarded as being equal to the percentage of equationals, cross-overs of the reciprocal type being so rare as to render them negligible.

In order to compute the total number of cross-over *X* chromosomes in the gametes of *XXY-ClB* females, not only the viable classes must be taken into consideration, but the perishing classes of zygotes as well, represented by the 3*X* superfemales and by the *ClB* males. The following figures are then obtained (Table VII).

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As can be seen from this table, the crossing-over values in the offspring of *XX* and *XXY-CLB* females are approximately equal. This allows us to interpret the mechanism of secondary non-disjunction in the *CLB* stock on the ground of Anderson's hypothesis. This fact taken

TABLE VII.

	Percentage of crossing-over in the left non-inverted region	Percentage of double crossing-over in the inverted region
Data from <i>XX</i> mothers	0.77 ± 0.11	0.15 ± 0.08
Data from <i>XXY</i> mothers (corrected)	1.29 ± 0.23	0.08 ± 0.06
Difference	0.52 ± 0.27	0.07 ± 0.09

in connection with the data of Dobzhansky (1933) concerning another closely related genetical phenomenon, the non-disjunction of autosomes in flies heterozygous for translocations, seems to indicate that Anderson's conception is generally applicable to all cases of chromosome disjunction in *Drosophila*.

IV. PRIMARY NON-DISJUNCTION IN THE *CLB* STOCK.

According to the hypothesis of Darlington (1931, 1932), the regular pairing and disjunction of chromosomes is invariably dependent on chiasma formation, and every chiasma represents a crossing-over. Since the great majority of gametes produced by *CLB* females belongs to the non-cross-over classes, we should expect on Darlington's view, that such females would show in their offspring a very high percentage of primary exceptions, due to the irregular distribution of their *X*-chromosomes during meiosis.

The percentage of primary exceptions in the offspring of *CLB* females is presented in Table VIII, which includes the offspring of *XX-CLB*

TABLE VIII.

Series	Regular ♀♀	Exceptional ♀♀	Total ♀♀	Regular ♂♂	Exceptional ♂♂	Total ♂♂	Total flies	Percentage of exceptional ♀♀	Percentage of exceptional ♂♂ (corrected)	Percentage of exceptional flies (corrected)
♀ $\frac{CLB}{sc\ w^e\ ec}$ × ♂ $w^e\ bb^t$	2605	9	2614	1116	—	1116	3730	0.34	—	0.34
♀ $\frac{CLB}{y\ cl\ f\ cr}$ × ♂ $y\ v\ bb$	1739	3	1742	843	9	852	2594	0.17	0.53	0.35
Total	4344	12	4356	1959	9	1968	6324	0.28	0.53	0.34

regular females obtained from exceptional *CLB* mothers and mated to *bb* or *bb^t* males. It shows that such females produce primary exceptions

in a ratio of about 1 : 300. The ratio of primary exceptions is evidently about seven times higher than in the case of normal *X*-chromosomes; but nevertheless the percentage of exceptions is incomparably lower than should have been expected on the basis of Darlington's hypothesis. About 99 per cent. of the *X*-chromosomes carried by the gametes of *XX-ClB* females belong to the non-cross-over classes¹, and if they were distributed at random, the frequency of exceptions in the offspring of such females should have been not 1 : 300, but approximately 1 : 3.

Thus, it is evident that the *X*-chromosomes of a female of *D. melanogaster* can disjoin regularly during meiosis without undergoing detectable crossing-over, *i.e.* crossing-over in their genetically active parts. This means that the mechanism of chromosome disjunction in the female of *Drosophila* can be regarded as similar in principle to that in the male; a conclusion, which confirms the somewhat less direct evidence of Dobzhansky (1933), who arrived at analogous ideas in his work on the rôle of inversions in the meiotic processes of flies carrying translocations.

On the basis of Darlington's hypothesis concerning the relation of chiasmatisation and chromosome disjunction, the only explanation of the regular distribution of the *X*-chromosomes in *ClB* females must be essentially the same as that proposed by him for the case of *Drosophila* males (Darlington, 1931, 1932, 1934); that is, it must be assumed that in such females crossing-over does actually take place between the *X*-chromosomes, but is restricted to their inert parts alone, located near the spindle fibre and remains therefore unobserved.

Though this supposition cannot be directly tested on our material, it seems nevertheless to be highly improbable. The works of Stern (1929), Muller (cited by Muller and Painter, 1932), Bridges and Anderson (1925), Bedicsek and Muller (cited by Muller and Painter, 1932) and Rhoades (1931) have shown that the region of the *X*-chromosome between the locus of *bobbed* and the point of attachment of the spindle fibre is devoid or practically devoid of crossing-over. Moreover, even if a small amount of crossing-over could take place in this region, it is hardly possible that such crossing-over can take place when the *X*-chromosomes synapse in

¹ Single crossing-over in the inverted region gives rise to inviable chromosomes and cannot be detected directly. Cross-overs of this type could not be therefore taken in view in the above calculations of crossing-over shown by *ClB* females. Nevertheless, the following facts make it evident that this type of cross-overs is so rare as to be negligible. Firstly, this is seen from the absence of XO patroclinous males in the offspring of *XXY-ClB* females (in the series with *bb* males). Secondly, extensive egg counts in crosses of *XX* and *XXY-ClB* females show that the percentage of perishing eggs is practically equal to that of perishing *ClB* males, *3X* superfemales and *YY* zygotes.

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a reversed fashion; and the occurrence of double crossing-over in the inverted part of the *CLB* chromosome (Tables V and VI) shows that this type of synapsis is probably not at all infrequent in *CLB* females. On the ground of all these data it seems evident that Darlington's conception concerning the absolute dependence of the regular disjunction of chromosomes upon crossing-over between them, meets with grave difficulties when applied to the mode of distribution of the *X*-chromosomes in *Drosophila*.

V. SUMMARY.

1. *XXY* females heterozygous for the *CLB* chromosome give among their offspring about 35 per cent. of secondary exceptions. The percentage of secondary exceptions is equal in the male and female offspring. The *Y*-chromosome disjoins from the *CLB* chromosome and from the normal *X*-chromosome with equal frequency.

2. In *CLB* females crossing-over between the *X*-chromosomes is greatly inhibited. The percentage of cross-over *X*-chromosomes carried by the gametes of *XX-CLB* females is equal to that of *XXY-CLB* females, indicating the correctness of Anderson's conception of the mechanism of secondary non-disjunction.

3. *XX-CLB* females give among their offspring about 0.35 per cent. of primary exceptions. The comparison of the number of non-cross-over *X*-chromosomes produced by such females, with the frequency of non-disjunction in their offspring shows that the mechanism of chromosome disjunction in the male and female *Drosophila* is essentially similar, and speaks against Darlington's views concerning the relation between crossing-over and the disjunction of chromosomes.

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