CROSSING OVER AND HETEROCHROMATIN IN THE X CHROMOSOME OF DROSOPHILA MELANOGASTER*

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Received, December 24, 1938

INTRODUCTION

IT IS well known that the frequency of crossing over per unit cytological length, either mitotic or salivary, is not constant along the chromosomes of *Drosophila melanogaster*. Furthermore there is ample evidence to show that the centromeric, or spindle attachment, regions of the chromosomes have certain peculiar properties in this respect. Two of the most important of these characteristics are:

(a) Crossing over per unit cytological length near the centromere is lower than elsewhere.

(b) Crossing over is most variable near the centromere, as shown by the effects of temperature changes, age and irradiation.

Now the centromeric regions of the three long chromosomes are also peculiar in another respect; they are largely heterochromatic and "inert," the more distal regions being euchromatic and "active." It is important to know how far the special properties in crossing over of these regions are related to their heterochromatic nature, or on the other hand whether their low and variable crossing over is conditioned by proximity to the centromere. Such information is of value not only in connection with the theory of crossing over but also from the standpoint of the nature of heterochromatin, and of inertness.

This problem has been attacked in the past by OFFERMAN and MULLER (1932) who used two X chromosome inversions, sc^8 and $\delta 49$, and several X-IV translocations. Their method was to follow crossing over in females homozygous for these structural changes. It was concluded that though the frequency of crossing over of the euchromatic regions was related to their proximity to the centromere, that of the heterochromatin was independent of its position in the chromosome. Mention was made of attempts to follow the variability of crossing over in these changed chromosomes, but no specific results or conclusions are noted.

The many X chromosome inversions now available make it possible to attempt a more extensive analysis of the relations of crossing over, the centromere and heterochromatin. It seems desirable to confine attention to such inversions rather than to consider translocations, as the spatial

* The cost of composition of tabular matter has been defrayed by the GALTON and MENDEL Memorial Fund.

distribution of crossing over in any chromosome is apparently related to the length of the arm in question (MATHER 1936). Certain data of this kind have been obtained by STONE and THOMAS (1935), GRÜNEBERG (1935, 1937) and STURTEVANT and BEADLE (1936), though these authors were largely concerned with other aspects of inversion behavior. The experiments described below are in many ways an elaboration and extension of certain crosses made by these earlier investigators.

I wish to express my gratitude to PROFESSOR T. H. MORGAN for granting me facilities for this work at the California Institute of Technology, while I was holding a Rockefeller International Research Fellowship. I am also indebted to DR. A. H. STURTEVANT and others of the genetical staff of that Institute for their interest and guidance during these investigations. A number of the flies used in the study of the y^4 inversion were kindly recorded for me by DR. P. CH. KOLLER.

EXPERIMENTAL MATERIAL

Crossing over was followed in flies homozygous for X chromosomes having (a) normal sequence, (b) the sc^8 inversion (c) the rst^3 inversion (d) the sc^4 inversion (e) the y^4 inversion. Certain other inversions would have been valuable for the work, but were either not readily available or difficult to use on technical grounds.

The lengths and positions of the inversions are shown, together with the loci used, in figure 1. The locations of the breakage points and gene loci in the euchromatic left section of the chromosome (shown by a solid line) are based on BRIDGES' (1938) salivary map of this element. These loci may be placed on such a map with some precision. The heterochromatic right end of the chromosome (shown as a wavy line) is, however, not portrayed with any attempt at accuracy, other than as to the positions of the breaks relative to each other and to the *bb* locus. Information about the length and structure of this heterochromatin is not good, and in any case the relative length is not the same in salivary and mitotic chromosomes.

Descriptions of the inversions and their phenotypic effects, together with the evidence for the location of their breaks in the places chosen, will be found in the papers cited in the introductory section.

It will be convenient at this point to give the meanings of the gene symbols used in this paper. The inversions are scute-8 (sc^8), scute-4 (sc^4), roughest-3 (rst^3) and yellow-4 (y^4). The name and symbol in each case depends on the mutation or position effect accompanying the inversion. The genes used are yellow (three alleles, y, y^2 and y^{31d}), apricot (w^a), echinus (*ec*), crossveinless (*cv*) cut-6 (ct^6), vermilion (*v*), dusky (*dy*), garnet-2 (g^2), forked (*f*), Bar (*B*), carnation (*cr*) and bobbed (two alleles, *bb* and bb^1).

Their positions on the standard map are shown in figure 1 and also given numerically in table 14.

In nearly all cases the crossover data were obtained from single females which were mated with from one to five males in a vial for the first day after emergence. These single females were then allowed to lay in separate bottles for two-day periods. After four such periods, that is, on the ninth day, the surviving females were removed and killed. In the temperature experiments, the females were raised from an early larval stage through pupation and emergence at the chosen temperature. They were further mated and allowed to lay at this same temperature. Thus the data on crossing over at any temperature are from progeny of females whose whole life, after the first few days as larvae, was spent at that tem-

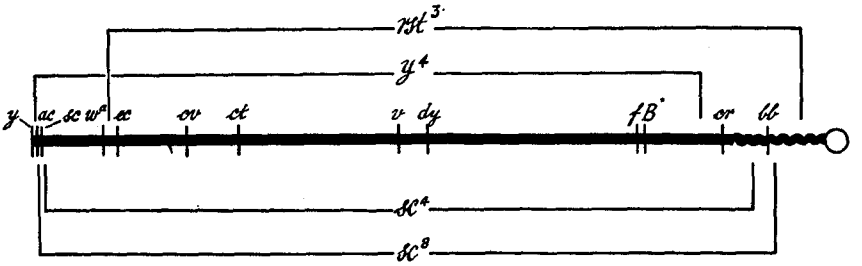

FIGURE 1.—Diagram showing the relative positions of the inversion breaks and of the genes used in these experiments. The solid line indicates euchromatin, and the wavy line heterochromatin. The positions of the various loci in the euchromatin are taken from BRIDGES (1938) map. The loci in the heterochromatin are not accurately placed. Only their relative positions are shown.

perature. The actual progeny of these females, after the removal of the mother, was always raised at 25°C in order to minimize death from overheating.

In all cases it proved possible to obtain adult females at each of the temperatures tried. Sometimes however these females were sterile, or nearly so, at the higher temperatures and so no crossing over data could be obtained. These observations may be of interest and so they are set out in tabular form in table 1. "Partially fertile" indicates that the number of offspring, though considerably reduced, was still appreciable.

TABLE 1

SEQUENCE	19°C	25°C	28°C	30°C.
Normal	Fertile	Fertile	Fertile	Fertile
sc^{8}	Fertile	Fertile	Fertile	Fertile
rst^{3}	Fertile	Fertile	Fertile	Partially fertile
y^{4}	Fertile	Fertile	Partially fertile	Sterile
sc^{4}	Fertile	Fertile	Almost sterile	Sterile

The results are shown in tables 2–13 in the form of recombination percentages together with the number of flies counted. Details of segregations cannot be given as they would occupy too much space.

The statistical analyses were concerned with the frequencies of recombination, each marked section of the chromosome being treated individually. In all cases the χ^2 test was used, χ^2 being calculated either by the method appropriate to a 2×2 contingency table or from BRANDT and SNEDECOR'S formula (MATHER 1938). The χ^2's were analyzed into two parts, one concerned with variation in recombination following temperature changes and the other relating to age variation. Table 2, for example, contains the results of an experiment involving three temperatures with four ages at each temperature. Thus there are 12 groups, and hence 11 degrees of freedom. Now if we sum all the data for any one temperature regardless of age (given as Totals in table 2) we have three groups separated solely on the basis of temperature. A test of this "temperature variation" may be made by calculating a χ^2 for two degrees of freedom. Within each temperature are four age groups, which may be compared by calculating a χ^2 for three degrees of freedom. We may however sum the three age χ^2's, one from each temperature, and obtain a general "age variation" χ^2 for 9 degrees of freedom. Thus the 11 degrees of freedom between the 12 recombination determinations are divided into two for temperature and nine for age variation. Below each table are given the χ^2 analyses and the appropriate probabilities as determined by reference to a table of χ^2 such as that given by MATHER (1938).

OBSERVATIONS

(a) *Control data.* Three different crosses made using the normal chromosome sequence were

1. $$\frac{y^2\ w^a}{cv\ v\ f} \times y^2\ w^a\ cv\ v\ f$$

2. $$\frac{v\ B \quad bb}{cr} \times v\ cr\ bb^1$$

3. $$\frac{v\ f}{bb} \times v\ f\ bb^1$$

In both (2) and (3) other genes were segregating but were disregarded for various reasons. There is no reason to believe that any of these extraneous segregations would upset the results from the genes followed.

The cross (1) was made three times. On each occasion, if more than one

temperature was used, the heterozygous females used were all sisters, though raised at different temperatures. Hence their results are strictly comparable, if it is granted that age of mother or father has no effect on the frequency of crossing over in the daughter. Both male and female progeny were recorded in each case, with the exception of the 30°C cross, where only males were used. In this case the males used in the cross were of a slightly different constitution (table 4). Their sisters were of type 3.

The heterozygous females used at the different temperatures in crosses 2 and 3 were also progeny of the same parents.

TABLE 4

Cross $\frac{y^2\ w^a \qquad bb}{cv\ \ v\ \ f} \times y^2\ cv\ v\ f\ cr\ bb^1$ *[Males recorded] Percentages of Recombination.*

REGION	30°C				
	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL
y^2–w^a	3.24	1.52	2.06	3.92	3.26
w^a–*cv*	18.77	16.46	15.10	18.14	16.92
cv–*v*	24.92	25.57	26.78	26.96	25.97
v–*f*	32.68	29.62	5.36	29.41	29.15
Number of flies	309	395	351	204	**1295**

The control data are summarized in tables 2–6. Results for different temperatures where shown in the same table, were obtained from sister flies, as noted above. Where on different tables the mother flies were not sisters. In general the results are much as expected, though recombination throughout is higher than would be expected from the standard map. The data confirm STERN'S (1926) conclusions in showing that the region *f-bb* shows temperature variations in recombination, and even go further in narrowing down this effect to the *cr-bb* region. The other regions also show significant age variations, particularly in the data of table 3, but as these are not consistent from one group of data to the next it seems safe to suppose that these are not due to genuine age and temperature effects. Their cause is not obvious.

The data of table 4 are particularly aberrant in showing very high recombinations for all the regions. These results are, however, not strictly comparable with any of the others, except for the *v-f* region. For this region they may be compared with their sisters recorded in table 6. This comparison shows the figures of table 4 to be unduly high; they should therefore be treated with considerable caution. In general the age and temperature effects occur consistently only in the *f-bb* region.

One technical point may properly be considered here. In the crosses of

tables 5 and 6, segregation for the extreme bobbed character was observed and as was expected, the extreme bobbed females failed to equal their not bobbed sisters in number. Now MORGAN, BRIDGES and SCHULTZ (1935) advocate treating the *bb* locus as a lethal and discarding all the extreme bobbed flies. Equal numbers of flies are then raised from crosses having *bb* in coupling and in repulsion with the other genes, in order to balance the effect. I have not followed this practice as there is internal evidence which shows that the bobbed flies may be used. The extreme bobbed flies will not give different recombination values for the various regions unless their mortality is affected differentially by the other genes.

This question may be approached statistically. If we make a 2×2 table having recombination and non-recombination for the region in question along one margin and the numbers of *bb* and not-*bb* flies along the other, we may calculate a χ^2 which will detect differences in recombination between *bb* and not-*bb* flies. They should, of course, give the same recombination value. The χ^2 values, calculated separately for each age group at each temperature, wherever the numbers are sufficiently large, may then be summed for each region. In this way we obtain a χ^2 testing the agreement, over all ages and temperatures, of the *bb* and not-*bb* flies in the recombination shown for either the *v-f* region or the *f-bb* region or any other region followed. Each 2×2 table in the analysis will contribute one degree of freedom (MATHER 1938). The results of this analysis are:

REGION	DATA FROM CROSS GIVEN IN TABLE	χ^2	DEGREES OF FREEDOM	PROBABILITY
v–f	6	15.4941	11	0.20–0.10
f–bb	6	14.8671	11	0.20–0.10
v–B	5	14.9245	11	0.20–0.10
B–cr	5	5.7476	9	0.80–0.70
cr–bb	5	9.4679	9	0.50–0.30

It will be observed that in no case is the χ^2 significant. Hence for the purpose of determining the recombination values, we may add the *bb* flies to the not-*bb* flies, and need not discard them as has been advocated. Further results illustrating the same point will be given in the section dealing with the *rst*[3] crosses.

(b) *Inversion sc*[8]. This inversion was used quite extensively. All the genes employed in the experiments were introduced by double crossing over, with the exception of the yellow markers. Two ways of achieving this were used. In the first place the *sc*[8] deficiency chromosome was employed. This is *sc*[8] with the uninverted left section and a small amount

of the inverted heterochromatin missing. The deficiency covers yellow and may be used to mark that locus. The second marker was the mutant y^{31d}, introduced into the sc^8 chromosome by X-irradiation. The sc^8 deficiency chromosome requires further comment. The deficiency for the inverted heterochromatin was detected by showing that it was deficient for *bb*. This was done by crossing to *bb* and bb^l stocks. With *bb* it gives extreme bobbed females and with bb^l it is lethal. Secondly this chromosome shows a very peculiar type of segregation from normal sc^8 chromosomes. In the daughters of a sc^8/sc^8 def. female the deficiency is recovered just twice as frequently as the corresponding portion of the other chromosomes. The segregation for other loci in such flies depends on the closeness of their linkage to the yellow locus. The reasons for this behavior are not fully known, but it seems to be bound up with a maternal effect. However, the recombination values shown by such females agree reasonably well with those shown by sc^8/sc^8 flies and so may be used (tables 7–9).

The sc^8 recombination values are given in tables 7–9. In each case the females used at different temperatures, where shown in the same table, were from the same parents, as in the case of the control data.

It is unfortunately true that in these crosses, as in the controls, a certain amount of inconsistent variation is shown by nearly all the regions followed. The only region to show consistent variability in recombination with temperature is however the *y-f* region. There is a suggestion of variability with temperature in the *cv-w*^{a} section but it is not particularly clear and cannot be taken with any emphasis. The variability of the *y-f* section with temperature is further checked by the rst^3 data given in the next section.

In table 10 are the results of a more complex determination of crossing over in the sc^8 chromosome. This was done solely at 25°C, and no account was taken of age of mother. Eight loci were followed and this allowed a rather detailed analysis of the distribution of crossing over in this chromosome to be made. An attempt was made to follow the sable (*s*) gene in addition; but for a number of reasons, notably the difficulty of classification of *s* on y^{31d}, complicated by the apparent segregation of some autosomal modifiers of *s*, the attempt was given up. Sable did not segregate visibly in the daughters as the recessive fathers were not carrying this gene. The bb^l of the test males had no effect as the mothers were homozygous not-*bb*.

(c) *Inversion* rst^3. Only one type of cross was used with this inversion. This was

$$\frac{rst^3 \quad y\ bb\ cr}{rst^3 \quad\ bb\ \ f\ v\ cv} \times y^2\ cv\ v\ f\ cr\ bb^l$$

present purpose. This was then done and no further distinction was drawn between the normal males and the extreme bobbed females. This agrees with the absence of effect of extreme *bb* on apparent recombination in certain regions of the normal chromosome.

The *rst*^{3} data are given in table 11. They are quite unambiguous in showing a temperature variation in the frequency of recombination in the $y-cr$ region but in no other section of the chromosome. This is in general better shown here than by the *sc*^{8} results. Certainly the data from both inversions taken together bring out the variability of the $y-f$ or $y-cr$ region very well.

TABLE 10

$$Cross\ \frac{sc^8\ y^{31d}\quad f\quad dy\quad cv\quad w^a}{sc^8\quad cr\quad s\quad ct^6\quad ec} \times y^2\ w^a\ ec\ cv\ ct^6\ dy\ f\ cr\ bb^1\ [Females\ recorded].$$

REGION	y^{31d}–cr	cv–f	f–dy	dy–ct^6	ct^6–cv	cv–ec	ec–w^a	NUMBER OF FLIES
RECOMBINATION	4.99	10.81	22.47	14.89	3.86	3.02	0.98	1424

The other striking feature of the *rst*^{3} results is the large recombination value of the $y-cr$ region (see also GRÜNEBERG 1935, 1937). This will be discussed later.

(d) *Inversion sc*^{4}. The cross

$$\frac{sc^4\ y\qquad\qquad w^a}{sc^4\ y\ \ g^2\ ct^6\ ec} \times B$$

was used, only the males being recorded (table 12). It proved impossible to obtain many flies at 28°C, so no attempt to follow the variability of the recombination values was made. The results are of some interest in connection with the so-called spindle fibre effect.

(e) *Inversion y*^{4}. A number of flies were raised from the cross

$$\frac{y^4}{y^4\ f\ v\ cv} \times y^2\ cv\ v\ f$$

at 25° and 28°C. The results are given in table 13. Little need be said about them. There is no evidence of age or temperature variation in either region. This is not unexpected, in view of the length and position of the inversion.

DISCUSSION

The "spindle fibre effect"

Table 14 shows a summary of the frequencies of recombination observed in the different regions at 25°C. The standard map differences are also

given for comparison. It will be seen that the normal sequence results agree reasonably well with the standard, though they are in general slightly higher. The proximal regions are slightly more variable than the rest, as might be expected from the results of GOWEN (1918).

On turning to the results from the sc^8 chromosome we find that the region w^a-ec, normally distal, on being placed close to the centromere becomes genetically much shorter. It shows about a quarter of its normal frequency of crossing over. This is a clear manifestation of the so-called spindle fibre effect. The effect decreases in potency as we proceed distally along the chromosome. The region $ec-cv$ is about 37 percent of normal, the $cv-ct$ region 61 percent of normal and the rest almost unchanged, except for $f-cr$, which is now nearly twice as long as usual. In this case it is the reverse of the spindle fibre effect, observed when a normally proximal region is displaced distally. Are these effects on crossing over genuinely caused by the centromere or are they the effect of heterochromatin? The

TABLE 12

Cross $\frac{sc^4\ y}{sc^4\ y}\ \frac{w^a}{g^2\ ct^6\ ec}\times B$ *[Males recorded] Percentages of Recombination.*

REGION	g^2-ct^6	ct^6-ec	$ec-w^a$	NUMBER OF FLIES
TOTAL	24.38	12.60	2.19	365

answer is obtained by comparison with the sc^4 results. In the latter, the w^a-ec region is about 50 percent normal, that is, it is showing a smaller spindle fibre effect than in sc^8. Now it is known that the right break of sc^4 is much distal to the right break of sc^8 (GERSHENSON 1935). In the latter, w^a-ec is nearer to the centromere but adjacent to a smaller section of heterochromatin than in sc^4. Now the greater reduction in sc^8 as compared with sc^4 is easily explained as due to a stronger centric effect, following greater proximity to the centromere. On the other hand if the spindle fibre effect is supposed to be due to the heterochromatin, this result is difficult to understand, as it would mean that the smaller portion of heterochromatin is having a greater effect than the larger one while the $f-cr$ region at the other end is showing the reverse, in that $f-cr$ is genetically longer in sc^8 than in normal sequence. Thus the supposition that the spindle fibre effect is a genuine centric action seems more reasonable. The behavior of the $ec-ct^6$ region is in agreement with this view.

The rst^3 data support those from sc^8 in showing that proximal regions displaced distally have increased crossing over, $f-cr$ being here 8.8. This value probably does not differ in reality from the 10.8 of sc^8.

The y^4 results are of negative interest in helping to demonstrate the

absence of any effect when the right break of the inversion is not close to the centromere.

Crossing over in the heterochromatin

If the low crossing over per unit cytological length in the proximal euchromatin is due to an effect of the centromere and not to some property of the adjacent heterochromatin, the question immediately arises as to whether the heterochromatin itself fails to show crossovers because it is near to the centromere or whether it is by nature incapable of free crossing over. The behavior of the sc^8 and rst^3 chromosomes supplies information on this point.

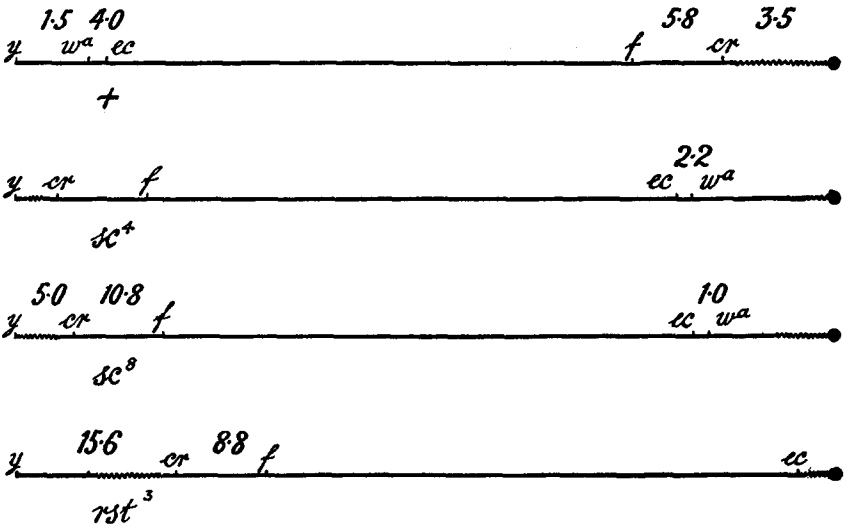

FIGURE 2.—Diagram to illustrate the "spindle fibre" effect. The solid line indicates euchromatin and the wavy line heterochromatin. The numbers above the symbols are the recombination percentages observed.

In the case of sc^8 the left break of the inversion is between achaete and scute (STURTEVANT and BEADLE 1936) and so *y* serves as a good marker of this point. If crossing over between *y* and *cr* is followed in the sc^8 chromosome, a comparison with the value of 3.5 between *cr* and *bb*, which genetically speaking practically coincides with the centromere, yields information of this type. The value observed in experiment was 5.0 percent (table 10) which shows a very low excess over the standard 3.5 percent. Such an excess could be accounted for by the increase of crossing over observed in distally displaced proximal regions, as in the case of *f*—*cr*. The situation is somewhat different in the case of rst^3. Here the left break lies between w^a and *ec*, probably nearer the former. Thus there is a section of euchromatin undisturbed near yellow. Its length is at most 5.0 crossover units and probably much less. At 25°C, 15.6 percent re-

combination was observed between *y* and *cr* in homozygous *rst*^{3} females (GRÜENBERG, 1937, gave even larger values). Allowing 5 percent for the included remnant of euchromatin, this leaves nearly 11 units for crossing over between *cr* and the breakage point. It would then seem almost beyond doubt that, by comparison with the 3.5 of the normal and the 5.0 of *sc*^{8}, some crossing over must be taking place in the distal heterochromatin of *rst*^{3}. The cause of the discrepancy between *sc*^{8} and *rst*^{3} is not clear, as *sc*^{8} certainly includes, according to GERSHENSON, a considerable piece of inverted heterochromatin. It would be of great interest to obtain a cross-over between *rst*^{3} and *sc*^{8} or *rst*^{3} and *sc*^{4} to see how much more heterochromatin is inverted in *rst*^{3} than in *sc*^{8}. It is doubtful if the excess is sufficient to account for the difference in the observed recombination values.

Another possibility exists. If there is a small distal region of low crossing over near yellow, the heterochromatin of the *sc*^{8} inversion might lie wholly or largely in this area and show little if any more crossing over than in its normal place; whereas in *rst*^{3} the remaining euchromatin between *y* and the inversion would keep the inverted heterochromatin in the region of high crossing over. This combined with some excess of inverted heterochromatin in *rst*^{3} as compared with *sc*^{8} might perhaps account for the difference.

Such a distal region of low crossing over, which CHARLES (1938) has supposed to be equal to the centric region, must in fact be shorter than the latter. The evidence from *sc*^{8} shows this well. The region *f*—*cr* is clearly nearer to *y* in *sc*^{8} than it is to the centromere in the normal sequence because the inversion, while almost involving *y*, does not involve all the heterochromatin. Yet this region shows more crossing over in *sc*^{8} than in the normal chromosome.

Whatever the situation with regard to the distal region of low crossing over and the difference between *rst*^{3} and *sc*^{8}, there can be little doubt that in *rst*^{3} the heterochromatin is showing some crossing over, though whether heterochromatin can cross over so freely as euchromatin is an unanswered question. It then follows that the frequency of crossing over in heterochromatin, as in euchromatin, is dependent on the distance from the centromere.

The effect of temperature on crossing over

We have seen above that most probably the "spindle fibre" effect is governed by the centromere and that heterochromatin can most likely cross over when displaced from its position near the centromere to one where crossing over is more favoured. The heterochromatin is thus similar to the euchromatin in respect of some of its behavior in crossing over.

We must now turn to the question of the cause of the high temperature

sensitivity of crossing over near the centromere in the normal chromosome. Here the answer is different.

The data of tables 2–6 confirm STERN's (1926) conclusion that temperature affects the frequency of crossing over in the $f-bb$ region and nowhere else, in the normal chromosome. The data of table 6 further narrow down the seat of the main disturbance to the $cr-bb$ section. This could clearly be either another effect of the centromere or a peculiar property of the heterochromatin, which may also be expressed by adjacent euchromatin.

Turning to the sc^8 and rst^3 data once more we possibly find slight evidence of variability with temperature of crossing over in the now proximal w^a-cv region in sc^8. On the other hand the distal $y-f$ region of sc^8 and $y-cr$ region of rst^3 show a marked and consistent variability of crossing over with temperature changes. The intermediate regions show some spasmodic variability which is unrelated to temperature.

Thus we here have clear evidence of a property peculiar to heterochromatin. Whether proximally or distally placed, its frequency of undergoing crossing over is very much more affected by environmental conditions than is the case with the euchromatin. There is no real evidence of any relation between variability and distance from the centromere, as the possible sensitivity of w^a-cv in sc^8 may be ascribed to its being in juxtaposition to the inverted heterochromatin. (It may be noted that the results of GRAUBARD (1932) on temperature effects in chromosome II fall into line if we suppose his inversion included no heterochromatin.)

Thus of the two peculiarities with regard to crossing over of the centric region of the normal chromosome, one, the low frequency, is an effect of the centromere while the other, the sensitivity to environmental changes, is a property of the heterochromatin. Further, though the heterochromatin most probably resembles the euchromatin in showing a similar relation in crossing over to position with respect to the centromere, it is distinct from the euchromatin in its susceptibility to environmental conditions.

SUMMARY

Crossing over was followed in females homozygous for the normal sequence and four inversion sequences in the X chromosome, different temperatures being used.

The results indicate that:

(a) crossing over in the euchromatin is dependent upon the distance from the centromere.

(b) the heterochromatin can show crossing over, the frequency obtained being dependent on distance from the centromere.

(c) high sensitivity of crossing over to temperature is a property of the heterochromatin.

Thus of the two special properties of the centric region in crossing over, that of the low frequency is a property of adjacency to the centromere, while that of variable frequency is a property solely of the heterochromatin.

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