

THE EFFECT OF TEMPERATURE ON MEIOTIC LOSS OF THE Y CHROMOSOME IN THE MALE DROSOPHILA¹

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GERSHENSON (1933) and SANDLER and BRAVER (1954) have clearly shown that following nondisjunction of X and Y in the *Drosophila melanogaster* male, the frequency of recovery of XY sperm is markedly lower than that of nullo-X-nullo-Y sperm. Their suggestion to account for this discrepancy was to suppose that a chromosome which fails to pair with its homologue may be lost at meiosis. Such loss was found to be most pronounced in cases in which the X chromosome was deficient for a large portion of the basal heterochromatin ordinarily involved in pairing with the Y chromosome.

Preliminary data (ZIMMERING 1962 and unpublished) have suggested that (1) from situations in which the homology between X and Y is greatly reduced, the rate of chromosome loss at meiosis can be appreciably decreased by temperature treatment, and furthermore, that (2) such a decrease in the rate of loss need not necessarily involve an increase in the frequency of X-Y synapsis prior to anaphase I separation. A detailed account of these and subsequent experiments is presented below.

MATERIALS AND METHODS

Males possessing a modified X chromosome, *Ins(1) γ sc^t-sc^s*, and the *sc^s-Y* of MULLER (1948) were employed in the initial temperature experiments. *In(1) γ sc^t*, a long inversion of the X chromosome marked distally with the mutant γ (yellow body), leaves most of the basal heterochromatin including *bb⁺* and Block A at the proximal region, while *In(1)sc^s*, also a long inversion of the X chromosome, transposes most of the basal heterochromatin including *bb⁺* and Block A distally. Thus, an X chromosome which carries the distal region of *In(1) γ sc^t* and the proximal region of *In(1)sc^s*, i.e., *Ins(1) γ sc^t sc^s*, is deficient for a considerable portion of the basal heterochromatin including *bb⁺* and Block A and (for at least some) regions normally involved in synapsis with the Y chromosome (GERSHENSON 1940; COOPER 1951; LINDSLEY and SANDLER 1958). The *sc^s-Y*, a fully fertile Y chromosome, carries *U1⁺*, $\gamma⁺$, and *ac⁺* derived from the tip of *In(1)sc^s*.

The experimental setup was as follows. Males carrying the *γ sc^t sc^s* chromosome and the *YB^s*, a modified Y chromosome synthesized by BROUSSEAU and

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LINDSLEY (1958) carrying the dominant mutant Bar of STONE (STONE 1934) were crossed with females carrying the reversed acrocentric compound X of MULLER (1943) homozygous for the mutant γ and f (forked bristles) and possessing the sc^s -Y. The flies were placed at 18°C in the experimental series and at 26°C in the control. Male offspring from these crosses carrying in addition to the γ sc^t sc^s chromosome, the sc^s -Y but not the YB^s , were collected within a period of 24 hours after eclosion. Single males were then mated with three γ ν (vermilion eye) free-X females at 26°C for two days. The males were then transferred to fresh cultures with three virgin females for an additional two-day period. Males were then discarded. The critical difference between the two experiments is presumed to be the temperature at which the meiotic divisions took place in the two groups of γ sc^t sc^s / sc^s -Y males.

RESULTS

The results from two experiments of the kind described above are given in Table 1. The following points may be made. In the controls, the X class constitutes 48.8 percent and 49.7 percent of the total number of offspring in Experiments Ia and IIa, respectively. Since without loss its expected frequency is 50 percent, there appears to be no evidence of X chromosome loss under these conditions. The values do not change significantly after temperature treatment. On the other hand, there is an appreciable deficiency of the Y class since its frequency, 23.6 percent and 23.5 percent, respectively, in Experiments Ia and IIa, is only about one-half that expected under normal conditions. There is, however, a considerable increase in its frequency after temperature treatment, being 42.2 percent and 41.5 percent in Experiments Ib and IIb, respectively. The frequency of the Y class is therefore increased by 18.6 percent (42.2 percent–23.6 percent) in Experiment I and by 18.0 percent (41.5 percent–23.5 percent) in Experiment II. Finally, there is a marked discrepancy in the recovery of the XY *vs.* the O-class, 2.3 percent *vs.* 25.3 percent, and 1.7 percent *vs.* 25.1 percent in Experiments Ia and IIa, respectively. After temperature treatment, the O-class

TABLE 1

Progeny from the crosses of γ sc^t sc^s / sc^s -Y males with free-X γ ν females.
These males were raised at 26°C or 18°C until eclosion and then mated at 26°C

Male gametes			X	Y	XY	O	
F ₁ phenotypes			γ ♀	ν ♂	+ ♀	γ ν ♂	N
I.	a.	26°C	2,373 (49.7%)	1,123 (23.5%)	82 (1.7%)	1,196 (25.1%)	4,774
	b.	18°C	3,539 (50.0%)	2,936 (41.5%)	238 (3.4%)	363 (5.1%)	7,076
II.	a.	26°C	5,414 (48.8%)	2,624 (23.6%)	260 (2.3%)	2,805 (25.3%)	11,103
	b.	18°C	5,699 (48.3%)	4,971 (42.2%)	411 (3.5%)	711 (6.0%)	11,792

declines sharply dropping by 19.3 percent (from 25.3 percent to 6.0 percent) in Experiment I, and 20.0 percent (from 25.1 percent to 5.1 percent) in Experiment II. There is a small but significant increase in the XY class, from 2.3 percent to 3.5 percent in Experiment I, and from 1.7 percent to 3.4 percent in Experiment II. These observations suggest that the temperature treatment regularizes the transmission of the *sc^s*-Y chromosome.

One question of interest that arises in connection with these results is the following. Does the more normal rate of recovery of the Y chromosome at the cooler temperature depend upon an increase in the frequency of cells in which the Y chromosome is synapsed with the X such as to permit the Y chromosome to disjoin more normally, or is a Y chromosome capable of near-normal transmission at the cooler temperature independent of synapsis with the X chromosome? One way to approach this problem is to test the effects of a cooler temperature on the transmission of a Y chromosome which has not been provided with any pairing partner, that is, one which is present in the cell as a univalent.

For this purpose, males of a modified Bar-Stone translocation type, $T(1;4)B^s$ were employed. Ordinarily, diploid males carrying this translocation possess as one pair of homologues a fourth chromosome attached to a large distal piece of the X chromosome (X^D) and the normal fourth chromosome (IV) and as another pair, the remaining proximal part of the X chromosome (X^P) marked with the dominant B^s , and the Y chromosome (Y). In the modified form used in the present experiments, the proximally located heterochromatin in X^P is largely replaced by a fully fertile Y chromosome (BROSSEAU and LINDSLEY 1958) arising from a crossover between X^P and PARKER's $XY^L\cdot Y^S$ chromosome (PARKER and McCURONE 1958), where X represents the euchromatic region of the X chromosome, Y^L the long arm of the Y, the raised dot the position of the centromere, and Y^S the short arm of the Y chromosome. The modified X^P will be symbolized as $X^PY^L\cdot Y^S$, and the genetic composition of the Bar-Stone male carrying $X^PY^L\cdot Y^S$ but not an additional free Y chromosome as X^D/IV , $X^PY^L\cdot Y^S/O$, where X^D/IV represents paired "homologues," and $X^PY^L\cdot Y^S/O$ the modified X^P without a homologue, and hence present as a univalent. The X^D/IV , $X^PY^L\cdot Y^S/O$ males were mated with attached-X females homozygous for $\gamma v bb$ and not carrying a free Y, i.e., $\gamma v bb/O$, at 26°C or 18°C and the offspring allowed to develop at these respective temperatures. Within a period of 24 hours after eclosion, male offspring were mated singly at 26°C with three virgin γv free-X females for a period of two days and then transferred to fresh cultures with three virgin females and allowed to mate for an additional two-day period. Males were then discarded. The following gametic types are produced by the male: $X^D + X^PY^L\cdot Y^S$ and its reciprocal $IV + O$, and $X^D + O$ and its reciprocal $IV + X^PY^L\cdot Y^S$. From the crosses with γv free-X females, $X^D + X^PY^L\cdot Y^S$ is recovered as a B^s female and $IV + O$ as a γv male. Without loss of the univalent, these classes are expected with equal frequency. The gametic type $X^D + O$ is not recoverable from these crosses since females heterozygous for this deficient X are inviable, while the gametic type $IV + X^PY^L\cdot Y^S$ is recovered as a male hyperploid for the univalent. These hyperploid males are considerably less viable than the B^s females and the γv males

and their recovery is very likely influenced by culture conditions, etc. Hence, while their recovered numbers are shown, they probably represent a poor indicator of the degree of difference in the frequency of recovery of the $X^P Y^L Y^S$ chromosome at the two different temperatures. Consequently, only the classes representing the recovery of the gametic types $X^D + X^P Y^L Y^S$ and $IV + O$ are considered in the calculations included in Table 2. It is clear that in the controls ($26^\circ C$) there is a pronounced deficiency in the B^S female class with frequencies of 17.3 percent and 24.8 percent in Experiments IIIa and IVa respectively. The expected frequency of this class without chromosome loss is 50 percent. In contrast, the results from the experiments at a cooler temperature indicate that under these conditions the gametic type $X^D + X^P Y^L Y^S$ is recovered with virtually normal frequency, i.e. 46.5 percent and 47.5 percent, respectively, in Experiments IIIb and IVb.

An alternative reason which would partially account for the deficiency of the gametic type $X^D + X^P Y^L Y^S$ in the control experiment described above is that the components X^D and IV are not segregating in a 1:1 ratio, those gametes carrying IV being produced with an appreciably greater frequency than those carrying X^D . This is the kind of result reported by NOVITSKI and SANDLER (1957) and ZIMMERING (1960) from Bar-Stone males of the A -type, in which the segregation ratio of $X^D:IV$ was found to be as low as 0.37:0.63.

To determine the segregation ratio of $X^D:IV$, males of the composition of $X^D/IV; X^P Y^L Y^S/O$ were mated with three attached- X females homozygous for $\gamma v f$ and carrying the proximal fragment of the ordinary Bar-Stone translocation, X^P , for a period of two days and then transferred to fresh cultures with three virgin females for an additional two-day period. The males were then discarded. Sperm of the composition $X^D + O$ are recovered upon fertilization of an egg carrying X^P , while a sperm of the composition $IV + O$ is recovered upon fertilization of an egg carrying the attached- X chromosome. The former gives rise to phenotypically B^S males and the latter to phenotypically $\gamma v f$ females. A third class, $\gamma v f B^S$ females, is recovered when a sperm of the composition $IV + X^P Y^L Y^S$ fertilizes an egg carrying the attached- X chromosome. Of particular interest here are the relative rates with which B^S males and $\gamma v f$ females are recovered since they reflect directly the rates with which X^D - and IV -bearing sperm are produced, on the reasonable assumption that the univalent,

TABLE 2

Progeny from the crosses of $X^D, X^P Y^L Y^S/O$ males with free- $X \gamma v f$ females.
These males were raised at $26^\circ C$ or $18^\circ C$ until eclosion and then mated at $26^\circ C$

		Male gametes F_1 phenotypes	$X^D + X^P Y^L Y^S$ $B^S \text{♀}$ (1)	$IV + O$ $\gamma v \text{♂}$ (2)	$IV + X^P Y^L Y^S$ $\gamma v B^S \text{♂}$	$N = (1) + (2)$	(1) (1) + (2)
III.	a.	$26^\circ C$	1,719	5,217	899	6,936	24.8%
	b.	$18^\circ C$	4,310	4,951	1,423	9,261	46.5%
IV.	a.	$26^\circ C$	2,784	13,255	1,622	16,039	17.3%
	b.	$18^\circ C$	4,995	5,506	1,657	10,501	47.5%

TABLE 3

Progeny from the crosses of $X^D, X^{PY^L}Y^S/O$ males with $\gamma v f$ attached-X females carrying X^P of the Bar-Stone translocation

Male gametes F_1 phenotypes	X^D+O $B^S\sigma$ (1)	$IV+O$ $\gamma v f\sigma$ (2)	$IV+X^{PY^L}Y^S$ $\gamma v f B^S\sigma$	$N=(1)+(2)$	Ratio of classes (1):(2)
I.	726	844	270	1,570	0.46:0.54
II.	1,061	1,011	504	2,072	0.51:0.49

$X^{PY^L}Y^S$, is assorting at random with respect to X^D and IV. As may be seen, the ratio of the gametic types $X^D + O:IV + O$ turns out to be 0.46:0.54 in the first run and 0.51:0.49 in the second. Thus, there is no evidence of a significant departure from the expected 1:1 ratio.

Some experimental evidence has been obtained suggesting no marked deviation from the expected random assortment of the univalent with respect to X^D and IV. Matings were made of $X^D, X^{PY^L}Y^S/O$ males with $\gamma v f/Y$ attached-X females. In this case, B^S male offspring represent the recovery of the univalent with X^D and $\gamma v f B^S$ female offspring the recovery of the univalent with IV. A third class, $\gamma v f$ females, represents the recovery of the gametic type $IV + O$. Of 1,735 offspring representing the recovery of the gametic types $X^D + X^{PY^L}Y^S$ and $IV + X^{PY^L}Y^S$, 792 or 45.6 were of the former type and 943 or 54.4 of the latter.

CONCLUSION

Briefly, the following points may be made: (1) In $sc^4-sc^8/sc^8.Y$ males, the $sc^8.Y$ chromosome is lost at a markedly higher rate when such males are raised at 26°C than is the case when males are raised at 18°C. The question arose as to whether this difference was the result of an increase in the frequency of synapsis between X and Y at the cooler temperature; and (2) from the observations on the behavior of the univalent Y chromosome, $X^{PY^L}Y^S$, it was found that (a) there is a marked difference in the frequency of loss of this univalent at the higher and lower temperatures, paralleling the behavior of the $sc^8.Y$, and (b) that in the case of this univalent chromosome, these differences cannot depend upon a change in the frequency of synapsis with a homologue since, in fact, no homologue is present. It follows, then, that no special assumptions about frequencies of synapsis are necessary to account for the difference in the rate of loss of the $sc^8.Y$ at the different temperatures. A suggestion may be proposed that a Y chromosome undergoes a high or low rate of meiotic loss independent of synapsis.

Still puzzling is the finding that the sc^4-sc^8 X chromosome is not lost in the $sc^4-sc^8/sc^8.Y$ male. This is in agreement with the observations made by SANDLER and BRAVER (1954). Yet, the sc^4-sc^8 X chromosome does appear to be lost in $sc^4-sc^8/Y/Y$ males as judged by the fact that the frequency of recovery of XY-bearing gametes is only about one-half that of Y-bearing gametes (SANDLER and BRAVER 1954). Preliminary data (ZIMMERING unpublished) suggests that such XY-bearing gametes are recovered appreciably more frequently when males are

raised at 18°C instead of 26°C. An account of these experiments will be the subject of a later publication.

SUMMARY

When males carrying the sc^1-sc^8 X chromosome and the sc^8 -Y chromosome are raised at 26°C and 18°C, respectively, and then tested at 26°C the sc^8 -Y is lost with a markedly higher frequency at the higher than at the lower temperature. A question posed was whether this difference was the result of an increase in the frequency of synapsis between X and Y at the cooler temperature. Experiments were therefore carried out testing the response to temperature of a modified univalent Y chromosome composed of the proximal fragment of the Bar-Stone translocation to which is appended proximally the equivalent of a Y chromosome. The behavior of this univalent paralleled that of the sc^8 -Y in exhibiting an appreciably higher rate of loss at the higher than at the lower temperature. In the case of the univalent, then, it was concluded that the differences in the rates of loss could obviously not depend upon a change in the frequency of synapsis with a homologue since, indeed, no homologue was present. It followed, then, that an explanation which would account for the observation that the sc^8 -Y was lost with markedly different frequencies at the two temperatures did not require any assumptions about synapsis. The suggestion was made that a Y chromosome undergoes a high or low rate of meiotic loss independent of synapsis.

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