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Tests for an effect of the *Y*-chromosome on the mutagenic action of formaldehyde and X-rays in *Drosophila melanogaster*

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1. INTRODUCTION

Formaldehyde is a highly specific mutagen. Whether injected as aqueous solution into adult flies or added to the medium of the larvae, it is strictly sex-specific, producing mutations only in the males. Experiments by Sobels & Simons (1956) indicate that the injection method acts via the formation of organic peroxides from formaldehyde and metabolically produced hydrogen peroxide: the mutagenic effect in males was enhanced twofold when catalase was poisoned by cyanide gas. In females, pretreatment with cyanide gas yielded a very small percentage of mutations, suggesting that at least part—although probably not all—of the recalcitrance of female germs cells to injected formaldehyde may be due to an excess of catalase (Sobels, 1956).

When formaldehyde is mixed into the food of Drosophila it probably acts in quite a different way. Alderson (1961) has proposed an attractive model according to which the actual mutagen is dimerized adenylate. In any case, the sex difference in response to formaldehyde feeding is so striking (up to 15% sex-linked lethals in males, none beyond control value in females: Herskowitz, 1950; Auerbach & Moser, 1953) that it can hardly be attributed to a difference in catalase content between male and female germs cells. Moreover, there is striking selectivity even within the male germ tract. Only young larval spermatocytes respond to treatment (Auerbach & Moser, 1953), although labelling experiments have shown (Kaplan & Pelc, 1956) that formaldehyde penetrates into all germ-cell stages of larvae and adults that ingest the treated food. Results by Nafei & Auerbach (1964) strongly suggest that the treatment acts only on replicating DNA; Alderson's model fits with this conclusion. This would explain why post-meiotic stages do not respond, but leaves the recalcitrance of spermatogonia unexplained.

Most of these specificities could be explained if some action of the *Y* chromosome were required for the mutagenic effectiveness of formaldehyde-food. The lack of mutations in females would then be readily accounted for. In males the *Y* chromosome is present in all premeiotic stages of the germ cells. However, only in the early premeiotic spermatocytes does it unfold a striking activity, detected cytologically in the formation of lampbrush loops (Hess & Meyer, 1963). This is the stage that responds to formaldehyde-food mutagenesis. It seems possible, therefore, that it is

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not simply the presence of the Y chromosome which is necessary for formaldehyde-food mutagenesis, but a special activity of it is also involved. In order to test the above hypothesis I have compared the effects of formaldehyde-food on males and females with and without an extra Y chromosome.

Previous workers have found no differences between the frequencies of spontaneous sex-linked mutations in XY and XYY males (Kershner, 1949) or in XX and XXY females (Valencia & Valencia, 1951). There is, however, one previous report of the effect of the Y-chromosome on induced mutation frequency. Kershner (1949) observed a significant reduction of X-ray-induced mutation frequencies by an extra Y in mature sperms. A Y-chromosome in the female was without effect. A repeat of this test under similar conditions, but only for males, was made as a comparison to the formaldehyde experiments.

2. MATERIALS AND METHODS

(i) *Sex-linked lethals in* XY *and* XYY *males*

Phenotypically distinguishable XY (mottled-eyed) and XYY (vermilion-eyed) males with the same genetic background were obtained from a v; bw^{VA}/BlL^2 stock (vermilion on the X chromosome; dominant brown variegated on one of the second chromosomes and the dominant markers Bristle and Lobe^2 on the other; bw^{VA} and L^2 act as balanced lethals). An extra Y chromosome obtained through non-disjunction suppresses the mottling effect of bw^{VA} and gives vermilion-eyed flies. This phenotype is also simulated by rare cross-over flies which have lost bw^{VA} and are therefore vermilion-eyed but have no extra Y-chromosome (Kelsall, 1963). When this has happened in a culture bottle, mottled-eyed flies without BlL^2 should also be present. No such flies were detected in the bottles from which the XY and XYY males were collected for the experiments reported in this paper. Even if some XY males had been present among the vermilion-eyed XYY males, they would have been too few to have seriously affected the result.

Both sets of males were treated as first-instar larvae by rearing them on formaldehyde-food (FF) as described by Auerbach & Moser (1953). The emerging males (fifty of each type) were then mated individually to 3–5 $ysc^{81}In49sc^8$ virgin females every 3 days to obtain four broods, and the F_2 of each brood was scored for sex-linked lethals in the usual manner.

For the X-ray experiments 1-day-old XY and XYY males were irradiated with 4000 r (140 KVP, 5 MA) and mated immediately to three $ysc^{81}In49sc^8$ virgin females for 3–4 days only. Kershner (1949) used 4000 r and made pair matings for 3 days. An untreated control was run parallel to the X-ray experiments.

(ii) *Autosomal lethals in attached-*X ($\widehat{XX}$Y) *females*

Attached-$Xywf$ (yellow, white, forked) females were mated to Cy/BlL^2 (Curly balanced against Bristle Lobe^2) males and their larvae were reared on FF as described above. The treated $\widehat{XX}Yywf$; $Cy/+$ females were mated as virgins singly to 2 Cy/BlL^2 males and transferred every 3 days into fresh vials to obtain

four broods. The F_3 of each brood was scored for second chromosome lethals. Treated $Cy/+$ males were mated to M-5 (Muller-5) females in a ratio of 1 to 3 for 3 days only and their sex-linked lethal frequency was scored in the F_2 to obtain a measure of the effectiveness of the treatment.

3. RESULTS

(i) *Effect of* Y *on* FF *mutagenesis*

(*a*) *Males.* The results of two experiments on FF-induced sex-linked lethal frequencies in the four broods of XY and XYY males are summarized in Table 1. Both experiments show the typical brood pattern for FF-induced sex-linked lethals. Neither experiment showed an effect of the extra Y on mutation frequency.

Table 1. *Effect of larval feeding of formaldehyde (0·18 %) on the sex-linked lethal frequency in* XY *and* XYY *males of* Drosophila melanogaster

		Type of male					
		XY			XYY		
Brood	Days	n	l	% l	n	l	% l
		Experiment I					
A	0–3	211	14	6·64	260	17	6·54
B	4–7	376	8	2·13	331	7	2·11
C	8–11	271	6	2·21*	432	4	0·93*
D	12–15	527	3†	0·57	539	1	0·19
		Experiment II					
A	0–3	582	55	9·45	515	49	9·51
B	4–7	641	27	4·21	607	29	4·77
C	8–11	673	10	1·48	712	10	1·40
D	12–15	591	4	0·68	652	6	0·92

n, Number of chromosomes tested; l, number of lethals.

* $\chi^2_{(1)}$ for difference in lethal frequency between XY and XYY males in brood C of Expt I = 0·116 (with Yates Corr.).

† Cluster from one male.

(*b*) *Females.* Auerbach & Moser (1953) had found no lethals in 366 second chromosomes from FF-treated females without an extra Y. In a pilot experiment on $\widehat{XX}Y$ females reared on FF (0·18 % formaldehyde), 0·8 % autosomal lethals occurred in 705 chromosomes spread over four broods, compared to a sex-linked lethal frequency of 6·6 % in the first brood of treated males. The spontaneous mutation frequency on the second chromosome is about 0·6 %. In a second experiment, with untreated $\widehat{XX}Y$ females as the control, the lack of effect of the Y in females was confirmed (Table 2).

(ii) *Effect of* Y *on X-ray mutagenesis in the male*

The results of two X-ray experiments with an unirradiated control are summarized in Table 3 along with Kershner's (1949) data. Her finding that an extra Y

significantly *decreased* the sex-linked frequency could not be confirmed. On the contrary, in both experiments, there was an *increase* of mutation frequency in *XYY* males, but it was not statistically significant in either.

Table 2. *Effect of larval feeding of formaldehyde (0·20 %) on the autosomal lethal frequency in attached-*X (X$\hat{\text{X}}$Y) *females of* Drosophila melanogaster

		Treated			Control		
Broods	Days	*n*	*l*	% *l*	*n*	*l*	% *l*
A	0–3	146	1	0·68	188	2	1·06
B	4–7	173	2	1·10	151	1	0·66
C	8–11	225	2	0·89	126	1	0·79
D	12–15	220	2	0·91	203	3	1·47
Total		772	7	0·91	668	7	1·05
Sex-linked lethals in males (brood A)		186	16	8·6	871	4	0·46

n, Number of chromosomes tested; *l*, number of lethals.

Table 3. *Effect of X-rays (4000 r) on the sex-linked lethal frequency in* XY *and* XYY *males of* Drosophila melanogaster

		Experiments		Unirradiated control	Kershner (1949)
		I	II		
XY males	*n*	130	239	1023	286
	l	16	20	5	35
	% *l*	12·30	8·32	0·48	12·24
XYY males	*n*	174	258	1048	344
	l	25	31	4	22
	% *l*	14·37	12·01	0·38	6·40
$\chi^2_{(1)}$ test for effect of extra *Y*		0·27	1·79	—	6·4
		$P > 0{\cdot}50$	$> 0{\cdot}10$	—	$< 0{\cdot}014$

n, Number of chromosomes tested; *l*, number of lethals.

4. DISCUSSION

The experiments on females show that their refractoriness to FF mutagenesis cannot be due to lack of a *Y*-chromosome. The experiments on males do not allow such a clear conclusion to be drawn. It is true that in spermatocyte nuclei two *Y*-chromosomes produce twice as many lampbrush loops as does one (Hess, 1966), but for formaldehyde–food mutagenesis there might be a threshold effect which is reached already by one *Y*. It is also possible that the behaviour of the *Y* in early spermatocytes is an expression of a unique metabolic condition which independently promotes FF mutagenesis.

The lack of confirmation of Kershner's (1949) results might be due to differences

in the strains used or in other experimental conditions. In any case the above experiments show that an effect of the *Y*-chromosme on X-ray mutagenesis is not a general phenomenon.

5. SUMMARY

An additional *Y*-chromosome in either male or female did not have an influence on the mutagenic action of formaldehyde–food. It is concluded that the lack of response of female germs cells to this treatment is not due to lack of a *Y*-chromosome, and that the specific response of spermatocytes among germ cells is probably not due to the striking activity of the *Y* in these cells.

Kershner's (1949) observation that the sex-linked lethal frequency in X-rayed males was significantly reduced by an additional *Y*-chromosome could not be confirmed.

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