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CHROMOSOMAL BASIS OF DOSAGE COMPENSATION IN *DROSOPHILA*

1. HYPERACTIVITY OF THE POLYTENE X-CHROMOSOME IN MALE *DROSOPHILA KIKKAWAI* AND *DROSOPHILA BIPECTINATA*[1]

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FOUR TEXT-FIGURES

ABSTRACT

The functional morphology and the transcriptive activity of the male X-chromosome in *Drosophila kikkawai* and *D. bipectinata* have been examined. In both these species of the *melanogaster* species-group, the X-chromosome in the salivary glands of the male larvae is enlarged and pale stained as in *Drosophila melanogaster*. ^{3}H-uridine autoradiography shows that in both the species, the relative rate of RNA synthesis by the single X of the male is similar to that by the two X's of female.

The results indicate that: (*a*) the enlargement and pale staining of the single X in the larval salivary glands of the male is of general occurrence in the genus *Drosophila*, and (*b*) despite the changes in the configuration and organization of the X-chromosome in these species (that have taken place during their evolution), the hyperactivity of the male X, and therefore, dosage compensation for X-linked genes, has remained unchanged.

INTRODUCTION

In the course of evolution in the genus *Drosophila* there have been many chromosomal rearrangements involving the sex-chromosomes. While in some cases merely the configuration of the original X-chromosome is changed, in several instances such rearrangements have modified the sex-determining mechanism (Sturtevant and Novitski, 1941; Patterson and Stone, 1952). Previous studies (Mukherjee, 1966; Mukherjee *et al.*, 1968, 1969; Lakhotia and Mukherjee, 1969, 1970; Lakhotia, 1970, 1971) have indicated that in *Drosophila melanogaster*, dosage compensation operates by hyperactivity of the single X in the male, that this hyperactivity shows a cellular autonomy in sex mosaics, that it is functionally related to the replicative organization of the male X, and finally, that the hyperactivity of the male X is not a 'position effect' (Lakhotia,

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1970). In view of these findings, a cytological and gene-physiological study of dosage compensation in different species of *Drosophila* should be obligatory for a better understanding of the chromosomal basis of dosage compensation in this genus.

In the present work, the functional morphology and transcriptive activity of the male and female polytene X-chromosomes in *D. kikkawai* Burla, 1954, and *D. bipectinata* Duda, 1923, are presented. These two species have been selected because in the course of evolution of these species the original X-chromosome has undergone reorganization (Patterson and Stone, 1952). In *D. kikkawai* the X is acrocentric like that in *D. melanogaster* but the proximal heterochromatic segment of the original *melanogaster*-type X has been translocated to the 4th in *kikkawai*, while in *D. bipectinata* in addition to this translocation of the basal X-heterochromatin to the 4th, a pericentric inversion has occurred in the X, producing a submedian centromere in the X-chromosome (Patterson and Stone, 1952). The present work aims at showing that in these *Drosophila* species too, dosage compensation operates by X-hyperactivity in the male and that despite the evolutionary reorganization of the X, there are no disturbances in dosage compensation mechanism.

MATERIAL AND METHOD

Functional morphology:

Wild strains of *Drosophila kikkawai* (from Brazil) and *Drosophila bipectinata* (from Calcutta) were used for these studies. The flies and larvae were reared in the standard *Drosophila* food at 24° ± 1°C. The salivary glands from their late third instar larvae were dissected out in *Drosophila*-Ringer at pH 7·0 (prepared after Berendes *et al.*, 1963) and squash preparations made following the usual technique (Lakhotia and Mukherjee, 1969). From the preparations, the widths of the X-chromosome and a particular autosome (for details see under OBSERVATIONS) in the male and female of these two species were measured by the technique described earlier by us (Mukherjee *et al.*, 1968; Lakhotia and Mukherjee, 1969) and the autosome/X-chromosome width ratios calculated to examine the enlargement of the single X in the male.

^{3}H-uridine labelling:

For ^{3}H-uridine labelling the excised salivary glands from the late third instar male and female larvae were incubated in *Drosophila*-Ringer containing ^{3}H-uridine (100 μCi/ml; Sp. Act. 5·6 Ci/mM, obtained from Bhabha Atomic Research Centre, Trombay) for 10 minutes. The preparations were processed for autoradiography using Kodak AR 10 Stripping Film. The autoradiographic processings were the same as described earlier by us (Lakhotia and Mukherjee, 1969). Exposure time was 20 days.

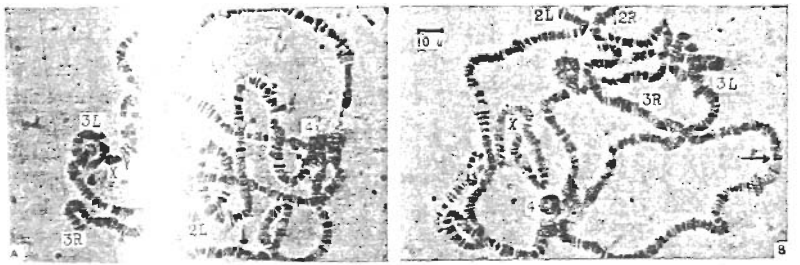
OBSERVATIONS

FUNCTIONAL M[illegible]OLOGY OF THE POLYTENE X-CHROMOSOME IN MALE AND FEMALE:

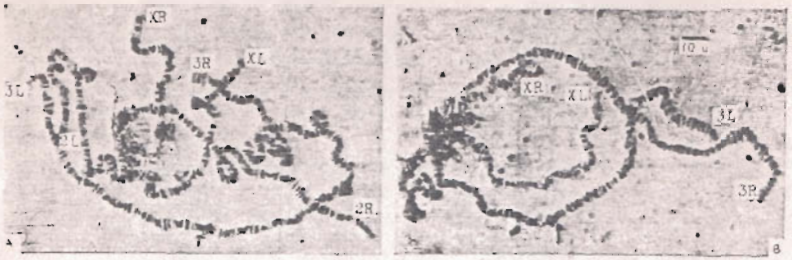
A detailed d[illegible]ption of the salivary gland chromosomes of *D. kikkawai* and *D. bipectinata* [illegible]quired for the present work, is not available from the literature; as such [illegible]different chromosomes had to be provisionally identified before undert[illegible] the studies from the point of view of dosage compensation.

Drosophila [illegible]awai: This species is a close relative of *D. montium* de Meijere, 191[illegible] Burla, 1954). Kikkawa (1936) found that in *D. montium* salivary gland[illegible]i, six strands branch off from the chromocentre and the largely heter[illegible]matic and shortest arm remains attached at its tip also to the chromoce[illegible]. In *D. kikkawai* (Text-fig. 1) it was observed that there are five long and [illegible]mall heterochromatic arms. The small arm often assumes a 'U'-shaped [illegible]ration due to its both ends sticking into the chromocentre. This smalles[illegible] probably represents the 4th chromosome. Among the five longer arms[illegible] presents the X (acrocentric in metaphase, Patterson and Stone, 1952) [illegible]the remaining four represent two arms of each of the two metacentric [illegible]es. The X was identified in the male by its pale staining and then hi[illegible]d in the female by comparing the banding pattern. The largest autos[illegible]m has been termed 3R; it is probably homologous to the longest 'elem[illegible] of Sturtevant and Novitski (1941). The autosome arm which was s[illegible]st often associated with the 3R upon its separation from the common [illegible]nocentre, was termed 3L and the other two arms as 2L and 2R.

The chron[illegible]d arm 3R is easily recognized in the salivary gland nuclei by its longes[illegible]and by the presence of two adjacent puffs of characteristic appearance [illegible]fig. 1: the puffs are just proximal to the arrow on 3R). This arm ha[illegible] selected for comparing the width of the autosome and the X-chromoson[illegible] the male and female.



TEXT-FIGURE 1. [illegible]romicrographs of polytene nuclei in larval salivary glands of female (A) and male (B) *Drosophila kikkawai*.
Different chrom[illegible]e arms are indicated by the provisional nomenclature (see text). The two characte[illegible] puffs on 3R are just proximal to the dark band indicated by arrow.



Text-figure 2.—Photomicrographs of polytene nuclei in larval salivary glands of female (**A**) and male (**B**) *Drosophila bipectinata*.
Different chromosome arms are identified by the provisional nomenclature (see text). The two characteristic puffs on 3L are just proximal to the dark band indicated by arrow.

The data presented in Table 1 on the relative width of the X-chromosome in the male and female (shown as 3R/X width ratios) make it clear that as in *D. melanogaster*, the single X in the male *D. kikkawai* is also nearly as wide as the two X's in the female or as the paired autosomes (Text-fig. 1B). As in *D. melanogaster* (Lakhotia and Mukherjee, 1969), the autosome/X width ratios in the male and female *D. kikkawai* are close to 1·0.

Drosophila bipectinata: The chromosome complement of *D. bipectinata* (Text-fig. 2) is very similar to that of *D. ananassae* (Patterson and Stone, 1952). In salivary glands, the X-chromosome shows two comparatively short arms—XL and XR, XR being the smaller of the two. There are four large autosomal arms representing the two metacentric autosomes. As in *D. kikkawai*, the longest autosomal arm has been termed 3R and the arm most often associated with 3R as the 3L; the other two autosomal arms have been provisionally identified as 2L and 2R respectively. A 4th chromosome could not be positively identified. As in *D. ananassae* (Dutta Gupta, 1969), in *D. bipectinata* both XL and XR frequently show (one on each arm) large interstitially situated heterochromatic blocks. In this species, the 3L arm was taken as the reference autosome since this could be easily identified by the two adjacent puffs situated at the proximal one-third of this arm (Text-fig. 2: the puffs are just proximal to the arrow on 3L).

Both the arms of the X-chromosome (XL and XR) in *D. bipectinata* exhibit light staining and enlarged width in ths male (Text-fig. 2B). The 3L/X chromosomal width ratios (3L/XR and 3L/XL) in the male and female presented in Table 1 show that they are very close to 1·0.

RNA synthesis by X-chromosome in male and female salivary gland in D. kikkawai and D.bipectinata:

^{3}H-uridine autoradiography was carried out in these two species to examine

Table 1.—*Autosome-to-X-chromosome width ratio in polytene nuclei of male and female nuclei of* Drosophila kikkawai *and* D. bipectinata.

Species	Mean chromosomal width ratios	
Drosophila kikkawai	3R/X	
Female	1·01 ± 0·02 (20)*	
Male	1·08 ± 0·03 (20)	
Drosophila bipectinata	3L/XL	3L/XR
Female	1·02 ± 0·02 (20)	1·00 ± 0·03 (20)
Male	1·05 ± 0·02 (25)	1·04 ± 0·02 (25)

*Figures in parentheses indicate the number of nuclei measured.

the relative rate of RNA synthesis by the X-chromosome in the male and female salivary glands of late third instar larvae.

In *D. kikkawai* the total number of silver grains present on the entire X-chromosome was scored. A part of the 3R was also selected for grain counting; this region extended from the dark band immediately following the two characteristic puffs on this arm (pointed out by arrow in Text-fig. 1) to its tip. The absolute grain numbers scored on the X and the 3R in the male and female nuclei and the means of ratios of silver grains on the 3R and that on the X of a nucleus are presented in the Table 2. A direct comparison of the absolute grain numbers in the male and female has not been made since the general degree of labelling is different in the two sexes (as judged by differences in the labelling intensity on the 3R segment in the male and female nuclei, see Table 2); labelling in the male nuclei seems to be slightly higher than in the female nuclei observed. However, a comparison of the 3R/X ^{3}H-uridine grain ratios in the male and female nuclei examined shows that the relative incorporation of ^{3}H-uridine in the single X of the male and the two X's of the female is very similar (male 3R/X grain ratio = 0·63; female 3R/X grain ratio = 0·61; $P > 0·8$; see Table 2).

A similar analysis of the grain numbers has been made in *D. bipectinata*. The total number of grains present on the XL and XR and that on a region of an autosomal arm (3L) were scored in the male and female. The region on the 3L extended from the dark band immediately following the two puffs on the proximal part of 3L (indicated in Text-fig. 2 by arrow on the 3L) to the tip. Table 2 shows the mean numbers of grains recorded on the XL, XR and 3L and the mean 3L/XL and 3L/XR grain ratios in the two sexes. In

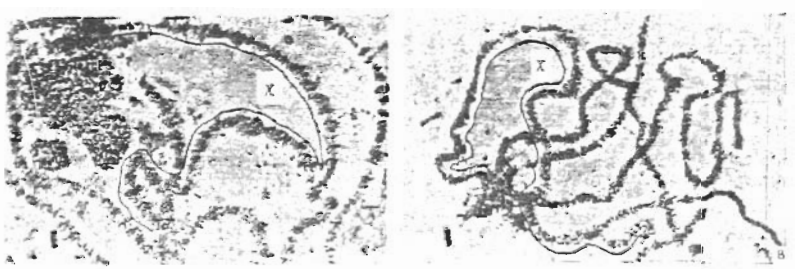
TABLE 2.—*^{3}H-uridine incorporation in male and female salivary gland nuclei of* Drosophila kikkawai *and* D. bipectinata.

Sex	Total no. of grains on		Mean grain ratio
	X	3R	3R/X
Drosophila kikkawai			
Female	233 ± 22 (16)[a]	141 ± 13 (16)	0·61 ± 0·017
Male	293 ± 33 (18)	195 ± 24 (18)	0·65 ± 0·016
't'			0·18
P>			0·30

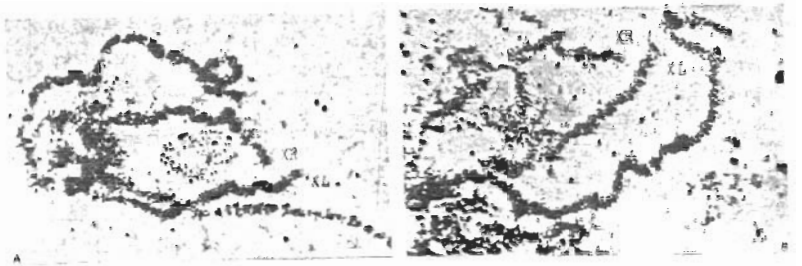
Drosophila bipectinata	XL	XR	3L	3L/XL	3L/XR
Female	242 ± 25 (11)	162 ± 27 (11)	257 ± 27 (11)	1·05 ± 0·03	1·56 ± 0·07
Male	115 ± 19 (12)	72 ± 12 (12)	113 ± 22 (14)	0·98 ± 0·04	1·62 ± 0·06
't'				0·40	0·20
P>				0·60	0·80

[a]Figures in parentheses indicate the number of chromosomes examined.

this case too, a direct comparison of the mean numbers of grains on XL and XR in the male and female nuclei could not be made due to a very high difference in the labelling intensity in the male and female nuclei analysed (compare the grain numbers on 3L in the male and female in Table 2). It



TEXT-FIGURE 3.—Photomicrographs of ^{3}H-uridine labelling of polytene chromosomes of female (**A**) and male (**B**) *Drosophila kikkawai*.
The solid line indicates the orientation of the X-chromosome.



TEXT-FIGURE 4.—Photomicrographs of ^{3}H-uridine labelling of polytene chromosomes of female (**A**) and male (**B**) *Drosophila bipectinata*.

may be pointed out here that the number of nuclei examined for ^{3}H-uridine grain counting in the two sexes in this species is rather low. The main reason for this is the fact that in *D. bipectinata*, very few nuclei in any squash preparation remain intact to allow a comparison of the labelling on a particular autosome and the X-chromosomal arms of a nucleus. Nevertheless, a comparison of the mean 3L/X grain ratios (3L/XL and 3L/XR) in the male and female nuclei shows that the relative uptake of ^{3}H-uridine by the single X in the male and the two X's in the female is similar. The 3L/XL and 3L/XR grain ratios in the male and female are not significantly different (P>0·6).

Text-figures 3 and 4 present photomicrographs of ^{3}H-uridine autoradiograms of the salivary gland chromosomes of the male and female *D. kikkawai* and *D. bipectinata* respectively. In both the species, the relative incorporation of ^{3}H-uridine is similar for the X-chromosome and the autosomes in the two sexes.

DISCUSSION

In both *D. kikkawai* and *D. bipectinata*, the single X-chromosome in the salivary glands of the male larvae is seen to be pale stained and nearly as wide as the paired chromosomes in the nucleus. Pale staining and the enlargement of the single X in the male glands of many other *Drosophila* species and several other Diptera have been reported earlier (Dobzhansky, 1957; Cordeiro and Winge, 1964; Berendes, 1966; Roberts *et al.*, 1967; Mukherjee *et al.*, 1968; Lakhotia and Mukherjee, 1969). Thus, it seems that the enlargement and pale staining of the male X in polytene nuclei are of general occurrence in the genus *Drosophila* and possibly also in other Diptera, and in view of the evidence presented earlier (Lakhotia and Mukherjee, 1969, 1970; Lakhotia, 1970) this enlargement of the male X may be considered to be related to the dosage compensation mechanism.

The rate of RNA synthesis on the X-chromosome in the male and female, as judged by ^{3}H-uridine autoradiography, has been found to be equal in

D. melanogaster (Mukherjee, 1966; Lakhotia and Mukherjee, 1969; Kaplan and Plaut, 1968) and in *D. hydei* (Mukherjee *et al.*, 1968, 1969). The present results extend this observation to *D. kikkawai* and *D. bipectinata*. As mentioned in the introduction, *Drosophila melanogaster*, *D. kikkawai* and *D. bipectinata* differ in the configuration of their X-chromosomes. In the origin of the X's of *D. bipectinata* and *D. kikkawai* no new autosomal region (at least not major) is believed to have been involved (Patterson and Stone, 1952). Since the dosage compensation in *Drosophila* is not a 'position effect' as in mammals (Lakhotia, 1970*a*, *b*), it is to be expected that the evolutionary rearrangements that have led to the origin of the reorganized X in these species would not disturb the piecemeal regulatory system of dosage compensation in *Drosophila*. The single X in the males of *D. kikkawai* and *D. bipectinata* seems to be as hyperactive as in *D. melanogaster*. Unfortunately, no genetic studies are available in these species to demonstrate the existence of dosage compensation at phenotypic level. The results obtained here suggest that the X chromosome in the male and female larval salivary glands in *D. kikkawai* and *D. bipectinata* is compensated with respect to RNA synthesis, and comparing with the situation in *D. melanogaster* (Lakhotia and Mukherjee, 1969, 1970; Korge, 1970; Lakhotia, 1971) this would indicate that at phenotypic level too, there would be, in general, a full compensation in these *Drosophila* species.

More detailed studies on different aspects of dosage compensation in these different species of *Drosophila* would be very important to have a better understanding of the chromosomal basis of dosage compensation mechanism. First, extensive genetic studies in these species are required to show that as at chromosomal level, there is a compensation at the phenotypic level too. Secondly, at the chromosomal level, a locus- or puff-wise analysis of RNA synthesis is necessary to show that all the loci on the X are equally activated in the two sexes. Thirdly, an examination of the activity of the heterochromatic part of the original X, which now has become translocated to the 4th chromosome, is necessary to find out the dosage relationships of the genetic factors located in this region; thus the *bb* locus is represented in *D. bipectinata* and *D. ananassae* by three doses in the male, but only by two doses in the female (Kaufmann, 1936; Kikkawa, 1938; Patterson and Stone, 1952; Atwood, 1969). And finally, a study of the dosage compensation at chromosomal level in *D. pseudoobscura* and *D. miranda*, with partial dosage compensation at genetic level (Muller, 1950) is expected to provide a better and clear idea of the dosage compensation mechanism in *Drosophila*.

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