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SHORT PAPER

Reduction of wild-type *X* chromosomes with the Y^{bb-} chromosome of *Drosophila melanogaster*

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SUMMARY

The Y^{bb-} chromosome has been previously shown to induce reduction of *X* chromosome ribosomal genes in X^{bb}/Y^{bb-} or X^{bb+}/Y^{bb-} flies. These reduction events are presumed to arise as one of the two products of unequal sister chromatid exchanges, which result in both magnified and reduced products. Bobbed reduced chromosomes may also arise as products of other recombinative events such as intrachromatid deletions. In this report we use the Y^{bb-} chromosome to reduce the number of ribosomal genes present on *X* chromosomes from two wild-type stocks under 'non-magnifying' conditions. We then show that the bobbed reduced *X* chromosomes show no detectable difference in their Southern blot rDNA patterns when compared with the parental wild-type *X* chromosome. This indicates that reduction events do not preferentially delete certain repeat classes, and supports previous observations that the repeat types present in the *D. melanogaster X* chromosome nucleolus organizer are not significantly clustered.

1. INTRODUCTION

Reductions in the number of *X* chromosome ribosomal genes have been observed to occur in the germline of X^{bb}/Y^{bb-} and X^{bb+}/Y^{bb-} males (Locker & Prud'homme, 1973; Tartof, 1974), although reduction is not observed in males which do not carry Y^{bb-}. Flies bearing a reduced number of ribosomal genes are thought to arise as one of the two products of an unequal sister chromatid exchange event (Tartof, 1974). In addition to arising as a reciprocal product of unequal sister chromatid exchange, bobbed reduced chromosomes may also arise as products of other recombinative events such as intrachromatid deletions.

We have examined two laboratory strains of *Drosophila melanogaster* which were derived from single pair matings in 1977 and 1979 (Endow & Glover, 1979; Endow, 1980) for the presence of X^{bb} chromosomes and have found none. This suggests that the starting pairs did not contain bobbed *X* chromosomes and that the chromosomes are stable in the isolated stocks. We do observe, however, that reduction of X^{bb+} chromosomes can

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occur with significant and reproducible frequencies if the X chromosome is first associated with the Y^{bb-} chromosome. The Y^{bb-} chromosome contains a large deletion of Y^S which includes almost the entire nucleolus organizer region, and possibly other genetic loci (Bridges & Brehme, 1944). The Y^{bb-} chromosome exhibits several unusual genetic properties including the ability to induce a rapid reversion of X^{bb} mutants to wild-type or near wild-type when maintained in X^{bb}/Y^{bb-} flies over a period of several generations (Ritossa, 1968; Ritossa *et al.* 1971; Tartof, 1973), and the ability to induce a disproportionate replication of ribosomal genes in an X or X^{bb} chromosome (Tartof, 1973). We have examined the ribosomal genes present on three independently isolated reduced X^{bb} chromosomes using Southern blot analysis, in order to determine whether differences between the parental wild-type chromosome and the reduced chromosomes can be detected. Differences in rDNA blot pattern might arise as a result of deletion endpoint 'hotspots' within the ribosomal gene cluster or as a result of clustering of genes with the same spacer or intron length. Our results show no detectable difference between the wild-type and reduced chromosome blot patterns, suggesting that reduction does not specifically delete a particular set or class of ribosomal gene repeats.

2. MATERIALS AND METHODS

(i) Drosophila *stocks and crosses*

Wild-type stocks of *Drosophila melanogaster* OK-1 and Ore R A have been described previously (Endow & Glover, 1979; Endow, 1980). Stocks of $y\ car/B^S Y^{bb-}$ and $w\ sn/Y^{bb-}$ were obtained from the laboratories of Drs K. Atwood and F. Ritossa, respectively. The $B^S Y^{bb-}$ chromosome was constructed by Dr D. Komma as described in Endow (1982*a*) and was maintained with the Ore R A X chromosome. Wild-type OK-1 or Ore R A X chromosomes were tested for bobbed as X/O males or $X/\underline{X}_{NO}$ females before or after association with the $B^S Y^{bb-}$ or Y^{bb-} chromosome. X/O flies were generated by mating X/Y males to $\widehat{XX}/O$ females carrying the $C(1)RM$, $y^2\ w^a$ chromosome, while $X/\underline{X}_{NO}$ females were obtained by mating X/Y males to $In(1)sc^{4L,8R}$, $y\ cv\ v\ B/In(1)dl\ 49$, $y\ Hw\ mg^4$ females. X/O or $X/\underline{X}_{NO}$ progeny were scored for bobbed reduced alleles based on bristle length, delay in time of emergence and abdominal etching. Reduced *bb* alleles found in $X/\underline{X}_{NO}$ females were stocked as homozygous X^{bb}/X^{bb} females together with $X^{bb}/B^S Y$ males. This was accomplished by crossing the initial $X/\underline{X}_{NO}$ females to $\underline{X}_{NO}/B^S Y$ males as a test cross to ensure *bb* phenotype. $X^{bb}/\underline{X}_{NO}$ daughters were then crossed to $X^{bb}/B^S Y$ sons and the X^{bb}/X^{bb} daughters and $X^{bb}/B^S Y$ sons were used to establish stocks. Several such stocks were established for each *bb* mutant and followed for several generations to test for the appearance of *cv* or *v* males which would indicate a double cross-over between the X^{bb} and $\underline{X}_{NO}$ chromosomes. Three stocks which corresponded to independent reduced *bb* alleles and which did not segregate *cv* or *v* were kept for this study.

(ii) *Southern transfers*

Analysis of rDNA patterns by Southern blot analysis was as described previously (Endow & Glover, 1979). Briefly, DNA from diploid tissue of five female individuals from each of three X^{bb} reduced stocks was prepared, digested with Eco RI restriction endonuclease, fractionated on 0·8% agarose gels and blotted on to nitrocellulose. Hybridization was with the gel-purified 11·5 kb rDNA insert from pDm r·a51 ♯ 1 (Dawid, Wellauer & Long, 1978; Endow, 1982*b*).

(iii) *Quantitative hybridization*

Quantitative hybridization experiments were carried out using the dot blot method (Kafatos, Jones & Efstratiadis, 1979) and co-hybridization with the ^{3}H-labelled 11·5 kb

Table 1. *Frequency of* X^{bb} *chromosomes before and after association with the* $B^{S}Y^{bb-}$ *or* Y^{bb-} *chromosome*

I. X^{OK} chromosome; results of crossing males of the indicated genotype to $C(1)RM$, $y^2\ w^a/O$ females

		Progeny					
Parental genotype	Type of cross	y^2w^a ♀♀	$y^2w^aB^S$ ♀♀	bb^+ ♂♂	B^S ♂♂	bb ♂♂	Frequency of bb ♂♂ (%)
X^{OK}/Y	Single pairs	932	0	1024	0	0	< 0·10
X^{OK}/Y	Mass	567	0	635	0	0	< 0·16
X^{OK}/B^SY^{bb-}	Single pairs	1	817	858	1	3	0·35
X^{OK}/Y^{bb-}	Single pairs	440	0	559	0	0	< 0·18

II. X^{Or} chromosome

A. Results of crossing males of the indicated genotype to $C(1)RM$, $y^2\ w^a/O$ females

		Progeny					
Parental genotype	Type of cross	y^2w^a ♀♀	$y^2w^aB^S$ ♀♀	bb^+ ♂♂	B^S ♂♂	bb ♂♂	Frequency of bb ♂♂ (%)
X^{Or}/Y	Single pairs	582	0	852	0	0	< 0·12
X^{Or}/Y	Mass	435	0	614	0	0	< 0·16
X^{Or}/B^SY^{bb-}	Single pairs	1	667	834	1	3	0·36
X^{Or}/Y^{bb-}	Single pairs	472	0	617	0	1	0·16

B. Results of crossing males of the indicated genotypes to $In(1)sc^{4L,sR}$, $y\ cv\ v\ B/In(1)dl\ 49$, $y\ Hw\ mg^4$ females

		Progeny				
Parental genotype	Type of cross	bb^+B ♀♀	bbB ♀♀	bb^+B ♂♂	bbB ♂♂	Frequency of bb ♀♀ (%)
X^{Or}/Y	Mass	1830	0	849	0	< 0·05
X^{Or}/B^SY^{bb-}	Mass	427	6	0	0	1·4

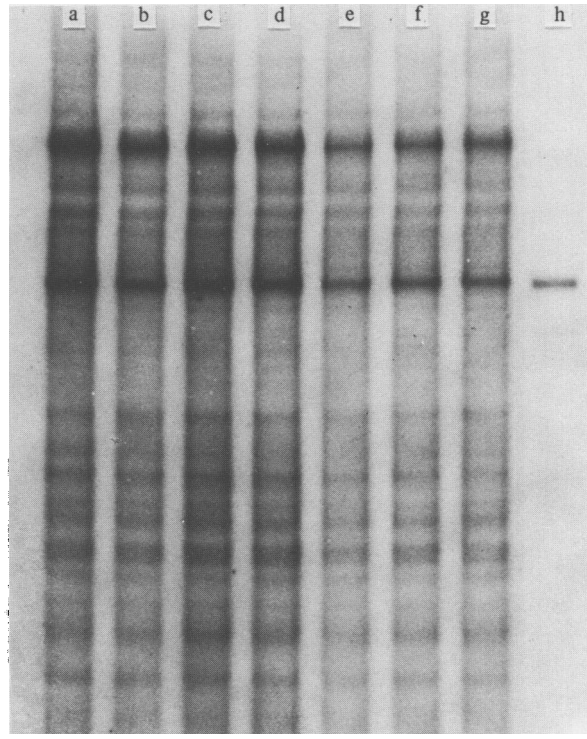
insert from pDm r·a51 # 1 and the ^{32}P-labelled insert from a cloned *Drosophila* actin gene (a gift of E. Fyrberg), as described previously (Endow, 1983). The actin gene insert corresponded to the 1·8 kb + 1·6 kb actin-coding sequences from the gene at 5C on the *X* chromosome. DNA samples were prepared from diploid tissue (larval brains and imaginal disks) from homozygous X^{bb}/X^{bb} females which had been derived from the wild-type Oregon R *X* chromosome. Hybridization to X^{bb}/X^{bb} DNA samples was compared with hybridization to DNA from diploid tissue of wild-type *X*/*X* Oregon R females. Hybridization of the actin gene probe was used to determine the amount of DNA present on the filter. After hybridization samples were autoradiographed, dots of ^{32}P hybridization were cut out, avoiding regions of non-specific ^{32}P binding to the filter, and then counted in toluene fluor for ^{3}H and ^{32}P. Samples were hybridized in triplicate in three or four separate experiments.

3. RESULTS AND DISCUSSION

X chromosomes from two wild-type strains of *D. melanogaster* were tested for bobbed reduced before or after association with the $B^S Y^{bb-}$ or Y^{bb-} chromosomes. Control crosses in which the *X* chromosomes had not been associated with the $B^S Y^{bb-}$ or Y^{bb-} chromosome resulted in the detection of no bobbed flies after scoring a total of 1659 *X*/*O* flies carrying the OK-1 *X* chromosome, 1466 *X*/*O* flies carrying the Ore R A *X* chromosome or 1830 $X/\underline{X}_{NO}$ flies carrying the Ore R A *X* chromosome (Table 1). After association with the $B^S Y^{bb-}$ chromosome, however, the frequency of bobbed *X*/*O* males was found to be 0·35 % for the OK-1 *X* chromosome. Similarly, the frequency of bobbed reduced flies was 0·36 % for the Ore R A *X* chromosome in one experiment and 1·4 % in a second experiment (Table 1). The higher frequency of reduction observed in the last experiment may be a consequence of the reduced penetrance of *bb* alleles in X^{bb}/O males compared with X^{bb}/X^{bbl} females (Lindsley & Grell, 1968). The OK-1 and Ore R A *X* chromosomes were also tested after association with the Y^{bb-} chromosome. This resulted in the finding of 0·16 % bobbed reduced flies for the Ore R A *X* chromosome and no bobbed reduced fly for the OK-1 *X* chromosome out of 559 flies scored (Table 1). These numbers are not statistically significant.

These genetic data demonstrate that a low but reproducible number of bobbed reduced chromosomes arise from two wild-type *X* chromosomes after association with the $B^S Y^{bb-}$ chromosome. The Y^{bb-} chromosome may also induce the formation of *X* bobbed reduced chromosomes, perhaps at a lower frequency than the $B^S Y^{bb-}$ chromosome.

The rDNA patterns for three independently isolated bobbed reduced *X* chromosomes were examined by Southern blot analysis of females which were homozygous for each X^{bb} chromosome. The X^{bb} chromosomes carried by these females were confirmed as bobbed reduced by phenotype when heterozygous with the $In(1)sc^{4L,8R}$ chromosome which is $\underline{X}_{NO}$, including delay in time of emergence relative to bb^+ males from the same vial and, in two cases, abdominal etching. In addition, thoracic bristle length measurements were carried out for one of the three *bb* reduced alleles, bb^{72}, and the number of ribosomal genes present in the bb^{72} chromosome was estimated by quantitative hybridization. Scutellar bristle length was found to be reduced by 50 % in $bb^{72}/In(1)sc^{4L,8R}$ females (192 ± 18 μm) compared with $bb^+/In(1)sc^{4L,8R}$ females (380 ± 8 μm). This is consistent with a 30–40 % reduction in the ribosomal gene content compared with the parental *X* chromosome. In one experiment DNA from bb^{72}/bb^{72} diploid tissue showed 72·9 % of the rDNA hybridization observed for Ore R *X*/*X* diploid DNA (bb^{72} [^{3}H]rDNA/[^{32}P]actin = 9·67 ± 0·18 cf Ore R [^{3}H]rDNA/[^{32}P]actin = 13·26 ± 0·39, $n = 3$); in a second experiment, rDNA hybridization to bb^{72} DNA was 57·0 % of that observed for Ore R DNA (bb^{72} [^{3}H]rDNA/[^{32}P]actin = 4·05 ± 0·12 cf Ore R [^{3}H]rDNA/[^{32}P]actin = 7·11 ± 0·32, $n = 3$). The rDNA content of the other two less severely reduced *bb* alleles could not be accurately determined using the small DNA



Ribosomal gene patterns for parental and *bb* reduced chromosomes. Southern blot analysis of DNA from brains and imaginal discs of Ore R A X/X female larvae (lanes a, b) or bb^{72}/bb^{72} female larvae (lanes c–g). DNA was prepared from tissue of individual larvae, digested with Eco RI restriction enzyme, fractionated on a 0·8% agarose gel and blotted on to nitrocellulose filter paper. Hybridization was with the gel-purified 11·5 kb rDNA insert from pDm r·a51 ♯ 1 (Dawid *et al.* 1978; Endow, 1982*b*), which is also present in lane h.

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samples and methods used here. DNA from diploid tissue of five X^{bb}/X^{bb} females homozygous for each of the three X^{bb} reduced alleles was examined by Southern blot analysis. The Southern blot patterns after digestion of total DNA from diploid tissue with Eco RI were indistinguishable from the Eco RI rDNA pattern for the parental Ore R A *X* chromosome (Plate 1). No difference in either the rDNA fragment pattern or the relative molar amounts of the rDNA bands present in the blot patterns could be detected. This suggests that specific classes of ribosomal gene repeats are not preferentially deleted in rDNA reductions mediated by the Y^{bb-} chromosome. A similar conclusion for spontaneously occurring *bb* alleles was obtained by Tartof & Dawid (1976). No change in the Southern blot pattern was observed for the bb^{72} reduced allele, which is deleted for 30–40 % of the parental rDNA genes. This supports the conclusion based on other lines of evidence (Tartof & Dawid, 1976; Wellauer & Dawid, 1977; Pellegrini, Manning & Davidson, 1977; Hawley & Tartof, 1983) that intron$^+$ and intron$^-$ genes are randomly interspersed in the *D. melanogaster* nucleolus organizer.

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