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Exchange between the ribosomal RNA genes of *X* and *Y* chromosomes in *Drosophila melanogaster* males

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SUMMARY

The genes coding for the 18s and 28s ribosomal RNA (rRNA) are present on both the *X* and *Y* chromosomes of *D. melanogaster* at a site known as the *bobbed* locus. Exchange was observed in males between a normally orientated *X* and *Y* chromosome (*Dp(1; 1)* sc^{V1} and $B^S Y\ y^{31d}$) with a frequency of 0·079 %. One-quarter (7 in 27) of these exchange products between two + chromosomes which both carried sufficient rRNA genes for a bb^+ phenotype exhibited a *bb* phenotype. Evidence is presented that one-half, and possibly all, of the exchanges involved the repetitive *bb* genes. These results together with those reported by Palumbo, Caizzi & Ritossa (1973) imply that the repeated *bb* genes of either (or both) the *X* or *Y* chromosome are not arranged with uniform polarity and, further, that spermatogonial exchange between the *X* and *Y* chromosomes may be restricted to the *bb* loci.

1. INTRODUCTION

The genes coding for the 18s and 28s ribosomal RNA associated with the nucleolus organizer regions are present in most eukaryotes as a series of tandemly repeated genes. The genetic properties of this region have been elucidated from studies in *D. melanogaster* (Ritossa, 1976) where extensive biochemical analyses are also in progress (Pelligrini, Manning & Davidson 1977; Wellauer & Dawid, 1977). In *D. melanogaster* this region is known as the *bobbed* locus, though in fact it is a sequence of from 200 to 250 genes (Tartof, 1971) or 300 genes (Ritossa, 1976), referred to hereafter as the *bobbed* complex. A substantial deficiency of *bobbed* genes results in a mutant (*bb*) phenotype and in the extreme is lethal (bb^1), Ritossa. Atwood & Spiegelman (1966). Although recombination between *X* and *Y* chromosomes is uncommon (Cooper, 1959), recombination between the bobbed genes would be anticipated, since they make up a large proportion of the DNA of both the *X* heterochromatin and of the short arm of the *Y* (Hilliker & Appels, 1980), Recombination between the bobbed genes of the *X* chromosomes has been demonstrated to occur in females (Schalet, 1969).

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Palumbo *et al.* (1973) observed recombination within the *bobbed* complexes of *X* and *Y* chromosomes only when the *X* and *Y* were inverted in relationship to one another. They concluded that the bobbed genes on the *X* and *Y* chromosomes are orientated in the opposite sense to each other with respect to the centromere. Lindsley (1955) also reported exchange within the bobbed complexes of *X* and *Y* chromosomes when the *X* was inverted. By contrast Neuhaus (1937) did find recombination between *X* and *Y* chromosomes in both males and females where the normal orientation of the bobbed complex had not been altered. At that time they were interpreted as exchanges either to the left or right of the *bobbed* locus, which was thought to be a single indivisible gene, but they could equally well have been exchanges within the *bobbed* complex.

Evidence is presented in this paper for the occurrence of exchange within the bobbed complexes of normally orientated *X* and *Y* chromosomes. It is argued that in the experiments conducted by Palumbo *et al.* (1973) this was not observed because of the poor viability of the *bb* recombinant progeny in their experiments. An outline of the method and results has appeared in Abstract form (Maddern, 1979).

2. MATERIALS AND METHODS

Recognition of exchange between the *X* and *Y* chromosomes was facilitated by both the *X* and *Y* being marked distally on both arms. The *X* chromosome was *Dp(1:1)sc*^{v1}, *y Hw v f bb*$^{+}$*.sc*^{v1} *ac*$^{+}$ *y*$^{+}$ and differs from that used by Ritossa *et al.* (1973) only in the markers carried. This chromosome had a complete or near-complete complement of bobbed genes since *X/O* males did not have a *bb* phenotype and X-ray-induced centric fragments are *bb*$^{+}$ in combination with *C(1)DX*, *y f bb*^{0}, a compound *X* chromosome devoid of *bobbed* genes (Ritossa, 1976). The *Y* chromosome was $B^{S}Y^{L}.Y^{S}y^{31d}$ (Lindsley & Grell, 1968). The long arm (Y^{L}) was marked by two euchromatic regions from the *X* chromosome, the more distal included B^{S} and *pdf*$^{+}$ and the more proximal the basal *X* euchromatin including *su(f)*$^{+}$ (Schalet & Lefevre, 1973). The short arm (Y^{S}) was marked by a distal *X* chromosome tip carrying y^{31d} and *l(1)*^{d} (Maddern, 1977). From the origin of this *Y* chromosome the *bobbed* complex is expected to be in its normal relationship to the centromere and in combination with *C(1)DX*, *y f bb*^{0} it produces a *bb*$^{+}$ phenotype.

Dp(1:1)sc^{v1}, *y Hw v f bb*$^{+}$*.sc*^{v1} *ac*$^{+}$ *y*$^{+}/B^{S}Y^{L}.Y^{S}y^{31d}$ males were crossed to *C(1)RM, y ct*^{6} *v f car su(f)*/O virgins. Twenty pairs of parents were held in 125 ml cultures for 1 day, the males discarded and the females transferred for two consecutive 2-day periods. The regular male progeny *y Hw v f bb*$^{+}$*.sc*^{v1} *ac*$^{+}$ *y*$^{+}$*/0* were essentially *y*$^{+}$ but have a slight *y* mottling of the black chitin caused by a position effect on the *y*$^{+}$ allele in *Dp(1; 1)sc*^{v1}. By contrast the $X^{L}.Y^{L}/0$ exceptional males are recognized by their *y* body colour and the B^{S} eye shape. Due to the absence of Y^{S} these flies are expected to be sterile, preventing analysis of these chromosomes.

All F_1 females were scored for *y*, *y*^{31d}, or *y*$^{+}$; *su-f*, or *su-f*$^{+}$; both eyes for B^{+} or B^{S}; and *Hw*. The thorax was examined on both sides for extra hairs just below

the humeral bristles. Exceptional flies were also examined for hairs on the wing veins, wing cells, and beneath the scutellum. The absence, presence and number of hairs in these areas is indicative for various combinations of sex-chromosome tips (Traut, Scheid & Wind, 1970). The y^{31d} tip causes a slight *Hw* phenotype and two doses produce a more extreme effect. The sc^{V1} duplication had a very slight *Hw* effect. This system enables the sex chromosome tips to be recognized, though $y^{+}(X^{R})$ is epistatic to y^{31d}. Two doses of X^{L} can be recognized by the extreme *Hw* phenotype, and two doses of B^{S} by the extreme reduction of eye size.

All exceptional female progeny were backcrossed to $XY^{L}.Y^{S}/0$ males. Transmitted chromosome fragments were held in stock and tested for coverage of *l(1)2870* (a deficiency which exposes all known lethals distal to and including *l(1)J1*), *y*, *ac*, *sc*, *l(1)87* (a lethal proximal to *sc*) *su-s*, *pdf*, *su-f* and *bb*. In addition the presence of Y^{S} was tested for and the '*Hw*' expression assessed from sibships in which there was a normal amount, excess ($X.Y$) or a deficiency ($In(1)sc^{4L+6R}$, $C(1)DX, y\ w\ f$ and $C(1)RA, y\ ac\ v\ f\ mal^{1}\ su\text{-}f$) for sex chromosome heterochromatin.

3. RESULTS

Though X and Y chromosomes are known to pair in two ways X^{L} with Y^{L} and X^{L} with Y^{S} (Cooper, 1964) no evidence of exchange between X^{L} and Y^{L} was found, although such exchange products ($Y^{L}.X^{R}$ and $X^{L}.Y^{S}$) would have been both recoverable and recognizable. By contrast, pairing of X^{L} with Y^{S} followed by exchange between the *bobbed* complexes of X^{L} and Y^{S} will produce $Y^{S}.X^{R}$ ($y^{31d}.y^{+}$) and $X^{L}.Y^{L}$ ($y\ Hw\ v\ f.B^{S}$) both of which were observed. The finding of exchange only between X^{L} and Y^{S} and not between X^{L} and Y^{L} is in agreement with previous work (Palumbo *et al.* 1973; Cooper, 1959; Neuhaus, 1937).

Eighteen presumptive $Y^{S}.X^{R}$ exchange chromosomes were recovered in $C(1)R\ M$ females of which 11 bred, and their genotypes were confirmed by progeny testing. The presence of Y^{S} was confirmed in all, and the presence of y^{31d} inferred by observing the '*Hw*' phenotype from sibships in several genetic backgrounds with varying amounts of heterochromatin. All were $y^{31d}\ Y^{S}.y^{+}$. The bobbed phenotype of each of the 11 $Y^{S}.X^{R}$ chromosomes was examined in $X, bb^{2r1\ 6}/Y^{S}.X^{R}$ males, and in $C(1)DX$, $y\ f\ bb^{0}/Y^{S}.X^{R}$ females. The $bb^{2r1\ 6}X$ chromosome X is a bb^{l}, with approximately 25 *bobbed* genes (A. Schalet, personal communication). Nine appeared bb^{+} and 2 showed a strong *bb* phenotype.

Thirteen of the reciprocal $X^{L}.Y^{L}$ exchange chromosomes were recovered as $X^{L}.Y^{L}/0$ males which were sterile, presumably owing to the absence of the fertility loci on Y^{S}. None of the single exceptional males expressed a strong *bb* phenotype, and the author would probably have failed to recognize a single exceptional male with a slight *bb* phenotype in the presence of *y*. However, any extreme *bb* or bb^{l} alleles would have been inviable in the absence of a duplicated bb^{+} complex; such exchange products also arose at least once during stock-keeping in the presence of an additional Y chromosome which carried bb^{+}, and from this occurrence the genotype was confirmed to be $y\ Hw\ v\ f\ bb^{+}.Y^{L}\ B^{S}$.

In addition to the 18 $Y^S.X^R$ and 13 $X^L.Y^L$ exchanges (Table 1) further instances of $X-Y$ exchanges were observed during experiments in which the parental males were irradiated (Maddern & Leigh, 1976). Since radiation was administered to mature sperm it would not have influenced the $X-Y$ exchange. Radiation did however, generate y^+ fragments from X^R which could only be differentiated from $y^{31d}\ Y^S.y^+$ exchange chromosomes by progeny testing. Of 41 y^+ exceptions recovered 21 were tested, 16 of which proved to be $y^{31d}\ Y^S.y^+$. Therefore an estimated 31 of the 41 y^+ elements would have been $y^{31d}Y^S.y^+$. A further 10 presumptive $X^L.Y^L$ exchanges ($y\ Hw\ v\ f.Y^LB^s/0$ males) were also observed, all of which were sterile.

Table 1. *Frequency of spontaneous exchange between* X *and* Y *chromosomes in males*

Treatment	No. of exchanges $X^L.Y^L$	No. of exchanges $Y^S.X^R$	Total no. ♀	Total no. ♂	Proven $y^{31d}\ Y^S.X^R$ exchanges bb^+	bb	Total
0 R	13 (0·080)*	18 (0·12)	14491	16287	9	2	11 (0·076)
1000 or 3000 R	10 (0·059)	31†	16085	16980	11	5	16 (0·099)
Total	23	49	30576	33267	20	7	27

* Figures in parentheses indicate percentage recovery of exchanges among the total progeny of that sex.

† Estimated, see text

Taking both the sets of data after irradiation and without irradiation together, a total of 23 presumptive $X^L.Y^L$ exchanges were observed, and without progeny testing were assessed as bb^+. From an estimated 49 presumptive $Y^S.X^R$ exchanges 27 were confirmed genetically. From progeny tests twenty of these were bb^+, 6 were bb and one was bb^l. The two reciprocal exchange classes differed in frequency (23:49) and in the proportion which were bb (0 in 23 *versus* 7 in 27). The latter difference is attributable, at least partly, to the inviability of bb^1 or extreme bb visible phenotypes in $X^L.Y^L/0$ males, whereas bb^1 complements were recoverable in $Y^S.X^R$ chromosomes, obtained as duplications in $C(1)RM\ bb^+$ females. Therefore the absence of bb flies amongst the $X^L.Y^L$ products is due in part to their inviability, and this is reflected in their lower frequency of recovery compared to the reciprocal $Y^S.X^R$ class, and in part to a failure to recognize slight bb phenotypes in single exceptional y flies. Further, the y phenotype of the $X^L.Y^L/0$ males also placed them at a competitive disadvantage compared with their y^+ male siblings, while the y^+ phenotype of the $Y^S.X^R$-bearing females placed them at an advantage over their y female siblings.

The recovery of exchange chromosomes was not distributed independently between cultures, that is, there was a clustering of such events within the one culture and the data did not fit a Poisson distribution (1 culture with 6, 2 with 3, 9 with 2, 20 with 1 and 172 with 0 exchanges: $P < 0{\cdot}001$). However, the distri-

bution of $Y^S.X^R$ exchanges was at random (3 cultures with 2, 21 with 1 and 150 with 0 exchanges: $0{\cdot}20 > P > 0{\cdot}10$) and although the distribution of $X^L.Y^L$ exchanges did not fit a Poisson (1 culture with 4, 5 with 2, 9 with 1 and 190 with 0 exchanges: $P < 0{\cdot}001$) this was largely attributable to the culture with 4. As 20 pairs of parents were used per culture such a result could have been fortuitous. It is the recovery of reciprocal exchange classes that deviates most significantly from random. As 15 out of 204 cultures produced $X^L.Y^L$ exchanges and 24 out of 204 cultures $Y^S.X^R$ exchanges, 1·8 cultures ($15/204 \times 24/204$) would be expected to produce both if the two events were independent. Seven cultures were observed with reciprocal exchanges. The simultaneous recovery of pairs of reciprocal products does not distinguish between mitotic and meiotic exchange. However, the small size of the majority of clusters and the infrequent clustering of like exchanges does suggest that the recovery of exceptional sperm is due to spermatogonial exchange.

4. DISCUSSION

The recovery of $X–Y$ recombinant chromosomes with a *bb* phenotype from an X and Y that were bb^+ demonstrates unequal exchange within the bobbed complex. Seven of 27 $Y^S.X^R$ exchanges must have occurred within the bobbed complex. However, this is an underestimate of the frequency of $X–Y$ exchange involving the *bobbed* complex. First a portion of such exchanges would lead to phenotypically undetectable reductions in the number of *bobbed* (rRNA) genes. Secondly, half of all unequal exchanges in the *bobbed* complex would result in an increase in the number of bobbed genes, which would also be phenotypically undetectable. Therefore, since 1/4 of the exchanges resulted in a reduction in the number of *bobbed* genes and 1/4 would be expected to produce an increase, at least half, and possibly all of the 27 $Y^S.X^R$ products arose by exchange within the *bobbed* complex.

These conclusions differ from those of Palumbo *et al.* (1973), who failed to detect exchange within the *bobbed* complexes of normally oriented X and Y chromosomes (see Fig. 1). In their experiments the $Y^S.X^R$ product analogous to those recovered here as duplications in females was not recognizable, and only the reciprocal class $X^L.Y^L/Y\ bb^l$ analogous to the $X^L.Y^L/O$ males observed here could be recovered. Only one of the two reciprocal classes could be recovered, and this was the one where the *bobbed* complex of the exchange chromosomes was not covered by a bb^+ complex elsewhere in the genome. Thus any exchanges producing an extreme *bb* phenotype are unlikely to have been observed because of their reduced viability. By contrast, when the order of the *bobbed* complex on the X chromosome was reversed, Palumbo *et al.* did observe exchange within the complex. Again only one of the reciprocal classes, this time the $X^{distal}.Y^L$ element (equivalent in size to $Y^S.X^R$) was recovered as a duplication in $C(1)RM\ bb^+$ females, that is the bobbed complex of the exchange element was covered by a bb^+ complex elsewhere. As in the present paper, on progeny testing, a proportion of the exceptions were

bb, indicating exchange between the bb^+ of the *Y* chromosome and bb^l of the *X*. Thus there is no conflict between the results of Palumbo *et al.* (1973) and those reported here, demonstrating that exchange between bobbed genes of *X* and *Y* chromosomes in normal as well as reversed sequence can occur.

Regardless of the evolutionary forces maintaining the similarities and dissimilarities between the rRNA genes on *X* and *Y* chromosomes (Tartof & Dawid, 1976

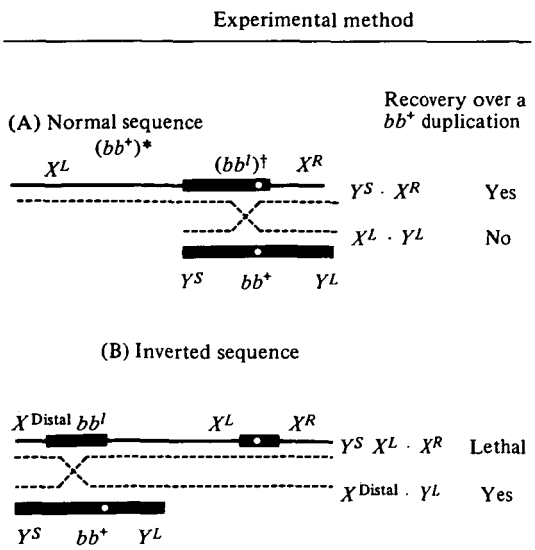

Fig. 1. Comparison of *X*–*Y* exchange between a chromosome in normal sequence A, and an inverted sequence *X*, B. In Palumbo *et al.* (1973) one product could not be recognized in A, and was lethal (*X* deficiency) in B.

and Palumbo *et al.* 1973) recombination does occur between the bobbed genes, though unless a double exchange occurs such products (Y^S. and X^L. Y^L) will be eliminated. As exchange does occur between the *bobbed* loci of *X* and *Y* chromosomes both in the inverted and normal sense, the genes on at least one and perhaps both are not of uniform polarity. It is envisaged that the individual repeating unit comprising the *bb* 'gene' consists of coding, non-transcribed and transcribed spacer and variability intervening sequences all of which may be the actual site of the exchanges.

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