X-Y Exchange and the Coevolution of the X and Y rDNA Arrays in *Drosophila melanogaster*

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ABSTRACT

The nucleolus organizers on the *X* and *Y* chromosomes of *Drosophila melanogaster* are the sites of 200–250 tandemly repeated genes for ribosomal RNA. As there is no meiotic crossing over in male *Drosophila*, the *X* and *Y* chromosomal rDNA arrays should be evolutionarily independent, and therefore divergent. The rRNAs produced by *X* and *Y* are, however, very similar, if not identical. Molecular, genetic and cytological analyses of a series of *X* chromosome rDNA deletions (*bb* alleles) showed that they arose by unequal exchange through the nucleolus organizers of the *X* and *Y* chromosomes. Three separate exchange events generated compound $X{\cdot}Y^L$ chromosomes carrying mainly *Y*-specific rDNA. This led to the hypothesis that *X-Y* exchange is responsible for the coevolution of *X* and *Y* chromosomal rDNA. We have tested and confirmed several of the predictions of this hypothesis: First, $X{\cdot}Y^L$ chromosomes must be found in wild populations. We have found such a chromosome. Second, the $X{\cdot}Y^L$ chromosome must lose the Y^L arm, and/or be at a selective disadvantage to normal X^+ chromosomes, to retain the normal morphology of the *X* chromosome. Six of seventeen sublines founded from homozygous $X{\cdot}Y^L bb$ stocks have become fixed for chromosomes with spontaneous loss of part or all of the appended Y^L. Third, rDNA variants on the *X* chromosome are expected to be clustered within the X^+ nucleolus organizer, recently donated ("*Y*") forms being proximal, and *X*-specific forms distal. We present evidence for clustering of rRNA genes containing Type 1 insertions. Consequently, *X-Y* exchange is probably responsible for the coevolution of *X* and *Y* rDNA arrays.

THE ribosomal RNA genes on the *X* and *Y* chromosomes of *Drosophila melanogaster* show similarities and differences that pose important problems in understanding the evolution of multigene families. The *X* and *Y* chromosomes each carry 200–250 genes for the major ribosomal RNAs (TARTOF 1975; RITOSSA 1976). These genes are tandemly repeated at the nucleolus organizers (RITOSSA and SPIEGELMAN 1965). The tandem array consists of alternating spacer and rRNA coding regions. Spacers vary in size (LONG and DAWID 1979a), such variation being found both among chromosomes, and within single nucleolus organizers (WELLAUER, DAWID and TARTOF 1978; BONCINELLI *et al.* 1983).

A portion of the rDNA repeats is interrupted in the 28S coding region by one of two types of nonhomologous insertion sequence. Type 1 (T1) insertions are restricted to the *X* chromosome where they interrupt about 50% of the rDNA repeats. The DNA sequences at rDNA/T1 junctions and the location of sequences homologous to T1 insertions away from the nucleolus organizer suggests these elements may be transposable (KIDD and GLOVER 1980; PEACOCK *et al.* 1981; ROIHA *et al.* 1981; RAE 1981).

Type 2 (T2) insertions interrupt about 15% of the rDNA repeats on both the *X* and *Y* chromosomes. These insertions are not homologous to T1 insertions and have a slightly different insertion point in the 28S rRNA coding region (ROIHA and GLOVER 1980; LONG, REBBERT and DAWID 1980). Repeats containing T1 or T2 insertions do not appear to contribute significantly to the production of mature rRNA (LONG and DAWID 1979b; LONG *et al.* 1981).

In the absence of meiotic crossing over in male *Drosophila* the *X* and *Y* chromosomal rDNA arrays should be evolutionarily independent and diverge. Despite the heterogeneity outlined above, the rRNAs transcribed from the uninterrupted (active) rDNA repeats on the *X* and *Y* chromosomes are very similar. The 28S rRNAs are identical (MADEN and TARTOF 1974), and only a single base difference has been reported between the 18S rRNA transcribed from the *X* and *Y* chromosomes (YAGURA, YAGURA and MURAMATSU 1979).

How do the *X* and *Y* rDNA arrays coevolve? Reduced rRNA gene copy numbers in the selection lines of FRANKHAM, BRISCOE and NURTHEN (1978, 1980) were generated by unequal *X-Y* exchanges (translocations) through the *X* and *Y* chromosomal nucleolus

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organizers (COEN and DOVER 1983). This has led to the hypothesis that the coevolution of the *X* and *Y* chromosomal rDNA is mediated by *X-Y* exchange.

This hypothesis has the following predictions:

1. *X-Y* exchange through the nucleolus organizers must occur.

2. The $X \cdot Y^L$ product of *X-Y* exchange must be present in natural populations for the mechanism to be generally applicable.

3. To account for the normal karyotype, the $X \cdot Y^L$ chromosome must be at a selective disadvantage to the normal *X* chromosome, and/or the $X \cdot Y^L$ chromosome must be unstable.

4. rDNA variants on the *X* chromosome should be clustered. The *X-Y* exchange should result in forms donated by the *Y* being predominantly on the proximal side of the *X* nucleolus organizer, while variants specific to the *X*-chromosomal rDNA should be clustered distally.

5. *X* and *Y* chromosomes in long-established stocks should share some rDNA spacer classes, and should not have fixed differences in rRNA coding sequence variants.

6. As T1 insertions do not exist on the *Y* chromosome, they may transpose into *X* chromosomal rDNA from the nearby heterochromatin or they might eventually be eliminated from *X* chromosomes by the mechanics of *X-Y* exchange.

7. The heterochromatin of the proximal X^+ and proximal Y^S must be homologous, as this region of the *Y* chromosome is donated to the *X* chromosome by *X-Y* exchanges.

This paper is concerned with the evaluation of the first four of these predictions. We present evidence for the occurrence of three *X-Y* exchanges through the rDNA, for the loss of part or all of the Y^L arm from $X \cdot Y^L$ chromosomes in some lines, for clustering of rDNA variants on the *X* chromosome and for the presence of an $X \cdot Y^L$ chromosome in a wild population.

MATERIALS AND METHODS

Lines: The control and abdominal bristle selection lines analyzed in this paper all arose from an isogenic stock (derived from the Canberra population) (LATTER 1964) into which the fourth chromosome recessive spa^{pol} had been substituted. These stocks are described in detail by FRANKHAM, BRISCOE and NURTHEN (1978, 1980), and FRANKHAM (1980). Their relationships are shown in Figure 1. CD and CF are unselected control lines. All other lines are derived from the low abdominal bristle selection lines LA or LC by continued selection, reverse selection or relaxation of selection. LA and LC fixed for *X* chromosomal *bb* alleles that arose *de novo* in the lines. LA fixed for an extreme *bb* allele between generations 33 and 37, while LC fixed for a mild *bb* allele between generations 14 and 21.

Stocks: The following stocks were used in analyses of the lines.

C(1)DX, y f/X·Y^L, In(1)dl-49, v^of B/Y^S

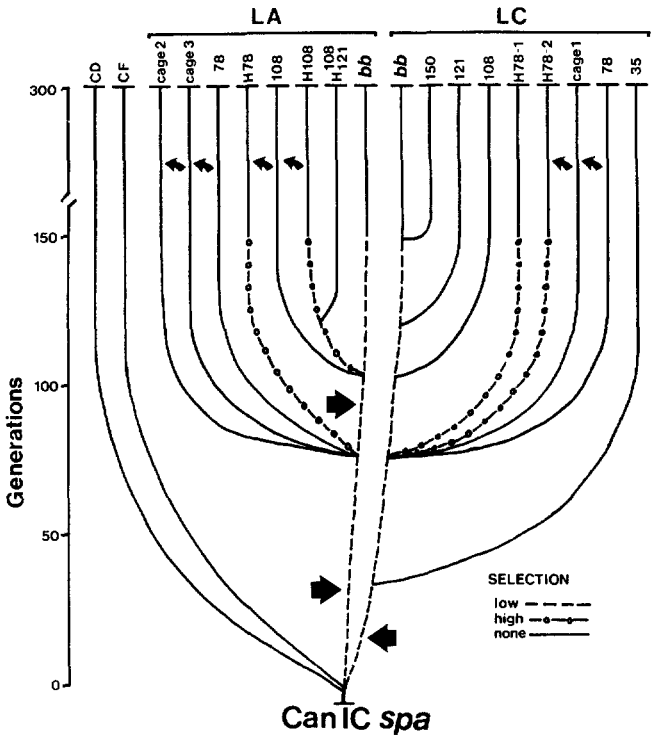


FIGURE 1.—Family tree of the abdominal bristle number selection lines. Broad arrows indicate major *X-Y* exchange events and fixation of the resultant $X \cdot Y^L$. Small arrows indicate lines that have subsequently lost all or part of the appended Y^L.

C(1)DX, y f/X·Y^L, In(1)dl-49, v^of f B/Y^S
C(1)DX, y f/X·Y^S, In(1)dl-49, v^of f B/Y^L·y^3
C(1)DX, y f/X·Y^S, In(1)sc^8L sc^4R, y sc-/Y^L·sc^S1
C(1)DX, y f/X·Y^S, y ac w^a ct^6 f/Y^L·sc^S1
X·Y^L, y cv v/y^+ Df(Y) kl-1-/C(1)DX, y f
X·Y^L, y cv v/y^+ Df(Y) kl-2-/C(1)DX, y f
X·Y^L, y cv v/y^+ Df(Y) kl-3-/C(1)DX, y f
X·Y^L, y cv v/y^+ Df(Y) kl-5-/C(1)DX, y f
X·Y^L Y^S, y w^a/y^+ Df(Y) kl-3-, 4-/C(1)RM, y v bb

Stocks are described by LINDSLEY and GRELL (1968) and by KENNISON (1982).

Generation of specific genotypes: *X*/O* males were generated by crossing males from the stock of interest to *C(1)RM, y v bb/O* females. To analyze *Y* chromosomal rDNA, males from the stock of interest were crossed to $B^S Y/C(1)DX$, $y f bb^-$:= females and female progeny utilized.

Y chromosome fertility factor complementation tests: Complementation analysis was used to test for the presence of Y^S or Y^L chromosome arms appended to the *X* chromosome (*X**). Males from each stock were crossed to $C(1)DX/Y^S$ or $C(1)DX/Y^L$ virgin females. The resultant X^*/Y^S or X^*/Y^L males were tested for fertility.

Y^L fertility factor point testing: Mapping of individual fertility factors on $X \cdot Y^L$ chromosomes was accomplished by a procedure similar to that above. *C(1)DX/Df(Y)* or *C(1)RM/Df(Y)* virgin females were crossed to males from the line of interest, and the F_1 male progeny tested for fertility by mating to their sisters. That the F_2 progeny were fathered by the F_1 males was confirmed by the segregation of the recessive spa^{pol} marker in the F_2.

Mapping of the *Stellate* control (*Ste^c*) region: The presence of Ste^c, a *Y* chromosomal locus mapping between *kl-1* and *kl-2*, was scored by inspection of primary spermatocytes of *X/O* or $X \cdot Y^L/O$ males. The absence of Ste^c in *X/O* males or by loss of the region in $X \cdot Y^L/O$ males results in the

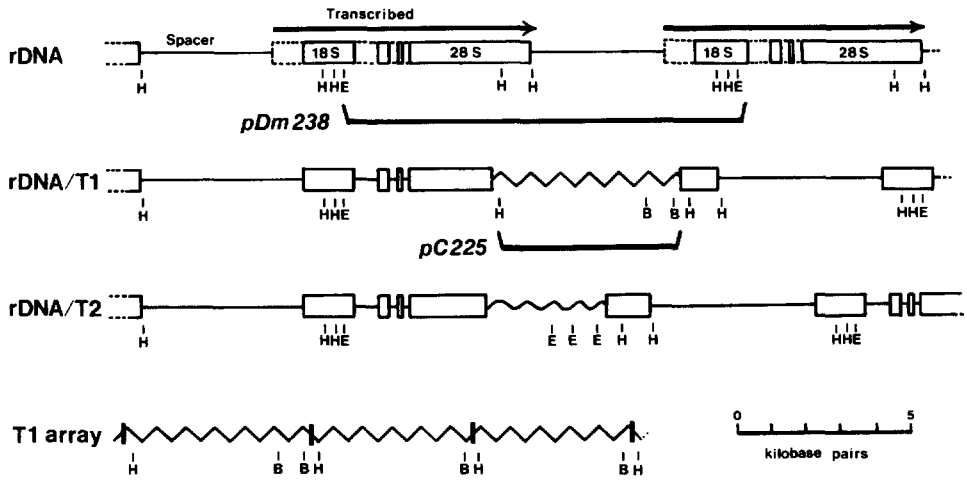


FIGURE 2. Organization of rDNA and related sequences in *Drosophila melanogaster*. Symbols: Boxes = rRNA coding regions, dotted boxes = transcribed spacer regions, straight lines = spacer regions, zig-zag lines = T1 insertion, wavy lines = T2 insertion. Restriction enzyme sites: B = *Bam*HI, E = *Eco*RI, H = *Hin*dIII. Fragments held as recombinant plasmids are indicated below the restriction maps.

appearance of crystals in the spermatocytes of these males (HARDY *et al.* 1984; LIVAK 1984).

Cytology: Larval brain squashes were prepared after GATTI and PIMPINELLI (1983) and stained with 0.25 μg/ml Hoechst 33258 (Calbiochem).

DNA extraction and analysis: Flies (100–150) were ground in a 2 ml Dounce homogenizer with 1 ml of ROBB's (1969) phosphate buffered saline (minus Mg salts). A crude nuclear pellet was obtained by low speed centrifugation. Nucleic acids were extracted from this pellet by resuspending in 200 μl 1 × SSC (SSC = 0.15 M NaCl, 0.015 M trisodium citrate), and incubating with 400 μl phenol [0.1% (w/v) 8-hydroxyquinoline and equilibrated in 1 × TE = 10 mM Tris-HCl, 1 mM EDTA pH 7.5], and 200 μl of a lysis solution (0.15 M NaCl, 0.5 M Na perchlorate, 2% SDS, 0.1 M EDTA, pH 8.0). After centrifugation, the aqueous phase was extracted with 400 μl of chloroform/isoamyl alcohol [24:1 (v/v)] and nucleic acids precipitated by the addition of 2 volumes of cold (−20°) absolute ethanol. After 0.5 hr on ice, the precipitate was collected, briefly dried under vacuum, and resuspended overnight in 100 μl TE. The solution was brought to 50 mM [Na^+] and 10 μl of preboiled RNAase A (1 mg/ml) were added. The mixture was incubated 1 hr on ice, when 200 μl of SSC were added and the temperature raised to 37° for 10 min. The preparation was extracted as above, ether extracted, precipitated and resuspended as above. All manipulations were in 1.5 ml tubes on ice, using 15-min microfuge runs, unless otherwise indicated.

DNA was digested with *Hin*dIII, *Eco*RI or *Bam*HI according to the manufacturer's instructions. Enzymes were from BRL (Lyphozymes). λDNA was included in all genomic digests as an internal control. Genomic DNA digests (2–4 μg) were separated by electrophoresis through 0.75% agarose and transferred to nitrocellulose (S & S BA85) by SMITH and SUMMER's (1980) modification of SOUTHERN's (1975) original procedure.

Hybridization probes (Figure 2) were pC225 (T1 rDNA insertion sequence clone; ROIHA and GLOVER 1980) and pDm238 (rDNA repeat; ROIHA *et al.* 1981). A 5.4 kilobase pair (kb) *Hin*dIII fragment, gel purified (TAUTZ and RENZ 1983) from pDm238 was used as a probe for genomic spacer sequences. This fragment contains all the rDNA spacer and a small portion of the 18 S rRNA gene (Figure 2). The presence of 18 S sequences does not interfere with the analysis of spacer variation. *Eco*RI and *Hin*dIII digests of pC225 and pDm238 were included as flanking marker tracks on all transferred gels.

Gel purified plasmids or restriction fragments were labeled by nick-translation (RIGBY *et al.* 1977) using α^{32}P-labeled dCTP.

Hybridization was a modification of WAHL, STERN and STARK's (1979) procedure, the major change being the inclusion of 5% dextran sulfate in the prehybridization. Hybridization and subsequent washes were under stringent conditions. Treated filters were used to expose preflashed X-ray film (Fuji RX) with intensifying screens (LASKEY and MILLS 1977). Transfers were recycled, where necessary, by stripping the bound probe (MANIATIS, FRITSCH and SAMBROOK 1982) before rehybridization with other probes.

RESULTS

Restriction analysis of rDNA in the selection lines: The *bb* alleles in the two selection lines, LAbb and LCbb, arose by *X-Y* exchange through the nucleolus organizers. This generated compound $X \cdot Y^L$ chromosomes visible at metaphase (Figure 5) (COEN and DOVER 1983).

This section deals with the rDNA restriction patterns of all extant lines founded from LAbb or LCbb. The initial survey was conducted using the restriction enzyme *Eco*RI. This enzyme cuts all rDNA repeats once in the 18S rRNA coding region, and also has three internal sites in the T2 insertion, allowing the three classes of rDNA repeat (uninterrupted, rDNA/T1 and rDNA/T2) to be easily separated by agarose gel electrophoresis. rDNA repeats interrupted by T2 insertions are cleaved into a coding fragment (5.8 kb) and a variable fragment (7–9 kb) largely composed of spacer sequences (Figures 2 and 3). Uninterrupted rDNA repeats produce larger fragments (11–12 kb), while rDNA repeats containing T1 insertions produce still larger fragments (17 kb) (Figure 3). The identity of each fragment class shown in Figure 3 was confirmed using probes specific to coding, spacer, T1 or T2 sequences (GILLINGS 1986).

The rDNA restriction patterns of representative $X \cdot Y^L$ chromosomes are shown in Figure 3. These patterns are largely characteristic of *Y*-chromosomal rDNA. Repeats containing T1 insertions are not found on the Y^+ chromosomes, and are present in

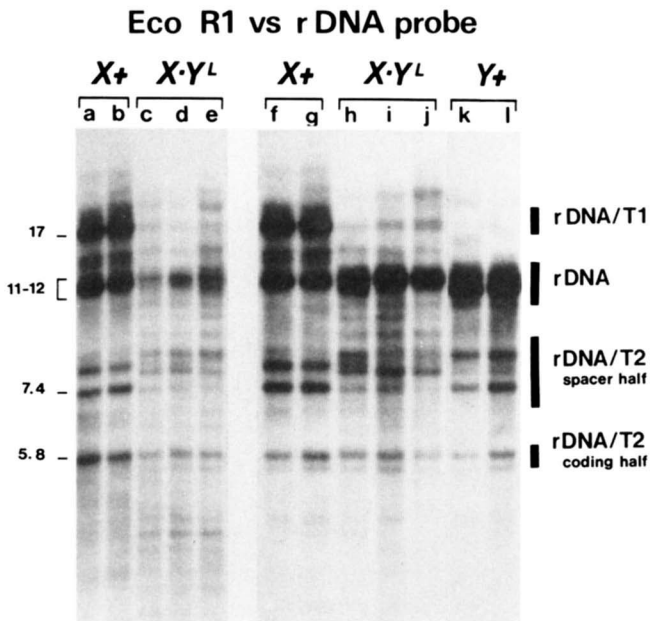


FIGURE 3.—*Eco*RI digests of genomic DNA probed with rDNA sequences (pDm238). Tracks a–j, females from the following lines: (a) CF, (b) CD, (c) LAbb, (d) HLA78, (e) HLA108, (f) CD, (g) CF, (h) LA108, (i) HLC78-1, (j) HLC78-2. Tracks k and l, *C(1)DX bb-/Y* females, *Y* chromosome from lines (k) HLC78-1, (l) HLC78-2.

only small quantities on the $X \cdot Y^L$. There is however, a strong rDNA/T1 band in the X^+ tracks, demonstrating that large numbers of these repeats were deleted by the *X-Y* exchange (Figure 3).

The X^+, $X \cdot Y^L$ and Y^+ chromosomes do share some rDNA/T2 fragments. All carry the invariant 5.8-kb coding fragment and the smallest spacer fragment of 7.4 kb. Above 7.4 kb, the X^+ and Y^+ chromosomes carry rDNA/T2 fragments of differing mobilities.

The $X \cdot Y^L$ chromosomes carry elements of both X^+ and Y^+ patterns, those elements characteristic of the *Y* being in the majority (Figure 3). Since elements of both X^+ and Y^+ rDNA are present on the $X \cdot Y^L$, the original exchange breakpoints must have been within the nucleolus organizers of both X^+ and Y^+ chromosomes (see Figure 7).

Analysis of a series of exposures of the autoradiographs presented in Figure 3 (and other experiments not shown) yields the following observations. The X^+ chromosome carries a single uninterrupted rDNA repeat of 11.6 kb, while the Y^+ has a prominent doublet of about 11.9 and 11.2 kb. The $X \cdot Y^L$ chromosomes of the LC lines carry the 11.9 kb *Y*-chromosomal band (Figure 3, tracks i and j). All extant LC lines exhibit this *Eco*RI restriction profile, with only minor variations.

The rDNA restriction pattern of LA lines founded before generation 108 (Figure 3, tracks c and d) is similar, but not identical to that of the LC lines. The LA lines carry different ratios of rDNA/T2 fragments, establishing that the original *X-Y* exchanges in LA and LC were independent and different events.

All LA sublines founded at, or after, generation 108 carry an additional rDNA band at 11.2 kb (Figure 3, tracks e and h). This band is *Y*-chromosomal in origin, but was not transferred to the $X \cdot Y^L$ by the initial exchanges in LA or LC. A second exchange between the LA $X \cdot Y^L$ and a *Y* chromosome must have occurred (and fixed) in the main LA line between generations 78 and 108 (see Figure 1).

The number of different restriction fragments between 11 and 12 kb precludes an absolute assessment of the proportion of $X \cdot Y^L$ rDNA that is *Y*-chromosomal in origin. These minor size differences are probably caused by polymorphism for the numbers of a 240-base pair (bp) repeat module within the spacer region of each full length repeat (WELLAUER, DAWID and TARTOF 1978; BONCINELLI *et al.* 1983). The same is also true of rDNA containing T2 insertions, the variation in this case being more fully resolved by the *Eco*RI digest (Figure 3, T2 spacer halves).

Since the spacer region is apparently polymorphic, the spacers of X^+, $X \cdot Y^L$, and Y^+ chromosomes were investigated. DNA was digested with the restriction enzyme *Hin*dIII and the transferred fragments probed with the *Hin*dIII fragment of pDm238 that contains the spacer region (Figure 2). This procedure confirmed that variation is generated by differing numbers of 240-bp subrepeats. Both $X \cdot Y^L$ and Y^+ chromosomes exhibit a ladder of *Hin*dIII spacer fragments with a periodicity of about 240 bp (Figure 4).

The X^+ chromosomes of the control lines, CD and CF, carry a major spacer fragment of 5.7 kb (Figure 4, tracks a and b). Consequently, most rDNA repeats on the X^+ chromosome carry this spacer class. A range of larger spacers is also present, these being the "long" spacers described by INDIK and TARTOF (1980). The X^+ chromosome does not share any spacer classes with the Y^+.

In contrast to the X^+, the Y^+ chromosome carries five major spacer classes and a similar number of minor spacer variants (Figure 4, tracks i and j). Consideration of the *Eco* and *Hin* restriction sites within rDNA (Figure 2) suggests that the doublet observed in the *Eco*RI digests of Y^+ rDNA (Figure 3) is in fact composed of the five major classes, rDNA with 6.25 and 6.0-kb spacers (*Hin*) comigrating as the "11.9"-kb band (*Eco*), and rDNA with 5.5-, 5.25-, and 5.0-kb spacers (*Hin*) comigrating as the "11.2"-kb band (*Eco*).

The $X \cdot Y^L$ chromosomes carry mainly *Y* rDNA spacer classes. Only small quantities of X^+ spacer classes (5.7 and 6.7 kb) remain on the $X \cdot Y^L$, demonstrating that most X^+ rDNA was deleted during the *X-Y* exchanges. The LC lines all have a similar *Hin*dIII spacer profile (Figure 4, representative tracks g and h). LA lines founded before generation 108, have a profile similar, but not identical, to that of the LC lines (Figure 4, tracks c and e). LA lines founded at,

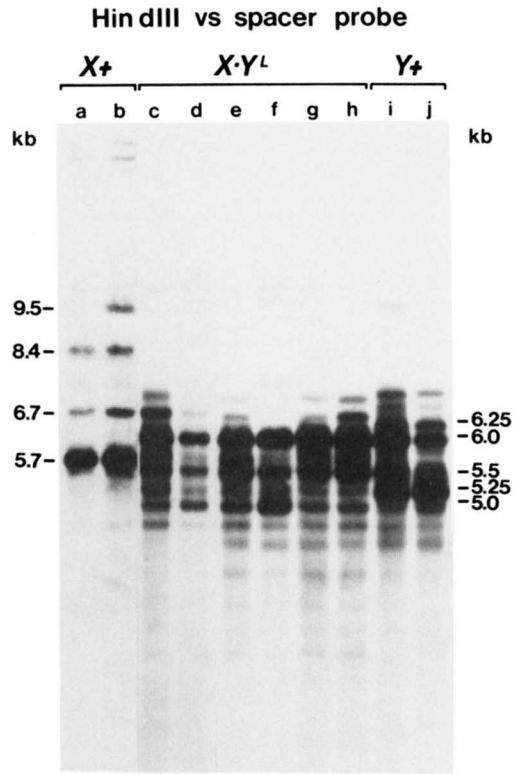


FIGURE 4.—*Hin*dIII digests of genomic DNA probed with a *Hin*dIII fragment gel-purified from the spacer region of pDm238. Tracks a–h, females from the following lines: (a) CD, (b) CF, (c) LA3, (d) LA108, (e) HLA78, (f) LA108, (g) HLC78-1, (h) HLC78-2. Tracks i and j, *C(1)DX bb-/Y* females. *Y* chromosomes from the following lines: (i) HLA78, (j) HLA108.

or after, generation 108 show evidence of a second exchange event as suggested by the *Eco*RI digests. Changes in the restriction pattern after generation 108 include the deletion of most of the remaining X^+ spacers (5.7 and 6.7 kb), and the increase in molarity of a Y^+ spacer class (5.0 kb) (Figure 4, track f). Such observations can only be explained by a further interchromosomal exchange ($X \cdot Y^L$-Y^+) in the main LA line between generations 78 and 108. The *Hin*dIII digests therefore confirm the interpretation suggested by the *Eco*RI digests.

In summary, the restriction analysis has shown that the initial events in LA and LC were unequal *X-Y* exchanges through the nucleolus organizers. The resultant $X \cdot Y^L$ chromosome carried mainly *Y* rDNA. A second exchange ($X \cdot Y^L$-*Y*) occurred in the main LA line between generations 78 and 108. These events are noted on Figure 1.

Loss of Y^L material from $X \cdot Y^L$ chromosomes: complementation analysis: *X-Y* exchange through the nucleolus organizers produces compound $X \cdot Y^L$ chromosomes (Figure 7). If such exchanges are to allow Y^+ rDNA to infiltrate the X^+ nucleolus organizer, the resultant $X \cdot Y^L$ chromosomes must undergo further exchanges with X^+ chromosomes, or must lose the Y^L arm to regenerate the normal acrocentric *X* chromosome morphology. Since LAbb and LCbb fixed for $X \cdot Y^L$ chromosomes, sublines founded after this fixation should all carry $X \cdot Y^L$ chromosomes. To test this supposition, males of each stock in Figure 1 were crossed to C(*1*)*DX*/Y^S females. The F_1 males ($X \cdot Y^L$?/Y^S) were tested for fertility. Males of each stock were also crossed to C(*1*)*DX*/Y^L females. This tested for $X \cdot Y^S$ chromosomes and acted as a negative control. No "*X*" chromosome was fertile against Y^L in any of the tests.

$X \cdot Y^L$? chromosomes from 12 of the 17 LA and LC sublines were fertile against Y^S. The Y^L arm of these $X \cdot Y^L$ chromosomes is therefore unchanged from that on the normal *Y*. The remaining five sublines carried $X \cdot L^L$? chromosomes that were completely sterile against Y^S. There are three explanations for the recovery of $X \cdot Y^L$?/Y^S sterile *X* chromosomes from lines with known $X \cdot Y^L$ founders. There may be a point mutation in a Y^L fertility factor, a deletion of one or more fertility factors, or contamination of the stock with normal X^+ chromosomes that have subsequently gone to fixation. Contamination can be ruled out, as all the lines are still homozygous for the recessive *spa*^{pol} mutant, are electrophoretically monomorphic and carry a Y^+ rDNA restriction pattern on the $X \cdot Y^L$? chromosome (GILLINGS 1986).

To map these mutations or deletions, a series of *Y* chromosomes with known lesions in the fertility factors was employed. The principle of the complementation test was to construct $X \cdot Y^L$?/*Y-tester* males and assay them for fertility. Rescue of the sterile *Y-tester* chromosome showed that the $X \cdot Y^L$? chromosome carried that Y^L fertility factor deleted from the *Y-tester* chromosome.

As expected, $X \cdot Y^L$ chromosomes fertile against Y^S in the first test were also fertile against all the *Y-tester* chromosomes (Table 1). Based on the complementation tests, the $X \cdot Y^L$? chromosomes of the three cage lines, LA2, LA3 and LC1, have lost *kl-2*, *kl-3* and *kl-5* from the Y^L arm. The reverse selection line HLA78 has lost *kl-1*, *kl-2* and *kl-5*, whereas the reverse selection line HLC78-2 has lost all the Y^L fertility factors from the $X \cdot Y^L$ (Table 1). The $X \cdot Y^L$ chromosomes that have lost fertility factors from the Y^L will be referred to as $X \cdot Df(Y^L)$.

Some of the $X \cdot Df(Y^L)$ chromosomes have lesions between *kl-1* and *kl-2*, the region containing the *Stellate* control (*Ste*^{c}) locus. The results of tests for this locus (in *X/O* males) are presented in Table 1. Of the lines carrying a full Y^L arm, all except LA108 also carry *Ste*^{c}. LA108 still carries all the Y^L fertility factors. Of the $X \cdot Df(Y^L)$ lines, only LA2 and LA3 still carry *Ste*^{c}. All other $X \cdot Df(Y^L)$ lines (LC1, HLA78 and HLC78-2) are deficient for *Ste*^{c}.

Cytological analysis: To confirm the physical deletion of Y^L sequences, and to map their extent, mitotic

TABLE 1

Complementation tests between X or $X \cdot Y^L$ chromosomes and a series of Y chromosomes with lesions in the Y^L fertility factors

Stock	Y^L Function					
	Ykl-1$^-$	*Ste*^{c} [a]	*Ykl-2*$^-$	*Ykl-3*$^-$	*Ykl-3,4*$^-$	*Ykl-5*$^-$
Control lines						
CD	S[b]	N	S	S	S	S
CF	S	N	S	S	S	S
LA sublines						
LAbb	F[c]	+	F	F	F	F
LA78	F	+	F	F	F	F
LA108	F	N	F	F	F	F
HLA78	S	N	S	F	F	S
HLA108	F	+	F	F	F	F
HLA108/121	F	+	F	F	F	F
LA2	F	+	S	S	S	S
LA3	F	+	S	S	S	S
LC sublines						
LCbb	F	+	F	F	F	F
LC35	F	+	F	F	F	F
LC78	F	+	F	F	F	F
LC108	F	+	F	F	F	F
LC121	F	+	F	F	F	F
LC150	F	+	F	F	F	F
HLC78-1	F	+	F	F	F	F
HLC78-2	S	N	S	S	S	S
LC1	F	N	S	S	S	S

[a] Results of cytological tests for *Ste*^{c}, in $X \cdot Y^L/O$, $X \cdot Df(Y^L)/O$ and X/O males. + = *Ste*^{c} region present on the X chromosome, N = needle shaped crystals.

[b] S = sterile combination.

[c] F = fertile combination.

chromosomes of all lines were examined by Hoechst fluorescence microscopy. The results presented here agree entirely with the cytological analysis of the Y chromosome published by GATTI and PIMPINELLI (1983), in both the number and placement of Hoechst fluorescent blocks on the Y^L, and the location of particular fertility factors. The numbers used to refer to Y chromosomal landmarks in Figure 5 are those suggested by GATTI and PIMPINELLI (1983).

The normal X^+ chromosome is an acrocentric with a strong Hoechst positive knob over the centromere. The compound sex chromosomes of LAbb and LCbb (and all other $X \cdot Y^L/Y^S$ fertile sublines) clearly demonstrate that the appended arm is the Y^L. The loss of *KL* fertility factors from the $X \cdot Y^L$ to generate the $X \cdot Df(Y^L)$ chromosomes is accompanied by the loss of corresponding regions of the Y^L arm (Figure 5). The $X \cdot Df(Y^L)$ chromosome of HLC78-2 has lost all the Y^L fertility factors and consequently the whole Y^L arm. It is morphologically similar to the X^+ chromosome (Figure 5).

The deletions of Y^L material from the $X \cdot Y^L$ chromosomes as determined by complementation and cytological analysis are summarized at the base of Figure 5. Most, if not all the deletions have at least two breakpoints, retaining the Y^L telomere on the newly generated $X \cdot Df(Y^L)$. All the Y^L deletions were spontaneous, and each newly formed $X \cdot Df(Y^L)$ chromosome replaced the original $X \cdot Y^L$ in the lines where the events occurred.

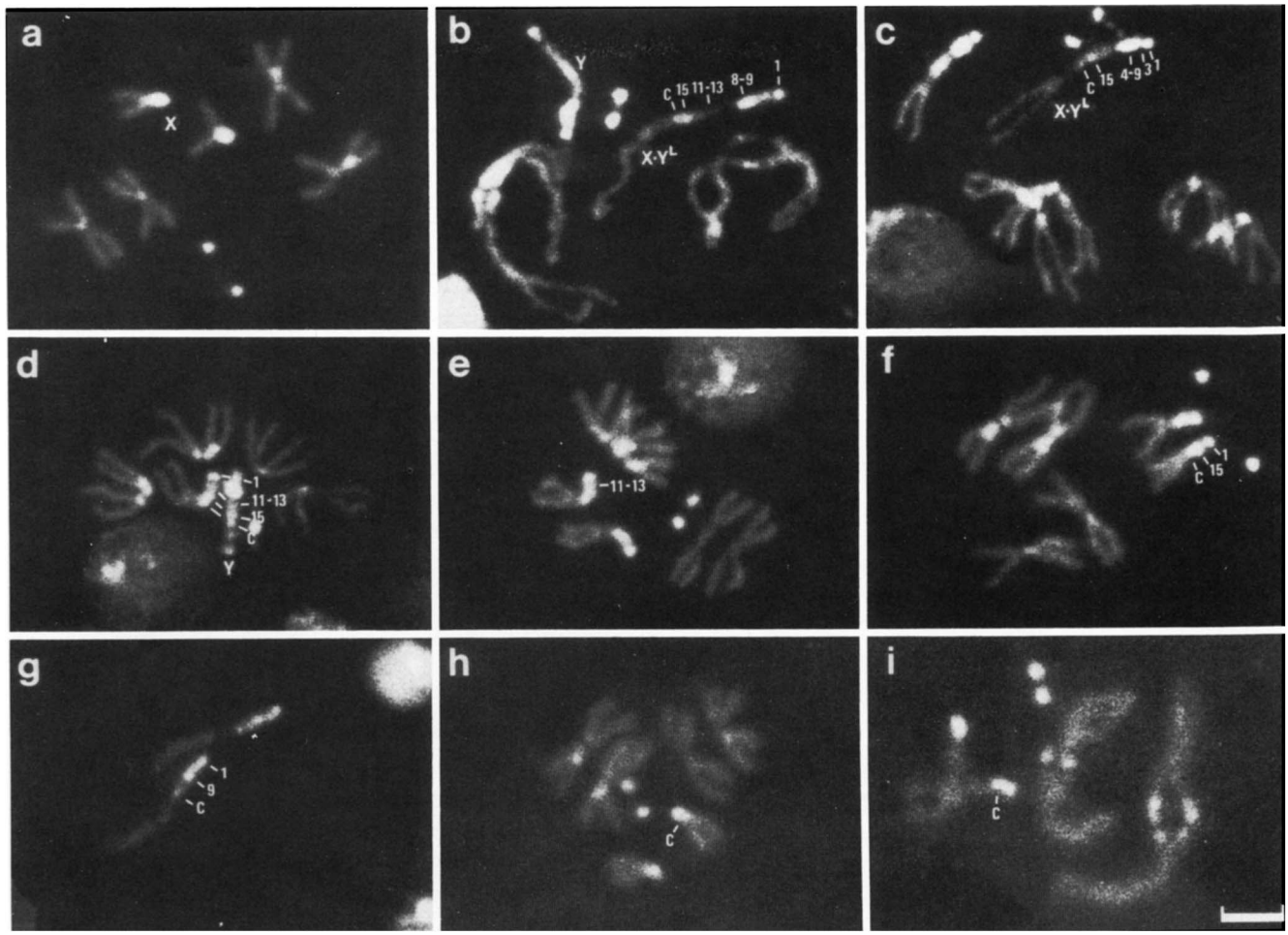
$X \cdot Y^L$ chromosome in a wild population: To test if $X \cdot Y^L$ chromosomes could be found in natural populations, males from a wild type Sydney population were mated to *C(1)DX, y f/ y*$^+$ *Y*$^{kl\text{-}1-}$ females, and the F_1 males tested for fertility. F_1 males of one bottle were fertile, showing that at least one wild male carried an $X \cdot Y^L$ chromosome, or produced such a gamete. This $X \cdot Y^L$ chromosome was obtained after sampling only 110 wild males. The chromosomes of this stock were cytologically indistinguishable from the $X \cdot Y^L$ of the LA and LC lines.

Clustering of rDNA repeat types: *X-Y* exchange results in the donation of the proximal Y^+ nucleolus organizer to the $X \cdot Y^L$. Consequently we would predict that rDNA/T1 repeats should be clustered in the distal X^+ nucleolus organizer.

Analysis of *Bam*HI and *Eco*RI digests of X^+ rDNA indicates that rDNA repeats containing the major T1 insertion are significantly, if not totally, clustered within the X^+ nucleolus organizer. *Eco*RI digestion of X^+ rDNA generates a single major band at 17 kb that is homologous to rDNA and T1 probes (Figure 6). This band is generated from all rDNA repeats containing the 5.4-kb T1 insertion.

*Bam*HI does not cut rDNA or T2 insertion sequences, but does have two sites in the T1 insertion. Hence discrete *Bam*HI bands homologous to rDNA must have T1 insertions (or unrelated sequences containing *Bam*HI sites) on either end of the fragment. The major *Bam*HI fragment homologous to rDNA and T1 sequences is 16.1 kb (Figure 6). The only way this fragment can be generated is from two neighboring rDNA repeats, both containing T1 insertions (Figure 2). Since the 16.1-kb fragment is the only major band homologous to rDNA and T1 probes, most, if not all, rDNA/T1 repeats must be adjacent to other repeats also containing T1 insertions. The numbers of adjacent rDNA/T1 repeats can be gauged by comparison with the response to the *Bam*HI fragments (5.4–4.0 kb) generated from tandem T1 arrays in the X heterochromatin (~100 copies) (HILLIKER, APPELS and SCHALET 1980).

The restriction analyses in this paper also show that other rDNA elements must be clustered within the X^+ nucleolus organizer. A 9.5-kb *Hin*dIII spacer class is totally missing from the CD X^+ chromosome, while it is a prominent band in the sister control line, CF (Figure 4, tracks a and b). Such an observation is most easily explained by a single exchange event deleting all of these 9.5-kb spacers, which must then of neces-



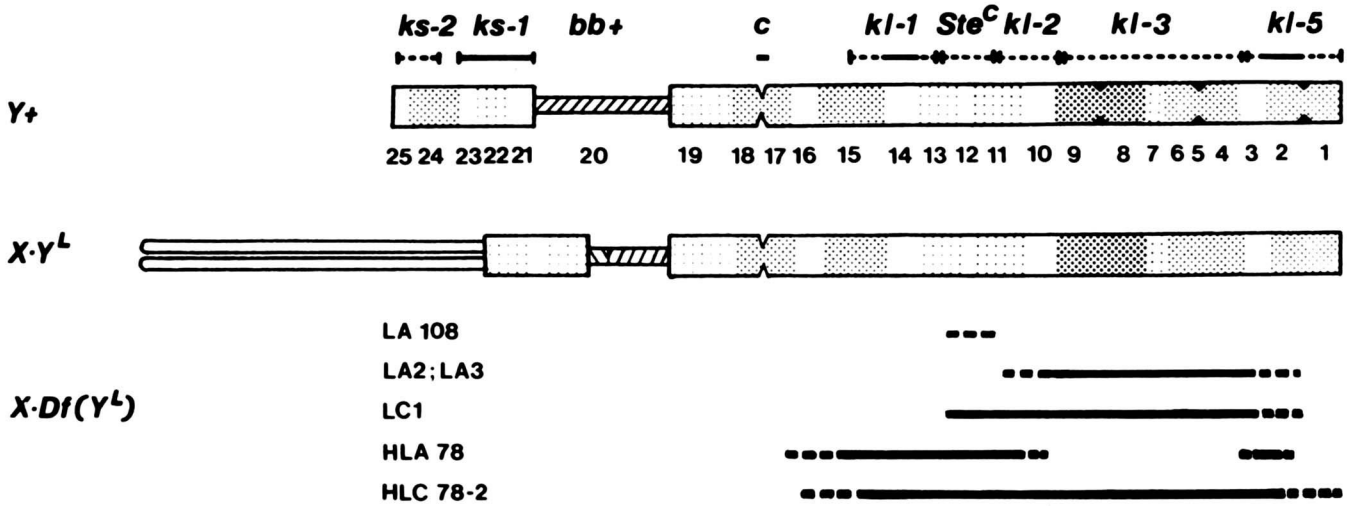


FIGURE 5.—Neuroblast chromosomes from X^+, $X \cdot Y^L$, and $X \cdot Df(Y^L)$ lines stained with Hoechst 33258. (a) CD female; (b) LAbb male; (c) LCbb female; (d) LA2 male; (e) LA3 female; (f) LC1 female; (g) HLA78 female; (h) and (i) HLC78-2 females. Bar = 5 μm. Below the photographs are maps of the Hoechst banding patterns of Y^+ and $X \cdot Y^L$ chromosomes and the genetic functions thereon. Numbers used to indicate chromosomal landmarks are those suggested by GATTI and PIMPINELLI (1983). Intensity of stippling corresponds to intensity of fluorescence when preparations are stained with 0.25 μg/ml Hoechst 33258. c = centromere. Regions of the Y^L deleted from the $X \cdot Y^L$ chromosomes of some lines (as determined by complementation and cytological analysis) are summarized at the base of the figure.

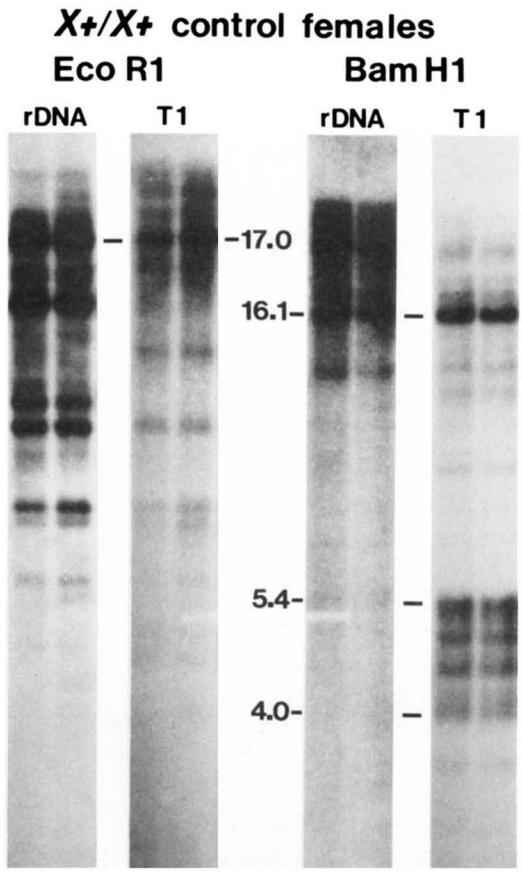


FIGURE 6.—Analysis of rDNA clustering in X^+/Y^+ females. Genomic DNA was digested with *Eco*RI or *Bam*HI and sequentially probed with rDNA (pDm238) or T1 (pC225) sequences. CD females; left hand tracks of each pair, CF females; right hand tracks. Sizes of fragments are in kilobase pairs.

sity be clustered, as there is little corresponding reduction in other spacer classes.

Spacer classes on the Y^+ chromosome must also be significantly clustered. Since some spacer classes on the Y^+ (5.25 and >6.25 kb) were not transferred to the $X \cdot Y^L$ (Figure 4), these spacer classes must be restricted to the distal Y^+ nucleolus organizer. Those Y^+ spacer classes present in large numbers on the $X \cdot Y^L$ must be primarily located in the proximal Y^+ nucleolus organizer.

DISCUSSION

A model illustrating the events by which *X-Y* exchange may mediate coevolution of the rDNA arrays is shown in Figure 7. Such exchanges result in the donation of *Y* rDNA, the proximal Y^S heterochromatin, the *Y* centromere and the Y^L to the newly formed $X \cdot Y^L$ chromosome. Infiltration of *Y* rDNA into the X^+ rDNA pool can then occur by $X \cdot Y^L$-X^+ exchange or by loss of the Y^L arm to generate a pseudo *X* chromosome. Evidence concerning predictions arising from this model is reviewed below.

If *X-Y* exchange is responsible for the coevolution of *X* and *Y* rDNA, exchange through the nucleolus

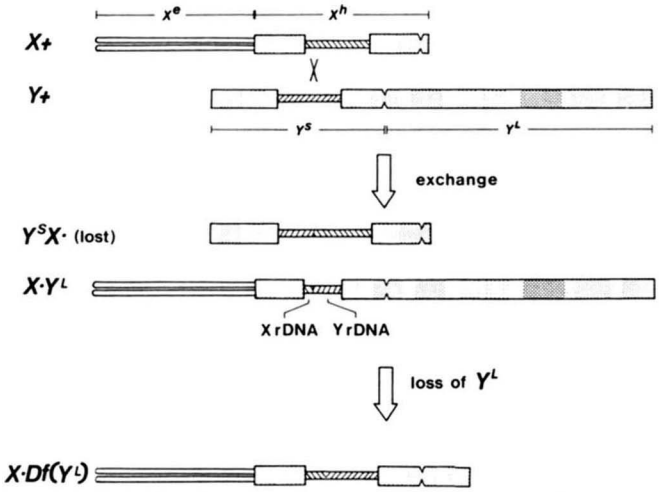


FIGURE 7.—Model of *X-Y* exchange through the nucleolus organizers and subsequent loss of the Y^L arm.

organizers of these chromosomes must occur. We have documented three *X-Y* exchanges involving *X* and *Y* rDNA. Two of these events have been described previously (COEN and DOVER 1983), the third was detected by analysis of a more extensive set of lines. The frequency of *X-Y* exchange through the rDNA and subsequent fixation of the resultant $X \cdot Y^L bb$ chromosome has been estimated at about 2×10^{-4} per gamete per generation (FRANKHAM, BRISCOE and NURTHEN 1980). This is a minimum estimate; rates of exchange through the rDNA may be four times higher than this (MADDERN 1981). We have also detected an $X \cdot Y^L$ chromosome in a natural population, demonstrating that *X-Y* exchange is not confined to laboratory stocks.

X-Y exchange through the nucleolus organizers produces a compound $X \cdot Y^L$ chromosome. To account for the normal *X* chromosome morphology, the $X \cdot Y^L$ chromosome must be unstable, and/or be at a selective disadvantage to the normal X^+ chromosome. Six of seventeen lines founded from homozygous $X \cdot Y^L$ stocks have become fixed for $X \cdot Y^L$ chromosomes with loss of all or part of the Y^L arm ($X \cdot Df(Y^L)$ chromosomes). This shows that the $X \cdot Y^L$ chromosome is prone to spontaneous loss of the Y^L. Fixation of the resultant $X \cdot Df(Y^L)$ chromosomes suggests that the $X \cdot Y^L$ was eliminated by natural selection. There is now direct evidence that the $X \cdot Y^L$ chromosome is at a selective disadvantage to the same chromosome lacking the Y^L arm (R. FRANKHAM, unpublished data).

X-Y exchange should result in recently donated rDNA (*Y* forms) being predominantly on the proximal side of the *X* nucleolus organizer and *X* forms being clustered in the distal *X* nucleolus organizer. Since rDNA repeats containing T1 insertions are only found on *X* chromosomes, this class of repeats should be clustered in the distal *X* nucleolus organizer. Analysis of rDNA/T1 repeats on the X^+ chromosomes of our control lines shows that the rDNA/T1 repeats are

clustered. Published analysis of other X^+ chromosomes is, however, equivocal. Various authors report clustering of repeats containing T1 insertions (RENKAWITZ-POHL, GLATZER and KUNZ 1981; SHARP, GANDHI and PROCUNIER 1983; KALUMUCK and PROCUNIER 1984; SALZANO and MALVA 1984), while others maintain repeats containing T1 insertions are randomly distributed within the X^+ nucleolus organizer. The lack of change in rDNA restriction pattern during rDNA magnification/reduction (DE CICCO and GLOVER 1983; PALUMBO, ENDOW and HAWLEY 1984) or compensation (DUTTON and KRIDER 1984) has been used to argue that different rDNA repeats are randomly dispersed within the X^+ chromosomal nucleolus organizer. However, these interpretations are subject to assumptions about the mechanisms of magnification and compensation that are still open to question. In fact, M. R. GILLINGS (unpublished data) has also failed to find changes in restriction patterns during magnification and compensation experiments using one of our lines, even though rDNA variants carried by this line are significantly clustered (GILLINGS 1986).

Analysis of *Eco*RI and *Bam*HI rDNA restriction patterns led HAWLEY and TARTOF (1983) to suggest that rDNA/T1 repeats were randomly distributed. However, they did not confirm that their high molecular weight rDNA bands were in fact homologous to type 1 probes. We have shown that most of the high molecular weight material is not significantly homologous to type 1 sequences. Close examination of HAWLEY and TARTOF's data shows that the 17-kb *Eco*RI band and the 16.1-kb *Bam*HI band account for equal proportions of the total rDNA response. Since the 17-kb *Eco*RI bands are generated from rDNA repeats containing the 5.4-kb T1 insertion, but the 16.1-kb *Bam*HI band is only produced from clusters of these repeats, the equality of response to the *Bam* and *Eco* bands in HAWLEY and TARTOF's experiment shows that most rDNA/T1 repeats are adjacent to other similar repeats. Despite assertions to the contrary, rDNA/T1 insertions are most probably clustered in all X^+ nucleolus organizers. *In situ* hybridization indicates that most rDNA repeats containing type 1 insertions are located in the distal region of the X^+ nucleolus organizer (HILLIKER and APPELS 1982), as predicted by the *X-Y* exchange hypothesis.

The *X-Y* exchange hypothesis predicts that *X* and *Y* chromosomes in long established stocks should share some rDNA spacer classes, coding sequence classes and classes of type 2 insertions. The X^+ and Y^+ chromosomes studied here carry *no* spacer classes in common. While this made the *X-Y* exchange easy to detect, it conflicts with the prediction. However, these sex chromosomes came from unrelated stocks, perhaps explaining this anomaly. In other stocks, *X* and *Y* chromosomes do share rDNA spacer classes (WELLAUER, DAWID and TARTOFF 1978; COEN, THODAY and DOVER 1982; BONCINELLI *et al.* 1983), internal transcribed spacers (COEN, STRACHAN and DOVER 1982) and classes of T2 insertions (ROIHA *et al.* 1983) so this prediction may be satisfied.

YAGURA *et al.* (1979) reported a single base difference in the 18 S rRNA transcribed by *X* and *Y* chromosomes. The *X* and *Y* chromosomes used in their work also came from unrelated stocks, so this may simply reflect stock differences in 18 S rRNA coding sequences. Further, the 28 S rRNAs transcribed by *X* and *Y* chromosomes are reputed to be identical (MADEN and TARTOF 1974).

The prediction above does not preclude the existence of rDNA variants unique to either the *X* or *Y* chromosomes of wild-type stocks. We would predict that such unique variants should lie in regions not regularly participating in *X-Y* exchange (i.e., in the distal portion of *X* and *Y* nucleolus organizers).

As T1 insertions do not exist on the *Y* chromosome, we must predict that they transpose into *X* chromosomal rDNA from the nearby T1 arrays in the *X* heterochromatin, otherwise they would eventually be eliminated by the mechanics of *X-Y* exchange. No evidence for transposition of T1 sequences from the *X* chromosomal heterochromatin to the rDNA has been found in our $X \cdot Y^L$ lines (GILLINGS 1986), conflicting with expectations. However, the Y^L arm may be inhibiting transposition, thereby also preventing transposition into Y^+ rDNA in X^+/Y^+ males. Alternatively, the rate of transposition may be too low to have been detected, insertions may have occurred but not altered the restriction pattern by a detectable amount, or "dysgenic" crosses may be required to mobilize these elements.

The proximal heterochromatin of the X^+ and Y^s must show homology, as this region of the *Y* chromosome is donated to the X^+ chromosome during *X-Y* exchange. *X* and *Y* chromosomes do share satellite sequences in these regions (PEACOCK *et al.* 1978; STEFFENSEN, APPELS and PEACOCK 1981). However, sequences influencing nucleolar dominance in *D. melanogaster* × *D. simulans* hybrids have been mapped to the proximal *X* heterochromatin, but to a different region of the *Y* chromosome (DURICA and KRIDER 1978). Close examination of the data in their paper suggests that such dominance is more easily explained by dependence on the rDNA region itself. This is in accord with what is now known about the behavior of the rDNA spacer in cross-species transcription systems (COEN and DOVER 1982; KOHORN and RAE 1982; MILLER, HAYWARD and GLOVER 1983; REEDER and ROAN 1984). Whatever the case, DURICA and KRIDER's experiments merit reinvestigation.

The 1.688 satellite of *D. melanogaster* was originally

reported as being located primarily on the sex chromosomes (PEACOCK *et al.* 1978). A more recent report using a cloned 1.688 sequence as a probe suggests that this satellite may be restricted to the proximal *X* heterochromatin (D. L. BRUTLAG personal communication, cited in HILLIKER and APPELS 1982), an observation that would be fatal to the *X-Y* exchange hypothesis as presented in Figure 7. If this were the case, for the hypothesis to still be tenable, the coevolution of *X* and *Y* rDNA would have to proceed by further exchange of $X \cdot Y^L$ chromosomes with normal *X* chromosomes, thereby transferring some *Y* rDNA into the *X* nucleolus organizer. However, the resolution of *in situ* hybridization to *Drosophila* mitotic chromosomes is less than ideal, and the discrepancies between cloned probes and those purified from gradients may be caused by sequence complexity of the 1.688 satellite. While adjacent repeats are highly homologous, independent 1.688 satellite clones can have as little as 60% homology (MIKLOS and GILL 1982). If variants of the 1.688 satellite were present on the *X* and *Y* chromosomes divergence of these sequences would be expected, with resulting complications in hybridization analysis.

This paper presents evidence that the coevolution of *X* and *Y* rDNA proceeds via *X-Y* exchange. Are there other mechanisms that could explain this coevolution? While the processes of natural selection and gene conversion undoubtedly influence rDNA evolution, neither can adequately explain the coevolution of *X* and *Y* rDNA. It is highly unlikely that selection could fix the same mutants within *X* and *Y* rDNA arrays, and the presence of T1 rDNA insertions on the *X*, but not the *Y* chromosome argues against gene conversion operating between these chromosomes. We suggest that occasional *X-Y* exchange events allow *Y* rDNA to infiltrate the *Y* chromosome nucleolus organizer via an $X \cdot Y^L$ intermediate. We have confirmed several predictions arising from this hypothesis. Other predictions are currently being evaluated.

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