Genet. Res., Camb. (1990), **58**, *pp.* 7–13 *With 3 text-figures Printed in Great Britain*

Population genetics of the Y chromosome of *Drosophila melanogaster*: rDNA variation and phenotypic correlates

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(*Received 13 July 1990 and in revised form 16 October 1990*)

Summary

One means of examining the evolutionary significance of molecular variation on the Y chromosome is to identify phenotypes specifically affected by Y-linked genes, and to quantify the phenotypic variation and its correlation to the molecular variation. The functional importance of the Y-linked array of rRNA genes is demonstrated by the ability of Y chromosome to rescue X-linked *bobbed* lethal alleles, whose lethality is seen in homozygous females. Because low numbers of X-linked rDNA gene copies result in increased developmental time and shortened bristles, and because there is considerable natural variation in Y-linked copy number, a careful examination of Y-linked variation in these two traits may uncover a mode of selection acting on the multigene family. In this study, 36 Y-chromosome replacement lines were tested to detect subtle variation in bristle phenotypes and developmental rates. Correlations among these traits, rDNA gene copy number, and intergenic sequence length were quantified. The absence of significant correlations between phenotypic characters and rDNA copy number or intergenic sequence length suggests that the extant molecular variation in Y-linked rDNA can have at most very small selective effects.

1. Introduction

A prerequisite for Darwinian evolution is the presence of heritable variation affecting survival or reproduction. If variation among Y chromosomes can be shown to result in phenotypic variation, this would open the possibility of selective differences among Y chromosomes. One of the classes of genes on the Y that may admit this possibility is the rDNA array. Deficiencies of X-linked rDNA have been associated with short bristles (the 'bobbed' phenotype), longer developmental time, and in extreme cases, abnormal segregation. Observation of the short bristle phenotype led to the discovery of the *bobbed* locus, and the early identification of X and Y-linked factors affecting *bobbed* (Stern, 1927). Posterior macroscutellar bristle length is now known to be highly correlated with the number of functional X-linked rDNA copies (Dutton & Krider, 1985), and the effect of variation in X-linked rDNA copies on abdominal bristle number is influenced by the number of Y-linked rDNA copies (Frankham *et al.* 1980).

The expression of rDNA might be regulated by differential replication and/or transcription. Several lines of evidence suggest that the rDNA array is not dosage compensated. X^{bb}/X^{bb} females have longer bristles than X^{bb}/O males, and when X/X and X/O flies have the same total copy number of rDNA, their bristle lengths are indistinguishable (Dutton & Krider, 1985). Over-replication of rDNA may also be important in the expression of the gene family, since X/O and X/X^{bb} may have 50–100% more rDNA than predicted from copy numbers of X/X genotypes. This phenomenon has been called compensation (Tartof, 1971, 1973). The rate of transcription of rDNA depends on the efficiency of the promoter, and there is strong interspecific conservation of promoter sequences in the intergenic spacer (Tautz *et al.* 1987; Hayward & Glover, 1989). Whether differences between the expression of X- and Y-linked rDNA is due to different efficiencies of transcription initiation is not known.

Selection experiments have been very useful in providing information about the expression and rates of change in the rDNA array. Frankham *et al.* (1980) selected for high and low abdominal bristle number in an essentially homozygous base population, and got very good response for decreased bristle number in females. This response was shown to be due to the occurrence of a bobbed mutation, generated by

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unequal exchange in the rDNA array. The lack of response in males, and the maintenance of Ybb^+ in the selected lines, suggests that the Y chromosome may be less prone to expression, and hence less susceptible to adaptive evolutionary change. Cluster *et al.* (1987) selected lines for rapid and slow developmental rates, and observed a remarkable response in the X-linked rDNA array. Among the 104 X chromosome arrays examined, all had a 5·1 kb spacer, and the fast-selected lines had a high frequency of long (9+ kb) spacers, while the slow-selected lines tended to have a shorter mean spacer length. There were 88 Y-chromosomal arrays that were also assayed by Southern analysis, and they neither had the 5·1 kb spacer common to all X chromosomes, nor was there a significant tendency for the longer spacers to be found in the faster developing lines. This also suggests that while the Y-linked rDNA array is expressed, it is less likely to be involved in response to selection that targets the rDNA array.

The Y chromosome replacement lines that we have constructed provide an ideal opportunity to test whether variation among Y-linked rDNAs have similar phenotypic effects on bristle length and developmental rate as does variation among X-linked rDNA genes. In this study, developmental rates, bristle lengths, and Southern blots were examined in order to test correlations among phenotypic traits, rDNA spacer length, and rDNA gene copy number. Existence of such correlations would provide evidence for the opportunity for selective differences among Y chromosomes.

2. Materials and methods

(i) Drosophila *stocks*

The 72 Y-chromosome replacement lines were constructed following the procedure described in Clark (1987). There were a total of 36 distinct Y chromosomes among these lines, with each Y chromosome in two different lines having different genetic backgrounds. Flies whose only rDNA was Y-linked were produced by crossing males from the replacement lines to inbred virgin females bearing the $Df(1)bb^{l\cdot158}y$ chromosome (obtained from the Pasadena stock centre and sib-mated eight generations before test-crossing).

(ii) *Bristle phenotype*

Macroscutellar bristle length of a minimum of ten $Xbb^{l\cdot158}$ males from each of two vials for each line were measured with an ocular micrometer on a dissecting microscope. The data table consisted of measurements from an average of 12·43 flies from each of (36 Y chromosomes) × (2 backgrounds) × (2 replicate vials), giving a total of 1790 measurements. These data were analysed by a random effects analysis of variance:

$$L_{ijkl} = \mu + Y_i + B_j + YB_{ij} + R_{ijk} + \epsilon_{ijkl},$$

where L_{ijkl} is the bristle length of the lth male from the kth replicate vial of the jth background and ith Y chromosome line. The grand mean is μ, and effects of Y_i for the ith Y chromosome, B_j for the jth background, YB_{ij} for interactions between Y chromosomes and the genetic background, R_{ijk} for the kth replicate vial nested in the ith line and jth background. The error term is ϵ, and is assumed to be normally distributed with mean 0.

(iii) *Analysis of developmental time*

Virgin 3- to 4-day-old females of $bb^{l\cdot158}$ were crossed to males of both sets of Y replacement lines. Inseminated females were placed in vials, five females per vial, and transferred every day for 9 days. The first vial was discarded, and progeny of the remaining vials were scored by sex and yellow body phenotype every day beginning on the eighth day after eggs were laid. Vials were maintained in an incubator at 25 °C with a 12 h light/dark cycle. The data consisted of counts of progeny organized as (36 Y chromosomes) × (2 genetic backgrounds) × (2 replicate vials) × (8 transfers), with counts of these 1152 vials scored every day from day 8 to day 15 (when virtually all had emerged). The data were examined using the following random effects ANOVA model:

$$D_{ijkl} = \mu + Y_i + B_j + LB_{ij} + R_{ijk} + \epsilon_{ijkl},$$

where D_{ijkl} is the egg-to-adult developmental time of the lth fly in the kth replicate ($k = 1, 2$), with the jth background and the ith Y chromosome. The developmental rate is viewed to be a consequence of effects of the ith Y chromosome, the jth background, an interaction between the Y chromosome and background, a replicate effect, R_{ijk}, nested within the line and background effect, and a random error ϵ. Significance of these effects was determined by constructing an F statistic, using the appropriate compound term in the denominator.

(iv) *Southern analysis of rDNA spacer length*

Total genomic DNA was isolated from males of each line (bearing the $bb^{l\cdot158}$) following the protocol of Bender, Spierer & Hogness (1983), with modifications described in Clark & Lyckegaard (1988). Restriction digestion with *Hae* III, agarose gel electrophoresis, capillary transfer and probing of membrane-bound DNA was done following the protocols described in Clark & Lyckegaard (1988). The probe that was used was *pDm103HH2* (provided by Scott Williams) and contains the rDNA intergenic sequence flanked by a *Hind* III and *Hae* III site. Autoradiographs were scanned with an LKB Ultroscan XL laser densitometer. Fragment lengths were estimated from migration distances with the regression approach of Schaffer & Sederoff (1981). Mean intergenic sequence lengths were estimated from two independent samples

Table 1. *Means of bristle lengths, developmental times, and IGS spacer lengths. Bristle lengths are the lengths of the macroscutellar bristles in millimetres. An average of 49·7 flies were measured for each mean. Developmental times are the difference in fractions of a day between the egg-to-adult time of* yellow *males (whose only rDNA is Y-linked) and wild male sibs. An average of 1540 flies were scored for each line. Spacer lengths were estimated from densitometric scans of Southern blots of genomic DNA digested with* Hae *III and probed with the IGS sequence*

Y-line	Bristle length	Developmental time	Mean IGS length (bp)
R1	0·396 ± 0·005	0·209 ± 0·066	6150
R2	0·395 ± 0·004	0·075 ± 0·038	5266
R3	0·400 ± 0·003	0·251 ± 0·059	5443
R4	0·401 ± 0·003	0·148 ± 0·038	5057
R5	0·389 ± 0·004	0·131 ± 0·085	5079
R6	0·395 ± 0·002	0·177 ± 0·050	6025
R7	0·381 ± 0·006	0·101 ± 0·050	6042
R8	0·394 ± 0·003	0·333 ± 0·089	5633
R9	0·391 ± 0·003	0·171 ± 0·122	5542
R10	0·393 ± 0·002	0·176 ± 0·038	5215
R11	0·396 ± 0·004	0·178 ± 0·071	7203
R12	0·401 ± 0·003	0·087 ± 0·061	6124
R13	0·398 ± 0·002	0·100 ± 0·058	6135
R14	0·407 ± 0·003	0·121 ± 0·058	5103
R15	0·404 ± 0·003	0·189 ± 0·087	5936
R16	0·402 ± 0·003	0·116 ± 0·089	6865
R17	0·391 ± 0·004	0·032 ± 0·065	6691
R18	0·401 ± 0·002	0·207 ± 0·060	5383
R19	0·393 ± 0·002	−0·022 ± 0·074	5496
R20	0·407 ± 0·003	0·083 ± 0·058	6375
R21	0·404 ± 0·003	0·089 ± 0·104	6250
R22	0·404 ± 0·003	0·008 ± 0·049	5662
R23	0·398 ± 0·002	0·159 ± 0·037	5588
R24	0·394 ± 0·004	0·033 ± 0·050	5132
R25	0·390 ± 0·003	0·100 ± 0·051	4520
AH41	0·412 ± 0·003	0·177 ± 0·052	5431
AH198	0·400 ± 0·003	0·342 ± 0·081	4579
Egål	0·406 ± 0·002	0·124 ± 0·045	5436
Fan6	0·403 ± 0·002	0·181 ± 0·052	6270
GB13	0·394 ± 0·003	0·101 ± 0·067	4449
GB41	0·403 ± 0·004	0·094 ± 0·032	5411
Hikone	0·384 ± 0·004	0·215 ± 0·187	5711
Samarkand	0·392 ± 0·003	0·124 ± 0·079	7161
Still-4	0·398 ± 0·003	0·159 ± 0·038	6028
Wd-4	0·388 ± 0·003	0·161 ± 0·050	6859
Wd-7	0·389 ± 0·003	0·027 ± 0·059	4321

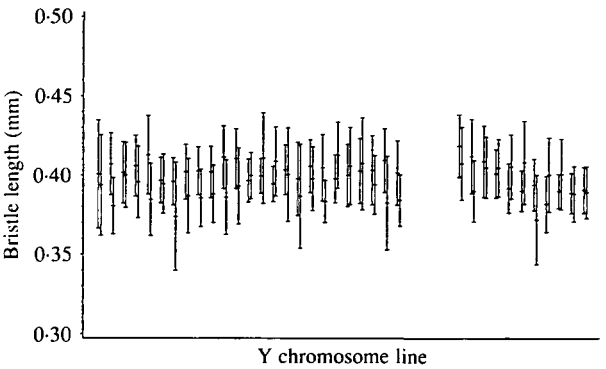

Fig. 1. Lengths of macroscutellar bristles of 72 Y-chromosome replacement lines. The 36 pairs of bars represent the 36 Y chromosomes that are in the same two co-isogenic backgrounds. Means ± S.E. are plotted.

by weighting the fragment size by the density of the autoradiograph and summing over fragment lengths.

3. Results

(i) *Bristle length variation*

Table 1 reports the mean macroscutellar bristle lengths for males in each line. Untransformed measures had variances that were homogeneous and independent of the means, so subsequent analyses were performed on untransformed data. The analyses of variance, given in Table 2, indicate a consistent lack of significant contribution of Y chromosomes to bristle length. There was a significant effect of the genetic background on bristle length in the Rothrock lines and the pooled

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Table 2. *Analysis of variance of bristle length. Y refers to a Y-chromosome replacement line effect, and Background refers to an effect of the genetic background. Units are those of the ocular micrometer (78 units = 1 mm)*

	Rothrock		Diverse		Pooled	
Source	D.F.	MS	D.F.	MS	D.F.	MS
Y	24	5·34	10	17·97	35	8·86
Background	1	90·51***	1	0·01	1	65·12**
Y × Background	24	7·32	10	9·96	35	8·78
Replicate	50	16·07***	20	8·08**	70	13·79***
Error	1203	2·26	445	2·16	1648	2·21

** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

Table 3. *Analysis of variance of developmental time, as defined in Table 1*

	Rothrock		Diverse		Pooled	
Source	D.F.	MS	D.F.	MS	D.F.	MS
Y	24	0·168	10	0·154	35	0·164
Background	1	2·189***	1	0·274	1	2·369***
Y × Background	24	0·188	9	0·094	34	0·165*
Replicate	46	0·081	19	0·095	65	0·085
Error	602	0·110	242	0·087	844	0·129

* $P < 0{\cdot}05$; *** $P < 0{\cdot}001$.

data, which serves to show that the experiment was sufficiently replicated to have the sensitivity to detect genetic variation in bristle length. In fact, the mean square for the pooled background effect was 7·35 times that for the Y chromosome effect. Despite this sensitivity, there were neither Y chromosome effects nor interactions between the Y chromosomes and the genetic background that were significant. Figure 1 shows the means ± 2 S.E. of the bristle lengths for each line on each genetic background.

(ii) *Developmental time*

Mean developmental times are given in Table 1, and results of the analysis of variance are given in Table 3. The developmental times can be analysed in terms of either the absolute time from egg to adult or in terms of the time relative to other genotypes within the same vial. The analyses reported in Table 3 are for the difference between the developmental time of the *yellow* males minus the developmental time of wild siblings. The analyses of Table 3 shows that the Y chromosome did not have a significant effect among either the Rothrock lines, the diverse lines nor in the pooled analysis, however in the pooled analysis, there was a significant Y-background interaction. The magnitude of the background effect was 14·44 times the Y chromosome effect, according to the mean squares of the pooled analysis. Figure 2 shows the

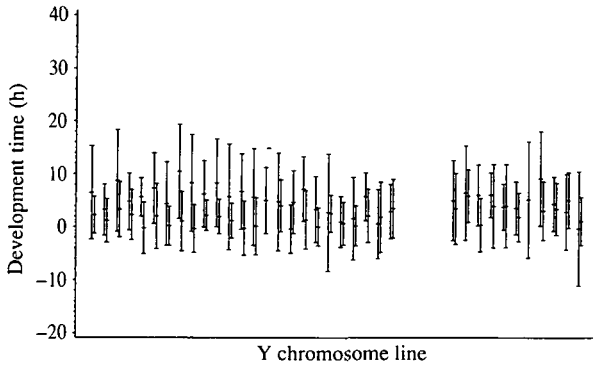

Fig. 2. Relative developmental time from egg to adult of 72 Y-chromosome replacement lines. The 36 pairs of bars represent the 36 Y chromosomes that are in the same two co-isogenic backgrounds. Means ± S.E. of the difference in developmental time between $bb^{t\text{-}158}$ males (which have the yellow body phenotype are deficient in X-linked rDNA) and wild male siblings are plotted.

means ± 2 S.E. of the difference in developmental times of *yellow* males (whose only rDNA is Y-linked) and wild siblings. When the analysis of variance was performed on the absolute developmental time, none of the Y chromosome effects were significant (table not shown). Replicates of the developmental rate tests were successive broods of the same female, so it was important to test for a maternal age effect on developmental rate. Another analysis of variance indicated that successive broods were homogeneous in developmental time.

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(iii) *Length variation of rDNA intergenic sequence*

Densitometric scans of the Southern blots of genomic DNA digested with *Hae* III and probed with the cloned IGS sequence (*pDm103HH2*) were used to calculate the mean intergenic sequence length, as illustrated in Figure 3. Because the genomic DNA was obtained from males that carried the $bb^{l\text{-}158}$ rDNA deficiency, the variation that is seen reflects Y-linked rDNA copies. Table 1 gives the means of two independent assays of mean IGS length.

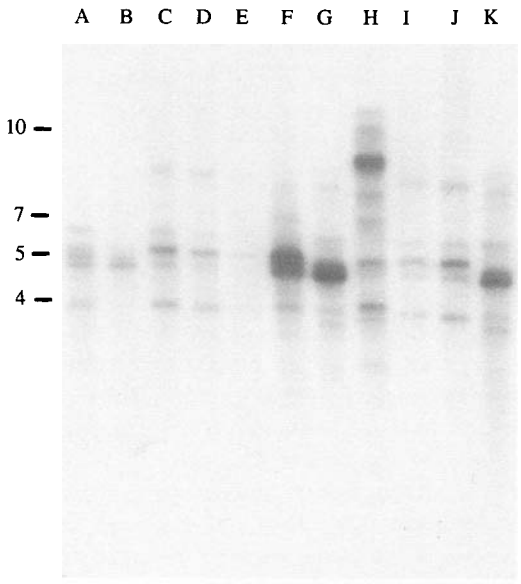

Fig. 3. Southern blot of genomic DNA from $bb^{l\text{-}158}$ males digested with *Hae* III and probed with the intergenic sequence clone *pDm103HH2*. This is one of the autoradiographs that were scanned to produce estimates of average intergenic sequence length. Lanes A–K represent lines R3, R4, R5, R6, R7, R8, R9, R10, R11, R12, and R13. The scale on the left is in kilobases, determined from known standards (not shown).

(iv) *Correlations among bristle traits, fertility, copy number and intergenic sequence length*

Table 4 reports the Pearson product-moment correlations among line means of six traits, including bristle length, fecundity, segregation, copy number, developmental time, and intergenic sequence length. Male fecundity estimates were taken from Clark (1990), segregation estimates from Clark (1987), and copy number estimates were obtained from Lyckegaard & Clark (1989). There was a significant positive correlation between developmental time and fecundity, implying that lines with a longer mean developmental time had higher fecundity. Developmental time and fecundity were measured in completely independent tests, but the two may be physiologically related. Density was not strictly regulated in the developmental time assay, and the densities of vials in the developmental time test were not significantly heterogeneous. Consequently, there was no significant correlation between density and developmental time ($r = -0{\cdot}064$, calculated from only the developmental rate data). The significant positive correlation between male fecundity and developmental time found in Table 4 was not an experimental artifact of retarded development in more fecund lines.

The only other significant correlation was between copy number and mean intergenic sequence length, and this correlation was also positive. An artifactual correlation could be produced in this case by the fact that the rDNA arrays with greater IGS lengths may have a stronger signal when the slot blot is probed with the complete rDNA sequence. We re-probed the slot blots of Lyckegaard & Clark (1989) with the 1·7 kb *Hind* III–*Eco*R I fragment of *pDmr.a51#1* (provided by Sharyn Endow) corresponding to the 18S rDNA gene, and estimated the relative copy numbers from densitometric scans of these autoradiographs. The correlation between estimates of copy number from the two probings was 0·848 ($P <$

Table 4. *Correlations among line means of Y-replacement lines. The upper figure is the Pearson product-moment correlation coefficient among the line means, and the lower figure is the probability of obtaining a value of greater magnitude by chance under the null hypothesis of zero correlation*

	Male fecundity	Segregation	Copy number	Developmental time	IGS length
Bristle length	−0·211 (0·074)	0·026 (0·830)	−0·077 (0·530)	−0·203 (0·090)	−0·046 (0·700)
Male fecundity		−0·060 (0·615)	−0·050 (0·688)	0·457 (< 0·001)	−0·061 (0·608)
Segregation			0·007 (0·954)	0·001 (0·990)	−0·016 (0·891)
Copy number				0·115 (0·351)	0·284 (0·019)
Developmental time					−0·004 (0·973)

https://doi.org/10.1017/S0016672300029554 Published online by Cambridge University Press

0·0001), and the correlation between the copy number estimates from the 18S probing and the IGS spacer length was 0·289 ($P = 0·019$). We conclude that the positive correlation between copy number and spacer length is not an experimental artifact of the hybridizations.

For the purposes of this study, the most important point of Table 4 is that there were no significant correlations between the molecular variation and phenotypic traits like bristle length or developmental time. When Spearman rank correlations were calculated, only fecundity and developmental time were significantly correlated.

4. Discussion

The observation that the Y chromosome of *Drosophila melanogaster* has several functions provides an opportunity for natural selection to operate. There is extensive sequence variation in Y-linked rDNA, in terms of restriction site variation, length of the intergenic sequence (Coen *et al.* 1982; Williams *et al.* 1987), copy number (Lyckegaard & Clark 1989), and presence of type II inserts (Long & Dawid 1980). The fact that wild Y chromosomes can rescue Xbb^{l} chromosomes shows that the Y-linked rDNA genes can be active, and the fact that low copy number Y chromosomes can produce a bobbed phenotype opens the possibility that the Y chromosome may serve as a target for natural selection. The germline increase in rDNA copy number, thought to be mediated by unequal sister chromatid exchange, requires at least part of the Y chromosome (Komma & Endow 1987). Because magnification occurs before the adult phenotype of bobbed is expressed, the low copy number must be sensed by abnormal or failed pairing at an earlier stage (Hawley & Marcus 1990). The importance of rDNA for sex chromosome pairing was dramatically demonstrated by McKee & Karpen (1990) when they rescued normal pairing in an rDNA-deficient X chromosome that had a single copy of the rDNA repeat inserted by P-element-mediated germline transformation. Deficiency mapping has shown that, the bulk of the chromosome is necessary for male fertility (Gatti & Pimpinelli 1983), providing an obvious target for natural selection. Another function borne by the Y chromosome is the suppressor of *Stellate*, which has the dual function of regulating the steady state levels and splicing of transcripts of the X-linked *Ste* array (Livak, 1990). Finally, the Y chromosome has elements involved in the control of position effect variegation (Dimitri & Pisano, 1989).

Despite the array of essential functions carried by the Y chromosome of *D. melanogaster*, a number of studies have failed to find evidence for phenotypic variation. No quantitative trait has been sited to have an important component attributed to the Y chromosome (Williamson, 1976), and a massive attempt to find additive genetic variance in sex ratio found none (Toro & Charlesworth, 1982). Our studies of Y-replacement lines have indicated a remarkably low level of phenotypic variation associated with the Y chromosome, including male fertility (Clark, 1990), sex ratio (Clark, 1987), and net fitness (Clark & Lyckegaard, 1990).

The approach of this study in detecting Y-linked phenotypic variation was to target a phenotype with a clear mechanistic relation to genes known to occur on the Y. Males with a sufficiently low number of rDNA copies on both the X and Y chromosome will exhibit the bobbed phenotype. The purpose of this study was to determine whether naturally occurring variation in Y-linked rDNA copy number was associated with the bobbed phenotype. In order to maximize the sensitivity of the test, the males that were tested carried the X-linked $bb^{l\text{-}158}$ deficiency, and both bristle length and developmental time were measured. The lack of variation in bristle lengths, and the lack of a Y chromosome effect on developmental time indicate that even the Y chromosome with the lowest copy number produces sufficient rRNA to alleviate a phenotypic defect. This contrasts with *D. mercatorum*, which has phenotypically significant variation attributable to the Y (Templeton *et al.* 1985). The lack of Y-linked phenotypic variation that we observed in *D. melanogaster* is consistent with the results of the artificial selections that Cluster *et al.* (1987) performed. Selection for fast development results in X-linked rDNA arrays with longer intergenic spacers on average than lines selected for slow development, while differences seen in the frequencies of Y-linked rDNA patterns were compatible with the expectation under neutral genetic drift. Similarly, the selections of Frankham *et al.* (1980) for bristle number resulted in a greater response in females than males, and compound X–Y translocation chromosomes were recovered (Coen & Dover, 1983). It appears that although the Y-linked rDNA can be expressed, its variants do not exhibit phenotypic variation (except in rare deficiencies), and is hence less likely than the X-linked array to be the target of natural selection. Despite these negative findings, there is one report of an rDNA deficient Y chromosome that appeared to cause an increased rate of rRNA synthesis (Clark *et al.* 1977), demonstrating the potential for a Y chromosome effect on rRNA expression. Similarity between X- and Y-linked rDNA arrays may be the result of exchange events, even if those events occur very rarely (Ohta & Dover 1983). England *et al.* (1988) demonstrated that X–Y exchange events influence the structure of variation along the rDNA array when they showed that proximal rDNA repeats of the X chromosome are the most Y-like, and that the type I inserts, which are found only on the X chromosome, are clustered toward the centromere. The survey of Williams *et al.* (1987) identified greater geographic diversity in Y-linked rDNA than in X-linked rDNA, which they interpreted as evidence for

selective constraints operating only on the X-linked array.

The work of McKee & Karpen (1990), demonstrating the importance of the rDNA arrays for sex chromosome pairing in *D. melanogaster*, implies that Y-linked rDNA is constrained to retain sequence similarity to the X-linked array. This constraint may be of greater evolutionary significance than the expression of rRNA by the Y-linked genes. The observation that the Y chromosome of *D. simulans* contains a tandem array of just the spacer (Lohe & Roberts, 1990) is consistent with the idea that the role of Y-linked rDNA in pairing is more important than its transcriptional potential.

We thank Sharyn Endow and Scott Williams for sharing plasmid clones, and Nancy Dougherty, Sharyn Houtz, and Susan Sweeney for technical assistance. This study was supported by grants HD00743 and HD21963 from the National Institutes of Health.

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