Title

Correlations between development rates, enzyme activities, ribosomal DNA spacer-length phenotypes, and adaptation in Drosophila melanogaster.

Journal

Proceedings of the National Academy of Sciences of the United States of America, 84(2)

ISSN

0027-8424

Authors

Cluster, PD
Marinković, D
Allard, RW
et al.

Publication Date

1987

DOI

10.1073/pnas.84.2.610

Correction. In reference to the article "Correlations between development rates, enzyme activities, ribosomal DNA spacer-length phenotypes, and adaptation in *Drosophila melanogaster*" by P. D. Cluster, D. Marinkovic, R. W. Allard, and F. J. Ayala, which appeared in number 2, January 1987, of *Proc. Natl. Acad. Sci. USA* (**84**, 610–614), the following error should be noted. The first author's initials are correctly cited on the paper (P. D. Cluster) but are incorrect in the index (p. v) and the Table of Contents (p. iv), where P. E. should be changed to P. D.

Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 610–614, January 1987
Population Biology

Correlations between development rates, enzyme activities, ribosomal DNA spacer-length phenotypes, and adaptation in *Drosophila melanogaster*

(restriction fragment-length polymorphisms/regulatory gene variation/evolution/population genetics)

P. D. CLUSTER, D. MARINKOVIĆ*, R. W. ALLARD, AND F. J. AYALA

Department of Genetics, University of California, Davis, CA 95616

Contributed by R. W. Allard, September 26, 1986

ABSTRACT **Selection for "fast" preadult development rate among the progeny of flies collected in a natural population of *Drosophila melanogaster* produced a line that developed more rapidly than a line selected for "slow" preadult development rate. Assays for enzyme activity levels showed that the activities of α-glycerophosphate dehydrogenase, alcohol dehydrogenase, and malic enzyme were higher in the fast than in the slow line, but that the activity of superoxide dismutase was lower in the fast line. Differences in the frequencies of spacer-length phenotypes of X chromosome-linked rRNA genes (rDNA), which developed between the lines during the selection process, are larger than can be explained on the basis of genetic drift alone. Long rDNA spacers had high frequency in the fast line; short spacers, in the slow line. We conclude that enzyme levels affected adaptation under the selective regimes imposed and that the different X-linked rDNA spacer-length phenotypes are either adaptive in themselves or that they mark chromosomal segments carrying genes relevant to adaptation.**

Interest in the role that regulatory genes play in adaptation has increased in recent years. In insects, regulatory variation for enzyme activities has frequently been found both within and among individuals (1–6); thus, for example, variation in the activity of six among seven enzymes studied has been reported to be correlated with preadult development rates in *Drosophila melanogaster* and *Drosophila subobscura* (7, 8). Although allozyme variants have been implicated as sources of variation for enzyme activities (2, 5, 7, 8) and for development rates (7–9), variation in enzyme activity has also been reported in stocks that are identical to each other for the chromosome carrying the structural locus but differ in other chromosomes, which demonstrates that regulatory genes other than structural allozymes may also be responsible for variation in enzyme activities (1, 5).

rRNA gene (rDNA) mutants have been reported to be associated with retarded development in *D. melanogaster* (10), *Drosophila hydei* (11), and *Drosophila mercatorum* (12, 13), and it has been suggested that the slower rates of development may be due to decreased metabolic activity resulting from reduced numbers of functional rRNA genes. The transcriptional efficiency of the rDNA might also affect development rates. There is evidence that rDNA spacers may modify this transcriptional efficiency (14–19).

In *D. melanogaster*, natural populations are usually highly polymorphic for rDNA spacer-length phenotypes; also different phenotypes often differ sharply in frequency in different ecogeographic regions (P.D.C., R.W.A., and A. A. Hoffmann, unpublished data). In *D. melanogaster* 150–250 rRNA genes form tandem arrays in both X and Y chromosomes that segregate as allelic Mendelian units (ref. 20; P.D.C., R.W.A., and A. A. Hoffmann, unpublished data). Coding sequences from which 18S and 28S rRNAs are transcribed are interspersed with intergenic spacers (Fig. 1). In the experiment reported in this paper, we selected for "fast" and "slow" development rates among the progeny of flies collected in a natural population and assayed the fast and slow development lines produced by selection for enzyme activities and for rDNA phenotypes. We found that enzyme activity levels and frequencies of X-linked rDNA spacer-length phenotypes were correlated with the selection regimes imposed. We interpret these results as indicating that enzyme activity levels and also the rDNA phenotypes or genes associated with them are implicated in adaptation to the environments created by the selective regimes.

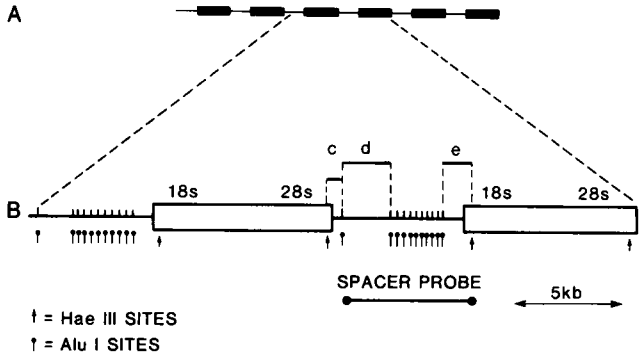

FIG. 1. (*A*) Diagram of six tandem rDNA segments of *D. melanogaster*. Solid bars represent coding sequences and connecting lines represent intergenic spacers. (*B*) Enlarged diagram of two rDNA segments. Regions coding for 18S and 28S rRNAs are labeled above open rectangles. Known *Hae* III and *Alu* I restriction sites in and near spacer regions are shown (21). Spacer regions contain internally repetitious (subrepeating) 240-bp elements, the number of which largely determines spacer length. In this diagram, each spacer contains a cluster of 10 240-bp elements. Each 240-bp element contains an *Alu* I site and a duplicate transcription initiation sequence (14, 15). Digestion of rDNA with *Hae* III produces complete spacer fragments. Double digestion of rDNA with *Hae* III/*Alu* I produces spacer fragments c, d, and e plus many copies of 240-bp elements.

MATERIALS AND METHODS

The Fast and Slow Selection Lines. The selected lines of this study were derived from more than 1000 wild flies collected in September 1984 at Jastrebec Mountain, Yugoslavia. Thirty male and 30 female wild-collected flies were placed in each of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: rDNA, ribosomal DNA (DNA encoding rRNA); kb, kilobase(s); bp, base pair(s); α-GPDH, α-glycerophosphate dehydrogenase; ADH, alcohol dehydrogenase; SOD, superoxide dismutase; ME, malic enzyme.

*Permanent address: Faculty of Science, University of Belgrade, 11000 Belgrade, Yugoslavia.

16 culture bottles (200 ml); after 6 hr the flies were removed, and the bottles were incubated at 24°C. Emergence of adult flies, which began about day 8 after egg laying, was allowed to continue until day 25, at which time ≈200 progeny had been produced in each of the 16 bottles. Thirty pairs of flies selected from adults that had emerged by day 10 were placed in each of 8 bottles to initiate the "fast development" line; similarly, 30 pairs of adults from those that emerged after day 18 were placed in each of 8 bottles to initiate the "slow development" line. Selection of flies that had emerged by day 10 was continued for 24 generations in the fast line, and selection of flies that emerged after day 18 was continued for 15 generations in the slow line. Flies were intercrossed among the 8 fast-line bottles every third generation and among the 8 slow-line bottles every second generation to reduce inbreeding and genetic drift. In the first intercrossing generation, 30 males from culture 1 were placed in a bottle with 30 virgin females from culture 2, 30 males from culture 2 were placed in a bottle with 30 virgin females from culture 3, and so on. In subsequent intercrossing generations, flies from different replicate cultures were intercrossed in such a way as to provide eventually for interchanges among all replicate cultures. Flies collected at the Jastrabec Mountain site 1 yr later were used to establish a control line; the control line was maintained by mass culture in the laboratory without intentional selection. The first half of the selection process was carried out at the University of Belgrade. The remaining generations of selection and the analyses of enzyme activities and the rDNA polymorphisms were carried out at the University of California, Davis.

Sampling Lines and Extracting Chromosomes. Three-day-old males were sampled randomly from the fast, slow, and control lines. Each male was mated with two virgin females having compound (C) chromosomes: (*i*) a C(1)RMyw female, which produces some gametes containing no sex chromosome, and (*ii*) a C(1)DXywf female, which produces gametes containing double X (DX) chromosomes in which the rDNA arrays have been deleted. X chromosome-linked rDNA spacer-length variants from sample males were assayed in X0 progeny from the first cross, and Y chromosome-linked spacer-length variants were assayed in Y(DX) progeny from the second cross.

Enzyme Activity Assays. After the above matings, each male sampled was homogenized in 0.5 ml of 10 mM KH_2PO_4/1 mM EDTA, pH 7.4, and activity was determined for two chromosome 2 enzymes [α-glycerophosphate dehydrogenase (α-GPDH; E.C. 1.1.1.8), encoded by *α-Gpdh* located at 2-20.5, and alcohol dehydrogenase (ADH; E.C. 1.1.1.1), encoded by *Adh* at 2-50.1] and two chromosome 3 enzymes [superoxide dismutase (SOD; E.C. 1.15.1.1), encoded by *Sod* at 3-34.6, and $NADP^+$ malate dehydrogenase (malic enzyme, ME; E.C. 1.1.1.40), encoded by *Men* (also called *Mdh-NADP*) at 3-51.7].

Table 1. Mean activity levels of four enzymes measured in individual males from fast, slow, and control development lines

Enzyme	Mean activities, ΔOD/2 min			*t*
	Fast (F)	Slow (S)	Control	(F vs. S)
α-GPDH	37.6	29.6	31.2	2.26*
ADH	25.9	20.4	18.9	3.14†
ME	32.5	30.5	31.1	2.33*
SOD§	22.7	28.3	27.9	3.76‡

*Significant at probability level 0.05.
†Significant at probability level 0.01.
‡Significant at probability level 0.001.
§SOD activities are ΔOD/1 min.

Homogenates were centrifuged for 5 min at 12,000 × *g* and 4°C. Enzyme assays were performed as described (3, 4) at 30°C in 0.1 ml of homogenate per 0.9 ml of reaction mixture in a Gilford model 250 spectrophotometer.

DNA Procedures. DNA was isolated from groups of four identical X0 or Y(DX) progeny and electrophoretic banding patterns of rDNA spacer-length variants were produced by established methods (21, 22). DNA isolates were restriction-digested with *Hae* III, electrophoresed in 1.2% agarose gels, transferred to Biodyne filters (Pall Ultrafine Filtration, Glen Cove, NY), probed with a ^{32}P-labeled spacer clone (pDMHH-3102), and exposed to x-ray film. Intact rDNA spacers resulting from *Hae* III digestion of single rDNA arrays fell into 15–25 length classes, which ranged from 3 to 30 kb, thereby producing diagnostic phenotypes.

Estimating Average Spacer Length of rDNA Arrays. Average spacer length was estimated for rDNA arrays by using data from densitometer scans. A weighted average spacer-length index, *I*, was calculated for each X- and Y-linked rDNA sample by a modification of the diversity index,

$$I = \sum_{i=1}^{n} S_i P_i,$$

in which *n* is the number of spacer bands in a lane, S_i is the fragment size of each band, and P_i is the proportion of signal density. S_i was estimated by comparison with standards, and P_i was calculated for each band by dividing its signal density by total signal density in its lane. Units of the indices were kilobases (kb). Mean spacer-length indices of rDNA phenotypes and of fast and slow lines were calculated as arithmetic averages.

RESULTS

The length of preadult development in the slow- and fast-selected lines was compared in three replicated experiments.

Table 2. Phenotype frequencies and length indices (SEM in parentheses) of X-linked rDNA spacer-length variants in the fast, slow, and control development lines and in the combined data set

Lines	Length index, kb	X-linked spacer phenotype																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
		Length index, kb																
		5.02 (0.05)	5.06 (0.06)	5.20 (0.11)	5.20 (0.16)	5.22 (0.05)	5.26 (0.15)	5.32 (0.13)	5.51 (0.06)	5.53 (0.04)	5.68 (0.10)	5.86 (0.12)	6.00	6.28	6.35 (0.10)	6.40 (0.12)	6.76	7.21
		Frequency																
Fast	5.80 (0.08)	0.03	0.03			0.08		0.08		0.45		0.03	0.03	0.03	0.08	0.20		
Slow	5.12 (0.05)	0.20	0.40		0.05	0.03	0.15		0.08	0.10								
Control	5.54 (0.11)	0.04	0.25	0.13		0.08	0.04		0.08	0.04	0.08	0.04				0.13	0.04	0.04
Combined	5.45 (0.05)	0.10	0.22	0.03	0.02	0.06	0.07	0.03	0.05	0.22	0.02	0.02	0.01	0.01	0.03	0.11	0.01	0.01

The number of X-linked rDNA samples was 40, 40, 24, and 104 in fast, slow, and control lines, and the combined data set, respectively. All samples from fast, slow, and control lines are included in the combined data set. Standard errors are not given when the number observed was one.

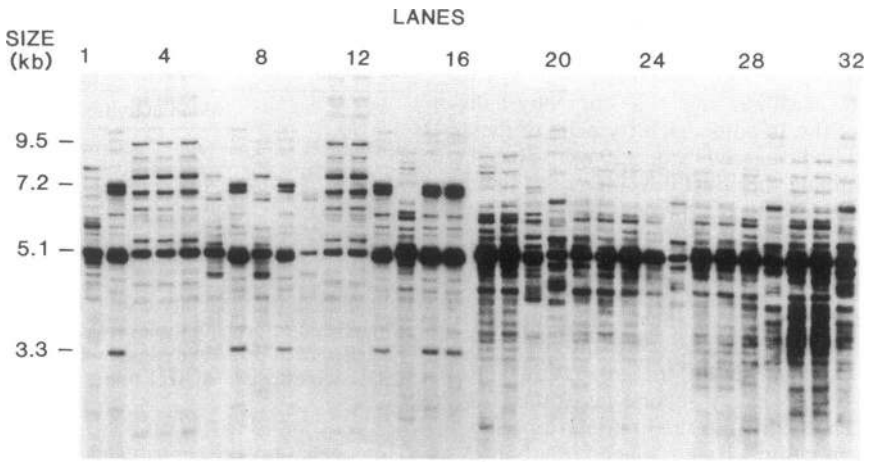

FIG. 2. Autoradiogram of Southern blot containing 32 samples of X-linked rDNA spacer-length variants; the samples were obtained randomly from the fast development line (lanes 1–16) and from the slow development line (lanes 17–32). Note the prevalence of large rDNA spacers (6.8–9.5 kb) having high copy number (dark intensity) in samples from the fast line (lanes 1–16). X-linked phenotypes (XP) of rDNA space-length variants in lanes: 1, XP5; 2, 7, 9, 13, 15, and 16, XP9; 3, 4, 5, 11, and 12, XP15; 6, 8, and 10, XP7; 14, 17, 23, 24, and 28, XP1; 18, 21, 22, 26, 27, 30, and 31, XP2; 19, XP4; and 20, 25, 29, and 32, XP6.

The weighted mean emergence time of all flies (n = 8,775) in the fast line was 11.19 days, whereas the flies (n = 6,530) in the slow line emerged in 11.74 days. Thus, flies in the fast line emerged about 13 hr earlier, on the average, than flies in the slow line; t tests showed that differences between the fast and slow lines were significant ($P < 0.001$) in all three experiments.

Mean activities of four enzymes, measured in 36, 35, and 37 males from fast, slow, and control lines, respectively, are given in Table 1. Values of t indicate that the activities of all four enzymes were significantly different in the fast vs. slow lines; activities of three of the enzymes (α-GPDH, ADH, and ME) were higher in the fast line, but the activity of one enzyme, SOD, was lower in the fast line, which is in accord with earlier unpublished results (D.M.).

rDNA was examined from individual X and Y chromosomes, and spacer-length phenotypes were scored for 104 X-linked rDNA arrays from fast, slow, and control lines. Seventeen phenotypic classes were distinguishable among the X-linked samples: these were numbered 1 through 17 in the order of increasing mean spacer-length index (X-linked phenotypic class 1, XP1, was assigned the smallest index, and class XP17, the largest index). Frequencies of X-linked phenotypes, together with mean indices of X-linked phenotypic classes in the fast, slow, and control lines are given in Table 2; indices for all data from the fast, slow, and control lines pooled into a "combined" set are also given. Ten X-linked phenotypes were observed in the fast line, 7 in the slow line, and 12 in the control. All rDNA arrays with X-linked phenotypes contained a prominent 5.1-kb spacer band. The fast line also often contained X-linked rDNA arrays with phenotypes characterized by prominent longer spacer bands, whereas the slow line did not (Fig. 2). Frequencies of X-linked phenotypes were different in fast vs. slow lines—e.g., frequencies of the two shortest X-linked phenotypes (XP1 and XP2) were low in the fast line and high in the slow line, whereas the frequencies of longer spacer phenotypes (XP9 and XP15) were high in the fast line and low in the slow line. Frequencies of these four phenotypes were generally intermediate in the control line between those in the fast and slow lines.

Spacer-length phenotypes were also scored for 88 Y chromosomes in the fast, slow, and control lines. The observed rDNA spacer phenotypes fell into 16 distinguishable classes, numbered 1 (smallest index) through 16 (largest index). Frequencies of the Y-linked phenotypes and their mean indices in the fast, slow, and control lines and combined data from all three lines are given in Table 3. Six Y-linked phenotypes were observed in the fast line, 8 in the slow line, and 6 in the control line; no band, such as the 5.1-kb band that was found in all rDNA with X-linked phenotypes, was universally present. Variances were generally larger for the Y-linked than the X-linked phenotypes, indicating that Y-linked phenotypes were less uniform for spacer-length variants than were X-linked phenotypes. Frequencies of Y-linked phenotypes differed in the fast and slow lines. The two most frequent Y-linked phenotypes (YP7 and YP12) in the fast line were not observed in the slow line, whereas the most frequent

Table 3. Phenotype frequencies and length indices (SEM in parentheses) of Y-linked rDNA spacer-length variants in the fast, slow, and control development lines and in the combined data set

Lines	Length index, kb	Y-linked spacer phenotype															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
		Length index, kb															
		5.41 (0.06)	5.56	6.32	6.33 (0.37)	6.54 (0.28)	6.60 (0.10)	7.61 (0.15)	7.64 (0.33)	7.88	7.93	8.27 (0.24)	8.47 (0.28)	8.57 (0.16)	8.72	9.48 (0.25)	10.43 (0.99)
		Frequency															
Fast	7.73 (0.15)			0.03			0.16	0.44		0.03	0.03		0.31				
Slow	7.61 (0.23)	0.09			0.06	0.03	0.22		0.06					0.34	0.03	0.16	
Control	8.84 (0.33)		0.04			0.13						0.13		0.17		0.46	0.08
Combined	7.99 (0.14)	0.03	0.01	0.01	0.02	0.05	0.14	0.16	0.02	0.01	0.01	0.03	0.11	0.17	0.01	0.18	0.02

The number of Y-linked rDNA samples was 32, 32, 24, and 88 in fast, slow, and control lines, and the combined data set, respectively. Standard errors are not given when the number observed was one.

Y-linked phenotype (YP13) in the slow line was not observed in the fast line. The fast and slow lines, in fact, carried only a single Y-linked phenotype (YP6) in common. The control line shared three Y-linked phenotypes (YP5, YP13, and YP15) with the slow line but none with the fast line.

The estimate of phenotypic frequencies in the original collection obtained by pooling all data from the fast, slow, and control lines into the combined data set was used as a standard in goodness-of-fit tests of the statistical significance of changes in rDNA phenotypic frequencies that occurred over the course of the fast and slow selection processes (Table 4). Observed and expected numbers of X-linked phenotypes did not differ significantly between the control line and the combined data set, and they differed only slightly, but significantly ($P = 0.05$), for the Y-linked phenotypes. However, differences between the combined set and the fast line, and also between the combined set and the slow line, were significant ($P = 0.001$ and 0.005, respectively) for the X-linked and Y-linked phenotypes.

To determine the effect of genetic drift on frequencies of rDNA phenotypes during the course of selection, expected standard deviations in frequencies were calculated from the binomial distribution,

$$\Delta p = \sigma_p = (pq[1 - (1 - 1/n)^t]^{1/2},$$

in which p and q are initial allelic frequencies, n is the number of chromosomes sampled in each generation, and t is the number of generations. In *Drosophila* populations, X chromosomes outnumber Y chromosomes 3:1; consequently, genetic drift is more likely to be a factor in the differentiation of the Y-linked than the X-linked phenotypes. Ninety X and 30 Y chromosomes were sampled per bottle during the selection process and, by assuming that the intercrossing among the eight slow-line and the eight fast-line cultures led to free gene exchange, sample sizes per generation were 720 X and 240 Y chromosomes. The X and Y chromosomes are not homologs, and they share no rDNA phenotypes in common; consequently, X-linked rDNA phenotypes were treated as alleles of one locus, and Y-linked rDNA phenotypes were treated as alleles of a different locus. In estimating frequencies, the frequencies of rDNA phenotypes 1 through 8 were pooled to estimate the frequency of one allele, p, at each locus, and the remaining rDNA phenotypes were pooled to estimate q. Observed changes in frequency, Δp, were calculated as the difference between p in the pooled set and p in the fast selection line or the slow selection line.

Observed and expected values of Δp for the X-linked phenotypes are 0.37 and 0.09 in the fast line and 0.33 and 0.07 in the slow line. Thus, allelic frequencies in both the fast and slow lines diverged from the original frequencies by ≈4 times the standard error; both departures are much larger than expected from genetic drift alone. Observed values of Δp for the Y-linked phenotypes are 0.18 and 0.03 in the fast and slow lines, respectively, whereas expected values are 0.15 and 0.12. For Y-linked rDNA, the observed changes in p in the fast and slow lines are within the limits compatible with genetic drift; therefore, there is no evidence that the changes observed in the Y-linked phenotypic frequencies are due to selection.

Table 4. χ^2 tests of "goodness of fit" of observed numbers of rDNA phenotypes in fast, slow, and control development lines with expected numbers in the combined data set

	X-linked phenotypes		Y-linked phenotypes	
	χ^2	P	χ^2	P
Combined vs. fast	22.57	<0.001	32.62	<0.001
Combined vs. slow	16.83	<0.005	19.80	<0.005
Combined vs. control	4.95	NS	8.25	<0.05

Data from two or more classes were combined in some cases to increase expected numbers in each class to five or more. NS, not significant.

Table 5. Pairwise comparisons of mean spacer-length indices in fast, slow, and control development lines

	X-linked phenotypes			Y-linked phenotypes		
	t	df	P	t	df	P
Fast vs. slow	7.16	78	<0.0001	0.43	62	NS
Fast vs. control	1.94	62	<0.06	3.30	54	<0.01
Slow vs. control	3.93	62	<0.001	3.15	54	<0.01

Mean X-linked spacer-length indices for fast, slow, and control lines were 5.80, 5.12 and 5.54 kb, respectively. Mean Y-linked spacer-length indices for the same lines were 7.73, 7.61, and 8.84 kb, respectively. See Tables 2 and 3 for SEMs. *df*, degrees of freedom.

Mean spacer-length indices for X-linked rDNA phenotypes in the fast, slow, and control lines were 5.80, 5.12, and 5.54 kb, respectively. Tests of significance (Table 5) show that the differences between the fast and slow lines and the slow line and control are highly significant ($P < 0.0001$ and 0.001, respectively) but that the difference between the fast line and control is only marginally significant ($P = 0.06$). Mean indices for Y-linked rDNA phenotypes in the fast and slow lines are 7.73 and 7.61 kb, respectively; the difference between these values is not significant. However, the index for the control line (8.84) is significantly larger than those for both the fast and slow lines ($P < 0.01$ in both cases); much of the difference is due to the presence in high frequency of a long Y-linked phenotype (YP15) in the control line.

Although intact rDNA spacers are highly variable in length (e.g., Fig. 2), variability collapsed into the simple pattern seen in Fig. 3 after double digestion with *Hae* III/*Alu* I. This reduction in spacer-length variability featured decreases in the numbers of the longer spacers and concomitant increases in 240-base-pair (bp) elements, suggesting that the longer spacers contain more copies of 240-bp elements and that these elements are released by *Alu* I digestion.

DISCUSSION

Strong associations were observed between enzyme activities and rDNA spacer-length phenotypes in fast and slow developing lines of *D. melanogaster* that we developed by imposing artificial selection on flies collected in a natural population. Higher enzyme activity was usually associated (three cases in four) with fast development rates and lower

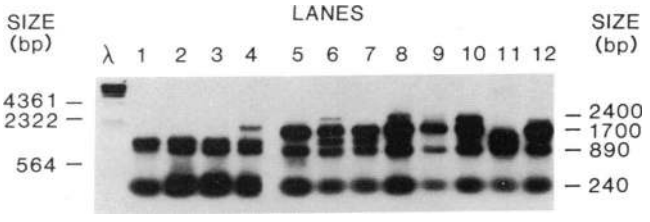

FIG. 3. Autoradiogram of Southern blot containing spacer fragments after double digestion with *Hae* III and *Alu* I of rDNA spacer-length variants with 10 frequent phenotypes (lanes 1–10). *Alu* I-digested subrepeats are isolated in the 240-bp band. Residual spacer fragments, ranging between 890 bp and 2400 bp, correspond to fragments d and e diagrammed in Fig. 1. Phenotypes of rDNA spacer-length variants in lanes: 1–4, Y-linked phenotypes (YP), YP6, YP2, YP12, and YP7, respectively; 5–10, X-linked phenotypes (XP) XP9, XP15, XP14, XP2, XP1, and XP6, respectively; 11 and 12, X-linked rDNA phenotypes from an unrelated natural population.

enzyme activity with slower development; SOD activity was an exception, being lower in the fast developing line. Correlations were positive between development rates and particular X-linked rDNA phenotypes; X-linked rDNA containing long spacer variants were more frequent in the fast development line and less frequent in the slow development line than in the original population. Although significant divergence between the fast and slow lines was also found for Y-linked phenotypes, the extent of divergence was substantially smaller than that observed for the X-linked phenotypes. Therefore, it appears that the observed changes in the frequency of X-linked rDNA phenotypes were due to selection; particular rDNA phenotypes evidently enhance ability to survive and to reproduce when early emergence was made an imperative for survival. However, a different set of rDNA phenotypes appears to be adaptive when late emergence is favored by selection.

Three conspicuous differences were observed between spacer-length patterns in X- vs. Y-linked rDNA arrays: (*i*) long X-linked spacer variants were associated with fast development, whereas no similar association was detected for Y-linked spacer variants; (*ii*) a prominent spacer band at 5.1 kb was associated with all X-linked phenotypes, whereas no single band was present in all Y-linked phenotypes; and (*iii*) X-linked rDNA arrays were more uniform for spacer length than were Y-linked arrays. Thus, the forces that determine the distribution of spacer-length variants within rDNA arrays apparently do not have the same effect in X-linked phenotypes as in Y-linked phenotypes. The way or ways in which factors such as the ones we have discussed affect adaptation are not known. Longer spacers have more copies of a subrepeating 240-bp element, each containing a duplicate gene promoter sequence, and it has been suggested that such elements enhance the rate of rDNA transcription, and hence, development; thus, the advantage of long spacers may be manifested through greater rDNA transcriptional efficiency. If this is the case, ribosome production, translational capacity, enzyme activities, general metabolism, developmental rates, and adaptation might all be expected to change in correlated fashion.

We are grateful to M. Milošević for work with selection lines, R. A. Jorgensen for advice and technical expertise, A. A. Hoffmann for assistance with preliminary work, G. Dover for the spacer probe, and S. Goodwin for the index program. This work was supported in part by National Institutes of Health Grant GM 32429 to R.W.A. and by Department of Energy Contract PA 200-14 MOD4 with F.J.A.

1. Ayala, F. J. & McDonald, J. F. (1980) *Genetica* **52/53,** 1–15.
2. Gibson, J. (1970) *Nature (London)* **227,** 959–960.
3. Avise, J. C. & McDonald, J. F. (1976) *Comp. Biochem. Physiol. B* **53,** 393–397.
4. Stam, L. F. & Laurie-Ahlberg, C. C. (1982) *Insect Biochem.* **12,** 537–544.
5. Laurie-Ahlberg, C. C., Wilton, A. N., Curtsinger, J. W. & Emigh, T. H. (1982) *Genetics* **102,** 191–206.
6. Milošević, M. (1982) Dissertation (University of Belgrade).
7. Marinković, D., Milošević, M. & Milanović, M. (1984) *Arh. Biol. Nauka* **36,** 13–24.
8. Marinković, D., Milošević, M. & Milanović, M. (1986) *Genetica*, in press.
9. Cavener, D. R. (1983) *Genetics* **105,** 105–133.
10. Ritossa, F. (1976) in *The Genetics and Biology of Drosophila*, eds. Ashburner, M. & Novitski, E. (Academic, London), Vol. 1B, pp. 801–846.
11. Franz, G. & Kunz, W. (1981) *Nature (London)* **292,** 638–640.
12. Templeton, A. R., Crease, T. J. & Shah, F. (1985) *Genetics* **111,** 805–818.
13. Desalle, R., Slightom, J. & Zimmer, E. (1986) *Genetics* **112,** 861–875.
14. Kohorn, B. D. & Rae, M. M. (1982) *Nucleic Acids Res.* **10,** 6879–6886.
15. Coen, E. S. & Dover, G. A. (1982) *Nucleic Acids Res.* **10,** 7017–7026.
16. Miller, J. R., Hayward, D. C. & Glover, D. M. (1983) *Nucleic Acids Res.* **11,** 11–18.
17. Reeder, R. H. (1984) *Cell* **38,** 349–351.
18. Reeder, R. H. & Roan, J. G. (1984) *Cell* **38,** 39–44.
19. Moss, T. (1983) *Nature (London)* **302,** 223–228.
20. Boncinelli, E., Borghese, A., Graziani, F., La Mantia, G., Manzi, A., Mariani, C. & Simeone, A. (1983) *Mol. Gen. Genet.* **189,** 370–374.
21. Coen, E. S., Thoday, J. M. & Dover, G. (1982) *Nature (London)* **295,** 564–568.
22. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).