Bipartite Structure of the 5S Ribosomal Gene Family in a *Drosophila melanogaster* Strain, and Its Evolutionary Implications

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ABSTRACT

Knowledge of multigenic family organization should provide insight into their mode of evolution. Accordingly, we characterized the 5S ribosomal gene family in the *Drosophila melanogaster* strain ry^{506}. The 5S genes in this strain display a striking *Hin*dIII restriction difference compared to the "standard" *D. melanogaster* 5S genes. The sequence of three ry^{506} 5S genes was determined. We show that the *Hin*dIII restriction site heterogeneity within the ry^{506} 5S family most probably results from the same point mutation, suggesting that a single 5S variant was propagated into the 5S cluster of this strain. Furthermore, we demonstrate that the structural organization of the 5S genes in ry^{506} is a bipartite structure, i.e., that about 40% of the 5S genes constitute a *Hin*dIII$^+$/*Hin*dIII$^-$ mixed cluster, while those remaining constitute an homogeneous *Hin*dIII$^-$ cluster. The events which might lead to such an heterogeneous pattern are discussed from an evolutionary point of view.

MULTIGENIC families constituted by repeated sequences are present in all eukaryotic species. Although unit structure and family organization are highly variable among species, a high level of homogeneity is maintained within each, the different units of the family evolving, apparently, in unison. This process is usually referred to as concerted evolution (ZIMMER *et al.* 1980).

Nonreciprocal exchanges, mainly unequal crossing-over and conversion events, are commonly thought of as phenomena affecting the evolution of repeated sequences (SMITH 1976; NAGYLAKY and PETES 1982). These events are able to modify the frequency of a variant in a multigene family, leading thus to its spread or elimination. For example, an experimental determination of the rate of unequal mitotic crossing-over in *Saccharomyces cerevisiae* shows that it would be high enough to homogenize the 140 tandemly arranged 18S–28S ribosomal units (SZOSTAK and WU 1980). The authors calculate that there is a 0.5 probability that a ribosomal locus containing two equally abundant variants would be homogenized after 48,000 generations. However, in most cases, the rate of homogenization resulting from these events is too low to explain the observed levels of homogeneity (DOVER 1982). Thus, at least some of the phenomena affecting the structure of multigene families are faster, and/or are not strictly random, thus keeping only very similar copies as family members. Processes such as nonrandom recombination, transposition and RNA-mediated correction may be involved in concerted evolution (ARNHEIM 1983; STRACHAN, WEBB and DOVER 1985; MORZYCKA-WROBLEWSKA *et al.* 1985). For instance, the dispersed structure of the *Neurospora crassa* 5S ribosomal gene family is best explained by transposition (SELKER *et al.* 1981). The occurrence of directional gene conversion has been demonstrated at the *Ascobolus immersus b2* locus, where it leads to a preferential conservation of determined alleles, depending on the molecular nature of the mutation (HAMZA *et al.* 1986; HAMZA, NICOLAS and ROSSIGNOL 1987).

Experimental approaches to the study of concerted evolution dynamics are difficult. Based on available structural criteria, multigenic families appear homogeneous and seem to have reached a state of equilibrium. Depending on the family, *i.e.*, of nature of functional and structural constraints, different degrees of homogeneity are observed. Noncoding families, such as the 360 and 500 Drosophila satellites, display high levels of heterogeneity (STRACHAN, WEBB and DOVER 1985). This is not surprising since there are in this case few or no functional constraints. On the contrary, high levels of homogeneity are observed in the case of the coding families (ARNHEIM 1983).

The *Drosophila melanogaster* 5S ribosomal genes constitute a multigenic family clearly displaying concerted evolution. In this species, the haploid genome contains approximately 160 clustered 5S genes (PRO-

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CUNIER and TARTOF 1975; PROCUNIER and DUNN 1978). The 5S genes are tandemly arranged (PROCUNIER and TARTOF, 1976) and are located in region 56F (PRENSKY, STEFFENSEN and HUGHES 1973). Each is 373 ± 7-bp long, divided into a 135 bp long transcribed region (JACQ, JOURDAN and JORDAN 1977) and a 238 ± 7-bp long nontranscribed spacer (TSCHUDI and PIRROTTA 1980). This size heterogeneity is due to the variable copy number (4, 5 or 6 copies) of the heptamer GCTGCCT downstream of the transcribed region. TSCHUDI and PIRROTTA (1980) sequenced four *D. melanogaster* 5S genes and reported three point differences between the sequenced copies. Three other base changes and one duplication of three bases were found by TSCHUDI, PIRROTTA and JUNAKOVIC (1982) when sequencing another 5S gene. A two-nucleotide deletion in the coding region has also been reported by SHARP *et al.* (1984), who partially sequenced three 5S genes. All of these data demonstrate that the degree of homogeneity within the *D. melanogaster* 5S gene family is high, but that some heterogeneity of the family exists.

In this paper, we analyze the heterogeneity of the Drosophila ry^{506} strain, in which approximately 20% of 5S units share a *Hin*dIII restriction site. This analysis provides some insight into the processes involved in the concerted evolution of the Drosophila 5S family. We will refer to the consensus 5S sequence determined by TSCHUDI and PIRROTTA (1980) as "standard."

MATERIALS AND METHODS

Drosophila strains: All strains were from the Gif collection. Several *D. melanogaster* isolates were used (Canton-S, π2, Charolles, Gruta, and Oregon-R), as well as the mutant strains *min* (PROCUNIER and TARTOF 1975), ry^{506} (COTÉ *et al.* 1986) and the balancer stock *CyO;TM3/T(2;3)ap*^{Xa} (LINDSLEY and GRELL 1968).

Molecular analyses: DNA from populations of adults was prepared by homogenizing about 100 flies in 4 ml of extraction buffer (100 mM EDTA, 200 μg/ml pronase, 50 mM Tris-HCl, pH 7.8) at 4°. SDS was added to a concentration of 1% prior to incubation at 65° for 30 min. The pronase concentration was increased to 400 μg/ml, and the preparation was incubated for 3 hr at 37°. After phenol-chloroform extraction, DNA was precipitated by adding 3 ml of isopropanol at room temperature. The precipitate was rinsed with ethanol and redisolved in 500 μl of 1 mM EDTA, 10 mM Tris-HCl, pH 8.

DNA was prepared from individual flies using an adaptation of the technique described by JUNAKOVIC, CANEVA and BALLARIO (1984). Each fly was homogenized with a glass stick in a 1.5-ml Eppendorf tube. Extraction buffer (500 μl of 0.2 M sucrose, 0.1 M Tris-HCl, pH 9.2, 50 mM EDTA, 0.5% SDS) was then added and the mixture heated for 10 min at 65°. The mixture, after addition of potassium acetate (120 μl of a 5 M stock solution, pH 9), was kept 10 min on ice, then spun for 10 min. The supernatant, transferred to a fresh tube, was spun again for 10 min. DNA was then precipitated at −20° after addition of 400 μl of isopropanol. The precipitate was ethanol rinsed and allowed to redissolve overnight in 50 μl 10 mM Tris-HCl, pH 8, 1 mM EDTA prior to restriction digestion.

Restriction digests were analyzed on 0.6% agarose gels. Southern analyses were performed under standard conditions (WAHL, STERN and STARK 1979; SMITH and SUMMERS 1980), except that nitrocellulose filters were baked for 4–5 hr at 80° in order to improve the retention of short restriction fragments (less than 600 bp).

Scanning of 5S patterns obtained by *Hin*dIII digestion of the ry^{506} DNA was performed on several autoradiograms corresponding to different experiments and to various times of exposure.

Genetical localization of 5S sequences in ry^{506}: ry^{506} virgin females were crossed to *CyO;TM3/T(2;3)ap*^{Xa} males and the F_1 males carrying *CyO* and *TM3* balancers were mated with *min* virgin females. Eight phenotypically distinguishable classes of F_2 flies were recovered. They respectively bore all possible combinations of the first, second and third chromosomes of the ry^{506} strain. All were tested by Southern analysis for the presence or the absence of ry^{506} specific 5S sequences.

In situ hybridizations were performed according to SPIERER *et al.* (1983). The 5S DNA probe (pBR7A including ten copies of *D. melanogaster* 5S genes, provided by V. PIRROTTA) was labeled with [^{3}H]dGTP by nick translation.

Cloning and sequencing of *Hin*dIII restriction fragments containing 5S sequences: Fifty micrograms of genomic DNA from the ry^{506} strain were cut with *Hin*dIII. The resulting restriction fragments were fractionated on a 0.6% agarose gel. Two size classes of DNA fragments, respectively around 375 and 750 nucleotide long, were electroeluted into dialysis bags, and then cloned into the *Hin*dIII site of pUC8. Screening for clones containing 5S sequences was performed using a *D. melanogaster* 5S probe, purified from pBR7A.

Plasmid DNA was prepared according to the method described by BIRNBOIM and DOLY (1979). Drosophila sequences contained in the clones were purified on acrylamide gels (MAXAM and GILBERT 1980). A set of fragments with various 5′ protruding ends were generated by cutting the inserts with appropriate restriction enzymes. Klenow labeling and additional restriction cuts provided fragments with a unique ^{32}P-labeled 3′ end. These fragments were used in sequencing reactions as described by MAXAM and GILBERT (1980).

RESULTS

Heterogeneity of the *D. melanogaster* 5S cluster: To study the structural heterogeneity of the 5S locus, we digested the genomic DNA of different strains with restriction enzymes which do not cut standard 5S units, digestions with *Hin*dIII yielding the most informative patterns. In addition to a fragment of high M_r (>30 kb) corresponding to clustered standard 5S sequences, we found in all strains, with the exception of Charolles, other fragments hybridizing to 5S sequences (Figure 1). As shown in Figure 1, each strain has a specific *Hin*dIII 5S restriction pattern. The number of *Hin*dIII fragments usually is low (1–3), with the exception of ry^{506} (Figures 1 and 2).

The ry^{506} 5S locus: As shown in Figure 2A, the sizes of the ry^{506} *Hin*dIII 5S fragments correspond

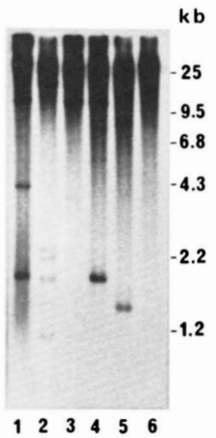

FIGURE 1.—Autoradiogram of Drosophila DNA prepared from populations of adults, digested with *Hin*dIII, fractionated on a 0.6% agarose gel and hybridized to a ^{32}P-labeled 5S probe. (1) Canton-S, (2) ry^{506}, (3) Gruta, (4) Oregon-R, (5) π2, (6) Charolles. M_r markers are indicated on the right.

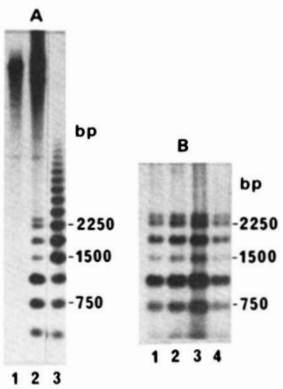

FIGURE 2.—A, Drosophila DNA prepared from populations of flies was digested with *Hin*dIII and analyzed as in Figure 1: (1) min, (2) ry^{506}, (3) *in vitro* polymerized *D. melanogaster* 5S genes, *i.e.* multimers of 375-bp long fragments. B, ry^{506} DNA was digested with *Hin*dIII and analyzed as in Figure 1. Monomeric units were run out of the gel. Only the bottom of the gel is shown. Samples from single individuals were run in the first three lanes: (1) ry^{506} male, (2) ry^{506} female, (3) ry^{506} female, (4) ry^{506} population.

to *n*-mers (n varying from 1 to 6) of a 375-bp long fragment, the size of a standard 5S unit. An additional band (2.4-kb long), not included within the 375-bp ladder, is also detected (Figure 2A). The same pattern is obtained when analyzing DNA from various preparations or from individual flies (Figure 2B).

The genetic localization of 5S sequences in ry^{506} was performed according to the strategy described in Materials and Methods. The results are summarized in Table 1. The seven *Hin*dIII restriction fragments specific for ry^{506} (Figure 2) are present only in DNA of flies bearing a ry^{506} chromosome *2*.

This result was confirmed by *in situ* hybridization to salivary gland polytene chromosomes. Hybridization of 5S probe was detected on the second chromosome, in section 56 F, which is the location of the

TABLE 1

Genetic localization of 5S genes in ry^{506}

F2 flies	Chromosomes from ry^{506} strain				*Hin*dIII$^+$ 5S sequences
	1	*2*	*3*	*4*	
Females, *CyO;TM3*	+	−	−	U	−
Females, *CyO*	+	−	+	U	−
Females, *TM3*	+	+	−	U	+
Females, wild type	+	+	+	U	+
Males, *CyO;TM3*	−	−	−	U	−
Males, *CyO*	−	−	+	U	−
Males, *TM3*	−	+	−	U	+
Males, wild type	−	+	+	U	+

F_2 flies were obtained in genetic crosses as described in MATERIALS AND METHODS. The presence or absence of ry^{506} specific sequences was checked by Southern analysis. These sequences are present only in ry^{506} DNA. They are absent in the two other strains which were involved in the crosses, *i.e. min* and *CyO;TM3/T(2;3)ap^{Xa}*. The following symbols have been used: +: present, −: absent, U: unknown.

5S cluster in *D. melanogaster* (PRENSKY, STEFFENSEN and HUGHES 1973). No additional signals were detected.

Structure and organization of "nonstandard" ry^{506} 5S sequences: Clones of 5S genes bearing *Hin*dIII restriction sites were isolated for sequencing. Two clones, p002 and p003, respectively, containing 373- and 749-bp long inserts, were selected. The sequencing results are shown in Figure 3. The two cloned genomic inserts correspond to a whole 5S gene in p002, and to two tandemly arranged 5S units in p003. Their sequences are very similar to the *D. melanogaster* standard 5S sequence published by TSCHUDI and PIRROTTA (1980), but differ at some locations. The single 5S sequence contained in p002 differs from the standard 5S unit at two positions located in the spacer segment (nucleotides 1 and 316, respectively, Figure 3). The two 5S units from p003 show a total of five differences when compared with the standard 5S sequence. Three point mutations are found in the nontranscribed spacer of the gene (nucleotides 1, 403 and 692, Figure 3), in addition to a duplication of a preexisting trinucleotide (ATT, nucleotides 369–371 and 372–374, Figure 3). The point mutation at position 403 and the duplication of the trinucleotide have also been found by TSCHUDI, PIRROTTA and JUNAKOVIC (1982) in another 5S unit. The difference located at position 578 in one of the two transcribed regions of the p003 insert has previously been described as a variant position in *D. melanogaster* 5S genes (TSCHUDI and PIRROTTA 1980; TSCHUDI, PIRROTTA and JUNAKOVIC 1982; SHARP *et al.* 1984).

The *Hin*dIII restriction site which distinguishes the cloned 5S genes from the standard sequence results from a T to G point mutation (Figure 3, nucleotide 1) for both clones. This modification is located at the

p002

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T
GCTTTTATAT CTTTATTACG ATATCTGAAA CCCAATGGTA CAAAAACAGT CTATTTCAGT
CTATGGGCAT AACTGAATAT CAGAGTATAA GGACACTGTT TAGCCCCTCG ACTTTCGCCA
ACGACCATAC CACGCTGAAT ACATCGGTTC TCGTCCGATC ACCGAAATTA AGCAGCGTCG
GGCGCGGTTA GTACTTAGAT GGGGGACCGC TTGGGAACAC CGCGTGTTGT TGGCCTCGTC
CACAACTTTT TGCTGCCTGC TGCCTGCTGC CTGCTGCCTG CTGCCTTCTT AGTTTTTATT
TTAGCATTAT TGGCTGCAAA TCAGAATGAA AACTTTGTTC ACCTAATTTC AAATTTTGTC
TTTCACTCAT TAA
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p003

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T
GCTTTTATAT CTTTATTACG ATATCTGAAA CCCAATGGTA CAAAAACAGT CTATTTCAGT
CTATGGGCAT AACTGAATAT CAGAGTATAA GGACACTGTT TAGCCCCTCG ACTTTCGCCA
ACGACCATAC CACGCTGAAT ACATCGGTTC TCGTCCGATC ACCGAAATTA AGCAGCGTCG
GGCGCGGTTA GTACTTAGAT GGGGGACCGC TTGGGAACAC CGCGTGTTGT TGGCCTCGTC
CACAACTTTT TGCTGCCTGC TGCCTGCTGC CTGCTGCCTG CTGCCTTCTT AGTTTTTATT
TTAGCATTAT TGGCTACAAA TCAGAATGAA AACTTTGTTC ACCTAATTTC AAATTTTGTC
                                                    G
TTTCACTCAT TATTAATCTT TTATATCTTT ATTACGATAT CTAAAACCCA ATGGTACAAA
AACAGTCTAT TTCAGTCTAT GGGCATAACT GAATATCAGA GTATAAGGAC ACTGTTTAGC
CCCTCGACTT TCGCCAACGA CCATACCACG CTGAATACAT CGGTTCTCGT CCGATCACCG
                                                G
AAATTAAGCA GCGTCGGGCG CGGTTAGTAC TTAGATGAGG GACCGCTTGG GAACACCGCG
TGTTGTTGGC CTCGTCCACA ACTTTTTGCT GCCTGCTGCC TGCTGCCTGC TGCCTGCTGC
                                          A
CTTCTTAGTT TTTATTTTAG CATTATTGGC TGCAAATCAG AATGAAAACT TTGTTCACCT
AATTTCAAAT TTTGTCTTTC ACTCATTAA
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FIGURE 3.—Nucleotide sequences of the ry^{506} *Hin*dIII$^+$ 5S units inserted in p002 and p003 (noncoding strand). The sequences corresponding to the transcribed regions are underlined. Arrows at positions 1 indicate the point mutations generating the *Hin*dIII site. The corresponding position is not changed in the middle of p003 (open arrow). The point differences with the standard 5S sequence (see text) are written above each sequence. Hyphens above nucleotides indicate that they are missing in the standard sequence.

same position in each case, *i.e.* 116 nucleotides upstream from the first transcribed nucleotide. On the basis of the multimeric pattern that we observe (Figures 1 and 2) and the primary structure of p002 and p003 inserts, we assume that the other multimeric fragments detected on Southern blots have the same overall structure, *i.e.* that they are composed of *Hin*dIII$^-$ 5S genes surrounded by two *Hin*dIII$^+$ 5S units.

To obtain insight into the organization of the *Hin*dIII$^+$ 5S sequences, we performed partial *Hin*dIII digests of ry^{506} DNA. Results are shown in Figure 4. Partial restriction cuts lead to a range of 5S hybridizing fragments whose sizes correspond to multimers, up to approximately 20 × 375 bp units. This result indicates that the *Hin*dIII$^+$ 5S blocks constitute a cluster of adjacent sequences. The 2.4-kb long fragment (Figure 2) detected in all *Hin*dIII digests of ry^{506} DNA, is not a multimer of a 5S unit and could be one of the outside borders of the

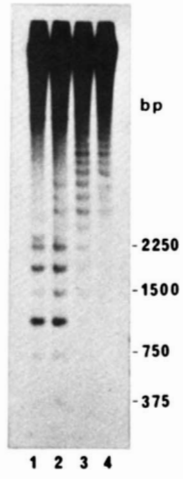

FIGURE 4.—Autoradiogram of DNA prepared from a population of ry^{506} flies, digested with various amounts of *Hin*dIII and analyzed as in Figure 1. Lane 1, total *Hin*dIII digest (one unit of enzyme per μg of DNA). Lanes 2 to 4, partial *Hin*dIII digests (½, ¼ and ⅛ unit per μg of DNA, respectively).

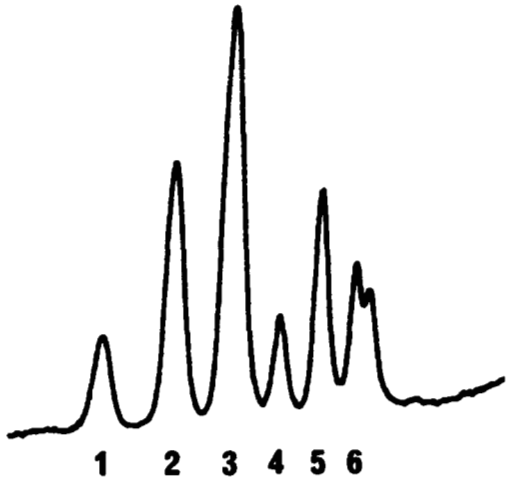

FIGURE 5.—Scanning of the autoradiogram shown in Figure 2A, lane 2. The six multimers are marked from 1 to 6. The 2.4-kb fragment appears as a shoulder of the hexamer.

*Hin*dIII$^+$/*Hin*dIII$^-$ mixed cluster. Southern analyses of *Hin*dIII restriction digests show high M_r fragments in addition to the smaller multimeric fragments (Figure 2A), indicating that while the *Hin*dIII$^+$ 5S genes can be interspersed with *Hin*dIII$^-$ genes, a significant portion of the *Hin*dIII$^-$ genes remain clustered independently.

Quantifying *Hin*dIII$^+$ 5S units in ry^{506}: Densitometric analyses of the 5S pattern from several *Hin*dIII digests of ry^{506} DNA were performed (Figure 5). Relative hybridization intensities of the six restriction fragments whose size is proportional to 375 bp (Figure 2) were determined by measuring the areas of the peaks (Table 2). As shown in Figure 2, relative hybridization intensities are identical, using both DNA from populations of flies or from single flies. Within the range of their M_r (375–2250 bp), we can reasonably postulate that the relative hybridization intensities of the multimeric fragments are directly related to their relative proportions. The absolute copy number of *Hin*dIII$^+$ 5S genes was thus calculated by considering that the less abundant multimers, *i.e.*, the tetramers and the hexamers, are present only as one single copy (Table 2). This was determined using a ry^{506} strain bearing a single copy of a 3.2-kb *Hin*dIII fragment (nine 5S genes of *Drosophila teissieri*) introduced by transformation (manuscript in preparation). As shown in Table 2, the *Hin*dIII$^+$/*Hin*dIII$^-$ cluster would consist of approximately 66–68 5S genes (Table 2). The corresponding number of *Hin*dIII sites, if all *Hin*dIII fragments are clustered, would be around 28–29 (Table 2).

The copy number of the 5S genes has been determined to be approximately 160 per haploid genome in *D. melanogaster* (PROCUNIER and DUNN, 1978). Although gene redundancy varies from strain to strain

TABLE 2

Determination of number of 5S units forming the *Hin*dIII$^+$/*Hin*dIII$^-$ mixed cluster

Size of restriction fragments (bp)	Area of peaks	Relative abundance	Redundancy	5S copy No.
375	7.9 ± 0.3	7.9	5(10)	5(10)
750	20.7 ± 0.3	10.3	6–7	12–14
1125	39.6 ± 0.4	13.2	8	24
1500	5.8 ± 0.2	1.5	1	4
1875	14.5 ± 0.5	2.9	2	10
2250	11.5 ± 0.3	1.9	1	6

Autoradiograms corresponding to *Hin*dIII restriction digests of ry^{506} were scanned, and the area of the peaks corresponding to the ry^{506} specific 5S bands was determined. We present measurements performed on a single autoradiogram (mean of nine scans). Areas are expressed as the percentage of the total surface of the six peaks. Relative abundances are obtained by dividing the area of the peaks by the corresponding sizes of the 5S fragments. We calculated the redundancy of the different kinds of multimers by considering that tetramers and hexamers are single copy within the cluster. The absolute 5S copy number (*Hin*dIII$^+$ and *Hin*dIII$^-$) can thus be deduced. Redundancy of *Hin*dIII$^+$ monomeric units, due to the difficulty of binding small fragments to the nitrocellulose filter (375 bp), is underevaluated in this experiment. Measurements from other experiments give a more probable value of ten *Hin*dIII$^+$ monomeric units (number given in parentheses).

(PROCUNIER and DUNN 1978), we can deduce that the mixed *Hin*dIII$^+$/*Hin*dIII$^-$ locus contains approximately 40% of the entire 5S cluster.

DISCUSSION

Heterogeneity within the Drosophila 5S clusters: In *D. melanogaster*, 5S genes are known to be tandemly arranged (PROCUNIER and TARTOF 1976; HERSHEY *et al.* 1977; ARTAVANIS-TSAKONAS *et al.* 1977). Digestion of genomic DNA with enzymes which do not cut standard 5S units should thus give rise to a single fragment containing the clustered 5S genes (PROCUNIER and TARTOF 1976). However, 5S clusters can be split in some cases into one or more fragments by such restriction enzymes (JUNAKOVIC 1980; TSCHUDI, PIRROTTA and JUNAKOVIC 1982; SAMSON and WEGNEZ 1984; this report), shown to be due to the presence of rare restriction site variants or to the integration of the *B104* element within the 5S cluster (TSCHUDI, PIRROTTA and JUNAKOVIC 1982). In this paper, we tested six *D. melanogaster* strains with *Hin*dIII, an enzyme which does not cut the standard 5S gene (TSCHUDI and PIRROTTA 1980). We found that *Hin*dIII splits the 5S locus in five of them, giving rise to a specific restriction pattern for each of the strains (Figure 1). In all cases except ry^{506}, the number of *Hin*dIII restriction sites within the 5S cluster is low. In ry^{506}, the *Hin*dIII restriction pattern includes six restriction fragments whose sizes correspond to 5S gene multimers (Figure 2). On the basis of frag-

ment sizes, this pattern is similar to the ladder resulting from partial digestion with a restriction enzyme whose recognition sequence is present in all 5S units. Sequencing data strongly suggest that all *Hin*dIII sites are located at the same position (Figure 3). By analysis of *Hin*dIII partial digestions (Figure 4) we found that the six types of multimeric blocks constitute a cluster of adjacent sequences.

Origin and spread of the *Hin*dIII$^+$ sites: The 5S locus in ry^{506} includes 28–29 *Hin*dIII$^+$ sites (Table 2). Only two point substitutions, one of them corresponding to that found in our sequencing, could generate a new *Hin*dIII site in a 5S unit (T to G or A to G substitutions, nucleotides 1 and 332, respectively, Figure 3). As shown in Figure 1, several Drosophila strains bear an occasional *Hin*dIII site. Thus, the probability that all of these sites are located at the same position as found in the ry^{506} 5S genes is very high. This is supported by the work of TSCHUDI, PIRROTTA and JUNAKOVIC (1982), who located a *Hin*dIII variant site at this position by restriction analysis in the Oregon-R Yale strain. One may thus hypothesize that a point mutation leading to a *Hin*dIII site occurred in the 5S cluster in the common ancestor of all of the studied strains. Spreading of this variant did not occur, with the exception of the ry^{506} chromosome.

We can thus describe the ry^{506} 5S gene cluster as a bipartite locus, with about 40% of the 5S genes constituting a *Hin*dIII$^+$/*Hin*dIII$^-$ mixed cluster, while those remaining constitute a homogeneous *Hin*dIII$^-$ cluster. Similar clustering was already described for recurrent mutations localized in the nontranscribed spacers of ribosomal RNA genes (DVORAK, JUE and LASSNER 1987).

Starting with a single 5S variant, how many steps were required to reach a situation in which 28–29 variants have invaded the 5S locus? This surely required several rounds of conversion or unequal crossing-over. An important point is the fact that *Hin*dIII$^+$ genes are clustered. If unequal crossing-over and/or conversion events are involved in the process, this means that these genetic exchanges concern 5S genes not more than six units apart, since *Hin*dIII$^+$ fragments longer than six units were not observed in the 5S ladder (Figure 2). This result is surprising, but perhaps explains why the size of the 5S cluster does not vary to any large extent among Drosophila strains. Similar observations were reported in the case of the *S. cerevisiae* ribosomal locus, where the average displacement during unequal crossing-over involves only six to eight ribosomal units (SZOSTAK and WU 1980).

An alternative model is provided by the finding of PONT, DEGROOTE and PICARD (1987) who reported the existence of nonchromosomal 5S sequences in Drosophila embryos. These 5S sequences are present in supercoiled DNA molecules whose size corresponds to multimers of 1–16 5S genes. The copy number of these molecules is between 200 and 1000 per embryo. The integration of such circles including several variants within the 5S cluster might increase the variant copy number and, through localized amplification, lead to an asymmetric 5S pattern as found in ry^{506}.

The future of the ry^{506} 5S locus: The main difficulty encountered when discussing phenomena related to concerted evolution is the lack of knowledge about absolute rates of evolution. What is the absolute rate of unequal crossing-over within the Drosophila 5S cluster, for example? The ry^{506} 5S locus could be very informative in this respect. Its asymmetric organization is fortuitously revealed by the point mutation leading to a *Hin*dIII restriction site. The locus has been invaded by a 5S variant, and displays a characteristic *Hin*dIII 5S restriction pattern. What will happen in the future to that locus? As a consequence of concerted evolution, the number of the *Hin*dIII 5S variants might be either increased or decreased. Recalling that the 5S pattern is the same when DNA from populations or from single flies is analyzed, we can surmise that the rate at which the distribution of *Hin*dIII sites within the 5S cluster is changed is slow. However, the heterogeneous structure of the ry^{506} locus itself demonstrates that occasional exchanges do occur. A survey of the ry^{506} 5S locus over several years should bring some interesting information about the absolute rate of evolution within Drosophila 5S genes.

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