J Mol Evol (1995) 41:615–621

JOURNAL OF MOLECULAR EVOLUTION

Structural Evolution of the *Drosophila* 5S Ribosomal Genes

F. Pâques,* M.-L. Samson, P. Jordan, M. Wegnez**

Laboratoire d'Embryologie Moléculaire, Unité de Recherche Associée 1134, Centre National de la Recherche Scientifique, Université Paris XI, Bâtiment 445, 91405 Orsay CEDEX, France

Received: 31 January 1995 / Accepted: 28 April 1995

Abstract. We compare the 5S gene structure from nine *Drosophila* species. New sequence data (5S genes of *D. melanogaster, D. mauritiana, D. sechellia, D. yakuba, D. erecta, D. orena,* and *D. takahashii*) and already-published data (5S genes of *D. melanogaster, D. simulans,* and *D. teissieri*) are used in these comparisons. We show that four regions within the *Drosophila* 5S genes display distinct rates of evolution: the coding region (120 bp), the 5′-flanking region (54–55 bp), the 3′-flanking region (21–22 bp), and the internal spacer (149–206 bp). Intra- and interspecific heterogeneity is due mainly to insertions and deletions of 6–17-bp oligomers. These small rearrangements could be generated by fork slippages during replication and could produce rapid sequence divergence in a limited number of steps.

Key words: *Drosophila* — Ribosomal genes — Sequence data

Introduction

The genome of eukaryotes contains many families of repeated sequences, including the 5S ribosomal genes. Lower and higher eukaryotes show different modes of 5S gene organization. In *Neurospora crassa,* the majority of the 100 copies of 5S genes are dispersed throughout the genome (Selker et al. 1981; Metzenberg et al. 1985). In the yeast *Saccharomyces cerevisiae,* most 5S genes are tandemly arranged as an integral part of the genetic unit coding for the ribosomal RNAs, although some are found clustered at another chromosomal location as an independent tandem array (McMahon et al. 1984). In the oomycete genus *Pythium,* 5S genes are linked or unlinked to the rDNA locus depending on the species (Belkhiri et al. 1992). Interdispersion of 5S and histone genes within a tandemly arranged gene cluster is found in the crustacean *Artemia salina* (Andrews et al. 1987). In higher eukaryotes, clustering of 5S genes away from larger ribosomal genes or other genes is the usual organization of this gene family (Long and Dawid 1980). However, inclusion of 5S genes within the rDNA intergenic regions occurs in several arthropod lineages (Drouin et al. 1992).

These marked differences in genetic organization for genes sharing the same function are puzzling in terms of evolution. Nevertheless, 5S genes from all species demonstrate a common feature, i.e., concerted evolution. This mode of evolution, which is also observed in all other multigene families, is characterized by the fact that the different members of a gene family display high levels of structural similarity within every species (Coen et al. 1982; McMahon et al. 1984; Samson and Wegnez 1984; Morzycka-Wroblewska et al. 1985). The molecular grounds of concerted evolution are not yet clearly understood.

The *Drosophila* 5S genes offer many interesting opportunities to approach experimentally the concerted evolution process. The 5S gene unit of *D. melanogaster*

**Present address:* Rosenstiel Center, Brandeis University, 415 South Street, Waltham, MA 02254-9110, USA
***Present address:* Rutgers University, Waksman Institute, P.O. Box 759, Piscataway, NJ 08855-0759, USA
Correspondence to: M. Wegnez

is short (coding + spacer regions: 375 bp; Tschudi and Pirrotta 1980), which allows visualization on Southern blots of single or multimeric 5S units as well as of the entire 5S cluster (Junakovic 1980; Tschudi et al. 1982; Samson and Wegnez 1984). The homogeneity/heterogeneity of the cluster in different strains of *D. melanogaster* can then be easily assessed by restriction analysis. The analysis of *Hin*dIII 5S variants in the ry^{506} strain, for example, allowed us to hypothesize that genetic exchanges within the 5S locus involve 5S genes that are located, in general, not more than six units apart (Samson and Wegnez 1988).

Structural comparison of 5S genes from closely related species provides a way to gain information on the evolutionary trends of this gene family. For this reason, we began a study of the 5S genes from several of the species most closely related to *D. melanogaster*. Sequences of several 5S gene units from *D. melanogaster, D. simulans,* and *D. teissieri* are already known (Tschudi and Pirrotta 1980; Samson and Wegnez 1984; Sharp et al. 1984; Samson and Wegnez 1988), as is the sequence of one 5S unit of *Calliphora erythrocephala,* another dipteran species (Rubacha et al. 1984). We report in this paper the sequence of eleven 5S genes from *D. melanogaster, D. mauritiana, D. sechellia, D. yakuba, D. erecta, D. orena,* and *D. takahashii.*

Comparison of all *Drosophila* 5S gene sequences reveals some features of their mode of evolution. The most striking observation is that 5S gene structure differences are mainly due to insertions and deletions of short duplicated oligomers within the spacer region. Thus, the cumulative effect of some of these events would significantly change the overall 5S gene spacer structure.

Materials and Methods

Drosophila *Stocks.* All *Drosophila* stocks were provided by J. David and F. Lemeunier from the laboratory Populations, Génétique et Evolution at Gif-sur-Yvette, France. The following strains have been used: *D. erecta,* str. 220-5, *D. mauritiana,* str. 163-1, *D. orena,* str. 188-1, *D. sechellia,* str. 228, *D. takahashii,* str. 227-5, *D. yakuba,* str. 115.

DNA Extraction and Analysis. DNA was prepared from adult flies as described previously (Samson and Wegnez 1989). Fractionation of restriction fragments was performed on vertical 1-mm-thick, 40-cm-long polyacrylamide gels (5.2% or 6.2%) in 25 mM EDTA, 89 mM boric acid, 89 mM Tris-borate, pH 8.3. Electrophoreses were carried out for 36 h at 150 V. Standard conditions were used for DNA transfer and hybridization.

*Cloning and Sequencing of 5S Units. Mbo*I genomic DNA fragments containing 5S units of *D. mauritiana, D. sechellia, D. yakuba, D. erecta, D. orena,* and *D. takahashii* were cloned as previously described (Samson and Wegnez 1984). We also cloned one 5S locus border from the ry^{506} *D. melanogaster* strain. The corresponding 2.4-kb *Hin*dIII genomic fragment (Samson and Wegnez 1988) was cloned in *pUC18.* Subclones were generated with the Erase a base kit of Promega. We used the method of Maxam and Gilbert (1980) to sequence *D. sechellia* and *D. takahashii* 5S genes. The method of Sanger et al. (1977) and the Sequenase system (US Biochemical Corporation, Cleveland, OH) was used for all other 5S genes. EMBL accession numbers: X87873 to X87880.

Sequence Comparisons. We used the BESTFIT program, from the University of Wisconsin Genetics Computer Group. BESTFIT finds optimal alignments between two sequences by inserting gaps. We chose a gap weight of 5.0 and a gap length weight of 0.3.

Results

Sequence Polymorphism of D. melanogaster *and* D. sechellia *5S Genes*

Polymorphism within the *D. melanogaster* 5S gene family is due to variable copy numbers of the GCTGCCT heptamer downstream of the transcribed region and to point mutations (Tschudi and Pirrotta 1980; Tschudi et al. 1982; Sharp et al. 1984; Samson and Wegnez 1988). These data were obtained from individually cloned 5S units. In order to determine the level of heterogeneity of adjacent 5S units, we cloned and sequenced a 2.4-kb *Hin*dIII genomic fragment of a ry^{506} strain thought to correspond to one of the two borders of the 5S locus (Samson and Wegnez, 1988). This genomic fragment indeed contains four tandemly arranged 5S units and about 1 kb of non-5S sequences (Fig. 1). Three other 5S genes from the same ry^{506} strain were previously sequenced (Samson and Wegnez 1988). These seven 5S genes differ from one another by at least one point mutation in the spacer region and by variable copy numbers of the GCTGCCT heptamer (four to six copies, position 136, cf. Fig. 1 and 2).

Polymorphism is also found in the 5S coding region. Four sequenced 5S units of ry^{506} (three sequenced in this work, Fig. 1) are pseudogenes, according to Sharp et al. (1984). This is due to a single base substitution in the coding region (G to A, position 86, Fig. 1). The fourth 5S unit of the locus border (Fig. 1) is also probably a pseudogene since it contains a GG insertion at the position 88.

Two 5S genes from *D. sechellia* were cloned and sequenced (*D.se.1* and *D.se.2,* Fig. 2). *D.se.2* differs from *D.se.1* by three single substitutions (positions 178, 247, and 321, *D.se.2*) and by a CT/AG substitution (positions 365–366, *D.se.2*). Both genes differ also by a duplication of the TTA trinucleotide (positions 250–252, *D.se.2*) and by the deletion of one duplicated heptamer (TTGGCTA, positions 185–191, *D.se.1*).

Comparison of 5S Genes from D. melanogaster, D. simulans, D. mauritiana, *and* D. sechellia

The 5S genes of the sibling species *D. simulans, D. mauritiana,* and *D. sechellia* cannot be distinguished by restriction mapping (Samson and Wegnez 1984), and only

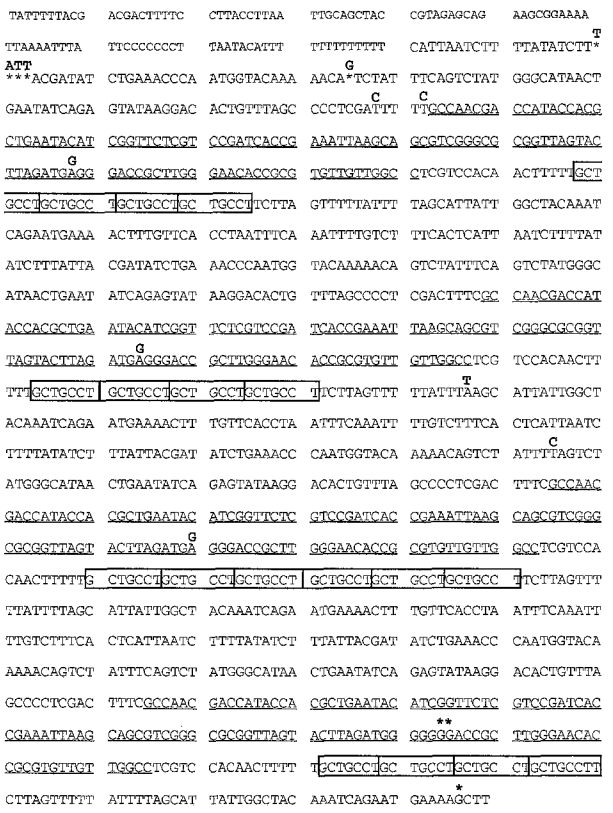

Fig. 1. Sequence of one border of the *D. melanogaster* 5S locus. The sequence encompasses 100 bp of non-5S sequences (*small letters*) and four 5S units of one border of the ry^{506} strain 5S locus. Sequences corresponding to the mature 5S RNA are *underlined. Bold letters* above variant sites correspond to the 5S "consensus" sequence defined by Tschudi and Pirrotta (1980).

some differences are found when they are compared with the *D. melanogaster* 5S gene. The comparison of 5S gene sequences of these four *Drosophila* species is shown in Fig. 2. Pairwise comparisons do not allow detection of more than 11 point differences. Two types of events are found: point mutations and duplications/deletions of short oligomers (trimers and heptamers). Most of these events are found in several 5S units from the same or from different species—i.e., they are not species-specific.

Two identical 5S genes were found, surprisingly cloned from different species, i.e., *D. sechellia* and *D. melanogaster* (respectively, *D.se.2* and one of the 5S genes included in p003: Samson and Wegnez 1988). The *D.se.1* sequence is identical to that reported for one 5S unit of *D. simulans* (Samson and Wegnez 1984), with the sole exception of position 321 (Fig. 2).

Comparison of 5S Genes from D. yakuba *and* D. teissieri

D. yakuba and *D. teissieri* are closely related species of the *D. melanogaster* subgroup (Lemeunier et al. 1986). The sequence of one *D. yakuba* 5S gene is compared in Fig. 3 with one previously sequenced 5S unit of *D. teissieri.* These genes differ by 22 substitutions, two deletions/insertions of one nucleotide, and four deletions/insertions of 7, 8, 14, and 16 nucleotides, respectively (Fig. 3). Hence, substitutions and deletions of single base pairs modify 24 nucleotides while the deletions/insertions of oligomers modify 45 nucleotides.

Comparison of 5S Genes from D. erecta *and* D. orena

D. erecta and *D. orena* are also closely related species (Lemeunier et al. 1986). The coding regions of both genes and the functional *D. melanogaster* 5S unit are identical, with the exception of one T insertion within the *D. orena* sequence at position 38 (not shown). The differences between *D. erecta* and *D. orena* 5S units are mainly due to deletions/insertions of small oligomers (Fig. 3). Within the spacer, four insertions/deletions of 6- or 8-bp oligomers account for 28 nucleotide differences, while substitutions or deletions/insertions of a single nucleotide occur only at five positions.

Overall Comparison of Drosophila *5S Genes*

Coding Regions

The 5S coding regions (nucleotides 1–120) of all sequenced *Drosophila* 5S genes are almost completely identical. Differences relative to the canonical *D. melanogaster* sequence are present at positions 86 and 88 in several *D. melanogaster* 5S variants, at position 38 in *D. orena,* and at position 24 (C instead of T) in *D. takahashii,* a member of the *D. takahashii* subgroup.

Noncoding Regions

Noncoding regions encompass the spacer and the transcribed 15–16-nucleotide segment specific to the 5S RNA precursor. The two regions flanking the coding sequence are very similar in all 5S sequences and can be aligned easily.

5′-flanking Sequences. A region of 54–55 nucleotides upstream the coding region displays high levels of interspecific similarity when *Drosophila* 5S genes are compared. Alignments of 5′-flanking sequences are shown in Fig. 4A. These regions, almost identical in *D. melanogaster, D. mauritiana, D. simulans,* and *D. sechellia,* display 67–71% similarity when compared to the corresponding region of *D. teissieri* and 75–76% similarity when compared to that of *D. takahashii.* The lowest interspecies similarity (62%) is found when comparing *D. teissieri* and *D. takahashii* 5S sequences. One 8- and one 10-bp conserved block (positions −1 to −8 and positions −21 to −30, respectively, Fig. 4A) are found in all known *Drosophila* 5S sequences.

3′-flanking Sequences. A region of 21–22 bp is also highly conserved downstream of the coding region in

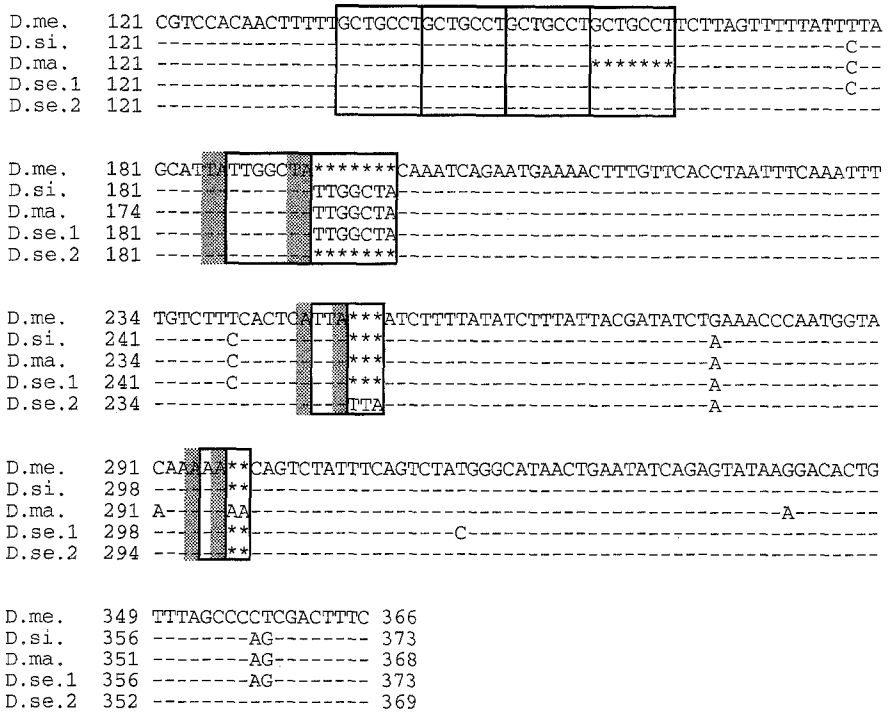

Fig. 2. Comparison of *D. melanogaster, D. simulans, D. mauritiana,* and *D. sechellia* 5S gene sequences. The *D. melanogaster* 5S "consensus sequence" (*D.me.,,* Tschudi and Pirrotta 1980) was used as a reference. Only positions differing from this sequence are shown for the other 5S units. The *D. simulans* sequence (*D.si.*) is taken from our previous work (Samson and Wegnez 1984). The *D. mauritiana (D.ma.)* and the *D. sechellia (D.se.1* and *D.se.2)* sequences are new data. The sequence comparison starts at position 121 (5S coding region: positions 1–120). The *asterisks* indicate that a position is missing in a sequence. One or two identical nucleotides highlighted in *gray* are found on each side of the repeats.

Drosophila 5S genes (Fig. 4B). Interspecies similarities range from 75 to 100%. *D. melanogaster, D. simulans, D. mauritiana,* and *D. sechellia* share the same sequence for this region. The corresponding regions of the other 5S genes differ from this sequence by some substitutions and by a deletion/insertion of 1 bp (Fig. 4B).

Internal Spacer. The internal spacer is the most variable region of the 5S genes when all known *Drosophila* sequences are compared. However, interspecies alignments of the entire noncoding regions of all the 5S genes from the *melanogaster* subgroup are possible when introducing some gaps. In the sequence alignments proposed by BESTFIT, regions surrounding the gaps (6 to 17 nucleotides) display generally significant levels of similarity. Such an alignment of the *D. melanogaster* and *D. erecta* 5S noncoding sequences is shown in Fig. 5.

In the same conditions, alignment of the noncoding regions of the 5S gene from *D. takahashii* can be done with the corresponding regions of the 5S genes from *D. melanogaster, D. orena,* and *D. erecta.* However, in these cases, no significant similarity is evident between some spacer regions when comparing the alignments proposed by BESTFIT. For example, sequences extending from positions 267 to 347 in *D. takahashii* and 288 to 383 in *D. erecta* are aligned by BESTFIT but clearly do not display high levels of similarity (Fig. 5).

Discussion

Sequence comparison of homologous genes in closely related species is a way to uncover molecular features implicated in their evolution. We used this strategy to study the 5S gene family of *Drosophila.* The *melanogaster* group is composed of more than 150 species divided among 10 subgroups. One of these, the *melanogaster* subgroup, includes eight species, i.e., *D. melanogaster, D. simulans, D. mauritiana, D. sechellia, D. teissieri, D. yakuba, D. erecta,* and *D. orena.* In this study, we compare 5S genes of these eight species and of *D. takahashii,* one species belonging to the *takahashii* subgroup, closely related to the *melanogaster* subgroup (Lemeunier et al. 1986).

The phylogeny of the *melanogaster* subgroup has been established according to the chromosome banding patterns (Ashburner et al. 1984), according to the sequence of *Adh* genes (Bodmer and Ashburner 1984), and according to enzyme electrophoretic properties (Cariou 1987). All of these studies show that three subsets of species can be considered. The first subgroup includes *D. melanogaster* and three very closely related species, i.e., *D. simulans, D. mauritiana,* and *D. sechellia.* The second subgroup unites *D. yakuba* and *D. teissieri* and the third *D. erecta* and *D. orena.* Our 5S gene data (Figs. 2 and 3) are in complete agreement with this classification.

The term "concerted evolution" (Zimmer et al. 1980) refers to the observation that members of a gene family display high levels of similarity within every species. In all species so far studied, the repeated 5S genes have been shown to display high levels of intraspecific homogeneity, as opposed to high levels of interspecific heterogeneity (McMahon et al. 1984; Samson and Wegnez 1984; Morzycka-Wroblewska et al. 1985). When comparing 5S genes from closely related species, the differ-

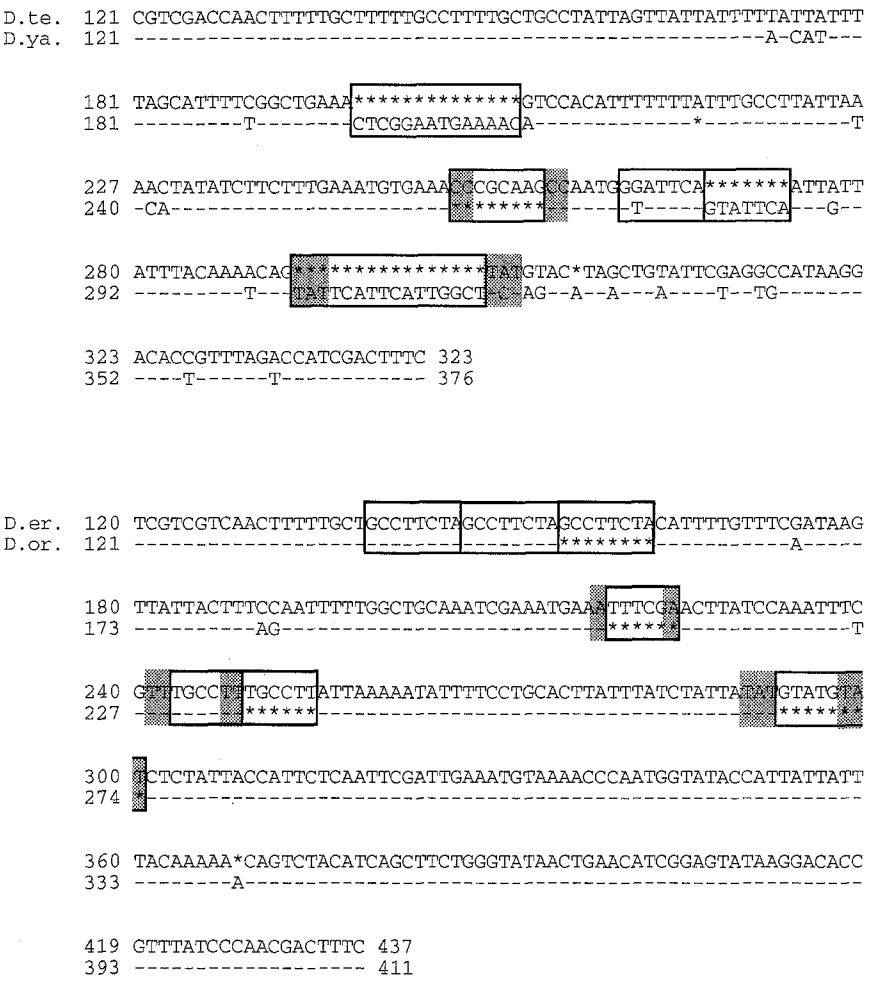

Fig. 3. Pairwise comparisons of *Drosophila* 5S gene sequences. The sequences are aligned according to the rules defined in Fig. 2. The *D. teissieri* 5S sequence is taken from our previous work (Samson and Wegnez, 1984). Identical nucleotides found on each side of *boxed* oligomers are highlighted in *gray. D.te.: D. teissieri, D.ya.: D. yakuba, D.er.: D. erecta, D.or.: D. orena.*

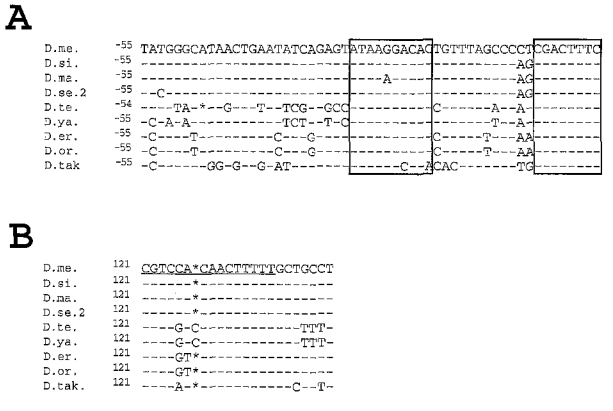

Fig. 4. Comparison of 5' and 3' flanking regions of *Drosophila* 5S genes. The sequences are aligned according to the rules defined in Fig. 2. **A** Alignments of 5'-flanking regions. Two highly conserved subregions are *boxed.* **B** Alignments of 3'-flanking regions. The 15–16 nucleotides transcribed in the 5S RNA precursor are *underlined. D.me.: D. melanogaster, D.si.: D. simulans, D.ma.: D. mauritiana, D.se.: D. sechellia, D.te.: D. teissieri, D.ya.: D. yakuba, D.er.: D. erecta, D.or.: D. orena, D.tak.: D. takahashii.*

ence between intra- and interspecific variability is less obvious. This is particularly striking when comparing the 5S genes from *D. melanogaster* with those of *D. sechellia, D. simulans,* and *D. mauritiana* (Fig. 2).

Evolutionary Conservation of the 5S Coding Region in Drosophila

Constraints modulating the evolution of *Drosophila* 5S genes are not the same along the length of the units. The slowest-evolving segment of the 5S gene is the coding region. In *D. melanogaster,* several kinds of variants within the coding region have been characterized (Tschudi and Pirrotta 1980; Sharp et al. 1984; Samson and Wegnez 1988; Fig. 1). Sharp et al. (1984) demonstrated that such variants are nontranscribed pseudogenes. In the other *Drosophila* species, the 5S coding regions we sequenced are identical to the *D. melanogaster* functional sequence, with the exception of *D. orena* and *D. takahashii* units that differ by one substitution, suggesting they may be pseudogenes. A high proportion of pseudogenes within the 5S locus would thus be a general feature among the *Drosophila* species. A similar situation has been found in *Aspergillus* (Gniadkowski et al. 1991). It is interesting to observe that the 5S coding regions of *Drosophila* species and of *Calliphora erythrocephala,* another dipteran, are identical with the sole exception of position 119, which is A in *Calliphora* and T in *Drosophila* (Rubacha et al. 1984).

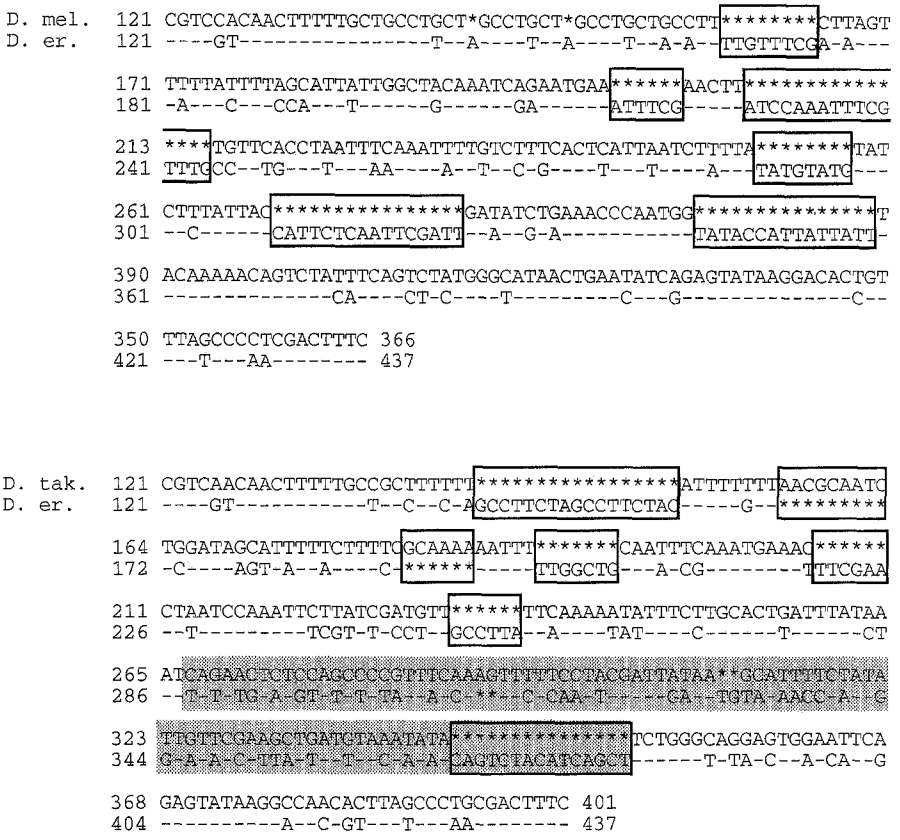

Fig. 5. Pairwise comparisons of *Drosophila* 5S gene spacers. The sequences were aligned by BESTFIT choosing a gap weight of 5.0 and a gap length of 0.3. Alignments are shown according to the rules defined in Fig. 2. Large insertions/deletions are *boxed.* Regions displaying low sequence similarities are highlighted in *gray. D.me.: D. melanogaster, D.er.: D. erecta, D.tak.: D. takahashii.*

Three Regions of the 5S Noncoding Sequence Evolve at Different Rates in Drosophila

In the noncoding region, a high level of heterogeneity is observed when comparing all known *Drosophila* 5S sequences. However, within this region, three subregions clearly evolve at different rates. The 3′-flanking region, as shown in Fig. 4B, evolves slowly. This 22–23-bp region includes the 5S precursor-specific segment (15 nucleotides: Rubin and Hogness 1975; Jacq et al. 1977). Strong selective pressure, probably related to the correct termination of 5S gene transcription, evidently works against modifications of this region.

The 54–55 nucleotides on the 5′-flanking side of the 5S coding region also evolve slowly (Fig. 4A). The first five nucleotides of the 8-bp conserved block (positions −1 to −8) are present in the corresponding region of the *C. erythrocephala* 5S gene (Rubacha et al. 1984). This short tract could be a positioning signal for the initiation of 5S gene transcription. Another conserved block, ATAAGGACAC, is found at positions −21 to −30 (Fig. 4A). It is interesting to observe that one conserved TATAAG block was found at a similar position in almost all 5′-flanking sequences of eukaryotic 5S genes (Rubacha et al. 1984). Conservation of this sequence suggests a functional role, probably in transcription. Sharp and Garcia (1988) indeed demonstrated that the region located between coordinates −26 and −39 is essential for transcription initiation. In *Bombyx mori,* deletion of the corresponding 5′-flanking TATATAG sequence of 5S genes lowers gene transcription significantly (Morton and Sprague 1984).

The internal spacer is the most divergent region of *Drosophila* 5S genes. Low structural constraints probably allow broad variations within the primary structure of this region. Duplications and deletions/insertions of small oligomers account for the intraspecific but also for the interspecific divergence of *Drosophila* 5S gene spacers (Figs. 1–3 and 5). It is noteworthy that the region downstream of the transcribed sequence seems to be a hotspot for oligomer duplications. In *D. teissieri* and *D. yakuba,* a GCTTTTTGCCTTTT sequence present in this region could have diverged from a GCTTTTT or a GCCTTTT dimer by a point mutation (Fig. 3). The accumulation of mutational events probably does not allow one, when comparing species not closely related, to detect all the "old" duplications and deletions/insertions (Fig. 5).

Origin of the 5S Spacer Duplications and Deletions/Insertions

As shown in Figs. 2 and 3, most of the duplicated and deleted/inserted sequences are flanked by small repeats (1 to 3 nucleotides). Fork slippage, one of the molecular processes known to play a significant role in genome turnover (Dover 1993), could explain the origin of these

structures. When comparing 5S spacers from two mice species, Suzuki et al. (1994) found that large size variations were due to the deletion of about 160 bp in *M. spretus* and to the duplication of 68 bp in *M. musculus*. They also observed intraspecific small size variations due to changes in the copy number of microsatellites. Schlötterer et al. (1994) showed that, within the *Drosophila* genus, deletions/insertions account for about half of the mutational events observed in the ITS region of the rDNA. Unique genomic sequences, like the *Adh* genes from *Drosophila* species, on the contrary, differ mainly in nucleotide substitutions (Bodmer and Ashburner 1994). The main type of rearrangement observed when comparing *Drosophila* 5S genes (deletions/insertions due to fork slippage) could thus be a general feature of repeated-sequences evolution. However, overall shaping of 5S gene clusters probably involves several other molecular processes (gene conversion, unequal crossing-over, retrotransposition, transposition . . .). Gene conversion not associated to crossing-over, in this context, could play an essential role in the concerted evolution of *Drosophila* 5S genes (Pâques and Wegnez 1993; Pâques et al., unpublished results).

Acknowledgments. We thank Anne-Marie Pret and Michel Veuille for their helpful comments on the manuscript.

References

Andrews MT, Vaughn JC, Perry BA, Bagshaw JC (1987) Interspersion of histone and 5S RNA genes in *Artemia*. Gene 51:61–67

Ashburner M, Bodmer M, Lemeunier F (1984) On the evolutionary relationships of *Drosophila melanogaster*. Dev Genet 4:295–312

Belkhiri A, Buchko J, Klassen GR (1992) The 5S ribosomal RNA gene in *Pythium* species: two different genomic locations. Mol Biol Evol 9:1089–1102

Bodmer M, Ashburner M (1984) Conservation and change in the DNA sequences coding for alcohol dehydrogenase in sibling species of *Drosophila*. Nature 309:425–430

Cariou ML (1987) Biochemical phylogeny of the eight species in the *Drosophila melanogaster* subgroup, including *D. sechellia* and *D. orena*. Genet Res Camb 50:181–185

Coen E, Strachan T, Dover G (1982) Dynamics of concerted evolution of ribosomal DNA and histone gene families in the *melanogaster* species subgroup of *Drosophila*. J Mol Biol 158:17–35

Dover GA (1993) Evolution of genetic redundancy for advanced players. Curr Opin Genet Dev 3:902–910

Drouin G, Sévigny JM, McLaren IA, Hofman JD, Doolittle WF (1992) Variable arrangement of 5S ribosomal genes within the ribosomal DNA repeats of arthropods. Mol Biol Evol 9:826–835

Gniadkowski M, Fiett J, Borsuk P, Hoffman-Zacharska D, Stepien PP, Bartnik E (1991) Structure and evolution of 5S rRNA genes and pseudogenes in the genus *Aspergillus*. J Mol Evol 33:175–178

Jacq B, Jourdan R, Jordan BR (1977) Structure and processing of precursor 5S RNA in *Drosophila melanogaster*. J. Mol Biol 117: 785–795

Junakovic N (1980) Variability in the molecular organization of the 5S RNA genes among strains of *Drosophila melanogaster*. Nucleic Acids Res 8:3611–3622

Lemeunier F, David J, Tsacas L, Ashburner M (1986) The *melanogaster* species group. In: Ashburner M (ed) The genetics and biology of *Drosophila*. Academic Press, New York, Vol 3e, pp 147–256

Long EO, Dawid IB (1980) Repeated genes in eukaryotes. Annu Rev Biochem 49:727–764

Maxam AM, Gilbert W (1980) Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol 65:499–560

McMahon ME, Stamenkovich D, Petes TD (1984) Tandemly arranged variant 5S ribosomal RNA genes in the yeast *Saccharomyces cerevisiae*. Nucleic Acids Res 12:8001–8016

Metzenberg RL, Stevens JN, Selker EU, Morzycka-Wroblewska E (1985) Identification and chromosomal distribution of 5S rRNA genes in *Neurospora crassa*. Proc Natl Acad Sci U S A 82:2067–2071

Morton DG, Sprague KU (1984) *In vitro* transcription of a silkworm 5S RNA requires an upstream signal. Proc Natl Acad Sci USA 81: 5519–5522

Morzycka-Wroblewska E, Selker EU, Stevens JN, Metzenberg RL (1985) Concerted evolution of dispersed *Neurospora crassa* 5S RNA genes: pattern of sequence conservation between allelic and nonallelic genes. Mol Cell Biol 5:46–51

Pâques F, Wegnez M (1993) Deletions and amplifications of tandemly arranged ribosomal 5S genes internal to a P element occur at a high rate in a dysgenic context. Genetics 135:469–476.

Rubacha A, Sumner III W, Richter L, Beckingham K (1984) Conserved 5' flank homologies in dipteran 5S RNA genes that would function on 'A' form DNA. Nucleic Acids Res 12:8193–8207

Rubin GM, Hogness DS (1975) Effect of heat shock on the synthesis of low molecular weight RNAs in *Drosophila:* accumulation of a novel form of 5S RNA. Cell 6:207–213

Samson ML, Wegnez M (1984) The 5S ribosomal genes in the *Drosophila melanogaster* species subgroup. Nucleotide sequence of a 5S unit from *Drosophila simulans* and *Drosophila teissieri*. Nucleic Acids Res 12:1003–1014

Samson ML, Wegnez M (1988) Bipartite structure of the 5S ribosomal gene family in a *Drosophila melanogaster* strain, and its evolutionary implications. Genetics 118:685–691

Samson ML, Wegnez M (1989) An approach to study the evolution of the *Drosophila* 5S ribosomal genes using P-element transformation. J Mol Evol 28:517–523

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 74:5463–5467

Schlötterer C, Hauser MT, von Haeseler A, Tautz D (1994) Comparative evolutionary analysis of rDNA ITS regions in *Drosophila*. Mol Biol Evol 11:513–522

Selker EU, Yanofsky C, Driftmier K, Metzenberg RL, Alzner-DeWeerd B, RajBhandary UL (1981) Dispersed 5S RNA genes in *N. crassa:* structure, expression and evolution. Cell 24:819–828

Sharp SJ, Garcia AD (1988) Transcription of the *Drosophila melanogaster* 5S RNA gene requires an upstream promoter and four intragenic sequence elements. Mol Cell Biol 8:1266–1274

Sharp S, Garcia A, Cooley L, Söll D (1984) Transcriptionally active and inactive gene repeats within the *D. melanogaster* 5S RNA gene cluster. Nucleic Acids Res 12:7617–7632

Suzuki H, Moriwaki K, Sakurai S (1994) Sequences and evolutionary analysis of mouse 5S rDNAs. Mol Biol Evol 11:704–710

Tschudi C, Pirrotta V (1980) Sequence and heterogeneity in the 5S RNA gene cluster of *Drosophila melanogaster*. Nucleic Acids Res 8:441–451

Tschudi C, Pirrotta V, Junakovic N (1982) Rearrangements of the 5S RNA gene cluster of *Drosophila melanogaster* associated with the insertion of a *B104* element. EMBO J 1:977–985

Zimmer EA, Martin SL, Beverley SM, Kan YW, Wilson AC (1980) Rapid duplication and loss of genes coding for the α chains of hemoglobin. Proc Natl Acad Sci U S A 77:2158–2162