Evolutionary conservation and molecular characteristics of repetitive sequences of *Drosophila koepferae*

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Thirteen middle repetitive DNA clones obtained from the genome of *Drosophila koepferae* have been tested for their evolutionary conservation in the other seven species of the *buzzatii* and *martensis* clusters (*repleta* group). All but two of these clones exhibit qualitatively similar patterns of hybridization in the eight species. The average interspecific hybridization signal is 85 per cent of that found intraspecifically, ranging from 73 to 93 per cent. Partial sequencing of six of these clones has shown sequences related to the retrotransposon *Gypsy*, first characterized in *D. melanogaster*, as well as to the *Anopheles gambiae* LINE elements *T1Ag* and *Q*. A fragment of a hitherto unknown, short inverted repeat transposable element has also been found. The evolutionary conservation of repetitive *D. koepferae* sequences seems to be related to the high proportion of simple DNA and inactive mobile elements in the genome of this species.

Keywords: *Drosophila*, *Gypsy*, LINE, repetitive DNA, *repleta* group.

Introduction

Middle repetitive sequences obtained from *Drosophila melanogaster* frequently show a low hybridization signal or are absent in some of its closest relatives, the other species of the *melanogaster* species subgroup (Young, 1979; Dowsett & Young, 1982; Dowsett, 1983). These studies also demonstrated that mobile sequences are the main middle repetitive DNA component of the genome of *D. melanogaster*. Interestingly, the repetitive sequences obtained from *D. simulans*, a sibling of *D. melanogaster*, are much more conserved in the other *melanogaster* subgroup species, and the genome of *D. simulans* is characterized by a low number of mobile elements (Dowsett, 1983). This relationship between mobility and evolutionary instability, particularly loss of mobile sequences in closely related species, has been predicted theoretically and is well supported by experimental evidence. Provided there is a long evolutionary time, theoretical models predict frequent stochastic losses of mobile elements in finite populations, especially

under certain conditions such as small population sizes, high excision rates, or generation of defective elements that compete with active ones (Charlesworth, 1985; Kaplan *et al.*, 1985; Charlesworth & Langley, 1989). In good agreement with these expectations, when 11 characterized mobile elements (10 from *D. melanogaster* and one from *D. mauritiana*, also a species of the *melanogaster* subgroup) were tested for their phylogenetic distribution in other species of the *Drosophila* genus, all showed a patchy distribution. Moreover, nine of these elements have a low hybridization signal in one, or frequently several, of the *D. melanogaster* subgroup species (Martin *et al.*, 1983; Brookfield *et al.*, 1984; Bucheton *et al.*, 1986; Stacey *et al.*, 1986; Silber *et al.*, 1989; Daniels *et al.*, 1990a,b; Mizrokhi & Mazo, 1990; Maruyama & Hartl, 1991).

In a previous study (Marín *et al.*, 1992), we characterized the middle repetitive component of *D. koepferae* and *D. buzzatii*, two sibling species of the *repleta* group. Our results showed three interesting features. First, when hybridized against genomic DNA, a high number of clones showed a smeared pattern, similar to those produced by simple DNA sequences. These sequences are so abundant that the percentage of repetitive DNA in these two

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species is estimated to be higher than that found in any other species of the genus. Secondly, out of more than 100 clones tested, all but two showed similar hybridization patterns against the genomic DNA of both species. Finally, although all those clones which showed a pattern of hybridization against genomic DNA compatible with mobile elements (a limited number of bands with similar hybridization intensities) were tested, only two from *D. koepferae* and one from *D. buzzatii* showed a dispersed pattern of *in situ* hybridization on polytene chromosomes. The conclusion of our preliminary work was that the more abundant repetitive component of these species is quite different from that of *D. melanogaster*, and is probably simpler and more repetitive. Sequences related to mobile elements, with only a few exceptions, were predicted to be inactive and concentrated in the centromeres.

In this study we have tested for the evolutionary conservation of certain repetitive sequences of *D. koepferae* in other related species of the *repleta* group. In addition, by sequencing different fragments of a number of clones, we have recognized sequences with clear relationships with previously described mobile elements, the retrotransposon *Gypsy* of *D. melanogaster* (Marlor *et al.*, 1986) and the nonviral retrotransposons *TAg* and *Q*, first characterized in *Anopheles gambiae* (Besansky, 1990; Besansky *et al.*, 1994). A fragment of a new short inverted repeat mobile element has also been detected. Simple DNA sequences were also found, and we have an example of a scrambled arrangement of one of these sequences with a defective mobile element. These results help to clarify the differences between the repetitive components of *D. koepferae* and *D. melanogaster*.

Materials and methods

Drosophila strains

Strains from the eight species that form the *marten-sis* and *buzzatii* clusters (*buzzatii* complex, *mulleri* subgroup, *repleta* group; Ruíz & Wasserman, 1993) were used. These will be referred to as follows. (a) *buzzatii* cluster: KO4 (*D. koepferae*, Vipos, Argentina); BSL (*D. buzzatii*, San Luis, Argentina); SD14 (*D. serido*, Cafarnaum, Brazil, Bowling Green stock no. 15081–1431.4); BM1 (*D. borborema*, Cafarnaum, Bowling Green stock no. 15081–1281.0). (b) *marten-sis* cluster: SM3 (*D. starmeri*, Curaçao, Netherland Antilles); VZ8 (*D. venezolana*, Curaçao); UN5 (*D. uniseta*, La Boca, Venezuela); MA4 (*D. martensis*, Guaca, Venezuela).

Characteristics of the clones

Thirteen clones obtained from stock KO4 were used in this study. The sizes, patterns of hybridization against genomic DNA, and results of *in situ* hybridization on polytene chromosomes of 11 of these clones were described in Marín *et al.* (1992). The other two, cDk13 (4.6 kb) and cDk32 (6.1 kb) share their patterns of genomic DNA (one main band plus smeared hybridization) and *in situ* hybridization (one euchromatic band).

Southern blot analysis

Total genomic DNA from adult flies, obtained according to Piñol *et al.* (1988), was cut with *Hind*III, electrophoresed in 0.6 per cent agarose gels and transferred to Hybond-C extra nitrocellulose membranes (Amersham International, Bucks., U.K.). In each electrophoretic run, lanes were loaded with approximately the same amount of DNA (3–5 µg). Preparation of the probes, hybridization and developing procedures were performed as described by Marín *et al.* (1992). The stringency of hybridization (5 × SSC, 50 per cent formamide, 42°C) and posthybridization washes (0.16 × SSC, 50°C) used allows detection of sequences with at least 80 per cent identity (supposing G–C content = 50 per cent and a paired sequence length of 0.5 kb; Wetmur, 1991).

Quantification of the hybridization signals

The intensity of the hybridization signals was measured using a Vinix (Vicom–VDP) image analysis system. Images from each lane were digitized using a Hamamatsu c2400/01 camera equipped with a Canon 35–70 mm macro zoom lens under constant lighting, converting them into an array of pixels, each with grey values ranging from 0 to 128. For each lane, the average intensity per pixel, calculated after dividing the integrated hybridization intensity in that lane by its area, was corrected by subtracting the average intensity per pixel in a blank area of the same filter. The relative abundance of DNA hybridization (Dowsett & Young, 1982; Dowsett, 1983) was calculated by dividing the average intensity per pixel in the experimental lane by that of the control lane (KO4 strain), after subtracting the background signal, and eventually correcting for differences in DNA quantity. DNA amounts were estimated by a similar procedure using Polaroid photographs of the ethidium bromide-stained electrophoresis gel taken before Southern transfer. Under our current system

of quantification there was a linear relationship between DNA quantity and signal intensity in these gels up to a saturation value (75 grey intensity units/pixel). Eighty-seven out of the 91 estimations (96 per cent) were included in the linear part of the curve.

DNA sequencing and sequence analysis

Plasmid pBluescript KS⁺ (Stratagene) was used for subcloning procedures. High-quality supercoiled plasmid DNA for sequencing was obtained using Magic Minipreps columns (Promega, Madison, WI). Nucleotide sequences were determined by using the dideoxynucleotide chain-termination method (Sanger *et al.*, 1977). For the determination of similarities and sequence alignments, the GCG software package (Genetics Computer Group, 1991) was used. The FASTA program was used for nucleotide searches against the GenBank and EMBL databases. The nucleotide sequences were translated in the six possible frames and the predicted proteins were compared with the PIR and Swiss-Prot databases using TFasta. The BESTFIT and PILEUP programs were used for establishing the best alignments of the zones that gave positive results in the FASTA or TFasta searches. Although most of the sequences were determined only in one strand, this does not affect any of the conclusions concerning the homologies found (they extend along hundreds of base pairs) or the activity of the copies of the elements

analysed (we have considered inactive those sequences with large insertions or deletions).

Results

Most of the D. koepferae repetitive sequences tested are shared by the other seven species of the buzzatii and martensis clusters

Table 1 summarizes the estimations of the intensity of hybridization for the 13 clones. The average for all comparisons was 0.85 ± 0.04 . Eleven of the 13 repetitive sequences, the exceptions being cDk210 and cDk254, showed similar overall patterns of hybridization in the eight species of the *buzzatii* and *martensis* clusters (some examples are shown in Fig. 1). Differences found for some species in the clones cDk2, cDk13 and cDk665 were quantitative, but their hybridization patterns were similar to that of *D. koepferae*. This result confirms our previous findings: out of more than 100 repetitive clones obtained from the siblings *D. koepferae* and *D. buzzatii*, only cDk210 and cDk254 revealed significant differences in hybridizations against genomic DNA of these two species (Marín *et al.*, 1992). When our results are compared with those obtained for *D. melanogaster* (Dowsett, 1983), it can be seen that the distributions of the relative abundance of hybridization are qualitatively different (Fig. 2). When *D. koepferae* sequences were used as probes, only three out of 91 estimates (3.3 per cent) had

Table 1 Relative abundance of hybridization of the thirteen *Drosophila koepferae* clones in the other species of the *buzzatii* and *martensis* clusters. The control value for *D. koepferae* is 1.00. BSL, *D. buzzatii*; SD14, *D. serido*; BM1, *D. borborema*; SM3, *D. starmeri*; VZ8, *D. venezolana*; UN5, *D. uniseta*; MA4, *D. martensis*

Clone	BSL	SD14	BM1	SM3	VZ8	UN5	MA4
cDk2	1.03	1.20	0.98	0.56	1.29	0.53	0.28
cDk13	0.67	0.37	0.51	0.37	0.24	0.50	0.49
cDk27	0.68	0.87	0.63	0.79	0.90	0.89	0.92
cDk32	0.96	0.84	0.83	1.01	1.06	0.81	0.81
cDk72	0.89	0.67	0.75	1.01	1.15	1.38	1.32
cDk210	0.25	0.65	0.66	0.60	0.58	0.48	0.59
cDk254	0.10	0.19	0.54	0.49	0.49	0.41	0.01
cDk265	0.72	0.71	0.71	0.66	0.64	0.81	0.86
cDk270	0.80	0.96	1.12	0.83	0.67	0.66	0.48
cDk271	1.68	1.80	1.91	0.71	1.23	1.39	1.13
cDk323	1.31	1.01	1.76	0.73	1.54	1.85	1.73
cDk596	1.11	1.09	1.01	1.27	1.38	1.16	1.31
cDk665	0.77	1.01	0.73	0.48	0.39	0.41	0.29
Means $\pm$ SEM	0.84 ± 0.11	0.87 ± 0.11	0.93 ± 0.12	0.73 ± 0.07	0.89 ± 0.12	0.87 ± 0.13	0.79 ± 0.14

Fig. 1 Examples of hybridization patterns of *Drosophila koepferae* repetitive sequences in the species of the *buzzatii* and *martensis* clusters. (a) cDk596; (b) cDk323; (c) cDk2; (d) cDk27. Lanes: 1, KO4 (*D. koepferae*); 2, BSL (*D. buzzatii*); 3, SD14 (*D. serido*); 4, BM1 (*D. borborema*); 5, SM3 (*D. starmeri*); 6, VZ8 (*D. venezolana*); 7, UN5 (*D. uniseta*); 8, MA4 (*D. martensis*).

Fig. 2 Distributions of the values of the relative abundance of hybridization. *Drosophila koepferae*: 91 comparisons (this study). *D. melanogaster*: 112 comparisons (Dowsett, 1983).

values lower than 0.20. However, using *D. melanogaster* repetitive sequences, this percentage was 48.2 per cent (54 out of 112 comparisons).

Partial sequencing of selected clones reveals fragments of three different mobile elements

The sequence of one of the clones studied here (cDk27, 0.7 kb) was shown by Marín *et al.* (1992). It carries two stretches of low-complexity DNA. In this study we have sequenced 8.4 kb, including different fragments of the other six clones, accounting for 37 per cent of their length. These clones were chosen because their pattern of hybridization against genomic DNA and/or their *in situ* pattern on polytene chromosomes suggested that they could carry mobile sequences. Our strategy has been to sample for the types of sequences in these clones by sequencing randomly several zones, starting from some of the available restriction sites. A summary of these results is as follows.

cDk2 (4.5 kb) In this centromere-hybridizing clone, 1.9 kb have been sequenced. Two different parts have shown similarity to mobile elements that have

been described previously. The predicted protein for one zone is related to those of the *TIAg* and *Q* nonviral retrotransposons (Besansky, 1990; Besansky *et al.*, 1994). In particular, a fragment related to its reverse transcriptase has been detected. A second interesting region shows significant similarity to the *Gypsy* retrotransposon as well as to other *Gypsy*-related sequences. Inside this sequence a 350 bp simple DNA stretch is found, consisting in partially degenerate TAA repeats (see below).

cDk210 (1.8 kb) Marín *et al.* (1992) found that this clone has a dispersed repetitive sequence with variable cytological positions in different *D. koepferae* stocks. This clone was used as a probe to obtain three homologous recombinant phages and the dispersed sequence has been characterized. It consists of a new short inverted repeat mobile element that we have named *Gandalf* (Marín & Fontdevila, 1995). Sequencing of cDk210 showed that it carries 0.5 kb of the putative 3' end of this element.

cDk254 (2.6 kb) Approximately 2.0 kb of this clone has been sequenced. cDk254 hybridizes predominantly on the telomeric regions (Marín *et al.*, 1992). Comparisons with sequence data banks did not show significant DNA similarities.

cDk270 (4.9 kb) This clone bears a dispersed repetitive sequence and also hybridizes in the centromeres (Marín *et al.*, 1992). 1.0 kb have been sequenced. Similarity to the *TIAg* and *Q* elements has been observed, corresponding to the same regions found in cDk2.

cDk323 (5.1 kb) This clone is mainly centromeric, although a few euchromatic bands have been found in some stocks (Marín *et al.*, 1992). 1.4 kb have been sequenced. A very high similarity to the reverse transcriptase of *Gypsy* and other *Gypsy*-type elements has been found.

cDk665 (3.9 kb) Of this clone, 1.5 kb has been sequenced without significant findings.

Gypsy-related defective sequences in the genome of D. koepferae

cDk2 and cDk323 bear sequences closely related to the *Gypsy* element. Similarity is found both at the DNA and protein levels. Comparisons with the *D. melanogaster* and *D. virilis* elements show that the *Gypsy*-related sequence of cDk2 is divided into two

fragments (A and B) by a 350 bp sequence of unknown origin, composed mainly of degenerate (TAA)_n repeats (Fig. 3). Regions A and B of cDk2 are 300 bp apart in the *Gypsy* sequences of *D. melanogaster* and *D. virilis*, in the same order as they are found in cDk2. Clearly they comprise a single *Gypsy*-like sequence in which this 300 bp inner fragment has been substituted by the TAA repeat. There are no signs of any (TAA)_n sequence in the homologous zones of the *D. melanogaster* or *D. virilis* *Gypsy* elements.

Database searches revealed that region A (265 bp) shows significant nucleotide identity with five different sequences; that is, the *Gypsy* elements of *D. melanogaster* and *D. virilis* (Marlor *et al.*, 1986; Mizrokhi & Mazo, 1991), a third sequence (accession number X15707) found in association with different rRNAs in a *D. melanogaster* clone (V. A. Kokoza, N. G. Kholodilov and I. B. Rogozin, unpublished data) and two sequences obtained from the Y chromosome of *D. hydei* (accession numbers X74538 and X74539; Hochstenbach *et al.*, 1994). Region B (357 bp) has been found to be related to four of these five sequences, and to another one, a piece of a *Gypsy*-related element inserted in a *Micropia* retrotransposon of *D. hydei* (clone DhMiF8; accession number X13305; Lankenau *et al.*, 1990). The protein alignments of these two regions are shown in Fig. 4. The *Gypsy* sequences of cDk2 are similarly related to all these sequences, irrespective of the phylogenetic proximity of their host species. Their nucleotide identities range between 50 and 60 per cent. These results contrast with the fact that the active elements of *D. melanogaster* and *D. virilis* show a 75 per cent nucleotide identity in the same zones. At the protein level, the similarity between the active elements (over 80 per cent, identity over 75 per cent) is also higher than the values for the cDk2 sequence (identity 40 per cent, similarity 60 per cent).

Two features of the *Gypsy*-related sequence of cDk2 are worthy of mention. First, this sequence corresponds to the *env*-like gene characteristic of the *Gypsy*-type retrotransposons (Smith & Corces, 1991). This gene, which codes for the envelope protein in retroviruses, is known to change rapidly (McClure *et al.*, 1988). No other characterized retrotransposon has shown homology with the *env*-related sequence of cDk2. A second important point is that the cDk2 *Gypsy*-related sequence is clearly nonfunctional. Not only does it have a 350 bp insertion, but also frameshifts have been found in the predicted coding region. A similar situation is found in the *Gypsy*-related sequence in cDk323. The character-

ized sequence is related to the carboxyl terminal end of the *Gypsy* reverse transcriptase enzyme. This sequence is more conserved among elements than those found in cDk2, as expected for a protein that

is much more functionally constrained than the *env*-related sequences. Fig. 5 shows the best alignments of cDk323, indicating the domains 4–7 of the reverse transcriptase enzyme, as defined by Xiong &

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ACAGTAGATCTTTTAATAAGAGACACCAAAAGAAAGCAAATAGACACGGAACTTTGTATGAAAC
T V D L L I R D T K R K Q I D T E L L Y E T

ACTGCTCTCTAGAAACAGAATAAGTATTTAAGAATTTAAAAACCTAATGCTATCTGTAACCTAATG
L L S R N R I S I * E F K N L M L S V T N A

CGAAAGTTGGAATTGTAACCCCCCATATGGACTCTTTTGNNTTTAAAAAAATATTGCTAAC
K V G I V N P P I L D

GNAACACCCATATAATATTACTATTACTAACCTAATTAAGTATCCCGTTGGTTTTTTTAAATAA
TAATAATAATAATANTNATAATAACAATAAATAATAATAATATTAGTTAAATAATTAGTTTTGTT
AAATAATAAAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATA
GTTTTGTTAAATAATAATAACAATAATAATAATAATAATAATAATAATAATAATAATAATAATA
ATAACAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATA
CCTCAAAATAACGCTCTGCAAAAAAATACCTTACACTTCGTGCGCAGAACAAATGCACTCCGG
H S G

TGGCACCGCACATTGCCCACTAGACCTAGGCATCTAGAACTTCTAACCATCATCGACGATGGCG
G T A H C P T R P R H L E L L T I I D D G V

TTATCGTCATCAACGACAGACGGATATAGTTGACGGGCAAATGATAAAAGGGACATTACTCCTT
I V I N D R A D I V D G Q M I K G T L L L

ACCTTTGACGATGGGATAAAGGTAAATGNCACCGTATACAAAAATCCAAAAGCCTACATGAGGAA
T F D D G I K V N X T V Y K N P K A Y M R K

ATCCCCG
S P
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REGION A

cDk2	TVDLLIRDTKRKQIDTELLYETLLSRNRISIXEFKNLMLSVTNAKVGINVPPILD
GYPY1	TINKVINARKGLVDTPHLYEALLARNRMLSTEIQNLILITILVKSNIINPTILD
GYPY2	TINKIISRKGLVDTPHLYETLLARNRILNSEIQNLVLTITLAKASIVNPTILD
X15707	NRQNFSEISQTSQIDTGHLYETLLARNRMFMAELQNLMLAITLAKINIVSSNILD
X74538	TVNEILKAWKAIQIDTSHLYETLLARNRMLTLEIENLIVAITLATANIINPVIIN
X74539	TINKIISRKGLVDTPHLYETLLARNRILNTEIWNLMITITLAKGNIVNPTILD
CONSENSUS	T-N--I--K---DT-HLYETLLARNR-L--EI-NL-L-ITLAK-NIVNP-ILD

REGION B

cDk2	HSGGTAHCPTPRHLELLTIIDDGIVIVIND---RADIVDG---QMKGTLTLLTFD
GYPY1	HAGNAAQCHTQPSHLREINPVDDGVVINEAAHVSTDGS-PETLIEGTYLVTFE
GYPY2	HAGNIANCHTQPSHLKAIMPVDDGVVINEATAHVRTDD- AEVTVSGTFLITFE
X15707	HAGGVVACHRVQESDLHPITYVDEGIILINDRSKVRVDNGY/KSWTHGTHLITFD
X13305	QAGGTARCHTQPSHLKPITVDDGIVVNESPARISTDDS-PEIAVN----VTFE
X74538	HAGKTAHCNTQPNELAVVTLVDDGILVLNDSPARVTIDDG-PENTITGTYLVTFD
CONSENSUS	HAG--A-C-TQPSHL--IT-VDDG--VIN--A-V--DD---E----GT-L-TF-

cDk2	DGIEKVNXTVYKPKAYMRKSP
GYPY1	RTATINGSEFVN----LRKTL
GYPY2	RSATINGTEFVN----LRKSL
X15707	KQATINDTPPSSTTITPRREL
X13305	RTAFINGSKYVN----RREIL
X74538	RFAYVNGTRFTNNNGIVKMIIP
CONSENSUS	R-A-INGT---N-----R--L

Fig. 3 Regions related to *Gypsy* in the *Drosophila koepferae* clone cDk2: the two zones with similarity (referred to as regions A and B in the text) and their predicted proteins. They are separated by a 300 bp sequence with numerous TAA repeats (underlined).

Fig. 4 Protein sequence similarity among the predicted product of the *Drosophila koepferae* clone cDk2 and *Gypsy* sequences. Bold letters represent amino acids identical to those in cDk2. Some gaps (–) and frameshifts (/) have been added to improve the alignments. The consensus sequence includes those positions where at least four sequences have the same amino acid. GYPY1, *D. melanogaster* *Gypsy* element; GYPY2, *D. virilis* *Gypsy* element; X15707, *D. melanogaster* *Gypsy*-related sequence; X13305, X74538 and X74539, *D. hydei* *Gypsy*-related sequences (see text).

	Domain 4	Domain 5
cDk323	EF CRLLP F GLKNAPS I FOR S I-DVLREQ I G K I	CYVYVDDV I I-----
GYPSY1	EF CRLLP F GLRNASS I FORALDDVLREQ I G K I	CYVYVDDV I I F SENE S D
GYPSY2	EF CRLLP F GLRNASS I FORAIDDLREH I G K I	CFVYVDDV I I F SKNET E
X74538	EF CRLLP F RSRPDGS I FOR T IDDVLRA R IG K T	CYVYVDDV I I F SKSEL D
X74539	EF CRLLP F GLRNAS G IF Q RA/DDILREH I G K I	CFVYVDDV I I F SKNER E
X15707		
CONSENSUS	EF CRLLP F GLRNA-S I FOR--DD-LRE-IG K I	C-VYVDDV I I F S--E--

Fig. 5 Alignment of the predicted product of the sequence found in the *Drosophila koepferae* clone cDk323 with the retrotranscriptases of the *Gypsy*-related sequences (domains 4–7 according to Xiong & Eickbush, 1990). Consensus sequence obtained as in Fig. 4. Bold letters represent amino acids identical to those found in cDk323. Gaps (–) and frameshifts (/) have been included to improve alignment. GYPSY1, *D. melanogaster* *Gypsy* element; GYPSY2, *D. virilis* *Gypsy* element; X74538, X74539, *D. hydei* *Gypsy*-related sequences; X15707, *D. melanogaster* *Gypsy*-related sequences (see text).

	Domain 6	Domain 7
cDk323	----- K NLRDAGMRVL K /K S K F	F K SVEYL G IT V
GYPSY1	HVRHIDTVLKCLIDANMRVSQ E K T R F	F K ESVEYL G F I V
GYPSY2	HLQHINIVLKCKIDANMRVG P E K T R F	F K ESIEFL G F I V
X74538	H*QG I KQVLECLRDANMRVS K E K S H F	F K KSVEFL G F V V
X74539	HLQHLNIVLKCLIDANMRVG P E K K R F	F K ESIEFL G F T V
X15707		S VSFL G F I V
CONSENSUS	H -----V L E K L-DANMRV--E K -- F	F K -SVEFL G F-V

cDk323	C RGG I Q T SADK V RA I K D F Q P P K T LFN L RS F LG X S Y Y R C F IAG F AK I A
GYPSY1	SKD G T K SD P E K V K AI Q E Y PE P DCV Y K V RS F LG L AS Y Y R V F IK D FA A IA
GYPSY2	TKD G ATSD P K V K A I Q E F PE P KNV S VS R FL G L A NY R V F IK D FA A IA
X74538	SS S G T RT S Q E K V HA I KN F A E PT N L F AV S FL G L A S Y Y R C F VR D FA A IA
X74539	TKD G ATSD P K V K A F Q E F PK P KS V HS V RS F LG L AS Y Y R V F IK D FA A IV
X15707	TC N GAT T D P E K V H A I KE F PE P KS V FE I RS F LG L AS Y Y R FI K GF S KL A
CONSENSUS	---G---D P - X V- A I-E F PE P --V--V S FL G L A S Y Y R -FI K DFA A IA

Eickbush (1990). As this figure shows, cDk323 domains 5 and 6 have been partially deleted. Moreover, a frameshift mutation is found in domain 6. As it occurred in cDk2, the *Gypsy*-related sequence of cDk323 is defective. cDk323 is similarly related to the *D. melanogaster* and *D. virilis* elements (average nucleotide identities, 62 per cent; average protein similarities, 77 per cent), whereas the comparison between these active elements shows values of 75 per cent nucleotide identity and 90 per cent protein similarity in the same zones.

D. koepferae has sequences related to T1Ag and Q, two *Anopheles gambiae* nonviral retroposons

Similar sequences have been found in clones cDk2 and cDk270. Database searches have shown that the putative proteins encoded by these sequences are related to those of the elements T1Ag and Q, two nonviral retroposons first found in the very distantly related dipteran *Anopheles gambiae* (Besansky, 1990; Besansky *et al.*, 1994). However, DNA identities are below 50 per cent in the whole sequences (58 per cent in the best aligned regions).

Two different stretches showing similarity to these elements have been sequenced for each clone (Fig. 6). There was no *a priori* reason for expecting any similarity between these clones, because their

restriction maps are completely different. In fact, the nucleotide sequences in these clones are only 82 per cent identical in the determined zones. The cDk2 and cDk270 sequenced zones most probably correspond to (1) region A: a zone of unknown function typical of some nonviral retroposons (a low similarity has been found with the *D. melanogaster* elements *F*, *G*, *Doc* and *Jockey*, and especially with the *Aedes aegypti* element *Juan-A*; data not shown) placed at the beginning of the second open reading frame of these elements, amino terminally to the sequences related with the capsid proteins of retroviruses (McClure, 1991) and (2) region B: the 'Z' region, immediately amino terminal to the reverse transcriptase (McClure, 1991), together with the first, and in cDk2, the second domains of this protein (Xiong & Eickbush, 1990). As happened in the case of the *env*-related proteins, selective constraints acting on these regions are expected to be rare. Protein comparisons have shown that cDk2 and cDk270 are likewise related to T1Ag and Q (cDk2/T1Ag, 32 per cent identity and 78 per cent similarity; cDk2/Q, 34 per cent identity and 75 per cent similarity; cDk270/T1Ag, 31 per cent identity and 79 per cent similarity; cDk270/Q, 36 per cent identity and 78 per cent similarity). Comparisons between these sequences established that region B of cDk270 has a short deletion including part of the

REGION A

cDk2 LVYDII**VL**TETWLTONVHNSEIMSSN-**FTIYRLDR**-----LNRRGGGV**FI**AV
 cDk270 SSYNLI**AL**TETWLSHSIHNSEILSSN-**FTIYRLDR**-----LNRRGGGV**XI**AV
 T1Ag SGFEM**LA**TETWLNESIPSNMVLDSDSY**NIYRC**DRSRLNNERS**RG**GGV**LL**AC
 Q ADFDLI**AT**TETWLVNDNIPSALLFNNN-**FSVYRC**DRS-LSGSSSRGGGV**LL**AV

REGION B

cDk2 CIRRFRI SRPGFKDSLNLNRNIKSRFYKKYKRSCLAVDWVKYIAKSNFNLLN
 T1Ag IFRSPNPFPWSNRTLRLNLKKDRMKYLRRLNRSANFRLFKYAASAHRLYN
 Q VQRPKPNPFWADRTLKRLKRVKRAAYRHYQTRRCQRSRSIYFDTHSLYCSYN

cDk2 SRCYENYLIRVKRQFTSDPKQFYTFVNS**KRK**SSGLPTSFCEGTEKSCEDQV
 cDk270 **KSKRK**SSXLPTSFFFGLKKSCEDQE
 T1Ag RARFEAYSSRLQSRFRSDPASFWQFVR**IRRG**CNTLPNEMVLDSTRASTPVE
 Q RFRYRRLSKIQRNLCRWPDSEFWRFYNS**SKTK**STHTPKSITYKGATSANTNE

cDk2 **SSDLFAKFF**QSTYSS----VDCFNNSYPFSFSETFSIDVPRVSDSLAAAI
 cDk270 **SSDLFAKFF**QSTYSS----VDSINYSNTYSFSEFYSVNIPKISADCLVAAI
 T1Ag **ICELFSAHF**SQMFEPSPVDPNLIIEGGLLYTPENLINLSDISVSSETVVQVL
 Q **MCNLFADRF**ADCFSPAMNDTDTIDAALVNTPAGAINMSTPFIDSEIVLSAL

cDk2 **RTLKSSFS**PGADNIPSCVKECSASLLSPLLKLFNL**SLDTSVFPSCWK**
 cDk270 **RTLKSSFS**PGADNIPSCVKECSAALLSPLL-----**TSVFPLSWX**
 T1Ag **FGLKRSFT**PGPDGIPASVLINCKDVLAPHLAKIFNL**SLSLGVFPALWK**
 Q **AQLKPSFAP**PGPDGIPSTVLKRCQTTPAVILAKLFN**ASLANGYFPKAWR**

cDk2 **ESFIIP**LHKKGKSDISNY**RGIA**KL SVIPKLSEKI
 cDk270 **ESFIIP**LHKKGGRS
 T1Ag **SCWLF**FPVHKKGCRSIVSNY**RGIT**TOTCAT**AKTFELV**
 Q **KSWMVPI**YKKGDRDADAINY**RGIT**SLCAIAK**VFELV**

Fig. 6 Alignment of the *T1Ag/Q*-related sequences found in the *Drosophila koepferae* clones cDk2 and cDk270 with the sequences of the *Anopheles gambiae* elements. Bold: blocks of conserved amino acids. The two zones underlined in region B correspond to domains 1 and 2 of the reverse transcriptase (Xiong & Eickbush, 1990).

first domain of the reverse transcriptase. This deletion includes a frameshift, so, most probably, the sequence in cDk270 is not functional. The sequence determined in cDk2 does not allow us to eliminate the possibility that this clone contains part of a functional mobile element.

Discussion

Conservation of D. koepferae repetitive sequences

Thirteen clones, obtained from an Argentinian stock of *D. koepferae*, were selected in order to test whether the repetitive sequences of this species are evolutionarily conserved in its closest relatives, in a study parallel to that of Dowsett (1983) on the *melanogaster* subgroup. No other groups have been similarly studied. A few sequences from *D. subobscura* (Felger & Sperlich, 1989), *D. algonquin* (Hey, 1989) and *D. virilis* (Riede *et al.*, 1983) have been tested in related species, but their degree of conservation was not analysed. Our results differ qualitatively from those found for *D. melanogaster* and its relatives (Fig. 2). It is difficult to find a single *D.*

melanogaster repetitive sequence shared by all the *melanogaster* subgroup species, and the average intensity of interspecies hybridization is 0.39 ± 0.05 for *D. melanogaster* clones (Dowsett, 1983). On the other hand, most of the *D. koepferae* repetitive sequences are present in all of its closest relatives (average hybridization signal 0.85 ± 0.04).

This result can be explained in two ways. It may be argued that (i) the *melanogaster* subgroup species are less inter-related than the species of our study, and (ii) the proportions of the different classes of repetitive DNA in the two sets of species are different. The first possibility is unlikely, according to genetic distances as measured by allozymic differentiation. Cariou (1987) showed that Nei's genetic distances among the eight species of the *melanogaster* subgroup range from 0.28 (*simulans*–*sechellia*) to 1.74 (*melanogaster*–*erecta*). A similar study with the eight species of the *buzzatii* and *martensis* clusters produced estimates of Nei's distances ranging from 0.43 (*starmieri*–*venezolana*) to 2.02 (*buzzatii*–*venezolana*) (Sánchez, 1986; Fontdevila *et al.*, 1988). These data suggest that both clusters split several million years ago. Moreover, differentiation among

species within a given cluster is also high, suggesting that speciation events are ancient (Sánchez, 1986; Fontdevila *et al.*, 1988). However, we have only exceptionally found reductions in the hybridization signals, not only in species of the *buzzatii* cluster but also in those of the *martensis* cluster, a result difficult to reconcile with their phylogenetic relationships.

The most likely explanation is therefore that there are differences in the molecular properties of the repetitive sequences. The analysed clones can be classified into two main groups. Clones cDk13, cDk32, cDk72, cDk265 and cDk596 are representative of sequences that show characteristic 'smeared' hybridization patterns against genomic DNA (see for example, Fig. 1a). These sequences are very frequent both in *D. koepferae* and in its sibling species *D. buzzatii*, accounting for 80 per cent of the repetitive clones obtained (Marín *et al.*, 1992). These clones most likely bear simple DNA sequences, that would explain both their patterns of hybridization and their evolutionary conservation (Hamada *et al.*, 1982; Tautz & Renz, 1984; Morris *et al.*, 1986; Tautz *et al.*, 1986; Huijser *et al.*, 1987; Pardue *et al.*, 1987). Stretches of simple DNA sequences are frequent in our clones. We have found them in cDk27 (Marín *et al.*, 1992) and cDk2 (Fig. 3), as well as finding in other clones near copies of the *Gandalf* element (Marín & Fontdevila, 1995, and unpublished data).

A second group is formed by the other eight clones: cDk2, cDk27, cDk210, cDk254, cDk270, cDk271, cDk323 and cDk665. These clones were selected because they were the only candidates to bear mobile elements among more than 100 *D. koepferae* clones analysed by Marín *et al.* (1992). As expected, fragments of mobile element-related sequences have been recognized in cDk2, cDk210, cDk270 and cDk323. However, only in cDk210 have we been able to detect part of an active element (Marín & Fontdevila, 1995). With the possible exception of the *TLAg/Q*-related sequence in cDk2, the transposable element-related sequences found until now in cDk2, cDk270 and cDk323 are unambiguously inactive. They are structurally abnormal, suffering deletions and frameshifts in their coding regions. More work is needed in order to test whether active *TLAg/Q*-related sequences exist in *D. koepferae*. However, the centromeric hybridization of cDk2 suggests that this is unlikely. Although cDk270 also carries *TLAg/Q*-related sequences, its dispersed pattern on polytene chromosomes, different from that of cDk2, can be explained by the combination in this clone of two or more repetitive sequences, with at least one of them (other than the *TLAg/Q*

sequence and which has still not been characterized) being an active transposable element. Because our sequencing analysis has been partial and as difficulties exist in detecting zones (as long-terminal repeats, as well as other noncoding or rapidly evolving coding regions) or even types of mobile elements (as short inverted repeat elements), it is likely that other sequences derived from transposons exist in these clones.

The centromeric origin of most of these clones is important for an understanding of their evolutionary conservation. *Drosophila* pericentromeric β -heterochromatin is known to be formed by a complex mixture of repetitive sequences, interspersed with some single copy DNA (Healy *et al.*, 1988; Miklos *et al.*, 1988; Vauray *et al.*, 1989; Shevelyov *et al.*, 1989; Devlin *et al.*, 1990; Russell *et al.*, 1992). Thus, centromere-derived clones can bear several different repetitive sequences, so their combined signals will be detected. Our best characterized centromeric clone, cDk2, is an example of this type of arrangement. It contains at least two transposon-related sequences and one simple DNA sequence. Because of these findings, we favour the hypothesis that the apparent evolutionary stability of the *D. koepferae* repetitive sequences is related to the low number of active transposable elements in this species. This is a similar to what probably explains the higher relative abundance of hybridization of *D. simulans* repetitive sequences (0.61 ± 0.09), when compared to those of *D. melanogaster* (0.39 ± 0.05) in the same species of the *melanogaster* subgroup (Dowsett, 1983). Dowsett & Young (1982) found that *D. simulans* repetitive clones came mainly from the centromeric regions.

Gypsy- and T1Ag/Q-related sequences in D. koepferae: common origin or horizontal transfer?

Sequences that hybridize with the *Gypsy* element have been found in a large number of *Drosophila* species, showing one of the more extended distributions among the mobile elements tested in the genus (Stacey *et al.*, 1986). So far, *Gypsy* sequences have been detected in four species of the *repleta* group; *D. repleta*, *D. mercatorum*, *D. mulleri* (Stacey *et al.*, 1986) and *D. hydei* (Lankenau *et al.*, 1990; Hochstenbach *et al.*, 1994). *Drosophila mulleri* is the closest relative to *D. koepferae* (Wasserman, 1992). In that species, *Gypsy*-related sequences are detected only under low stringency conditions when the *Gypsy* element of *D. melanogaster* is used as a probe (Stacey *et al.*, 1986). Our *D. koepferae* *Gypsy*-related nucleotide sequences are 50–60 per cent

identical to *D. melanogaster* Gypsy, in good agreement with these data.

The relationships among the Gypsy sequences found so far are interesting. The pattern that emerges is that active copies (in *D. melanogaster* and *D. virilis*) are quite similar, much more so than the inactive copies found (cDk2 and cDk323 sequences, X15707 sequence of *D. melanogaster* and sequences characterized in *D. hydei*), irrespective of the phylogenetic relationships among their host species. Horizontal transmission may be invoked to explain the similarity among active copies (Mizrokhi & Mazo, 1991). Recent evidence suggesting that Gypsy may behave as an infectious virus (Kim *et al.*, 1994; Song *et al.*, 1994) makes this hypothesis very attractive. The characterization of active elements in other species will eventually explain the evolutionary history of this element.

The finding of sequences related to the *TLAg* and *Q* elements can be interpreted as evidence that they are ancient components of the genome of these species or, alternatively, as a new suggestion that horizontal transmission is an unexpectedly frequent phenomenon. Data on sequences related to known *Drosophila* transposons in other insect species are quickly accumulating. First, sequences related to *P* and *mariner* elements, both of the DNA-transposing type, were found in *Scaptomyza* and *Zaprionus*, respectively, two genera closely related to *Drosophila* (Daniels *et al.*, 1990b; Maruyama & Hartl, 1991). After that, sequences related to *mariner* were first found in the Lepidopteran *Hyalophora cecropia* (Lindholm *et al.*, 1991) and later in a large number of other insects (Robertson, 1993). *P*-related sequences have also been found in *Lucilia cuprina* (the blowfly) (Perkins & Howells, 1992). In parallel studies on retrotransposable elements, Martin *et al.* (1983) found hybridization signals of two retrotransposons, *copia* and *412*, in the *Zaprionus vittiger* genome. Finally, a report of sequences related to the *D. melanogaster* Jockey nonviral retroposon in the species *Apis mellifera* and *Bombyx mori* has appeared, although this evidence is also indirect, obtained by hybridizations with a *D. melanogaster* probe (Kimura *et al.*, 1993). Our study is the second direct demonstration, by means of sequencing, of retrotransposable sequences found in very distant species, after the finding of related *R1* and *R2* elements (ribosomal DNA insertions) in *B. mori* and *D. melanogaster* (Burke *et al.*, 1987; Jacubczak *et al.*, 1990) and later in other insect species (Jacubczak *et al.*, 1991). Although the low similarity at the nucleotide level is compatible with an old origin of both sequences, the protein similarities (75–79 per cent,

identities ranging from 31 to 36 per cent) are quite high, if the fact that the sequenced regions are expected to be diverging rapidly is taken into account. Some proteins have been found to be divergent to this extent when the homologous genes of species of the *Drosophila* genus are compared (O'Neil & Belote, 1992). Only a detailed study of the phylogenetic distribution and the similarity of *TLAg*-related sequences may clarify whether horizontal transfer is a plausible explanation.

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