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Characterization of *Gandalf*, a new inverted-repeat transposable element of *Drosophila koepferae*

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Abstract The cloning and characterization of *Gandalf*, a new DNA-transposing mobile element obtained from the *Drosophila koepferae* (*repleta* group) genome is described. A fragment of *Gandalf* was found in a middle repetitive clone that shows variable chromosomal localization. Restriction, Southern blot, PCR and sequencing analyses have shown that most *Gandalf* copies are about 1 kb long, are flanked by 12 bp inverted terminal repeats and contain subterminal repetitive regions on both sides of the element. As with other elements of the DNA-transposing type (known as the '*Ac* family'), the *Gandalf* element generates 8 bp direct duplications at the insertion point. Coding region analysis has shown that the longer open reading frame found in *Gandalf* copies could encode part of a protein. However, whether or not the 1 kb copies of the element are actually the active transposons remains to be elucidated. *Gandalf* shows a very low copy number in *D. buzzatii*, a sibling species of *D. koepferae*. An attempt to induce interspecific hybrid dysgenesis in hybrids of these two species has been unsuccessful.

Key words *Drosophila* · Transposable elements · *repleta* group · *Ac* family · Hybrid instability

Introduction

Finnegan (1989) classified eukaryotic transposable elements into two classes depending on whether they do or do not code for a reverse transcriptase (Class I or Class II respectively). Class II transposable elements, also known as inverted-repeat transposable elements or DNA-transposing elements, are characterized by the presence of terminal inverted repeats, usually of less than 100 bp; exceptions include the foldback elements and the *Minos* element of *Drosophila hydei* (Templeton and Potter 1989; Franz and Savakis 1991). The best characterized elements belonging to Class II code for at least one protein, which is usually known as transposase and is involved in the transposition of the element (Jacobson et al. 1986; Rio 1990; Frey et al. 1990). Although in some cases it has been found that the transposases of different elements are related (Harris et al. 1988; Hehl et al. 1991; Calvi et al. 1991; Doak et al. 1994), no close relatives have been found for some of the best-known element proteins, such as the *P* transposase. Moreover, because defective elements are often predominant, the products of several elements have not been defined (Ueda et al. 1986; Wobus et al. 1990).

In a previous study (Marín et al. 1992), over 100 DNA clones from *D. koepferae* (*repleta* group) and a similar number of clones from its sibling species *D. buzzatii* were analysed for their content of repetitive DNA. An unexpected number of clones contained non-satellite repetitive sequences. Around 80% of the clones carried highly repetitive DNA, probably simple sequence DNA (Marín et al. 1992). However, only a few clones were obtained that showed the characteristics of mobile elements, that is, middle repetitive sequences with a dispersed and variable pattern of in situ hybridization of polytene chromosomes. When these latter clones were studied, it was found that most of them carried Class I elements. Homologies with the well-known *D. melanogaster* retrotransposon *Gypsy*, as well as with the *Anopheles gambiae* non-viral retroposon *TIAg*, have been found in *D. koepferae* clones (Marín and Fontdevila, submitted). Moreover, Labrador and Fontdevila (1994) have characterized a new retrotransposon, *Osvaldo*, first found in a *D. buzzatii* clone.

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Additionally, sequences related to the *D. melanogaster Copia* retrotransposon have been found in both species (Francino et al. 1994). Only one clone of *D. koepferae* (cDk210) was shown to carry a fragment of a short inverted-repeat mobile element, which we have named *Gandalf* (Tolkien 1954). In this work, we describe the molecular characterization of this new Class II element.

Research on the mobile elements of *D. koepferae* and *D. buzzatii* was stimulated by the finding of high rates of chromosomal instability in hybrids of these two species (Naveira and Fontdevila 1985). Under the simplest assumptions, this phenomenon could be due to a hybrid dysgenesis syndrome, similar to those found intraspecifically in *D. melanogaster*. These syndromes are related to the high rates of transposition of one of several mobile elements (*P. hobo* and *I*: Rubin et al. 1982; Bucheton et al. 1984; Blackman et al. 1987) in the genome of peculiar strains normally devoid of elements or lacking active ones (Engels 1989). Some time ago, we discovered that the mobile sequence included in the cDk210 clone revealed a very small number of bands when hybridized with genomic DNA of *D. buzzatii*, while in its sibling *D. koepferae* and in other closely related species, a characteristic middle repetitive pattern was found. This result suggested that this mobile sequence could be related to the interspecific phenomenon previously described by Naveira and Fontdevila (1985), a possibility that is investigated in the present work. Although we have been unsuccessful in detecting transposition in our hybrids, this could be because the introgressed *Gandalf* copies were inactive or gave transposition rates of less than 3×10^{-3} transpositions/gamete per generation.

Materials and methods

Drosophila stocks

Various stocks of 22 different species of the *repleta* group were used. *D. koepferae*: KSL (San Luis, Argentina), KO2 (San Luis, Argentina), KO3 (San Luis, Argentina), KO4 (Vipos, Argentina), KO5 (Quilmes, Argentina), KO6 (Mazán, Argentina), KO7 (Los Negros, Bolivia), KO9 (San Isidro, Bolivia), KO11 (San Isidro, Bolivia). *D. buzzatii*: BSL (San Luis, Argentina), BU10 (Melocotón, Chile), BU20 (Los Negros, Bolivia), BU24 (Comarapa, Bolivia). *D. serido*: SD14 (Cafarnaum, Brazil). *D. borborema*: BM1 (Cafarnaum, Brazil). *D. starmeri*: SM3 (Curaçao, Dutch Antilles). *D. venezolana*: VZ8 (Curaçao, Netherland Antilles). *D. uniseta*: UN5 (La Boca, Venezuela). *D. martensis*: MA4 (Guaca, Venezuela). *D. stalkeri*: SK3 (Little Cayman, Cayman Islands). *D. richardsoni*: RS1 (Fox's Bay, Montserrat). *D. mulleri*: MU2 (Lake Travis, Texas, USA). *D. aldrichi*: AL1 (Zuata, Venezuela). *D. wheeleri*: WH3 (Uruapán, Mexico). *D. huaylasi*: FP2 (Caraz, Peru). *D. nigrodumosa*: NG2 (Quiragua, Venezuela). *D. mayaguana*: MY4 (Beef Island, Tortola). *D. straube*: SB19 (Montecrist, Dominican Republic). *D. arizonae*: AR9 (Desemboque, Mexico). *D. navojoa*: NA2 (Navojoa, Mexico). *D. hydei*: HY10 (Zurich, Switzerland). *D. hydeoides*: HD1 (Tulacingo, Mexico). *D. mercatorum*: MC1 (Los Negros, Bolivia).

Basic molecular biology techniques

DNA extraction from *Drosophila* stocks, Southern blot and in situ hybridizations and DNA sequencing were performed as described in Marín et al. (1992).

Sequence analysis

GCG (Genetics Computer Group 1991; see Devereux et al. 1984) programs FASTA, TFASTA, BESTFIT and GAP were used to analyse the *Gandalf* sequences in the GenBank, EMBL, PIR and Swiss-Prot databases.

Polymerase chain reaction (PCR) primers and methods

Four different primers were used for PCR amplifications. 210.1 (5′-GCTGCCAGAATCTCCTAAGCAAA-3′) and 210.4 (5′-GCCA-GTTTGGCAATTTAGTGG-3′) correspond to nucleotides 975–952 and 8–28 of *Gandalf* 1 (see below). Adh1 (5′-AAGAATATCAT-CTTTGTCGCTGG-3′) and Adh2 (5′-CCCAGTGCTTGGTC-CATTCAAT-3′) were selected from the sequence of the *Adh2* gene of *D. buzzatii* (EMBL accession number M62743) based on their G–C content and conservation in species of the *repleta* group (The *Adh2* sequence of *D. buzzatii* was aligned with those of eight other *Drosophila* species of the *repleta* group, and the more conserved zones at the extremes of the *D. buzzatii* sequence were selected). The *Adh* primers were used to obtain a fragment of this gene, which was used as probe in the introgression experiments detailed in the next section.

Design of the introgression experiment

The stocks KO2 (*D. koepferae* from San Luis, Argentina) and BSL (*D. buzzatii* from the same locality) were selected because they interbreed easily (Marín et al. 1993), producing abundant F_1 and some, albeit not very abundant, offspring when F_1 females are backcrossed with *D. buzzatii* males (we designate the offspring of the successive backcrosses B_1, B_2, and so on). Sixty virgin KO2 females were crossed with 60 BSL males to generate 200 F_1 offspring. F_1 females were individually backcrossed with BSL males and 26 of these crosses produced B_1 offspring. The same scheme of individual crosses was applied for three further generations, selecting as progenitors those individuals that still carried *D. koepferae* chromosomal fragments. These individuals can be selected by detecting asynapsis between the homologous polytene chromosomes in the salivary glands of their offspring (Naveira et al. 1986). After analysis of 25 larvae of the KO2 stock, which showed a very low degree of polymorphism for *Gandalf* positions, suggesting high endogamy, we were able to select, by cytogenetic analysis, those individuals that carried *Gandalf* elements in the introgressed *D. koepferae* chromosome fragments.

In this experiment, we considered only those individuals (B_2, B_3 or B_4) whose mothers were hybrid for chromosomal segments that included at least one copy of the *Gandalf* element. The aim was to detect in their genomes new insertions of the element, produced by transposition in the germline of the mother. One thousand four hundred third instar larvae were dissected, and 920 individuals whose mothers were hybrid were selected for in situ hybridization analysis. The selected individuals carried asynaptic chromosomal fragments or were siblings of such carriers. Their salivary gland polytene chromosomes were hybridized simultaneously with two different probes. Firstly the PCR amplified fragment of the *Adh2* gene of *D. buzzatii*, obtained using the Adh1 and Adh2 primers, was used as a positive control for the hybridization. Labrador et al. (1990) have

demonstrated that this gene is located in the G1a band of the third chromosome of *D. buzzatii* (it is in the same position in *D. koepferae*, as we have found). The second probe was obtained by PCR amplification of most of the element (*Gandalf* 1, see below) contained in the sDk210.10 clone. Only 596 of the 920 preparations (64.8%) had the high quality needed confidently to detect the presence of new positions. Most of the other larvae showed the *Adh* control band, but the signal was low or the background high, so new positions might be missed.

To estimate the insertion rate, the number of opportunities for insertion was calculated as follows: opportunities for insertion in a B_n larva = (number of F_1 elements × probability of transmission of a new insertion from F_1 to B_n) + (number of B_1 elements × probability of transmission B_1–B_n) + ⋯ + (number of B_n elements). The pre-existing positions in the BSL stock were not taken into account in this calculation (see the Discussion). The number of elements in each generation was estimated from the detected positions and is an underestimate, because some of the elements present in the initial generations are lost by segregation without having been detected. The probability of transmission of a new insertion from a given generation to the next was considered to be $P = 0.5$. This is also an underestimate, because those elements inserted in the hybrid chromosomes that have been selected in each generation have a transmission probability higher than 0.5. However, these biases are not significant for our conclusions (see the Results).

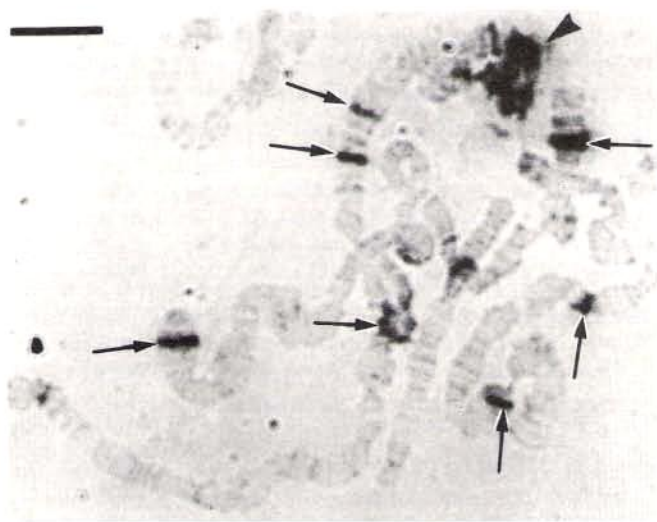


Fig. 1 In situ hybridization of the cDk210 clone shows a dispersed pattern. *Arrows* indicate euchromatic hybridizations and the *arrowhead* shows a centromeric hybridization. *Bar*, 30 μm

Results

cDk210 bears a mobile sequence of *D. koepferae* that is underrepresented in the sibling species *D. buzzatii*

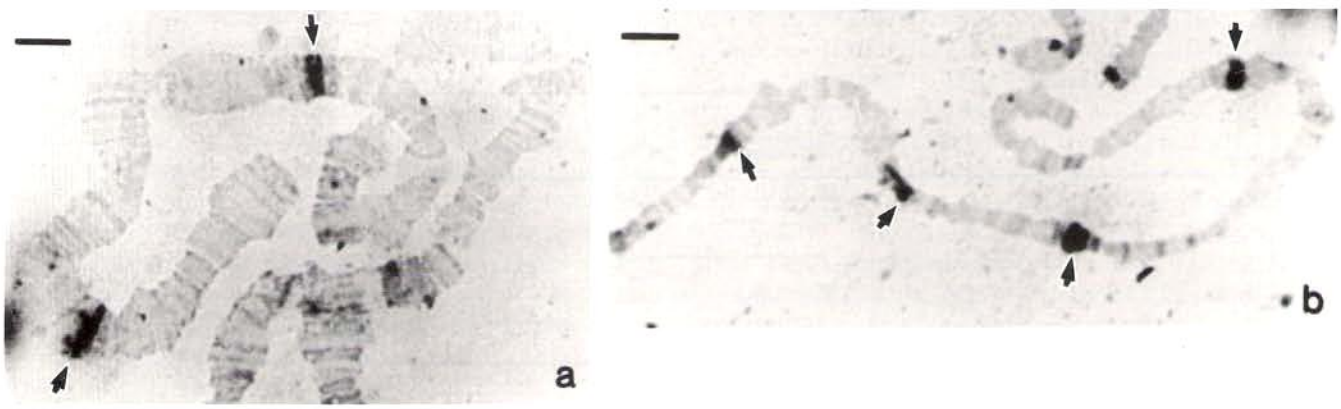
cDk210 (1.84 kb) is one of the forty-nine *D. koepferae* repetitive sequence-bearing clones detected by Marín et al. (1992). In situ hybridizations to polytene chromosomes showed that cDk210 contains a dispersed middle repetitive sequence (Fig. 1). The probe hybridized typically with 5–10 euchromatic bands per nucleus. When different stocks of *D. koepferae* were examined, it was found that the positions of this sequence were variable (see the examples shown in Fig. 2) suggesting that this clone carries at least a part of a mobile element.

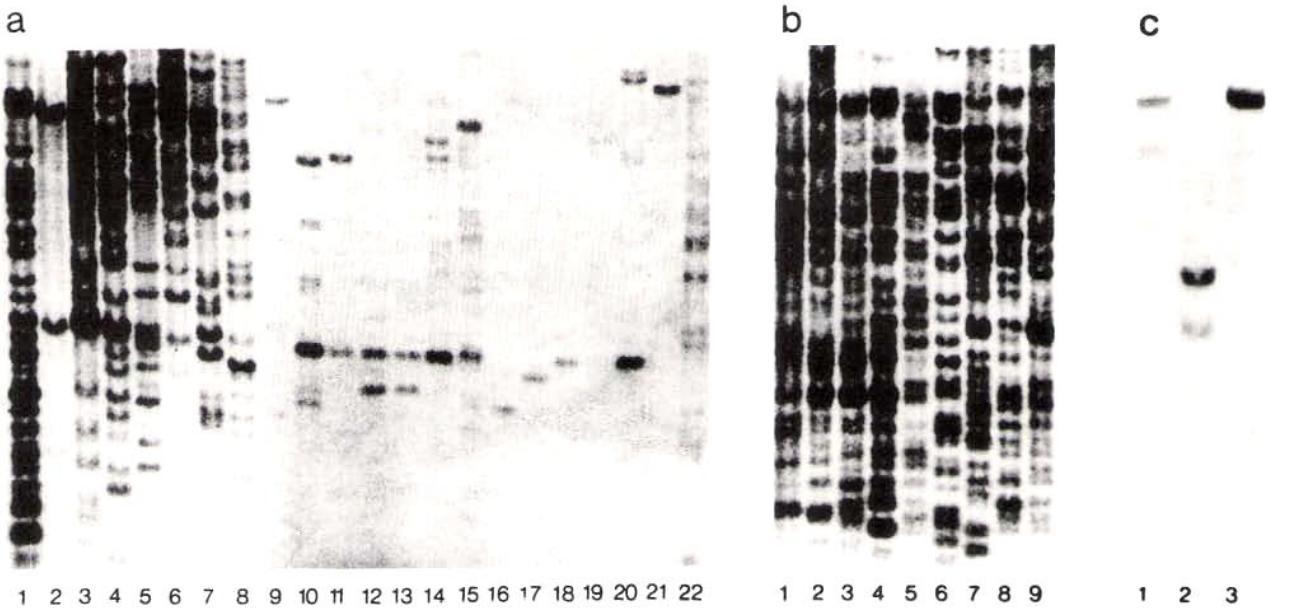
When cDk210 was hybridized with genomic DNAs of different species of the *repleta* group, middle repetitive patterns were found in a number of them (Fig. 3a). Such a pattern also appears in *D. mercatorum* (Fig. 3a, lane 22), which belongs to a different subgroup of the *repleta* group. A remarkable feature is that all but one of the closer relatives of *D. koepferae* (Fig. 3a, lanes 2–8) show similar degrees of repetitiveness of the cDk210 sequence. The single exception is *D. buzzatii* (Fig. 3a, lane 2), where the sequence hybridizes with only a few bands. There was a striking contrast between the high representation of this putative mobile element in stocks of *D. koepferae* (Fig. 3b) and the low number found in *D. buzzatii* (Fig. 3a, lane 2; Fig. 3c). These results suggested that it would be interesting to test whether this element is destabilized in interspecific crosses between these two species, in a manner similar to that seen for other elements in the intraspecific hybrid dysgenesis syndromes.

cDk210 carries a fragment of *Gandalf*, a new short-inverted-repeat element

cDk210 was used to screen a *D. koepferae* (KSL) genomic library and three complementary phages

Fig. 2a, b Variable localization of the cDk210 repetitive sequence in chromosome 4 of different *Drosophila koepferae* stocks. **a** KO2 (San Luis, Argentina). **b** KO6 (Mazán, Argentina). *Bar*, 10 μm



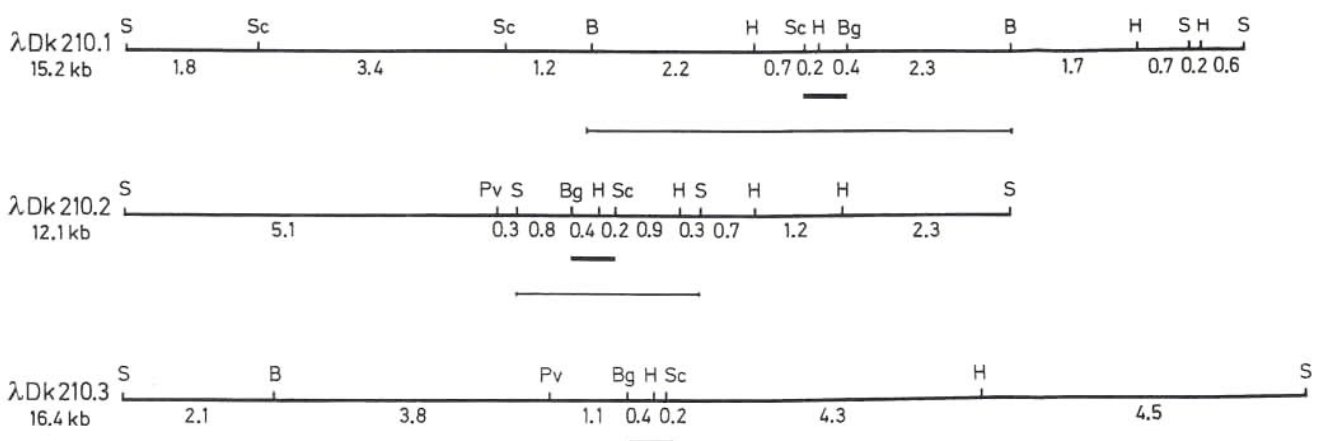


(λDk210.1–3) were isolated. Restriction and hybridization analyses showed that all three phages carry a 0.6 kb *Sca*I-*Bgl*II fragment that hybridizes with cDk210 (Fig. 4). This fragment contains an internal *Hin*dIII restriction site, generating a 0.2–0.3 kb *Hin*dIII-*Sca*I fragment. A similar 0.2–0.3 kb *Hin*dIII-*Sca*I fragment is found at one end of cDk210. These results suggested that the *Sca*I-*Bgl*II fragment could be part of the mobile element and that only the segment located beyond the *Hin*dIII site is present in the cDk210 clone. Moreover, because no other common restriction sites were found flanking the *Sca*I-*Bgl*II fragment, the maximum size of the element (assuming that those present in the λDk210 phages were complete copies) would be less than 2.0 kb. This small size is typical of Class II elements.

Subclones of two of the phages (named sDk210.1.3 and sDk210.10) were obtained, and the zones around the *Sca*I-*Bgl*II fragments, as well as the *Hin*dIII-*Sca*I end fragment of cDk210 were sequenced (Fig. 5). A more refined restriction analysis of the subclones

Fig. 3a–c Southern blot analysis using cDk210 as probe against genomic DNAs. **a** *repleta* group species. Lane 1, KO4 (*D. koepferae*); lane 2, BSL (*D. buzzatii* San Luis, Argentina); lane 3, SD14 (*D. serido*); lane 4, BM1 (*D. borborema*); lane 5, SM3 (*D. starmeri*); lane 6, VZ8 (*D. venezolana*); lane 7, UN5 (*D. uniseta*); lane 8, MA4 (*D. martensis*); lane 9, SK3 (*D. stalkeri*); lane 10, RS1 (*D. richardsoni*); lane 11, MU2 (*D. mulleri*); lane 12, AL1 (*D. aldrichi*); lane 13, WH3 (*D. wheeleri*); lane 14, FP2 (*D. huaylasi*); lane 15, NG2 (*D. nigrodumosa*); lane 16, MY4 (*D. mayaguana*); lane 17, SB19 (*D. straube*); lane 18, AR9 (*D. arizonae*); lane 19, NA2 (*D. navojoa*); lane 20, HY10 (*D. hydei*); lane 21, HD1 (*D. hydeoides*); lane 22, MC1 (*D. mercatorum*). **b** *D. koepferae* stocks. Lane 1, KSL (San Luis, Argentina); lane 2, KO2 (San Luis, Argentina); lane 3, KO3 (San Luis, Argentina); lane 4, KO4 (Vipos, Argentina); lane 5, KO5 (Quilmes, Argentina); lane 6, KO6 (Mazán, Argentina); lane 7, KO7 (Los Negros, Bolivia); lane 8, KO9 (San Isidro, Bolivia); lane 9, KO11 (San Isidro, Bolivia). **c** *D. buzzatii* stocks. Lane 1, BU10 (Melocotón, Chile); lane 2, BU20 (Los Negros, Bolivia); lane 3, BU24 (Comarapa, Bolivia)

Fig. 4 Restriction maps of the λDk210 phages. The *broad line* marks the conserved *Bgl*II- *Sca*I 0.6 kb fragment. Also represented *below* the maps are the origins of the subclones sDk210.1.3 (upper segment, 5.9 kb) and sDk210.10 (lower segment, 2.6kb). Restriction enzyme abbreviations: B, *Bam*HI; S, *Sal*I; Sc, *Sca*I; H, *Hin*dIII; Bg, *Bgl*II; Pv, *Pvu*II



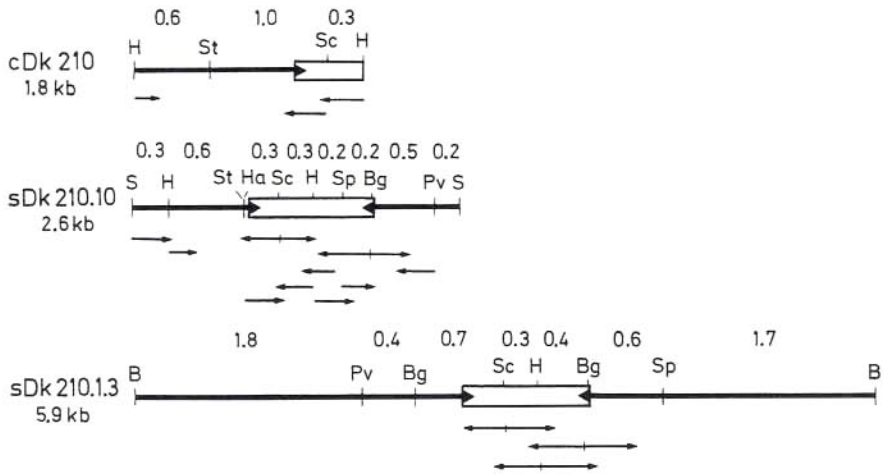


Fig. 5 Restriction maps and sequenced zones of the clones cDk210, sDk210.10 and sDk210.1.3. The *boxes* show the localization of the *Gandalf* element (only part of which was present in cDk210). Restriction enzyme abbreviations: St, *Sty*I, Sp, *Spe*I, Ha, *Hae*III, others are as in Fig. 4

showed that the common region had to be smaller than 1.5 kb in size, and sequencing has confirmed this assumption. The sequence similarity ends abruptly after approximately 1 kb of DNA sequence. In situ hybridizations on polytene chromosomes of larvae of the KO2 *D. koepferae* stock, using as a probe the *Sca*I-*Bgl*II fragment of sDk210.10, showed that this fragment was indeed part of the dispersed sequence first detected in cDk210. It hybridized to the same bands as cDk210, with the exception of the band at cytological position 5G5, from which the cDk210 clone itself derived. This single band appeared in all the in situ hybridizations performed using cDk210 as probe, irrespective of the *D. koepferae* stock tested. We interpret this result to indicate that 5G5 hybridizes with cDk210 due to the single-copy DNA present in this clone, but that there is no copy of the dispersed sequence at this site in the KO2 stock.

The common sequence has the structural characteristics of a DNA-transposing mobile element (Fig. 6) and, because no significant DNA similarities have been found, it is considered to be a new element and has been named *Gandalf* (Tolkien 1954). The analysed *Gandalf* copies have terminal inverted repeats of 12 bp showing obvious relationships with those of other transposable elements, including the CA dinucleotide at their ends: CA, sometimes TA, is at the end of the inverted repeats of most DNA-transposing eukaryotic elements. Moreover, the first eight nucleotides of the inverted repeat of *Gandalf*, CAGTGCTG, are identical to those of the *Caenorhabditis elegans* element *Tc1* (Rozenweig et al. 1983). Flanking the two inverted repeats, direct repeats of 8 bp are found. These repeats are produced when DNA transposing elements insert into the host genome. Finally, subterminal regions at both extremes of the element show 8 and 6 copies, respectively, of a sequence (consensus TTAGCAATT) in both orientations.

Such subterminal repeats are also frequently found in DNA-transposing mobile elements (Müller-Neumann et al. 1984; Pereira et al. 1985; Rhodes and Vodkin 1988; Wobus et al. 1990; Morgan and Middleton 1990; Nacken et al. 1991) The two completely sequenced copies have 979 bp (*Gandalf 1*, from sDk210.10) and 956 bp (*Gandalf 2*, from sDk210.1.3) respectively, while the fragment included in cDk210 (*Gandalf 3*) is 537 bp long. Considering the gaps as single mutations, the nucleotide sequence identity between the complete copies is 95.2%, while the fragment *Gandalf 3* is 96.3 and 96.4% identical to *Gandalf 1* and *Gandalf 2*, respectively. The main differences among copies occur in three zones of low sequence complexity, where small deletions/insertions occur (nucleotides 283–298, 474–488 and 698–710 of *Gandalf 1*).

Gandalf 1 is the longest complete copy sequenced, and was therefore the focus of our further work, although the following analysis is also valid for *Gandalf 2*. No open reading frames (ORFs) of more than 103 amino acids in length have been found in the *Gandalf* copies examined. The Testcode program, based on Fickett's (1982) algorithm considers that only the largest putative ORF (ORF1-*Gandalf*, 442–750 in *Gandalf 1*) has the characteristics of a true coding sequence. The 5′–3′ orientation in Fig. 6 has been selected because it contains the longest ORFs, including ORF1-*Gandalf*. Putative splicing junction consensus sequences have been found in positions 216–321, 428–533 and 699–777 of *Gandalf 1*. These putative introns include most of the sequences that differ in the *Gandalf* copies analysed. The spliced sequence could encode a protein of 134 amino acids. No significant homologies were found when the original ORFs and the reconstructed putative protein were compared with sequences in the databases (TFASTA program), or specifically compared with the proteins of other DNA-transposing elements (*Ac*, *Hobo*, *Tam3*, *P*, *Uhu*, *Tc1* and *Mariner*; BESTFIT and GAP programs).

To investigate whether *Gandalf* copies of longer size can be found in other *D. koepferae* stocks or even in other species, we used the primers 210.1 and 210.4 for

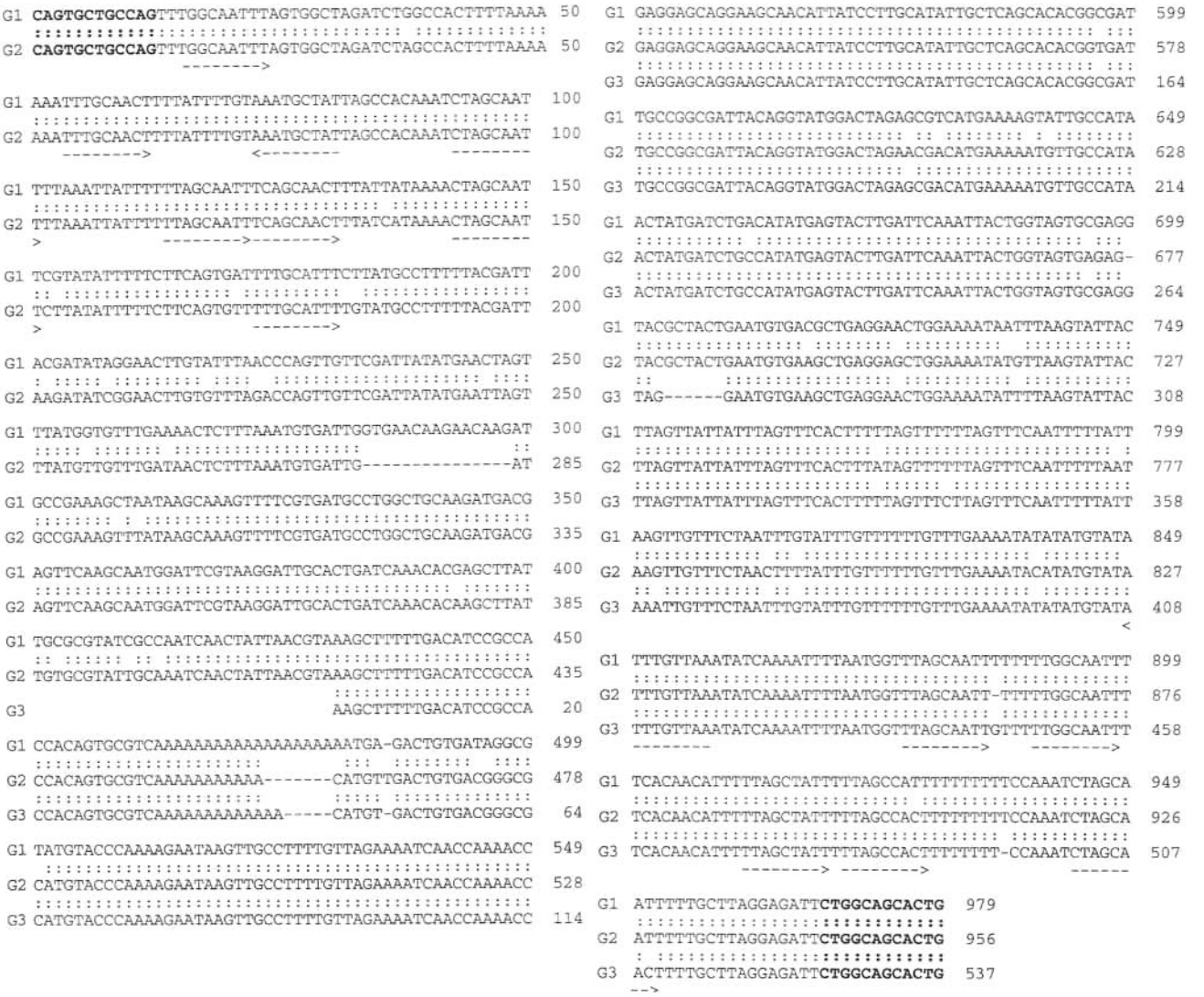


Fig. 6 Sequences of *Gandalf 1* (G1, from sDk210.10), *Gandalf 2* (G2, from sDk210.1.3) and *Gandalf 3* (G3, the fragment found in cDk210). *Bold letters* indicate inverted terminal repeats; *arrows* show subterminal sequences; *colons* indicate identical amino acids. Some gaps (*dashes*) have been included to improve the alignments

PCR amplifications. A prominent band of *c.* 1 kb and, less frequently, bands of smaller size were found in *D. koepferae* stocks (Fig. 7a). When the closer relatives of *D. koepferae* (Marín et al. 1993) were tested, bands were found in five out of seven species (exceptions were *D. buzzatii* and *D. venezolana*, Fig. 8b). However, those bands were similar in size or smaller than the *D. koepferae* main band. These experiments showed that the 0.9–1 kb is the most common *Gandalf* element size; no candidate for a longer element was found.

Test of the dysgenic potential of the predominant class of *Gandalf* elements in interspecific hybrids

The results of the introgression experiment are shown in Table 1. None of the 596 larvae tested showed new insertions of the element. In some of them, independently of their origin, one or two signals appeared in the *D. buzzatii* chromosomes, always in the same positions (near the centromere of chromosome 2 and in the *X* chromosome). We did not find these signals when the cDk210 clone or the internal *Sca*I-*Bgl*II fragment of the *Gandalf 1* element were used as probes. However these bands appeared also in non-introgressed larvae of the BSL stock when the amplified 965 bp fragment obtained from sDk210.10 was used as probe. When the *Adh* fragment alone was used as probe, the single expected band was detected at position Gla. Therefore, we consider the two *D. buzzatii* bands to be positions previously occupied in the BSL stock. The fact that they are not detected with smaller probes (or amplified using PCR) could mean that they are defective elements. In any case, these bands were not considered in the estimation of the transposition rate. Most of the tested larvae had a single copy of *Gandalf* (in 5A1). The reason for this bias is the higher fecundity of these lines

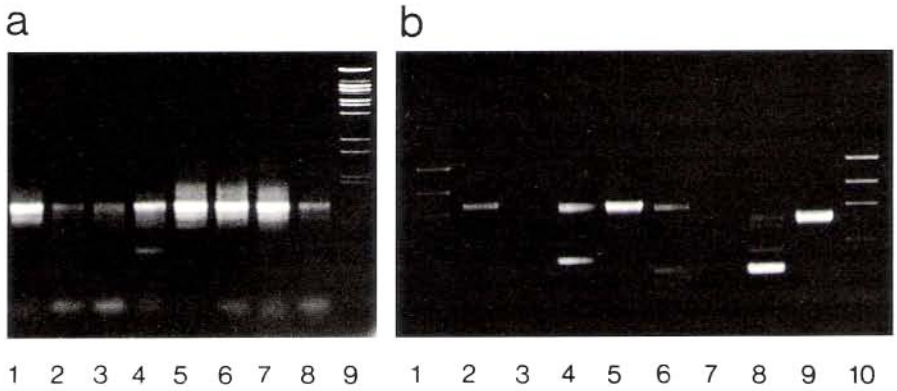


Fig. 7a, b Polymerase chain reaction (PCR) amplifications using as primers 210.1 and 210.4 (see text). **a** *D. koepferae* stocks. Lane 1, KO4; lane 2, KO5; lane 3, KO6; lane 4, KO7; lane 5, KO9; lane 6, KO12; lane 7, KO13; lane 8, KO14; lane 9, lambda phage cut with *Bst*EII. **b** *repleta* group species. Lanes 1, 10, Phage ϕX174 cut with *Hae*III (third band, 872 bp); lane 2, KO4; lane 3, BSL; lane 4, SD14; lane 5, BM1; lane 6, SM3; lane 7, VZ8; lane 8, UN5; lane 9, MA4

and the good quality of the preparations obtained from their larvae. PCR analysis showed that all the hybrid lines carried 0.9–1.0 kb *Gandalf* sequences.

By using the formula detailed in the Material and methods, the total of transposition opportunities estimated is 1327.5 for this set of preparations. Thus the transposition rate can be estimated to be lower than 3×10^{-3} transpositions/gamete per generation; the probability of obtaining at least one new position if the rate was higher is $> 95\%$. The fact that both the transposition opportunities and the probability of transmission have been slightly underestimated suggests that the maximum rate is even lower than this estimate.

Table 1 Results of the introgression experiment. Positions refer to the chromosome map of *Drosophila koepferae* according to Fontdevila et al. (1988). Centr (1, 2, 3) are three close bands near the centromere of the third chromosome. B_2, B_3, B_4 are backcrosses 2, 3 and 4 (see the Materials and methods)

Lines	Positions of the introgressed *Gandalf* elements	No. of individuals examined		
		B_2	B_3	B_4
1	5A1	41	212	88
2	5A1	11	21	69
3	5A1	–	–	66
	2G2	–	–	18
	2G2, 5A1	–	12	40
4	2G2	–	2	–
5	2G2, 5A1, 5B2, 5E5	–	2	9
6	3 centr (1, 2, 3)	3	–	–
7	3 centr (1, 2, 3)	2	–	–
Total		57	249	290

Discussion

Gandalf is a new Class II element, first characterized in species of the *repleta* group, and probably is of ancient origin

Examination of more than 200 DNA clones from the species *D. koepferae* and *D. buzzatii* has revealed only four mobile element-related sequences (Marín et al. 1992; Labrador and Fontdevila 1994; Marín and Fontdevila, submitted). Three of them, are Class I elements; sequences related to the *Gypsy* retrotransposon, first found in *D. melanogaster*, and the *T1Ag* non-viral retroposon, first characterized in *Anopheles gambiae* were found in *D. koepferae* (Marín and Fontdevila, submitted), and a new retrotransposon, which was named *Osvaldo*, has been found in *D. buzzatii* (Labrador and Fontdevila 1994). Independent experiments have shown that the *Copia* retrotransposon is also present in these species (Francino et al. 1994). Our results have unambiguously characterized *Gandalf* as a new Class II element, the first to be found in *D. koepferae*. The pattern of in situ hybridization, variable in different stocks, suggests that it is actively mobile in the genome of this species. The fact that extensive in situ hybridization analysis in both *D. koepferae* and *D. buzzatii* (Marín et al. 1992; Marín and Fontdevila, submitted; M. Labrador, unpublished data) have failed to show more dispersed sequences suggests that active elements of this type are scarce in these genomes. Only one other Class II element, *Minos*, has so far been described in a species of the *repleta* group, *D. hydei* (Franz and Savakis 1991).

Gandalf-related sequences have been detected in other *repleta* group species (Fig. 3a), including *D. mercatorum*, which belongs to a different subgroup of this group (Wasserman 1992). This result suggests that the element may be of ancient origin, although the occurrence of horizontal transmission cannot be ruled out. Because our hybridization experiments were performed at medium-high stringencies (allowing a maximum mismatch of 20%; Wetmur 1991), it is likely that *Gandalf*-related sequences are found in other *Drosophila* groups.

Relationships of *Gandalf* with other known elements

Several Class II elements can be grouped together based on protein homologies. For example, the *Tc1* family has been defined by the similarities of their encoded transposases. This family comprises, at least six elements from *Caenorhabditis* and *Drosophila* species (Harris et al. 1988; Brezinsky et al. 1990; Prasad et al. 1991; Franz and Savakis 1991; see also Doak et al. 1994). Elements of a second family, named 'CACTA', have been found in different plant species (Gierl et al. 1989; Gierl 1990). Moreover, *Hobo* (*D. melanogaster*), *Ac* (*Zea mays*) and *Tam3* (*Anthirrinum majus*) are also related, based on transposase sequence similarities (Hehl et al. 1991; Calvi et al. 1991). Although the proteins encoded by other elements are not known, or cannot be classified in one of these families, there are other structural similarities. Most of the terminal inverted repeats of these elements begin with the dinucleotide CA or, less frequently, TA, and weak similarities to these repeats are found in unrelated elements. Moreover, the size of the direct duplications of the host DNA sequence that result when the elements insert is identical for all the elements of a given family: the *Tc1* family elements induce 2 bp duplications, the CACTA family elements, 3 bp, and *Hobo* and its related elements, 8 bp. The production of 8 bp direct repeats upon insertion has been considered by Calvi et al. (1991) to be the key character for defining the '*Ac* family' of elements, which would include *P* and *Hobo* (*Drosophila*), *Ac* (*Z. mays*) and *Tam3* (*A. majus*) as well as the lesser known elements *TeCth1* (*Chironomus thummi*), *Ocr* and *1723* (*Xenopus laevis*), *Bg* (*Z. mays*), *Ips-r* (*Pisum sativum*) and *Tpc1* (*Petroselinum crispum*) (O'Hare and Rubin 1983; Kay and Dawid 1983; Müller-Neumann et al. 1984; Sommer et al. 1985; Streck et al. 1986; Gierl 1990; Morgan and Middleton 1990; Calvi et al. 1991).

Whether *Gandalf* is related significantly to other elements cannot be conclusively determined. *Gandalf* induces 8 bp duplications, and therefore can be classified as belonging to the *Ac* family. The small size of the inverted terminal repeats (12 bp) is also similar to the size of the repeats in the elements of this family (11–31 bp), while the elements of other families usually have longer inverted repeats (i.e. 30–255 for the *Tc1* family). However, the inverted repeats of *Gandalf* are more similar to those found in the elements of the *Tc1* family, particularly *Tc1*, *Tc2* and *Tcb2* (Rosenzweig et al. 1983; Prasad et al. 1991). Because no significant DNA or protein similarities have been found with the other Class II elements, it is possible that *Gandalf* is a member of a totally different class. A similar case is that of the *P* element: its inclusion in the absence of protein similarities in the *Ac* family, solely because it produces 8 bp duplications, is purely tentative and is probably more easily explained by functional convergence than by common origin.

Subterminal regions such as those found in *Gandalf* occur in only one other Class II invertebrate element, *TeCth1* (Wobus et al. 1990). *Ac/Ds*, *En/Spm*, *Tam1* and *Tgm*, all plant mobile elements, and *Ocr*, from *X. laevis*, also have subterminal repeats, and in the *P* element there is a single subterminal 11 bp sequence on both sides of the element (O'Hare and Rubin 1983; Müller-Neuman et al. 1984; Pereira et al. 1985; Rhodes and Vodkin 1988; Nacken et al. 1991). These repeats are unrelated in sequence but might be of similar function. It is known that the subterminal sequences are needed for excision in *P*, *En/Spm* and *Ac/Ds* (Coupland et al. 1988, 1989; Gierl et al. 1989; Rio 1990). It is supposed that the transposase protein (or one of the proteins coded by the element if, as occurs in *En/Spm*, there are two) binds to the subterminal repeats facilitating interaction of a second protein (element-encoded or host-encoded) with the terminal inverted repeats to induce, directly or indirectly, breakage of the DNA strand (Frey et al. 1990; Rio 1991). Several lines of evidence favour this hypothesis. Firstly different transposases have high affinity for DNA and bind to the subterminal regions (Gierl et al. 1988; Kaufman et al. 1989; Kunze and Starlinger 1989). Furthermore, host proteins or, as in the case of *En/Spm*, a second element-encoded protein, are known to bind to the terminal inverted repeats (Rio and Rubin 1988; Frey et al. 1990). There is a noteworthy similarity between the subterminal sequences of *Gandalf* (consensus: TTAGCAATT) and the CCAAT box commonly found in eukaryotic genes. At least one transcription factor (C/EBP; Ryden and Beeman 1989) binds to sequences with a consensus that coincides with that of *Gandalf* subterminal sequences. It is suggested that the proteins that bind to these sequences could regulate the transcription of the element. An important precedent is that of the *P* element, where the transposase represses in vitro the transcription of the element by blocking the TATA box of its promoter (Kaufman and Rio 1991).

Autonomy of the characterized *Gandalf* copies

For Class II elements, it is difficult to determine which of the copies are active elements and able to transpose by themselves, as opposed to inactive or defective elements, which transpose only in the presence of active ones. In a few cases (*P* and *Hobo* in *D. melanogaster*, *Mariner* in *D. mauritiana*, *Ac/Ds* and *En/Spm* in *Z. mays* and *Tc1* in *C. elegans*), information on the activity of the different copies is available. This derives from the molecular characterization of complete and defective copies of the element and ultimately led to the induction of transposition in controlled in vitro or in vivo assays (Karess and Rubin 1984; Scavarda and Hartl 1984; Brennan et al. 1984; Daniels et al. 1985; Rio et al. 1986; Van Sluys et al. 1987; O'Brochta and Handler 1988; Mori et al. 1988; Blackman et al. 1989; Li and

Starlinger 1990; Frey et al. 1990; O'Brochta et al. 1991; Garza et al. 1991; Medhora et al. 1991; Capy et al. 1992). In other cases, however, very little is known about the characteristics of the active elements or even whether they exist in the species in which the element was first found. Some authors have suggested that some elements could transpose using exclusively cellular proteins (Wobus et al. 1990).

The main problem in the characterization of active elements has been the structural similarity of active and inactive elements (Jacobson et al. 1986; Mori et al. 1988 Medhora et al. 1991; Maruyama et al. 1991) and the small number of active elements (Medhora et al. 1988; Mori et al. 1988; Chomet et al. 1991). In a number of cases, the significance of the differences among active and defective elements was revealed only following the development of a functional assay in which active elements are mobilized at high rates. These considerations all have to be taken into account in the interpretation of our results. All *D. koepferae* stocks analysed contain a predominant PCR product of a similar size to that of the cloned *Gandalf* elements (Fig. 7a). Moreover, the sequenced elements are structurally similar, although the percentage of nucleotide sequence divergence between copies (4.8%) is quite high and activity differences could exist among them.

Gandalf 1, in the 5′–3′ orientation as shown in Fig. 6, has a structure appropriate for an active form of the element. Firstly, the sequence of the subterminal regions is related to the CCAAT box and some of these sequences could be part of the *Gandalf* promoter. Moreover, a consensus TATA box sequence is found in positions 136–142 of *Gandalf 1*. The first AUG triplet downstream of this point, where protein synthesis would be expected to begin (Kozak 1983), is found at nucleotides 185–187. In this 5′–3′ orientation, the longest ORFs are found, among them ORF1-*Gandalf*, considered as putatively coding by the Testcode program (Fickett 1982). Finally, a protein can be reconstructed by splicing out of the sequence three regions of 105, 105 and 78 bp. In *Drosophila*, the average size of the small introns is 79 bp (Mount et al. 1992). However, it would be premature to conclude that *Gandalf 1* is an example of active copy. The putative protein coded by this element would be 134 amino acids, much smaller than the transposases coded by the best characterized Class II elements: *P* (751 residues), *Ac* (807), *Hobo* (658), *Mariner* (346) or *Tam3* (748) among others. Moreover, the *Gandalf* protein lacks homology with other transposases. This uncertainty stimulated our attempt to develop a functional test, based on the search for transpositions in interspecific hybrids under presumptively dysgenic conditions. Our negative result does not rule out the possibility that *Gandalf* is implicated in this phenomenon. It is likely that the introgressed *Gandalf* copies, especially that found in 5A1, which account for most of our results, are inactive or transpose at such a low rate that our experiments were not sufficiently extensive to detect a single new position. However, the absence of transpositions in our experiments suggests that the probability of interspecific transmission following species hybridization is low.

Is there a relationship between the small number of mobile sequences and higher rates of recombination?

Our work in the sibling species *D. koepferae* and *D. buzzatii* suggests that the genomes of these species are organized differently from the paradigmatic *D. melanogaster* genome. The percentage of repetitive sequence-bearing clones is very high and most of them bear non-mobile, simple DNA sequences (Marín et al. 1992), a characteristic that seems to be common to other species of the *Drosophila* subgenus (Lowenhaupt et al. 1989). Moreover, when potentially mobile sequences have been found, most appear to be inactive and restricted to the centromeric zones (Marín et al. 1992; Francino et al. 1994; Labrador and Fontdevila 1994; Marín and Fontdevila, submitted). It is interesting to consider whether a single factor could account for these two features. One possible mechanism could be that the recombination rates in these species are higher than in *D. melanogaster*. Experimental evidence suggests that simple DNA sequences could influence the recombination rate (Treco and Arnheim 1986; Bullock et al. 1986; Hellman et al. 1988). In a recent paper, Schafer et al. (1993) summarized the map length of the *X* chromosome in 13 different *Drosophila* species, and showed that it ranges from 70 map units (*D. melanogaster*) to 171 map units (*D. virilis*). The map lengths of all the species of the *Drosophila* subgenus considered are 40–130% larger than that of *D. melanogaster* and, particularly, the *D. buzzatii X* chromosome has 109 map units, although only 14 markers have been found to date. Whether or not these differences are due to the changes in simple sequence DNA content among these species remains to be determined. The scarcity of mobile elements may be a byproduct of this apparent increase in recombination rate. It is known that homologous and ectopic interchanges are positively correlated (Montgomery et al. 1991) and ectopic exchange between copies of transposable elements is considered to be the main factor that controls the transposon number in eukaryotic species (Charlesworth et al. 1986; Langley et al. 1988). It is possible that mobile elements exist in a very delicate equilibrium between accumulation by transposition and elimination by ectopic recombination. Thus an increase in recombination rates would cause the elimination of active copies in a whole species, while defective copies could remain in centromeres and other zones without undergoing recombination. Under these assumptions, a negative correlation between recombinogenic and mobile sequences is

expected, and the differences in the genomic organization between *D. koepferae* or *D. buzzatii* and *D. melanogaster* could reflect this indirect relationship.

Note added in proof: The GenBank numbers of the *Gandalf* sequences are U29466-8.

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