**bilbo, a Non-LTR Retrotransposon of *Drosophila subobscura*: A Clue to the Evolution of LINE-like Elements in *Drosophila***

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We used the repetitive character of transposable elements to isolate a non-LTR retrotransposon in *Drosophila subobscura*. *bilbo*, as we have called it, has homology to TRIM and LOA elements. Sequence analysis showed a 5' untranslated region (UTR), an open reading frame (ORF) with no RNA-binding domains, a downstream ORF that had structural homology to that of the I factor, and, finally, a 3' UTR which ended in several 5-nt repeats. The results of our phylogenetic and structural analyses shed light on the evolution of *Drosophila* non-LTR retrotransposons and support the hypothesis that an ancestor of these elements was structurally complex.

**Introduction**

Most transposable elements (TEs) described so far in the genus *Drosophila* have been detected through their phenotypic effects (Fawcett et al. 1986; O’Hare et al. 1991) or through other indirect methods like searching for homologous elements in other species (Mizrokhi and Mazo 1990). Almost all elements have been described in *Drosophila melanogaster* (Arkhipova, Lyubomirskaya, and Ilyin 1995). These two aspects are likely to have produced a bias in the type of TE known in this genus. Thus, it is necessary to search for new TEs by using more direct methods and to describe them in other *Drosophila* species in order to understand their organization, evolution, and transposition mechanisms.

One class of TEs is the superfamily non-LTR retrotransposons, long interspersed nuclear element (LINE)-like or poly(A) retrotransposons (see Eickbush 1992 and Arkhipova, Lyubomirskaya, and Ilyin 1995 for recent revisions). There is not an established nomenclature for RNA-mediated transposons. Some authors use the term "retrotransposon" for LTR-containing elements and "retroposon" for non-LTR elements. Other authors, as we do in this paper, use the term "retrotransposon" for all of them. Although we focus on *Drosophila* non-LTR retrotransposons, these elements are found in most eukaryotes. Most non-LTR elements are widely dispersed in their host genomes, but others are located in *Drosophila* telomeres (Pardue 1995) or in specific sites like the 28S rRNA gene (Xiong and Eickbush 1988; Jakubczak, Xiong and Eickbush 1990). Some non-LTR elements have an internal promoter for RNA polymerase II, and a few of them have been shown to transpose via an intermediate RNA. Non-LTR retrotransposons typically have two translational open reading frames (ORFs) whose products resemble retroviral proteins. The protein encoded by the upstream ORF of many non-LTR elements (ORF1) has several copies of zinc-finger motifs, which have been also found in retroviral nucleocapsid proteins. ORF2 has a region with homology to retroviral reverse transcriptases (RTs) (Xiong and Eickbush 1990), an RNA-dependent DNA polymerase whose activity has been shown. Other ORF2 domains are: a domain that is possibly related to proteases described in the LOA element (Felger and Hunt 1992), an RNase H activity that has been postulated for only a few elements (McClure 1991), and another zinc-finger motif that can be found in several *Drosophila* non-LTR retrotransposons at the end of ORF2. Recently, an endonuclease (EN) domain has been shown to be encoded by the human L1 element (L1Hs) in the N-terminal region of ORF2; the EN domain is found in a diverse collection of non-LTR retrotransposons (Feng et al. 1996). The R2 element of *Bombyx mori*, which encodes a sequence-specific endonuclease, lacks this EN domain. Non-LTR retrotransposons lack terminal repeats, but they usually have a polyadenine (poly(A)) tract, preceded in a few cases by a polyadenylation signal.

One of the defining features of TEs is their repetitive character, and we can take advantage of this feature for the isolation of new elements (Felger and Hunt 1992; Ogiura et al. 1994). In this paper, we use this approach and describe a new family of non-LTR elements in *Drosophila subobscura* that we have termed *bilbo* (after the main character of Tolkien’s *The Hobbit* [1937]). *Drosophila subobscura* presents a high amount of inversion polymorphisms. The involvement of TEs in causing specific chromosome rearrangements in *D. melanogaster* has been shown (Lyttle and Haymer 1992), a fact we considered when we chose *D. subobscura*. Well-known transposable elements like the P element (Paricio et al. 1991) and gypsy (Alberola and de Frutos 1993) have been characterized in this species, and some others have been detected as well (de Frutos, Peterson, and Kidwell 1992).

*bilbo* has sequence and structural similarity to other *Drosophila* non-LTR retrotransposons, but particularly to TRIM (Steinemann and Steinemann 1991) and LOA (Felger and Hunt 1992) elements. One of the sequences described here, named *bilbo1*, seems to be a defective full-length element, with structural homology to I factor (with EN, RT, and RNase H domains and zinc-finger motifs). Our phylogenetic and structural analyses of
D. subobscura was used (H271: Helsinki, Finland; BA: Bariloche, Argentina; RA1: Canarias, Spain; PC: British Columbia, Canada; COL: Valencia, Spain; EU: Eureka, Calif., U.S.A.; MA: Madeira, Portugal; and B1: Bizerte, Tunisia). These strains were selected because of their diverse geographical origins. These locations have different inversion polymorphism frequencies. The D. subobscura H271 genomic DNA library, provided by Marfany (1991), was based on the EMBL4 phage vector (10–20 kb fragments).

Detection and Cloning of bilbo Sequences

The procedure we used to clone a transposable element from D. subobscura was the following: In our laboratory, a number of clones had been isolated from the D. subobscura genomic library using as probes the P element and the antennapedia and Hsp83 genes, all of them from D. melanogaster. Nineteen of these clones were EcoRI-digested and analyzed by Southern blot hybridization using D. subobscura (H271 strain) genomic DNA digested with EcoRI as a probe. Seven of these 19 clones developed rapid hybridization signals in some of their restriction fragments (indicative of repetitive sequences). These seven clones were then digested with several restriction endonucleases and a second Southern blot analysis with the same probe was performed. This step allowed us to detect a small, easy-to-study restriction fragment including a repetitive sequence for every clone. In order to analyze the repetitiveness of the sequences of these small restriction fragments, each of them was used as a probe in a Southern blot hybridization with D. subobscura H271 genomic DNA digested with several restriction endonucleases. In this analysis, a 2.0-kb Sac I restriction fragment of one of these seven clones, the F11, gave a typical pattern of moderately repetitive sequences as shown in figure 1A. F11 had been isolated with a probe from the P element but the sequences of the P element and the 2.0-kb Sac I fragment were 7 kb apart. The 2.0-kb restriction fragment was cloned into the Sac I site of the plasmid pUC18 and named DsS112. DsS112 was used to screen 70,000 clones of the D. subobscura H271 genomic library. After analyzing 30 of approximately 300 positive clones, the clone D7 was selected. We selected D7, and stopped analyzing further positive clones, because it contained all the restriction fragments detected as strong signals in the D. subobscura genomic DNA Southern blot analysis (see fig. 1A). Probe labeling with digoxigenin and the procedure for Southern blot analysis are described in “the DIG system user’s guide” of Boehringer Mannheim, Germany. The stringency washes of these hybridizations were performed at 0.1 × SSC, 0.1% SDS, 65°C. Other molecular biological techniques were performed using standard procedures (Ausbuhl et al. 1988).

In Situ Hybridization

Polytene chromosomes of the D. subobscura strains H271, COL, BI, MA, RA1, and PC were prepared following Atherton and Gall (1972) with minor modifications. DsF112 was radiolabeled with 3H-dCTP following the oligolabeling method (Feinberg and Vogelstein 1983). Hybridizations of one slide of each strain and washes were performed as described in Terol, Perez-Alonso and de Frutos (1991).

Sequence Analysis

Both strands of DsF112 and DsD765 (derived from the D7 clone) were sequenced by nested deletion strategy (Henikoff 1984). Sequences were compiled using DNASTAR (Lasegene, London) and analyzed with the Genetics Computer Group (GCG) programs. Drosophila_high.cod (GCG) and etd89.cod (from Ashburner 1989, table 6.4, p. 76) codon usage tables were used with CODONPREFERENCE. The complete deduced amino acid (aa) sequences encoded by the second ORF

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**Materials and Methods**

**Drosophila Strains and Genomic Library**

Eight strains of D. subobscura were used (H271: Helsinki, Finland; BA: Bariloche, Argentina; RA1: Canarias, Spain; PC: British Columbia, Canada; COL: Valencia, Spain; EU: Eureka, Calif., U.S.A.; MA: Madeira, Portugal; and B1: Bizerte, Tunisia). These strains were selected because of their diverse geographical origins. These locations have different inversion polymorphism frequencies. The D. subobscura H271 genomic DNA library, provided by Marfany (1991), was based on the EMBL4 phage vector (10–20 kb fragments).
of *Drosophila* non-LTR elements were compared using the program PILEUP. The alignments shown here (see below) were extracted from this comparison. A manual refinement was necessary in the EN, RNase H, and zinc-finger motif alignments. The phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei 1987) using the program MEGA (Kumar, Tamura, and Nei 1993). Distances were calculated using the Poisson correction for all pairwise comparisons of the aa sequences. References to the sequences used are: Doc (O’Hare et al. 1991), F (Fw; Di Nocera and Casari 1987), G (Di Nocera 1988), jockey (*Drosophila funebris*; Mizrokhi and Mazo 1990), BS1 (Udomkit et al. 1995), TART-B1 (Sheen and Levis 1994), R1Dm (*D. melanogaster*; Jakubczak, Xiong, and Eickbush 1990), LOA (Felger and Hunt 1992), TRIM (Steinemann and Steinemann 1991), I (*Drosophila teissieri*; Abad et al. 1989), R2Dm (*D. melanogaster*; Jakubczak, Xiong, and Eickbush 1990). We chose non-*D. melanogaster* sequences when possible in order to increase the number of species in the analysis. The LIHs (Dombroski, Scott, and Kazazian 1993) as a non-*Drosophila* element and HIV-1 (Wain-Hobson et al. 1985) as an outgroup to the non-LTR retrotransposons were used.

**PCR Determination of the Insertion Limits of the Isolated Element**

The following assay was used to test if the element detected in D7 was an actual insertion. Oligonucleotide primers (5′-TCAACTACAGATGTTGCTCG-3′, 5′-CGAAGGATGTCACCAACGC-3′) were designed from the sequence of DsD765. Their sequences were presumed to be adjacent to the insertion point of the characterized element (see below). Amplification was carried out in 50-μl reactions that included 0.4 μg of *D. subobscura* EU strain genomic DNA, 80 pmol of each primer, 3 units of Taq DNA polymerase, and buffer supplied by the manufacturer (Boehringer Mannheim). Amplification was conducted in a Perkin-Elmer GenAmp PCR System 2400 following the Touchdown PCR program. The resultant 234-bp product was gel-purified and cloned into pCR-Script vector (Stratagene, La Jolla, Calif.). Four clones were sequenced.

**Results**

Isolation of a New Transposable Element

We obtained the DSF112 clone as described in Materials and Methods. We tested whether DSF112 could be a TE partial sequence by using Southern blot analysis and polytene chromosomes in situ hybridization of *D. subobscura* strains. The Southern blot analysis revealed specific signals for each of the strains (fig. 1B), and in the in situ hybridization, the signals in the chromosome arms differed in position and number among strains (figs. 2 and 3). The number of signals varied between 24 and 30, and roughly half of them were located close to the centromere (chromosome photographs are available upon request). The occurrence of signals that appear to be restricted to only one of the homologous chromosomes could indicate recent transposition events. We named this new family of TEs *bilbo*.

**Fig. 2.—In situ hybridization of *D. subobscura* PC strain. Each chromosome has been identified at its distal end (A, J, U, E, O, Dot). Arrowheads point out those hybridization signals that are present in all nuclei (116× magnification).**

The restriction map of the F11 phage clone showed that it lacked some restriction fragments observed as intense signals on the Southern blot using DSF112 as a probe (fig. 1A), particularly the 3.6-kb *Sna* I and 1.8-kb *Hind* III. The 2.0-kb *Sac* I, from which we obtained DSF112, is the same as that shown in figure 1A and its sequence is contained within the 2.6-kb *Ace* I fragment shown in figure 1A and B. Therefore, we supposed that F11 contained a truncated copy. However we assumed that there might be a subpopulation of *bilbo* elements containing all these internal restriction fragments in the *D. subobscura* genome, we decided to look for another element of this family in a genomic library of *D. subobscura*. We selected phage D7 (see Materials and Methods) because it contained restriction fragments of the expected sizes, indicating that we had one element of this subpopulation, probably a full-length element. We detected a 6.5-kb *Sal* I fragment containing all of the repetitive sequence, hybridizing D7 with *D. subobscura* genomic DNA as a probe. The plasmid subclone of this fragment was called DS765. Using the sequence adjacent to DS765 as a probe, this phage clone maps on the U chromosome of *D. subobscura* H271 (data not shown) at 45A.

**Sequence Analysis**

We sequenced the inserts of DSF112 (GenBank accession number U73800) and DS765 (GenBank accession number U73803), 2,017 bp and 6,556 bp in length,
Their sequence identity is 96.3%, and transition:transversion ratio and codon position analysis of substitutions suggest that both sequences have been under selective pressure. A Blast search for similarity in the GenBank and EMBL databases revealed homology of both sequences to the TRIM element of *Drosophila miranda* (Steinemann and Steinemann 1991) (61.7% on 3,046 bp) and the LOA element of *Drosophila silvestris* (Felger and Hunt 1992) (50.9% on 3,372 bp), both non-LTR retrotransposons.

We found the sequence 5'-TTTCTATTTACG-3' two times in DsD765, at positions 524 and 6066. To test the hypothesis that these repeats were in fact the duplication of the target site sequence generated by a transposition event, we performed PCR using *D. subobscura* EU strain genomic DNA and oligonucleotides designed from positions 381 and 6157 of the DsD765 sequence. The result was a 234-bp amplified sequence (GenBank accession number U73801) expected from an amplification of the insertion site without the transposable element. In this amplified sequence there is only one copy of the sequence given above. This result confirms the hypothesis that this repeat was a product of the duplication of the target site sequence and defines the *bilbol* element as the sequence of 5,530 bp between positions 536 and 6065 of DsD765.

The sequence of the insertion site of *bilbol* is probably a gene (which we have called genxDs, GenBank accession number U73802). Clone DsD765 contains 1014 bp of this gene. The major putative ORF comprises 169 aa; it begins at position 507 and continues at the other side of *bilbol*, without interruption, to the end of the cloned fragment in DsD765. A CODONPREFERENCE analysis of genxDs showed a high probability of coding capacity for this ORF from its first codon for methionine (position 585 of the 1,014 bp of genxDs). The upstream sequence to this ORF shared the features of TATA-less promoters of *Drosophila* (Arkhipova 1995), with a possible RNA start site around positions 503-507, a few bases upstream of the target site of *bilbol* (position 524). This gene has not been previously described in the databases (GenBank, EMBL).

**Fig. 3.**—Scheme of the subdivision map of *D. subobscura* (Kunze-Mühl and Müller, 1958). The centromeric region of each chromosome (A, J, U, E, O) is found at the left side of the map. We have localized all hybridization signals that appear in at least five nuclei. Underline means a continuous hybridization signal over several subdivisions. Asterisks indicate hybridization signals that are not precisely localized. 54D in PC strain and 68A in MA strain are hybridization signals seemingly restricted to only one of the homologous chromosomes.

**Fig. 4.**—Scheme of the cloned *bilbol* sequences. Open boxes represent ORFs. Thick lines under ORFs represent putative proteins of a possible active element. Black triangles indicate the positions of possible initiation methionines. Vertically striped boxes represent conserved EN, RT, and RNase H domains. Vertical bars indicate zinc-finger motifs (Cys). Abbreviations: UTR, untranslated region; ORF, open reading frame; EN, endonuclease; RT, reverse transcriptase; RH, RNase H.

**Comparative Analysis of *bilbol* with *Drosophila* non-LTR Retrotransposons and Human LINE-1**

Several regions can be recognized in the *bilbol* sequence (fig. 4): a 5' UTR, three ORFs, and a 3' UTR that lacks both a polyadenylation signal and a poly(A) tract.
In the 5’ UTR of *bilbol*, there is no sequence homology to other promoters of *Drosophila* non-LTR elements. Next to this region, the *bilbol*-ORF1 sequence had no homology to non-LTR retrotransposon ORF1 or to currently available database sequences (BLASTX, GB release 96.00), but TESTCODE and CODONPREFERENCE programs indicate a high coding capacity. Although its nucleotide sequence was aligned to the an-
database, at the aa level, the percent identity is just 18.1% (for comparison, aa identity between *bilbol*-ORF1 and LOA-ORF1 is 17.0%).

We have evidence for a single ORF2, which would include ORF2a and ORF2b of *bilbol*, in the putatively active elements of the *bilbol* family. These two ORFs were probably generated after a deletion of 2 bp. In the first place, the sequence of DsF112 presents the 2 bp deleted in *bilbol*, and this clone shows a continuous reading frame in this region. Eight different sequences obtained by PCR from *D. subobscura* also contain continuous reading frames (data not shown). Moreover, it would necessitate an uncommon +1 frameshift to generate the putative RT domain where the deletion is located. Finally, the conjunction of ORF2a and ORF2b generates a single ORF2 similar in structure to that of I factor. Thus, henceforth, we will refer to the second and third ORFs (ORF2a and ORF2b) of *bilbol* as *bilbol*-ORF2.

We analyzed *bilbol*-ORF2 and those of DsF112 generating a multiple alignment using PILEUP. In this alignment, we included ORFs encoding for RT from all sequenced *Drosophila* non-LTR retrotransposons and L1Hs. This alignment showed several regions of conserved aa. First, we found the EN domain in *bilbol*. This domain is located at the beginning of the elements and about 270 aa preceding the RT domain in all *Drosophila* non-LTR retrotransposons except R2Dm (fig. 5A). This is the first time that the EN domain has been described for the LOA element. Second, DsF112 and *bilbol* have a region showing sequence similarities to RT. Both sequences have the seven conserved regions of aa found in other retroelements (Xiong and Eickbush 1990). Moreover, like in the RT of non-LTR retrotransposons there is another conserved region between the second and the third of the retroelements (Xiong and Eickbush 1988). A consensus of this region shows higher similarity of *bilbol* to TRIM and LOA (data not shown). Third, the RNase H in *bilbol* is found 170 aa after the RT (fig. 5B). This is the first time that RNase H has been described for the LOA element. Finally, in *bilbol*-ORF2, 84 aa after RNase H, we found two putative zinc-finger motifs, one of them similar to that found in other non-LTR elements with the structure CX₂CX₁₀HX₃CX; the other one has a CX₂CX₄HX₂H structure (fig. 5C). The latter is also found, almost complete, in the TRIM element. Most elements in figure 5C conserve the two histidines.

The *bilbol* 3’ UTR does not present a canonical polyadenylation signal and, like other *Drosophila* elements (R1, LOA, TRIM, and I), lacks a terminal poly(A) tract. *bilbol* ends in the sequence 5’-CCGCCTCA-
ACCTAACCTAACCT-3’ (the last thymine could be part of the duplication of the target site). In the TRIM element, the same sequence is found five bases before the duplication of its target site, with the exception of the seventh base, which is a T instead of a C. Thus, both elements end in a series of TAACC or AACCT repeats.

Evolution of *Drosophila* Non-LTR Retrotransposons

We performed an alignment with the RT sequences of non-LTR retrotransposons of *Drosophila*. In the elements *bilbol* and TRIM, we generated a single sequence by frameshift, if necessary. To this alignment we manually added the sequence of HIV-1 RT as it appears in Xiong and Eickbush (1990). With this alignment, we made a dendrogram with the neighbor-joining (NJ) method (fig. 6). The topologies of trees obtained using UPGMA (Sneath and Sokal 1973) and NJ were similar (data not shown). The HIV-1 sequence was used to root the tree of the non-LTR retrotransposons.

There is a consistent relationship between the structures of *Drosophila* elements and their positions in the tree. Most of the elements from the branch of jockey (Doc, F, G, jockey, and BS, which we have named “jockey-like” elements) have only the EN and RT in their ORF2, whereas those of the *bilbol* branch plus the I factor (“I factor-like” elements: LOA, TRIM, *bilbol*, and the I factor) have EN (except TRIM, which could be a truncated copy), RT, RNase H, and zinc-finger motifs. In addition, jockey-like elements have a poly(A) tail, while I-factor-like elements end in direct repeats, (TAₐ)ₙ for I and LOA and (TAACC)ₙ for TRIM and *bilbol*. The remaining elements of the tree, TART-B1, R1Dm, and R2Dm, not included in these two groups, have specialized features as large UTRs or specific insertion sites for their retrotransposition.

Discussion *bilbol* Elements

*bilbol* is a new TE with the features of non-LTR retrotransposons. There is a major subpopulation of *bilbol* elements that have some internal restriction fragments in common. These fragments are shown as high hybridization signals in figure 1A. *bilbol* and DsF112 belong to this major subpopulation, but whereas *bilbol* might be a defective full-length element, DsF112 seems to be a truncated copy. As homologous sequences to DsF112 were present in all *D. subobscura* strains analyzed and in all species studied in the *obscura* group (unpublished data), this family was probably present in the common ancestor to these *Drosophila* species.

In some *Drosophila* non-LTR elements, a promoter sequence is located around position 30 of the 5’ UTR. Unfortunately, the promoters of LOA and TRIM, the elements more similar to *bilbol*, have not been characterized. The sequence data of the *bilbol* element alone are insufficient to propose a possible promoter region. *bilbol*-ORF1 and -ORF2 are similar in size and position to the ORFs of other non-LTR retrotransposons (Arkhipova, Lyubomirskaya, and Il-
We interpret that both *bilbol*-ORF2a and -ORF2b should correspond to a single ORF2 in an active *bilbo* element. The structural similarity between the ORF2 of the I factor and *bilbol*-ORF2 indicates that *bilbol*-ORF2 can be the complete structure of the ORF2 of the active *bilbo* elements. Finally, *bilbo*, like other *Drosophila* elements (R1, LOA, TRIM, and I) or elements in other species (Burch, Davis, and Haas 1993; Teng, Wang, and Gabriel 1995), lacks a terminal poly(A) tract and allows us to question the poly(A) tract as a non-LTR elements and LOA. 

Proteins encoded by retroviral ORF1 have the ability to bind RNA. The predicted protein for *bilbol*-ORF1 contains neither zinc-finger motifs nor other RNA-binding motifs (Burd and Dreyfuss 1994). The product of LOA-ORF1 also lacks these motifs; however, it forms a ribonucleoprotein complex with LOA RNA (Hohjoh and Singer 1996); the same could happen with *bilbo* elements. A 2-bp deletion in the *bilbol*-ORF2 results in two reading frames (ORF2a and ORF2b). They overlap in the RT domain, which probably makes the *bilbol* copy a defective element.
Evolution of *Drosophila* Non-LTR Retrotransposons

According to the relationship observed between the phylogenetic tree and the structure of *Drosophila* non-LTR elements (fig. 6), we put forward an hypothesis and an evolutionary pathway for non-LTR retrotransposons in this genus. We suggest the occurrence of an ancestral element, which would encode for an EN domain, an RNA-dependent DNA polymerase (RT) domain, an RNase H domain, and one or more nucleic-acid-binding motifs (zinc-fingers). Furthermore, this element had no terminal poly(A) tracts but several nucleotide repeats. We base this hypothesis on the most parsimonious explanation for the location of I-factor-like elements in two different basal branches of the tree, whereas jockey-like elements (the classic example of non-LTR retrotransposons) are located in a single branch. Our hypothesis implies the conservation of a structure more similar to that of the ancestor elements in LOA, TRIM, *bilbo*, and the I factor. Furthermore, it implies the appearance of an element lacking RNase H and zinc-finger motifs, an ancestor of jockey-like elements, and the evolution of specialized elements with unique features such as large UTRs (TART, HeT-A) or more specific endonucleases as in R2 (Luan et al. 1993) and, possibly, R1. Our hypothesis depends on an early divergence of the I factor from the remaining elements. Other published phylogenetic trees (Xiong and Eickbush 1990; Eickbush 1992; Burch, Davis, and Haas 1993; Burke, Muller, and Eickbush 1995), in which TRIM and LOA were not included, show the differentiation of the I factor from jockey-like elements. Following our hypothesis, a loss of the 3′ region could have generated an ancestor of the jockey-like group. This ancestral element could have undergone a rapid evolutionary radiation in the primitive host and given origin to the now existing elements of this group.

A model of the transposition mechanism of non-LTR retrotransposons should be able to explain and integrate the features of I-factor-like elements (including *bilbo*): RNase H activity (although this activity needs to be experimentally confirmed), zinc-finger function in the C-terminal region of ORF2, and the origin or role of final repeats in these elements.

Here, we have described a new non-LTR retrotransposon family in *D. subobscura*. We propose a structurally complex ancestral non-LTR retrotransposon in *Drosophila* which evolved mainly through loss of different domains but whose origin is unknown. The phylogenetic and structural analysis of the remaining non-LTR retrotransposons would be a test for the validity of this hypothesis and whether this ancestral element can be generalized to the whole group of non-LTR elements. This view of non-LTR retrotransposons helps to partially clarify the evolution of retrosequences (Xiong and Eickbush 1990; McClure 1991), but it also poses new questions about the evolution of non-LTR retrotransposons and their transposition mechanisms.

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