Intra- and Interspecies Variation Among *Bari-1* Elements of the Melanogaster Species Group

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ABSTRACT

We have investigated the distribution of sequences homologous to *Bari-1*, a *Tc1*-like transposable element first identified in *Drosophila melanogaster*, in 87 species of the Drosophila genus. We have also isolated and sequenced *Bari-1* homologues from *D. simulans*, *D. mauritiana*, and *D. sechellia*, the species constituting with *D. melanogaster* the melanogaster complex, and from *D. diplacantha* and *D. erecta*, two phylogenetically more distant species of the melanogaster group. Within the melanogaster complex the *Bari-1* elements are extremely similar to each other, showing nucleotide identity values of at least 99.3%. In contrast, *Bari-1*-like elements from *D. diplacantha* and *D. erecta* are on average only 70% similar to *D. melanogaster Bari-1* and are usually defective due to nucleotide deletions and/or insertions in the ORFs encoding their transposases. In *D. erecta* the defective copies are all located in the chromocenter and on chromosome *4*. Surprisingly, while *D. melanogaster Bari-1* elements possess 26-bp inverted terminal repeats, their *D. diplacantha* and *D. erecta* homologues possess long inverted terminal repeats similar to the terminal structures observed in the *S* elements of *D. melanogaster* and in several other *Tc1*-like elements of different organisms. This finding, together with the nucleotide and amino acid identity level between *D. diplacantha* and *D. erecta* elements and *Bari-1* of *D. melanogaster*, suggests a common evolutionary origin and a rapid diversification of the termini of these Drosophila *Tc1*-like elements.

TRANSPOSABLE elements constitute a significant fraction of the repeated DNA present in the genome of virtually all organisms so far studied. Many families of transposons have been isolated and characterized and, depending on structure and mechanism of transposition, they are grouped into two main classes. Class I transposons move via an RNA intermediate while class II elements transpose directly from DNA into a new DNA target site (Finnegan 1989). The wide distribution of transposons raises interesting questions about their origin and about the roles they play in the evolution of host genomes and in regulating host structural genes (for reviews, see Berg and Howe 1989; Kidwell 1992a,b; McDonald 1993).

Some families of transposons are present in very distantly related taxa. A well-known example is represented by the *Tc1-mariner* superfamily comprising *mariner*-like and *Tc1*-like elements (for reviews, see Robertson and Lampe 1995; Plasterk 1996). Detailed analysis of copies isolated from different organisms suggests that the *Tc1-mariner* elements have an ancient origin (see, for example, Kidwell 1993; Radice *et al.* 1994) and that horizontal transmission events play a substantial role in their diffusion, probably because their transposition mechanism is independent of host factors (Lampe *et al.* 1996; Vos *et al.* 1996).

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Three *Tc1*-like elements have been isolated in *Drosophila melanogaster*: *HB1*, *S*, and *Bari-1*. *HB1* was the first element identified as a *Tc1*-like element of *D. melanogaster* (Henikoff and Plasterk 1988). It possesses 28-bp inverted terminal repeats and on the basis of DNA sequencing of the analyzed copies it appears to be a defective element (Brierly and Potter 1985). The *S* element was originally discovered during the characterization of a spontaneous *suppressor of sable* mutation (Merriman *et al.* 1995). In *D. melanogaster*, where both defective and complete copies exist, the complete *S* element (pS3) possesses 234-bp-long inverted terminal repeats. *S*-like elements, partially complementary to the melanogaster probe, are present in *D. simulans* and *D. mauritiana*, the two species most closely related to *D. melanogaster*. The third *Tc1*-like element of *D. melanogaster*, *Bari-1*, was originally discovered in a molecular analysis of the heterochromatic h39 band (Caizzi *et al.* 1993). It is present as an array of tandem repeats in the h39 region and as single copies in a few euchromatic polytene bands. By DNA sequencing, ORFs of heterochromatic and euchromatic copies of the element proved to be identical (Caizzi *et al.* 1993). The apparent homogeneity of *Bari-1* in the genome of *D. melanogaster* resembles that of *Tc1* in *Caenorhabditis elegans* and of *mariner* in *D. mauritiana* rather than the variability in length and sequence of the *D. melanogaster HB1* and *S* elements. *Bari-1* is present in all strains of *D. melanogaster* and *D. simulans* so far analyzed (Caggese *et al.* 1995). This suggests a much wider diffusion in nature than that of the *S* elements.

To further study the evolution of the *Bari-1* element and its phylogenetic relationship with other *Tc1*-like elements we carried out hybridization experiments to detect the presence of *Bari-1* homologues in species representative of the Sophophora and Drosophila subgenera. DNA sequence analysis of elements isolated from all species of the melanogaster complex and from *D. erecta* and *D. diplacantha* suggests an evolutionary origin of these elements by diversification of a common ancestor element in different lineages.

MATERIALS AND METHODS

Fly stocks: Lines of *D. sechellia*, *D. erecta*, *D. orena*, *D. teissieri*, and *D. simulans* (Bordeaux strain) were obtained from the collection of R. Costa, University of Padova, Italy. All other strains were obtained from the National Drosophila Species Resource Center, Bowling Green State University, Bowling Green, Ohio. The species examined for the presence of sequences homologous to *Bari-1* are listed in Figure 2.

DNA blotting experiments: Genomic DNA for Southern blot hybridization experiments was prepared from 50 to 100 flies immediately after the stock was received. DNA extraction, restriction digestion, gel electrophoresis, and transfer to nylon membranes were performed according to Maniatis, Fritsch and Sambrook (1982). The 1.3-kb *Hin*dIII-*Bgl* II internal fragment of *Bari-1* was labeled to high specific activity with [^{32}P]dATP by the method of Feinberg and Vogelstein (1983). The hybridization mixture was 0.8 m NaCl, 0.05 m Na_2PO_4, 0.8% SDS, 0.005 m EDTA, and 0.1% sodium pyrophosphate. Two filter hybridization and washing conditions were used: (1) high stringency conditions (hybridization at 67° and final wash in 0.1× SSC, 0.1% SDS at 65°) for DNAs from species of the *melanogaster* complex; (2) reduced stringency conditions (hybridization at 60° and final wash in 0.5× SSC, 0.1% SDS at 56°) for DNAs from other species. Filters hybridized under reduced stringency conditions were exposed to X-ray films for 16–60 hr.

Genomic DNA libraries and cloning of PCR fragments: Lambda genomic libraries of *D. simulans*, *D. erecta*, and *D. diplacantha* were constructed using the Lambda GEM-12 *Xho*I half-site arms cloning system (Promega, Madison, WI) following the protocol supplied by the manufacturer. In each case, roughly 30,000 recombinant phages were screened with the same probes and conditions used in the Southern blot experiments. Positive plaques were subjected to three rounds of purification. Appropriate restriction fragments recognized by the *Bari-1* probe were gel eluted and ligated to the pUC19 vector for further analysis. *Bari-1* elements from *D. mauritiana* and *D. sechellia* were isolated by PCR amplification using 50-ng genomic DNA as template, the two 26-bp terminal inverted repeats as primers (the inverted repeats have four mismatches), and 1 unit of Taq DNA polymerase (Promega) in the following conditions: 1′, 95°; 1′, 60°; 1′, 72° for 35 cycles and 5′, 72° as final extension. With both DNA templates a single 1.7-kb band was obtained which was gel-purified and ligated to the pGEM-T vector (Promega) for cloning.

DNA sequencing and computer analysis: Plasmid DNAs harboring a complete *Bari-1* element from each species of the *melanogaster* complex, or subclones obtained on the basis of the restriction maps shown in Figure 6, were sequenced using the appropriate vector primers. Synthetic primers (Genset, France) constructed on the basis of the published *Bari-1* sequence (AC X67681) were also used. Sequencing of *D. erecta* and *D. diplacantha* plasmids was mainly performed by subcloning fragments generated on the basis of their restriction maps and using pUC vector primers. To complete the sequence of *Er-1* and *Di-3* clones three specific synthetic primers were also constructed. DNA sequencing was carried out with the dideoxy chain termination method (Sanger *et al.* 1977) using either Sequenase or Thermosequenase (Amersham, Arlington Heights, IL) cycle sequencing. Comparisons of homologues were done using the 1991 GCG Sequence Analysis Software Wisconsin Package V7. The sequence flanking the *Bari-1* homologue in the *Di-7* clone was identified as a *copia*-like element (70% similarity) in a Blast search of the EMBL databank. The DNA sequence of *Di-3* and *Er-1* clones have been archived under accession numbers Y13852 and Y13853, respectively.

***In situ* hybridization:** Salivary gland chromosomes from third instar larvae of *D. erecta* were prepared essentially as described in Pardue (1986). The plasmid pEr/PK0.3, carrying the 380-bp *Pst*I-*Kpn*I fragment of the *Er-1* element, was labeled by nick-translation with the fluorescent Cy3-dCTP precursor (Amersham). Chromosomes were stained with DAPI. Digital images were obtained using a Leica DMRXA epifluorescence microscope equipped with a cooled CCD camera (Princeton Instruments, NJ). Gray-scale images obtained separately recording Cy3 and DAPI fluorescence by specific filters were computer colored and merged for the final image using the Adobe Photoshop software.

RESULTS

Genomic analysis of *Bari-1* elements in species of the melanogaster complex: In a previous paper we showed that *Bari-1* elements were present in all stocks analyzed from the two cosmopolitan sibling species *D. melanogaster* and *D. simulans* (Caggese *et al.* 1995). A striking difference between the two sibling species is the presence of a cluster of roughly 80 copies of *Bari-1* elements in the heterochromatic band h39 in every *D. melanogaster* strain analyzed. This cluster was not observed in any *D. simulans* strain. These findings are suggestive of the presence of *Bari-1* elements before the evolutionary splitting of the two species and of an origin of the h39 cluster of *D. melanogaster* after the separation of the two species but before the world-wide diffusion of *D. melanogaster*. We have tested this suggestion in *D. mauritiana* and *D. sechellia*, the two remaining species of the melanogaster complex, which are endemic to the Mauritius Island and to the Seychelles Archipelago, respectively.

Figure 1 shows the results of a Southern blot analysis of the genomic DNAs from the four species of the melanogaster complex when probed with an internal *Bari-1* fragment after single and double digestions. Digestion with *Hin*dIII, which recognizes a restriction site present only once in the *Bari-1* sequence, produces in *D. melanogaster* (Canton-S strain) a strong 1.7-kb band deriving from the monomerization of the 80 heterochromatin clustered copies, plus eight bands coming from single copies of the element inserted into different regions of the genome. *D. simulans*, *D. mauritiana*, and *D. sechellia* only produce discrete hybridization bands of similar intensity. Analogous patterns of hybridization are observed after digestion with *Bgl* II, *Sma*I, or *Kpn*I (data

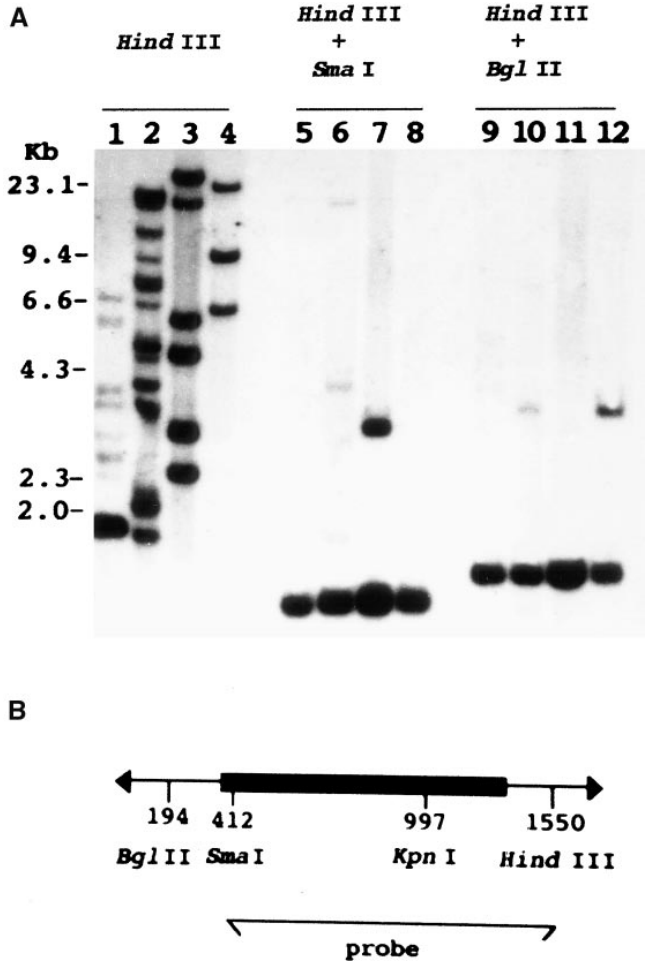

Figure 1.—Genomic analysis of *Bari-1* elements in species of the *melanogaster* complex. (A) DNAs from *D. melanogaster* (*Canton-S*, lanes 1, 5, 9), *D. simulans* (*Bordeaux*, lanes 2, 6, 10), *D. mauritiana* (lanes 3, 7, 11), and *D. sechellia* (lanes 4, 8, 12) were digested with *Hin*dIII (lanes 1–4), *Hin*dIII plus *Sma*I (lanes 5–8) or *Hin*dIII plus *Bgl* II (lanes 9–11). The amount of *D. melanogaster* DNA loaded was 10 times less than that of DNAs from other species to better visualize the position of the 1.7-kb *Hin*dIII band deriving from the heterochromatic cluster and the identical size of the hybridizing fragments from double digestions. (B) Restriction map of the *D. melanogaster Bari-1* element showing the extension of the fragment used as a probe in A.

not shown). These results provide further evidence that a cluster of tandem repeats is present only in *D. melanogaster* while single elements inserted in different genomic regions are present in all the four species of the melanogaster complex. From the single digestion patterns it is also possible to estimate the number of elements present in each genome. *D. simulans*, Bordeaux strain, contains 15–18 *Bari-1* copies, the analyzed *D. mauritiana* strain contains six copies, and the analyzed *D. sechellia* strain contains three copies.

*Hin*dIII-*Sma*I or *Hin*dIII-*Bgl*II double digestions of all analyzed DNAs produced a main hybridization band at the same molecular weight, suggesting that *Bari-1* is conserved in structure in all species of the complex. Hybridization bands appearing at higher molecular weights are presumably due to polymorphisms in one of the restriction sites used. From the intensity of the hybridization bands and from the size of fragments produced by double digestions we conclude that most *Bari-1* copies present in *D. simulans*, *D. mauritiana*, and *D. sechellia* are very similar to the copies present in *D. melanogaster*.

Taxonomic distribution of *Bari-1* elements: The presence of sequences homologous to *Bari-1* in many species of the Sophophora and Drosophila subgenera and in *Zaprionus tuberculatus* was investigated by Southern blotting experiments under conditions of moderate stringency. The results obtained with respect to 87 different species are summarized in Figure 2 and representative examples of the gel hybridizations are shown in Figure 3, A and B. The phylogenetic relationship of the species analyzed are shown in Figure 4. According to the banding patterns observed, and taking into account the relative amounts of DNA loaded on the gel, each species was assigned in Figure 2 a number of plus signs (+, ++, or +++) proportional to the intensity of the signal when hybridization bands were clearly visible after short exposure, the letter "F" when faint bands appeared only after long exposure, or the minus (−) sign when no signal was observed under either condition. Within the melanogaster subgroup only *D. yakuba* apparently lacks *Bari-1* elements. Except for *D. ficusphila*, all species of each group of the Sophophora subgenus produced hybridization signals, although of variable intensity. Among species of the Drosophila subgenus, hybridization was observed in species of the virilis, robusta, and melanica groups and in some species of the other groups. In general, cross hybridization between species was less intense in the Drosophila subgenus than in the Sophophora subgenus. *Z. tuberculatus*, belonging to a different genus, also produced faint hybridization bands. These results indicate that, unlike the *S* elements, *Bari-1* is widespread in the Drosophila genus.

DNA analysis of *Bari-1* elements from species of the melanogaster complex: The sequence of a *D. melanogaster Bari-1* element cloned from the insertion site at polytenic band 47D has been previously reported (Caizzi *et al.* 1993). In order to define the level of homogeneity of *Bari-1* elements present in different species, we have cloned and sequenced elements from each species of the melanogaster complex. *Bari-1* elements of *D. simulans* were isolated by screening genomic λ libraries, whereas *Bari-1* clones from *D. mauritiana* and *D. sechellia* were obtained by PCR amplification using two 26-bp primers representing the inverted terminal repeat of the *D. melanogaster* element. We present here the sequence of five additional *D. melanogaster* clones: two independent heterochromatic clones (clones h19/5 and h1/1, both coming from the cluster located at the h39 region), and three clones from euchromatic regions

Subgenus *Sophophora*
Group *melanogaster*
Subgroup *melanogaster*
+++ *melanogaster*
+++ *mauritiana*
+++ *sechellia*
+++ *simulans*
++ *erecta*
+ *orena*
F *teissieri*
– *yakuba*
Subgroup *takahashii*
++ *lutescens*
++ *takahashii*
Subgroup *suzuki*
+ *mimetica*
Subgroup *ananassae*
++ *ananassae*
++ *malerkotliana*
Subgroup *ficusphila*
– *ficusphila*
Subgroup *eugracilis*
F *eugracilis*
Subgroup *elegans*
F *elegans*
Subgroup *montium*
++ *auraria*
++ *bicornuta*
+ *birchii*
++ *lacteicornis*
++ *diplacantha*
+ *serrata*
++ *triauraria*
++ *vulcana*

Group *obscura*
Subgroup *obscura*
+ *ambigua*
+ *miranda*
+ *persimilis*
+ *pseudoobscura*
Subgroup *affinis*
+ *affinis*
++ *algonquin*
+ *azteca*
+ *bifasciata*
+ *narraganset*
+ *tolteca*

Group *willistoni*
+ *capricorni*
+ *equinoxialis*
+ *nebulosa*
F *sucinea*
+ *paulistorum*
+ *tropicalis*
+ *willistoni*

Group *saltans*
Subgroup *cordata*
+ *neocordata*
Subgroup *elliptica*
+ *emarginata*
Subgroup *sturtevanti*
+ *sturtevanti*
Subgroup *parasaltans*
+ *subsaltans*
Subgroup *saltans*
+ *lusaltans*
+ *saltans*

Subgenus *Drosophila*
Group *virilis*
+ *americana americana*
+ *borealis*
+ *flavomontana*
+ *lacicola*
+ *littoralis*
+ *lummei*
+ *montana*
+ *novamexicana*
+ *virilis*

Group *robusta*
– *robusta*
F *sordidula*

Group *melanica*
+ *euronotus*
F *melanica*

Group *annulimana*
F *aracatacas*
– *gibberosa*

Group *dreyfusi*
F *camargoi*

Group *mesophragmatica*
+ *pavani*

Group *repleta*
Subgroup *mulleri*
+ *arizonensis*
+ *buzzatii*
+ *meridiana rioensis*
F *mojavensis mojavensis*
+ *mojavensis baja*
+ *mulleri*
+ *peninsularis*
Subgroup *mercatorum*
+ *mercatorum mercatorum*
+ *paranaensis*
Subgroup *melanopalpa*
F *limensis*
+ *repleta*
Subgroup *hydei*
+ *eohydei*
F *hydei*

Group *polychaeta*
+ *polychaeta*

Group *immigrans*
Subgroup *immigrans*
F *formosana*
F *immigrans*
Subgroup *nasuta*
F *albomicans*
– *nasuta*
– *sulfur albostringata*
– *sulfur.sulfurigaster*
Subgroup *lineosa*
– *lineosa*

Group *funebris*
F *macrospina macrosp.*

Genus *Zaprionus*
F *tuberculatus*

Figure 2.—Species examined for the presence of sequences that hybridize with *Bari-1*. The number of + signs indicates the relative strength of the hybridization signal. F, one or more bands were faintly discernible on the blot after long exposure; –, a complete absence of signal.

41AB, 55F and 91F. Heterochromatic clones can be distinguished from the euchromatic ones since they are present in λ phages carrying at least five *Bari-1* copies in tandem repeat. Figure 5 summarizes the nucleotide differences between the new sequences obtained and the *Bari-1* element from *D. melanogaster* band 47D taken as a reference.

The *Bari-1* copies at 55F and at 91F were singled out for cloning and sequencing because they appear not to be mobile (Caggese *et al.* 1995). Since both elements have normal terminal repeats, their lack of mobility is likely to be due to some effect of the flanking regions or to differences in internal sequences. In contrast, both the heterochromatic clones sequenced lack the first two nucleotides (CA) of the terminal inverted repeats. It should be noted that the sequences of the heterochromatic clones, although reported as coming from isolated elements, are really composed of parts of two adjacent elements since the 1.7-kb *Hin*dIII fragments, subcloned from two different λ phages into the pUC vector, represent the joining of the right terminus of one element (from the *Hin*dIII site to the right inverted repeat) to the left part of another one (from the left terminus to the adjacent *Hin*dIII site). Therefore, the sequences of heterochromatic clones given in Figure 5 do not represent the entire sequence of a single element and no nucleotide difference can be unambigously assigned to a specific heterochromatic copy. Nevertheless, the heterochromatic elements clearly are very similar in nucleotide sequence to the euchromatic ones.

The two recovered *D. simulans Bari-1* clones both have the typical TA duplication at the ends of their inverted repeats. Since the flanking regions are different, they are from two different (but unidentified) chromosomal sites. Both clones have inverted repeats identical to the reference sequence and possess intact ORFs.

One of the two sequenced *Bari-1* elements of *D. mauritiana*, *D.mau* 9b, has a frameshift in the coding region,

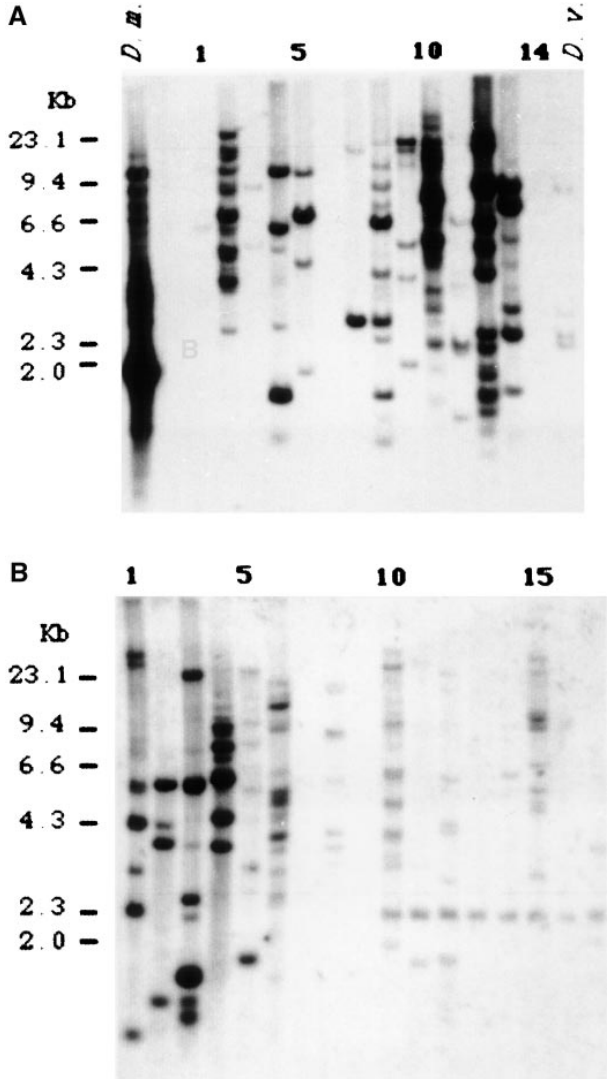

Figure 3.—Genomic analysis of the species containing *Bari-1* homologues. (A) Species from melanogaster group: 1, *D. teissieri*; 2, *D. erecta*; 3, *D. orena*; 4, *D. lutescens*; 5, *D. takahashii*; 6, *D. eugracilis*; 7, *D. mimetica*; 8, *D. auraria*; 9, *D. birchii*; 10, *D. diplacantha*; 11, *D. serrata*; 12, *D. ananassae*; 13, *D. malerkotliana*; 14, *D. elegans*. *D. melanogaster*, *D.m.*, and *D. virilis*, *D.v.* DNAs were used to establish the strength of the hybridization signal. Exposure was overnight. (B) Comparison of hybridization intensity (species of the Sophophora subgenus, lanes 1–6 and species of the Drosophila subgenus lanes 7–17). 1, *D. bicornuta*; 2, *D. lacteicornis*; 3, *D. triauraria*; 4, *D. vulcana*; 5, *D. equinoxialis*; 6, *D. tropicalis*; 7, *D. robusta*; 8, *D. melanica*; 9, *D. gibberosa*; 10, *D. arizonensis*; 11, *D. meridiana rioensis*; 12, *D. mojavensis baja*; 13, *D. mulleri*; 14, *D. peninsularis*; 15, *D. mercatorum*; 16, *D. repleta*; 17, *D. eohydei*. Exposure was 24 hr. All DNAs were *Hin*dIII digested. The probe was the same as in Figure 1. Note that the 2.3 kb-band in lanes 10–17 of B is shared by all species of the *repleta* group.

whereas the *D.mau* 6 clone has eight nucleotide substitutions, five of which are in the coding region. Finally, the *D.sec* 0 element from *D. sechellia* has a stop codon in the ORF.

The comparison of the 11 *Bari-1* elements sequenced does not permit construction of a clear phylogenetic relationship since very few informative sites were found. In general, the nucleotide variations appear randomly distributed over the length of the element and, within the ORFs, are equally distributed in the three codon positions.

The pairwise divergence values from a comparison of *Bari-1* sequences from the four species are shown in Table 1. Within *D. melanogaster* elements the highest divergence is between *D.mel 55F* and *D.mel 19/5*, both non-autonomous elements. Comparison between elements from any two species of the complex yields low divergence values with a maximum of 1% between *D. sechellia* and *D. mauritiana*. This low divergence makes *Bari-1* similar to the *mariner* elements (Maruyama and Hartl 1991; Capy *et al.* 1992) rather than to the *Tc1*-like *S* elements (Merriman *et al.* 1995).

Cloning and characterization of *Bari-1* homologues from *D. erecta* and *D. diplacantha*: In order to investigate whether the structure and the DNA sequence of the *Bari-1* elements were also conserved in species relatively distant from the melanogaster complex, we isolated *Bari-1* homologues from *D. erecta*, a species of the melanogaster subgroup, and from *D. diplacantha*, a species of the montium subgroup. These species were chosen for detailed analysis because of the high level of hybridization with the *Bari-1* probe observed in the Southern blotting experiments. In fact, when lambda libraries were screened with an internal *Bari-1* fragment several positive plaques were found, most containing only a limited portion of the element. From each species three random clones were characterized. Figure 6 shows a preliminary restriction analysis of these clones and summarizes the most relevant features revealed by sequencing. The *D. erecta Er-1* clone is the only one that contains a complete element flanked on both sides by the usual TA dinucleotide. The *D. diplacantha Di-3* clone appears almost complete, lacking only a few nucleotides at the left end. The remaining cloned elements from *D. erecta* and *D. diplacantha* appear either to have been truncated during the cloning procedure or to be flanked by unrelated sequences. In one case, the *Di-7* clone, the flanking DNA is related (70% similarity) to *copia* like sequences present in the databases. Comparison of the physical maps of the cloned elements shows that all of them share some restriction sites and that they are related to each other.

Nucleotide sequence variations in *D. erecta* and *D. diplacantha* elements: The relationship between the isolated *D. erecta* and *D. diplacantha* elements was confirmed by sequencing. Figure 7 aligns the nucleotide sequences of *Er-1* and *Di-3*, the two almost complete elements, with the reference *D. melanogaster Bari-1* copy. Several deletions and insertions affecting both coding and noncoding regions are present in the two elements and in the sequences of the incomplete clones. Thus, it appears that most *Bari-1* homologues present in the

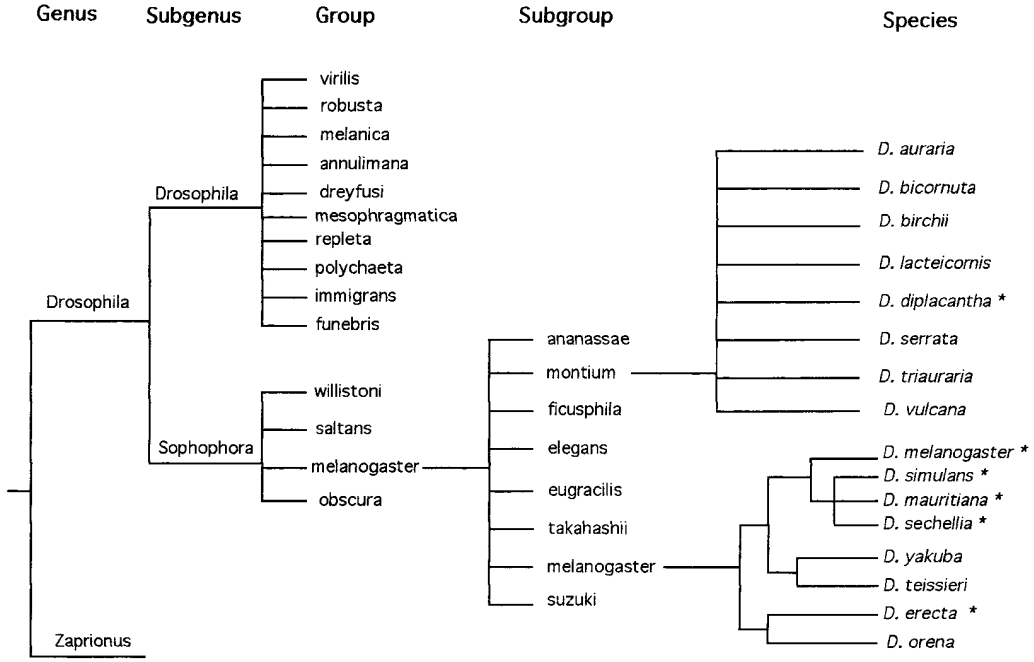

Figure 4.—Relationships between genera, subgenera, groups and subgroups of the Drosophila species utilized in this study. Only the melanogaster group is detailed, and it is drawn according to Lachaise *et al.* (1988). Asterisks indicate the Drosophila species from which *Bari-1* elements were sequenced.

genome of *D. erecta* and *D. diplacantha* are defective elements. Table 2 shows pairwise similarities of the sequences compared. The three clones from *D. erecta* have on average 95% identity to each other and approximately 70% identity with *Bari-1* of *D. melanogaster*. Among clones from *D. diplacantha* we can distinguish a subclass, represented by the *Di-3* sequence, which is 93% similar to elements of *D. erecta* and 70% to *Bari-1*, from an apparently different one represented by clones *Di-1* and *Di-7*, which are only ~70% similar to *Di-3* and to the *D. erecta* elements. Interestingly, elements of the latter subclass show higher similarity, ~88%, to the *D. melanogaster* element. This evidence suggests that at least two different members of the *Bari-1* family exist in *D. diplacantha*.

Optimal alignment of the *D. diplacantha Di-3* ORF with the *D. melanogaster* ORF is obtained by introducing three single nucleotide gaps and deleting two nucleotides. The best alignment of the *Er-1* ORF with the *Bari-1* ORF is obtained by introducing three gaps of 1, 15

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                  123689991123  4  4  5  5  6  6  7  8  8  8  9  9  9  9  9  1  1  1  1  1  1  1  1  1  1  1  1  1  111111111111
                    98515969820  1  6  2  9  0  8  0  6  9  9  0  4  5  6  9  0  0  0  0  0  0  1  1  2  2  2  2  3  444445556666
                          09022  9  2  9  8  6  2  4  9  6  7  8  3  4  2  4  0  2  3  3  4  6  6  7  3  7  8  9  5  016665562267
                                                                              2  3  4  9  3  1  2  9  6  9  2  0  9  190291866719

D.mel 47D         CAGAGCACCTGA  T  G  C  G  G  C  A  C  T  A  T  G  T  A  T  G  G  T  T  C  T  T  C  C  T  A  G  G  TACAGGACACAG
                                Gly Ala Thr Gly Ala Ser Lys Tyr Asn Asn His Gly Phe Gly Val Glu Gly Leu Leu Asn Ser Leu Pro Gln Ile Glu Glu Ala

D.mel 41AB       CAAAGCACCTGA  T  G  C  G  G  C  A  C  T  A  T  G  T  A  T  A  G  T  T  C  T  T  C  C  T  A  G  G  TACAAGACACAA
                                Gly Ala Thr Gly Ala Ser Lys Tyr Asn Asn His Gly Phe Gly Val Lys Gly Leu Leu Asn Ser Leu Pro Gln Ile Glu Glu Ala

D.mel 91F         CAGAGCACCTGC  C  G  C  G  G  C  A  C  T  A  T  G  T  A  T  A  G  T  T  C  T  T  C  C  T  A  G  A  TACAGGA▽ACAA
                                Gly Ala Thr Gly Ala Ser Lys Tyr Asn Asn His Gly Phe Gly Val Lys Gly Leu Leu Asn Ser Leu Pro Gln Ile Glu Glu Thr

D.mel 55F         CAGAGCACCTGA  T  G  C  G  G  C  A  C  T  A  T  G  T  A  T  G  G  T  T  C  T  T  C  T  T  T  G  G  T+CAGGACACAG
                                Gly Ala Thr Gly Ala Ser Lys Tyr Asn Asn His Gly Phe Gly Val Glu Gly Leu Leu Asn Ser Leu Pro  *  Ile Val Glu Ala

D.mel h19/5       --GAGCACCTGA  T  T  C  G  G  C  A  C  T  A  T  G  T  A  G  G  G  G  T  C  T  T  T  T  C  T  A  G  G  TACAGGACACAG
                                Gly Ser Thr Gly Ala Ser Lys Tyr Asn Asn His Gly Phe Gly Gly Glu Gly Leu Leu Asn Ser Leu Ser Gln Ile Glu Glu Ala

D.mel h1/1        --GAGCACCTGA  T  G  C  G  G  C  A  C  T  A  T  G  T  A  T  G  G  T  T  C  T  T  C  C  T  A  G  G  TACAGGACACAG
                                Gly Ala Thr Gly Ala Ser Lys Tyr Asn Asn His Gly Phe Gly Val Glu Gly Leu Leu Asn Ser Leu Pro Gln Ile Glu Glu Ala

D.sim S1          CAGAGCAC▽CGA  T  G  C  G  G  C  T  C  T  A  T  G  T  A  T  G  G  T  T  C  T  T  C  C  T  A  G  G  TACAGGACACAG
                                Gly Ala Thr Gly Ala Ser Asn Tyr Asn Asn His Gly Phe Gly Val Glu Gly Leu Leu Asn Ser Leu Pro Gln Ile Glu Glu Ala

D.sim S9          CAGAACACCTAA  T  G  C  G  G  C  A  C  T  A  A  G  T  A  T  G  G  T  T  G  T  T  C  C  G  A  G  G  TAA-GAACACAG
                                Gly Ala Thr Gly Ala Ser Lys Tyr Asn Asn Gln Gly Phe Gly Val Glu Gly Leu Leu Lys Ser Leu Pro Gln Ser Glu Glu Ala

D.sec 0           CAGAGCACCTGA  T  G  C  G  G  C  A  A  T  A  T  G  G  G  T  G  A  T  T  C  -  A  C  C  T  A  A  G  TACAGGGCACGG
                                Gly Ala Thr Gly Ala Ser Lys  *  Asn Asn His Gly Val Gly Val Glu Gly Leu Leu Asn  #  Gln Pro Gln Ile Glu Lys Ala

D.mau 6           CAGAGCACCTGA  T  G  A  A  A  A  A  C  T  A  T  A  T  A  T  G  G  T  T  C  T  T  C  C  T  A  G  G  CACAGGACCTAG
                                Gly Ala Lys Glu Thr Tyr Lys Tyr Asn Asn His Gly Phe Gly Val Glu Gly Leu Leu Asn Ser Leu Pro Gln Ile Glu Glu Ala

D.mau 9b          CAGGGAC-CTGA  T  G  C  G  G  C  A  C  A  +  T  G  T  A  T  G  G  T  C  C  T  T  C  C  T  A  G  G  TACAGGACCTAG
                                Gly Ala Thr Gly Ala Ser Lys Tyr Lys  #  His Gly Phe Gly Val Glu Gly Leu Ser Asn Ser Leu Pro Gln Ile Glu Glu Ala
```

Figure 5.—Alignment of eleven *Bari-1* sequences from the melanogaster species complex. Only differing sites are shown. The numbers at the top indicate the positions of variations with respect to the reference *D.mel* 47D sequence. Single nucleotide deletions and insertions are indicated by − and + signs respectively. Nucleotide and, when applicable, amino acids variations are boldfaced. # indicates a frameshift and *, a stop codon. ▽ indicates in the *D.mel* 91F sequence the presence of the 18-bp TTTATCATCTTATCTTAT insertion, in the *D.mel* 41AB sequence the substitution of the GTTGAGTG with AA, and in the *D.sim* S1 sequence that CTGTTC is deleted.

TABLE 1

Pairwise divergence ten *Bari-1* sequences from species of the melanogaster complex

D.mel 47D	*D.mel* 41AB	*D.mel* 91F	*D.mel* 55F	*D.mel* h19/5	*D.sim* S1	*D.sim* S9	*D.sec* 0	*D.mau* 6	*D.mau* 9b
mel 47D	0.002	0.003	0.001	0.003	0.001	0.004	0.005	0.004	0.005
	mel 41AB	0.003	0.004	0.005	0.004	0.007	0.007	0.007	0.007
		mel 91F	0.005	0.007	0.005	0.008	0.008	0.008	0.008
			mel 55F	0.005	0.003	0.006	0.007	0.006	0.007
				mel h19/5	0.005	0.008	0.008	0.008	0.008
					sim S1	0.006	0.007	0.006	0.007
						sim S9	0.009	0.009	0.009
							sec 0	0.009	0.010
								mau 6	0.007

The divergence was calculated as the number of nucleotides differing between any two sequences divided by the total number of nucleotides in *Bari-1*. The 18-bp insertion in *D.mel* 91F and the 6-bp deletion in *D.sim* S1 were treated as single substitutions.

and 23 nucleotides respectively. The deduced proteins putatively encoded by *Di-3* and *Er-1* comprise 338 and 325 amino acids, respectively. As shown in Table 3, the deduced *Di-3* protein is 86% identical to the *Er-1* protein and 67% identical to the *Bari-1* protein. Such high levels of identity clearly demonstrate that *Er-1*, *Di-3* and *Bari-1* belong to the same family of transposable elements.

The inverted repeats of *Er-1* and *Di-3* elements: Careful analysis of the termini of the *Er-1* element, the only cloned element that apparently maintains both ends (see Figure 6), revealed a different organization with respect to the ends of *Bari-1*. The *Er-1* element has 254-bp terminal inverted repeats that are 96.4% identical to each other, with six gaps. In the *Di-3* element a similar long inverted repeat could also be postulated since its left end, although presumably truncated during the cloning, still possesses a 198-bp sequence that is repeated in opposite orientation at its right end (97.9% identity without gaps). The 254 bp at the right end of the *Er-1* and *Di-3* elements show 96.4% identity without gaps. Within each long inverted terminal repeat two 18-bp direct repeats are present: the outer direct repeat starts 8 bp from the end of the element and the inner one terminates the inverted repeat. This structure, called IR-DR, is also found in other *Tc1*-like transposable elements of invertebrates (Franz and Savakis 1991; Merriman *et al.* 1995; Petrov *et al.* 1995) as well as in *Tc1*-like transposable elements from some fishes (Radice *et al.* 1994; Izsvàk *et al.* 1995).

Table 3 lists all the invertebrate *Tc1*-like transposases which have been compared to the reconstructed transposase of *D. diplacantha* and *D. erecta* elements. While the overall homology of the ORFs strongly relates *Er-1* and *Di-3* to *Bari-1*, the structure of their terminal repeats is more related to other *Tc1*-like elements than to *Bari-1*. However, when the 26-bp inverted repeat of *Bari-1* is compared with the *Er-1* 26-bp terminal sequence comprising the terminal 8 bp of the element plus the 18-

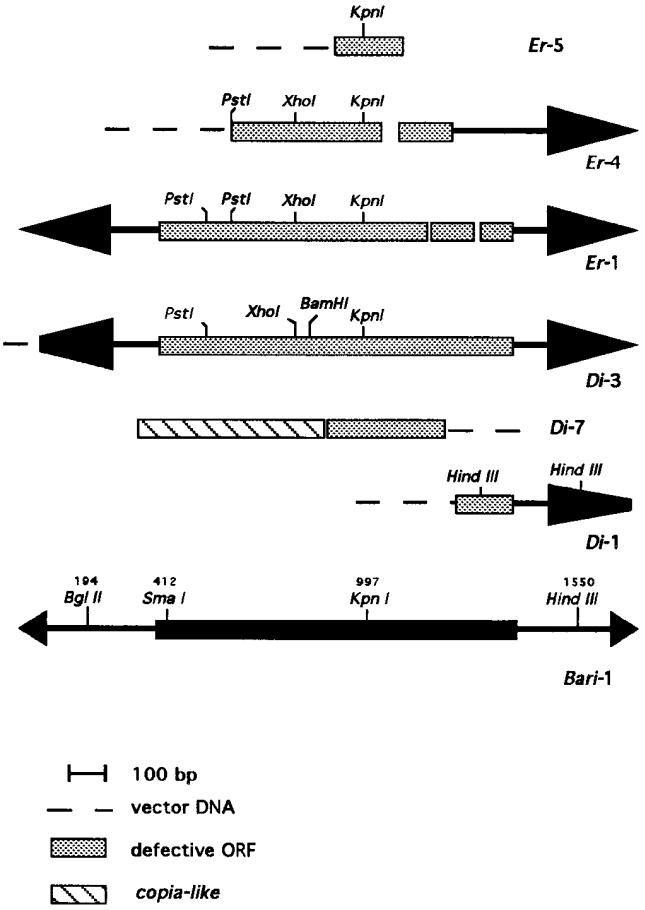

Figure 6.—Structure of *Bari-1* elements of *D. erecta* and *D. diplacantha*. The physical maps of *Bari-1* elements isolated from *D. erecta* (*Er-1*, *Er-4*, *Er-5*) and from *D. diplacatha* (*Di-1*, *Di-3*, *Di-7*) are compared with the restriction map of the *D. melanogaster Bari-1* element (bottom line). The structure of the long inverted terminal repeat (large arrows) and the presence of defective transposases (gray boxes) in *D. erecta* and *D. diplacantha* clones were revealed by DNA sequencing.

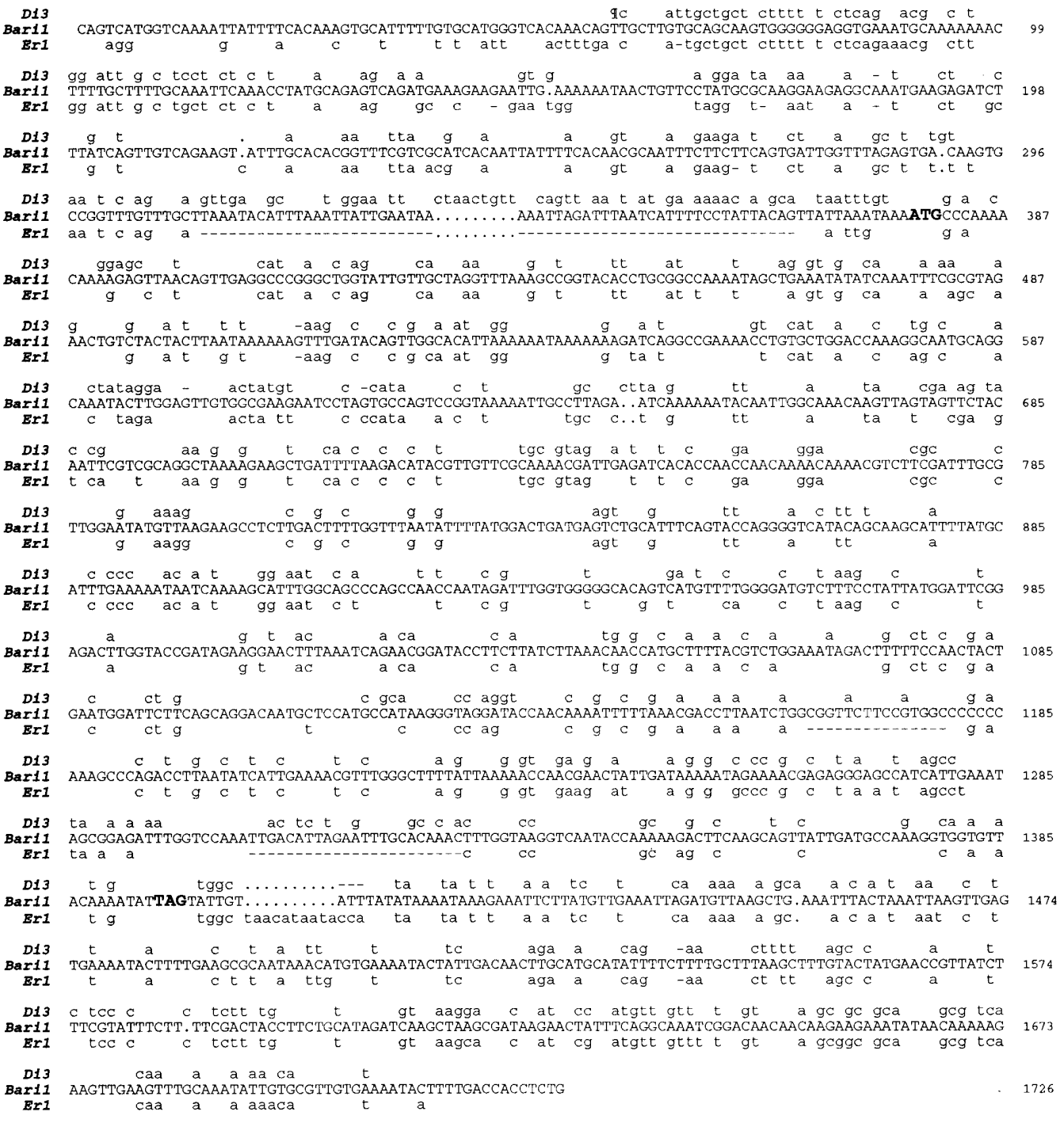

Figure 7.—Comparison of the *Bari-1 D. melanogaster* sequence with its homologues from *D. diplacantha* and *D. erecta*. The complete DNA sequences of *Di-3* (AC Y13852) and *Er-1* (AC Y13853) clones are aligned to the standard *D.mel* 47D sequence. The differing nucleotides are shown in lower case letters. (—) Deletion; (.) introduced to optimize the alignments. The start and stop codon of the *Bari-1* standard transposase are boldfaced. (¶) A cloning site.

bp outer direct repeat, a strong similarity is found. Moreover, the last eight nucleotides of the outer direct repeat are identical to the corresponding nucleotides of the *S* elements. The relationships among terminal sequences are shown in Figure 8.

Copy number and distribution of *Bari-1* related elements in *D. erecta* and *D. diplacantha*: We have estimated the copy number of *Bari-1*-like elements in *D. erecta* and *D. diplacantha* by Southern hybridization of genomic DNAs probed under stringent conditions with the 380-bp *Pst*I-*Kpn*I internal fragment of the *Er-1* element. Within this region *Er-1* and *Di-3* share 96% nucleotide identity. From the number of hybridization bands we estimate that 12–15 copies of the element are present

TABLE 2

Percent similarities among *D. erecta* and *D. diplacantha* elements

	Er-5	*Er-4*	*Er-1*	*Di-3*	*Di-1*	*Di-7*	*Bari-1*
Er-5	100	94.7	95.4	93.3	N.A.	72.6	74.8
Er-4		100	94.6	92.5	69.3	74.3	68.3
Er-1			100	94.9	66.0	74.0	68.0
Di-3				100	65.6	72.8	69.5
Di-1					100	N.A.	87.2
Di-7						100	89.5
Bari-1							100

The similarities were computed by the Bestfit program of the GCG software package. N.A., not applicable because the sequences do not overlap.

in *D. diplacantha* and 18–25 in *D. erecta* (Figure 9A). Furthermore, by *in situ* hybridization experiments in *D. erecta*, the elements appear to be located in the pericentromeric heterochromatin and/or the Y chromosome. Heavy labeling sites are also present on chromosome *4* (Figure 9B).

DISCUSSION

***Bari-1*-like elements in species of the melanogaster group:** Within the melanogaster species group *Bari-1*-like elements show several interesting features. First, the elements are widely distributed in the Sophopora subgenus (Figure 2), with only a few apparent cases of taxonomic discontinuity. Second, different copies of the element are extremely similar, at least within the species of the melanogaster complex. All *Bari-1* copies so far isolated from *D. melanogaster*, *D. simulans*, *D. mauritiana*, and *D. sechellia* are similar in size and physical map. Third, sequences isolated from *D. erecta* and *D. diplacantha*, two species phylogenetically quite distant from the melanogaster complex, are clearly related to the melanogaster *Bari-1* elements. However, their terminal repeats have a different organization.

The taxonomic distribution of many families of transposable elements can be explained by vertical transmission because the phylogeny of the transposon closely follows the phylogeny of the host species (see, for example, Maruyama and Hartl 1991; Radice *et al.* 1994). However, both vertical and horizontal transmission appear to have contributed to the distribution of some families of mobile elements (see, for example, Abad *et al.* 1991; Robertson 1993; Lohe *et al.* 1995). The wide distribution of *Bari-1*-like elements in the Sophophora subgenus could simply indicate that they have an ancient origin and have been able to remain in the genome of this group of organisms for a long time.

Our data indicate that in Drosophila two subclasses of *Bari-1*-like elements exist, strongly related by sequence similarity but differing by the structure of their terminal

TABLE 3

Comparison of the *D. diplacantha Di-3* element to other *Tc1*-like elements

Element	Accession no.	Species	Size (bp)	Length of IR	Presence of IR-DR structure	Length of ORF protein	Amino acid similarity	Amino acid identity
Di-3	Y13852	*D.diplacantha*	1732[a]	254	Yes	338[b]	1.000	1.000
Er-1	Y13853	*D.erecta*	1639	253	Yes	325[b]	0.910	0.866
Bari-1	X67681	*D.melanogaster*	1726	27	No	339	0.801	0.671
S (pS3)	U33463	*D.melanogaster*	1736	234	Yes	345	0.486	0.320
Quatzal	L76231	*A.albimanus*	1680	236	?[c]	341	0.465	0.320
Uhu	X63029	*D.heteroneura*	1646	46	No	335[d]	0.534	0.305
Minos2	Z29098	*D.hydei*	1773	255	Yes	361	0.491	0.305
Paris	U26938	*D.virilis*	1730	242	Yes	348	0.496	0.304
Tc1	X01005	*C.elegans*	1611	54	No	343[e]	0.475	0.268
HB1	X01748	*D.melanogaster*	1643	27	No	320[f]	0.476	0.247

[a] First 56 nucleotides deduced from comparison of left and right inverted repeats.
[b] Protein deducted after correction of frame shifts and stop codons.
[c] Presence of direct repeats within the long inverted repeats but with different properties.
[d] Protein deduced by Brezinsky *et al.* (1990).
[e] Based on the cDNA identified by Vos *et al.* (1993).
[f] Protein deduced by Henikoff and Plasterk (1988).

repeats. The IR-DR structure is found in the subclass represented by the *Er-1* and *Di-3* elements and the simple IR structure is found in the subclass represented by *D. melanogaster* elements. Similar observations have been reported for fish *Tc1*-like elements (Radice *et al.* 1994; Izsvàk *et al.* 1995). However, in several fish species both types of elements coexist and IR-DR elements from different species are more similar than elements with simple IR termini within a species. Subfamilies of the *mariner* element with independent evolutionary histories have been also reported (Robertson and Lampe 1993; Lohe *et al.* 1995). Peculiar to the *Bari-1*-like elements of Drosophila, however, is the higher level of nucleotide similarity between IR-DR and non IR-DR elements. Moreover, no Drosophila species has as yet been identified possessing elements of both *Bari-1*-like subclasses. Assuming that IR-DR *Bari-1*-like elements were present in an ancestor of the melanogaster subgroup, then they must have been lost in the lineage leading to the melanogaster complex, presumably by a stochastic process (Lohe *et al.* 1995), while being maintained in *D. erecta* and *D. diplacantha*.

The *Bari-1* elements that lack IR-DR are present only in the melanogaster species complex. Thus, either they were not present in the ancestor of the melanogaster subgroup or they were lost in the erecta-orena and yakuba-teissieri lineages. Their existence in all strains of *D. melanogaster*, *D. simulans*, *D. mauritiana*, and *D. sechellia* suggests that they were present before these species split. However, DNA sequencing reveals a low rate of divergence among the elements in the melanogaster complex. Repeated invasion of *Bari-1* in the common ancestor of the simulans/mauritiana/sechellia lineage and in the ancestor of the melanogaster lineage could explain the low observed level of divergence. Interestingly, the rate of divergence for *Bari-1* elements falls in the range reported for *mariner* elements in the simulans complex (Maruyama and Hartl 1991; Capy *et al.* 1992). Phylogenetic results suggest that the mauritiana subfamily of *mariner* was probably present before the melanogaster subgroup diverged and was then lost in some lineages. Amplification of a few ancestral elements is thought to be responsible for the sequence homogeneity. We cannot exclude that in the melanogaster complex *Bari-1* had a rapid amplification similar to the *mariner* elements.

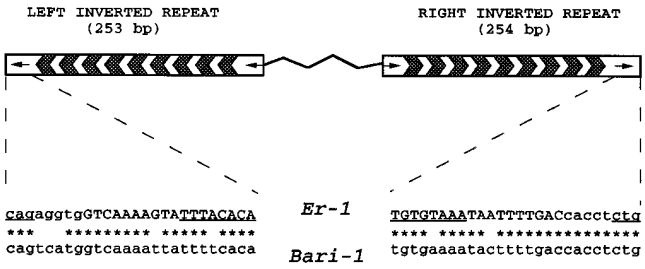

Figure 8.—Comparison of terminal repeats of *Bari-1* and its *Er-1 D. erecta* homologue. The upper diagram represents the long inverted repeats at both ends of the *Er-1* element. The small arrows within each box represent the outer and inner direct repeats. The terminal 26 nucleotides of *Er-1* and *Bari-1* are compared to show the high level of similarity. Identical nucleotides are shown by asterisks. The 18-bp sequences of the outer direct repeats of *Er-1* are in uppercase letters. The underlined nucleotides perfectly match the corresponding nucleotides in the terminal repeats of the *S* element.

Relationship between the *Bari-1* inverted repeats and the inverted repeats of *Er-1* and *Di-3*: Although strongly related to *Bari-1* by sequence similarity, the *Er-1* and *Di-3* elements are surprisingly different from this element in the structure of their terminal repeats. *Bari-1* possesses short 26-bp inverted repeats while *Er-1* and *Di-3* possess a terminal IR-DR structure. This type of structure is present in many families of *Tc1*-like elements, but it is likely not to be an essential feature since it is not found in other members of the *Tc1* superfamily (Ivics *et al.* 1996). As shown in Figure 8, the terminal 26 bp of *Er-1*, which include the 18-bp outer direct repeats of the IR-DR structure, are almost identical to the terminal 26 bp of *Bari-1*, suggesting that the 26 bp *Bari-1* sequence originated from the *Er-1* IR-DR structure. The loss of the long inverted repeat in *Bari-1* can-

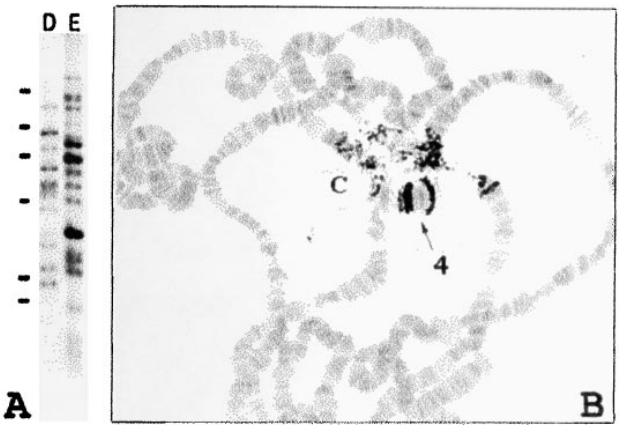

Figure 9.—Copy number and *in situ* hybridization of *D. erecta* elements. (A) Southern blot of genomic DNAs from *D. diplacantha* (D) and *D. erecta* (E) digested with *Pst*I and probed with the O.35-kb *Pst*I/*Kpn*I internal fragment of *Er-1* clone. Bars on the left represent the position of λ *Hin*dIII marker fragments. (B) Salivary glands chromosomes of *D. erecta* showing the hybridization of the same probe used in A over chromocenter (C) and on the fourth chromosome (4).

not be explained simply by an unequal recombination event between the direct repeats within each long inverted repeat because the product of such an event would be about 450 bp shorter than the original element; however, *Er-1* and *Bari-1* are similar in length.

At present it is not clear why two kinds of termini exist in the *Tc1*-like superfamily nor what mechanisms are responsible for maintaining the inverted repeats in the transposon (for review see Plasterk 1996). The sequences of the direct repeats in IR-DR *Tc1*-like elements from very distant species are clearly related, while the surrounding sequences diverge substantially (Izsvàk *et al.* 1995; Merriman *et al.* 1995). The DR motifs represent the binding site of the transposase (Vos *et al.* 1993; Ivics *et al.* 1997) and in *Tc3*, which has an internal repeat in a different position with respect to the IR-DR *Tc1* subclass, the removal of the internal DR binding site does not reduce the rate of transposition (Colloms *et al.* 1994). This suggests that the internal repeats do not have a functional significance directly linked to the mobility of the element, but they could have regulating purposes (Ivics *et al.* 1997).

The relationship between the defective elements of *D. erecta* and the homogeneous elements of the melanogaster complex: All elements as yet isolated from *D. erecta* and *D. diplacantha* are defective. Moreover, the 18–25 copies in the *D. erecta* genome are localized either in the chromocenter or on chromosome *4*, which, by analogy with the *D. melanogaster* chromosome *4*, can be considered heterochromatic (Miklos *et al.* 1988). Thus, it is reasonable to suppose that the *D. erecta Bari-1* homologues are the evolutionary relics of an autonomous ancestor possessing IR-DR terminal structures. The lack of any relics of the element in euchromatic regions of the genome may be the result of loss by genetic drift or elimination by natural selection (reviewed in Charlesworth *et al.* 1994). The defective heterochromatic elements are unlikely to have been present before the evolutionary splitting of the melanogaster subgroup, since they are not found in any species of the subgroup except *D. erecta*. According to the model proposed by Hartl *et al.* (1997) the dynamics of transposon mobility and mainteinance within a host genome lead inevitably to loss of the element, whose survival is only possible by invasion of a new host. We suggest that the transposition machinery of a primitive IR-DR mobile element was error-prone in *D. erecta*, and possibly also in *D. diplacantha*, due, for example, to the inability of the transposase to recognize the termini of the element correctly because of identical direct repeats present within each long inverted repeat. From an evolutionary standpoint, therefore, survival of the transposon required either modification of the transposase so as to acquire more specificity, or modification of the sequence responsible for the transposase's error-propensity. This second strategy may have been responsible for the generation of *Bari-1*-like elements that lack terminal IR-DR.

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