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Genetics

Transposable and nontransposable elements similar to the I factor involved in inducer–reactive (IR) hybrid dysgenesis in *Drosophila melanogaster* coexist in various *Drosophila* species

(evolution/vestiges of transposable elements/*melanogaster* group)

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ABSTRACT **The I factor is a transposable element controlling inducer–reactive (IR) hybrid dysgenesis in *Drosophila melanogaster*, which occurs when males from the class of inducer strains are crossed with females from the class of reactive strains. Inducer strains contain several copies of the complete 5.4-kilobase (kb) I factor at various sites on the chromosomal arms; reactive strains contain no complete I factor. Incomplete and defective I elements occur at constant locations in pericentromeric heterochromatin of both types of strains. The 5.4-kb I factors transpose, whereas incomplete I elements do not transpose. The constant location of defective I elements in all strains indicates that they were in the genome before the spread of *D. melanogaster* throughout the world. Sequences homologous to I occur in other *Drosophila* species, and their distribution correlates with the phylogenetic relationships between species. We have studied the organization of I homologues in *Drosophila simulans* and *Drosophila teissieri*. These species seem to contain both transposable I elements, even though their structure may differ from that of the 5.4-kb I factors of the inducer strains of *D. melanogaster*, and nontransposable I elements, which are always at the same place in the genome when different stocks of the same species are compared. These results suggest that both mobile and nonmobile I elements are very old components of the Drosophilidae genome.**

Dispersed moderately repetitive sequences represent about 10% of the *Drosophila* genome (1). Many of them are mobile elements that probably play a role in genomic evolution and could be involved in speciation. Little is known about their origin and evolutionary history. Sequences homologous to transposable elements of *Drosophila melanogaster* occur in other species. The study of their distribution in various species indicates that some of them have appeared or disappeared during the diversification of the Drosophilidae (2–4). This unusual behavior raises the question of their formation, spread, and maintenance in wild populations. A mobile-element family might originate in a species by either horizontal transmission from another species, or by rearrangements of existing sequences.

Among the various mobile-element families known in *D. melanogaster*, two have particular status—the I- and P-element families. They possess properties allowing the integration of molecular, population, and evolutionary studies. Mobilization of these elements results in a set of phenotypic traits named hybrid dysgenesis (5).

Inducer–reactive (IR) hybrid dysgenesis is controlled by transposable elements called I factors (6). All *D. melanogaster* strains belong to one of these two categories: (*i*) inducer, which possess complete and active I factors, and (*ii*) reactive, which do not contain complete I factors. The phenomenon appears in the progeny of crosses between females from reactive strains and males from inducer strains. Various genetic abnormalities occur in the germ line of F_1 females from such crosses (6, 7). Inducer strains contain about 15 copies of the complete 5.4-kilobase (kb) I factor dispersed on the chromosomal arms (8); the factors are stable in these strains but are affected by hybrid dysgenesis and transpose with unusually high frequencies in the germ line of dysgenic females (9). Active I factors have a particular structure (10) with no terminal repeats, but they are terminated at their 3′ end by a number (4–7) of TAA triplets. They possess two long open reading frames, one of them encoding a polypeptide with similarity to reverse transcriptases. So, I factors share structural similarities with the F elements of *D. melanogaster* (11) and with the L1 family of transposable elements that are repeated several thousand times in mammalian genomes (12, 13). They are thought to transpose by reverse transcription of an RNA intermediate (10).

In situ hybridization to the salivary gland chromosomes and Southern transfer experiments have shown that incomplete and nontransposable I elements are present in the pericentromeric regions of both categories of strains. Most of these incomplete and defective I elements are at constant locations in both classes of strains, suggesting that they are old components of the genome (8). Other studies indicate that the complete I factor appeared in the 1930s and then invaded natural populations of *D. melanogaster* (14, 15). So, all natural populations are now inducer, and reactive strains come from flies caught in the wild before invasion of the I factor. To investigate the evolutionary origin of this element, various *Drosophila* species have been studied for sequences homologous to I factor. Such sequences are found in many species, and their distribution correlates with the phylogenetic relationships between species (16). This indicates that I elements are old components of the *Drosophila* genome. Further analysis has shown that I elements with a structure strikingly similar to that of the complete I factor occur in the species most closely related to *D. melanogaster*—i.e., *Drosophila simulans*, *Drosophila mauritiana*, and *Drosophila sechellia* (16).

To learn more about the evolutionary history of the I factor and to study relationships between complete I factors and defective I elements, we designed experiments with two species, *D. simulans* and *D. teissieri* that contain I homologues. *D. melanogaster, D. simulans*, and *D. teissieri* are three of eight closely related species constituting the *mela-*

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Abbreviation: IR, inducer–reactive.

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nogaster subgroup (17). *D. simulans* is more closely related to *D. melanogaster* than is *D. teissieri* (18).

MATERIALS AND METHODS

Bacterial Strains. Plasmids were propagated in *Escherichia coli* strains HB101 (19) or NM522 (20), and λ bacteriophages were grown in *E. coli* strains C600 (21) or Q359 (22).

***Drosophila* Strains.** *D. melanogaster* stocks were reactive and inducer strains maintained in our laboratory. *D. simulans* strains were from the Bowling Green (Ohio) and Umea (Sweden) stock centers, the Laboratoire de Génétique Evolutive of the Centre National de la Recherche Scientifique (CNRS; Gif-sur-Yvette, France), and the Laboratoire de Génétique des Populations, University of Paris VI (France). Many were derived from flies caught in the wild during the past 20 years. *D. teissieri* stocks came from the Laboratoire de Génétique Evolutive of the CNRS at Gif-sur-Yvette and were derived from flies recently caught in Central Africa.

Enzymes and Isotopes. Enzymes were purchased from various companies and used as recommended by the manufacturers. [α-^{32}P]dATP and [^{3}H]dTTP were purchased from Amersham.

DNA Preparation. Plasmid, phage, and *Drosophila* DNAs were prepared as previously described (8, 23, 24).

Agarose Gel Electrophoresis. Horizontal slab gels were run in 40 mM Tris/20 mM sodium acetate/1 mM EDTA, pH 8.2, at about 1 V/cm. DNA was transferred to nitrocellulose filters by a modification (25) of the method of Southern (26).

***In Vitro* Labeling of DNA, Hybridization, and Autoradiography.** These procedures were done as described (8).

Construction of a Library of *D. teissieri*. A random library of the *D. teissieri* strain 128.2 was constructed in phage λEMBL4 (27) as follows. Four micrograms of λEMBL4 DNA was digested with both *Bam*HI and *Sal* I, and the resulting polylinker fragments were eliminated by precipitating them twice with ethanol. This vector DNA was dissolved, together with 1 μg of 15- to 25-kb-selected fragments produced by partial digestion of genomic DNA with *Sau*3A in 100 μl of 100 mM Tris·HCl, pH 7.2/10 mM EDTA/100 mM $MgCl_2$/100 mM dithiothreitol/10 mM ATP. Ligation was done overnight at 10°C in this buffer. After ethanol precipitation, ligated DNA was packaged *in vitro* (28), and the resulting phages were plated on *E. coli* strains Q359 and C600.

RESULTS

D. simulans. I elements of *D. simulans* have a structure strikingly similar to that of the complete I factors of the inducer strains of *D. melanogaster*. Indeed, Southern blot experiments that have been reported (16) indicate that internal restriction fragments of the I factor are present in several copies in the genome of *D. simulans*. The only noticeable difference is an additional *Hin*dIII restriction site in some I elements of this species. However, we had previously analyzed only one stock of *D. simulans*, and whether all stocks of this species were identical, or two categories of strains like inducer and reactive of *D. melanogaster* existed, was unanswered. Inducer and reactive strains can be distinguished by the genomic presence or absence of particular internal fragments of I, such as the 2.3-kb *Hin*dIII–*Pst* I or 4.1-kb *Ava* I–*Pst* I fragments (8, 16) (see Fig. 1). In the first experimental series we looked for these fragments in DNA from 19 *D. simulans* strains, most of which were recently isolated from natural populations in different parts of the world.

DNAs from these stocks and from reactive and inducer strains of *D. melanogaster* were digested with *Ava* I and *Pst* I and hybridized with clone pI901, which contains the large 4.1-kb *Ava* I–*Pst* I internal fragment of the I factor. The

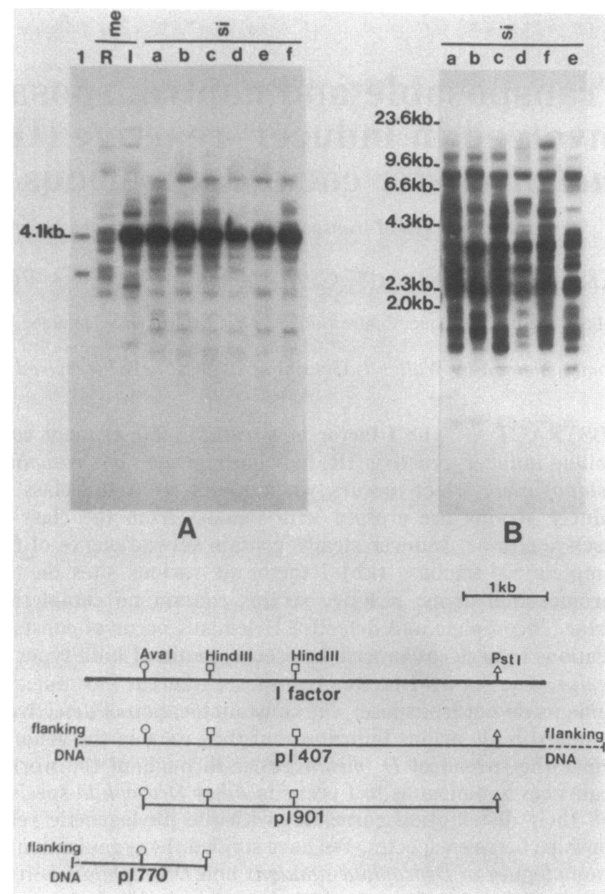

FIG. 1. Hybridization of two digests of DNAs from various stocks of *D. simulans* with two different parts of the I factor. Four micrograms of DNA of each *D. melanogaster* and *D. simulans* strain was digested with appropriate restriction enzymes, electrophoresed on 1% agarose gels, transferred to nitrocellulose filters, and hybridized with probes corresponding to various parts of the I factor. (*A*) *Ava* I–*Pst* I digests probed with pI901. (*B*) *Hin*dIII digests probed with pI770. R, reactive strain; I, inducer strain; me, *D. melanogaster*; si, *D. simulans*; lanes a, b, c, d, e, and f, different stocks of *D. simulans*. Restriction maps of the I factor and of clones bearing different parts of I factor that were used as probes are given below. Flanking DNA in pI407 and pI770 is from the *white* gene. Lane 1 contains an *Ava* I–*Pst* I digest of pI901 in amount equivalent to about two copies of the 4.1-kb *Ava* I–*Pst* I fragment per haploid genome.

results, shown in Fig. 1*A*, indicate that this large *Ava* I–*Pst* I fragment, which is not found in the reactive strains of *D. melanogaster*, is present in several copies (10–15) per haploid genome in all stocks of *D. simulans* as in the inducer strains of *D. melanogaster*. Because the same result is seen for the 19 stocks studied, most strains in this species probably possess I elements similar to the complete I factor. Thus, all these stocks belong to the same class of strains equivalent to the inducer category.

A similar experiment looked for the 2.3-kb *Hin*dIII–*Pst* I internal fragment of I factor. This fragment occurs in many copies per haploid genome in inducer strains of *D. melanogaster* but is absent in reactive strains. The experimental probe was a plasmid containing the 2.3-kb *Hin*dIII–*Pst* I fragment cloned from a *D. melanogaster* I factor. All stocks of *D. simulans* possess a 2.3-kb *Hin*dIII–*Pst* I fragment in several copies per haploid genome, as well as two smaller fragments of 1.7- and 0.6-kb, which hybridize strongly to the probe (data not shown). As described (16), these two small fragments result from the presence in some elements of an additional *Hin*dIII restriction site within the *Hin*dIII–*Pst* I

fragment. So, two types of I elements very similar to the complete I factor coexist in *D. simulans*, and this polymorphism is found in all stocks studied.

Inducer strains of *D. melanogaster* are characterized by active I factors able to transpose and inserted at various locations in different stocks. We were therefore curious whether I elements of *D. simulans*, which have a structure similar to the complete and mobile I factors of *D. melanogaster*, are also transposable. To answer this question, DNAs of several stocks of *D. simulans* were digested with *Hin*dIII and probed with a clone corresponding to the left part of the I factor, pl770 (see Fig. 1). Fig. 1*B* shows that many bands comigrate in all stocks; some bands probably correspond to internal fragments of I elements, but others correspond to I sequences localized at similar positions in the genomes of different stocks, suggesting that they are not transposable. Such immobile I elements are found in both inducer and reactive strains of *D. melanogaster*; they are at constant locations in the genome of all the stocks of this species, and therefore are thought to be old components of the genome. As the same is true in *D. simulans*, these defective I elements can be imagined as present in the genome before the divergence between *D. simulans* and *D. melanogaster*.

The results presented in Fig. 1*B* also show that some additional bands are specific to each stock, bands which could correspond to I elements localized at different positions in the genome of the different stocks. This suggests that some I elements in *D. simulans* can transpose. Further, we compared distribution of I elements in two stocks of *D. simulans* by *in situ* hybridization to salivary gland chromosomes of the larvae, using clone pI407 (see Fig. 1) as probe. Results (Fig. 4) show that in both stocks, I homologues are found in the pericentromeric regions and chromosomal arms and that number (12–16) and location of insertion sites on the chromosomal arms differ between these two stocks—again suggesting strongly that these I elements can transpose. These results indicate that all stocks of *D. simulans* used resemble the inducer strains of *D. melanogaster*. Indeed, they all contain I elements with a structure strikingly similar to that of the complete I factor, and these elements seem mobile.

***D. teissieri*.** We also studied the I elements of *D. teissieri*, another species of the *melanogaster* subgroup more distant from *D. melanogaster* than *D. simulans*. From previous experiments (16), the structure of I homologues in *D. teissieri* appears to differ from that of the I factor of *D. melanogaster* because most internal restriction fragments of the I factor are not found in the *D. teissieri* genome. Therefore, we analyzed these I elements in more detail: we studied their structure and tried to determine whether they were defective I sequences similar to those of the reactive strains of *D. melanogaster*, or if they were active elements able to transpose.

A random library of the *D. teissieri* 128.2 stock was therefore made in the bacteriophage λEMBL4, and it was screened with clone pI407 as probe. This plasmid contains the complete I factor of *D. melanogaster* (see Fig. 1). Six clones hybridizing strongly with the probe were selected for detailed analysis. Because two of these clones were not organized similarly to the genome of *D. teissieri* 128.2 strain, they were not further analyzed (da a not shown). This result was not surprising because rearranged clones are frequently obtained during cloning of I elements (8).

Fig. 2 shows the four other clones. Restriction maps of the cloned I sequences differ from each other and from that of the I factor of *D. melanogaster*. Only two restriction fragments of phage λIT190 are identical to those of the I factor on the criteria of comigration and cross-hybridization; these fragments are the 1-kb *Hin*dIII fragment corresponding to the internal part of I and the 0.66-kb *Acc* I–*Hin*dIII fragment localized to the right of the previous one.

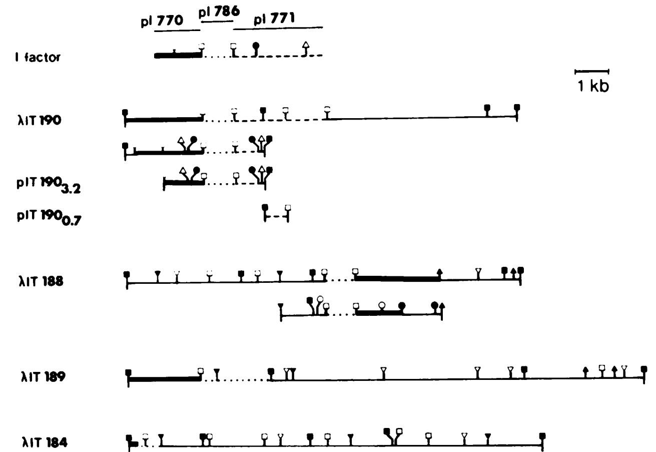

FIG. 2. Restriction maps of four cloned I elements from *D. teissieri*. At top is indicated the parts of the I factor cloned in plasmids pI770, pI786, and pI771. Homologies are indicated by thick lines (to pI770), dotted lines (to pI786), and broken lines (to pI771). *Eco*RI sites at the phage borders are from λEMBL4 (phage arms are not represented). Fragments of λIT190 and λIT188 subcloned in vector pUC13 are presented under the corresponding phages. ●, *Acc* I; ○, *Ava* I; ▲, *Bam*HI; ■, *Eco*RI; □, *Hin*dIII; △, *Pst* I; ▽, *Sal* I; and ▼, *Xho* I.

Moreover, these four I elements are associated with other repeated sequences as inferred by hybridization of various digests to total genomic DNA of the *D. teissieri* 128.2 stock (data not shown). This suggests that these I elements resemble those in the genome of the reactive strains of *D. melanogaster*, which are defective and localized in the pericentromeric heterochromatin that is mainly constituted of repeated sequences. To study organization of I elements in the genome of *D. teissieri*, we subcloned in plasmid pUC13 the 3.2-kb *Ava* I–*Eco*RI and 0.7-kb *Eco*RI–*Hin*dIII fragments of λIT190, which are two internal fragments of I. These two subclones were respectively called pIT190$_{3.2}$ and pIT190$_{0.7}$ (Fig. 2). They were used to probe digests of DNAs from different stocks. Results are given in Fig. 3.

In Fig. 3*A*, DNAs of nine stocks of *D. teissieri* were digested with *Ava* I and *Eco*RI and hybridized with clone pIT190$_{3.2}$. In all stocks, a 3.2-kb fragment comigrates and hybridizes strongly with the 3.2-kb *Ava* I–*Eco*RI fragment of the I element cloned in IT190. The intensity of hybridization corresponds to several copies of the fragment per haploid genome. The 3.2-kb *Ava* I–*Eco*RI fragment is evidently conserved in several I elements in the different stocks studied.

In another experiment, DNAs of the same nine stocks were digested with *Hin*dIII and hybridized with both clones pIT190$_{3.2}$ and pIT190$_{0.7}$. Results (Fig. 3*B*) show several bands that comigrate. Most of them probably correspond to nontransposable and identical I sequences located at the same position in the genome of all nine stocks. But the autoradiograms also show some other bands specific for each *D. teissieri* stock and that could correspond to I elements having recently transposed.

To confirm the presence of mobile I elements in the genome of *D. teissieri*, *in situ* hybridization experiments to the salivary gland chromosomes of the larvae were done with two different stocks. Results, shown in Fig. 4, indicate that both *D. teissieri* stocks contain I sequences in the pericentromeric regions and chromosomal arms. The number (2–4) and location of the insertion sites on the chromosomal arms differ between the two stocks, suggesting transposition by some of these elements.

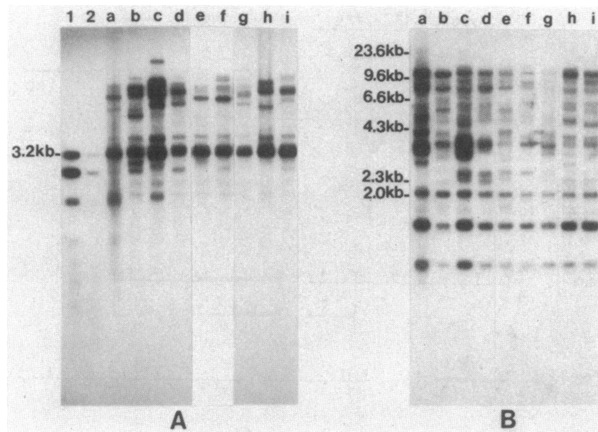

FIG. 3. Hybridization of two digests of DNAs from various *D. teissieri* stocks with two internal fragments of an I element of this species. Four micrograms of genomic DNAs from nine stocks of *D. teissieri* was digested with restriction enzymes and electrophoresed on 1% agarose gels. Fragments were transferred to nitrocellulose filters and hybridized with ^{32}P-labeled pIT190$_{3.2}$ and/or pIT190$_{0.7}$ (see Fig. 2). (*A*) *Ava* I-*Eco*RI digests probed with pIT190$_{3.2}$. (*B*) *Hin*dIII digests probed with a mixture of pIT190$_{3.2}$ and pIT190$_{0.7}$. Lanes a–i, different stocks of *D. teissieri*. Lanes 1 and 2 were loaded with *Bam*HI-*Eco*RI digests of pIT190$_{3.2}$ in amounts equivalent, respectively, to five and one copies of the 3.2-kb *Ava* I-*Eco*RI fragment per haploid genome (*Bam*HI is a restriction site of the polylinker of pUC13, in which the fragment was subcloned and is therefore 5 base pairs distant from the *Ava* I site).

Therefore, distribution of I elements in the genome shows that these nine stocks could be similar to inducer strains of *D. melanogaster*, because hybridization of I factor DNA in reactive strains is restricted to pericentromeric heterochromatin. Moreover, results suggest that some I elements of *D. teissieri* are mobile, although their structure differs from that of the active I factor of *D. melanogaster*.

DISCUSSION

These results indicate that probably all species analyzed thus far contain both transposable and nontransposable I elements.

Defective I sequences occur in all the species we studied (*D. melanogaster, D. simulans*, and *D. teissieri*). Indeed, Southern blot experiments show a background of bands common for all stocks within the same species. This result is also true in *Drosophila ananassae*, which belongs to another subgroup of species (unpublished results). These bands correspond to similar I sequences lying at constant locations in the genome of all strains and which therefore are probably nontransposable.

In situ hybridization experiments to salivary gland chromosomes of the reactive strains of *D. melanogaster* have shown that such defective and immobile I elements are located in the chromocenter of the nuclei (8). Similar studies of *D. simulans* and *D. teissieri* show that these species also contain I homologues in the pericentromeric regions, and the same results have been obtained with *D. mauritiana* (16). Therefore, as in reactive strains of *D. melanogaster*, most I sequences found in the chromocenter of these species could be defective and nontransposable, the hybridization sites seen on the chromosomal arms corresponding mainly to mobile elements.

Identical defective I elements at the same place in the genome of all strains within species indicates that these elements were present in the population from which the species originated. As such is true for all species that we studied, nontransposable I elements are suggested to have been in the genome before divergence between these species; thus, they are very old genomic components.

The other important finding from these experiments is that active and mobile I elements are not restricted to inducer strains of *D. melanogaster*.

Results from *D. simulans* strongly suggest that this species contains, in addition to defective I elements, I sequences

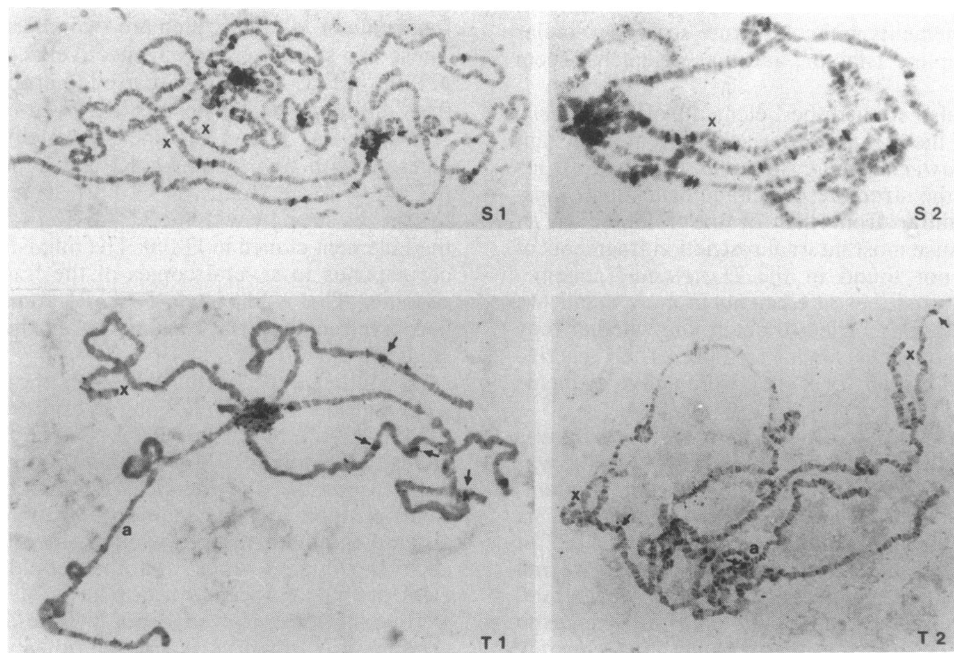

FIG. 4. *In situ* hybridization of I elements to polytene chromosomes of various stocks of *D. simulans* (S1, S2) and *D. teissieri* (T1, T2). Squashes and hybridization were done as previously reported (8). Probes were pI407 (see Fig. 1) for *D. simulans* and a mixture of pIT190$_{3.2}$ and pIT190$_{0.7}$ (see Fig. 2) for *D. teissieri*. The large interstrain variation of the insertion sites of the I elements in *D. simulans* is apparent on the X chromosomes (marked X) in S1 and S2. X chromosomes of strain S1 contain only two euchromatic I elements, which are located more distal than the five I elements of strain S2. Arrows point to the location of the six I element-insertion sites scored in *D. teissieri*. None of them is shared by both strains; the two euchromatic I elements of strain T2 are carried by two chromosomal arms marked X and a, which are unlabeled in strain T1.

able to transpose, as their locations differ from strain to strain. Structure of these mobile I elements, according to their restriction map, is strikingly similar to that of the complete I factors of *D. melanogaster*, except that some have an additional *Hin*dIII restriction site. All stocks of *D. simulans* studied contain a mixture of the two types of I elements, with and without this additional restriction site. So, these stocks all look like the inducer strains of *D. melanogaster*: they possess transposable I elements, the structure, copy number (10–25), and genomic distribution of which are similar to those of the I factors in the inducer strains. We have yet to find in this species a stock that would be similar to the reactive strains. However, note that the active I factors invaded *D. melanogaster* populations after 1930 (15) and that most of the *D. simulans* stocks used here were derived from flies caught in the wild after this date.

Our results also provide evidence for active and transposable I elements in *D. teissieri*. Indeed, I sequences are not restricted to the pericentromeric heterochromatin but are also distributed on the chromosomal arms. Their location on the arms of the chromosomes differs from strain to strain, as would be expected of transposable elements. In contrast to *D. simulans*, the structure of the mobile I elements of *D. teissieri* differs from that of the active I factors of *D. melanogaster*, according to homology and restriction maps. Most internal restriction fragments of the I factor are absent from the genome of *D. teissieri*; the only ones identified were the 1-kb *Hin*dIII central fragment of I and a small fragment next to it (see Fig. 2). Thus, I elements showing a sequence different from that of the active I factors of *D. melanogaster* may transpose. Determination of the most conserved parts of the elements between the two species should be interesting.

Another point is that the analyzed stocks of *D. teissieri* seem to belong to only one class of strains, equivalent to the inducer state in *D. melanogaster*, because they have transposable I elements. Moreover, they all contain a large 3.2-kb *Ava* I–*Eco*RI internal fragment of I repeated several times per haploid genome. This suggests that this fragment could be present in elements susceptible to replicative transposition.

Among the various species that have been thus far studied, the situation of the reactive strains of *D. melanogaster* appears rather exceptional, for these strains are presently the only stocks devoid of transposable I elements and containing only defective and immobile I sequences. In all other cases, the different stocks of the various species seem to have two types of I homologues: defective I sequences that are old components of the genomes, and active and mobile I elements.

These data could be interpreted using the hypothesis that transposable elements like I were subjected to cycles during evolution, a functional mobile element successively undergoing mutational inactivation and then reactivation.

Invasion of natural populations by transposable elements seems more frequent than expected; many results strongly suggest that natural populations of *D. melanogaster* were reactive before 1930 and that the I factor progressively invaded them after this date (15); all of them are now inducers. The same is probably true of the P factor involved in PM hybrid dysgenesis, which invaded populations after 1950 (15).

Therefore, the defective I elements in the genome of all species studied could result from inactivation of a transposable element in some ancestor. This active element, or another one which would be related, would have also been maintained in the species. The differences in the structural organization of the elements in various species, as seen, for example, in *D. melanogaster* and *D. teissieri*, would then be the consequence of their divergence. In this hypothesis, the reactive strains would result from loss of this transposable I element before distribution of *D. melanogaster* throughout the world. Recently (about 1930), the I factor would have reinvaded the *D. melanogaster* genome, giving rise to the inducer strains. The I factor could have originated by horizontal transfer from another species—for example, *D. simulans*, which contains I elements similar to the functional I factors of *D. melanogaster*. However, other more complex hypotheses could also explain our results.

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