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Properties of transgenic strains of *Drosophila melanogaster* containing I transposable elements from *Drosophila teissieri*

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Summary

I factors are transposable elements of *Drosophila melanogaster* similar to mammalian LINEs, that transpose by reverse transcription of an RNA intermediate and are responsible for the I–R system of hybrid dysgenesis. There are two categories of strains in this species: inducer, that contain about 15 I elements at the various sites on chromosomal arms, and reactive, that lack active I factors. I elements occur in various *Drosophila* species. Potentially functional I factors from *Drosophila teissieri* can transpose when introduced by P-element-mediated transformation in a reactive strain of *Drosophila melanogaster*. We have studied the properties of *Drosophila melanogaster* strains into which such an I factor from *Drosophila teissieri*, named *Itei*, was introduced. Typical hybrid dysgenesis is produced when males carrying *Itei* are crossed with reactive females. However, more than one copy of the element seems necessary to produce dysgenic traits, whereas only one I factor of *Drosophila melanogaster* seems to be sufficient. The copy number of *Itei* in transformed lines maintained by endogamous crosses increases rapidly and stabilizes at values similar to those observed in inducer strains. As *Drosophila teissieri* contains much fewer copies than the *Drosophila melanogaster* strains, this suggests that the copy number of I elements is not simply regulated by sequences present in the element itself.

1. Introduction

A large fraction of eukaryotic genomes is made up of transposable elements. Although their presence has many implications for genome organization and expression and in evolutionary processes, very little is known on the control of their maintenance in apparently almost all living organisms. The content of middle repetitive DNA sequences may vary considerably between closely related species, variations resulting mainly from differences in the copy number of mobile elements. For example, *Drosophila melanogaster* contains many more such sequences than the sibling species *Drosophila simulans* (Rubin, 1983). The mechanisms controlling the copy number of transposable elements are difficult to study because the conditions in which they transpose are unknown for most of them. I elements of *Drosophila melanogaster* are responsible for a hybrid dysgenesis system and are consequently interesting tools to study these mechanisms. Functional I elements can be introduced in the genome of strains that are usually devoid of such elements, and their evolution can be monitored, providing a good model to study interactions between transposable elements and the host genome.

There are two categories of strains in *Drosophila melanogaster* with respect to I–R hybrid dysgenesis, named inducer and reactive (for review see Bucheton, 1990). Crosses between females of reactive strains and males of inducer strains produce F1 females, called SF females, that are more or less sterile. Sterility results from low hatchability of their eggs. Reciprocal crosses (inducer females × reactive males) produce females, named RSF females, that are normally fertile (Bucheton *et al.* 1976). The inducer phenotype is due to the presence in the inducer strains of potentially functional transposable elements, the I factors, that are not present in the reactive strains. I factors are stable in inducer stocks but transpose at very high frequency in the germ-line of SF females and, to a lesser extent of RSF females. They never transpose in males (Picard, 1976).

I factors belong to the class of transposable elements known as LINEs (Fawcett *et al.* 1986). Complete I factors are 5·4 kb long and are devoid of terminal

repeats. They terminate at their 3′ end in several repeats of the sequence TAA. They contain two long open reading frames, one of which encodes a potential polypeptide showing strong similarity with reverse transciptases. They transpose replicatively by reverse transcription of an RNA intermediate (Jensen & Heidmann, 1991; Pélisson *et al.* 1991) that appears to be a full-length RNA (Chaboissier *et al.* 1990). They are dispersed at various sites on chromosomal arms of inducer strains. Both inducer and reactive strains contain defective incomplete I elements at similar locations in pericentromeric heterochromatin (Bucheton *et al.* 1984; Crozatier *et al.* 1988; Vaury *et al.* 1990).

Various results indicate that at the beginning of the century natural populations of *Drosophila melanogaster* were reactive, and that they were converted to the inducer state as a result of their invasion by active I factors between 1920 and 1970 (Kidwell, 1983). Now all natural populations are inducer, and reactive stocks are derived from flies caught in the wild before I factor invasion.

I elements are present in many *Drosophila* species (Bucheton *et al.* 1986; Stacey *et al.* 1986; De Frutos *et al.* 1992). Their distribution correlates with the phylogenetic relationships between species. The structure of the complete I factor of *Drosophila melanogaster* is very similar to that of the I elements of *Drosophila simulans*, the species most closely related (Bucheton *et al.* 1986; Simonelig *et al.* 1988). This suggests that functional I factors that spread recently in *Drosophila melanogaster* could have arisen by horizontal transfer from *Drosophila simulans*.

Drosophila teissieri also belongs to the *melanogaster* subgroup of species, although it is not as closely related to *Drosophila melanogaster* as *Drosophila simulans*. It contains active I factors having the same general organization as the I factors of the inducer strains. However the nucleotide sequences of the functional I factors of *Drosophila teissieri* and *Drosophila melanogaster* show about 15% divergence (Abad *et al.* 1989). When an active I factor from *Drosophila teissieri* (named *Itei*) is introduced into the germ-line of a reactive strain of *Drosophila melanogaster* by P-transposable-element-mediated transformation, it is able to transpose, resulting in an increase of the copy number of the element. Transgenic stocks behave as inducer strains after a few generations (Abad *et al.* 1989).

In the present paper we report experiments designed to compare the properties of active I factors from both species in the genome of *Drosophila melangoster*. They indicate that both I factors behave similarly in dysgenic crosses. However, more than one I factor of *Drosophila teissieri* seems to be necessary in order to induce SF female sterility after crosses with reactive females, whereas only one I factor of *Drosophila melanogaster* appears to be sufficient. Our results also show that the copy number of *Itei* is much higher in the transformed *Drosophila melanogaster* host than it is in the original *Drosophila teissieri* host.

2. Materials and methods

(i) Drosophila *strains and transgenic lines*

Cha-(R) and HJ_{30} are strong and weak reactive stocks respectively. *Canton-S* is an inducer strain.

128·2 and *Taï81* are two *Drosophila teissieri* strains derived from flies recently caught in the wild kindly provided by J. David.

Two transgenic lines of *Drosophila melanogaster* containing an active I factor from *Drosophila teissieri* were previously constructed by P-element-mediated transformation of the *Cha-(R)* reactive strain by Abad *et al.* (1989). They were obtained by using the P-element-transformation vector *pUChsneo* (Steller & Pirotta, 1985) that contains the selectable marker gene *neomycin*. G418–resistant individuals were selected and transgenic lines were constructed in two different ways: (1) some were made homozygous for the transgene after appropriate crosses with reactive stocks and then maintained by endogamous crosses; (2) the others were maintained by successive back-crosses of males resistant to G418 to females of the *Cha-(R)* stock. All stocks of *Drosophila melanogaster* used in the experiments were M in the P–M system of hybrid dysgenesis (for a review, see Engels, 1989) so that no transposition events could result from P-element activity.

G418-resistant individuals were selected by growing larvae on medium containing the drug to the concentration of 1 mg ml^{-1}.

(ii) *Measurement of the fertility of the females*

The fertility of the females was measured as the hatching percentage of their eggs. Eggs were collected from either individuals or groups of 2- to 4-day-old females at 20 °C except otherwise stated.

(iii) In situ *hybridization experiments to salivary gland chromosomes*

In situ hybridization experiments to salivary gland chromosomes of larvae were performed as described by Bucheton *et al.* (1984) using phage *λ4D* (Abad *et al.* 1989; see Fig. 2) as a probe.

(iv) *Southern blot analysis*

DNAs were extracted from adult flies as previously described (Bucheton *et al.* 1984), digested with restriction enzymes and the resulting fragments were separated on 1% agarose gels. They were transferred to Biodyne filters and hybridized to ^{32}P-labelled

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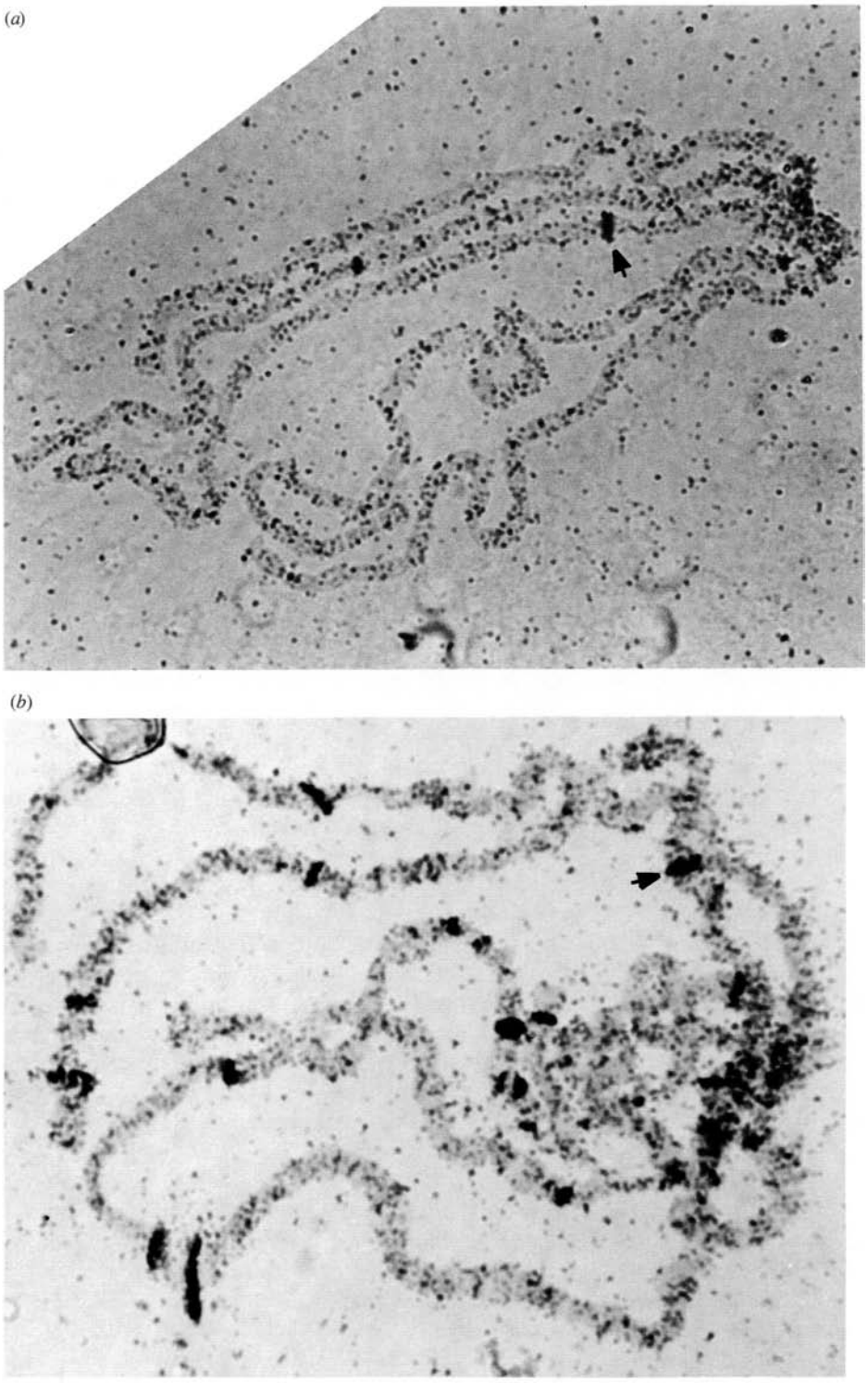

Fig. 1. *In situ* hybridization of phage *λ4D* to polytene chromosomes of larvae from lines BCT1 (*a*) and ET1 (*b*) (see text). The arrowheads indicate hybrization signals due to the sequences flanking the I element in *λ4D*.

probes. All procedures were carried out as the recommended by the manufacturers.

3. Results

(i) *I element copy number seems to be regulated by the host genome*

In order to study the behaviour of an active I factor from *Drosophila teissieri* (named *Itei*) in *Drosophila melanogaster*, we subcloned such an element from phage *λ4D* (see Fig. 2*a*) into the transformation vector *pUChsneo* (Steller & Pirrota, 1985). The resulting plasmid, *pπhsneo-Itei*, was microinjected together with plasmid *pπ25·7wc* as a source of transposase (Karess & Rubin, 1984) into embryos of the reactive strain *Cha-(R)*. G0 adults derived from these embryos were crossed with *Cha-(R)* flies and the larvae coming from these crosses were selected on

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G418 containing food. Two independent G1 flies resistant to G418, one male and one female, named T1 and T2 respectively, were recovered in this way (Abad *et al.* 1989). They resulted from insertions of the *πhsneo-Itei* transposon on the second chromosome. They were crossed with reactive flies, and different lines were established from each of them. Two lines, called ET1 and ET2, derived from T1 and T2 respectively, were maintained by endogamous crosses. The other two lines, referred to as BCT1 and BCT2 respectively, were made by crossing G2 males resistant to G418 to females of the *Cha-(R)* strain and were maintained by successive back-crosses of the male progeny selected on G418 containing food to *Cha-(R)* females.

For the different lines we have determined at various generations the number of I elements on chromosomal arms. For this the individuals to be studied were crossed with *Cha-(R)* females which are devoid of I elements on chromosomal arms as are all flies of reactive strains. Salivary gland polytene chromosomes of larvae coming from these crosses were hybridized with probe *λ4D*, and the sites of hybridization were numbered, giving an estimate of the number of I elements on chromosomal arms per haploid genome. Measurements were usually made using five larvae.

Fig. 1 shows some typical results. They shows that line BCT1 (Fig. 1*a*) contains only one hybridization site on chromosome 2. A probe of the *neomycin* gene hybridizes to the same site (result not shown), indicating that it corresponds to the *πhsneo-Itei* transgene. By contrast, line ET1 contains multiple copies of the I element dispersed on the arms of the chromosomes (Fig. 1*b*), resulting from transpositions of *Itei* in the female germ-line. In line BCT1 the transgene is transmitted only in the male germ-line and in line ET1 it is transmitted in both the female and male germ-lines. These results indicate that transposition of I elements of *Drosophila teissieri* is restricted to the female germ-line as is that of I elements of *Drosophila melanogaster* (Picard, 1976).

G2 individuals derived from the T2 transgenic female also contained several I elements on chromosomal arms indicating that transposition had occurred in the germ-line of the original G1 transformed female. Lines BCT2 and ET2 contain multiple copies of the I element on the arms of the chromosomes (data not shown).

We have studied the kinetics of invasion of lines ET1 and ET2 by *Itei* in *in situ* hybridization experiments on chromosomes of larvae produced by flies of generations 1, 9 and 54 as described above. The results are reported in Table 1. The number of sites follows a similar increase in both lines. The mean copy number is 3 in the progeny of the females of the first generation. It increases and reaches a stable value (about 14). Therefore the number of I elements from *Drosophila teissieri* in both transformed lines is of the same order of magnitude as the number of I elements usually observed in inducer strains of *Drosophila melanogaster* (Bucheton *et al.* 1984; Ronsseray & Anxolabehere, 1986).

Previous results obtained by *in situ* hybridization experiments indicate that the number of I elements dispersed on chromosomal arms is only two to four in *Drosophila teissieri* (Simonelig *et al.* 1988). We asked the question: is there an actual difference in the copy number of potentially complete I elements between the two species or is the difference restricted to the euchromatic part of the genome (the only one tested by *in situ* hybridization)? Equal amounts of DNA from the *128·2* and *Taï·81 Drosophila teissieri* strains were digested with enzymes *BgI* II and *Eco*R I and hybridized with fragment A of clone *λ4D* (see Fig. 2*a*). These two restriction enzymes generate an 1·6 kb internal fragment near the 3′ end of the complete I factors that is expected to be representative of potentially active elements (Bucheton *et al.* 1986; Crozatier *et al.* 1988; Simonelig *et al.* 1988). Clone *λ4D* was also digested with these two enzymes and loaded on the same gel in amounts corresponding to 2, 4, 8, 16 and 32 copies of the fragment per haploid genome. The I factor contained in this clone is flanked by a sequence that is unique in the genome of *Drosophila teissieri* (Abad *et al.* 1989) and can therefore be used to quantify the amounts of DNA (*Eco*R I–*Xho* I fragment B in Fig. 2*a*).

The filter was hybridized with a probe corresponding to fragment A (Fig. 2*b*). It appears that the copy number of the 1·6 kb *BgI* II/*Eco*R I fragment is between two and four per haploid genome in the two strains. This is in agreement with the copy number of I elements previously detected on chromosomal arms of *Drosophila teissieri* by *in situ* hybridization experiments (Simonelig *et al.* 1988). We checked that equal amounts of DNA were loaded for both strains and that the amount of DNA loaded in well 2 for phage *λ4D* actually corresponded to two copies of fragment B per haploid genome, by hybridizing the same filter with probe B (results not shown).

We conclude from these experiments that there are fewer copies of *Itei* in the genome of *Drosophila teissieri* than in the genome of transformed lines ET1 and ET2 of *Drosophila melanogaster*. The copy number in both transformed lines is similar to that of I elements in typical inducer strains of *Drosophila melanogaster*.

(ii) *Transformed lines ET1 and ET2 behave like typical inducer strains*

We have previously shown that the I factor cloned in *λ4D* is active in *Drosophila melanogaster* and can induce the female sterility usually observed in I–R hybrid dysgenesis (Abad *et al.* 1989). Both transformed lines ET1 and ET2 contain multiple copies of the I element from *Drosophila teissieri*. We have

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Table 1. *Mean copy number of I elements in lines ET1 and ET2*

	Larvae coming from generation		
	1	9	54
ET1	3±1	11±3	14±2
ET2	3±1	16±2	13±1

carried out experiments in order to study if these lines look like typical inducer strains of *Drosophila melanogaster* and to determine the major characteristics of hybrid dysgenesis induced by the *Drosophila teissieri* I factor in this species.

SF female progeny produced by crossing reactive females and inducer males show reduced fertility. They lay a normal number of eggs, a fraction of which does not hatch. The intensity of SF female sterility can be measured by determining the hatching percentage of their eggs. It depends largely on the reactive stock used in the dysgenic cross. So there are strong and weak reactive stocks that produce respectively strongly and weakly sterile SF females after crosses with standard inducer males (Bucheton & Picard, 1978). The degree of the sterility of SF females also depends on their age. The hatching percentage of eggs increases regularly when the females age (Bucheton, 1978 & 1979).

We determined the sterility level of SF females produced by crossing females from the *Cha-(R)* and

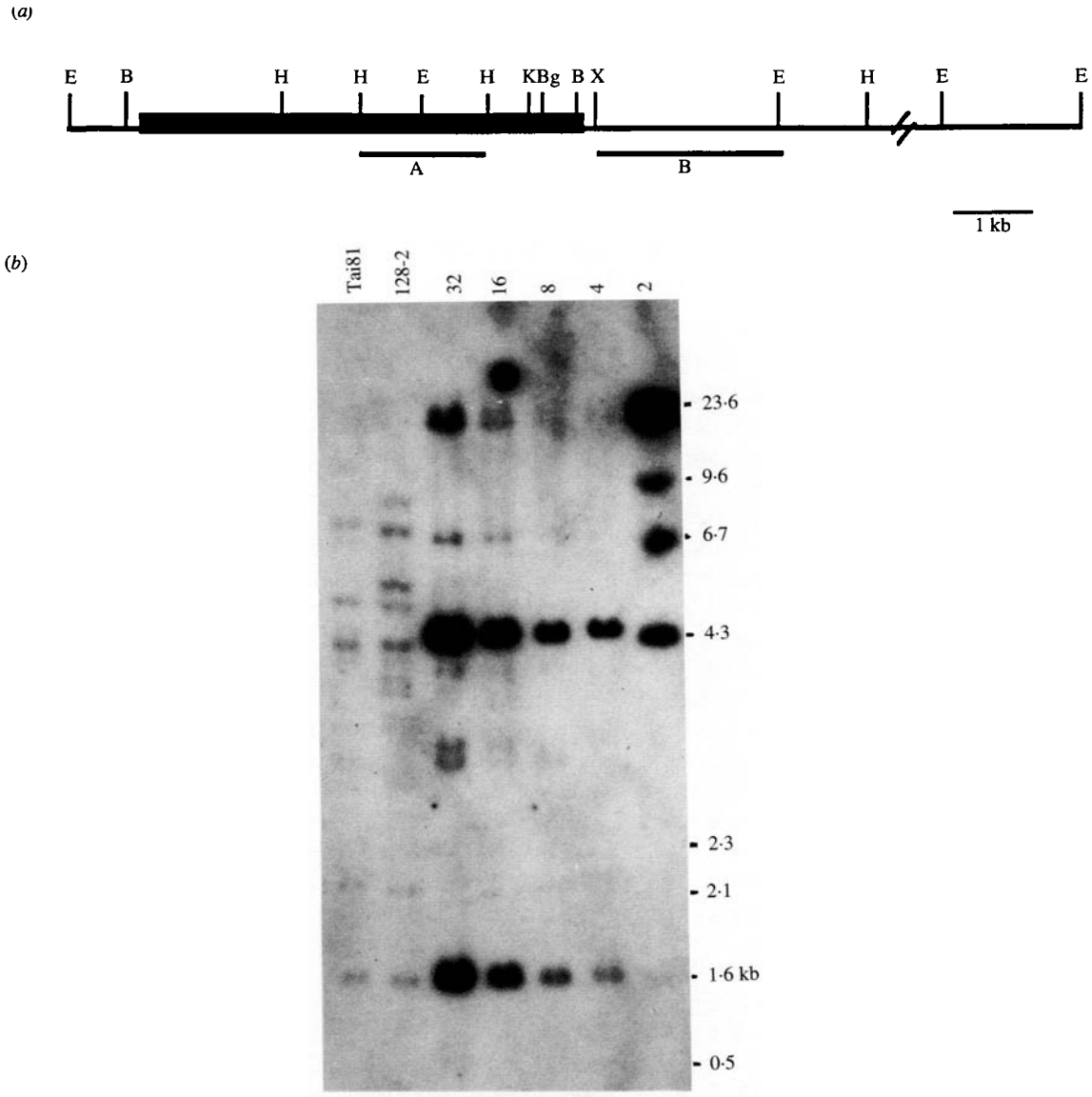

Fig. 2. Structure of phage *λ4D* (*a*) and determination of the copy number of putative functional I elements in two stocks of *Drosophila teissieri* (*b*). In (*b*) DNAs were digested with restriction enzymes, separated on an agarose gel, transferred to a filter and hybridized with probe A indicated in (*a*) as described in the text. 2, 4, 8, 16 and 32 correspond to equivalent copy numbers of phage *λ4D* per haploid genome. In (*a*) the I element is represented by a thick line whereas unique sequences are represented by thin lines. B indicates the sequence of *λ4D* used to quantitate the DNAs (see text). Abbreviations for the restriction enzymes are: B, *Bam*H I; Bg, *Bg*I II; E, *Eco*R I; H, *Hin*dIII; K, *Kpn* I; X, *Xho* I.

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Table 2. *Hatching percentages of the eggs from the female progeny from crosses involving lines ET1 and ET2*

Age of laying females (in days) . . .	2	22	35		2	22	35
Cha-(*R*) females × ET1 males	0	6±1	21±2	ET1 females × *Cha*-(*R*) males	41±1	73±2	75±2
Cha-(*R*) females × ET2 males	2±1	29±2	49±3	ET2 feamles × *Cha*-(*R*) males	71±2	93±1	90±2
HJ_{30} females × ET1 males	24±2	95±1		ET1 females × HJ_{30} males	58±2	96±1	
HJ_{30} females × ET2 males	36±2	91±2		ET2 females × HJ_{30} males	77±2	96±1	
ET1 females × ET1 males	63±2	76±2	82±2				
ET2 females × ET2 males	75±2	76±2	77±2				
Cha-(*R*) females × *Canton-S* males	0	77±2		*Canton-S* females × *Cha*-(*R*) males	95±1	98±1	
HJ_{30} females × *Canton-S* males	84±1	98±1		*Canton-S* females × HJ_{30} males	97±1	97±1	

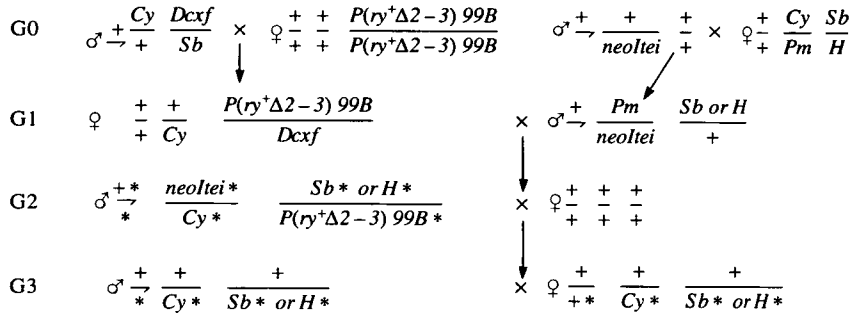

Fig. 3. Mobilization of the *πhsneo-Itei* (neoItei) transposon to new chromosomal positions using the chromosome *P*(*ry*$^+$ Δ*2–3*)(*99B*) as a donor of transposase. All stocks used in this experiment were reactive and M in the P–M system of hybrid dysgenesis. Stars indicate chromosomes on which transposition of *πhsneo-Itei* could have occurred in G2 males.

HJ_{30} strains, which are strongly and weakly reactive respectively, with males from the ET1 and ET2 lines after 15 generations of endogamous crosses. At the same time we measured as controls the reactivity of both *Cha*-(*R*) and HJ_{30} by crossing them with standard inducer males of the *Canton-S* stock. The fertility of the female progeny coming from all reciprocal crosses was also determined.

The hatching percentages of the eggs laid by SF females produced by crossing *Cha*-(*R*) and HJ_{30} females with *Canton-S* males confirm that *Cha*-(*R*) is strongly reactive while HJ_{30} is weakly reactive (Table 2). ET1 and ET2 behave as inducer strains when males from these stocks are crossed with reactive females. Sterility of SF females produced with males from these two lines is stronger when they are derived from strong reactive females and weaker when they are derived from weak reactive females, as usually observed in I–R dysgenic crosses (Table 2).

In some cases we also determined the hatching percentages of the eggs laid by 22- and 35-day-old females. Table 2 shows that fertility increases with age, indicating that these females have the main characteristics of typical SF females.

The behaviour of both ET1 and ET2 seems however slightly different from that of typical inducer strains. We may see in Table 2 that all crosses involving females from these lines and reactive males give daughters that exhibit moderately low fertility. This contrasts with the high fertility of RSF females resulting from crosses between typical inducer females and reactive males (95 and 97% of egg hatching for the RSF females obtained by crossing *Cha*-(*R*) and HJ_{30} males respectively with *Canton-S* females; see Table 2). The sterility of these RSF females does result from inducer-reactive interaction since it decreases when they age as shown in Table 2. This suggests that ET1 and ET2 retain some reactivity.

The fertility of females from the lines themselves is also low and as usual increases with age. This reduced fertility results probably from the fact that these lines exhibit some reactivity and contain simultaneously active I factors, resulting in hybrid dysgenesis occurring within the lines themselves. Similar results are observed in stocks derived from the offspring of SF and RSF females (Picard, 1978).

(iii) *More than one I factor from* Drosophila teissieri *seem necessary to induce hybrid dysgenesis*

One copy of the I factor of *Drosophila melanogaster* seems to be sufficient to induce SF female sterility (Pélisson, 1981). It is apparently not the same for the I factor of *Drosophila teissieri* since when males of line BCT1 are crossed with females of the *Cha*-(*R*) reactive strain, they produce fertile daughters (the hatching percentage of their eggs is 83), indicating that they are not inducer. BCT1 males have only one copy of *Itei*

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corresponding to the original insertion of the element in transformed male T1 (see Fig. 1). The fact that ET1, which contains multiple copies, behaves as an inducer stock indicates that this element is potentially active. Therefore, the non-inducer character of BCT1 males could result either from a position effect, the original insertion of *Itei* being silent, or to the fact that multiple copies of the I factor of *Drosophila teissieri* are necessary to induce SF female sterility.

In order to study the activity of *Itei* elements located at various positions in the genome, we moved the P element *πhsneo-Itei* from its original site in BCT1 males to new sites by mediating its transposition with the *P(ry*$^+$ *Δ2-3)(99B)* chromosome as a donor of P transposase (Engels, 1989). The mating scheme is presented in Fig. 3. The chromosome carrying the original *πhsneo-Itei* transposon was always transmitted to the next generation through male germ-line so that no I element transposition could occur. Transposition of *πhsneo-Itei* was allowed in G2 males and new positions were recovered at generation G3 by selecting flies of the *Cy* phenotype resistant to G418. 2600 Flies were screened in this way, and nine *Cy* females and five *Cy* males were isolated from independent crosses.

Eight of the G3 females produced males displaying the inducer character when crossed with reactive females: their female progeny laid eggs that did not hatch, and the hatching percentage of their eggs increased as the females aged. These results were expected since *Itei* could transpose in the germ-line of the G3 *Cy* females, giving rise to G4 males containing multiples copies of the element. *In situ* hybridization experiments of *λ4D* to salivary gland chromosomes of larvae coming from these males confirmed that *Itei* was present at 2–8 new sites (data not shown).

One of the G3 females (called 5R2) did not produce inducer G4 males. *In situ* hybridization experiments showed that it contained an unique insertion site located on the X chromosome.

The five G3 males were crossed with *Cha-(R)* reactive females, and the fertility of the daughters resistant to G418 was measured. Females produced by all five males were normally fertile, indicating that these males were not inducer. *In situ* hybridization experiments to salivary gland chromosomes of larvae coming from these males using *λ4D* as a probe showed that each of them contained only one I element, which was at a new position compared to the original insertion site observed in BCT1. Three new different sites were on chromosome 3 and two on chromosome 4.

The results with the five males together with male T1 that was not inducer and female 5R2 that produced non-inducer sons mean that we have studied the inducer activity of a single *Itei* element located at seven different chromosomal locations. In each case it induced no SF sterility. We showed that this did not result from inactivation of the I factor since for all seven cases, *Itei* invaded the genome in subsequent female generations and males carrying multiple copies were inducer.

All these results suggest that one copy of *Itei* is not sufficient to induce SF female sterility.

4. Discussion

We have studied the behaviour of an active I factor from *Drosophila teissieri*, *Itei*, introduced in a new genome: the genome of a reactive strain of *Drosophila melanogaster*. This element is able to transpose at high frequency in the germ-line of females and to induce the female sterility that is characteristic of I–R hybrid dysgenesis. For example, two lines, ET1 and ET2, have been obtained by endogamous crosses in the progeny of two flies transformed with *Itei* by P-element-mediated transformation. They contain several copies of *Itei*. When ET1 or ET2 males are crossed with reactive females, they produce F1 daughters that are sterile. A large fraction of their eggs do not hatch, and the hatching percentage increases with age. All these characteristics are typical of SF females, indicating that I factors from *Drosophila teissieri* can actually induce I–R hybrid dysgenesis in *Drosophila melanogaster*. In addition, they respond to variations of reactivity levels in the same way as I factors of the inducer strains of *Drosophila melanogaster*.

Our results also show that transposition of *Itei* does not occur in males and is restricted to the female germ-line. It is known that I factors of *Drosophila melanogaster* also transpose only in the female germ-line (Picard, 1976). Some results indicate that the tissue specificity of I factor activity is regulated at the level of transcription (Chaboisser *et al.* 1990). This suggests that the sequences responsible for the tissue specificity are conserved enough in the I factors of *Drosophila melanogaster* and *Drosophila teissieri*. This promoter specificity of the female germ-line could be located near the 5′ ends of these elements since 37 of their first nucleotides are identical with one exception (Abad *et al.* 1989).

A possible difference in the properties of these elements is that, while only one copy of the I factor of *Drosophila melanogaster* can be sufficient to induce SF female sterility after crosses with reactive females (Pélisson, 1981), more than one copy of *Itei* seems to be necessary. We have studied the inducer properties of males carrying one copy of *πhsneo-Itei* (a P transposon containing *Itei*) at various chromosomal sites. Of males carrying a single *Itei* at different insertion sites examined in this way, none was inducer. This suggests that the inability of *Itei* to induce female sterility in these conditions does not result from a position effect. For each of them, we have shown that the *Itei* element was not inactivated and was able to transpose and to induce hybrid dysgenesis after replicative transposition.

However, position effects cannot be totally excluded as an explanation for our results. Other results indicate that I factors inserted in silent sites in *Drosophila melanogaster* could be rather frequent. Non-inducer chromosomes have been isolated in inducer stocks (Picard & Pélisson, 1978). Some of them become fully inducer after passage through reactive females (Pélisson & Bregliano, 1981). The acquisition of the inducer property by such chromosomes could result from transposition of potentially active I factors from silent sites to new sites where their expression would no longer be repressed. The behaviour of the chromosomes carrying only one copy of *πhsneo-Itei* is strikingly similar to that of these non-inducer chromosomes.

The fact that the seven single *Itei* elements that we have studied behave in the same way and are not inducer suggests that one I factor from *Drosophila teissieri* is not sufficient to induce female sterility. Various genetic experiments suggest that one I factor of *Drosophila melanogaster* can produce SF females (Pélisson & Picard, 1979; Pélisson, 1981 & unpublished results). However it has never been shown in these cases that the inducer activity was actually associated with only one I factor. The one example for which it has been proven that a single copy is sufficient to induce hybrid dysgenesis is the I factor associated with the w^{IR1} mutation of *Drosophila melanogaster* (Pélisson, 1981; Bucheton *et al.* 1984).

The last hypothesis to explain the inability of the lines carrying only one I factor from *Drosophila tessieri* to induce female sterility would be that *Itei* expression would be repressed in some way by flanking sequences of the *pUChsneo* transformation vector that were present in all cases in which we have studied the inducer activity of a single *Itei*. Although sequences introduced in the genome using P-element transformation vectors usually show at least partial activity, we cannot exclude that expression of *Itei* is greatly reduced in *πhsneo-Itei*. One must notice however that all the stocks used in these experiments were resistant to G418, showing that the *neomycin* gene also contained in *πhsneo-Itei* was active. This suggests that expression of sequences present in this P transposon is not totally repressed.

An important result reported in this paper is that in lines maintained by endogamous crosses the number of I elements from *Drosophila teissieri* increases and reaches a stable value very rapidly (about 14 per haploid genome in lines ET1 and ET2). This value is very similar to that observed for the I elements in natural populations of *Drosophila melanogaster* (Ronsseray & Anxolabéhère, 1986). The copy number of I elements from inducer strains of *Drosophila melanogaster* has also been monitored after introduction of functional I factors in the genome of reactive strains by either appropriate dysgenic crosses (Pélisson & Bregliano, 1987) or P-element-mediated transformation (Pritchard *et al.* 1988). In all cases it appears that it increases very quickly and stabilizes at similar levels, between 7 and 17 according to the experiments. These data indicate that the number of I elements from either *Drosophila melanogaster* or *Drosophila teissieri* seems to be well regulated in *Drosophila melanogaster*.

These results contrast with those obtained for other mobile elements such as P (Engels, 1989) and with the regulation of the P element introduced in *Drosophila simulans* in which a steady state is reached only after more than 40 generations (Montchamp-Moreau, 1990).

The number of I elements on chromosomal arms of *Drosophila teissieri* also seems to be well regulated since it is about the same in various stocks of this species (Simonelig *et al.* 1988, and the present data). However it is comprised between two and four and is therefore much lower than that observed in *Drosophila melanogaster*. We conclude that the copy number of the I factor of *Drosophila teissieri* is not entirely controlled by the element itself but by other determinants contained in the host genome.

Three main hypotheses could explain the differences in the copy number of *Itei* in *Drosophila teissieri* and *Drosophila melanogaster*.

(1) The low copy number observed in *Drosophila teissieri* could be related to the history of the element in this species and result from progressive inactivation by mutation of positional effect. We suggested earlier that such events could lead to elimination of a family of transposable elements from euchromatic sites (Vaury *et al.* 1989) by recombination and natural selection as proposed by Montgomery *et al.* (1987). The few I elements remaining on chromosomal arms of *Drosophila teissieri* would be potentially active as *Itei* but repressed because inserted in silent sites.

(2) The copy number of I elements could be mainly regulated by the host genome. In this case some species could be more sensitive to invasions of mobile elements than others. However no specific genes affecting the number of copies of transposable elements have been described so far.

(3) All strains of *Drosophila melanogaster*, even those that are reactive, contain defective I elements located in the pericentromeric heterochromatin. They are old constituants of the genome of the species (Bucheton *et al.* 1984; Crozatier *et al.* 1988; Vaury *et al.* 1990). The same is true for *Drosophila teissieri* (Simonelig *et al.* 1986). We have previously suggested that these defective I elements could be involved in the regulation of I factor activity by producing partially active regulatory molecules (Crozatier *et al.* 1988; Bucheton, 1990). *Drosophila melanogaster* might produce less efficient or less abundant regulatory molecules than *Drosophila teissieri*, allowing transposition to occur more frequently. This hypothesis is very similar to the models proposed to explain regulation of P factor activity that involve combinations of functional and mutated elements as well

as their insertion sites (Simmons *et al.* 1987; Engels, 1989).

The present results show once more that transposable elements, when introduced in another species, can transpose and invade the genome. The I factor of *Drosophila teissieri*, which is present at low copy number in its natural host, can transpose in *Drosophila melanogaster* and induce characteristic dysgenic traits. Invasion of natural populations of *Drosophila melanogaster* by an active I factor occurred presumably at the beginning of this century (Kidwell, 1983; Vaury *et al.* 1990). It could have originated by horizontal transfer from another species. Such events appear to be rather frequent in the wild and their consequences for the species are unpredictable. Elucidation of the mechanisms controlling transposition would therefore be of considerable interest for evolutionary studies.

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