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The relationship between structural variation and dysgenic properties of *P* elements in long-established *P*-transformed lines of *Drosophila simulans*

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Ten lines of *Drosophila simulans* were investigated with respect to *P* activity, *P* susceptibility and the number and structure of their *P* copies, eight years after transformation with the *P* element. All 10 were found to have reached a steady state. They exhibited varying levels of *P* activity (from 0 per cent to 96 per cent GD sterility) and, with the exception of one line, were not *P*-susceptible. In contrast with *P* element behaviour in *D. melanogaster*: (i) no relationship was found between the molecular pattern of *P* copies in a line and its ability to induce or to repress *P* expression in *D. simulans*; (ii) peculiar *P* element derivatives were observed in this species; (iii) the average number of *P* copies per genome was only half of that in *D. melanogaster*. This may result from transposon–host genome interactions, which lead to a low invading power of the *P* element in *D. simulans*.

Keywords: *Drosophila simulans*, *P* element.

Introduction

The *P* transposable element was identified in the *Drosophila melanogaster* genome at the beginning of the 1980s. In this species, a syndrome of germline abnormalities, P–M hybrid dysgenesis, is associated with the mobilization of the *P* family of transposable elements (for a review see Engels, 1989). Historical and geographical data for *D. melanogaster* indicate that the *P* element first appeared in South American populations in the 1950s and subsequently spread worldwide (Anxolabéhère *et al.*, 1985, 1988). It is now accepted that the *D. melanogaster P* element arose by horizontal transfer from the neotropical species *D. willistoni* (Daniels *et al.*, 1990; Clark *et al.*, 1994). *P* homologous sequences are completely absent in the *Drosophila* species which are closely related to *D. melanogaster*, particularly in its sibling species *D. simulans* (Brookfield *et al.*, 1984).

Drosophila melanogaster and *D. simulans* are cosmopolitan species, sympatric over most of their range. Thus, the lack of *P* sequences in *D. simulans* may result either from a low probability of horizontal transfer or from an inability of the *P* element to invade *D. simulans*. *P* elements are able to transpose in the genomes of other species. *In vivo* experiments (O'Brochta & Handler, 1988; O'Brochta *et al.*, 1991) showed that the *P* element from *D. melanogaster* can be efficiently excised from plasmids in the subgenus *Sophophora*. In addition, autonomous *P* elements from *D. melanogaster*, introduced by microinjection into *D. simulans* embryos, were found to be able to transpose and multiply within their new host's genome (Scavarda & Hartl, 1984; Daniels *et al.*, 1985, 1987; Montchamp-Moreau, 1990) and to invade the *D. mauritiana* genome after interspecific crosses (Montchamp-Moreau *et al.*, 1991). However, differences in *P* element population dynamics have been reported between *D. simulans* and *D. melanogaster* which suggest that *P* element invasion might be less successful in the former species. Under the same experimental conditions, the *P* element transposition frequency is more than three times higher in *D. melanogaster* than in *D. simulans*, and *D. melanogaster* strains tend to evolve towards strong or moderately strong P types, whereas those of *D. simulans* evolve towards weak P or M′ types (Kimura & Kidwell, 1994). This agrees

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with our observations on *D. simulans* *P*-transformed lines (Montchamp-Moreau, 1990), which were found to reach a steady state with a moderate number of *P* copies (10–24 per haploid genome) and moderate *P* activity (10–35 per cent of GD sterility).

Here we present data on the long-term evolution of P–M hybrid dysgenesis in these transformed lines which led us to reconsider previous conclusions. The relationship between dysgenic properties of these lines and the molecular structure of their *P* copies has been investigated in order to understand the invasion dynamics of *P* elements in this species.

Materials and methods

Strains

SimO A wild strain of *D. simulans* collected in Nasrallah (Tunisia) in 1983. This strain is used as an M reference strain for the P–M system in *D. simulans*.

L3–2, W1–7, T1–4, Q8–4, S5–2, S5–3, C2–10 and C2–10b *P*-transformed isofemale lines obtained by injection of the *D. melanogaster* *P*-element into SimO embryos (Montchamp-Moreau, 1990). The first four correspond to independent initial *P*-insertion events. S5–2 and S5–3 are sublines originating from the same injected embryo. C2–10 and C2–10b came from the same transformation event. The L3–7, W1–7, T1–4, S5–2, S5–3 and C2–10 lines were cultured at 25°C and analysed after at least 220 generations, the Q8–4 and C2–10b lines were cultured at 18°C and analysed after at least 100 generations.

SimT A line maintained at 25°C, obtained by mixing five *P*-transformed lines at generation 30 (Montchamp-Moreau, 1990).

E2–3 *P*-negative control line which came from an unsuccessfully injected embryo and was cultured at 25°C.

Sw/W5 A line resulting from a dysgenic cross between females from the Seych *w* strain (a strain devoid of *P* sequences, bearing a spontaneous X-linked mutation, *white*) and males from a transformed line (W5–10) which had a high *P* activity potential (Montchamp-Moreau, 1990).

Gonadal sterility tests

P activity (A cross): 30 males from the line under test were mated with 30 SimO virgin females. *P* susceptibility (A*cross): 30 virgin females from the line under test were mated with 30 males from the C2–10 line or with 30 males from the Sw/W5 line, both of which exhibit strong *P* activity in the A cross. Intra-line cross: 30 males and 30 females from the line under test were crossed.

For each gonadal sterility test, crosses were performed at 28°C. After emergence the F_1 progeny were left to mature on fresh medium for 3–4 days at 25°C before the females were dissected. The frequency of gonadal sterility was calculated by dividing the number of dysgenic ovaries by the total number of ovaries scored.

Southern blot analysis

For each line, genomic DNA was extracted from 50 females using the method described by Junakovic *et al.* (1984). Restriction enzyme digestion of the DNA was performed according to the supplier's instructions. After gel electrophoresis, transfers were carried out on nitrocellulose filters (Schleicher and Schuell). Filters were prehybridized for 2 h at 65°C in a solution of 6 × SSC (3 M sodium chloride, 0.3 M trisodium citrate), 5 × FPG [1 g/L of Ficoll type 400 (Pharmacia), 1 g/L of polyvinylpyrrolidone and 1 g/L of glycine], 0.5 per cent sodium dodecyl sulphate (SDS), and 100 μg/mL of denatured salmon sperm DNA. Filters were then hybridized overnight at 65°C in the same solution, to which a DNA probe labelled with ^{32}P by nick translation had been added. Washes were performed at 65°C in 2 × SSC and 0.1 per cent SDS.

In situ *hybridization*

Squashes were prepared from the salivary glands of F_1 female larvae from crosses between transformed males and females of the SimO strain. The number of *P* elements per haploid genome of the transformed lines was estimated by hybridization of polytene chromosomes with nick-translated, ^{3}H-labelled pπ25.1 plasmid DNA (O'Hare & Rubin, 1983). The procedure was adapted from Pardue & Gall (1975).

Results

Dysgenic properties

Intraline crosses showed that 8 years after the introduction of *P* elements, all lines exhibited a stable or nearly stable state with regard to the P–M dysgenic system (Table 1). Intraline sterility is 5 per cent or less in all lines except C2–10 and C2–10b (9.24 and

Table 1 Gonadal sterility tests on *Drosophila simulans*. Results are given as percentage of dysgenic ovaries with the number of dissected females in brackets

Line	Intraline	A Cross: *P* activity	A* Cross: *P* susceptibility (a)	A* Cross: *P* susceptibility (b)
With *P* sequences				
Sw/W5	1.5 (100)	96.4 (250)		0.0 (50)
C2-10	9.2 (92)	69.0 (200)	3.0 (50)	
S5-2	0.0 (77)	71.0 (50)	3.0 (50)	4.0 (50)
T1-4	0.0 (80)	28.0 (50)	8.0 (50)	4.0 (50)
simT	1.0 (50)	20.0 (50)	1.0 (50)	0.0 (50)
C2-10b	9.0 (50)	13.0 (50)	89.5 (100)	40.0 (100)
W1-7	2.3 (43)	13.0 (50)	1.0 (50)	0.0 (50)
S5-3	2.0 (50)	8.0 (50)	3.0 (50)	0.0 (50)
Q8-4	2.0 (50)	1.0 (50)	2.0 (50)	0.0 (50)
L3-2	5.0 (50)	0.0 (80)	0.0 (100)	0.5 (100)
Without *P* sequences				
E2-3	0.0 (50)	0.0 (50)	100.0 (100)	65.5 (100)
simO	1.0 (250)			

(a) A* cross using S*w*/W5 males.
(b) A* cross using C2-10 males.

9.00 per cent GD sterility, respectively) which suggests a low *P* transposition rate in these lines.

P activity levels varied substantially between lines (Table 1), with *P*-induced GD sterility in excess of 69 per cent for three lines (S*w*/W5, C2–10 and S5–2) and at negligible levels in the L3–2 and Q8–4 lines. The five remaining lines showed moderate *P* activity (from 8 to 28 per cent).

The *P* susceptibility of the lines was tested in A* crosses with both C2–10 and S*w*/W5 as P tester strains. All lines except C2–10b were found to achieve *P* regulation (Table 1).

Using the classification previously defined for *D. melanogaster,* six of the lines would be described as P-type (more than 5 per cent GD sterility in the A cross and less than 5 per cent GD sterility in the A* cross). The lines Q8–4 and L3–2 would be described as Q-type (less than 5 per cent GD sterility in the A and A* crosses) and, as expected the two controls (SimO and E2–3) would be described as M-type lines. T1–4 is a quasi-P strain, with a moderate *P* activity (28 per cent GD) and a low *P* susceptibility (8 per cent GD). C2–10b can be considered as a quasi-M′ strain (13 per cent of *P* activity and 90 per cent of *P* susceptibility).

Molecular properties

The structure of the *P* sequences within the 10 lines was determined by Southern blot analysis, after separate digestion by *Ava*II, *Acc*I and *Hin*dIII endonucleases. Each filter was successively hybridized with three different probes (Fig. 1): *Hin*dIII (probe 1), *Hin*dIII-*Sal*I (probe 2) and *Sal*I (probe 3) fragments from pπ25.7 BWC (O'Hare *et al.*, 1992). These probes spanned the full-size *P* element of *D. melanogaster*. From the size of the restriction fragments detected and from their homology with the three probes used, the number of complete and derivative *P* elements was roughly estimated for each line (Table 2).

An estimation of the total number of *P* locations within lines was obtained from the results of both *Hin*dIII digestion (using probes 2 and 3), and *Acc*I digestion (using probe 3); it ranges from 14 (S5–3 line) up to 26 (S5–2 line). On the other hand, the maximum number of insertion sites per haploid genome observed by *in situ* hybridization of polytene chromosomes varies from 13 to 30 depending on the line. Values obtained by both methods are similar which suggests that the rate of insertion site polymorphism within a line is low. This is consistent

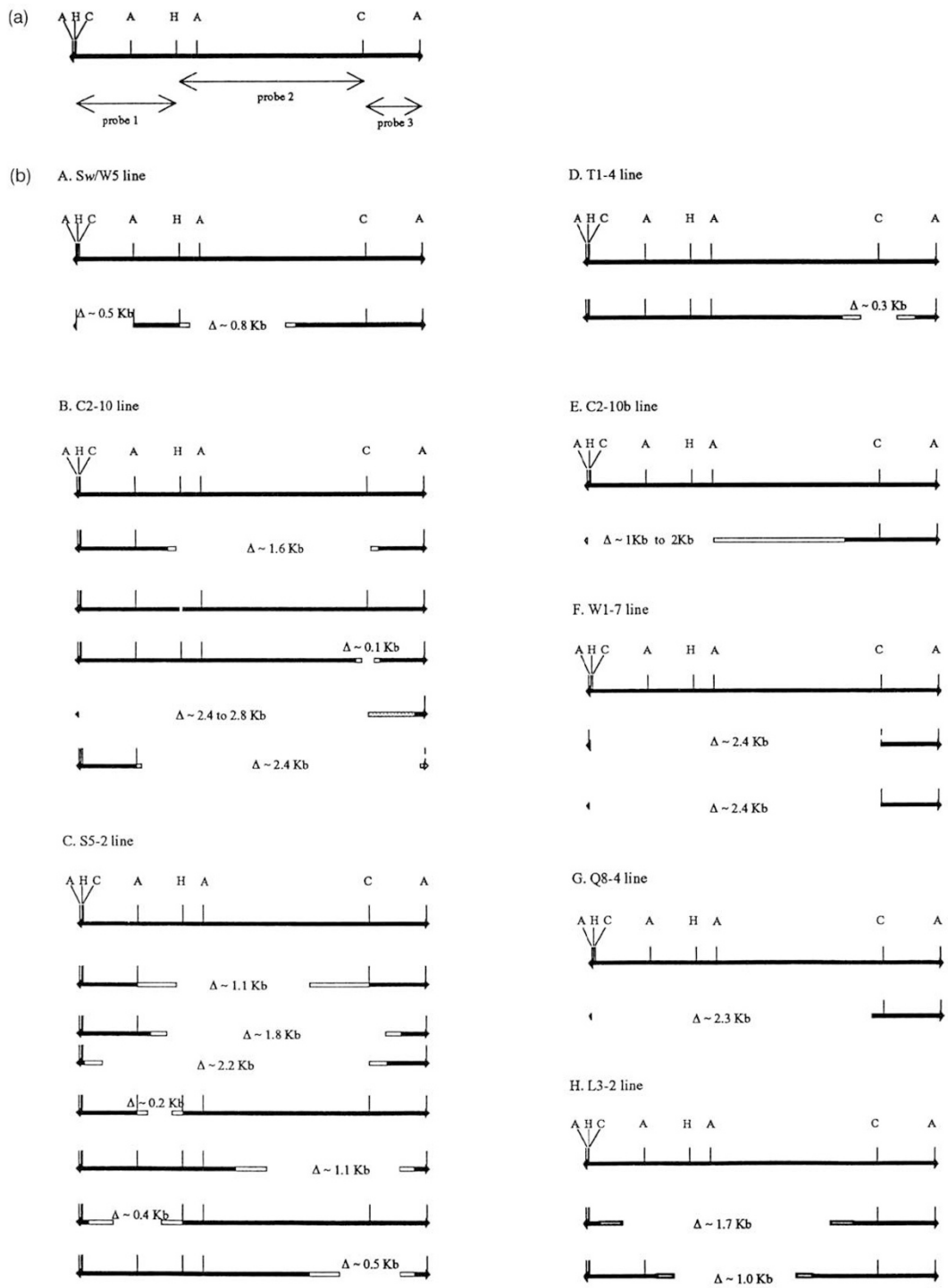

Fig. 1 (a) Full length *P* element of *Drosophila melanogaster*. Restriction map and probes used for the molecular analysis. (b) A 2.9 kb *P* element and its deletion derivatives in each of the eight transformed lines of *Drosophila simulans* for which derivatives were found. At the top is a map of the complete *P* element. Below this are maps of its apparent deletion derivatives. Sequences homologous to the complete *P* element present in a derivative are shown as a black horizontal bar; missing sequences are shown as a gap; and the region within which a deletion breakpoint lies is shown as an open bar. Each restriction site whose location was mapped is indicated by a vertical bar positioned below the analogous site in the complete *P* element (dashed vertical bar when the presence of a site is hypothetical). Endonuclease sites are denoted: (A) *Ava*II (C) *Acc*I and (H) *Hin*dIII.

Table 2 Estimation of the number of insertion sites in *Drosophila simulans* by Southern blot analysis and by *in situ* hybridization

	Southern blot analysis		*In situ* hybridization		
	Number of *P* copies			Number of sites	
Line	Total	Defective	*n*	Minimum	Maximum
Sw/W5	23	1		nd	nd
C2-10	25	20	6	22	30
S5-2	26	17	6	24	29
T1-4	16	1	7	13	15
simT	15	0	7	8	14
C2-10b	15	1		nd	nd
W1-7	18	2	7	20	25
S5-3	14	0		nd	nd
Q8-4	17	1		nd	nd
L3-2	16	10	6	10	13

n: number of haploid genomes examined in each line; nd: not determined.

with our *in situ* hybridization data on *P* location patterns which were often found to be similar between homologous chromosomes within a given line (data not shown).

Seven lines showed few or zero defective *P* copies, whereas in three lines more than 60 per cent of the copies were defective (Table 2). The deletions observed are widely variable in size as well as in position (Fig. 1). Both 5′ and 3′ regions may be affected whereas in *D. melanogaster* the *P* derivative elements described are generally deleted in the 3′ region (O'Hare *et al.*, 1992; Nitasaka & Yamazaki, 1993). In several cases, the deletion seems to include all or a part of the inverted terminal repeat (ITR). This is true at least in the W1–7 line as the *Ava*II site at position 23 is missing. Such ITR-spanning deletions are expected as unique copies given their inability to transpose. In fact they give faint hybridization signals: this can result from unique copies as well as from low hybridization ability resulting from deletion in the homologous region. On the other hand, copies bearing deletions spanning up to 1.6 kb in the middle of the element sometimes correspond to very strong hybridization signals which suggest that they are present in numerous copies. No recurrent deletion events were found between lines.

Discussion

Among the 10 transformed lines analysed here, five (C2–10, S5–2, T1–4, W1–7 and L3–2) had been extensively studied during the first 70 generations following transformation (Montchamp-Moreau, 1990). Since then, one of them (W1–7) appears to have decreased in its *P* activity but not in its *P* copy number; this decrease having in fact begun between generations 25 and 60. The other four lines have remained stable with regard to their *P* copy number as well as to their dysgenic properties. This stability is in agreement with the low level of insertion site polymorphisms inferred from both Southern blot and *in situ* hybridization results. Results of the molecular analysis suggest that some of the deleted *P* elements observed at generation 50 have been maintained and amplified. In addition, new deletions have appeared, particularly *P* elements with a deletion spanning the 5′ region (Fig. 1) which had not been previously detected. The correlation between the number of *P* sites and the *P* activity which had been observed between generation 5 and generation 60 was no longer apparent and thus appears to be restricted to the invasion period. Molecular characterization of our lines did not reveal a clear dichotomy between lines with high *P* activity and those with moderate or low *P* activity: when complete *P* elements are taken into account, low as well as high numbers of *P* copies are observed in both low and high *P* activity lines. For example C2–10 (69 per cent GD sterility; high *P* activity) and Sw/W5 (97 per cent GD sterility; high *P* activity) exhibit 5 and 22 full-size *P* copies, respectively. On the other hand, L3–2 and Q8–4, which do not exhibit *P* activity, bear 6 and 16 full-size *P* copies, respectively.

The dynamics of invasion in *D. melanogaster* and *D. simulans* appear clearly different. This is supported by several aspects of our data. The first is

the absence of correlation between *P* activity and the number of full-size *P* elements detected by Southern blot. In *D. melanogaster*, strains with high numbers of full-size *P* elements induce high *P* activity (Bingham *et al.*, 1982; Rubin *et al.*, 1982).

Several hypotheses can be proposed to explain the differences observed between the two species. First, some copies we describe as full-size may be in fact defective ones. To clarify this, we would have to check that full-size *P* elements detected by Southern blot hybridization are canonical *P* elements. Although in *D. melanogaster* no substitution polymorphism has been detected (except at positions 32 and 33; Black *et al.*, 1987; O'Hare *et al.*, 1992) such events could occur in *D. simulans* and lead to inactive *P* copies. In addition, the molecular analysis used was not able to detect short deletions unless they spanned the restriction sites studied.

Secondly, *P* element activity could be dependent on position effects. In *D. melanogaster*, position effects have been revealed through the expression of reporter genes (O'Kane & Gehring, 1987). Strong and/or frequent position effects in *D. simulans* could mask a correlation between *P* activity and the number of complete *P* elements. A further difference between *D. melanogaster* and *D. simulans* is the total *P* element copy number per genome. The average number observed in *D. simulans* is only around half that in *D. melanogaster* (Daniels *et al.*, 1987; Montchamp-Moreau, 1990). This may result in differences in the balance of transposase production and regulation. The regulation of *P* transposition according to the model of *P* cytotype proposed by Lemaitre *et al.* (1993) may be stronger in *D. simulans*. Under this assumption, for a given number of complete *P* elements, the level of pre-mRNA may be lower in *D. simulans* than in *D. melanogaster*. A higher production of the 66 kDa repressor in *D. simulans* would result in both a higher repression effect and a lower ability to transpose and consequently induce dysgenic traits. This may account for lines bearing mainly full-size elements which exhibit low *P* activity and high repression (T1–4, SimT, W1–7, L3–2 and Q8–4). However, this could not explain why the C2–10b line exhibits low *P* activity and no regulatory property despite a high number of full-size *P* copies.

The final difference concerns the molecular structure of deleted *P* elements. In several lines, we observed derivative *P* elements with deletions spanning the 5′ region up to the ITR whereas in *D. melanogaster* such deletions have not been described (O'Hare *et al.*, 1992; Nitasaka & Yamazaki, 1993). This could be fortuitous or it may result from differences in the transposition mechanism as well as in selective pressure on such derivative *P* elements.

Our data and those found in the literature both emphasize that an absence of horizontal transfer might not be the reason for the absence of *P* elements in the genome of *D. simulans*. Our results indicate differences in *P* element behaviour in *D. simulans* and in *D. melanogaster*. In particular, they reveal specific properties of *P* elements in *D. simulans* with regard to their molecular structures as well as to the relationship between classical structure and dysgenic properties. Thus, we conclude that our data support the contention of Kimura & Kidwell (1994) in that the primary reason for the absence of *P* elements in natural populations of *D. simulans* may be the difficulty of establishment of *P* elements in this genome rather than the absence of a mechanism for horizontal transfer. This low invading power of *P* elements in the *D. simulans* genome may result from transposon–host interactions.

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