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Transposable element insertion patterns as test of contamination of a *Drosophila melanogaster* inbred line

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Abstract. A highly inbred line of *Drosophila melanogaster*, stable for the insertion pattern of the transposable elements copia and mdg1, was experimentally contaminated by flies from another line. We show that the alien genome income is clearly detectable by the changes induced in the insertion profiles of transposable elements, even twenty generations later.
Key words. Drosophila melanogaster; transposable elements; inbred lines; insertion pattern; transposition rate.

Sudden bursts of activity of transposable elements have been reported in *Drosophila* either in progeny from special crosses[1-7] or independently of these crosses[8-11]. These rates of change in genomic location may involve only one kind of element[10,11] or many of them[1,2]. It is sometimes argued, however, that contamination by alien flies may explain the new insertion pattern observed. This hypothesis is usually ruled out when the change in pattern only concerns one element among many[10-12]. Indeed, in the case of contamination by alien flies, changes in the insertion pattern of one element are unlikely to occur without simultaneous changes in the pattern of others. To test the validity and generality of this assumption, we have contaminated a highly inbred line characterized by a stable insertion pattern with flies from another inbred line with a slightly different insertion profile. The new insertion pattern was followed through generations. We show that patterns of two mobile elements, copia and mdg1, were sufficiently modified to allow the unambiguous detection of the alien genome income.

Materials and methods

We worked on inbred lines (No. 2 and 16) established in 1984[13]. These lines were maintained by one sib every generation until generation 100 after which they were maintained by mass mating (ten couples per generation) to avoid losing them because of very low viability. At various generations, the lines were analyzed by in situ hybridization with biotinylated probes[14] for the localization of the copia and mdg1 elements on all the chromosomal arms: 2–6 larvae were checked. The line 16 which was used in many other experiments is particularly well known for its copia and mdg1 insertion profiles. At generation 135, we contaminated it by introducing two fertilized flies from line 2 into a vial containing ten couples of line 16. After this crossing the contaminated line 16, named 16C, was further maintained by small mass mating. The insertion patterns of the line 16C for the copia and mdg1 elements were then determined in the following generations.

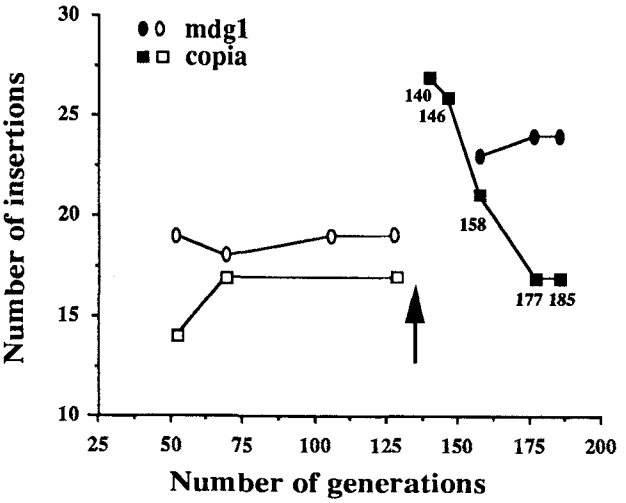
Results and discussion

As seen in the figure, the numbers of labelled insertion sites of both copia and mdg1 elements of line 16 were stable through time until the contamination event. When checked at generation 140 for copia and generation 158 for mdg1, the labelled insertion site numbers of line 16C were not the sum of the copy numbers of the two lines 16 and 2 (17 and 15 insertion sites for copia, 19 and 15 for mdg1, in lines 16 and 2, respectively) for two reasons: 1) because these two lines had many insertion sites in common (tables 1 and 2); we chose to cross these two lines with close insertion profiles so as to make the detection of contamination harder, and 2) because we checked the mobile element profiles a few generations after the crossing and thus the insertion sites in the contaminated line had time to segregate. The copia copy number then decreased gradually with the generations of small mass mating until generation 177 where it became stable again. This decrease concerned the non-common sites from lines 2 and 16 since the cross made them heterozygous and thus candidates for segregation. Similar results were observed for mdg1 although the copy number reached at generation 177 was higher than in each of the two initial inbred lines. Moreover, the very close numbers of mdg1 labelled sites seen at generations 158 and 177 (22 and 23 respectively, see the figure) do not mean that the 16C line was already stable. There were indeed some differences in the mdg1 insertion profiles of the 16C line between these 158 and 177 generations, as expected under segregation of heterozygous sites (table 1). Four new mdg1 sites were gained at generation 177 (4E, 44F, 92B from

Table 1. Mdg1 insertion profiles of the initial lines 16 and 2 (generation 128) and of the contaminated line 16C (generations 158, 177 and 185).

Chromosome arms	Lines 16	2	16C		
	128	128	158	177	185
X	3B	3B	3B	3B	3B
	-	4E	-	4E	4E
	7B	-	7B	7B	7B
	11C	-	11C	11C	11C
	19F	19F	19F	19F	19F
2L	25A	-	25A	25A	25A
	35CD	-	35CD	35CD	35CD
	36CD	-	-	-	-
	37A	-	37A	37A	37A
2R	42B	42B	42B	42B	42B
	-	44F	-	44F	44F
	-	-	46AB*	-	-
	49CD	49CD	49CD	49CD	49CD
	52A	52A	52A	52A	52A
	-	53A	-	-	-
	53C	-	53C	53C	53C
	59E	-	59E	59E	59E
3L	-	64C	64C	-	-
	73B	-	-	73B	73B
	-	75C	75C	75C	75C
	-	78A	78A	78A	78A
3R	-	85D	85D	85D	85D
	-	86B	-	-	-
	86C1	-	86C1	86C1	86C1
	86C2	-	86C2	86C2	86C2
	-	92B	-	92B	92B
	92DE	-	92DE	-	-
	96F	96F	96F	96F	96F
	97B	-	97B	97B	97B

The asterisk indicates a newly appeared insertion.



Number of labelled insertion sites of mdg1 and copia elements throughout generations of inbreeding: line 16 (white symbols), line 16C (black symbols). The arrow indicates the contamination event. Exact generation numbers are shown on the graph. We used the probe cDm5002 containing the copia element (5kb, refs 15, 16); the A fragment of the mdg1 element incorporated at the HindIII site of the pBR322 plasmid[17].

line 2, and 73B from line 16), and three sites were lost (64C from line 2, 92DE from line 16, and 46AB). The 46AB site was absent in both lines 16 and 2, suggesting a transposition event. Similar behaviour was observed for copia element at generation 140 (table 2) with the newly transposed site 35AF. None of these newly-appeared mdg1 and copia insertions were fixed in the 16C line since they disappeared over the next generations.

As seen in table 2, the decrease in copia copy number following the contamination process was very slow. Indeed, the copia copy number of line 16C remained stable with a similar insertion pattern between generations 140 (27 sites) and 146 (26 sites; mdg1 insertions were not checked at these generations). The only site lost was the newly transposed 35AF site (see generation 140). No other site changed during the period between generation 140 and 146. A large decrease in the number of insertions was observed only at generation 158. This maintenance of high copy number may reflect selection acting on flies with a high degree of heterozygosity and which may also have a high viability. It is only in the following generations that segregation associated with small effective population size significantly reduced the copia copy number.

Hence, if we compare the insertion profile of the line 16 in the early generations with the profile of the line 16C observed at the end of the experiment, and if we ignore the contamination event, we could conclude that during the generation period analyzed, copia had excised and transposed at similar rates while mdg1 had mainly transposed. This raises the problem of the historical characteristics of the line, strain or stock we are working on, and of the transposable element we are concerned with. Since estimations of transposition and excision rates are of great importance in determining which models of population biology of transposable elements may be accepted[18], values of these rates determined in laboratory strains should be used with caution to test models, especially if the history of these lines has not been regularly checked. We are thus clearly in need of reliable estimates of rates of movements in controlled populations. The picture we see in the genome of individuals may be only the reflection of a tendency towards a new equilibrium following an occasional, apparent, artefactual change in rate of movements.

These results clearly show that the contamination of an inbred line by another line with a closely related insertion profile leaves some signs still detectable 20 generations after the contamination. These signs will be even more detectable when the contamination comes from flies of completely different origin. This event is assumed to concern all transposable elements so that if one element insertion profile is modified, while the profile of others remains stable, a contamination hypothesis cannot be accepted. The recovery of the line as

Table 2. Copia insertion profiles of the initial lines 16 and 2 (generation 128) and of the contaminated line 16C (generations 140, 146, 158, 177 and 185).

Chromosome arms	Lines 16	2	16C				
	128	128	140	146	158	177	185
X	3F	-	3F	3F	3F	-	-
	12F	12F	12F	12F	12F	12F	12F
	-	15D	15D	15D	15D	15D	15D
2L	-	22A	22A	22A	22A	-	-
	25A	-	25A	25A	-	25A	25A
	-	-	26D	26D	26D	-	26D
	29AB	-	29AB	29AB	29AB	29AB	29AB
	30D	30D	30D	30D	30D	30D	30D
	-	-	35AF*	-	-	-	-
	-	38C	38C	38C	-	38C	38C
2R	-	42A	42A	42A	-	42A	42A
	42B	42B	42B	42B	42B	42B	42B
	50A	50A	50A	50A	50A	50A	50A
	57B	-	57B	57B	-	57B	57B
3L	67D	-	67D	67D	67D	67D	67D
	-	67E	67E	67E	67E	67E	-
	-	75A	75A	75A	75A	75A	75A
3R	84DE	-	84DE	84DE	84DE	84DE	84DE
	-	86D	86D	86D	86D	-	-
	-	88D	88D	88D	88D	-	-
	89A	-	89A	89A	89A	-	-
	89B	-	89B	89B	89B	-	-
	90A	-	90A	90A	90A	-	-
	90BC	-	90BC	90BC	90BC	90BC	90BC
	-	90E	-	-	-	-	-
	92D	-	92D	92D	92D	-	-
	-	92E	92E	92E	-	92E	92E
	98C	-	-	-	-	-	-
	100B	100B	100B	100B	100B	100B	100B

The asterisk indicates a newly appeared insertion.

a new homozygous one is, however, a long process that could be lengthened by selection for heterozygous flies. Following the insertion profiles of various transposable elements simultaneously is thus a powerful way of checking the events that shape the insertion pattern of a strain under study.

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