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Polymorphisms in the Genomic Distribution of Copia-like Elements in Related Laboratory Stocks of *Drosophila melanogaster**

Nikolaj Junakovic and Vincenzo Angelucci

Centro di Studio per gli Acidi Nucleici CNR, % Dipartimento di Genetica e Biologia Molecolare, Università degli Studi "La Sapienza," Piazzale A. Moro 5, 00185 Rome, Italy

Summary. The genomic distributions of the copia, 297, 412, mdg 1, and B 104 transposable elements have been compared by the Southern technique among two Oregon R and four Canton S *Drosophila* laboratory lines that have been maintained separately for defined periods of a few years. The heterogeneity of the autoradiographic patterns suggests that multiple transposition events have occurred during the time of separation. The hypothesis that transposition could be induced by variations of environmental parameters is discussed.

Key words: Transposons — Polymorphism — *Drosophila* — Southern technique

Introduction

The copia-like transposable elements represent a substantial fraction of the *Drosophila* middle repetitive DNA and are dispersed to many alternative sites in the genome (Finnegan et al. 1978; Ilyin et al. 1978). Mutations correlated with the insertion of these and other transposable elements have been genetically and molecularly characterized, showing that transposition can be a source of genetic variability (Rubin 1983; Levis et al. 1984). However, since little is known about the rate of transposition and the specificity of insertion, the evolutionary importance of transposition in generating variability is still poorly understood.

Previous studies aimed at estimating the rate of transposition in wild-type *Drosophila* were inconclusive. Cytological analyses in which the location of these transposons has been monitored for up to five years suggest that transposition is a rare event (Tchurikov et al. 1981; Young and Schwartz 1981). However, it has also been shown that individuals of laboratory as well as natural populations can be highly polymorphic in the location of copia-like elements, which suggests that transposition occurs at a measurable rate (Strobel et al. 1979; Montgomery and Langley 1983). Moreover, in a particular *Drosophila* mutant, multiple transposition bursts have been described, showing that transposition may occur at a high rate (Gerasimova et al. 1984, 1985).

The genomic location of copia-like transposable elements has been studied also by the Southern technique. The results showed that laboratory lines of a strain, when maintained separately for a few decades, exhibit substantially different DNA restriction patterns, a result that suggests that a significant number of transposition events occurred during the time since separation (Junakovic et al. 1984).

Here, the Southern approach has been used to compare the genomic distributions of five copia-like families in wild-type laboratory stocks of *Drosophila* that have been maintained separately for a shorter time. Pattern heterogeneity consistent with multiple transposition events is observed. The analysis of how this heterogeneity is distributed over time and in different populations suggests that transposon

Offprint requests to: N. Junakovic
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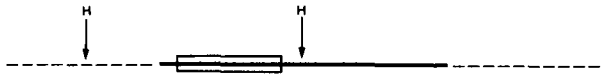

Fig. 1. Schematic representation of the Hind III restriction site (H) locations in the elements 297, 412, B 104, mdg 1, and copia (Finnegan et al. 1978; Tchurikov et al. 1978; Strobel et al. 1979; Dunsmuir et al. 1980; Scherer et al. 1982). The solid line represents a transposable element and the dashed line flanking genomic sequences. The boxed region indicates the portion internal to the transposable elements that was excised from the cloned transposons as previously described (Junakovic et al. 1984) and used as probe

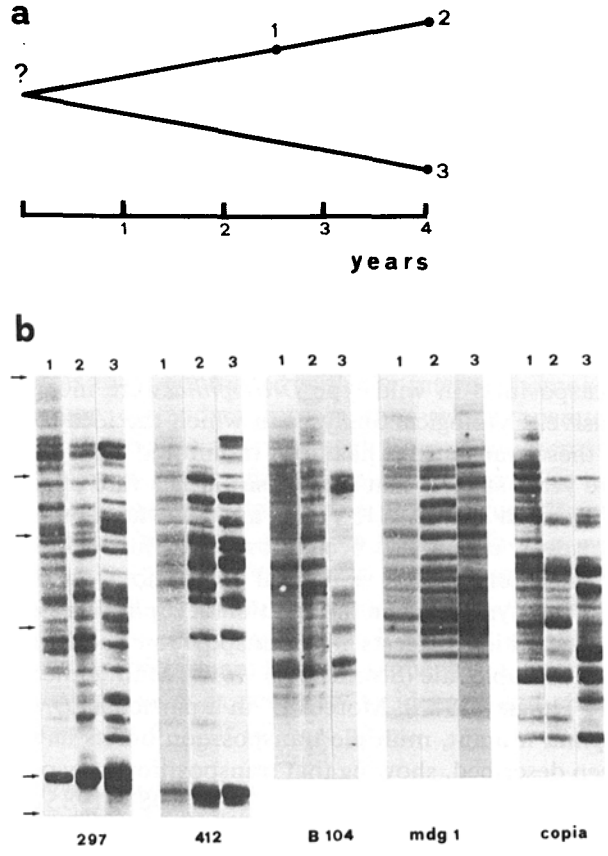

Fig. 2a,b. **a** Time relationship among the Oregon R laboratory stocks compared. An aliquot of the Oregon R strain was taken from the European Molecular Biology Laboratory (EMBL; Heidelberg) to Rome in 1980 and processed 2.5 and 4 years later (nodes 1 and 2). An additional aliquot was mailed from EMBL and processed in 1984 (node 3). The question mark indicates that the genomic distribution of the transposons was not analyzed in the population from which the two stocks were started. **b** Genomic distribution of the five transposon families in Oregon R (Heidelberg) laboratory lines at 2.5 and 4 years since separation. Lane numbers correspond to stocks in part a. DNA was extracted from pools of 100 flies, digested with Hind III, loaded in wide slots on agarose gels, run, and transferred to nitrocellulose filters, which were cut into five strips per slot and hybridized with the ^{32}P-labeled transposons. The arrows indicate the mobilities of the Hind III lambda DNA fragments of 23.7, 9.46, 6.66, 4.26, 2.30, and 1.96 kilobases

families can be rearranged at different rates and that transposition is discontinous in time.

Materials and Methods

Drosophila Stocks. The stocks of the Oregon R strain originate from the European Molecular Biology Laboratory (Heidelberg). An aliquot of about 200 flies was taken to the Institute of Genetics (University of Rome) in 1980. Samples of this line were analyzed 2.5 and 4 years later. In 1984, an additional aliquot was mailed from Heidelberg and processed. The Canton S strain originates from the International Institute of Genetics and Biophysics (IIBG; Naples). In 1977 an aliquot was taken to the Institute of Genetics (University of Naples). In 1982 another aliquot was taken to the Institute of Genetics (University of Rome). DNA was extracted and processed from the IIGB stock in 1982, and again from the IIGB stock and from the Institute of Genetics (Naples) and Institute of Genetics (Rome) stocks in 1985.

DNA Extraction. Individual adult flies were homogenized in 50 μl of cold 0.1 M Tris-HCl and 0.1 M ethylenediaminetetraacetate (EDTA), pH 9, with a Perspex rod shaped to fit the bottom of an Eppendorf tube. Fifty microliters of preheated 2% sodium dodecyl sulfate (SDS) was added and the mixture was incubated at 65°C for 1 h. After cooling at room temperature for a few minutes, 13 μl 8 M potassium acetate was added. After 30 min on ice, the samples were spun for 10 min in an Eppendorf centrifuge. Isopropanol (60 μl) was added to the supernatant and after 10 min at room temperature the samples were spun again for 10 min. The pellet was washed twice with 70% ethanol, dried under vacuum, and resuspended in 10 mM Tris, 1 mM EDTA, pH 8. For extractions from pools of flies the volume of the homogenizing buffer was adjusted to 10 μl per 1–2 flies and the volumes of the subsequent components were changed accordingly.

Restriction Enzymes. The enzymes were purchased from Biolabs and used according to the supplier's instructions.

Agarose Gel Electrophoresis. The Hind III digests were loaded in 5-cm-wide slots on a 0.7% agarose gel in phosphate buffer. Electrophoresis was carried out at 2 V/cm for 20 h. After transfer to nitrocellulose filters (Southern 1975), the filters were cut into five strips per slot. A single strip from each slot was then hybridized to one labeled transposon fragment. This experimental detail ensures that restriction patterns detected with different transposons are comparable, because it guarantees that quality of DNA preparation, extent of restriction enzyme digestion, and electrophoretic conditions are the same in all cases. It also allows different digests to be compared, provided they share at least one common pattern.

Hybridization. The restriction fragments of the cloned transposons were electroeluted from the agarose gels and labeled by nick translation to a specific activity of about 10^7 cpm/μg. Hybridization and washing conditions were as previously described (Junakovic et al. 1984). The films were exposed for 24–48 h at −70°C with an intensifying screen.

Results

A simplified restriction map of the common structure of the five transposons used (297, 412, B 104, mdg 1, and copia) is shown in Fig. 1. They all have

a Hind III restriction site in the central region. Digestion of the genomic DNA with this enzyme produces fragments that bear transposon sequences and that are heterogeneous in size because of the different locations of the Hind III sites in the external regions flanking the elements. After agarose gel electrophoresis and transfer to nitrocellulose filters, these fragments can be detected using as a probe an appropriate internal segment excised from the cloned transposons (Fig. 1). The resulting autoradiographic pattern is expected to change as transposition events take place, since the new insertion sites are associated with new flanking sequences in which the locations of the Hind III sites external to the elements are very likely to be different.

An important prerequisite for the validity of this approach is that the distribution of restriction sites internal to the genomic transposable elements be conserved. Comparisons among *Drosophila* species (Dowsett 1983), strains (Finnegan et al. 1978; Strobel et al. 1979), and laboratory stocks (Junakovic et al. 1984) have previously shown that although some variants can be detected, the majority of the genomic elements meet this requirement. Here, five laboratory lines of the wild-type Oregon R and Canton S strains, maintained separately for up to 8 years, have been compared by this approach (Figs. 2a and 3a). Populations already diverging have been analyzed because previous information indicated that 5 years was too short a time to detect transposition in the laboratory (Tchurikov et al. 1981; Young and Schwartz 1981). Thus, the initial stocks from which the lines were started could not be analyzed. However, preliminary experiments showed that the pattern heterogeneity detected was not equally distributed among the five transposon families analyzed. This indicated that a study of the relative mobilities of different transposon families was feasible. Since the elements of copia-like families are dispersed throughout the genome, both contamination and genetic drift should affect the different families equally.

In Fig. 2b the patterns obtained from the Oregon R lines are shown. DNA was extracted from groups of 100 flies and processed as described in Materials and Methods. A preliminary observation is that the patterns of the five transposon families exhibit many similarities. Since the genomic locations of transposable elements differ among *Drosophila* strains (Ilyin et al. 1978; Strobel et al. 1979), this similarity is an internal control, particularly evident for transposons 297 and mdg 1, of the common origin of the stocks compared. For the same reason, significant contamination by unknown flies can be ruled out, as the genomic distribution of the 297 and mdg 1 elements should have been the same in the contaminating and contaminated flies.

Pattern heterogeneity, which is correlated with transposition, is unevenly distributed among the five transposons. The 297 and mdg 1 families appear the least active in transposition. The copia patterns are the most heterogeneous ones. A number of changes in presence or absence and relative intensity of bands are detected at the 1.5-year interval and even more after 4 years of divergence. The 412 and B 104 families exhibit few changes between lanes 1 and 2. However, the 412 pattern in lane 3 is still reminiscent of the pattern in lane 2, whereas the B 104 pattern in lane 3 is substantially different from that in lane 2. This implies that during the separation time either the B 104 elements were involved in more cycles of transposition or at some stage the B 104 family underwent a particularly active burst of transposition.

The evidence suggesting that different transposon families are not equally mobile raises the question of whether this is due to structural differences among transposable elements that might code for more or less active "transposases." Alternatively, parameters such as the genomic location or the type of transposition-inducing signals could affect the mobility of transposable elements. In the first case, the relative order of mobility among the five transposons would be expected to be maintained in separate *Drosophila* populations. In the second case, the prediction is that the mobility order could vary among different populations. To distinguish between the two possibilities, we analyzed the genomic distributions of the above copia-like elements in four diverging laboratory stocks of the Canton S strain. Figure 3a outlines the time relationship among the populations compared, and the resulting autoradiographs are shown in Fig. 3b. Pattern heterogeneity is observed. This heterogeneity was quantitated as described in the legend to Fig. 3; Fig. 3c is a histogram summarizing the results. It appears that the order of mobility varies among *Drosophila* strains. The 297 patterns are the most heteogeneous here and the most homogeneous in the Oregon R strain (Fig. 2). This suggests that a particular transposon structure is not sufficient to determine the rate of transposition.

Additional information about the difference in mobility can be gained by comparing patterns obtained from individual flies. The relationship between polymorphism in genomic distribution of copia-like elements and transposition has been discussed in previous reports (Strobel et al. 1979; Montgomery and Langley 1983). Individuals of a population are expected to be more polymorphic for the location of more mobile elements and vice versa. In the Oregon R lines that we examined (Fig. 2), copia appears to be more mobile than mdg 1. We therefore processed individual flies from the

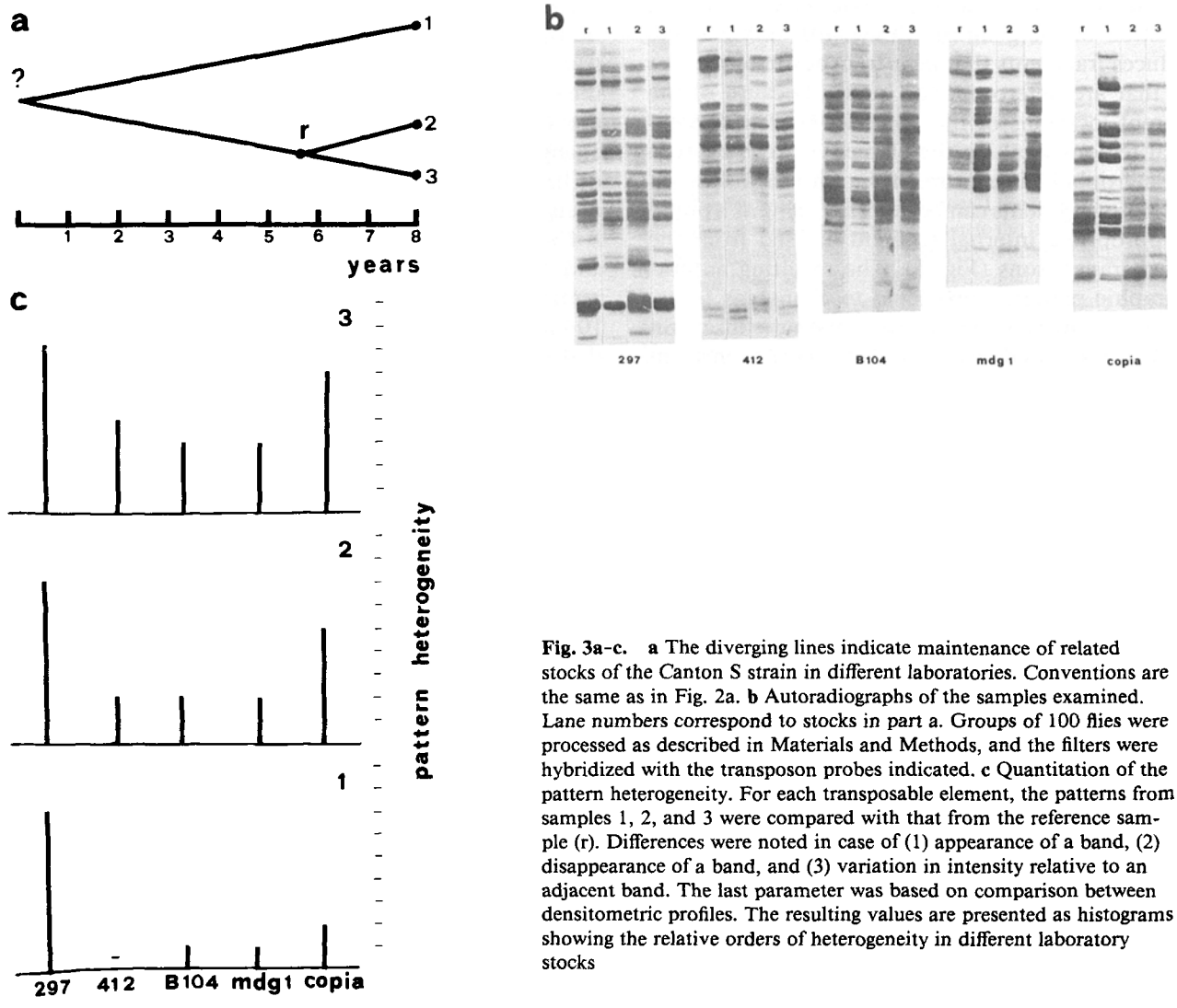

Fig. 3a–c. **a** The diverging lines indicate maintenance of related stocks of the Canton S strain in different laboratories. Conventions are the same as in Fig. 2a. **b** Autoradiographs of the samples examined. Lane numbers correspond to stocks in part a. Groups of 100 flies were processed as described in Materials and Methods, and the filters were hybridized with the transposon probes indicated. **c** Quantitation of the pattern heterogeneity. For each transposable element, the patterns from samples 1, 2, and 3 were compared with that from the reference sample (r). Differences were noted in case of (1) appearance of a band, (2) disappearance of a band, and (3) variation in intensity relative to an adjacent band. The last parameter was based on comparison between densitometric profiles. The resulting values are presented as histograms showing the relative orders of heterogeneity in different laboratory stocks

population termed 3 in Fig. 2 and hybridized the digests with copia. Subsequently the same filters were hybridized with mdg 1. As shown in Fig. 4, pattern polymorphism is detected with both probes. This is particularly evident in the high-molecular-weight region of copia and presumably accounts for the disappearance of some bands in that region of the copia patterns shown in Fig. 2b (lanes 2 and 3 with respect to lane 1). The polymorphism was quantitated as the ratio between the number of bands common to all individuals and total number of bands of different mobility. The ratios (0.47 and 0.25 for mdg 1 and copia, respectively) show that the individuals examined are more polymorphic with respect to the distribution of copia elements than with respect to that of the elements of the mdg 1 family. This is in accord with the evidence from Fig. 2 showing that the copia elements appear to be more mobile than the mdg 1 elements. The bands that account for the polymorphism between individuals appear to belong to discrete size classes, suggesting that a limited number of specific insertion sites have been involved in recent transposition events. Other bands, present in all the individuals, probably reflect more stable insertion sites. Polymorphic and stable insertion sites have been previously observed also by in situ hybridization (Strobel et al. 1979; Pierce and Lucchesi 1981).

Discussion

In the work reported here, the Southern technique was adopted for the study of transposition of copia-like elements in *Drosophila*. Obviously, in situ hybridization is more appropriate for locating transposon sequences along the chromosomes. However, detecting changes in location over short chromosomal distances is difficult or impossible by the cytological approach. In addition, if different transposon families are to be compared in different populations, it may be very laborious to process a

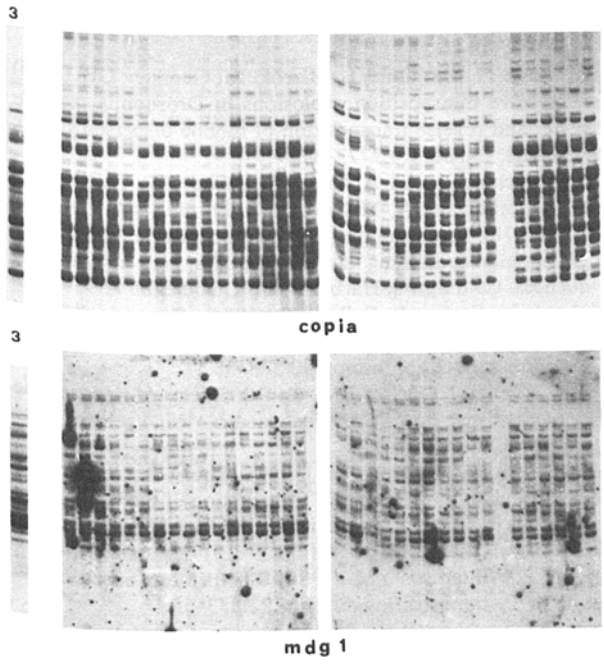

Fig. 4. Comparison between Southern-blot patterns from individual flies. DNA was extracted from individual adult flies of the population termed 3 in Fig. 2 and was digested with Hind III; the fragments were then analyzed by gel electrophoresis and transferred to nitrocellulose filters. The filters were hybridized with the ^{32}P-labeled copia internal fragment and exposed to x-ray film. The radioactivity was allowed to decay for 6 months and filters were rehybridized with mdg 1. This comparison shows that the individuals examined are more polymorphic for the locations of copia than for those of mdg 1 elements (see text). Partial digestion is an unlikely explanation for the copia polymorphism, as the two sets of patterns were obtained from the same digests. For ease of comparison, the corresponding pool patterns are reproduced at a convenient magnification

high enough number of samples to achieve a statistically significant comparison. These limitations of the cytological approach are greatly reduced in the Southern technique. Changes in location of a few kilobases can easily be detected. A general picture of a population can be obtained by simply analyzing DNA extracted from groups of flies. If relative mobility of different transposable elements is to be assessed, the same DNA digest can be probed with different labeled transposon sequences.

By this approach, we have detected pattern heterogeneity, which may be due to different causes: (1) Partial digestion can give rise to pattern heterogeneity. This is ruled out by the experimental design (see Materials and Methods) because portions of the same digest are present on the nitrocellulose strips probed with different transposons. Different digests can be compared because they exhibit one or more common patterns. (2) Contamination by unrelated *Drosophila* strains is another potential source of heterogeneity. However, it has been shown that in different *Drosophila* strains the genomic distributions of all the copia-like elements tested are polymorphic (Strobel et al. 1979), whereas the patterns observed in this report are heterogeneous for some transposon families only. (3) Selection for a particular genomic distribution does not seem to play a role either, because all the *Drosophila* strains and laboratory stocks of a strain compared so far differ in this trait (Tchurikov et al. 1978; Strobel et al. 1979; Junakovic et al. 1984). (4) Random genetic drift is not a source of population polymorphism. On the contrary, in the absence of migration and selection, random drift is expected to lead to homozygosity of laboratory *Drosophila* stocks. This implies that the polymorphisms observed are established and maintained by a process effective enough to counterbalance the homogenizing effect of genetic drift.

The finding that individuals of *Drosophila* laboratory populations can be highly polymorphic in the distribution of copia-like elements has been previously reported and the relationship between such polymorphism and transposition discussed (Strobel et al. 1979; Pierce and Lucchesi 1981). Here, these observations have been confirmed and a correlation has been established between the intrastock polymorphism and the apparent mobilities of different transposable elements.

Based on these criteria, we interpret the pattern heterogeneity observed here to be due to the association of transposable elements with new flanking sequences, that is, transposition. This phenomenon appears to be discontinuous in time and to vary in rate and timing among different transposon families.

Cytological observations consistent with this conclusion have been reported. Copia and mdg 1 appear to be more mobile than Dm2066 and mdg 4 (Ananiev et al. 1984). Transposition bursts, that is, variations in the rate of transposition, have been described over a 4-year period in genetically unstable *Drosophila* mutants (Gerasimova et al. 1984, 1985). In the latter case all the copia-like elements tested (as well as P and FB) appeared to have been mobilized synchronously.

Altogether, these data suggest that there is an inducer capable of triggering transposition of all the *Drosophila* transposable elements, as well as a number of other inducing signals (or gradations of the same signal) that act on particular transposon fam-

ilies only. The common trait of these transposition inducers would be discontinuous activity in an apparently homogeneous laboratory environment.

We believe that variations of environmental parameters can induce transposition. Temperature-induced transposition has been described in yeast (Paquin and Williamson 1984). In *Drosophila,* copia transcription, a putative intermediate step in transposition, is responsive to environmental stress (Strand and McDonald 1985), and preliminary evidence suggesting that heat shock promotes transposition has been reported (Barsanti and Palumbo 1985). Occasional induction of transposition could account for the previous reports of both homogeneity and polymorphism in location of transposable elements among individuals of different *Drosophila* populations.

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