

## Transposition of $P\{lacW\}^{\gamma Cop057302}$ into the germline of *Drosophila melanogaster* correlates with retaining of the original insertion

Received for publication, August 18, 2008

Accepted, August 27, 2008

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### Abstract

We performed a genetic and molecular screening in *Drosophila melanogaster* in order to investigate the mobilization pattern of  $P\{lacW\}^{\gamma Cop057302}$ , a lethal transposon insertion located in 5'UTR of  $\gamma Cop$  gene from 100C chromosomal region. New mutant lines, with modified levels of eye pigmentation, were obtained consequent to transposition in both male and female germline. A total of 153 founder individuals were preliminary screened, 68 of them descending from dysgenic males and 85 from dysgenic females. From a total of 114 reinsertion events occurring within the third chromosome, 98 were associated with an apparent conservation of the original insertion. A consistent tendency towards retaining of the original insertion has been also noticed in an older collection of 40 mutant lines harboring transposition events that also affect the third chromosome.

Our data show a strong correlation between transposition and the retaining of  $P\{lacW\}^{\gamma Cop057302}$  original insertion, a phenomenon that may unravel functional aspects of the P element's mobilization pattern. Conservation of the starting element may also represent an useful marker in genetic screenings aiming to detect local reinsertions of the P element.

Keywords: *Drosophila melanogaster*,  $P\{lacW\}^{\gamma Cop057302}$ , original insertion, germline, transposition pattern

### Introduction

The complete sequencing of *D. melanogaster* (the fruit fly, the vinegar fly) genome [1] opened new fields of investigation and the most promising one appears to be the functional genomics. One goal of *D. melanogaster* functional genomics is the understanding of the biological significance of P mobile element transposition. A consistent experimental advantage relies on observations revealing that P artificial derivatives seem to share the same mobilization characteristics as the natural P element.

Transposition of P mobile element is best described by the *cut-and-paste* mechanism, meaning that the mobile element is excised and then integrated as a discrete double stranded DNA fragment in a different genomic region [2, 3, 4]. According to this model, the canonical transposition implies an excision, a reinsertion and repairing of the excision site. If the process of excision repair employs as a template the sister chromatid still containing an

identical copy of the transposon (a probabilistic event) then the consequence will be the reintroduction of a new transposon copy exactly into the original donor site. Instead, if the repairing process uses the homologous chromosome as a template (in individuals heterozygous for the insertion) the site previously affected by the original insertion will be restored, equivalent with reversion of the insertional mutation [2, 4, 5].

The excised transposon may reinsert intra-chromosomally and/or inter-chromosomally and for the transpositions in the same chromosome the new insertion can target the donor chromatid or the sister one [2]. The local reinsertions in the donor chromatid (*in cis*) are often associated with a complete or partial repair of the original insertion [6, 7] but for some genetic experimental strategies, retaining of the starter mobile element can not be unequivocally linked with either one of the sister chromatids [8]. The term *primary local reinsertion* may be used to denote reintegration of a transposon copy precisely at the donor site as a result of the donor site restoration and the term *secondary local reinsertion* may be employed to define insertion of the excised transposon into a relative genomic neighborhood. The situation in which reinsertions are not in the relative vicinity of the original insertion is simply described as *reinsertion*. In the special case of a *local conservative transposition* (or *local conservative reinsertion*), both types of local reinsertions occur in the same germinal cell of a dysgenic individual.

Transposition is thought to be involved in the modulation of *D. melanogaster* genome evolution. Very often, the P element insertions are preferentially located in 5'UTR region and have an affinity for the inferred consensus GTCCGGAC sequence [9]. There were described both *hot spot* loci, in which a high frequency of insertion was observed [10] and *cold spot* loci which are refractory to insertion. Such an insertional asymmetry could have a functional meaning. Pertinent data suggest that a specific *P{EP}* artificial transposon insertion exhibits a biased mobilization pattern in male germline, but a comparative analysis of transposition in both male and female germlines is expected to offer more relevant data [8]. Similar studies were also performed in the pre-genomic era and revealed preferences for insertion of an engineered P transposon into enhancers depending on the germline origin [11]. The main goal of such studies is to identify a pattern for local conservative transpositions by hunting for reinsertions in genes which are transcriptionally active either in male or in female germline.

Here we present scientific results concerning conservation of the original insertion of a *P{lacW}* artificial transposon [12], consequent to its intra-chromosomal transposition in both female and male germline. For such a purpose, we used *l(3)S057302* transgenic line, containing a unique *P{lacW}* lethal insertion, located in 5'UTR of *gammaCop* gene, which is placed in 100C chromosomal region [13, 14, 15]. The insertion is denoted *P{lacW}<sup>gammaCop057302</sup>* and its genomic position is 27397925, according to Release 5.3 of *D.melanogaster* genome [15].

Mobilization of *P{lacW}<sup>gammaCop057302</sup>* transposon was induced in an attempt to obtain local reinsertions in genes located in 100 chromosomal region, into the relative vicinity of *gammaCop* gene. The experimental approach presented here represents a preliminary step of our endeavour towards investigation concerning the biological significance of conservative reinsertions of P element derivatives.

## Materials and methods

**Genetics:** The lines used in our experiments have a *w* background and are as it follows: *l(3)S057302/TM6TbHu* and *l(3)S057302/TM3SerGFP* as starter lines,  $\Delta 2\text{-}3\text{Sb}/\text{TM}2\text{Ubx}$  transposase source and *TM3SbSer/TM6TbHu* double balancer line. Lines were raised on an yeast-cornmeal-agar medium. All genetic crosses were performed and maintained at room temperature (20<sup>0</sup>-25<sup>0</sup> C). The flies were selected and scored for their phenotype using both *Carl Zeiss Jena* and *Olympus SZ61* binoculars.

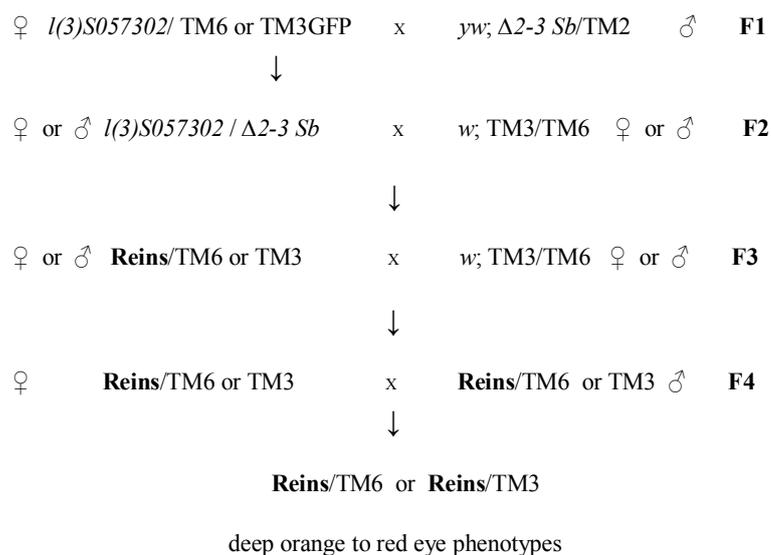
**Molecular analysis:** G1 and G3 primers were manually designed in our laboratory, and the sequence for MM11 primer was provided by Dr. Matyas Mink (Molecular Biology and Genetics Department, Szeged University, Hungary). G1 primer is 5'CTTCGTCACAATTCCGGC3' and anneal to a sequence located upstream from *gammaCop* gene and G3 is 5'GTTCTGGTACGCATTGCTC3' and targets a sequence located within *gammaCop* gene; MM11 specific primer has the sequence 5'CGACGGGACCACCTTATGTTATTTC3' and anneals in different orientations to both  $P\{lacW\}$  inverted repeat ends (IRs). Using modified versions of a classical protocol [16], we extracted genomic DNA either from 30 flies/line in the case of the lethal strains 1-40, or from each of the 154 mutant flies further described. For molecular confirmation of the primary local reinsertions we used a triplex PCR method. In order to standardize the experimental approach, PCR analysis was made with same primers combination (G1 + G3 + MM11). Triplex PCR technique using as a template the genomic DNA from *l(3)S057302/TM6TbHu* strain yields three amplicons with the following theoretically estimated dimensions: G1 + G3 = 819 bp (also a positive reaction control since is produced using the wild type template in heterozygous), G1 + MM11 = 538 bp and G3 + MM11 = 351 bp. Concentrations of the PCR reagents into a final volume of 20  $\mu\text{l}$  were: 0,2  $\mu\text{M}$  for G1 and G3 primers (for MM11 primer the concentration was doubled), 200  $\mu\text{M}$  dNTP, 0,05 u/ $\mu\text{l}$  Taq enzyme, 1X buffer, 1,5 mM  $\text{MgCl}_2$  (Promega reagents). The PCR program was: 94<sup>0</sup> C - 5'; (94<sup>0</sup> C - 30'', 52<sup>0</sup> C - 30'', 72<sup>0</sup> C - 1') x 30; 72<sup>0</sup> C - 5'; 4<sup>0</sup> - 5'. PCR reactions were performed in a gradient temperature *Corbett PalmCycler CGI-96*. The molecular weight marker that we used was *BenchTop 100 bp DNA Ladder* (Promega). Electrophoresis was performed in 1% TAE agarose gel.

**Bioinformatics:** For bioinformatics analysis we used *Apollo* software [17] and *FlyBase* database [15].

## Results and discussions

In our experiments we used the lethal chromosome *l(3)S057302* [15, 18] balanced in two different lines, each of them having a *w* genetic background. This lines harbors a single copy/genome of the  $P\{lacW\}$  artificial transposon, which is located in the 5'UTR region of *gammaCop* gene, placed in 100C chromosomal region [13]. This transposon has a copy of *miniwhite* allele as a genetic marker [12] and the eye color of flies heterozygous for the insertion is orange (in the absence of the transposon, because of the *w* genetic background, the eye would be white). The insertion is symbolized  $P\{lacW\}^{\gamma\text{Cop}057302}$  and determines *gammaCop*<sup>057302</sup> allele, which is essential for the normal embryo development [13, 14].

The mobilization of  $P\{lacW\}^{\gamma\text{Cop}057302}$  transposon (Fig. 1) was induced in dysgenic males or females in the presence of the artificial transposase source  $\Delta 2\text{-}3\text{Sb}$  [19].

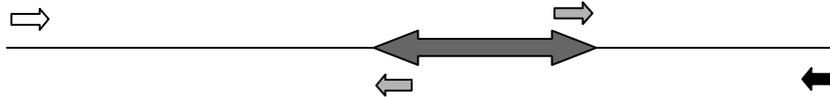


**Figure 1.** Genetic scheme used to obtain  $P\{lacW\}$  reinsertions. **Reins** symbol stands for reinsertions associated with deep orange to red eye phenotype.

By mating  $l(3)S057302/TM6$  or  $l(3)S057302/TM3SerGFP$  flies with  $\Delta 2-3Sb/TM2$  from the opposite sex we obtained dysgenic flies displaying mosaic eyes. Variegation of the eye color is a direct consequence of  $P\{lacW\}$  element mobilization. Mosaic eyes are an indirect proof that the mobilization is expected to take place in all fly cells, including the germline cells also. As a result, some gametes may carry conservative transpositions, equivalent with an increase in *miniwhite* copies number. These gametes were recovered by selecting deep orange and red eye progeny (phenotypes determined by the dose effect of *miniwhite* allele) obtained by mating dysgenics with *w* flies from the double balancer strain. We individually mated 22 dysgenic females (the underlined crosses) and 16 dysgenic males with the double balancer strain (for a total of 38 distinct crosses). This crosses were symbolized as it follows: *MZ2.1*; *MZ2.2*; *MZ2.A*; *MZ2.B*; *MZ2.C*; *MZ2.D*; *MZ3.A*; *MZ3.B*; *MZ3.C*; *FTMZA*; *FTMZB*; *FTMZC*; *FTMZD*; *FTMZ.1*; *FTMZ.2*; *FTMZ.3*; *MZ3.1*; *MZ3.2*; *MZ3.3*; *MZ3.4*; *MZ3.5*; *MZ4.1*; *MZ4.2*; *MZ4.3*; *MZ5.1*; *MZ4.A*; *MZ4.B*; *MZ4.C*; *MZ5.A*; *MZ5.B*; *MZ5.C*; *MZGFP-1*; *MZGFP-A*; *MZGFP-B*; *MZEM1*; *MZEM-A*; *MZEM-B*; *MZEM-C*. From *F1* descendants of these crosses we selected 159 flies with deep orange or red eyes phenotype which are likely to contain local conservative reinsertion phenomena. Each selected individual was mated with double balancer flies; 153 flies out of the total number of 159 had been fertile and allowed us to obtain new strains with putative local conservative reinsertions. When *F1* larvae, pupae or adults appeared, we selected 151 founder flies and extracted their genomic DNA using a one fly extraction protocol (see *Materials and Methods* section). The two exceptions are *FTMZ.A.F1* female which died so we used one of its female offspring instead, and fertile male *MZ2C.M2* which also died before extraction of its genomic DNA and the molecular analysis will be performed on its progeny. From the total of 159 flies, two males, symbolized *MZ3.4.M3* and *MZGFP.B.M1* were analyzed by triplex PCR, but they were sterile and no progeny was obtained. Thus, a total of 154 different flies were used for

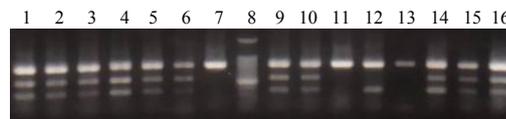
this molecular screening. *MZ2B.M1*, *MZ3.4.F6*, *MZ3.4.F8* and *MZ2C.M8* flies died before being analyzed or giving descendant lines.

By using a triplex PCR reaction with G1, G3 and MM11 primers (Fig. 2), we performed a molecular screening in order to verify if  $P\{lacW\}^{\gamma\text{Cop}057302}$  was retained in each of the selected individuals.

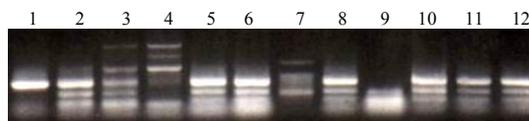


**Figure 2.** A schematic representation, from left to right, of the primers used in the triplex PCR reaction employed to detect conservation of  $P\{lacW\}^{\gamma\text{Cop}057302}$  original insertion (the double headed, deep grey arrow), located into the 5'UTR of *gammaCop* gene: G1 primer anneals to a genomic sequence located upstream to  $P\{lacW\}^{\gamma\text{Cop}057302}$  and is represented by a white arrow; MM11 primer is represented by two distinct grey arrows pointing to opposite directions and specifically anneals to both IRs of  $P\{lacW\}^{\gamma\text{Cop}057302}$ ; G3 primer (the black arrow) anneals downstream to  $P\{lacW\}^{\gamma\text{Cop}057302}$  and is specific for a sequence pertaining to the second exon of *gammaCop*.

Results presented in Fig. 3 and Fig. 4 (the complete results may be provided at request) illustrate a variety of specific phenomena associated with the transposition. For 12 transposition events located within different third chromosomes we obtained either only the G1 + MM11 amplicon or only the G3 + MM11 amplicon corresponding to the starter element location.



**Figure 3.** The molecular screening by triplex PCR for the identification of  $P\{lacW\}^{\gamma\text{Cop}057302}$  retaining in the following mutant flies: *MZ2D.M1* (gel electrophoresis lane 1); *MZ3B.M1* (lane 2); *MZ3C.M1* (lane 3); *MZ5A.M1* (lane 4); *MZ5A.M2* (lane 5); *MZ5B.M1* (lane 6); *MZ5B.M2* (lane 7); *MZ5C.M1* (lane 9); *MZ5C.M2* (lane 10); *MZ5C.M3* (lane 11); *FTMZ.B.M1* (lane 12); *FTMZ.D.MS* (lane 13); *MZGFP.B.M1* (lane 14; a sterile fly); *MZGFP.B.M2* (lane 15); positive control for triplex PCR with a template DNA from *l(3)S057303/TM6TbHu* strain (lane 16); lane 8 represents the 100 bp molecular weight marker (Promega). The wild type amplicon of 819 bp (the highest one) obtained with G1 + G3 stands also for a positive control for PCR.



**Figure 4.** The molecular screening by triplex PCR for the identification of  $P\{lacW\}^{\gamma\text{Cop}057302}$  conservation in the following mutant flies: *FTMZ.D.F1* (gel electrophoresis lane 1); *FTMZ.D.F2* (lane 2); *MZGFP.B.F2* (lane 3, the heavier supplementary amplicons suggest the occurrence of putative secondary local reinsertions); *MZGFP.B.F3* (lane 4, the supplementary amplicons also suggest the presence of putative secondary local reinsertions); *MZ4.C.F4* (lane 5); *MZ4.I.F1* (lane 6); *MZ2D.F2* (lane 8); *MZ2D.F3* (lane 9); *MZ3C.F1* (lane 10); *MZ3C.F2* (lane 11); *MZ5B.F3* (lane 12); lane 7 represents the 100 bp molecular weight marker (Promega). The wild type amplicon of 819 bp obtained with G1 + G3 stands also for a positive control.

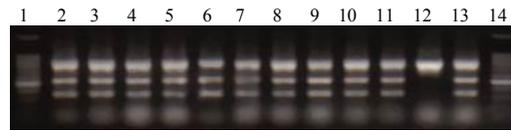
In Fig. 3, the gel electrophoresis lane 12 reveals such an example, where the lower band is specific for G3 + MM11 combination and accompanies the wild-type amplicon standing for a positive control of PCR reaction (see *Materials and Methods*). Such a situation may reflect a

failure during the original insertion repairing process [20], leading to reinsertion of an incomplete  $P\{lacW\}$  element at the original locus. Anyways, since the repairing process is error prone, one is not able to discriminate between an internally complete and an incomplete transposon copy only by molecular confirmation of IRs repair. Sequence analysis of several P elements revealed that a 138 bp sequence at the 5' end and a 216 bp sequence at the 3' end are necessary for transpositions [21]. These sequences include the perfect terminal inverted repeats of 31 bp and an 11 bp sequence found in inverted orientation at 130 bp apart from each end [22], both sequences being indispensable for the mobilization process. Since MM11 primer binds only to IRs, analysis of  $P\{lacW\}^{\gamma Cop057302}$  conservation in our experiments relies (aside from using *miniwhite* allele activity as a genetic marker) on detection of IRs repairing evidence, a method successfully used also in other studies [8].

We have counted for  $P\{lacW\}^{\gamma Cop057302}$  retaining in 153 out of the 154 different individuals since the female *MZ3.4.F7* did not yield clear results and its progeny was lost before we were able to perform their analysis. Out of them, 68 flies descended from dysgenic males and 85 flies descended from dysgenic females. Triplex PCR revealed that in 131 flies (where 61 are derived from dysgenic males and 70 from dysgenic females) both IR ends of the original insertion have been conserved. This means that 85.6% of the transpositions were accompanied by the apparent retaining of original insertion. Conservation of the starter mobile element occurred in different percentages in both dysgenic male and female flies (89.7% and, respectively, 82.3%). We also calculated the frequency of the original insertion's retaining for a total of 114 transposition events that occurred into the third chromosome (56 occurring in dysgenic males and 58 in dysgenic females). Out of them, 98 conserved both IRs, where 50 events descend from dysgenic males and 48 events descend from dysgenic females. The frequency of the original insertion retaining is about 86%, with similar differences as the above mentioned ones between events occurring in dysgenic males (89.3%) and in dysgenic females (82.7%).

The experimental approach allowed us to eliminate from our draft collection those lines giving only the wild type amplicon (Fig. 3, lanes 7 and 13; Fig. 4, lane 1), since they may have failed to preserve/repair the donor mobile element. Nevertheless, these lines are to be kept in a different collection and will help us to analyze the pattern of non-conservative reinsertions. For *MZ3.2.M5*, *MZ4C.M6*, *MZGFP.B.F2* and *MZGFP.B.F2* flies, triplex PCR generated more than three amplicons. We suppose that some of the supplemental amplicons could have been obtained with MM11 primer alone, since it anneals in two different orientations to the IRs.

In a previous small scale mutagenesis project, we mobilized  $P\{lacW\}^{\gamma CopS035702}$  original insertion in the male germline and obtained a number of 40 mutant lethal strains (symbolized 1-40) harboring transpositions events affecting the third chromosome. After a preliminary molecular screening proving that in most of the strains the original insertion was conserved, we performed the same triplex PCR with G1, G3 and MM11 primers (Fig. 5). The screening revealed that 33 strains (82.5%) have retained  $P\{lacW\}^{\gamma Cop057302}$  original insertion, a value that is close to the results previously presented. An unambiguous connection between primary local reinsertions and secondary local reinsertions into the same chromatid can not be described for some of the mutant chromosomes described in this paper. Exceptions are represented by the twelve above mentioned chromosomes for which only one IR of  $P\{lacW\}^{\gamma Cop057302}$  was repaired. Although recently was proposed that the excised transposon would land preferentially into the sister chromatid [20], reinsertions into the donor chromatid were also described [2].



**Figure 5.** The molecular screening by triplex PCR for the identification of  $P\{lacW\}^{\gamma Cop057302}$  retaining in the following mutant lines: strain 23 (gel electrophoresis lane 2); strain 24 (lane 3); strain 25 (lane 4); strain 26 (lane 5); strain 27 (lane 6); strain 28 (lane 7); strain 29 (lane 8); strain 30 (lane 9); strain 31 (lane 10); strain 32 (lane 11); strain 33 (lane 12); strain 34 (lane 13); lanes 1 and 14 represent the 100 bp molecular weight markers (Promega). The wild type amplicon of 819 bp obtained with G1 + G3 stands also for a positive control.

A model that explains local insertions through association of the excised transposon with specific proteins in a complex that diffuse closely or glides along the chromatin [3] suggests that secondary reinsertion may frequently hit the donor chromatid. Relevant results presented by other authors describe a robust connection between local insertions and conservation of the starting element [6, 7, 8]. Studies concerning mobilization of the  $P\{EP\}$  artificial transposon [23] in *D.melanogaster* germline were performed relying on the assumption that the original insertion used to induce local insertions is retained at the initial location [8]. The  $EP(3)3583$  viable and fertile insertion, located in 67 chromosomal region, was mobilized and 1157 descendant mutant strains were analyzed at molecular level in order to identify local reinsertion patterns associated with conservation of the original insertion. After a PCR screening, 45 strains with  $P\{EP\}$  reinsertions were identified and in 43 of them the original insertion was retained. Out of them, 12 strains were characterized at the level of genomic reinsertion situs sequence and none of them occurred within the coding sequence of the neighboring genes. Instead, some transpositions were located preferentially in the promotor region of genes that are transcriptionally active in male germline, as was shown by the analysis of EST's from a cDNA library derived from *D.melanogaster* testis [8].

In other species, the transposition patterns of mobile elements that transpose by a *cut-and-paste* mechanism involve the repair of the original insertions and a bias for the occurrence of secondary reinsertions in the relative proximity of the initial insertion site. When an engineered *Ac* element was mobilized in *Arabidopsis thaliana*, 50% of the transposition events had occurred within 1,700 kb on the same chromosome, with 35% taking place within a range of 200 kb. The mobile elements transposed in both directions on the chromosome, with a roughly equal probability. Even more relevant, analysis of *Ac* transposition in *A. thaliana* transgenic line *I-RS/dAc-I-RS#14* indicated that 13 reinsertions (8 to the left and 5 to the right side) were in the range of 14 kb from the original insertion situs [24]. Studies concerning *Ac* element mobilization in *Zea mays* revealed also that proximal transpositions are generated [25].

Although local insertions may not be associated with conservation of the starter element, allowing selection against reverse jumping [6], there is a bias toward restoration of the original insertion [6, 7, 8]. Thus, preliminary detection of the original insertion in the individuals harboring intra-chromosomal transposition revealed by genetic analysis could narrow selection of candidate chromosomes harboring local reinsertions, acting as a positive marker. We expect that some of the secondary reinsertions affecting mutants described in this paper might be located in the relative proximity of  $P\{lacW\}^{\gamma CopS035702}$  insertion and could impair genes located close to *gammaCop*. If such secondary local reinsertions will be demonstrated to affect genes that collaborate with *gammaCop* to fulfill molecular and cellular processes, a P transposon-dependent way that enables *D. melanogaster* to regulate its genome may be considered. A detailed analysis of the putative secondary reinsertions associated with conservation of  $P\{lacW\}^{\gamma CopS035702}$  is currently under performance, concomitant with the

enrichment of our collection of *D.melanogaster* lines harboring  $P\{lacW\}$  conservative reinsertions into the third chromosome.

## Conclusions

Conservation of  $P\{lacW\}^{\gamma CopS035702}$  lethal original insertion during transposition events in *D.melanogaster* male and female germline occurs with a high frequency. In a transposon mutagenesis experiment, we screened for  $P\{lacW\}^{\gamma Cop057302}$  maintaining in 153 different mutant flies and identified such events in 131 individuals (85.6%).

Apparent repairing/preserving of the original insertion was also observed in 82.5% of the lines pertaining to an older mutant lines collection. We are currently performing genetic and molecular experiments, in order to learn if the correlation between transposition events and retaining of the donor mobile element may reveal a biological role of the P element transposition pattern.

## Acknowledgments

We thank Dr. Janos Szidonya from Szeged Drosophila Stock Centre, Szeged, Hungary, for providing us *l(3)S057302* and *yw; Δ2-3 Sb/TM2Ubx* lines. We are also grateful to Dr. Matyas Mink from the University of Szeged, Szeged, Hungary for the MM11 primer sequence. This research was supported by MEC-CNCSIS, Romania, research grant PNII-IDEI 147/2007.

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