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[1]University of Belgrade, Faculty of Biology, Belgrade, F.R. Yugoslavia. [2]Institute for Biology of Gene, Russian Academy of Science, Moscow, Russia. Genetic analysis of transgenic *D. melanogaster* flies after injection of the foreign gene of esterase S.

In several studies successful interspecific gene transfer has been achieved (Scavarda and Hartl, 1984; Daniels *et al.*, 1989). The system of DNA segment transfer by a P-element is widely used in cloning of different genes in eucaryotes (Spradling and Rubin, 1983). A very effective vector is CaSpeR, a P-element in bacterial plasmid (Pirrotta, 1986). In this experiment, the continuing part of esterase S gene sequence from *D. virilis* was inserted into the CASPER vector which is then injected into *D. melanogaster* embryos. The final goal was to determine whether the gene for esterase S maintains its tissue-specific expression when transferred into the genome of other species. The experiment in this paper deals with obtaining the transgenic individuals of *D. melanogaster*.

CASPER vector includes a *white* gene sequence which serves as a visible marker (white eyes) in detection of the transformed flies. As the P-element in the CASPER vector can not produce its own transposase, a mixture with a "helper" vector p 25.7 was used in 5:1 ratio. Embryos of *D. melanogaster* strain $Df(1)^{w67c23(2)}$, *y w* were injected with this mixture. The flies that developed from the injected embryos were each crossed with (y^+w; *Cy/L; D/Sb*) flies to obtain transformants with the markers on all major chromosomes (X, 2, 3). About 33.9% of the progeny of the injected flies were transformants. The transformed progeny, (all flies with any eye color) were back crossed with the flies form the above strain and their progeny represent the transgenic flies. An analysis of these transgenic flies shows the location and number of insertions on chromosomes, (*e.g.*, if the offspring is *Cy/L* with white eyes, the insertion of the white gene was on the 2nd chromosome). The results of segregation analysis indicate the existence of multiple simultaneous insertion of CASPER vector on autosomes and X-chromosomes and that the quantity of eye pigment might depend on the number of insertion sequences inherited in each individual.

It is very rare that unusual phenotypes are obtained from the above cross. From the reciprocal cross with $Df(1)^{w67c23(2)}$, *y w*, all progeny should have been y^+. However, we obtained *y* males. Their frequency was 0.0503% and they appeared both in families with and without transformants in segregations. In order to check whether this happened as a result of our experimental procedure, a control cross was done, without the injection treatment. No unusual flies were recorded in that progeny, which indicates that the experimental procedure might have caused the phenomenon.

Further on, we crossed the unusual *y* females with y^+w; *Cy/L; D/Sb* males. Only one female gave progeny and it comprised *yw* females and *yw* and y^+w males. One explanation could be that the appearance of the unusual female phenotype is the result of a mutation that suppresses the y^+ locus. Another would be that this is a consequence of the linked-X chromosome that was unstable and could divide, with high frequency, into free X-chromosomes. The latter assumption is in contrast with the obtained data, as there were no y^+ females together with *y* females. Thus, three different crosses of *yw* females, with three different types of males (y^+w; *w*; yw^a/w^+Y) were carried out. In the progeny of these crosses unusual flies were obtained, *y* males and y^+ females, with frequencies of 3.43% and 0.11% respectively. This means that in the initial lines used the linked-X chromosome was present, while still unstable and segregating with a frequency of about 10^{-3}. However, it can be concluded that a linked-X chromosome can be obtained from the cross with yw^a/w^+Y males which have a dominant w^+ marker on Y chromosome. If the daughters from our crosses got their fathers' Y chromosome, than we could expect pure segregation in daughters with w^+ and sons with *w* eyes. Our data show such segregation. In our case with unusual *y* females, we have a non-disjunction of X chromosome. The phenomenon of unusual flies in our crosses with individuals with the injected plasmid could be explained by a non-disjunction of sex chromosomes, which might be a result of the influence of the P-element that is injected together with the CASPER vector as a helper. Studies show that preference exists of the P element towards certain sites of the chromosomes. This can induce some loci to have the effect on chromosomal pairing (Tower and Kurapati, 1994). Understanding the target specificity of the P element transposition, and the behavior of homologous chromosomes in nuclei after injection helps in utilizing a P element as an insertional mutagen.

References: Daniels, S.B., A. Chovnick, and M.G. Kidwell 1989, Genetics 121: 281-291; Dean, D., 1981, Gene 15: 99-102; Pirotta, V., H. Steller, and M.P. Bozzeti 1985, The EMBO Journal 4: 3501-3508; Scavarda, N.J., and D.L. Hartl 1984, P.N.A.S. U.S.A. 81: 7515-7519; Spradling, A.C., and G.M. Rubin 1982, Science 218: 341-347; Tower, J., and R. Kurapati 1994, Mol. Gen. Genet. 244: 484-490.