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Characteristics of w^{hd81b9} mutant demonstrate its M' cytotype**.**

Khromykh, Yu. M., E.R. Varentsova, S.V. Sarantseva, and L.V. Kotlovanova.
Petersburg Nuclear Physics Institute, Russian Academy of Sciences, Gatchina 188300, Russia. E-mail: khromykh@omrb.pnpi.spb.ru.

Induced by hybrid dysgenesis, the w^{hd81b9} mutation of *Drosophila melanogaster* (abbreviated w^{hd}) was derived from the Harwich P strain and selected by Rubin *et al.* (1982). It was characterized as a null allele with a 0.5-kb P-element insert in exon 6 of the *white* gene. The mutant was stable when kept in P cytotype but reverted at a high rate (4×10^{-3}) when kept in M cytotype, suggesting that an intact P element was present elsewhere in the genome of mutant strain.

The w^{hd81b9} strain used by our laboratory was obtained from the Mid America Stock Center (Bowling Green, Ohio). Based on the results of the above-mentioned work we supposed that this w^{hd} strain has M' cytotype and may be used as a source of nonautonomous P elements to induce the somatic dysgenesis in the experiments with the use of repair-defective mutants. To check this supposition some additional characteristics of w^{hd} strain were studied.

First, the number of P element insertions in the w^{hd} genome was determined by nonradioactive *in situ* hybridization technique (Ashburner, 1989) with the use of Vectastain ABC Kit (Vector Labs, USA). As illustrated by Figure 1, chromosomes of w^{hd} flies contain a big set of P elements.

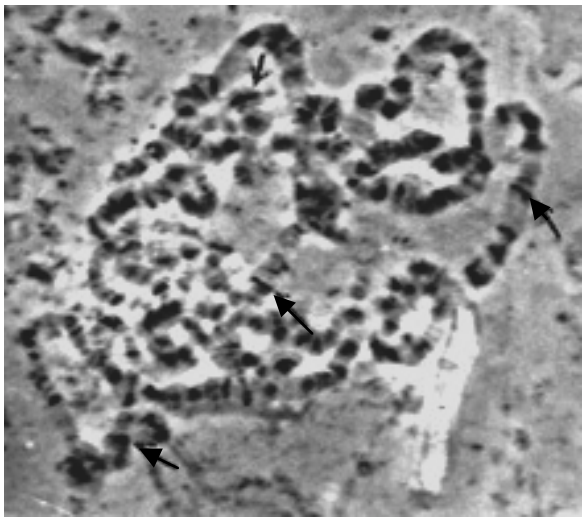

Figure 1. Photomicrograph showing total number of P element hybridization signals on the polytene chromosomes of w^{hd} strain. Some of signals are indicated by arrowheads.

Second, germ line and somatic cells (larval imaginal discs and nervous ganglia) of w^{hd} strain were tested in the presence of P repressor. For this purpose we applied the *P-lacZ* expression technique developed by Lemaitre and Coen (1991). *P-lacZ* constructs in which the *Esherichia coli lacZ* gene is fused in-frame with P element transposase gene was originally designed for the detection of genomic regulatory elements (O'Kane and Gehring, 1987) and they function as a reporter of P promoter activity. It was shown by Lemaitre and Coen (1991) that *P-lacZ* insertions have their expression repressed by P cytotype.

To construct the w^{hd}, *P-lacZ* strain, we used the *ABOO* strain obtained from Stephane Ronsseray. Chromosome 2 of ABOO flies contains an insertion of *P-lacZ* element, *(P[lac, ry^{+}]A)* that is expressed in germ line and soma (Lemaitre *et al.*,1993). One copy of this element was introduced into the w^{hd} genome by proper crosses of w^{hd} flies with flies of *ABOO* genotype. Similar crosses were performed with the *Lk-P(1A)* strain obtained from Stephane Ronsseray, in order to construct a *Lk-P(1A), P-lacZ* strain. The latter

Table 1. P element insertions are mainly distributed on the autosomes and show some cell-to-cell variations.

chromosome	number of P insertions
X	2
2L	11-12
2R	9-11
3L	7-9
3R	10-12
4	0
Total number: 39-46	

was used as the strain with known characteristics: it was shown by Ronsseray *et al*. (1991), that *Lk-P(1A)* strain fully represses germ line P element activity but does not fully repress transposase activity produced by an *in vitro* modified P element called *P[ry*$^{+}$ *Δ2-3]99B* (abbreviated Δ2-3). Ovaries of adult females and larval neural ganglia and imaginal discs were dissected and stained for ß-galactosidase activity as described by Lemaitre and Coen (1991). Results of staining are presented in Figure 2.

Judging from the results of X-gal staining, *Lk-P(1A), P-LacZ* strain, indeed, fully represses ovarian P-element activity and permits it on the middle level in somatic cells. At

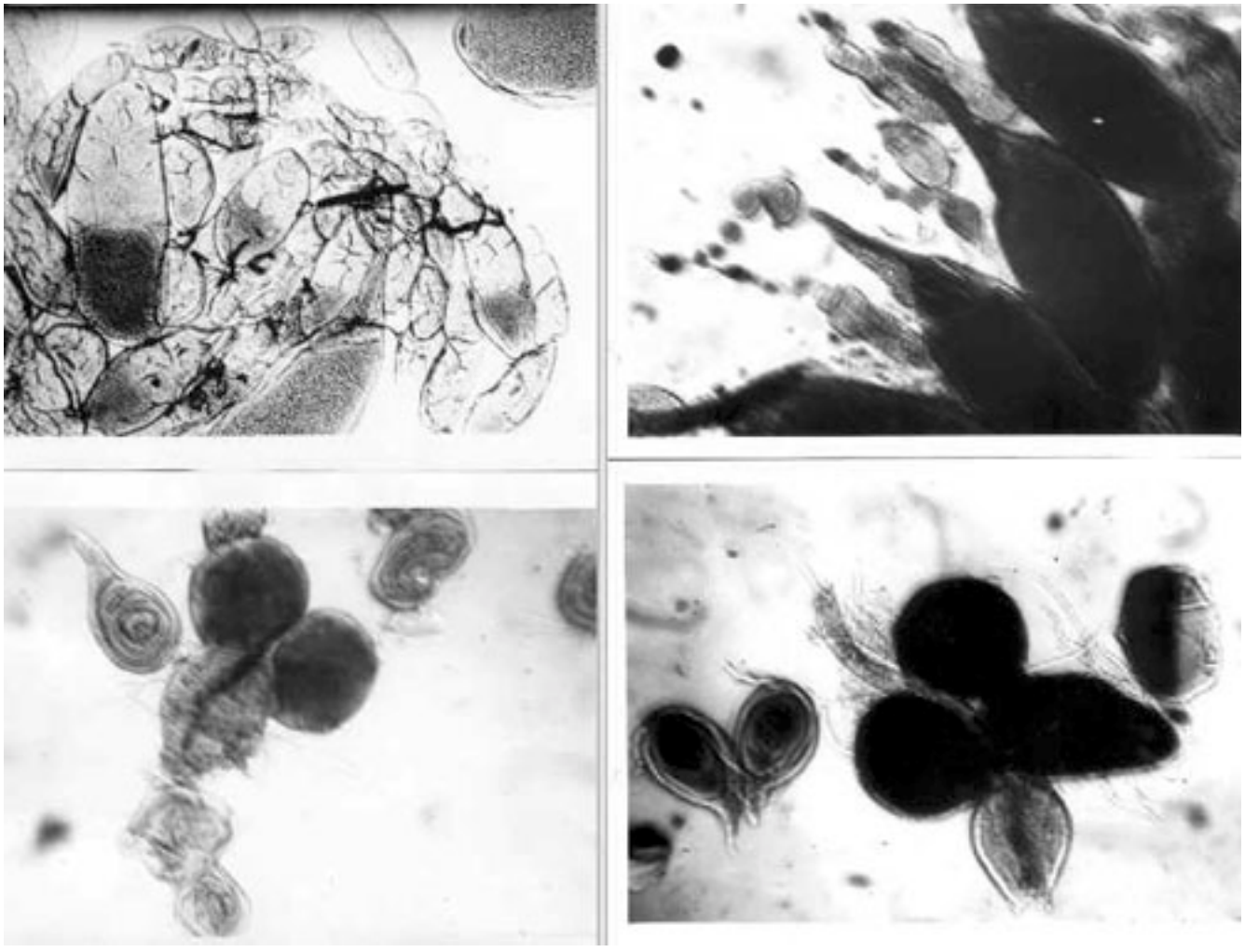

Figure 2. Photomicrographs showing X-gal staining of ovaries (above) and larval neural ganglia and imaginal discs (bottom) from the *Lk-P(1A), P-lacZ* and *w*^{hd}*, P-lacZ* strains.

the same time, *P-lacZ* strain demonstrates a high level of P element activity independently from the type of cells. Because the *w*^{hd} strain does not contain P repressor despite the large number of P element

insertions in the chromosomes, its cytotype may be classified as M'. Evidently, this strain contains defective P-elements and the role of intact P elements inserted elsewhere in the genome is unclear.

Third, w^{hd} strain cytotype was revealed by a test on the appearance of P element induced somatic mosaics. To do it, females of the w^{hd} genotype were crossed to males having the first chromosome with the yellow (nonessential to this report) and white markers and the third chromosome with the kinked marker and Δ2-3 source of P-transposase (*y^1 w^1; Ki^1 Δ2-3* strain was obtained from the Bloomington Stock Center, Bloomington, Indiana University). Females and males of w^{hd} strain itself and the F_1 generation of the mentioned cross were analysed in respect of red mosaic spots on the eyes. No mosaic eyes were registered among females and males of w^{hd} genotype. F_1 females showed the mosaic eyes at a rate 7% while the lack of mosaics was showed by males. This fact demonstrates that P element induced somatic mosaics arise as response of w^{hd} M' cytotype to the action of _2-3 P transposase. The differences between F_1 females and males are in good agreement with similar differences established by Engels *et al.* (1990) under analysis of w^{hd} germ line reversion and can be explained by the action of the same pairing dependent repair mechanism.

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