New Notch-like mutation in Drosophila virilis.

Ovcharenko, L.P., and I.A. Kozeretska. Department of General and Molecular Genetics, Biological Faculty, Taras Shevchenko Kyiv University, Vladimirskaya str, 64, Kyiv 01033, Ukraine. E-mail: leonst@ukr.net.

Abstract

A new dominant, sex-linked and lethal in the homozygote, wing mutation in Drosophila virilis was studied using a hybridological assay and light microscopy. The mutants have variable notches at the distal ends of the wings, parted wings thickening at the veins, and bended abdomen. The phenotype is also characterized by abnormal downiness of the wing edge and deformed eyes with reduced number of facets.

Organ size and shape are species-specific. Both parameters result from the coordination of cell proliferation, cell death, and arrangement of cells in specific patterns (Arias, 2003). During the last decades, our knowledge regarding the genetic basis of the cell cycle and cell survival has been greatly advanced, but the systemic relationships between gene expression patterns in cells and their proliferation only now are beginning to be established.

The wing of insects is an experimental model to study the genetic mechanisms of organ patterning and growth (Ren et al., 2005). The reason is its simple structure and a lot of data concerning genetic mechanisms of regulation of its development (Albagli et al., 2006). Due to extensive research work in this area a large number of genes, genetic interactions, several different signalling pathways that regulate strictly specific gene expression pattern in individual, and general wing morphogenesis programs in Drosophila were described (Baena-Lopez et al., 2006). Nevertheless, a lot of details in the wing morphogenesis process are still unclear (Wilkin et al., 2000).

Wing cells appear to be of neural (wing veins) or epidermal (interveins) origin according to (Molnar et al., 2006). Differentiation of wing disk cells into preveins and preinterveins is driven by Hedgehog and Decapentaplegic signal pathways that regulate the expression level of several transcription factors (Baena-Lopez et al., 2006). Then several genes of EGFR and Notch signal pathways are activated in prevein cells that cause differentiation of prevein cells into a central region that forms a vein and two rows of cells where Notch limits their proliferation. Distortion of expression patterns of genes of these signal pathways causes abnormal wing development (Artavanis-Tsakonas et al., 1995).

Results

The new Drosophila virilis mutation is dominant, sex linked, and lethal in homozygotes. The phenotypic display can be observed in females; all males of the mutant strain are wild type. Moreover, the number of males in progeny of mutant females is twice less than the number of females. It indicates the elimination of those males that get the mutation from their heterozygous
mothers. Also, the number of mutant females in the progeny of heterozygous females is 30% less than wild type females. This can be explained by the influence of the mutation on vitality.

The mutation is pleiotropic, it affects wing blade and hairs, eye, and abdomen structure.

![Figure 1. The wings of Notch-like mutants. The whole wing (a), notch (b), and thickening at the vein (c).](image1)

The wings of mutant flies are diverged, they have notches, extra vein material, and bundles of hairs near the veins ends see Figure 1. Each of these phenotypes can be characterized by incomplete penetrance. For example, 82.18% of specimens had two notches on each wing, 9.9% had only one notch on each wing, and 7.92% had an asymmetric display of this phenotype. All of these specimens had two or rarely one notch on the distal edge of the wing. Except for several occurrences, such notches were located on the inner side of the wing. The notches varied morphologically from small and slightly expressed to significant that inhibited the formation of wing edge structures. The wing hairs on the distal edge of the wing were often irregular in the mutant flies. The notch regions had no hairs; on the edges of notches wing hairs formed bundles (see Figure 1). All mutant specimens had thick veins with incrassation near the edge of the wing. Longitudinal veins, especially L5 (98.02%) and L4 (84.153%) were incrassated more often than cross veins (C1 24.752% – C2 26.733%). This phenotypic sign was characterized by asymmetric occurrence.

Phenotypic sign “diverged wings” occurred with high frequency; 66.3% of specimens had wings diverged with an angle of 70° relative to the anterioposterior axis.

53.46% of mutant flies had warped abdomen, mostly to the right side.

Another phenotypic sign was under-developed warped eyes with reduced number of facets (see Figure 2). This sign also was characterized by low penetrance (31.68% of specimens). This sign also had an asymmetric occurrence.

![Figure 2. The eyes of Notch-like mutant (a), and wild type Drosophila virilis (b) as example.](image2)

After the analysis of bilateral occurrence according to Астрауров (1927), it was shown that for all phenotypic signs the character of bilateral asymmetry was random.

For the localization of mutation on
the sex chromosome, the mutant strain was crossbred with a strain mutant for two genes: \textit{white} \((w, \text{1-105.0})\) and \textit{yellow} \((y, \text{1-2.9})\). It was shown that all females in the offspring of this cross that had \textit{Notch-like} phenotype simultaneously had white eyes but normal body color. It means that females with \textit{Notch-like} phenotype simultaneously are heterozygous for the white gene mutation, but as this mutation is recessive, the “white eyes” phenotype can only be observed if the mutant allele \(w\) is in the homozygous state. So the observed mutation and \(w\) are fully linked. It may be possible after the chromosome aberration that alters nucleotide sequences of both genes and makes crossover in this region impossible. An inversion or deletion can cause such an effect, moreover, if taking into account the localization of \textit{white} and \textit{Notchoid} (which has the similar phenotype and described earlier for \textit{Drosophila virilis} by Chino, 1941), on cytological and genetic maps of \textit{Drosophila virilis}, such an inversion has to be relatively small as it was shown by Gubenko and Evgenev (1984). The \textit{yellow} mutation was inherited independently from the observed mutation. This means that the distance between these two mutations is more than 40 cM.

For the genetic mapping, \textit{Notch-like} females were crossbred with \textit{miniature} \((mt, \text{1-78.0})\) strain males. All males in the offspring were wild type; females represented two groups – wild type ones and \textit{Notch-like}. All females were heterozygous for the \textit{miniature} allele. For the next cross, females with \textit{Notch-like} phenotype were chosen. These females were crossbred with \textit{miniature} males. The number of crossover females with both mutations in progeny was 10\%, so the genetic distance between \textit{Notch-like} and \textit{miniature} is 10 points.

\textbf{Discussion}

The described mutation of \textit{Drosophila virilis} is dominant, sex linked, and lethal in homozygotes. Its phenotypic display can be characterized by notches on the inner part of the distal edge of the wing blade, thick and incrassated veins, abnormal development of hairs on the edge of wing, reduced number of facets in the eye and its deformation, warped abdomen, and wings diverged at the angle of 70°.

Such phenotype was not described earlier for \textit{D. virilis}, but it resembles the abnormal function of \textit{Notch} gene of \textit{D. melanogaster}. Moreover, the new \textit{Notch-like} mutation of \textit{D. virilis} is located in the chromosomal region homologous to the chromosomal region of \textit{D. melanogaster}, where the \textit{Notch} mutation is located according to Whiting et al. (1989). That is why we state that the new wing mutation of \textit{D. virilis} damages the structure of gene orthologous to \textit{Notch}, so it is proposed to name it \textit{Notch-like}. It is also necessary to notice that for \textit{D. virilis}, \textit{Notched} gene \((N, \text{1-102.9})\) mutation is described earlier that locates in the same region, occurs to be dominant and lethal in homozygote. Mutant flies have diverged and thin wings, reduced number of hairs on head and thorax, and reduced L2 vein according to Chino (1941). So the phenotypic signs are similar to those described in this paper. It can be proposed that \textit{Notched} mutation described earlier is also another allele of the gene orthologous to \textit{Notch} gene of \textit{D. melanogaster}.

The only unclear moment is mismatch in chromosome localization of \textit{Notched} gene of \textit{D. virilis} and observed mutation, but as was mentioned before, the newly described mutation is a result of chromosomal aberration that locks crossover, such as inversion or deletion. Both of these aberrations can explain such a mismatch in localization of mutations.

It was shown that \textit{Notch-like} mutation is linked with the recessive mutant allele \textit{white} \((w, \text{1-105.0})\). The reason for such an occurrence is probably a chromosomal aberration that affects both of these genes \((w, \text{1-105.0}; N, \text{1-102.9})\), because the distance between them is insignificant according to Gubenko and Evgenev (1984). The smaller observed distance to the \textit{miniature} gene \((mt, \text{1-78.0})\) also can be explained by chromosomal aberration. So, at least two of the described effects, such as
linkage with \( w \) and smaller observed distance to \( mt \), indirectly indicate that the reason of mutant allele occurrence is a chromosomal aberration.

The final clarification of observed mutation nature and genetic features needs further research.

**Materials and Methods**

The new *Notch-like* mutation of *D. virilis* was obtained from the progeny of dysgenic crosses and was kindly presented by L. (Institute of Molecular Biology of RAS, Moscow). Not less than 100 females from progeny of all crosses were analyzed to estimate the phenotypic occurrence of the mutation. The principles of bilateral occurrence of phenotypical signs were according to Астауров (1927). If there are no dependence between sign occurrence on different sides of the body the frequency of its symmetrical occurrence (C) is equal to the product of frequencies of its occurrence (A) on the left (AL) and on the right (AR). The type of correlation between side of occurrence was estimated by comparing the theoretical frequency of symmetrical occurrence (cm) with the real frequency observed in the experimental sampling (ce). It was considered that there is no dependence if cm = ce.

For the localization of the newly observed mutation on the genetic map and for estimating the type of inheritance the crossbreds with strains mutant for genes *white* (\( w \), 1-105.0), *yellow* (\( y \), 1-2.9), and *miniature* (\( mt \), 1-78.0) were held. Two continuous crossbreds were held *miniature* strain males. The F2 specimens were analyzed in a number not less than 100. The distance between genes was estimated according to formula: \( D = 100*r \), when \( r \) is a frequency of crossover, \( D \) is a distance between genes in cM.

The flies were maintained in glass tubes using standard nutritional medium at 25°C temperature.

Also fly wing structure was analyzed with optical microscopes using preparation of wing in Canada balsam.

References:

**Novel mutants in Drosophila simulans.**

**Sousa-Neves, Rui.** Case Western Reserve University, Department of Biology, Cleveland, OH, 44106.

Here I report the isolation of 7 new spontaneous mutants in *D. simulans* identified recently and provide some updates about other mutants previously reported in Sousa-Neves *et al.*, 2009.