The *miniature* Gene in *Drosophila virilis*: Maternal Effect and Evolutionary Conservatism

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Abstract—A comparative study of two mutant alleles of the *miniature* gene of *Drosophila virilis* is carried out. The cytological position of the *miniature* locus on the polytene chromosomes of *Drosophila virilis* is determined using *Drosophila melanogaster* sequences that serves to confirm the high degree of evolutionary conservatism of the gene. It is demonstrated that the presence of the Min functional protein is required for fertilization, since females that are homozygous with respect to one of the alleles produce infertile eggs which are also situated anomalously on the surface of the nutrient medium. The temperature-sensitive influence of the other allele on the viability of the individuals is established. A search for interacting genes among the components of the Notch signal pathway is undertaken.

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Introduction. By comparison with the epidermis of vertebrates, the integument of insects is characterized by a rather simple organization. The epidermis of *Drosophila* is in the form of a monolayer of epithelial cells, whereas in vertebrates the epidermis is a multilayer formation with two tissues of differing origin [1]. Another important difference is the presence in insects of cuticle, a substance that produces rigidity in the integument and protection against external factors. In vertebrates this function is fulfilled by intermediate filaments (keratins) in conjunction with a number of other proteins. However, despite such major differences, evolutionary conservative signal systems have been involved in genetic control of the development of epithelial tissues. The same signal systems that control differentiation of the epidermis in *Drosophila* participate in a particular way in the morphogenesis of the epidermis of vertebrates [2]. It should also be noted that despite the rather simple organization of the epithelial tissues in insects, such characteristics as the polarity of the cells and the presence of intercellular contacts are preserved [1].

The wings of insects constitute two interacting layers of epithelium, the cells of which have repeatedly changed their form in the course of development, creating, in particular, a specific pattern of cuticular outgrowths on the outer surface [3]. Such a simple organization is the reason why the wing is widely used as a genetic model for studying the development and differentiation of epidermal tissues in *Drosophila*. A number of mutations that attest to the obvious role of certain proteins in differentiation of wing cells have been described, though it is not known what are the actual processes they are involved in. Two wing mutations, *miniature* and *dusky*, are known in *Drosophila virilis*, which in *Drosophila melanogaster* are combined into the *m-dy* complex. These genes are characterized by a similar phenotype of the mutations (reduced size of wing cells) and nearby position on the chromosome. They encode homologous transmembrane proteins that contain what is known as the zona pellucida (ZP) domain [4]. Usually, in other species ZP proteins function like structures (components of the intercellular matrix) or act like receptors. The Min and Dy proteins of *Drosophila* are involved in the formation of the wing cuticle and the reduced size of the cells is related to disruption in the reorganization of the apical membrane during differentiation, a process that includes changes to the actin cytoskeleton [4]. However, at the present time it is not known what role the Min and Dy proteins play in differentiation of the wing epithelium and which components of the known signal systems they interact with.

In the present study two mutant alleles of the *miniature* gene of *D. virilis* will be considered and a maternal effect established for one of the alleles. Moreover, the influence of the alleles on the viability of individuals will be studied, the cytological localization of the *miniature* and *dusky* genes will be determined, and a search for interacting mutations will be carried out.

Material and methods. *Drosophila virilis* strains. Strains obtained in the hybrid dysgenesis system in *D. virilis* contain different mutant alleles of the *miniature* gene—*m* and *mG*. The wild type strain 9 of the
natural population of Batumi with specific types of crossings was used to determine viability. Interactions between the genes were studied at the phenotypic level by obtaining double mutant strains on the basis of a number of strains, specifically, Delta (D1), gapL2 (gp), crossveinless (cv), and OddG1.

Investigation of maternal effect of the mutant allele mG1. Virgin females heterozygous and homозygous in mG1 were crossed with mG1 males. Following complete maturation the females were placed in Petri dishes containing a standard medium for 1 h. The parents were then removed and the development of the embryos observed under a stereoscopic microscope by transferring them into a compartiment with mineral oil [5]. The stages of development were determined according to Campos-Ortega [6]. An analysis of the reduction of the gonads of the adult females was conducted by scanning isolated ovaries under a stereoscopic microscope.

Cloning of fragments of the miniature and dusky genes of Drosophila melanogaster. Total mRNA was extracted from first- and second-stage wild-type larvae with the use of Trizol reagent (Invitrogen). A fragment of the miniature gene 875 bp in length was produced (using the primers 5'-TTGCCACTGCTGTTACCGT-3' and 5'-CGCAGGATTTCACACGCATA-3') and a fragment of the dusky gene 421 bp in length (using the primers 5'-CAaagcttAGCCCAACGAAAGGAAACGGAACG-3' and 5'-TTTgaattcCAATTCCGAATCTGAAAATC-3') by the RT-PCR method [7]. The fragments were cloned in EcoRI/HindIII-pBS (Strategene, USA) and sequenced using the PE Applied Biosciences Prism 310 Genetic Analyzer.

Hybridization in situ on polytene chromosomes. The salivary glands of third-stage wild-type larvae that had developed at a temperature of 18°C in a drop of 45% acetic acid were separated in order to prepare pressed samples of the polytene chromosomes [8]. Biotin-labeled fragments were obtained by means of nick translation with biotinylated dUTP and detected by avidine-bound horseradish peroxidase (ABC Vectastain kit (Vector Lab)) and subsequently displayed by means of treatment with diaminobenzidine. The chromosomes were prohybridized with each probe separately. Localization was performed on the basis of cytogenetic maps of the polytene chromosomes of D. virilis [9].

Results. Two mutant alleles of the miniature gene were obtained in the system of hybrid dysgenesis, which we have denoted m42 and mG1. There are a number of features that are common characteristics of both alleles. First, both mutations are recessive and sex-linked. Both alleles manifest themselves in a 1.4-fold reduction in the wing plate by comparison with the wild-type wing. For this type of mutation in D. melanogaster it is known that a decrease in plate size is associated with a decrease in the size of the wing cells. A count of the number of cells in each hair in particular sections of the wing of m42 and mG1 individuals showed that this result also occurs in D. virilis mutations. Moreover, unlike the wild type, the wing cells of the miniature mutants possess boundaries that are visible under a light microscope. The presence of rounded structures (nvs) in the veins is characteristic of both mutations. Studies of the nature of these structures by transmission electron microscopy demonstrated that they constitute nonuniform thickening of the cuticular layers [10]. In individuals that are mutant in m42 and mG1, the wing edge is undulated in shape, a circumstance that is probably related to the disparity between the amount of material in the veins and the intervenose space.

As a result of crossing of m42 females with mG1 males, females with the features of the miniature mutation were obtained in the first generation. Thus, the alleles that are being studied here are, in fact, mutations of one and the same gene complementary to each other.

A dependence of certain indicators on the development temperature was demonstrated for individuals that are mutant with respect to either of the m42 alleles. On the basis of crossings of several types it was shown that the viability of hemizygous mutant males is not reduced by comparison with wild-type males at 18°C (cf. table). However, at a temperature of 25°C it falls by 17.09 ± 2.72%. The viability of homozygous females and hemizygous males was the same at 18°C, but at 25°C the sex ratio shifted with an increase in the number of females. However, at 25°C there was no difference between the viability of heterozygous females and wild-type males, though such a difference appeared at 18°C. For the control (wild-type individuals) a shift in the sex ratio towards increasing number of females at both temperatures of development equally was characteristic. Whereas this trend was also observed for the m42 strain, it may be concluded on the basis of the ratios obtained here that the viability of the hemizygous males and the heterozygous m42 females at 25°C was reduced, and that the effect of the mutation is moderated over the more prolonged development of the mutant individuals.

The viability of the mG1 individuals was also analyzed on the basis of the two types of crossings and only at the temperature 25°C. Four classes of individuals were obtained from crossing of heterozygous females and hemizygous males (cf. table): heterozygous and homozygous females, hemizygous males, and wild-type males. A slight deviation in the number of females and males of the different types was observed, though this deviation was not statistically reliable. Crossings of the wild-type females with the mG1 males also did not demonstrate any reliable deviation in viability of the heterozygous females by comparison with wild-type