THE ROLE OF THE *LIMK1* GENE ON SHORT-TERM MEMORY FORMATION IN *DROSOPHILA MELANOGASTER*

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INTRODUCTION

Nowadays one of the topical problem of neurobiology is the research of the etiology and progression of different neurodegenerative diseases. One of the causes of neurodegenerative diseases is disturbance of actin remodeling cascade whose key enzyme is LIMK1. *Drosophila* constitutes a convenient model for studying the link between genome organization and chromosome architecture observed in cognitive disorders. The revealed association between *limk1* gene's mutational damage, changes in its expression and activity as well as cognitive impairment allows to use current model for the study of neurodegenerative and genomic diseases.

Drosophila melanogaster—the most convenient model object

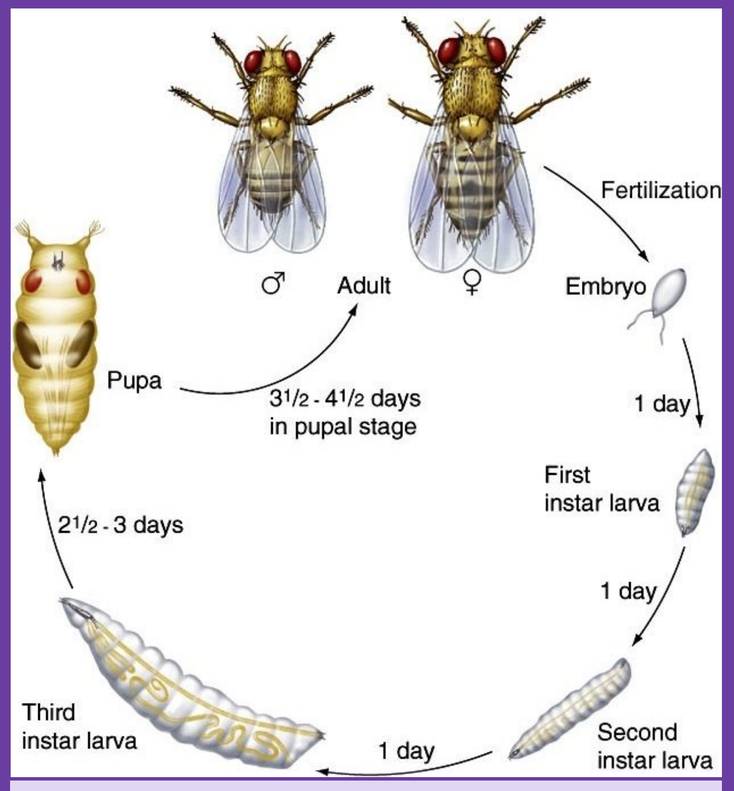

Figure 1. *Drosophila* life cycle

- Molecular-genetic studies of the human genome have emphasized the evolutionary conservation of homologous genes from different organisms.
- *limk1* gene *D. melanogaster* has 71% homology with the *limk1* gene *H. sapiens*
- *Drosophila* is especially advantageous for testing gene interactions and allows the usage of several mutant or transgenic models at a time.
- *Drosophila* mutations in such genes help to understand the putative function of a newly isolated human disease gene.
- *D. melanogaster* have short life span (Figure 1)
- Low maintenance costs
- No legislative limitations brought about by "animal defending laws"

<u>The main purpose:</u> to analyze the formation and dynamics of short-term memory in *D. melanogaster* stocks with *limk1* gene polymorphism: *Canton-S*, *Berlin* and *Oregon-R*

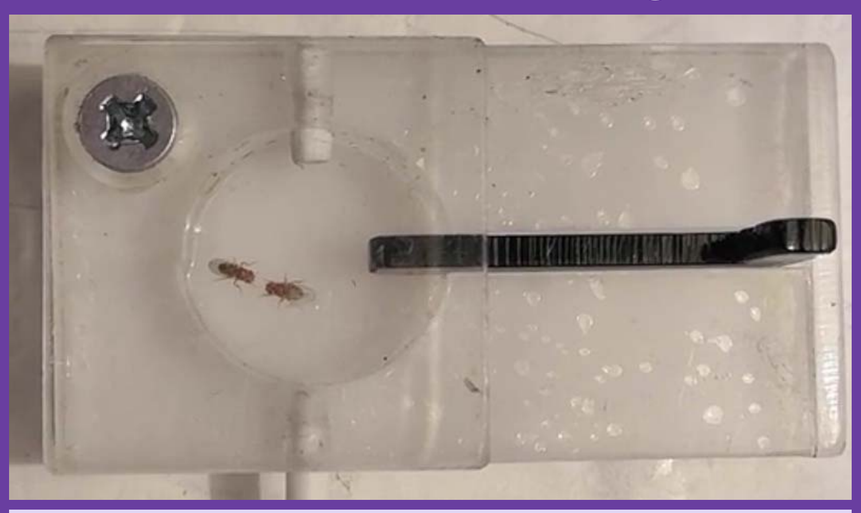

Figure 2. Special box for training and testing

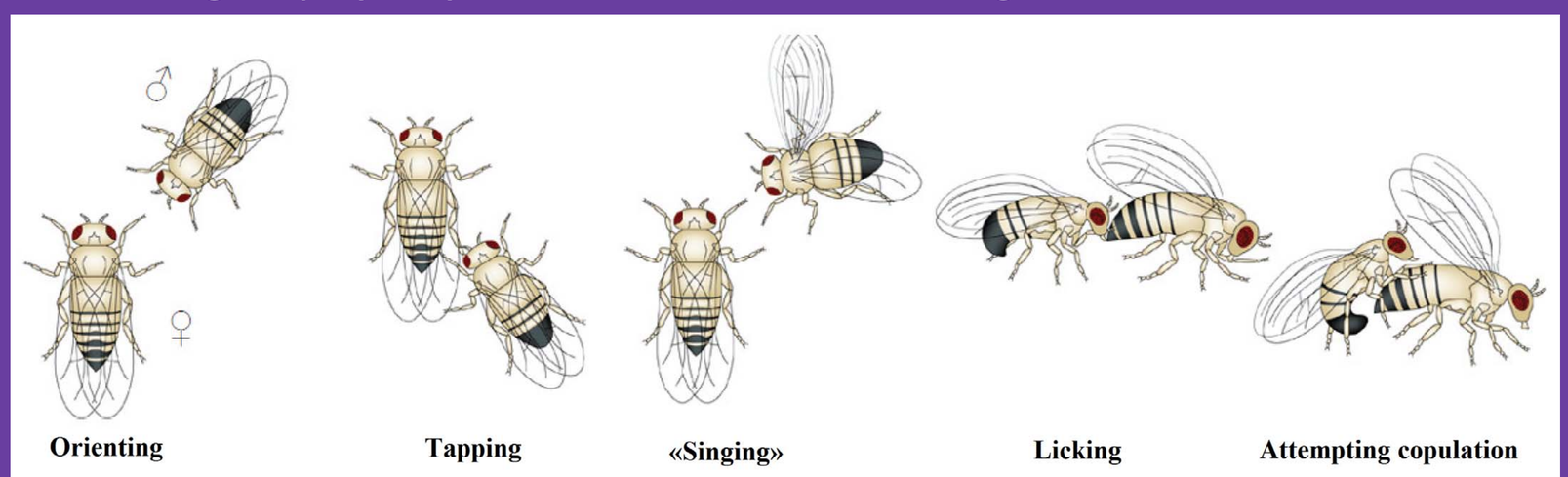

Figure 3. Individual courtship elements

MATERIALS AND METHODS

Canton-S stock has a single-nucleotide polymorphism C/T (+12 bp) in the binding site of the M1 transcription factor.

Berlin stock characterized by disturbances in primer binding in intron 2 and near introns 3 and 4. Also this stock has single-nucleotide polymorphisms in the N-terminal part of the gene (including the M1 binding site and the 1st part of intron 1), some of which correspond to *Canton-S* stock polymorphisms.

Oregon-R stock hasn't fragment between primers are limited the region with both LIM-domains and part of the PDZ-domain. Also this stock has multiple polymorphisms in the 1st intron:

- A/T-rich insert 28 bp;
- deletions of 8 bp and 9 bp
- Change the CAA/AGC

Conditioned courtship suppression paradigm was used to asses learning ability and short-term memory formation.

1. A 5-day-old virgin male was put in a special box with a fertilized female *Canton-S* and was left for 30 minutes (Figure 2).

2. Courtship behavior was analyzed in naive males and in males in 0, 15, 30 and 60 minutes after training to assess the formation and dynamics of short-term memory. The behavior of the male was recorded in a special program for 300 seconds. We recorded the start time of individual courtship elements (Figure 3) and the execution time of non-courtship elements (activity, prining, rest).

3. We calculated the learning index (LI) to assess the effectiveness of training. Randomization test was used to statistical analysis.

RESULTS

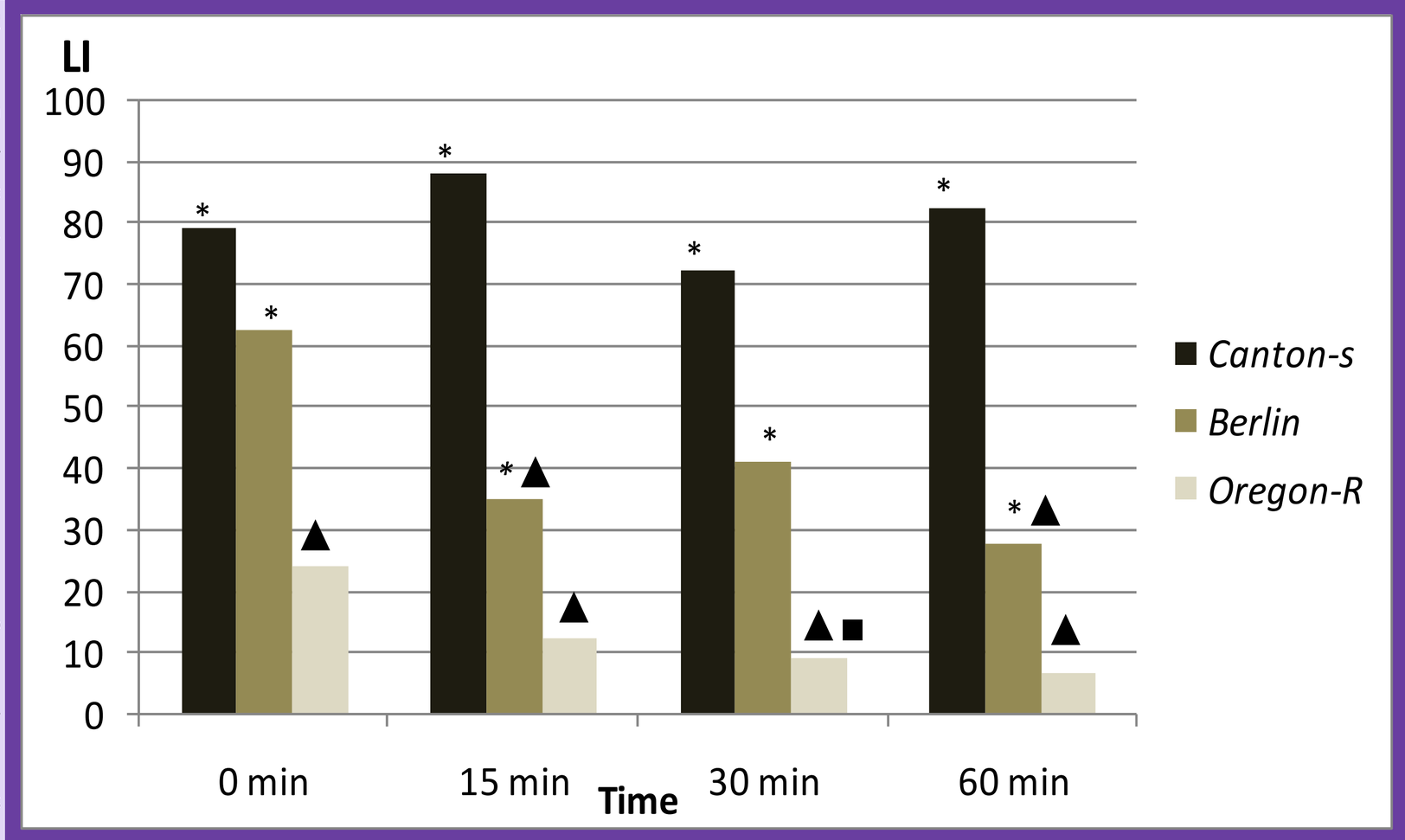

Figure 4. Learning index (LI) *Canton-S*, *Berlin* and *Oregon-R* stocks

* LI significantly differents from zero, $p<0,05$

▲ LI significantly differents from *Canton-S*

■ LI significantly differents from *Berlin*

Thus, *Canton-S* and *Berlin* stocks capable of formation short-term memory and to preserve it, while *Oregon-R* incapable of learning and formation short-term memory. Polymorphisms in the *limk1* gene apparently contribute to changes in the content and ratio of LIMK1 isoforms in the analyzed stocks, that influence in processes of learning and formation short-term memory.