

C

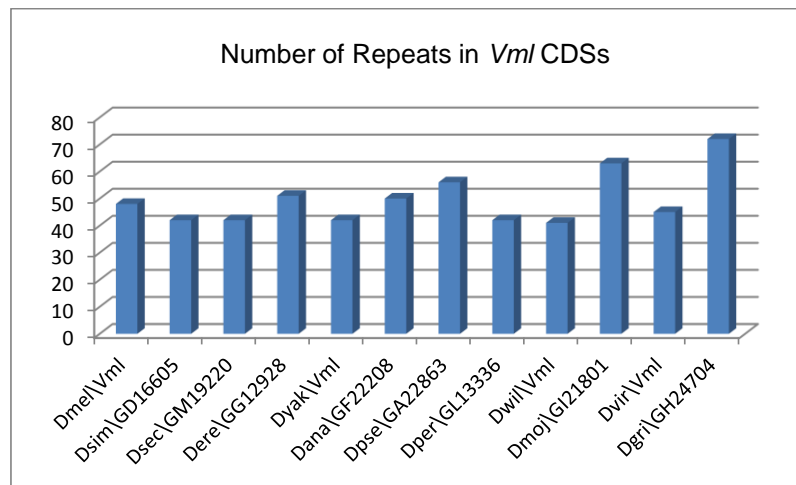


Figure 1. Structures of the VML orthologs. A, Domain structure of the VML protein: S, signal peptide; VM, domain specific for vitelline membrane proteins. B, Alignment of VM domain amino acid sequences of 12 orthologous VMLs. Orthologs found in this work are highlighted. Genome positions of orthologous genes in *D. yakuba*, *D. willistoni*, and *D. virilis* are given according to corresponding genome maps (FlyBase release FB2013_04). The VM domain sequence logo was created by WebLogo (Crooks *et al.*, 2004). C, Number of 24 bp DNA tandem repeats in orthologous *Vml* coding sequences.

Quantity of 24 bp tandem repeats in orthologous genes was determined by the TRF program (Benson, 1999). It was found that orthologs contain a diverse number of coding tandem repeats (Figure 1C). Thus, *Vml* orthologs in closely related species *D. pseudoobscura* and *D. persimilis* have considerable differences in repeat number, 56 and 42 copies, accordingly. A functional consequence of this diversity between species remains unclear. Possibly, changes in repeat number participate in species-specific changes of eggshell morphology.

References: Alatorsev, V.E., 2006, Mol. Biol. 40: 330-332; Clark, A.G., M.B. Eisen, D.R. Smith *et al.*, 2007, Nature 450: 203-218; Crooks, G.E., G. Hon, J.M. Chandonia, and S.E. Brenner 2004, Genome Res. 14: 1188-1190; Benson, G., 1999, Nucl. Acids Res. 27: 573-580; Scherer, L.J., D.H. Harris, and W.H. Petri 1988, Dev. Biol. 130: 786-788; Zhang, Z., L.M. Stevens, and D. Stein 2009, Curr. Biol. 19: 1200-1205.



Comparative analysis of the fragment of the Y chromosome gene *kl-2 1-beta* dynein heavy chain in *Drosophila virilis* species group (Diptera: Drosophilidae).

Andrianov, B.V., and D.A. Romanov. Vavilov Institute of General Genetics, Russian Academy of Sciences, 119991 Gubkin St.3, Moscow, Russia; E-mail: andrianovb@mail.ru

Introduction

Drosophila virilis species group is one of the best studied models of speciation and microevolution (Morales-Hojas *et al.*, 2011). We analysed Y chromosome DNA sequence variation

among all 12 *Drosophila* species of the *virilis* group for the first time in order to resolve uncertain aspects of the group phylogenesis. Sequence variation of genes locating on the Y chromosome provides a unique opportunity to elucidate the order of phylogenetic diversity in the group of closely related species due to the non-recombining nature of the Y chromosome.

Gene *kl-2 1-beta dynein heavy chain* was acquired by the Y chromosome of the ancestral *Drosophila* species before the split of the *Drosophila* and *Sophophora* subgenera between 260 and 63 Myr ago (Koerich *et al.*, 2008). *kl-2* gene remains Y-linked in all sequenced *Drosophila* species with the exception of *D. pseudoobscura* (Koerich *et al.*, 2008). In the case of *D. pseudoobscura* the ancestral Y chromosome became part of an autosome (Carvalho *et al.*, 2005).

In this study we present comparative analysis of the PCR fragment of the gene *kl-2* in all 12 species of the *virilis* species group.

Materials and Methods

Fly strains: All strains used in this work are from the collection of the Laboratory of Genetics, Kol'tsov Institute of Developmental Biology, Russian Academy of Sciences and National *Drosophila* Species Resource Center (Bowling Green, United States): *D. americana americana* (Spencer) 405, *D. americana texana* (Stone, Griffen, and Patterson) 423, *D. ezoana* (Takada and Okada) 572, *D. kanekoi* (Watabe and Higuchi) 1540, *D. lacicola* (Patterson) 0991.13, *D. littoralis* (Meigen) 06-17a - North population and AB54 - South population, *D. montana* (Patterson, Stone, and Griffen) 1021.13, *D. novamexicana* (Patterson) 424, *D. flavomontana* (Patterson) 0981.0, *D. lummei* (Hackman) 200, *D. borealis* (Patterson) 0961.00 East population, *D. virilis* (Sturtevant) B9. Each strain was founded by a single female fertilized in nature and maintained as a mass culture in vials on a standard *Drosophila* medium. The PCR amplification has been performed on the matrix of total DNA that was isolated from individual imago.

The primers used to amplify the *kl-2* sequences were as follows: the Dv-dy-f direct primer - 5' - GCTGCAGGCGGTAATAGAAG - 3' and the Dv-dy-r reverse primer - 5' - TTGCATTTGCGGATCAATAA - 3'. The length of the amplified fragment is 429 bp. A fragment of a genomic DNA was amplified by PCR in the volume of 25 µL using an Applied Biosystems PCR amplificator. The conditions of PCR were as follows: initial DNA denaturation at 94°C for 5 min; then, 35 cycles were performed as follows: denaturation at 94°C for 30 s, annealing at 60°C for 30 s, DNA synthesis at 72°C for 60 s, and finishing synthesis at 72°C for 7 min. The products of the amplification were separated by electrophoresis in 1.5% agarose gel. The obtained PCR fragments were sequenced without cloning.

Results and Discussion

Within the set of newly obtained sequences the number of nucleotide substitutions per site varies ten fold from 0.005 in the pair of *D. americana americana* and *D. americana texana* to 0.051 in the pair of *D. americana americana* and *D. borealis*. The overall mean distance for the whole group is 0.036. Based on these data we constructed median network of Y chromosome haplotypes of *Drosophila* of the *virilis* group with the TCS software (Figure 1). TCS is a program that implements the estimation of gene genealogies from polymorphic DNA sequences using statistical parsimony (Clement *et al.*, 2000). Results are, in general, consistent with previous studies based on the multilocus data (Morales-Hojas *et al.*, 2011) in recovering the four major lineages of the group: *D. virilis* phylada, *D. montana* phylada, *D. kanekoi* phylada and *D. littoralis* phylada. The most

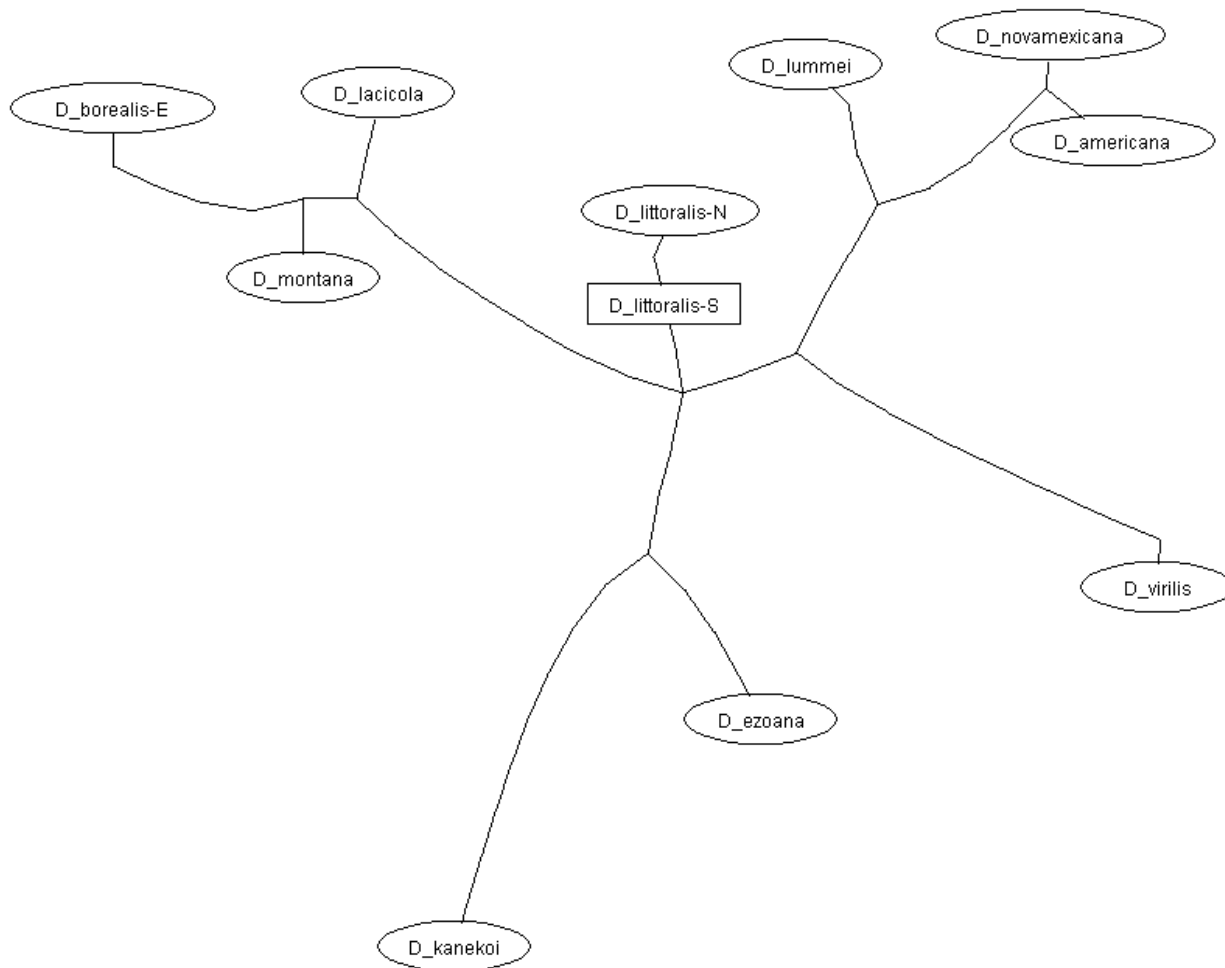


Figure 1. The median network of Y chromosome haplotypes of the *Drosophila virilis* group constructed using the TCS software (Clement *et al.*, 2000). The reconstruction is based on the nucleotide polymorphism of *kl-2* gene fragment (GenBank ID: KF600714 - KF600726). The lengths of the branches are proportional to the minimum number of nucleotide substitutions that are necessary for transformation of one haplotype into another. If there are synonymous haplotypes, only one synonym is indicated.

interesting new point concerns the order of phyladas filiation. Multilocus analysis based on six nuclear genes and two mitochondrial genes revealed that the last common ancestor of the group had a Holarctic distribution from which the North American and the Eurasian lineages evolved from 7.5 to 8.9 Mya. Two competing evolutionary hypotheses have been proposed and compared (Morales-Hojas *et al.*, 2011). First, simple consensus tree model for analysing multilocus data leads to the conclusion that *D. kanekoi* and *D. ezoana* form one lineage, and *D. littoralis* has a more ancient origin. More sophisticated method, using multispecies coalescent model (BEST), leads to the alternative conclusion. *D. kanekoi* was the first species to divide from the common ancestor of these three species. These conclusions are in agreement with localization of an ancient retrotransposon old *bilbo*-like non-LTR retroelement, which is present in *D. littoralis* and *D. virilis*, but absent in *D.*

kanekoi (Reis *et al.*, 2008). Our data strongly support the first simple model and indicate that *D. kanekoi* and *D. ezoana* form one lineage. Besides, it is clear that the speeds of nucleotide fixations in different species vary greatly. This may be due to the differences in the population size. Small island populations of *D. kanekoi* from Japan evolve much more rapidly than large continental populations of *D. littoralis*.

We found no difference between *kl-2* sequences of different laboratory lines of *D. virilis*. It is not surprising because *D. virilis* is a nearly monomorphic species (Mirol *et al.*, 2008). On the contrary *kl-2* sequences of two lines of *D. littoralis* from the South Caucasian population and from North European population (Andrianov *et al.*, 2010) are different. South population is ancestral in relation to North population. These data support a previously made proposal that South population of *D. littoralis* is a separate species.

We found no difference between *kl-2* sequences of Eastern *D. borealis* and *D. flavomontana*. Fly lines from Western population of *D. borealis* were unavailable to us. *D. borealis* comprises two different species, Eastern and Western. Eastern *D. borealis* is much more closely related to *D. flavomontana* (Morales-Hojas *et al.*, 2011). Our data support the hypothesis of hybrid origin of Eastern *D. borealis*. It may originate from hybridisation of *D. flavomontana* male with *D. borealis* female.

Acknowledgments: This research was supported by grants from the Russian Foundation for Basic Researches, Projects: 13-04-01250-a, 12-04-00926-a, 11-04-01630-a, and 13-04-90621.

References: Andrianov B.V., I.I. Goryacheva, N.S. Mugue, S. Yu Sorokina, T.V. Gorelova, and V.G. Mitrofanov 2010, Trends in Evolutionary Biology 2: e4.; Carvalho, A.B., and A.G. Clark 2005, Science 307: 108–110; Clement, M., D. Posada, and K.A. Crandall 2000, Mol. Ecol. 9: 1657–1659; Koerich, L.B., X. Wang, A.G. Clark, and A.B. Carvalho 2008, Nature 456: 949–951; Mirol, P.M., J. Routtu, A. Hoikkala, and R.K. Butlin 2008, BMC Evolutionary Biology 8: 59; Morales-Hojas, R., M. Reis, C.P. Vieira, and J. Vieira 2011, Molecular Phylogenetics and Evolution 60: 249–258; Reis, M., C.P. Vieira, R. Morales-Hojas, and J. Vieira 2008, Gene 425: 48–55.



A preliminary report and the frequency distribution of *Drosophila* species of Rabwah, Pakistan.

Tahir, Durr-e-Samin. Abdus Salam School of Science, Nusrat Jehan College, Rabwah, Pakistan; d.samin2@hotmail.com

To study the frequency distribution of some *Drosophila* species, a study was carried out in Rabwah, Pakistan (31° 32' 59"N, 74° 20' 37" E). Survey was made for the first time in this area and revealed seven species, which were already found before in Lahore, Pakistan. The collections were made twice, once during March–April and then during the month of September, 1992. It was observed that two *Drosophila* species, *D. immigrans* and *D. nepalensis*, were found only during the month of April. This observation is similar to the observation of Gupta (1973), who, while studying the fauna of Manipur, India, observed that these two species can be obtained only in the month of February. These collection data also reveal that all of these species are members of *melanogaster* and *immigrans* species groups of subgenera *Sophophora* and *Drosophila*, thus supporting the view of Bock and Wheeler (1973), who proposed that South East Asia is a fertile region for the rapid diversification and speciation of the members of the *immigrans* and the *melanogaster* species groups of *Drosophila*.