SUPPLEMENTARY MATERIALS

to the article B.V. Andrianov, D.A. Romanov, T.V. Gorelova "Genetic variation of the nuclear sequences of mitochondrial origin associated with retrotransposon *Tv1* insertions in *Drosophila* species of the *virilis* group"

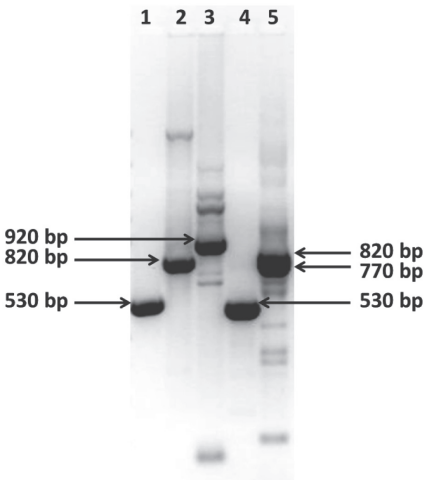
Supplementary material 1

List of *Drosophila* strains of the *virilis* group

Species	Strains	Locality	Year of capture
Drosophila americana americana	405	Myrtle Beach State Park, South Carolina, USA	1961
Drosophila americana texana	422	New Orleans, Louisiana, USA	1980
Drosophila borealis	0961.00	Itasca Park, Minnesota, USA	1950
Drosophila kanekoi	1061.00	Sapporo, Japan	1980
Drosophila lacicola	0991.13	Beaver Creek Camp Grounds, Manitoba, Canada	1949
Drosophila littoralis	06-17a	Rybniy, Rostov Oblast, Russia	2006
Drosophila lummei	200	Serebrianybor, Moscow, Russia	1969
Drosophila montana	1021.13	Kawasaki, Japan	1980
	1021.19	Mount Hood National Forest, Oregon, USA	1980
	20 OL8	Oulanka, Finland	2008
	KR 13-09	Biostation Raduga, Kamchatka, Russia	2013
Drosophila novamexicana	424	San Antonio, New Mexico, USA	1947
Drosophila virilis	B9	Batumi, Georgia	1965
	Dv1	Yerevan, Armenia	1969
	Dv40	Tashkent, Uzbekistan	1968
	L160	Laboratory obtained strain	1975
	Sa96	Sapporo, Japan	1996

Supplementary material 2

The result of PCR identification of chimerical sequences of numt-*Tv1* in the experiments: a-1, c-1, a-2 and c-2 on the template of genomic DNA of *Drosophila* of the *virilis* group



1. Experiment a-1

Fractionation of the PCR fragments formed by *atp6* numts associated with *Tv1* retrotransposon in direct orientation in an agarose gel stained by ethidium bromide.

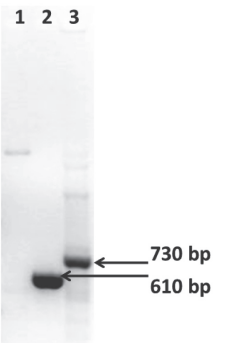
Lane 1 – PCR fragment, constituted by *Tv1* retrotransposon and *atp6* numt, of about 530 bp in size, obtained with the primers Dvir4.1F и Dvir7.1R on the *D. virilis* genomic DNA template.

Lane 2 – PCR fragment, constituted by *Tv1* retrotransposon and *atp6* numt, of about 820 bp in size, obtained with the primers Dvir6.2F and Dvir7.1R on the *D. virilis* genomic DNA template.

Lane 3 – PCR fragment, constituted by *Tv1* retrotransposon and *atp6* numt, of about 920 bp in size, obtained with the primers Dvir6.3F and Dvir7.1R on the *D. virilis* genomic DNA template.

Lane 4 – PCR fragment, constituted by *Tv1* retrotransposon and *atp6* numt, of about 530 bp in size, obtained with the primers Dvir6.1F and Dvir7.2R on the *D. lacicola* genomic DNA template.

Lane 5 – PCR fragment, constituted by *Tv1* retrotransposon and *atp6* numt, of about 770 and 820 bp in size, obtained with the primers Dvir6.2F and Dvir7.2R on the *D. lacicola* genomic DNA template.



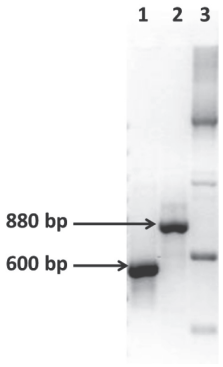
2. Experiment c-1

Fractionation of the PCR fragments formed by *cox3* numts associated with *Tv1* retrotransposon in direct orientation.

Lane 1 – Lack of expected PCR fragments, obtained with the primers Dvir8.1F and Dvir5.1R on the *D. virilis* flies genomic DNA template, which does not contain *cox3* numt and *Tv1* retrotransposon associations.

Lane 2 – PCR fragment, constituted by *Tv1* retrotransposon and *cox3* numt, of about 610 bp in size, obtained with the primers Dvir8.1F and Dvir5.1R on the *D. lacicola* genomic DNA template.

Lane 3 – PCR fragment, constituted by *Tv1* retrotransposon and *cox3* numt, of about 730 bp in size, obtained with the primers Dvir8.1F and Dvir5.2R on the *D. lacicola* genomic DNA template.



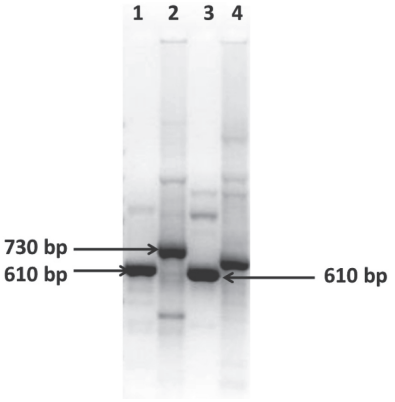
3. Experiment a-2

Fractionation of the PCR fragments formed by *atp6* numts associated with *Tv1* retrotransposon in opposite orientation.

Lane 1 – PCR fragment, constituted by *Tv1* retrotransposon and *atp6* numt, of about 600 bp in size, obtained with the primers Dvir6.1F and Dvir8.1F on the *D. montana* line 1021.13 genomic DNA template.

Lane 2 – PCR fragment, constituted by *Tv1* retrotransposon and *atp6* numt, of about 880 bp in size, obtained with the primers Dvir6.2F and Dvir8.1F on the *D. montana* line 1021.13 genomic DNA template.

Lane 3 – Lack of expected PCR fragments, obtained with the primers Dvir6.2F and Dvir8.1F on the *D. borealis*, genomic DNA template, which does not contain *atp6* numt and *Tv1* retrotransposon associations in opposite orientation.



4. Experiment c-2

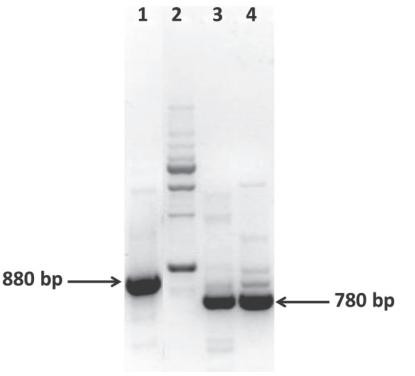
Fractionation of the PCR fragments formed by *cox3* numts associated with *Tv1* retrotransposon in opposite orientation.

Lane 1 – PCR fragment, constituted by *Tv1* retrotransposon and *cox3* numt, of about 610 bp in size, obtained with the primers Dvir7.2R and Dvir5.1R on the *D. montana* genomic DNA template.

Lane 2 – PCR fragment, constituted by *Tv1* retrotransposon and *cox3* numt, of about 730 bp in size, obtained with the primers Dvir7.2R and Dvir5.2R on the *D. montana* genomic DNA template.

Lane 3 – PCR fragment, constituted by *Tv1* retrotransposon and *cox3* numt, of about 610 bp in size, obtained with the primers Dvir7.2R and Dvir5.1R on the *D. borealis* genomic DNA template.

Lane 4 – PCR fragment, constituted by *Tv1* retrotransposon and *cox3* numt, of about 660 bp in size, obtained with the primers Dvir7.2R and Dvir5.2R on the *D. borealis* genomic DNA template.



5. Experiment a-2. *D. montana* interline polymorphism

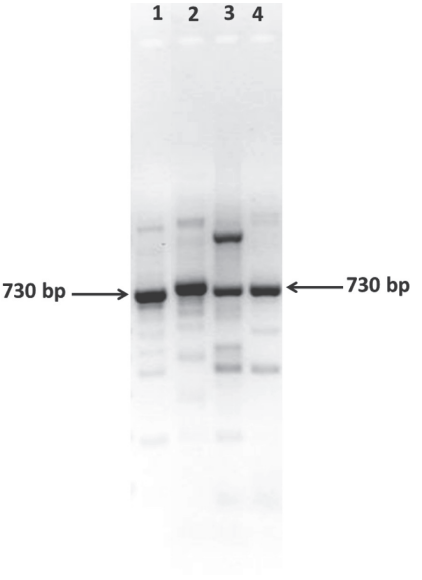
Fractionation of the PCR fragments formed by *atp6* numts associated with *Tv1* retrotransposon in opposite orientation, obtained with the primers Dvir6.2F и Dvir8.1F on the *D. montana* genomic DNA template.

Lane 1 – PCR fragment, of about 880 bp in size, obtained on the *D. montana* line 1021.13 genomic DNA template.

Lane 2 – The lack of expected PCR fragment in the case of *D. montana* line 1021.19.

Lane 3 – PCR fragment, of about 780 bp in size, obtained on the *D. montana* line KR 13-09 genomic DNA template.

Lane 4 – PCR fragment, of about 780 bp in size, obtained on the *D. montana* line 20 OL8 genomic DNA template.



6. Experiment c-2. *D. montana* interline polymorphism

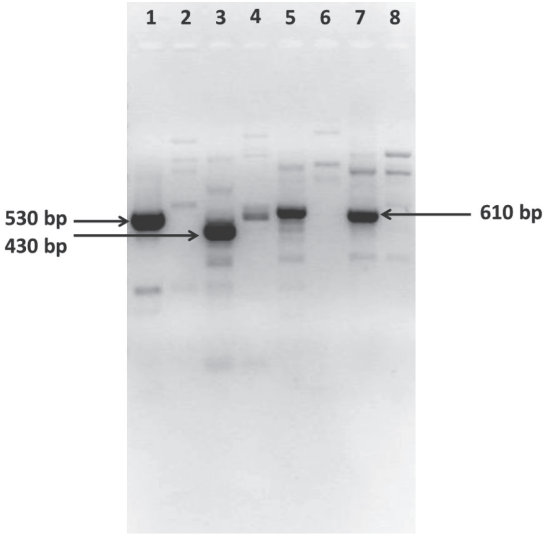
Fractionation of the PCR fragments formed by *cox3* numts associated with *Tv1* retrotransposon in opposite orientation, obtained with the primers Dvir7.2R and Dvir5.2R on the *D. montana* genomic DNA template.

Lane 1 – PCR fragment, of about 730 bp in size, obtained on the *D. montana* line 1021.13 genomic DNA template.

Lane 2 – PCR fragment, of about 730 bp in size, obtained on the *D. montana* line 1021.19 genomic DNA template.

Lane 3 – PCR fragment, of about 730 bp in size, obtained on the *D. montana* line KR 13-09 genomic DNA template.

Lane 4 – PCR fragment, of about 730 bp in size, obtained on the *D. montana* line 20 OL8 genomic DNA template.



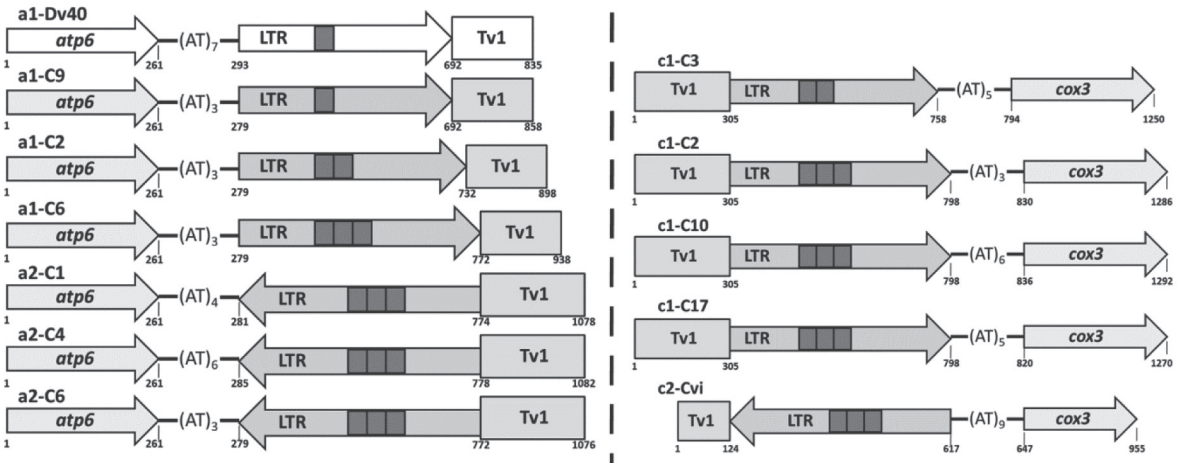
7. Comparison of males and females of *D. virilis*, *D. montana*, *D. lacicola* and *D. borealis* on the basis of the presence/absence of chimeric sequences of numt-*Tv1* shows their localization on the Y chromosome

Fractionation of PCR fragments, constituted by *Tv1* retrotransposon and numts.

Lane 1 – males and
Lane 2 – females of *D. virilis* – experiment a-1.
Lane 3 – males and
Lane 4 – females of *D. montana* line KR 13-09 (primers Dvir6.1F and Dvir8.1F) – experiment a-2.
Lane 5 – males and
Lane 6 – females of *D. lacicola* – experiment c-1
Lane 7 – males and
Track 8 – females of *D. borealis* – experiment c-2

Supplementary material 3

Schematic representation of the associations of *atp6* and *cox3* numts with *Tv1* retrotransposon in the cell culture line 79f7Dv3g of *D. virilis*



Schematic representation of *atp6* and *cox3* numts associated with the insertion of *Tv1* retrotransposon from the permanent cell culture 79f7Dv3g and from the *D. virilis* fly line Dv40. Arrows indicate the orientation of the genes and the long terminal repeats of *Tv1*.

Dark squares inside LTRs mark short direct repeats 40 bp in length.

Nucleotide sequences of *atp6* numts and *Tv1* from *D. virilis* fly lines B9, L160, Dv1 and Dv40 were deposited in GenBank under accession numbers JX560762–JX560765.

Nucleotide sequences of *atp6* numts and *Tv1* from *D. virilis* permanent cell culture 79f7Dv3g (experiment a-1) were deposited in GenBank under accession numbers JX560766–JX560769.

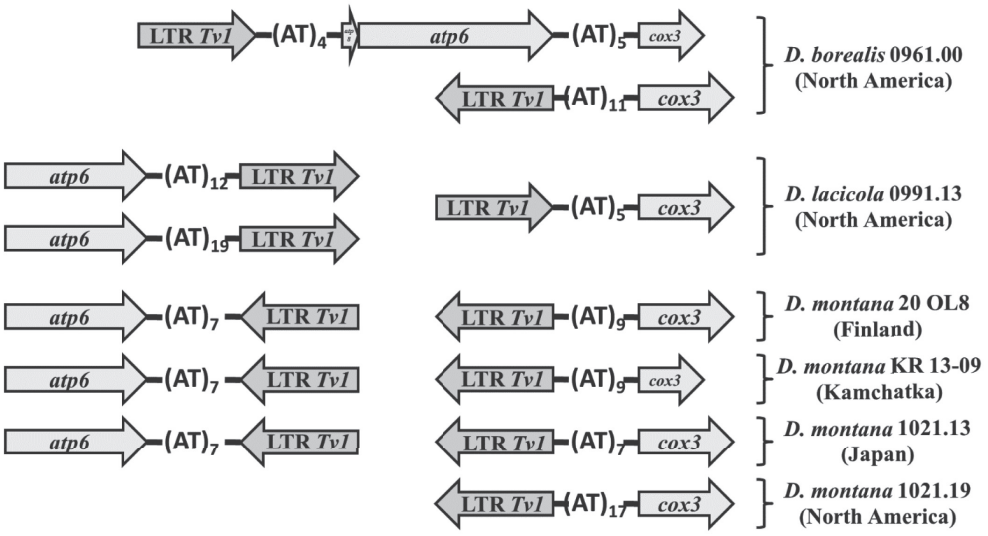
Nucleotide sequences of *atp6* numts and *Tv1* from *D. virilis* permanent cell culture 79f7Dv3g (experiment a-2) were deposited in GenBank under accession numbers KF669862– KF669864.

Nucleotide sequences of *cox3* numts and *Tv1* from *D. virilis* permanent cell culture 79f7Dv3g (experiment c-1) were deposited in GenBank under accession numbers KF669865–KF669868.

Nucleotide sequence of *cox3* numt and *Tv1* from *D. virilis* permanent cell culture 79f7Dv3g (experiment c-2) was deposited in GenBank under accession number FJ539165.

Supplementary material 4

Schematic representation of the associations of *atp6* and *cox3* numts with the insertions of retrotransposon *Tv1* from the genomes of three *Drosophila* species of the *virilis* group



Schematic representation of *atp6* and *cox3* numts associated with the insertion of *Tv1* from the genomes of three *Drosophila* species. Arrows indicate the orientation of the genes and the long terminal repeats of *Tv1*.

Nucleotide sequences of numts and *Tv1* from *D. borealis* genome were deposited in GenBank under accession numbers KX399473–KX399474.

Nucleotide sequences of numts and *Tv1* from *D. lacicola* genome were deposited in GenBank under accession numbers KX399470–KX399472.

Nucleotide sequences of numts and *Tv1* from *D. montana* genome were deposited in GenBank under accession numbers KX399475–KX399481.