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Gene Conversion in the Mitochondrial Genome on Interspecific Hybridization in Voles of the *Clethrionomys* Genus

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Abstract—The phenomenon of interspecific hybridization accompanied by transfer of the mitochondrial genome from the northern red-backed vole (*Clethrionomys rutilus*) to the bank vole (*Cl. glareolus*) in northeastern Europe is well known already for 25 years. However, the possibility of recombination between homologous segments of maternal and paternal mtDNAs of the voles during fertilization was not previously studied. Analysis of data on variability of nucleotide sequences of the mitochondrial gene for cytochrome *b* in populations of red-backed and bank voles in the area of their sympatry has shown that as a result of interspecific hybridization, the mitochondrial gene pool of bank voles contains not only mtDNA haplotypes of red-backed vole females, but also mtDNA haplotypes of bank voles bearing short nucleotide tracts of red-backed vole mtDNA. This finding supports the hypothesis that an incomplete elimination of red-backed vole paternal mtDNA during the interspecific hybridization between bank vole females and red-backed vole males leads to the gene conversion of bank vole maternal mtDNA tracts by homologous ones of mtDNA of red-backed vole males.

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Interspecific hybridization in areas of species sympatry is rather a common situation recorded in fishes, amphibians, and some mammals (house mice, voles, sables, and pine martens) [1-9]. According to the Haldane's rule [10], usually only hybrid females are fertile, and considering the maternal inheritance of the mitochondrial genome in vertebrates, during hybridization the mitochondrial gene pool of the acceptor species contains mitochondrial DNA (mtDNA) of both the acceptor and donor species. Bank and red-backed voles (respectively *Clethrionomys glareolus* and *Cl. rutilus*) whose sympatry area includes boreal coniferous forests of Fennoscandia and European Russia exemplify naturally hybridizing species. The introgression of mtDNA from red-backed to bank voles was shown 25 years ago in the Fennoscandian populations [3] and later also in Russian populations of the sympatry area of these vole species [5, 6]. The hybrid populations occupy the most northern parts of the bank vole areal [3, 4, 8]. Such direction of introgression can be associated with a prevalent mating of red-backed females (including hybrid ones) with bank vole males, because red-backed voles are believed to be better adapted to more severe ecological conditions [8, 11]. Although this hypothesis is not strictly proven, it is still very attractive.

However, another aspect of the interspecific hybridization in voles has not been studied at all, namely, the possibility of mtDNA transfer by the paternal line [12]. The mitochondrial genome in vertebrates is known to be inherited by the maternal line [13], and a system of paternal mtDNA elimination is supposed to exist on fertilization. However, on the interspecific mating of house mice a leak of the paternal mtDNA could occur, probably due to a strict species-specific system of elimination of the paternal mtDNA and a possibility of its failures in the case of interspecific hybridization [14-16]. In hybrids of the first filial generations, such failures manifest themselves as mtDNA heteroplasmy (i.e. coexistence of both maternal and paternal types of mtDNA), but later the strictly maternal inheritance of mtDNA is recovered in the repeated matings [15]. Under conditions of heteroplasmy, molecules of the paternal and maternal mtDNAs can recombine, although it occurs very seldom [17]. No cases of interspecific heteroplasmy of mtDNA have been reported in population studies on mtDNA diversity of red-backed and bank voles [3-6, 8, 9]. However, if "leaks" of the paternal mtDNA really occurred during interspecific hybridization, they could be recorded in the mitochondrial genomes as gene conversions of mtDNA fragments of one species by homologous fragments of

mtDNA of the other species. The purpose of the present work was to test this hypothesis.

METHODS OF INVESTIGATION

Molecular data. The analysis is based on data on the variability of nucleotide sequences of the cytochrome *b* gene mtDNAs obtained in four studies of bank vole *Cl. glareolus* populations in the European part of Russia [6], Finland [8], Norway, Sweden, Finland and Karelia (GenBank data HQ288328-HQ288418) and Poland [9].

Phylogenetic and statistical analysis of data. Phylogenetic analysis of nucleotide sequences of the cytochrome *b* gene was performed using the Neighbor-Joining (NJ) algorithm based on *p*-distances between the mtDNA sequences (MEGA 5.05 program package [18]). For the phylogenetic analysis, nucleotide sequences of the cytochrome *b* gene of red-backed and bank voles and also of the large-toothed red-backed vole *Cl. rufocanus* as outgroup were used.

Gene conversion was searched using an algorithm of the Gene conversion program in the DnaSP 5.10 program package [19], which on interpopulation comparisons allowed us to find the most probable DNA tracts with the gene conversion [20]. The influence of selection on mtDNA variability was determined using DnaSP 5.10, which allowed us to assess the ratio of numbers of nonsynonymous substitutions by nonsynonymous sites (K_A) to numbers of synonymous substitutions by synonymous sites (K_S). Under conditions of selective neutrality, the K_A value should correspond to the K_S value; K_A values higher than those of K_S suggest a positive selection effect, and the K_A values lower than those of K_S suggest negative (purifying) selection.

Search for selection on the level of individual amino acid residues. To reveal selection in the mtDNA of the cytochrome *b* gene, changes in the physicochemical properties of amino acids during evolution (i.e. following the topology of the phylogenetic NJ-tree) were analyzed using the TreeSAAP 3.2 program [21]. The algorithm of this program allowed us to compare the observed distribution of changes in physicochemical properties of amino acids (31 properties were analyzed) in the phylogenetic tree of mtDNA with distribution expected based on the hypothesis of random character of amino acid substitutions under conditions of selective neutrality. The *z*-test allowed us to evaluate the significance of amino acid substitutions and also to determine the selection type. According to the MM01 model [22], it was suggested that negative values of *z*-points should be determined by negative selection, whereas positive values should be determined by stabilizing selection in the case of conservative substitutions and by directed selection in the case of radical substitutions.

RESULTS AND DISCUSSION

Phylogenetic analysis of 392 sequences of the cytochrome *b* gene mtDNAs from different European populations of bank voles has shown that in three of four sets of the population data there was the transfer of mtDNA haplotypes of the red-backed vole into the gene pool of the bank vole (Table 1). The divergence between the mtDNA haplotypes of red-backed and bank voles is rather high (7.5%); therefore, the nucleotide sequences of these two species are characterized by clearly distinct profiles. The data are in agreement with the results of previous studies, which recorded for the first time interspecific hybridization in some regions of Europe [3-6].

However, comparative analysis of the data presented by the haplotypes of bank and red-backed voles within the gene pool of bank vole aimed to reveal interspecies recombination (the DnaSP 5.10 program packet [19]) revealed within two of four sets of population data analyzed gene conversion of tracts of mitochondrial lineages of bank voles by homologous tracts of red-backed vole mtDNA (Table 1).

Table 1. Distribution of cytochrome *b* gene haplotypes corresponding to *Cl. glareolus* (GLA) and *Cl. rutilus* (RUT) species and found in the population gene pools of the bank vole *Cl. glareolus*

Region, source of data	N	GLA	RUT	GLA_rec_RUT
European part of Russia [6]	95	56	30	9
Finland [8]	102	70	31	1
Fennoscandia (GenBank HQ288328-HQ288418)	91	80	11	0
Poland [9]	104	104	0	0

Note: N, size of general sample; GLA, number of mtDNA haplotypes of the *Cl. glareolus*; RUT, number of mtDNA haplotypes of species *Cl. rutilus*; GLA_rec_RUT, number of GLA haplotypes carrying the gene conversion with a haplotype RUT tract.

Fragment 1 (between positions 139 and 216 of the cytochrome *b* gene)

```
EU035648g     ACA GGA CTT TTC CTA GCA ATA CAC TAT ACA TCA GAC ACA TCA ACA GCA TTC TCA TCA GTT ACC CAC ATC TGT CGA GAC
EU035651g     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
JF929993g_rec ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
EU035699g_rec ... ... ... ... ... ..C ... ..T ..C ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ..A ... ...
EU035704g_rec ... ... ... ... ... ..C ... ..T ..C ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ..A ... ...
EU035705g_rec ... ... ... ... ... ..C ... ..T ..C ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ...
EU035639r     ... ... ... ... ... ..C ... ..T ..C ... ... ..T ... ... ... ... ... ... ... ..C ..T ... ... ... ... ...
JF930046r     ... ... ... ... ... ..C ... ..T ..C ... ... ..T ... ... ... ... ... ... ... ..C ..T ... ... ... ... ...
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Fragment 2 (between positions 529 and 606 of the cytochrome *b* gene)

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EU035648g     CGA TTC TTC GCA TTC CAC TTC ATC CTG CCT TTC ATC ATC ACA GCC CTC GTT TTC GTC CAC TTA TTA TTC CTA CAC GAA
EU035651g     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
JF929993g_rec ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ..T ... ... ... ... ... ... ... ... ... ...
EU035699g_rec ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
EU035704g_rec ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ...
EU035705g_rec ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
EU035639r     ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ..T ..C ... ... ..T C.. C.. ... ... ... ...
JF930046r     ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ..T ..C ... ... ..T C.. C.. ... ... ... ...
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Fig. 1. Nucleotide sequences of two fragments of the cytochrome *b* gene of bank and red-backed voles. The numbers indicate the sequences in GenBank; additional letters “g” and “r” mark, respectively, the species *Cl. glareolus* and *Cl. rutilus*. The *Cl. glareolus* haplotypes characterized by gene conversions of *Cl. rutilus* mtDNA fragments are indicated as g_rec.

Figure 1 shows nucleotide sequences of the cytochrome *b* gene fragments carrying the gene conversion. In the segment between positions 147 and 174 (according to nucleotide numbering in the cytochrome *b* gene) in bank voles from the European Russia population, nine cases of gene conversion were observed with three and four informative sites (specimen numbers in GenBank are: EU035689, EU035691, EU035699-EU035705). Figure 1 shows fragments of the three haplotypes EU036699, EU035704, and EU035705. In the Finnish population of bank vole one more case of interspecific hybridization was recorded in the segment located between positions 564-576 of the JF929993 haplotype (Fig. 1).

It was supposed that the mtDNA transfer of red-backed vole haplotypes into the gene pool of the bank vole could be significant for adaptation due to the better adaptation of red-backed voles to more severe ecological conditions (considering their inhabitance in the more northerly areas [11]); thus, the question of the role of selection of the mtDNA variants on the interspecific hybridization seemed to be rather important. Earlier studies revealed that the variability of mtDNA of bank voles within the interspecific hybridization areas seemed to be neutral, but this question was not analyzed in detail [8]. In the present work the K_A/K_S values have been obtained, and they show no differences between the haplotype groups of bank and red-backed voles in this parameter (Table 2). In both groups of mtDNA haplotypes, the K_A/K_S values are, on average, ~0.04, and thus are within the range of K_A/K_S values characteristic for vertebrates (0.03-0.05) [23-25]. Thus, analysis of distribution of the nonsynonymous and synonymous substitutions in the cytochrome *b* gene has shown the absence of difference between groups of haplotypes of bank and red-backed voles within the mitochondrial gene pools of the bank vole.

However, the organisms' adaptation can depend only on some important positions of amino acids, and then the general statistics of amino acid substitutions presented as K_A/K_S values becomes uninformative [21, 22]. To assess the selection effect on individual amino acid positions of cytochrome *b*, in the present work a TreeSAAP-analysis of nucleotide sequences of mtDNA was performed. This analysis allows a researcher to reveal conservative and radical changes in amino acids leading to changes in physicochemical properties of protein segments and to evaluate the role of various types of selection in structural rearrangements of DNA and proteins during evolution [21]. Obviously, substitutions accompanying formation of phylogenetic clusters and thus determining biochemical features of large groups of animals seem to be the most important.

Figure 2 shows the NJ-phylogenetic tree of the cytochrome *b* gene haplotypes of bank and red-backed voles with amino acid substitutions indicating phylogenetic differentiation stages. The determination of the whole group of red-backed vole haplotypes including those that are present in the bank vole mitochondrial gene pool is accompanied by a nonsynonymous substitution leading to the amino acid substitution I327V in the cytochrome *b* transmembrane domain G in accordance with the scheme of organization of the secondary structure of this protein in animals [26]. A further substitution V232A is observed in transmembrane domain E. Both substitutions are conservative; nevertheless, they result in significant ($p < 0.001$) changes in physicochemical characteristics of the protein segments and, according to the MM01 model [22] (TreeSAAP 3.2 program), indicated an influence of stabilizing selection on the cytochrome *b* gene. Analysis of mtDNA haplotypes specific for bank vole exemplifies the selection influence only in the case of haplotypes with the gene conversion of the 147-174 segment. The haplotypes shown in Fig. 2 as EU035699-EU035703 are characterized by T→A transversion in position 210 (Fig. 1), which leads to the amino acid substitution C70W in the cytochrome *b* Q_0-redox center *ab*-loop interacting with the cytochrome c_1 encoded by the nuclear genome [27, 28]. This segment is very important for functioning of the respiration chain; therefore, amino acid substitutions within it can be significant for adaptation [26-28]. The C70W substitution in the recombinant haplotypes suggests an influence of stabilizing selection ($p < 0.05$) on the cytochrome *b* gene. It should be noted that the transversion in position 210 seems to appear within the recombinant haplotype, which became a basis for the whole phylogenetic cluster of the mitochondrial tree of bank voles even after the gene conversion, because this substitution is specific only for members of this cluster. This is supported by results of analysis of the mutational spectrum of the cytochrome *b* gene of bank voles studied to date.

Table 2. Values of K_A/K_S ratio in the cytochrome *b* gene in bank voles with the GLA and RUT haplotypes from different populations of Europe

Region, source of data	K_A/K_S	
	GLA	RUT
European part of Russia [6]	0.040	0.039
Finland [8]	0.037	0.036
Fennoscandia (GenBank numbers HQ288328-HQ288418)	0.041	0.042
Poland [9]	0.038	–

Note: The sign "–" indicates the absence of RUT haplotypes in the sample.

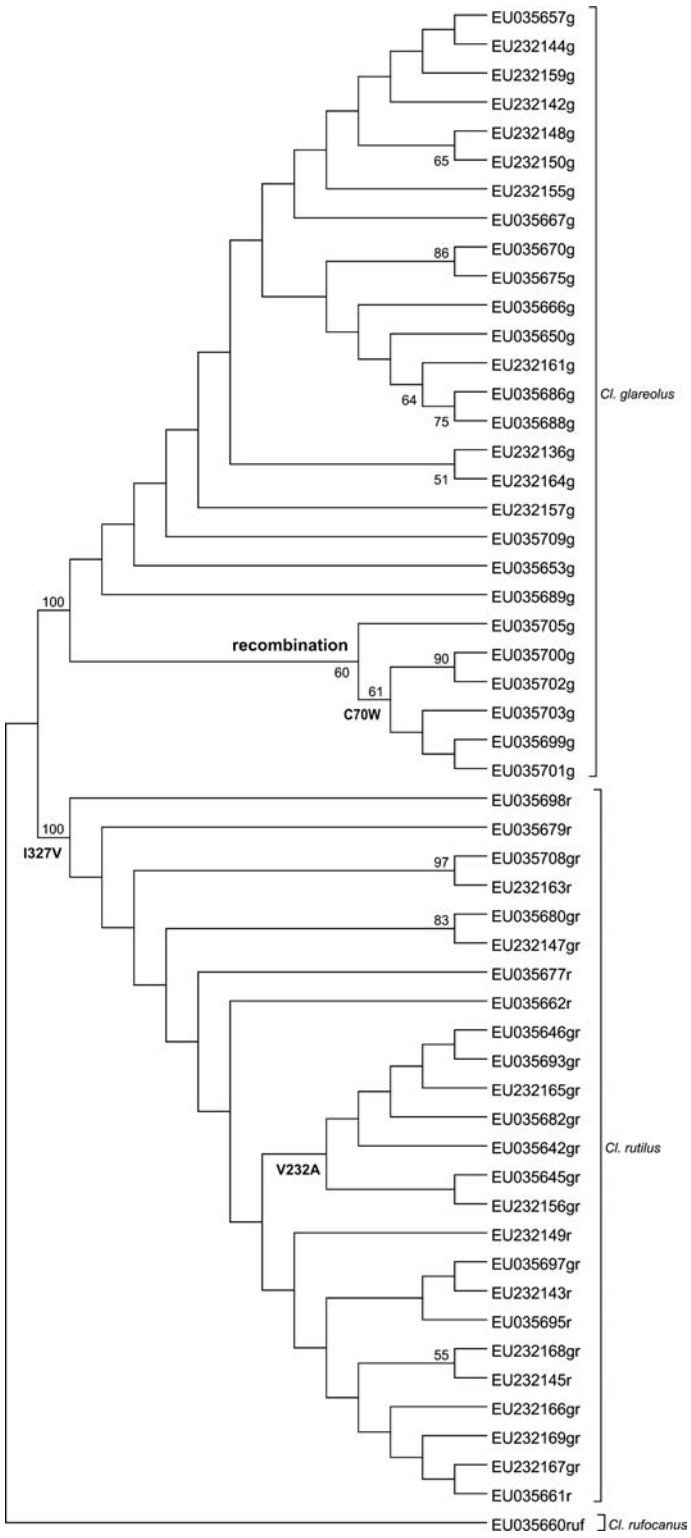

Fig. 2. NJ-phylogenetic tree of a segment of nucleotide sequences of the cytochrome *b* gene of bank and red-backed voles. On the branches, amino acid substitutions are shown which indicate the stabilizing selection influence on the mtDNA clusters by data of TreeSAAP-analysis. The sequence numbers correspond to those in GenBank, the letters added to the numerals indicate the species: *Cl. glareolus* (g) and *Cl. rutilus* (r). The mtDNA sequences found in the gene pool of bank voles but specified by the red-backed vole haplotypes are marked as "gr". A phylogenetic branch of mtDNA of recombinant origin is marked as "recombination". On the branches, values of bootstrap-indices (above 50%) are indicated which evaluate the statistical significance of the phylogenetic clusterization of mtDNA. Outgroup: the haplotype of mtDNA of the large-toothed red-backed vole *Cl. rufocanus*.

Thus, although there is no evidence in favor of selection associated with distribution of mitochondrial lineages of red-backed voles within gene pools of bank voles, positive selection influence is observed in mtDNA haplotypes specified by the gene conversion of bank vole mtDNA tracts by homologous tracts of red-backed vole. Overall, the data indicate that the interspecific hybridization of bank and red-backed voles is rather a complicated phenomenon that manifests itself not only by the unidirectional transfer of maternal haplotypes of mtDNA from red-backed voles to bank voles but also by a recombination of the maternal and paternal mtDNAs on the heteroplasmy stage arising on fertilization. The findings indicate that the interspecies barrier is overcome also by animals with recombinant haplotypes produced as a result of mating between bank vole females and red-backed vole males. The mtDNA haplotypes of red-backed males are eliminated by the system recognizing mitochondria of spermatozoa [14-16], which acts in oocytes of bank voles. However, the elimination seems to be incomplete, and as result the recombination between haplotypes of bank vole females and red-backed vole males has time to be finished, especially due to presence in the mitochondria of enzymes necessary for intermolecular recombination [12]. And it is quite possible that the recombinant haplotypes of mtDNA in the mitochondrial gene pool of bank vole is promoted by positive selection recorded in the present work. Note that up to now there are no data in favor of the opposite picture – the gene conversion of tracts of mtDNA of red-backed vole females by homologous tracts of mtDNA of bank vole males and the transfer of bank vole mtDNA into the gene pool of red-backed vole. To elucidate this, further studies are necessary on both the mtDNA variability in forest voles and biochemical features of systems responsible for the elimination of paternal mitochondria in these animals.

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