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Abstracts
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Interspecies interactions in the bank (*Clethrionomys glareolus*) and red-backed (*Clethrionomys rutilus*) voles: Introgression or ongoing hybridization?

Rodchenkova E.N.(1), Fokin M.V. (1), Rakitin S.B. (2), Abramson N.I. (1)

(1) Zoological Institute RAS, Sankt-Petersburg
Rodchenkovae@gmail.com
(2) Institute of Plant and Animal Ecology Ural Branch RAS
rakitin@ipae.uran.ru

Bank (*Cl. glareolus)* and red-backed (*Cl. rutilus)* voles are one of the most common and widely distributed rodents of forest zone with a very wide zone of sympatry which was formed gradually alongside with colonization of formerly glaciated area from different refugiums. Two species can produce hybrids in captivity and up to now there are a lot of data on individuals of *Cl. glareolus* that have the mitochondrial genome of *Cl. rutilus*. We applied molecular markers techniques both nuclear and mitochondrial to test the hypothesis on 1) such individuals may occur as a result of ancient hybridization event during postglacial colonisation in case when number of one of the species is small or 2) hybridization is randomly occurring now in sympatric populations.

First we applied the method of PCR-typing and screen 316 individuals of bank voles from 59 sites of European Russia and in result we defined the relative number and geographic distribution of bank voles with "foreign" *mt* genome. The distribution of such voles represents a curve from southern Urals to north-western Europe through Komi and Arkhangelsk region where their number is small related to "proper" bank voles to northern Karelia, Murmansk area and northern Finland where they reach 100% occurrence in the studied samples.t.

Second we sequenced cyt *b* (967 bp) in 28 individuals of *Cl. glareolus* with mt genome of red-backed vole and 23 *Cl. rutilus* from 7 localities where they occur together. Comparative analysis of haplotypes showed that most of them differ by 3-4 nucleotide substitutions what evidence for ancient hybridization event with a following introgression. However, we found one individual of the bank vole in the Middle Ural area with the identical haplotype with a red-bank vole from the same locality.

Thus the third step was in applying the nuclear loci in the survey: five microsatellite loci (MsCg4, MsCg9, MsCg19, LIST3-03) and LCAT gene, the latter discriminate these species reliably. We have used 5 microsatellite common for both species – MsCg4, MsCg9, MsCg19 (Ref1) and LIST3-03 (Ref2) for analysis of 4 populations of each species. Nearly three hundred voles were screened for all loci. Therefore we were able to evaluate species specific characteristics of each locus. Locus Cg9 was the most suitable for species discrimination since almost no overlap in allele lengths distribution. We have also found different parity of alleles lengths in bank and red voles.

In order to verify recent hybridization event we also examined F1 hybrids obtained in the laboratory alongside with "pure" bank and red-backed voles, and bank voles with "foreign" cyt *b* haplotype. The latter ones appear to be "pure" bank voles that come out from LCAT sequencing and microsatellite genotyping. Thus our assumption that "red-backed" cyt *b* haplotypes of bank vole is the result of the introgression is confirmed by nuclear genome data. All laboratory hybrids are heterozygotes in all species-specific sites of LCAT sequence, and four microsatellite loci. Exactly the same pattern was found in the only individual from Ural that was defined by the superficial appearance and skull morphology as bank vole and had a mitochondrial genome of the red-backed vole. Thus, both nuclear and mitochondrial molecular markers make it clear that this individual is a nature-born F1 hybrid.

Our results, confirmed by mitochondrial and nuclear genes survey, show that most voles possess "foreign" mitochondrial genome in result of the ancient hybridization and further introgression, but randomly hybridization occur and nowadays. Such cases most likely take place when the number of one of the species decreases and hybridization is more readily goes in crosses of red-backed females with bank vole males.

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