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MITOCHONDRIAL DNA POLYMORPHISM OF THE EUROPEAN ROE DEER, *CAPREOLUS CAPREOLUS* (ARTIODACTYLA, CERVIDAE), FROM THE SOUTH-WEST OF UKRAINE

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Mitochondrial DNA Polymorphism of the European Roe Deer, *Capreolus capreolus* (Artiodactyla, Cervidae), from the South-West of Ukraine. Zvychaynaya E. Yu., Volokh A. M., Kholodova M. V., Danilkin A. A. — Analysis of mtDNA control region (934 b. p.) and cytochrome b gene (1140 b. p.) polymorphism of the 33 roe deer samples from the south-west of Ukraine was carried out. 30 different haplotypes of mtDNA have been described and all of them are related to *Capreolus capreolus* Linnaeus, 1758. Two well differentiated haplogroups were discovered on the examined territory. The isolated subgroup of related haplotypes was revealed in the Crimea that is likely a result of the long-term geographical isolation of the Crimean roe deer populations.

Key words: European roe deer, Siberian roe deer, *Capreolus*, range, population, haplogroups, gene, control region, cytochrome *b* gene, mtDNA.

Полиморфизм митохондриальной ДНК европейской косули, *Capreolus capreolus* (Artiodactyla, Cervidae), юго-западной Украины. Звычайная Е. Ю., Волох А. М., Холодова М. В., Данилкин А. А. — Проведен анализ полиморфизма контрольного региона (934 н. п.) и гена цитохрома *b* мтДНК (1140 н. п.) 33 образцов косули юго-западной Украины. Описано 30 разных гаплотипов мтДНК, все они принадлежат *Capreolus capreolus* Linnaeus, 1758. На исследованной территории обнаружены две хорошо дифференцированные гаплогруппы с обособленной подгруппой близких гаплотипов в Крыму, что, скорее всего, является следствием длительной географической изоляции популяции косули Крымского полуострова.

Ключевые слова: европейская косуля, сибирская косуля, *Capreolus*, ареал, популяция, гаплогруппы, ген цитохрома *b*, мтДНК.

Introduction

In Holocene period the European (*C. capreolus* Linnaeus, 1758) and the Siberian (*C. pygargus* Pallas, 1771) roes inhabited Ukraine and the west of Russia. Remains of the Siberian roe, dating back to the end of the 1st — start of the 2nd millennium AD, were found in Orel, Kursk, Voronezh and Moscow regions, in the lower reaches of the Don river, in the peat layers near Zavorichi station in Kyiv region, in the Middle Dnieper, in the archeological sites of Poltava and Kharkiv regions. The skeletal remains about the same period belonging to the European form of the roe (roe deer) are recognized on the right bank of the Dnieper (the Right-Bank Ukraine). A zone of intergradation was, apparently, on the Left-Bank Ukraine. Both species of the roes were exterminated in the vast area from the Dnieper River to the Ural Mountains at the end of the XIXth and the start of the XXth centuries. Thus, the area of the genus *Capreolus* in Europe turned out to be divided on the west, Crimean, Caucasian and Ural parts.

A few intermediate, small habitats had only remained between them. Increase of the number and restitution of the roe habitat became noticeable from the 1930s and 40s, what is associated with a decrease in hunting pressure and a significant reduction in wolf number. The European roe deer populated almost the entire territory of Ukraine, as well as the western, central and southern regions of the European part of Russia in the second half of the XXth century. Restore of the Siberian roe area occurred at the same time. By the end of the 70s the Siberian roe moved further from the Ural foothills up to the borders of Mordovia and Penza region, came to the Hoper and Don rivers in the Volgograd region, where the European-Siberian

hybrid populations had formed (Heptner et al., 1961; Danilkin, 1999, 2006). Moreover, the European and the Siberian roes were settled intensively in the west of the Soviet Union during the XXth century. More than three thousand individuals have been released since 1925, mainly in the European part of Russia and in Ukraine (Kyiv region). 548 animals, including 72 animals of the Siberian roe caught in the Primorsky kray (Pavlov et al.., 1974; Pavlov, 1999) were brought there.

By analyzing the historical events, we can assume that mainly the European roe deer inhabits Ukraine at present. Meanwhile, it is quite possible that the Siberian roe and the hybrid individuals may live there. Such hypothesis is based on the reports about the discovery of the Siberian roe deer in the Samara forest of Dnipropetrovsk region at the beginning of the XXth century (Brauner, 1915) and the results of the karyotype analysis of the animals from this region (Danilkin, 1999).

The aim of our work is to provide the phylogeographic analysis of mitochondrial DNA sequences of the roe deer from the south-west of Ukraine, especially from the Crimea where its population was isolated for a long time.

Material and methods

It was carried out a molecular-genetic analysis of the roe deer muscle tissue samples that were collected in eight regions of the south-west of Ukraine (table 1) and preserved in alcohol. The control region and the cytochrome *b* gene of the mtDNA were used as molecular markers. The nucleotide sequences of the 33 samples served for the construction of the median haplotype network. Enough long chain of nucleotide sequences (936 bp for the control region and the 1140 bp for the cytochrome *b* gene) was received for them. The combined sequence of homologous fragments of the mtDNA of *Capreolus pygargus* from the Krasnoyarsk kray (sample 723; Zvychaynaya et al., 2011 a) was used as an outgroup in the phylogenetic analysis.

DNA was isolated with the help of a reagent kit "Diatom DNA Prep 200" (Isogene, Moscow). The amplification was performed in 10 μl of the solution composed of: 2 μl of the compound for polymerase chain reaction (PCR) X5MasterMix (Dialat, Moscow) comprising SmartTAQ polymerase (Dialat,), 1 μl of prepared deoxinucleotide solution, 1 μl of forward and 1 μl of reverse primers (5 pmol/μl), 5 μl of water. For the amplification of the control region were used primers tRNAProSai (5'-TCA ACA CCC AAA GCT GAA GT-3') and tRNAPheSai (5'-GCA TTT TCA GTG CCT TGC TT-3'), for the amplification of cytochrome b gene were applied primers Cytb-ung-F (5'-GAAAAACCATCGTTGT (C/T) ATTCA-3') and Cytb-ung-R (5'-TTTTCTGGTTTACAAGACCAGT (G/A) T-3').

The reaction was executed under such conditions: 94 ºC for 3 min (1 cycle), 94 ºC for 30 seconds, 62 ºC for 30 seconds, 72 ºC for 2 min (35 cycles) and 72 ºC for 6 min (1 cycle). PCR was carried out using the thermocycler "Tetrad2" (Bio-Rad, USA). Purification of the amplified product was performed by ethanol solution precipitation with 5M of sodium acetate. The electrophoresis and reading the nucleotide sequences of the amplified product were performed on the automatic sequencer ABI PRISM 3130 (Applied Biosystems, USA) using a reagent kit BigDye Terminator kit 3.1 (Applied Biosystems). Nucleotide sequences alignment was performed manually using the Bioedit (Hall, 1999). Analysis of

Table 1. List of the roe deer tissue samples from the south-west of Ukraine, included into molecular-genetic study

Таблица 1. Список образцов тканей косуль юго-западной Украины, включенных в молекулярно-генетическое исследование

Sample number *	Localization of the sample: region, district
586–588	Crimea
589, 594–596, 790–792, 796	Chernivtsi region, Storozhynets' district
592	Zaporizhzhya, Melitopil' district
593	Lviv region, Rava-Rus'ka
600, 835	Vinn itsa region
785, 786	Odesa region, Balta district
787, 788	Odesa environs
789	Odesa region, Kiliya district
793	Mykolaiv region, Veselinovka district
794, 795	Odesa region, Reni district
831	Odesa region, Frunzivkf district
834	Odesa region
797–800, 832	Ternopil' region
833	Kyiv region

* Numbers in the collection of tissues for genetic analysis of the Molecular Diagnostics Centre of the A. N. Severtsov IEE of the RAS.

sequences variability, estimation of nucleotide diversity (π) and the construction of the phylogenetic tree were performed using the Kimura two-parameter model in the programme MEGA 3.1 (Kimura, 1980; Saitou, Nei, 1987; Kumar et al., 2004). Construction of the median networks of examined haplotypes was done in the Network programme (Bandelt et al., 1999).

Results

All received nucleotide sequences of the mitochondrial DNA fragments belong to the European roe deer; characteristic sequences of the Siberian roe were not found in the studied samples.

The variability of the studied nucleotide fragments was estimated as relatively low. 23 variable positions (2.02 %) were just described for cytochrome *b* gene sequences, and 8 of them were single substitutions. The nucleotide variability was $\pi = 0.006$ (SE = = 0.001). Variability of the control region revealed twice higher: the amount of variable positions was 38 (4.07 %), from which single substitutions were 14. Nucleotide variability (π) for this fragment of the mtDNA was 0.009 (SE = 0.002).

The nucleotide variability is $\pi = 0.007$ (SE = 0.001) for combined alignment of the two fragments of the mtDNA. Altogether 30 haplotypes that form on the constructed median network of the haplotypes two distinct haplogroups were described (fig. 1).

These haplogroups are also well defined and are well notable on the phylogenetic tree (fig. 2).

Geographical distribution of selected mitochondrial lineages is as follows (fig. 3): haplogroup 1 has been found in Lviv, Odesa, Nikolaev, Chernivtsi and Ternopil' regions; haplogroup 2a has been found in Chernivtsi, Odesa, Kyiv, Vinnitsa and Ternopil' regions; haplogroup 2b has been found in Zaporizhzhya, Chernivtsi, Vinnytsia regions and in the Crimea. The Crimean population of the roe deer is represented only by the samples belonging to the haplogroup 2b.

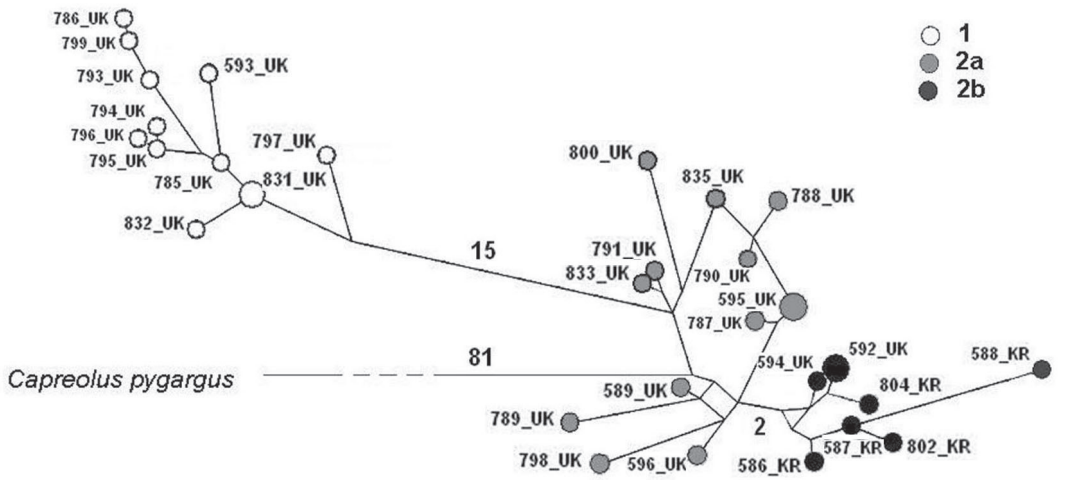

Fig. 1. Median network of roe deer mtDNA haplotypes from Ukraine, built in the Network programme on the basis of phylogenetic analysis of 33 cytochrome *b* gene and control region nucleotide sequences (2075 b. p. in total): 1 — the haplogroup 1; 2a and 2b — the haplogroup 2; numbers above the branches indicate the quantity of sinapomorphs that determine the branch length; "KR" indicates samples from the Crimean peninsula; "UK" indicates samples from the continental regions of Ukraine.

Рис. 1. Медианная сеть гаплотипов мтДНК косули Украины, построенная в программе Network на основании филогенетического анализа 33 объединённых нуклеотидных последовательностей гена цитохрома b и контрольного региона (2075 н. п.). 1 — гаплогруппа 1; 2a и 2b — гаплогруппа 2, цифрами над ветвями обозначено количество синапоморфий, определяющих длину ветви. «KR» обозначены образцы с Крымского полуострова, «UK» — образцы из континентальных районов Украины.

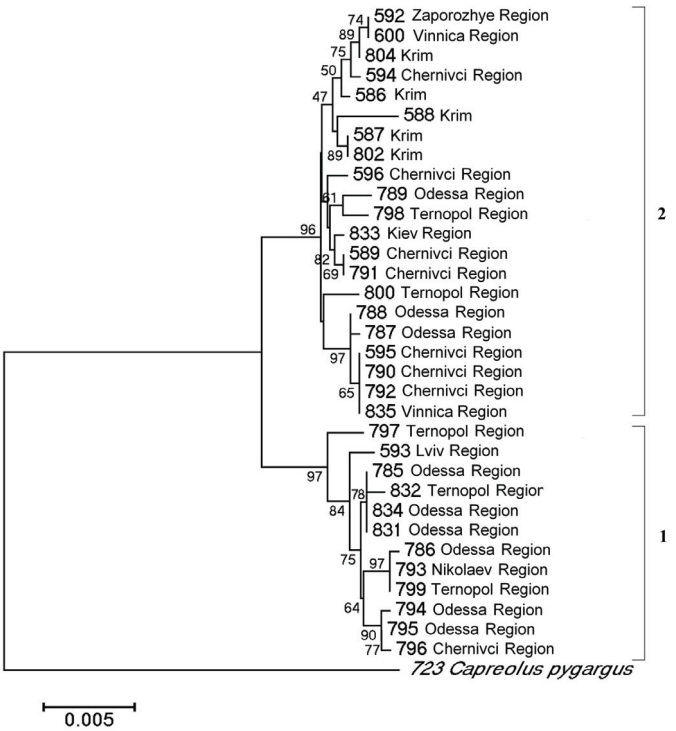

Fig. 2. Phylogenetic tree built according to the Neighbor-Joining method on the basis of analysis of 33 cytochrome *b* gene and control region of joined nucleotide sequences (2075 b. p. in total) of the roe deer from Ukraine: 1, 2 — certain haplogroups of *C. capreolus.*

Рис. 2. Филогенетическое древо, построенное методом ближайшего связывания (Neighbor-Joining) на основании анализа 33 объединённых нуклеотидных последовательностей гена цитохрома *b* и контрольного региона (2075 н. п.) косули Украины: 1, 2 — гаплогруппы *C. capreolus.*

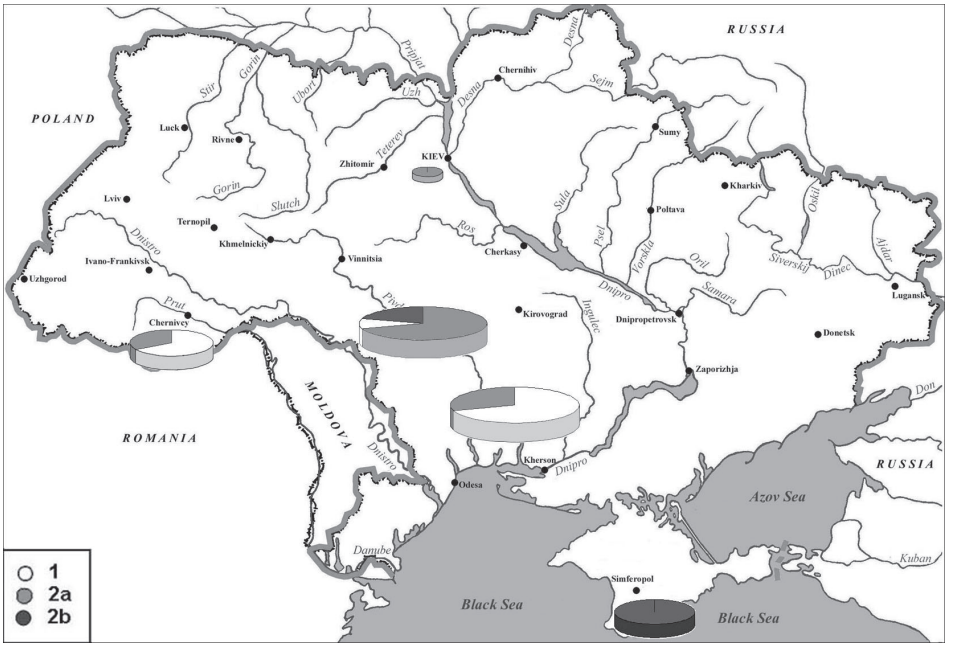

Fig. 3. Geographical distribution of the European roe deer mitochondrial lineages in the south-west of Ukraine: 1, 2a and 2b — mitochondrial lineages of *C. capreolus.*

Рис. 3. Географическое распределение митохондриальных линий европейской косули на юго-западе Украины: 1, 2a и 2b — митохондриальные линии *C. capreolus.*

Discussion

The formation of the phylogeographic structure of the roe deer in the south of Ukraine, undoubtedly, was affected by severe depression with decrease of animal number and fragmentation of the species habitat in the XIXth-XXth centuries. Monomorphism of the Crimean peninsula population and separation of the haplogroup 2b are obvious consequences of the geographic isolation and preservation of the Crimean refugium, what was proved by investigation of the Ukrainian roe deer craniology (Volokh, 2002). The distribution of the mitochondrial lineage from the Crimea to the north-west is likely to happen after the restitution of species abundance and aggregation of the separated parts of the species range, although the possibility of this lineage preservation outside the Crimean peninsula is not excluded.

Analysis of limited samples does not make possible to identify the factors that underlie the formation of two mitochondrial lineages (haplogroups 1 and 2) of the roe deer in Ukraine. Molecular genetic analysis of a larger number of Central and East European populations is required to describe the phylogeographic structure of the *C. capreolus* in the region and possible ways of its formation.

The mtDNA haplotypes belonging to the Siberian form are not detected in the populations of the roe deer from the south-west of Ukraine, although our study showed wide spread of its mitotypes in the European part of Russia and Belarus (Zvychaynaya et al., 2010). In vicinities of Moscow, particularly, 78 % of individuals carry the mtDNA of Siberian roe (Zvychaynaya et al., 2011 b). We suppose that the Siberian form or its genetic traces can be found on the Left Bank of Ukraine and in Kyiv region, where further research including nuclear DNA study of the roe deer are desirable.

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