

Siberian Roe Deer (*Capreolus pygargus* Pallas, 1771) in Ukraine: Analysis of the Mitochondrial and Nuclear DNA

A. A. Danilkin^{a, *}, D. A. Plakhina^a, E. Y. Zvychnaynaya^a, A. V. Domnich^b,
M. V. Kholodova^a, P. A. Sorokin^a, and A. M. Volokh^c

^aSevertsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, 119071 Russia

^bZaporozh'e State University, Zaporozh'e, 69000 Ukraine

^cTaurian State Agro-Technological University, Melitopol', 72312 Ukraine

*e-mail: ldan@mail.ru

Received May 16, 2016

Abstract—A molecular-genetic analysis of the nucleotide sequences of the cytochrome *b* gene (1140 base pairs) of the mitochondrial DNA and 17 microsatellite loci of eight samples of roe deer from the Samara forest of Dnipropetrovsk oblast (Ukraine) was carried out. For comparison, 212 corresponding mtDNA sequences of the Siberian and European roe deer and data on the variability of microsatellite markers in 49 representatives of these species were included in the study. It was noted that all the analyzed mitochondrial sequences of individuals from the Samara forest are characteristic of the Siberian roe *Capreolus pygargus* Pallas, 1771. Four haplotypes were described, all of which belonged to the haplogroup typical for the western part of the range of *C. pygargus*. A fragment analysis of the microsatellite loci of nuclear DNA confirmed the identification of the investigated group with the Siberian species.

DOI: 10.1134/S106235901706005X

INTRODUCTION

Does the Siberian roe *Capreolus pygargus* exist in Ukraine? This question has long been of interest to zoologists and game experts and has become particularly acute in recent years in connection with the discussion on the taxonomy of the genus *Capreolus* and the speciation and the boundaries of the historical habitats of the European *C. capreolus* L. and the Siberian roe.

Fossil remains of roe deer in Ukraine and southwestern Russia are known from early Quaternary sediments, but they are especially abundant in the Holocene strata (Gromov, 1948; Pidoplichko, 1956; Bibikova, 1963, 1975; Tatarinov, 1970; Timchenko, 1972; etc.). Beginning with the Pleistocene, the existence of *C. capreolus* and *C. pygargus* is clearly discernible. Remnants of the small European roe deer are common in many archaeological sites of Western and Central Europe, with remains of large Siberian deer in Asia and Eastern Europe (Korotkevich and Danilkin, 1992; Danilkin, 1992a, 1999, 2014). Remains of the Siberian roe deer, dating mainly to the last centuries B.C. and the beginning of the second millennium A.D., have been found in many settlements on the Upper Volga; in the Oka basin; in Moscow, Orel, Kursk, and Voronezh oblasts; in the lower reaches of the Don in the ancient settlement of Sarkel; in the middle Dnieper region; in the archeological monuments of

Poltava and Kharkov oblasts; and in the peat layers near the Zavorichi station of Kiev oblast (Gorbachev, 1915; Gromova, 1948; Korneev, 1952; Tsalkin, 1956, 1961, 1963; Vereshchagin, 1959; Timchenko, 1972).

In Ukraine, in the Holocene, the ranges of the European and Siberian roe deer were, apparently, in contact and superimposed one each other. During this period, some individuals or groups of the Siberian roe deer, most likely, reached Central Europe. Here, as in Ukraine, hybridization could have occurred, as a result of which large specimens with “Siberian-type” horns sometimes appeared in the populations of the European roe deer (Cotta, 1969; Lehmann, 1976).

In the 19th and early 20th centuries, roe deer were almost destroyed by man on the territory of Eastern Europe (Geptner et al., 1961; Kirikov, 1966; Danilkin, 1992a, 1999, 2014). In the vast area from the Dnieper to the Urals, only a few small foci have survived, including the Black Forest in Kirovograd oblast and the Samara forest in Dnepropetrovsk oblast, where the roe deer could have survived (Brauner, 1915, 1923, 1928; Migulin 1927, 1929; Sharleman', 1937). The famous zoologist Brauner (1915) reported the following: “Considering the number of skulls and horns of roe from the provinces of Podolia, Bessarabia, Kherston, Ekaterinoslav, and the Crimean mountains, you can see that the roes of Novomoskovsk, Alexandrov, and Pavlograd counties of Ekaterinoslav province

belong to the species of the Siberian goat (*Capreolus pygargus* Pall.), in other areas of Southern Russia and the Crimea, they belong to the European species (*Capreolus capreolus* L.).” The sizes of their skulls and horns given by him leave no doubt of the correctness of this conclusion.

Some experts considered the Siberian roe deer in these foci to be a glacial relic or a Siberian or Caucasian interloper (Migulin, 1927, 1929; Charleman, 1937; Korneev, 1952). Heptner (1961) wrote, however, that this “question requires special investigation, systematic as well; however, it is highly doubtful that the true Siberian roe lived in these places.” Kryzhanovskii (1965) and Karpenko (1977) believed that the forests of right-bank Ukraine and the Samara forest are inhabited by the European species, since the morphometric indices of the individuals studied do not exceed the species limits.

Special morphometric, karyological, and biochemical studies (Sokolov and Danilkin, 1981; Sokolov et al., 1986; Danilkin, 1992b) showed that four roe deer caught on the right bank of the Dnieper in Kirovograd oblast were the European species. On the left bank of the Dnieper, in the population of roe deer of the Samara forest, out of nine individuals studied, two did not have additional chromosomes (microchromosomes), which makes it possible for them to be considered European: three contained one, and four individuals had two B chromosomes; i.e., they had the traits of the Siberian roe deer. However, the latter, in terms of the biochemical parameters, color, size and weight of the body, the size of the skull, and for males, in the size and structure of the horns, did not differ significantly from the European roe deer obtained in Ukraine. It was suggested that the grouping of the Samara forest is mixed.

Volokh (2007) considered, however, that the existence of a “micro isolate” of the Siberian roe “within the range of the European species is even theoretically impossible. Therefore, it is not surprising that our research in Dnepropetrovsk oblast (2001–2003) showed a complete absence of the Siberian roe deer in all its administrative regions.”

The aim of this work is to solve the phylogenetic, taxonomic, and, to a great extent, hunting problems using molecular genetic methods.

MATERIALS AND METHODS

A molecular-genetic analysis of eight samples of muscular tissue of the roe deer inhabiting the Samara forest of Dnepropetrovsk oblast, Ukraine, was carried out. The cytochrome *b* gene was used as a mitochondrial marker. Complete nucleotide sequences (bp) of this gene (1140) were obtained. For comparison, 212 corresponding nucleotide sequences of mitochondrial (mt) DNA of the Siberian and European roe deer from the collection of the Severtsov Institute of Ecology

and Evolution, Russian Academy of Sciences, were included in the analysis (Table 1). As nuclear markers, we used 17 microsatellite loci (RT1, RT5, BM4513, RT27, RT9short, BM6506, NVHRT30, BMS1788, RT6, OheQ, BL42, NVHRT16, BMC745, Roe09, Roe01, RT24, and IDVGA8) (Buchanan and Crawford, 1993; Bishop et al., 1994; Moore et al., 1994; Stone et al., 1995; Kappes et al., 1997; Wilson et al., 1997; Roed and Midthjell, 1998; Jobin et al., 2008). For the comparison of the microsatellite loci of the nuclear DNA, 49 samples of the Siberian and European roe deer were analyzed: from Samara oblast (29) and Altai krai (8) in Russia and 12 samples from Chernivtsi, Odessa, and Ternopil oblasts of western and Southwestern Ukraine.

DNA was isolated using the Diatom DNA Prep 200 (Isogen, Moscow) and Invitek (Germany) kits and the KingFisher Flex automatic system (Thermo Fisher Scientific, United States). Amplification was performed in 10 μ L using 2 μ L of the 5 \times Master Mix kit for the polymerase chain reaction (PCR) (Dialat, Russia) with the addition of the Smart Taq polymerase (Dialat, Russia) at a concentration of 2.5 units/ μ L of 0.1 μ L per sample, 1 μ L of the obtained solution of DNA, and 1 μ L of forward and reverse primers (5 pmol/ μ L). For amplification of the cytochrome *b* gene, the following primers were used: Cytb-ung-F (5'-GAAAACCATCGTTGTYATTCA-3') and Cytb-ung-R (5'-TTTTCTGGTTTACAAGACCAG-TRT-3'). The reaction was carried out in the following mode: 94°C, 3 min (1 cycle); 94°C, 30 s; 62°C, 30 s; 72°C, 2 min (35 cycles); 72°C, 6 min (1 cycle). The amplification product was purified by precipitation with a solution of ethyl alcohol supplemented with 3 M sodium acetate. The polymerase chain reactions with microsatellite primers were performed under the following conditions: 95°C, 2 min 15 s; 60°C, 15 s (for primers BM6438 and BM203, 57°C); 72°C, 1 min; 60°C, 15 s (30 cycles) (for primers BM6438 and BM203, 57°C and 35 cycles); 72°C, 5 min (1 cycle); 15°C, 3 min 30 s. In addition, three multilocus PCRs were carried out (the first one: OheQ, BL42, NVHRT16, BMC745; the second: RT24, BM6506, NVHRT30, BMS1788, RT6; and the third one: RT1, RT5, BM4513, RT27, RT9short) (Buchanan and Crawford, 1993; Bishop et al., 1994; Moore et al., 1994; Kappes et al., 1997; Wilson et al., 1997; Roed and Midthjell, 1998; Jobin et al., 2008). A multiplex PCR reaction was carried out using 5 \times Master Mix (Dialat, Russia) in the following mode: 95°C, 15 min (1 cycle); 94°C, 30 s; 57°C, 1 min; 72°C, 1 min 20 s (35 cycles); 60°C, 30 min (1 cycle). PCR was performed using a Tetrad 2 Thermal Cycler (Bio-Rad, United States). Fragment analysis of the amplification product was performed on an 3130 Genetic Analyzer (Applied Biosystems, United States) using the LIZ500 size standard (Applied Biosystems). The results of the fragment analysis were deciphered using the GeneMapper 4.1 program (Applied Biosystems) and the

Table 1. List of samples of *Capreolus pygargus* and *C. capreolus* included in the analysis

Sample collection sites	Numbers of samples in the collection of the Institute of Ecology and Evolution, Russian Academy of Sciences, species status	Studied molecular-genetic markers	Source of information, numbers of haplotypes in GenBank (http://www.ncbi.nlm.nih.gov/)
Samara forest, Dnepropetrovsk oblast, Ukraine*	3483–3486, 3509–3512, <i>C. pygargus</i>	Cytochrome <i>b</i> gene of mtDNA, 17 microsatellite loci	Collection of the Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, new samples
Altai krai, Russia	1507–1514, <i>C. pygargus</i>	17 microsatellite loci	Plakhina et al., 2014
Samara oblast, Russia	2506, 2507, 2509, 2511–2515, 2517–2527, 2757–2763, <i>C. pygargus</i>	The same	The same
Western, Southwestern Ukraine	594–596, 786–792, 797, 798, <i>C. capreolus</i>	"	"
Kazakhstan, Russia: Orenburg, Sverdlovsk, and Kurgan oblasts, Altai and Krasnoyarsk krai, Tuva, Khakassia, Irkutsk oblast, Buryatia, Yakutia, Khabarovsk and Primorskii krai	149, 150, 154, 161, 179, 300–303, 310–325, 327, 333, 334, 493, 494, 496, 497, 685, 692, 721–725, 754, 765, 767–783, 1072–1074, 1156, 1158–1163, 1165–1170, 1172–1176, 1374–1376, 1508–1516, 1554, 1915, 1916, 1200, 1201, <i>C. pygargus</i>	Cytochrome <i>b</i> gene of mtDNA	Zvychnaynaya et al., 2011b
Krasnodar krai, Crimea	586–588, 802–804, 849, 851, 854–857, 858–863, 1234, 1237, 1243, 2396, <i>C. capreolus</i> , <i>C. pygargus</i>	The same	Kholodova et al., 2009; Zvychnaynaya et al., 2013
Moscow, Smolensk and Tula oblasts	1379, 1461–1465, 1472–1475, 1483–1485, 1487–1489, 1491–1495, 1497–1505, 1517–1523, 1525–1528, <i>C. capreolus</i> , <i>C. pygargus</i>	"	Collection of the Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, new samples
Stavropol krai	1909–1914, <i>C. pygargus</i>	"	Zvychnaynaya et al., 2014
Samara oblast	2504–2515, 2517–2520, 2522–2527, <i>C. pygargus</i>	"	Collection of the Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, new samples
Western, Southwestern Ukraine	589, 592–596, 600, 785–800, 831–835, <i>C. capreolus</i>	"	Zvychnaynaya et al., 2013

* According to the results of this study.

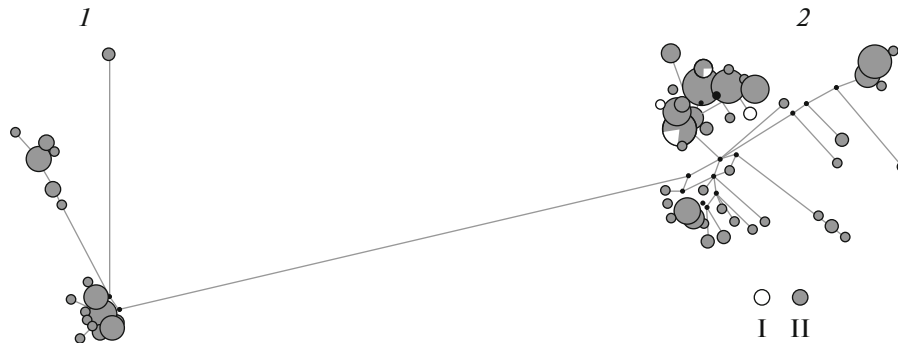


Fig. 1. Median network of haplotypes of mitochondrial DNA of roe deer built in the Network program on the basis of phylogenetic analysis of 1140 nucleotide sequences of the cytochrome *b* gene. (1 and 2) Haplogroups of *Capreolus capreolus* and *C. pygargus*, respectively. (I) Samples from the Samara forest, (II) other samples. The length of the branches is proportional to the number of mutations, and the size of the nodes is proportional to the number of samples; for Figs. 1 and 2.

genetic diversity indices were processed, including counting, using the MS-tools (Park, 2001) and GenAlEx 6.4 programs (Peakall and Smouse, 2006) for Microsoft Office Excel, and the Structure 2.3.4 (Pritchard et al., 2000) and Arlequin 3.5.1.3 (Excoffier and Lischer, 2010) programs, as well.

RESULTS

The variability of the cytochrome *b* gene of the mtDNA of the roe deer from the Samara forest in Dnepropetrovsk oblast was small. A total of 10 mutations (0.88%) were found, of which five (0.44%) were single for a sampling of eight samples.

All the sequences obtained were identical or very close to the fragments of mtDNA of the Siberian roe deer (Fig. 1), which were discovered earlier (Zvychnaynaya et al., 2011a, 2011b; Danilkin et al., 2012). Four haplotypes were found, all of which belonged to the mitochondrial line (Figs. 1, 2), typical of the western part of the range of *C. pygargus*, and also widespread in the artificially formed eastern European populations of *Capreolus*. Haplotype I was described in seven samples from the Urals (Orenburg, Sverdlovsk, and Kurgan oblasts), three from Moscow oblast, one from Altai krai, and one from Kazakhstan. Haplotype III was found in three samples from Moscow oblast. Haplotypes II and IV were unique: II was separated by a single substitution from the sequence of the gene common in the Urals ($n = 9$) and registered in Altai krai ($n = 1$), and IV was separated from the sequence obtained for ten samples from Samara oblast by two substitutions. The haplotypes were placed into the international NCBI database under the numbers KT964431–KT964433.

Fragment analysis of the microsatellite loci of the nuclear DNA confirmed the identification of the study group with the Siberian species. A compulsory separation of the mixed sample, which included all samples from the Samara forest of Dnepropetrovsk

oblast in Ukraine and representatives of *C. capreolus* from southwestern Ukraine and *C. pygargus* from Altai krai, into two clusters ($k = 2$, $\ln = 1064.3$) united the individuals of this group with representatives of *C. pygargus*. The smallest value of the logarithm of probability was found for $k = 3$ and equaled 966.7 (against $\ln = 1241$ for $k = 1$, $\ln = 1064.3$ for $k = 2$, and $\ln = 1006$ for $k = 4$) (Fig. 3). However, when a sampling of only Siberian roe deer was divided into two clusters, the samples from Dnepropetrovsk oblast showed a significant difference from those of Altai krai and Samara oblast (Fig. 4a). With an increase in k ($k = 3$), the picture remained the same (Fig. 4b): the grouping of the roe deer from the Samara forest of Dnepropetrovsk oblast retained its integrity as opposed to other representatives of *C. pygargus*, and the rest of the sampling tended to divide further. With a further step-by-step increase in the number of putative groups (parameter k) for the roe deer of Dnepropetrovsk oblast, the probability of falling into one common cluster remained at the level of 100%; i.e., the intrapopulation differentiation at the studied microsatellite loci was absent.

The observed heterozygosity (H_o) of the population under study is 0.32 ± 0.043 , while the expected one (H_e) is 0.44 ± 0.081 , whereas in the roe deer from Southwestern Ukraine and Altai krai of Russia, these parameters are higher (Zvychnaynaya et al., 2013) (Table 2). In the roe deer of the Samara forest of Dnepropetrovsk oblast, 57 alleles were found; in the animals from southwestern Ukraine, 75; from Altai krai, 73; and from Samara oblast, 117. In Dnepropetrovsk and other Ukrainian roe deer, 31 common alleles were found; in those from Dnepropetrovsk and from Samara oblast, there were 35; in Dnepropetrovsk and Altai deer, 30. In the studied animals of the Samara forest, 13 specific alleles (in seven loci) were found, according to which they can be distinguished from other Siberian roe deer.

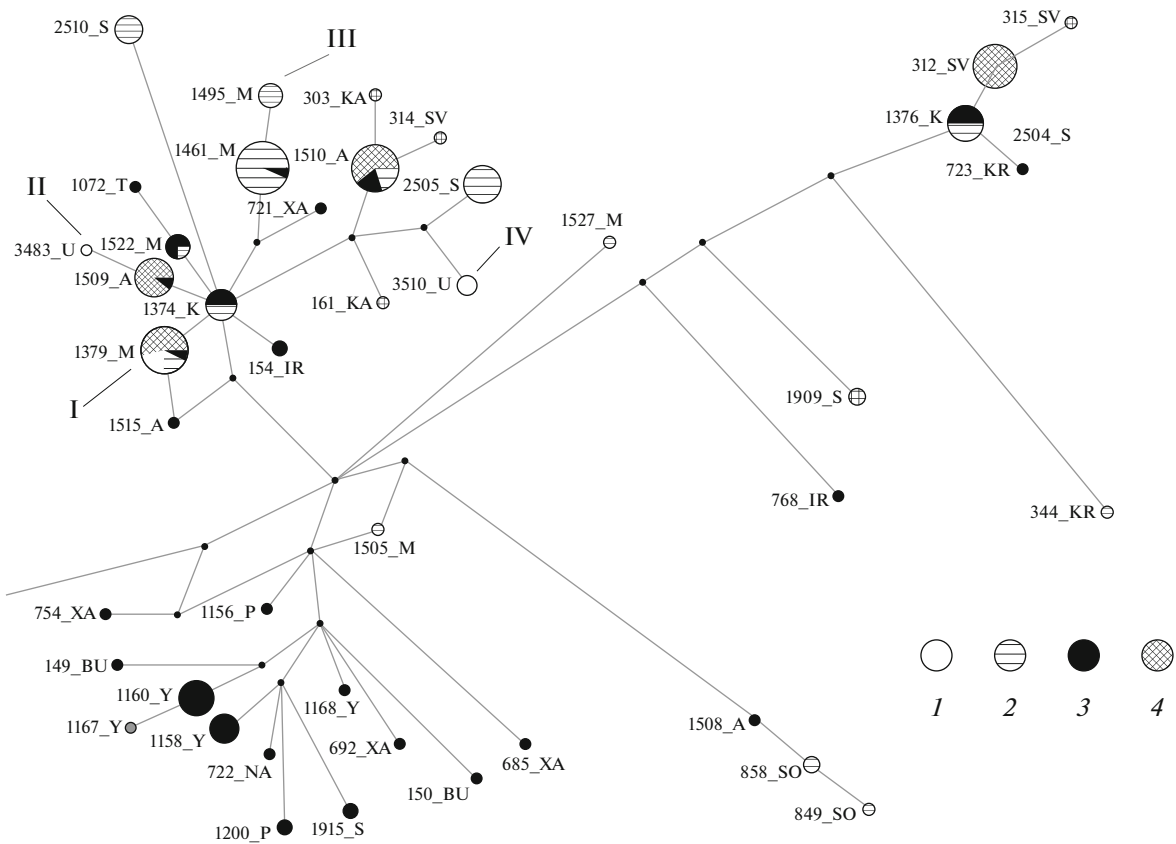


Fig. 2. Median network of haplotypes of the mitochondrial DNA of the Siberian roe deer, cytochrome *b* gene (1140 bp). (I–IV) Haplotypes of mtDNA of samples from the Samara forest; (1) Ukraine (Samara Forest), (2) European part of Russia (Moscow and Samara oblasts, Krasnodar and Stavropol kraia), (3) the Pre-Urals, the Urals, Kazakhstan, (4) Central and Eastern Siberia, Yakutia, Far East.

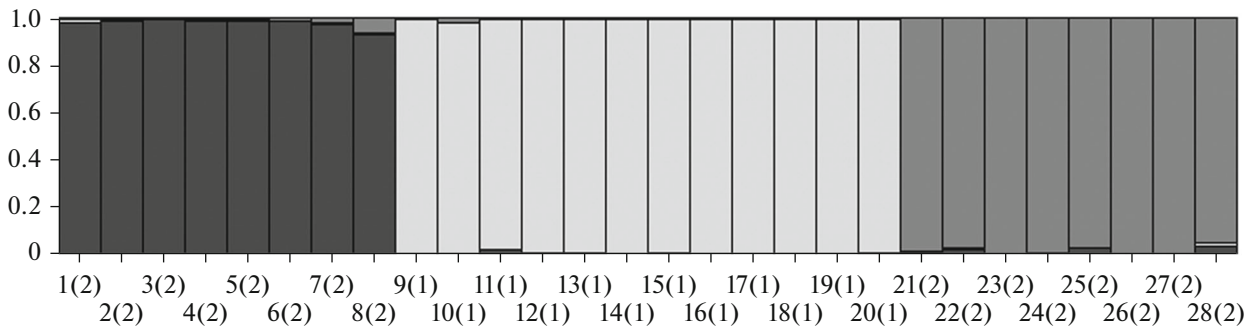


Fig. 3. Species differentiation of roe deer from Dnepropetrovsk oblast of Ukraine (1–8, *Capreolus pygargus*), Southwestern Ukraine (9–20, *C. capreolus*), and Altai krai (21–28, *C. pygargus*) according to the results of analysis of 17 microsatellite loci with $k = 3$. The histogram is built using the Structure 2.3.4 program. The abscissa axis shows the serial numbers of the samples, and the ordinate axis gives the probability of falling into one of the k clusters (Admixture model and allele frequencies are independent).

The statistical index F_{st} in the grouping from the Samara forest in Dnepropetrovsk oblast and Southwestern Ukraine turned out to be the maximum (0.51) for the described sampling of four populations (Table 3), which may indicate the genetic isolation of the Samara forest population.

DISCUSSION

The results obtained confirm the existence of a local grouping of the Siberian roe deer on the territory of the Samara forest in Dnepropetrovsk oblast in Ukraine. Its gene pool, apparently, was not subjected to significant transformations in historical time,

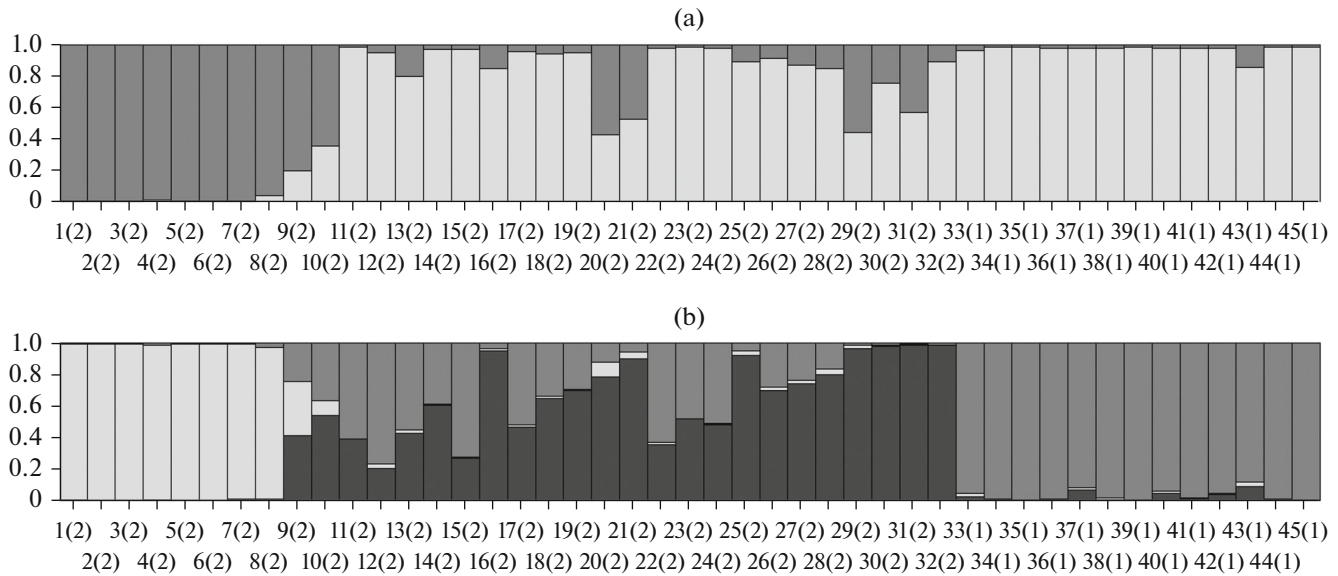


Fig. 4. Population differentiation of tissue samples of roe deer from Dnepropetrovsk oblast, Ukraine (1–8, *Capreolus pygargus*), Altai krai (9–16, *C. pygargus*), and Samara oblast (17–45, *C. pygargus*) based on analysis of 17 microsatellite loci for (a) $k = 2$ and (b) $k = 3$. The abscissa axis shows the serial numbers of the samples, and the ordinate axis is the probability of falling into one of the k clusters.

except for the inevitable loss of genetic diversity in the conditions of severe anthropogenic pressure. In the 1920s, the total number of roe deer in Samara forest was probably a few dozen (Barabash, 1928; Brauner, 1928; Volokh, 2007).

Related mitochondrial lines were found by us in the populations of the Siberian roe deer from the Trans-Volga region, the Urals, and Altai krai, as well as in the artificially formed population of Moscow oblast, where the descendants of individuals imported from the western part of the range of this species were preserved (Zvychnaynaya et al., 2011a, 2011b; Danilkin et al., 2012). It is possible that these haplotypes are a relict legacy of the Siberian roe, which has been preserved in Ukraine to the present day. The results of microsatellite analysis confirm this hypothesis and attest to the genetic originality of the Samara forest grouping.

Nevertheless, the question arises: could the grouping of the Samara forest have formed as a result of arti-

ficial introduction? No reliable information about releases of the Siberian roe deer in Ukraine in the 19th century or earlier is available, but this possibility cannot be excluded. In 1929, 1959, and 1968, 72 Siberian roe deer imported from Primorskii krai (Boldenkov et al., 1971; Pavlov et al., 1974; Pavlov, 1999) were released in Kiev oblast, and their descendants could have colonized Ukraine. In addition, in 1953, 20 individuals from the Caucasus were introduced into the Pavlograd hunting enterprise of Dnepropetrovsk oblast (Volokh, 2007). Our analysis, however, did not reveal mitochondrial genes in either the Far Eastern or Caucasian animals.

Interestingly, the investigated grouping of the Siberian roe deer has inhabited the modern range of the European roe deer for a long time (Danilkin, 1992a, 1999, 2014; Volokh, 2007), which is confirmed by molecular genetic analysis of the western Ukraine and Crimean populations (Zvychnaynaya et al., 2013). However, we did not find any “genetic traces” of confusion with the European species. The same situation

Table 2. Observed and expected heterozygosity values in the roe deer from the Samara forest of Ukraine, southwestern Ukraine, Samara oblast, and Altai krai in the Russian Federation

Sample collection sites	Species status	Heterozygosity	
		observed (H_o)	expected (H_e)
Samara forest, Ukraine	<i>C. pygargus</i> ($n = 8$)	0.32 ± 0.043	0.44 ± 0.081
Southwestern Ukraine	<i>C. capreolus</i> ($n = 12$)	0.379 ± 0.035	0.497 ± 0.078
Samara oblast	<i>C. pygargus</i> ($n = 29$)	0.455 ± 0.24	0.668 ± 0.56
Altai krai	The same ($n = 8$)	0.415 ± 0.044	0.598 ± 0.074

Table 3. F_{st} (fixation index, F -statistic) values for the roe deer from the Samara forest of Ukraine, southwestern Ukraine, Samara oblast, and Altai krai in the Russian Federation

	Samara forest, Ukraine	Altai	Samara oblast	Southwestern Ukraine
Samara forest, Ukraine	0	—	—	—
Altai	0.36017	0	—	—
Samara oblast	0.3304	0.03325	0	—
Southwestern Ukraine	0.51191*	0.36597	0.2914	0

* All values are significant ($p < 0.05$).

is typical of the Stavropol population of the Siberian roe (Zvychnaynaya et al., 2014). The Siberian roe deer, obviously, has advantages in the joint habitat with the European one, which allows its local groupings to persist (or preserve their gene pool), even within the habitat of the latter.

Nevertheless, mixed populations of European and Siberian roe deer also exist. In the Moscow oblast population formed as a result of artificial introduction of both species, 78% of the specimen with the Siberian mitotype prevail (Zvychnaynaya et al., 2011a). Whether the latter are purely Siberian or hybrids with the European roe deer or the introgression of the mitochondrial genome is observed, further studies will show. It has been revealed that the share of hybrid individuals in a sampling ($n = 42$) of roe deer groups in the European part of Russia is 4.8% (Plakhina et al., 2014). The mtDNA of the Siberian type has also been found in a number of individuals in the populations of roe deer from Belarus (Zvychnaynaya, 2010), eastern Poland (Matosiuk et al., 2014), and Lithuania (Lorenzini et al., 2014), which may indicate more significant genetic consequences of the artificial introduction of the Siberian roe deer than previously thought. However, it is very likely that the “Siberian” genome in individual populations is the result of the postglacial habitat of *C. pygargus* in Eastern and, probably, Central Europe.

The modern grouping of roe deer of the Samara forest in Dnepropetrovsk oblast is typically “Siberian” by genotype, but according to a relatively recent study, its phenotype is “European,” although only 100 years ago it was undoubtedly “Siberian” in the phenotype as well (Brauner, 1915). This fact does not lend itself to reasonable explanation. The phenotype could have changed during the critically low numbers at the beginning of the 20th century. In fact, it went through the notorious “bottleneck,” which theoretically could have led to inbreeding and the subsequent decrease in the size of animals. However, our results only indicate a slight decrease in the indicators of genetic diversity (heterozygosity and the number of microsatellite alleles). It can also be assumed that the reason for changing the phenotype of this “island” grouping could be the selective hunting elimination of large individuals. It is known, for example, that in the European part of Russia and in the south of Siberia and the

Far East, as a result of a prolonged selective shooting, the elk became smaller, the typical shovel-shaped horns in the males became a rarity, and its populations actually lost their trophy value. The populations of the Siberian roe deer in the Pre-Urals and Trans-Urals related to the genotype of the Samara forest individuals were also subjected to strong hunting pressure, but retained the “Siberian” phenotype. Nevertheless, local hunters claim that the roe deer of the Samara forest are large in size and weight, and the males have powerful widely spaced horns that are different from the horns of the European species (the survey was conducted by A.V. Domnich in April 2014).

Obviously, a careful study of the roe deer of both the Samara forest and neighboring populations in left-bank and right-bank Ukraine, using a large volume of material, is necessary. This can lead to unexpected findings and specification of the speciation hypotheses (Danilkin, 1999, 2014; Zagorodnyuk, 2002) and taxonomy of roe deer.

ACKNOWLEDGMENTS

The authors are grateful to zoologists, gamekeepers, and hunters who participated in collecting the samples of the European and Siberian roe deer.

This work was supported by the Presidium of the Russian Academy of Sciences, Program no. I.21P “Biodiversity of Natural Systems, Biological Resources of Russia: Status and Fundamentals of Monitoring”, and the Russian Foundation for Basic Research (grant no. 14-04-01135a).

REFERENCES

- Barabash, I.I., *Essay on the Fauna of the Steppe of the Dnieper* (Former Yekaterinoslav Region), Kharkiv: Derzhvidav Ukraïni, 1928.
- Bibikova, V.I., From the history of the Holocene vertebrate fauna of Eastern Europe, in *Prirodnyaya obstanovka i fauna proshlogo* (Natural Environment and Fauna of the Past), Kyiv: Izd. AN USSR, 1963, vol. 1, pp. 119–146.
- Bibikova, V.I., Change of some components of the ungulate fauna in Ukraine during the Holocene, *Byul. MOIP. Otd. Biol.*, 1975, vol. 80, no. 6, pp. 67–72.
- Bishop, M.D., Kappes, S.M., Keele, J.W., Stone, R.T., Sunden, S.L.F., Hawkins, P.G.A., Toldo, S.S., Fries, R.,

- Grosz, M.D., Yoo, J., and Beattie, C.W., A genetic linkage map for cattle, *Genetics*, 1994, vol. 136, pp. 619–639.
- Boldenkov, S.V., Krainev, E.D., and Galaka, B.A., On the introduction of the Far-Eastern boar, sika deer, and roe deer into Ukraine, in *Puti povysheniya effektivnosti okhotnich'ego khozyaistva. Mater. 3-i konf. okhotovedov Sibiri* (Ways to Improve the Efficiency of Game Management Units: Proc. 3rd Conf. of Game Managers of Siberia), Irkutsk, 1971, part 1, pp. 41–43.
- Brauner, A.A., Which species the roe deer of Southern Russia and the Crimea belong to?, in *Zapiski Krymskogo Obshchestva estestvoispytatelei i lyubitelei prirody* (Notes of the Crimean Society of Scientists and Nature Lovers), Simferopol': Tip. Tavrich. Gub. Zemstva, 1915, vol. 5, pp. 111–113.
- Brauner, A.A., *Sel'skokhozyaistvennaya zoologiya* (Farm Zoology), Kyiv: Ukrizdat, 1923.
- Brauner, A.A., Siberian roe deer, *Ukraïn. Misliv. Ribalka*, 1928, no. 10, p. 33.
- Buchanan, F.C. and Crawford, A.M., Ovine microsatellites at the OarFCB11, OarFCB128, OarFCB193, OarFCB266 and OarFCB304 loci, *Anim. Genet.*, 1993, vol. 24, no. 2, p. 145.
- Cotta, V., Asupra capriorului siberian (*Capreolus capreolus pygargus* Pallas) in Romania, *Rev. Padurilor.*, 1969, vol. 84, no. 7, pp. 345–348.
- Danilkin, A.A., Range, in *Evropeiskaya i sibirskaya kosuli: sistematika, ekologiya, povedenie, ratsional'noe ispol'zovanie i okhrana* (European and Siberian Roe Deer: Taxonomy, Ecology, Behavior, Sustainable Use, and Protection), Moscow: Nauka, 1992a, pp. 64–85.
- Danilkin, A.A., Cytogenetic variability, in *Evropeiskaya i sibirskaya kosuli: sistematika, ekologiya, povedenie, ratsional'noe ispol'zovanie i okhrana* (European and Siberian Roe Deer: Taxonomy, Ecology, Behavior, Sustainable Use, and Protection), Moscow: Nauka, 1992b, pp. 47–50.
- Danilkin, A.A., *Mlekopitayushchie Rossii i sopredel'nykh regionov. Olen'i (Cervidae)* (Mammals of Russia and Adjacent Countries. Cervidae), Moscow: GEOS, 1999.
- Danilkin, A.A., *Kosuli (biologicheskie osnovy upravleniya resursami)* (Roe Deer (Biological Bases of Management)), Moscow: KMK, 2014.
- Danilkin, A.A., Zvychainaya, E.Yu., and Kholodova, M.V., What roe deer inhabits Trans-Volga region?, *Vestn. Okhotoved.*, 2012, vol. 9, no. 2, pp. 200–208.
- Excoffier, L. and Lischer, H.E.L., Arlequin suite ver. 3.5: a new series of programs to perform population genetics analyses under Linux and Windows, *Mol. Ecol. Res.*, 2010, vol. 10, pp. 564–567.
- Geptner, V.G., Nasimovich, A.A., and Bannikov, A.G., *Mlekopitayushchie Sovetskogo Soyuza: Parnokopytnye i neparnokopytnye* (Mammals of the Soviet Union: Artiodactyla and Perissodactyla), Moscow: Vyssh. Shk., 1961, vol. 1.
- Gorbachev, S.N., Mammals of the Orel province according to new data of 1910–1914, *Mater. k poznaniyu prirody Orlovskoi gubernii* (Materials to the Knowledge of Nature of the Orel Province), 1915, no. 21, pp. 1–13.
- Gromov, V.I., Paleontological and archaeological substantiation of stratigraphy of continental deposits of the Quaternary period in the USSR (mammals, Paleolithic), *Tr. Inst. Geol. Nauk, Ser. Geol.*, 1948, no. 17, p. 521.
- Gromova, V.I., Remains of mammals from the early Slavic settlements near Voronezh, in *Materialy i issledovaniya po arkheologii SSSR* (Materials and Studies on the Archeology of the USSR), Moscow: Izd. AN SSSR, 1948, vol. 8, pp. 113–123.
- Jobin, R.M., Patterson, D., and Zhang, Y., DNA typing in populations of mule deer for forensic use in the province of Alberta, *Genetics*, 2008, vol. 2, no. 3, pp. 190–197.
- Kappes, S.M., Keele, J.W., Stone, R.T., McGraw, R.A., Sonstegard, T.S., Smith, T.P.L., Lopez-Corrales, N., and Beattie, C.W., A second-generation linkage map of the bovine genome, *Genet. Res.*, 1997, vol. 7, no. 3, pp. 235–249.
- Karpenko, A.V., Bioecological substantiation of the system of forest protection activities from the harmful effects of roe deer in the forests of the left-bank forest-steppe of the USSR, *Extended Abstract of Cand. Sci. (Biol.) Dissertation*, Kharkov: Khark. S.-Kh. Inst. im. V. V. Dokuchaeva, 1977.
- Kirikov, S.V., *Promyslovye zhitovnye, prirodnyaya sreda i chelovek* (Game Animals, Natural Environment, and the Man), Moscow: Nauka, 1966.
- Korneev, O.P., *Identification Guide to Animals of the Ukrainian SSR*, Kyiv: Rad. Shk., 1952.
- Korotkevich, E.L. and Danilkin, A.A., Phylogeny, evolution, and taxonomy, in *Evropeiskaya i sibirskaya kosuli: sistematika, ekologiya, povedenie, ratsional'noe ispol'zovanie i okhrana* (European and Siberian Roe Deer: Taxonomy, Ecology, Behavior, Sustainable Use, and Protection), Moscow: Nauka, 1992, pp. 8–21.
- Kryzhanovskii, V.I., Red deer and roe deer in the Ukraine, their environment and the prospects for economic use, *Extended Abstract of Cand. Sci. (Biol.) Dissertation*, Kyiv: Inst. Zoologii im. I.I. Shmal'gauzena, AN URSR, 1965.
- Lehmann, E., Einige Bemerkungen zum Sibirischen Reh (*Capreolus capreolus (pygargus) caucasicus* Dinnik, 1910) in Mitteleuropa, *Z. Jagdwiss.*, 1976, vol. 22, no. 2, pp. 75–84.
- Lorenzini, R., Garofalo, L., Xuebo, Q., Voloshina, I., and Lovari, S., Global phylogeography of the genus *Capreolus* (Artiodactyla: Cervidae), a Palaeartic meso-mammal, *Zool. J. Linnean Soc.*, 2014, vol. 170, pp. 209–221.
- Matosiuk, M., Borkowska, A., Swislocka, M., Mirski, P., Ratkiewicz, M., Borowski, Z., Krysiuk, K., Danilkin, A.A., Zvychainaya, E.Y., and Saveljev, A.P., Unexpected population genetic structure of European roe deer in Poland: an invasion of the mtDNA genome from Siberian roe deer, *Mol. Ecol.*, 2014, vol. 23, no. 10, pp. 2559–2572.
- Migulin, O.O., *Harmful and Useful Animals of Ukraine*, Kharkiv: Rad. Selyanin, 1927.
- Migulin, O.O., *Identification Guide to Animals of Ukraine*, Kharkiv: Darzhavne vid-vo Ukraini, 1929.
- Moore, S.S., Byrne, K., and Berger, K.T., Characterization of 65 bovine microsatellites, *Mamm. Gen.*, 1994, vol. 5, no. 2, pp. 84–90.
- Park, S.D.E., The Excel Microsatellite Toolkit (version 3.1), Animal Genomics Laboratory, UCD, Ireland, 2001. <http://animalgenomics.ucd.ie/sdepart/ms-toolkit/>.
- Pavlov, M.P., Korsakova, I.B., and Lavrov, N.P., *Akklimatizatsiya okhotnich'e-promyslovykh zveri i ptits v SSSR* (Acclimatization of Game Animals and Birds in the USSR), Kirov: Volgo-Vyatskoe Kn. Izd., 1974, part 2.
- Pavlov, M.P., *Akklimatizatsiya Okhotnich'e-promyslovykh zveri i ptits v SSSR* (Acclimatization of Game Animals and

- Birds in the USSR), Kirov: Volgo-Vyatskoe Kn. Izd., 1999, part 3.
- Peakall, R. and Smouse, P.E., GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research, *Mol. Ecol. Notes*, 2006, vol. 6, pp. 288–295.
- Pidoplichko, I.G., *Materials for the Study of the Former Faunas of the Ukrainian SSR*, Kyiv: Vid-vo AN URSSR, 1956.
- Plakhina, D.A., Zvychainaya, E.Yu., Kholodova, M.V., and Danilkin, A.A., Identification of European (*Capreolus capreolus* L.) and Siberian (*C. pygargus* Pall.) roe deer hybrids by microsatellite marker analysis, *Russ. J. Genet.*, 2014, vol. 50, no. 7, pp. 757–762.
- Pritchard, J.K., Stephens, M., and Donnelly, P., Inference of population structure using multilocus genotype data, *Genetics*, 2000, vol. 155, pp. 945–959.
- Roed, K.H. and Midthjell, L., Microsatellites in reindeer, *Rangifer tarandus*, and their use in other cervids, *Mol. Ecol.*, 1998, vol. 7, no. 12, pp. 1773–1776.
- Sharleman', M., *Zoogeografiya USRR* (Zoogeography of the Ukrainian SSR), Kyiv: Vid-vo AN URSSR, 1937.
- Sokolov, V.E. and Danilkin, A.A., *Sibirskaya kosulya: Ekologicheskie aspekty povedeniya* (Roe Deer: Environmental Aspects of Behavior), Moscow: Nauka, 1981.
- Sokolov, V.E., Shurkhal, A.V., Danilkin, A.A., Podogas, A.V., Rakitskaya, T.A., and Markov, G.G., Comparative analysis of the electrophoretic spectra of blood and muscle tissue proteins of European (*Capreolus capreolus* L.) and Siberian (*C. pygargus* Pall.) roe deer, *Dokl. Akad. Nauk SSSR*, 1986, vol. 288, no. 5, pp. 1274–1276.
- Stone, R.T., Pulido, J.C., Duyk, G.M., Kappes, S.M., Keele, J.W., and Beattie, C.W., A small-insert bovine genomic library highly enriched for microsatellite repeat sequences, *Mamm. Genome*, 1995, vol. 6, p. 714.
- Tatarinov, K.A., The fauna of Neogene and Anthropogene vertebrates of Podolia and Ciscarpathia, its history and current status, *Extended Abstract of Doctoral (Biol.) Dissertation*, Kyiv: Inst. Zoologii im. I.I. Shmal'gauzena, 1970.
- Timchenko, N.G., *K istorii okhoty i zhitovnovodstva v Kyivskoi Rusi (pozndnee Podneprov'e)* (On the History of Animal Husbandry and Hunting in the Kievan Rus (Later the Dnieper Region)), Kyiv: Nauk. dumka, 1972.
- Tsalkin, V.I., Materials on the history of animal husbandry and hunting in ancient Rus, in *Materialy i issledovaniya po arkheologii SSSR* (Materials and Studies on the Archeology of the USSR), Moscow: Izd. AN SSSR, 1956, vol. 51.
- Tsalkin, V.I., Mammals of the Oka and the Upper Volga basin at the beginning of Our Era, *Byul. MOIP. Otd. Biol.*, 1961, vol. 66, no. 1, pp. 23–39.
- Tsalkin, V.I., On the history of mammals of the Eastern European steppe, *Byul. MOIP. Otd. Biol.*, 1963, vol. 68, no. 2, pp. 43–62.
- Vereshchagin, N.K., *Mlekopitayushchie Kavkaza* (Mammals of the Caucasus), Moscow: Izd. AN SSSR, 1959.
- Volokh, A.M., The dynamics of roe deer (*Capreolus capreolus*) range in Ukraine, *Vestn. Okhotovedeniya*, 2007, vol. 4, no. 1, pp. 35–43.
- Wilson, G.A., Strobeck, C., Wu, L., and Coffin, J.W., Characterization of microsatellite loci in caribou, *Rangifer tarandus*, and their use in other artiodactyls, *Mol. Ecol.*, 1997, vol. 6, no. 7, pp. 697–699.
- Zagorodnyuk, I.V., Roe deer (*Capreolus*): the nature of the differences between them and the status of populations from Ukraine, *Visn. Lugansk. Derzh. Pedag. Univ.*, 2001, no. 12, pp. 206–222.
- Zvychainaya, E.Yu., Kholodova, M.V., and Danilkin, A.A., The consequences of the dispersal of the Siberian roe deer (*Capreolus pygargus*) in Eastern Europe. Molecular genetic analysis of mtDNA control region, in *Tselostnost' vida u mlekopitayushchikh: izoliruyushchie bar'ery i gibridizatsiya* (Species Integrity in Mammals: Insulating Barriers and Hybridization), Moscow: KMK, 2010.
- Zvychainaya, E.Yu., Kir'yakulov, V.M., Kholodova, M.V., and Danilkin, A.A., On the gene pool of the roe deer (*Capreolus*) in Moscow environs: the analysis of the variability of mtDNA control region, *Vestn. Okhotovedeniya*, 2011a, no. 2, pp. 168–172.
- Zvychainaya, E.Yu., Danilkin, A.A., Kholodova, M.V., Sipko, T.P., and Berber, A.P., Analysis of the variability of the control region and cytochrome *b* gene of mtDNA of *Capreolus pygargus* Pall, *Biol. Bull.* (Moscow), 2011b, vol. 38, no. 5, pp. 434–439.
- Zvychainaya, E.Yu., Volokh, A.M., Kholodova, M.V., and Danilkin, A.A., Mitochondrial DNA polymorphism of the European roe deer, *Capreolus capreolus* (Artiodactyla, Cervidae), from the South-West of Ukraine, *Vestn. Zool.* (Kyiv), 2013, vol. 47, no. 5, pp. 415–420.
- Zvychainaya, E.Yu., Plakhina, D.A., Danilkin, A.A., Kholodova, M.V., Trautvain, I.G., and Drup, A.I., On the gene pool of the Stavropol group of roe deer, *Vestn. Okhotovedeniya*, 2014, vol. 11, no. 1, pp. 31–36.

Translated by N. Smolina