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Comparative Genetic Characteristics of the Russian and Belarusian Populations of Wisent (*Bison bonasus*), North American Bison (*Bison bison*) and Cattle (*Bos taurus*)

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Abstract—A comparative study of the allele pool and the genetic structure of two Russian and one Belarusian populations of the wisent Bison bonasus against the representatives of the species of *Bison bison* and the genus *Bos taurus* was carried out. Russian populations were represented by samples of the Oka State Natural Biosphere Reserve ($n = 42$) and the Prioksko-Terrasny Nature Biosphere Reserve ($n = 69$), the Belarusian population was represented by samples of the Reserve "Belovezhskaya Pushcha" ($n = 42$), bison samples ($n = 8$) and cattle ($n = 55$) were used as an outgroup. The analysis of the mtDNA D-loop 630 bp fragment polymorphism for the presence of bison and cattle haplotypes, was performed. It was shown that there was a single haplotype for all wisent (*Bison bonasus*) which was different from the sequences of bison (*Bison bison*) and cattle. The analysis of population genetic parameters, calculated using 11 microsatellite markers, showed reduced diversity in wisent (*Bison bonasus*) groups compared to bison (*Bison bison*) and cattle. The largest number of monomorphic loci as well as the absence of private alleles were found in the group of Belarusian wisent. The analysis of the DJost pairwise genetic distances allowed us to establish clear genetic differentiation of the wisent from the outgroups. This fact was also confirmed by PCA analysis, carried out in the context of population identity and the analysis of population structure which demonstrated some proximity of bison (*Bison bison*) to wisent (*Bison bonasus*), which is due to their belonging to the same genus. The data obtained from this study showed differentiation between wisent (*Bison bonasus*) from bison (*Bison bison*) and cattle. It can be used in assessing the population genetic parameters of wisent, identifying and eliminating hybrid individuals. It also can be used in developing strategies and measures for the preservation and improvement of the wisent genetic resources.

Keywords: wisent (European bison), *Bison bonasus*, bison (North American bison, *Bison bison*), cattle (*Bos taurus*), mtDNA, microsatellites, allele pool

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INTRODUCTION

The problem of the reintroduction of various, practically exterminated representatives of the fauna, in particular, wisent, attracts close attention of the world community. The disappearance of wisent in the wild caused by anthropogenic factors: habitat destruction (deforestation and forest burning, forest conversion to agricultural land), and unlimited hunting [1]. Wisent population recruitment to 7180 individuals [2] is the result of effective international activities aimed at growth of the species population exterminated in the wild. Currently, the conservation of wisent as a species with the highest possible genetic diversity, functioning as a natural component of natural ecosystems, is strategically important.

Only 7 out of 52 individuals preserved in Western European zoos (4 males and 3 females) [3] became founders of Belovezhskaya line; 12 individuals (5 males and 7 females), including the Caucasian wisent male became founders of Caucasian-Belovezhskaya line [4, 5]. The use of the bull M 100 KAUKASUS saved the wisent population from complete loss of the Caucasian line gene [4].

Wisent recovery as a biological species was realized by reproduction of two genetic lines: the Belovezhskaya line with animals of the Belovezhskaya subspecies and the Caucasian-Belovezhskaya line with individuals of mixed origin [6, 7]. In the formed lines the greatest influence was made by the individuals M 45 PLEBEJER and F 42 PLANTA. Their genes predominate in both lines, accounting for more than 80% of the total number in the Belovezhskaya line, and 50% in the Caucasian-Belovezhskaya line [8]. Since a very limited number of founders took part in the restoration of both lines, the first generations of wisent had a very high degree of relatedness, consequently, the degree of inbreeding, was 44% in the Belovezhskaya line and 26% in the Caucasian- Belovezhskaya line [4, 8].

Another serious problem is the presence of only one male in the Belovezhskaya line, they all inherit the Y chromosome from M 45 PLEBEJER, while in the Caucasian- Belovezhskaya line three bulls were used as founders M 45 PLEBEJER, M 100 KAUKASUS and M 15 BERGRÜNDER [6, 9].

The population genetic parameters of the Belovezhskaya and Caucasian- Belovezhskaya wisent should be carefully studied. Different types of markers are used to assess biodiversity and analyze population characteristics. The first works devoted to the study of various wisent origin populations in order to assess the level of heterozygosity and the degree of inbreeding were based on the analysis of blood groups [10]. Thus, the study of 16 polymorphic systems (22 loci) of biochemical markers of blood in different wisent lines suggested that there were specific processes that prevent a decrease in the genetic diversity of the population, and the obtained estimates of the genetic distances between the lines of the European bison showed a close relationship between them [11].

Studies of the variability of the major class I histocompatibility complex (MHC) of European bison showed presence of three common haplotypes belonging to 1-3 classical loci of class I [12] have been conducted. The study of MHC-DRB3 polymorphism in populations of European bison showed low variability, which was a result of the bottleneck effect at the beginning of the 20th century [13, 14]. Researchers were also interested in searching for polymorphic variants by genes encoding milk proteins, for example, kappa-casein [15, 16].

The studies of the wisent mtDNA polymorphism were rather numerous [17–20]. It should be noted that the number of authors revealed the presence of hybridization between wisent (*Bison bonasus*) and cattle (*Bos taurus*). For the first time Ward et al. [18] detected cattle haplotype in one of the four Russian populations of wisent. The degree of genetic differentiation of wisent with cattle mtDNA from other studied wisent reached 16.91 ± 0.00%, while differences with the eight studied breeds of cattle were only 0.87 ± 0.44%. Yudin et al. [20] identified both the haplotype of pure wisent mtDNA and livestock mtDNA among individuals of the wisent population in Cherga. It was also found that one hybrid female with a complex pedigree (*B. bonasus* × *B. bison* × *B. taurus*) was delivered to Cherga in the 1980s (Yudin et al., 2012).

Microsatellite markers are also being used for estimating population genetic parameters, including wisent population [21–26].

Currently, for studying of biodiversity and demographic processes of various wisent populations SNP chips developed for cattle are used. Researches by Wecek and co-authors [27] using this technology to study demographic processes that occurred before and after the extermination of wisent in the wild are of undoubted interest. To date the complete wisent genome sequence were obtained using NGS [28].

The use of various methods of molecular genetic studies confirms the low level of genetic diversity in wisent populations, and, consequently, population genetic parameters (inbreeding coefficient, genetic diversity) should be under constant control. Identification of individuals which genome contains DNA of cattle or North American bison is also important. The purpose of this study was to determine the population genetic parameters in three groups of wisent, localized in the the Prioksko-Terrasny Nature Biosphere Reserve, Oka State Natural Biosphere Reserve and in Belovezhskaya Pushcha (territory of Belarus) using microsatellite markers, identifying haplotypes of the mtDNA D-loop region and conducting comparative studies with populations of North American bison and cattle.

MATERIALS AND METHODS

The material (blood or muscle tissue samples) for the research was taken from of the Prioksko-Terrasny Nature Biosphere Reserve (PTZ, $n = 69$), Oka State Natural Biosphere Reserve (OKZ, $n = 42$) and the Belovezhskaya Pushcha Reserve (Belarus) (BEL, $n = 42$). North American bisons (BIS, $n = 8$) and cattle (BOS, $n = 55$, 25 individuals of Holstein breed – HLST, 30 individuals of Simmental breed SIMM) were taken as outgroups.

To isolate the DNA the Nexttec columns (Nexttec Biotechnologie GmbH, Germany) were used, as well as the DNA Extran test kit (CJSC Syntol, Russia). For studying the polymorphism of the control region the Beth Big F ACCCCCAAAGCTGAAGTTCT were used and Beth 80r CAAGCATCCCCCAAAATAAA primers proposed by G. Larson (cited by [29]). Evrogen LLC (Moscow, Russia) provided sequencing services. Fragments of 630 bp length. were analyzed. Sequencing data analysis was performed using UGENE 1.21.0 software [30]. Median joining haplotype networks were constructed in PopART V1.7 [31].

Multiplex amplification of 11 STR cattle loci was performed (*TGLA227*, *BM2113*, *ETH10*, *SPS115*, *TGLA122*, *INRA23*, *TGLA126*, *BM1818*, *ETH225*, *BM1824*, *TGLA53*). PCR amplification was performed in a 10-μL reaction mixture, which consisted of 200 μM dNTPs, 1.0 mM $MgCl_2$, 0.5 mM mixture of primers, 1 unit of Taq DNA polymerase (Dialat Ltd, Russia) and 50–100 ng of genomic DNA. Reaction conditions consisted of initial denaturation at 95°C for 4 min followed by 35 cycles with denaturation at 95°C for 20 s, annealing at 63°C for 30 s and extension at 72°C for 1 min. Fragment analysis was performed on ABI3130xl genetic analyzer (Applied Biosystems, USA) using the software Gene Mapper v. 4 (Applied Biosystems, USA). The lengths of microsatellite alleles were standardized according to ISAG.

The average number of alleles per locus (*Na*) and Hardy-Weinberg genetic equilibrium testing were estimated using the GenAlEx 6.5 software [32].

The calculations of genetic diversity parameters (*Ho*, *He*, *Ar*, *Fis*, *uHe*, *uFis*) and D_{Jost} and F_{st} pairwise genetic distances were performed using R package "diversity" [33–35].

Neighbor-Net network based on Jost's *D* was constructed using SplitsTree version 4.14.6 [36].

Principal Component Analysis (PCA) analysis was performed using the R "adegenet" package [37] and visualized using the R package "gglot2" [38].

The population structure was estimated using the admix model in Structure 2.3.4 [39]. The analysis was performed using the following settings: the length of the burn-in period was 100000 and the model of the Markov Chain Monte Carlo (MCMC) was 100000 iterations. For each K value 10 iterations were performed. Visualization of clustering results in Structure was carried out using the R package "pophelper" [40].

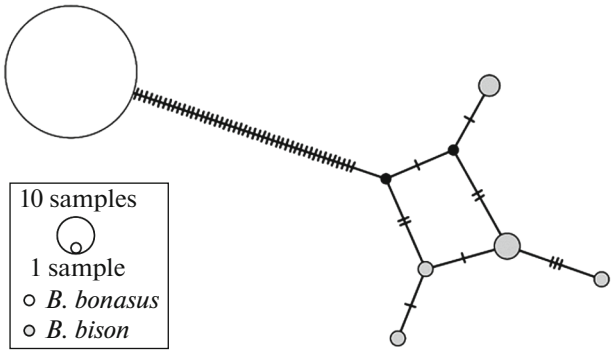

Fig. 1. The median network of *B. bonasus* and *B. bison* species.

RESULTS

Sequence analysis of the mtDNA 630 bp D-loop fragment showed that one haplotype identical to the sequence KY055664 (NCBI – National Center for Biotechnology Information) was found for all studied wisent. The mtDNA D-loop fragment of the studied bison and the cattle group was sequenced to identify the introgression of the North American bison and cattle in the wisent populations. Among the bison, five different sequences identical to deposited *Bison bison* sequences in the GeneBank database – JN632601, AY748700, AY748673, AY748477, AY748478 were identified.

For the studied cattle groups, genetic profiles of Bos taurus were determined. Thus, among the wisent (*Bison bonasus*) no sequences identical to bison (*Bison bison*) or cattle were identified.

A median network constructed using genetic profiles of mtDNA of wisent (*B. bonasus*) and bison (*B. bison*) is demonstrated on Fig. 1. The genetic profiles of the SIMM and HLST cattle groups were not included due to their considerable diversity in comparison with bison and wisent groups. This figure allowed us to illustrate the differentiation of wisent and bison. However, it was not possible to determine whether individuals belong to the Caucasian or Caucasian-Belovezhskaya line, due to the presence of a common genotype for all the studied wisent.

The study of polymorphism of 11 microsatellite loci made it possible to determine the parameters of the population diversity in the studied groups of animals.

Table 1 presents the loci for each population which had statistically significant deficit of heterozygotes or were monomorphic.

In all the wisent groups, the *BM2113* locus was monomorphic (Table 1). In the BIS, BEL and PTZ population, the *INRA23* and *TGLA227* loci were monomorphic, while in the OKZ population, the equilibrium in *TGLA227* locus ($P < 0.01$) towards

Table 1. Monomorphic and heterozygous deficient loci in the studied groups

Population	Locus	*DF*	χ^2	*P*
BIS	*TGLA227*	Monomorphic	–	–
BIS	*INRA023*	Monomorphic	–	–
BEL	*TGLA227*	Monomorphic	–	–
BEL	*BM2113*	Monomorphic	–	–
BEL	*INRA023*	Monomorphic	–	–
OKZ	*TGLA227*	1	9.476	0.002
OKZ	*BM2113*	Monomorphic	–	–
OKZ	*BM1824*	1	4.367	0.037
PTZ	*TGLA227*	Monomorphic	–	–
PTZ	*BM2113*	Monomorphic	–	–
PTZ	*SPS115*	3	9.421	0.024
PTZ	*INRA023*	Monomorphic	–	–
SIMM	*TGLA227*	21	50.265	<0.001
SIMM	*TGLA126*	10	34.309	<0.001
SIMM	*TGLA53*	28	89.799	<0.001

homozygous genotypes was deviated. The *SPS115* locus in the PTZ group and the *BM1824* locus in the OKZ group linkage equilibrium was deviated ($P < 0.05$). No monomorphic loci in the HLST and SIMM groups were found.

The calculation of genetic diversity parameters of the studied animal groups is presented in Table 2.

The lowest values of genetic diversity parameters in wisent were found in BEL, while in the OKZ and PTZ groups these values were slightly higher and similar to each other, except for a larger number of private alleles in the OKZ group. In the BEL group, no private alleles were identified. The BIS group was characterized with a higher diversity than wisent, but was inferior in all parameters to the SIMM and HLST groups.

In general, the data presented in Table 2 demonstrate a reduced genetic diversity in wisent group in comparison with a group of bison and cattle.

Dendrogram using the NeighborNet algorithm to visualize pairwise genetic distances D_{Jost}, was constracted (Fig. 2).

There was a clear separation of wisent, bison and cattle. The groups of wisent were strongly differentiated from bison and cattle. The differentiation among them was in the range of 0.030–0.084 and 0.002–0.008 by F_{st} and D_{Jost}, respectively. The greatest similarity between the OKZ and PTZ populations was observed.

This fact was confirmed by the PCA analysis carried out in the context of the population identity, which showed that the wisent were differentiated from the outgroups – bison and cattle (Fig. 3).

The first two principal coordinates (based on matrix of genetic distances between all studied animals), showed that there was a significant differentiation of populations according to their species, but there was no differentiation in the *B. bonasus* species depending on the population identity (Fig. 3).

Populations structure analysis (Fig. 4) showed a clear differentiation between representatives of the genus *Bison* and cattle belonging to the genus *Bos* with K = 2 for all the studied groups. At K = 3, there was a differentiation between individuals of bison and wisent. At K = 4 there was no separation of the BEL, OKZ, PTZ groups among themselves.

Table 2. Genetic diversity parameters of wisent, bison and cattle populations based on microsatellite loci

Parameter[1]	Population					
	BEL	OKZ	PTZ	BIS	SIMM	HLST
Na	1.818 ± 0.182	2.182 ± 0.182	2.182 ± 0.296	3.364 ± 0.49	5.818 ± 0.444	6.000 ± 0.447
Ne	1.489 ± 0.129	1.565 ± 0.141	1.566 ± 0.163	2.439 ± 0.430	3.473 ± 0.393	3.750 ± 0.416
Npr	0.00	0.182 ± 0.122	0.091 ± 0.091	0.818 ± 0.263	0.909 ± 0.368	1.273 ± 0.488
Ho	0.281 ± 0.067	0.302 ± 0.081	0.281 ± 0.077	0.429 ± 0.093	0.653 ± 0.059	0.676 ± 0.039
He	0.277 ± 0.068	0.286 ± 0.069	0.275 ± 0.072	0.427 ± 0.095	0.652 ± 0.045	0.686 ± 0.032
Fis[2]	–0.040 [–0.156; 0.076]	0.010 [–0.132; 0.152]	–0.011 [–0.13; 0.108]	–0.044 [–0.2; 0.112]	–0.002 [–0.12; 0.116]	0.013 [–0.067; 0.093]
Ar	1.771 ± 0.194	1.864 ± 0.18	1.871 ± 0.22	3.1 ± 0.482	4.147 ± 0.343	4.364 ± 0.276
uHe	0.280 ± 0.069	0.289 ± 0.07	0.277 ± 0.073	0.459 ± 0.102	0.672 ± 0.046	0.700 ± 0.032
uFis[2]	–0.026 [–0.14; 0.088]	0.020 [–0.12; 0.16]	–0.004 [–0.122; 0.114]	0.031 [–0.113; 0.175]	0.027 [–0.087; 0.141]	0.033 [–0.045; 0.111]

[1] *Na*—average number of alleles per locus; *Ne*—the number of effective alleles; *Npr*—the number of private alleles; *Ho*—observed heterozygosity, *He*—expected heterozygosity, *Fis*—inbreeding coefficient, *Ar*—allelic diversity; [2] CI—confidence interval (95%).

Fig. 2. Dendrogram based on pairwise genetic distances (D_{Jost}) using the NeighborNet algorithm.

DISCUSSION

Our studies allowed us to identify unique alleles in OKZ and PTZ wisent populations, and also showed their greater genetic diversity in comparison with Belovezhskaya line individuals (BEL), which were apparently due to the Russian wisent foundation from a larger number of individuals and their belonging to the Caucasian-Belovezhskaya line and their replenishment by animals from foreign breeding centers. This was confirmed by foreign authors studies. Thus, W. Olech [8] observed almost a half lower level of inbreeding in the Caucasian-Belovezhskaya line (approximately 26% versus almost 50% in the line of the Belovezhskaya line). But, despite the higher genetic potential of the Caucasian-Belovezhskaya line, symptoms of inbreeding depression affecting the parameters of reproduction and health of the Caucasian-Belovezhskaya line individuals were reported. It was probably due to the fact that the analysis based on animals of Russin reserves, while Belovezhskaya line individuals were obtained from wild life groups, where mutation process was higher. The relatively higher fitness of the Belovezhskaya line was probably the result of randomly selected high quality genomes of its seven founders. The supposedly reduced Caucasian-Belovezhskaya line fitness seems to be the result of unsuccessful genetic combinations inherited from one or several Belovezhskaya founders, or may be due to outbred depression between the Belovezhskaya and Caucasian founders [7]. In general, the wisent groups had a lower level of genetic diversity than bison and cattle, since a very limited number of founders took part in bison restoration.

The bison conservation strategy in the world provides for the purity of this species and the exclusion of introgression. Introgressive hybridization is one of the main threats to the preservation of species and is often caused by human influence on the natural habitat of wildlife species. The ability to accurately identify introgression is critical to understanding of its importance in the evolution and effective management of the species conservation [41]. Extensive studies were conducted on the North American bison. Cronin et al. [42] showed differentiation between *Bison bison* and *Bos taurus taurus* and *Bos taurus indicus* using 30 microsatellite loci, but could not accurately differentiate *Bison bison bison* and *Bison bison athabascae*. According to Halbert et al. [41], the use of one type of markers didn't allow an unambiguous conclusion about the presence of introgression. Thus, microsatellite analysis revealed its presence in 5 out of 12 groups of bison and a study of mtDNA in the same groups in 7 out of 12 groups.

In contrast to the North American bison, traces of introgression in the European bison were investigated to a lesser extent. In Russia, experiments on hybridization of *B. bonasus*, *B. taurus* and *B. bison* were carried out in the second half of XX century, as shown by some researchers who found evidence of introgression using mtDNA [18, 20]. With the use of different types of

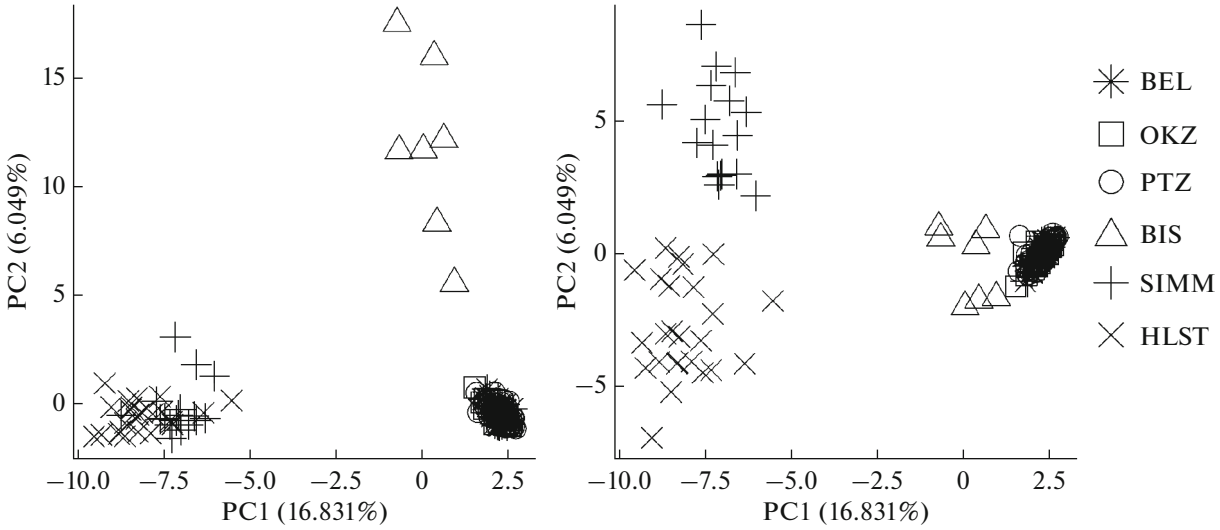

Fig. 3. Principal Component Analysis (PCA) based on genetic data for the studied animal groups.

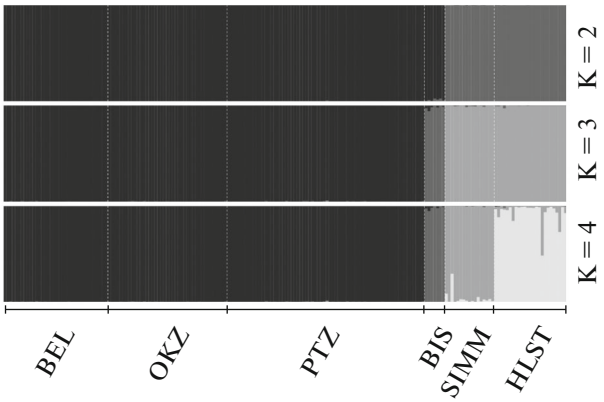

Fig. 4. Population affiliation of wisent, bison and cattle estimated using the program Structure 2.3.4.

molecular genetic markers (proteins, anonymous sequences of DNA) the comparative analysis of genetic structures of groups of *B. taurus*, *B. bonasus* and *B. bison* was carried out by Glazko et al. [43]. The expressed differences of estimations of similarity between species, depending on used markers were found. But, as shown by the example of bison, the most successful was the study of nuclear and mtDNA markers.

Our study demonstrated the successful use of these methods for the species identification of wisent, bison and cattle, and their clear differentiation from each other.

Thus, the use of molecular research methods allows both controlling population genetic parameters and identifying species that can be used in developing programs and procedures to increase the level of genetic diversity of the domestic bison and reduce the degree of inbreeding, identification of hybrid forms. Timely identification of hybrid animals in controlled groups, primarily in specialized reserves, will eliminate hybrid forms from reproduction and their reintroduction into wild life. Genetic studies of the wisent breeding stock in European breeding centers will make it possible to evaluate the population-genetic parameters of the bison as a biological species using modern methods, and to correct the actions for its recovery in wild life.

Compliance with ethical standards. All necessary international and national recommendations for the care and use of animals have been observed.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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