THE STUDY OF DNA-POLYMORPHISM OF EUROPEAN BISON BY PCR-ANALYSIS OF KAPPA-CASEIN GENE AND LOCI DQB AND DRB OF THE MAJOR HISTOCOMPATIBILITY COMPLEX

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SUMMARY

Using PCR-RFLP method allelic polymorphism of kappa-casein gene and polymorphic variants of of DRB (exon 2) and DQB (5'- flanking region) loci were studied in different European bison populations. Allelic variants A and B of kappa-casein (specific for cattle) were found in European bison with frequencies P(A) = 22,4 % and P(B) = 77,6%. The tendency towards the loss of A allele was revealed as a result of high level of inbreeding. Only few polymorphic variants of DQB and DRB loci were found, some of them were unique for European bison. Phylogeny trees for European bisons populations and related species of Bovidae family were obtained using cluster analysis of the estimates of similarity for these loci. European bison populations demonstrates low level of differentiation in comparison with cattle breeds. The comparative analysis of DNA polymorphism of related animal groups is useful for genetic monitoring of rare species.

INTRODUCTION

All European bisons or aurochs (Bison bonasus) are the descendants of 12 pure - blooded animals including last animals of Belovezhskyi subspecies (Bison bonasus bonasus) and unique animal of the exterminated Caucasian subspecies (Bison bonasus caucasicus). There are three lines of aurochs: Belovezhskaya, Caucasian-Belovezhskaya and Mountain (containing 5% of Bison bison blood). Extremely high level of inbreeding F = 0.24 (Olech,1987) influences viability and immune status of animals, and causes inevitable reduction of variability. The present study is devoted to investigation of DNA - polymorphism of different aurochs lines in order to estimate the level of their diversity. Comparative analysis of DNA - polymorphism of kappa-casein gene and DQB and DRB loci of the major histocompatibility complex(MHC) with other species of Bovidae family is fulfilled. Polymerase chain reaction (PCR) gives the possibility of amplification of copies of DNA fragments (Saiki et al., 1988).The oligonucleotide primers used in PCR with DNA probe of one species are suitable for heterologous PCR: for amplification of homologous genes of related species (Sommer & Tautz, 1989). Heterologous PCR has been used to study DNA polymorphism of kappa-casein gene in bison herds (Cronin and Cockett, 1993). Kappa-casein contains 169 amino acids, it has been shown two main protein variants (A and B) in cattle. Variant A and B can be distinguished by PCR-RFLP method using endonucleases Hind III, Hinf I, Pst I or Taq I (Sulimova et al., 1991). DNA polymorphism of MHC loci DRB was studied by PCR and DQB by PCR-RFLP method (Sulimova et al., 1992). The data of DNA - polymorphism was used for phylogeny analysis. We also consider blood groups (A, B, FV, C, L, M, S and Z systems) for analysis of diversity of different lines of European bison. This is neccessary for private genetics of the species and particularly for the analysis of genetic processes in the small populations.

MATERIALS AND METHODS

Characteristic of the investigated samples. We studied aurochs from Belovezhskaya line (n = 24), Caucasian-Belovezhskaya (n = 14) and Mountain (n = 2) ones, bisons (n = 3) and hybrids of European bison x bison (n = 2). In comparative analysis were included different cattle breeds: Black and White (n = 47), Kholmogorskaya (n = 60) and Hereford (n = 20) ones. The sample of Yakut cattle (Bos taurus turano-mongolicus) (n = 26) and Yaks (Bos poephagus) (n = 15) were

received from Institute of Biology RAS (Yakutsk). DNA extraction from blood specimen was fulfilled by method (Johns, Paulus-Thomas, 1989). We amplified DNA between 10809 and 11037 nucleotides of the k-casein gene (Alexander et al., 1988) by PCR using ligonucleotide primers: SGE (5'-TATCATTTATGGCCATTCCACCA-3') and SGO (5'-CTTCTTTGATGTCTCCTTAGAGTT-3') (Sulimova et al.,1991). To amplify DNA of BoLA-DQ and - DR loci we used primers (Udina et al.,1994) based on the DNA sequences (Groenen et al.,1990): 1) for 5'-ut of BoLa-DQ: Q1 (5'-CAGATGAAGTTTTTCCGCTCC-3') and QB2 (5'-ACTAATGGTAGTCAACACAGC-3') (length of fragment - 149 bp), 2) for of exon 2 of Bola-DR: R3 (5-' TGCCCACAGCACATTTGATGG-3') and RB4 (5'-TGAACTCAC-CCACATCGTTGC-3')(141 bp). PCR conditions were described by Sulimova et al. (1992). Sequence analysis of amplified fragments of k-casein, DQB and DRB for European bison were fulfilled using P-32-Sequencing kit ("Pharmacia") by the method of Sanger et al. (1977). The coefficient of similarity was calculated by the method of Serebrovsky (1970) and estimates of antigen diversity by the method of Zhyvotovskyi (1983). Cluster analysis was fulfilled by method of "average linkage".

RESULTS AND DISCUSSION

For genotyping by k-casein gene two pairs endonucleases were used HindIII- PstI or HindIII-HinfI for each sample, that increases the reliability of results. Obtained gene frequencies are shown in Table 1. Results of cluster analysis of similarity values are shown on Figure 1.

Table. 1 Distribution of allele frequences of k-casein

Group of animals	Allele frequencies %				
	n	A	B	F	O
Caucasian-Belovezhskiye aurochs	10*	11.0	75.0	–	14.0
Belovezhskiye aurochs	14*	23.7	60.5	–	16.8
Yaks	15	43.3	56.7	–	–
Yakut cattle	26	34.6	3.8	7.7	53.9
Black and White cattle	32	46.3	53.1	–	–

* Resulting sample was increased to 38 animals. The frequences in it were 22.4 % for A and 77.6% for B.

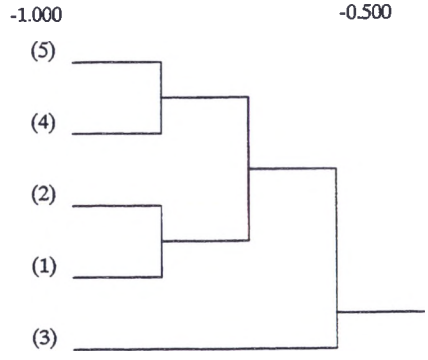

Figure 1. Phylogeny tree for the studied groups for k-casein gene polymorphism 1 - Caucasian-Belovezhskye aurochs; 2 - Belovezhskye aurochs; 3 - Yakut cattle; 4 - Yaks; 5 - Black and White cattle.

Cluster analysis reveals similarity between two lines of European bison. Yakut cattle is most divergent because it is an isolated group which lives in severe environment. New F allele found in this group differs from A by a meaning change in 148 codon (Sulimova et al., 1992) Joining of Yaks with black and white cattle in one cluster illustrates similarity of their gene frequences distribution. Yaks began its independent evolution about 2,3 mln years ago. However, the same alleles of k-casein gene are found in Yaks as in cattle. So this polymorphism is very ancient and can be supported by some selective processes.

After PCR analysis of the polymorphic region of BoLA-DRB with RB3-RB4 primers we obtained several gene fragments of different length because there are several gene copies of DRB

in the genome of cattle. Such results give an opportunity to study the whole spectrum of genotypes revealed by PCR, similar with molecular finger printing of genome (Jeffreys et al., 1985). 13 variants of genotypes in the samples were revealed, which are designated by large latin letters (Table 2). Each variant consists of several fragments of various length from 70 to 360 bp (Udina et al., 1994). In European bison after successful PCR we observe variants A and P, the last one is unique for this animals. Only few polymorphic variants in aurochs and Hereford cattle reveals their high homozygozyty level.

We obtained PCR-product of DQB - one fragment 149 bp in length in all animal groups, as expected. In cattle was shown polymorphism by the length of the amplified fragment: two animals has variant with fragment 156 bp, which is a result of insertion. Restriction site polymorphism of Hinf I (localizated in 47 position from 5'-end of fragment) was also demonstrated (Sulimova et al., 1992). In aurochs only fragment 149 bp in length was found and restriction site was absent. For comparison of the level of genetic differentiation of investigated animal groups estimates of genetic similarity are used in cluster analysis Figure 2. Clusters positions on given dendrogramme corresponds to phylogenetic data.

Obtained sequences for k-casein, DQB and DRB gene fragments of aurochs on the whole are similar to cattle ones and carry single base substitutions. Estimates of genetic similarity for European bison lines using blood group and biochemical markers, morphological variability and DNA - polymorphism gives 0.87-0.89 that is higher than estimates for different cattle breeds (0.70-0.82). Values of blood groups antigen diversity for Belovezhskaya line is 1.32, for Mountain - 1.43 and for Caucasian-Belovezhskaya one - 1.45. Similar estimates for cattle breeds - 1.51 - 1.63. Probably high level of inbreeding lead to poor diversity inside aurochs lines and their high similarity with each other. The lowest estimate of antigen diversity of Belovezhskaya line is in agreement with the fact that only five ancestors of this line existed.

Table 2. Genotypes of DRB loci in the studied groups of animals

Animal group	Genotype variants												
	O	A	B	C	D	E	F	G	H	I	J	K	P
European bison (n=26)	14*	1	-	-	-	-	-	-	-	-	-	-	11
	54,9	4,8											35,5
Kholmogorskaya breed (n=60)	6	28	9	7	5	5	-	-	-	-	-	-	-
	10	46,7	15	11,6	8,3	8,3							
Black and White cattle (n=47)	-	-	-	-	-	-	18	11	9	9	-	-	-
							38,3	23,4	19,1	19,1			
Hereford cattle (n=20)	2	12	-	-	-	-	-	-	-	-	4	2	-
	10	60									20	10	

* - the upper value - number of genotype variant in a sample, lower value - frequency of genotype variant.
Bisons and hybrids have A variant - fragment 141 bp. O - absense of amplification.

It is possible to distinguish lines of European bison by the applied methods. Obtained results can be used for fulfilling programs of concervation of the species aimed on the supporting of genetic polymorphism in the present European bison populations.

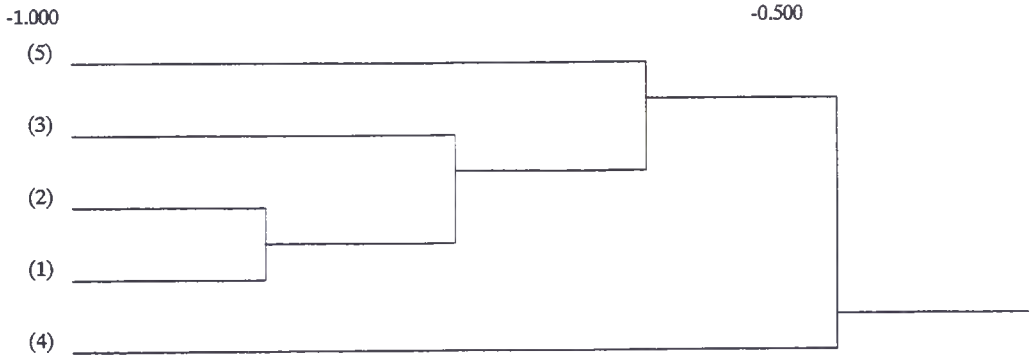

Figure 2. Phylogeny tree - a results of cluster analysis of genetic similarity values for DNA-polymorphism of DRB, DQB and k-casein genes combined. 1 - Belovezhskaya line, 2 - Caucasian - Belovezhskaya and 3 - Mountain lines, 4 - Black and White cattle, 5 - bison.

REFERENCES

ALEXANDER, L.J., STEWART, A.E., MACKINLAY A.G. et al. (1988) Eur. J.Biochem., 178: 395-401.

CRONIN, M.A. and COCKETT N. (1993) Animal Genetics, 24: 135-138.

GROENEN, M.A.M., VAN DER POEL, J.J., DIJKHOF, J.M., GIPHART, M.J. (1990) Immunogenetics, 31: 37-44.

JEFFREYS, A.J., WILSON, V. and THEIN, S.L. (1985) Nature, 316, N4: 76-79.

JOHNS, M.B. and PAULUS-THOMAS, J.e. (1989) Anal. Biochem., 80: 276-278.

OLECH, W. (1987) Acta theriologica, 32: 373-387.

SAIKI, R.K., GELFAND, D.H., STOFFEL, S. et al. (1988) Science, 239: 487-491.

SANGER, F., NICKLEN, S. and COULSON, A.R. (1977) Proc. National Acad. of Sci. USA, 74: 5463-5467.

SEREBROVSKYI, A.S. (1970) Genetic analysis, Moscow,Nauka: 342.

SOMMER, R. and TAUTZ D. (1989) Nuc. Acid. Res., 17: 6749.

SULIMOVA, G.E., SHAIKHAIV, G. O., BERBEROV E. M. et al. (1991) Genetika (Moscow), 27: 2053-2062.

SULIMOVA, G.E., SOKOLOVA, S.S., SEMIKOZOVA, O.P. et al. (1992) Tsytologia ang Genetika (Kiev), 26: 18-26.

UDINA, I.G., SIPKO, T.P., SOKOLOVA, S.S., SULIMOVA, G.E. (1994) Genetika (Moscow), 30: 340-344.

ZHYVOTOVSKYI, L.A. (1983) General genetics, Moscow, VINITI, V.8: 76-104.