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An attempt at understanding the genetic basis of domestication

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Based on protein polymorphism and results obtained with RAPD-PCR and ISSR-PCR methods, the domestic and wild *Artiodactyla* and *Perissodactyla* (14 and 7 species, respectively) were compared. The marker-specific species differentiation in domestic and wild species was observed, leading to the hypothesis of the "subgenome" existing in domestic species. It is assumed that "subgenome" contains certain genes encoding important proteins and enzymes. In the past, the high variation of "subgenome" could play an essential role in domestication, leading to the wide morphological differentiation of contemporary domestic species.

KEY WORDS: *Artiodactyla* / domestication / enzymes / *Perissodactyla* / polymorphism / proteins

Domestication is an unique area for studying the appearance of new forms of organisms under the pressure of selection. All domestic species possess specific morpho-physiological features, named domestication traits, distinguishing them from their wild ancestors. Domestication traits were described in mammals and birds by Bogolubski [1959] and in plants by Paterson *et al*. [1995].

Domesticated animals show wide phenotypic interspecies diversity and exist in about 4,000 varieties what is equal to about a half of a total currently known number of mammalian species [Cunningham 1996]. This suggests that the genetic background of all domestic species may be common, at least for some traits.

Recently, new methods are available in studies on interspecies genetic variation – randomly amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR). In both methods randomly chosen short sequences of DNA are used as primers for comparison the length of the sequences amplified between them. The interspecies differences in length of amplified DNA sequences are caused by differences in distribu-

tion of such sequences among the genomes.

In this report three types of genetic markers were used to compare the range of genetic variation in domestic and wild animal species. First type was the traditional protein markers. The second – UBC-85 and UBC-126 primers for RAPD-PCR as recommended for *Equidae* [Bailey and Lear 1994], and the third – microsatellite primers for ISSR-PCR analysis [Zietkiewicz *et al*. 1994].

All *loci* identified in this study may be divided into three main groups:

– conservative *loci* – showing no polymorphism within the species investigated;

– polymorphic *loci* participating in interspecies differentiation;

– polymorphic *loci* not involved in interspecies differentiation.

Genetic variation in domestic species was found to be of the same range as in wild ones.

Material and methods

Examined were 14 species and breeds of *Artiodactyla* and seven of *Perissodactyla* as listed below, all maintained at Askanya-Nova game reserve.

Artiodactyla

1. African buffalo (*Syncerus caffer*)
2. Aoudad (*Ammotragus lervia*)
3. Bison, american bison (*Bison bison*)
4. Black buck (*Antilope cervicapra*)
5. Blue buck, nilgay (*Boselaphus tragocamelus*)
6. Domestic cattle (*Bos taurus*):
    a. Ukrainian Grey
    b. Ukrainian Whiteheaded
    c. Watusi (*Bos taurus macroceros)*
7. Domestic pig (*Sus scrofa domestica*):
    a. Large White breed
8. Domestic sheep (*Ovis ariel*):
    a. Ascanian high-proficacy caracul
9. Eland (*Taurotragus oryx*)
10. European bison (*Bison bonasus*)
11. Gnu (*Connochaetes gnu*)
12. Mithan (*Bibos gaurus frontalis*)
13. Saiga (*Saiga tatarica*)
14. Snow buck (*Ovis canadensis*)

Perissodactyla

1. Domestic horse (*Equus caballus*)
    a. Arabian Horse
    b. Hutsul horse
    c. Orlov's trotter

d. Pony (mixed group)
e. Yakutian horse

2. Donkey (*Equus asinus*)
3. Chapman's zebra (*Equus (Hippotigris) chapmani*)
4. Grant's zebra (*Equus (Hippotigris) granti*)
5. Grevy's zebra (*Equus (Hippotigris) grevyi*)
6. Kulan (*Equus hemionus hemionus*)
7. Przewalski's Horse (*Equus przewalskii*)

Analysis of plasma and blood cell proteins polymorphism was performed with polyacrilamide grade electrophoresis (PAAG) and starch gel electrophoresis. Investigated was polymorphism of 30 following proteins:

Plasma proteins: albumin (ALB), ceruloplasmin (CP), transferrin (TF), vitamin D receptor (GC), alpha-1-B-glycoprotein (A1B), esterase (ES, E.C 3.1.1.1), amylase-1 (AM-1, E.C 3.2.1.1) and alkaline phosphatase (AP, E.C 3.1.3.1);

Blood cell proteins: sorbitol dehydrogenase (SORDH, E.C. 1.1.1.14), lactate dehydrogenase (LDH-1, LDH-2, E.C. 1.1.1.27), malate dehydrogenase (MDH, E.C 1.1.1.37), malic-enzyme (ME, E.C 1.1.1.40), 6-phosphogluconate dehydrogenase (6-PGD, E.C 1.1.1.44), glucose-6-phosphate dehydrogenase (G6PD, E.C 1.1.1.49), diaphorase (DP-1, E.C 1.6.4.3), superoxide dismutase (SOD-1, SOD-2, E.C 1.15.1.1), purine nucleoside phosphorylase (PN, E.C 2.4.2.1), glutamate oxaloacetate transaminase (GOT, E.C. 2.6.1.1), hexokinase (HK, E.C. 2.7.1.1), kreatine kinase (KK, E.C. 2.7.3.2), adenilate kinase (AK, E.C. 2.7.4.3), phosphoglukomutase (PGM, E.C. 2.7.5.1), carboanhydrase (CA, E.C. 3.1.2), leucine arylaminopeptidase (LAP, E.C. 3.4.11.1), peptidases A, B (PEP A, B, E.C 3.4.11 or 13), adenosine desaminase (ADA, B.C. 3.5.4.4), fumarate hydratase (FH, E.C. 4.2.1.2), glucose phosphate isomerase (GPI, E.C. 5.3.1.9), and mannose phosphate isomerase (MPI, E.C. 5.3.1.8).

Nuclear DNA was extracted from blood cells by a standard technique (Maniatis *et al.* 1984). RAPD-PCR was performed with primers UBC-85 (5'-GTGCTCGTGC-3') and UBC-126 (5'-CTTTCGTGCT-3') as the most convenient for inter- and intraspecies research of the genetic structure of *Equidae* [Bailey and Lear 1994]. ISSR-PCR analysis was carried out with primers containing the $(GA)_9C$ and $(AG)_9C$ dinucleotide repeats [Zietkiewicz *et al*. [1984].

The polymerase chain reaction (PCR) was performed in a reaction volume of 20 ml containing 20-50 ng DNA, 0.2 pM of each primer, 0.3 mM dNTP, 50 mM KCL, 10 mM TRIS-HCL (pH 9.0), 0.01 % triton X-100, 2 mM $MgCl_2$ (PROMEGA), and one unit of Taq polymerase (DIALAT Ltd, Moscow). Amplification was carried out under following conditions: initial five cycles with a denaturing step at 92°C for 1 min., annealing at 35°C for 1 min., and extension step at 72°C for 2.5 min. An initial amplification was followed by 35 cycles with a denaturing step at 92°C for 1 min., annealing at 42°C for 1 min., and extension step at 72°C for 2.5 min. (a total of 40 cycles). An additional extension step at 72°C for 10 min. was included at the end of 40-th cycle. ISSR-PCR

was carried out for 30 cycles: initial denaturation at 94°C for 2 min., denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 2 min. An additional extension step at 72°C for 10 min. was included at the end of 30-th cycle.

The PCR products were separated electrophoretically on 1.5 % agarose gel and visualised by ethidium bromide staining. The amplification products reproduced in 3-5 independently repeated PCRs with DNA of the same animals were considered for further analyses. The size of amplification products was estimated with molecular weight marker 0.1 kb DNA Ladder (GIBCO BRL). Mathematical analysis was performed using BIOSYS software package.

Results and discussion

The first method used was electrophoretical analysis of protein polymorphism. The results are shown in Table 1. Based on electrophoretic mobility of proteins, the genetic distances between species were estimated by programme BIOSYS-1 (Fig 1).

Table 1. Polymorphic enzyme systems found in domestic and closely related wild *Ungulata* species

Species, breed	n	Polymorphic *loci* (%)	Heterozygosity *locus*/animal	Polymorphic *loci*
			Artiodactyla	
African buffalo	3	10.0	0.077	LDH-1, G-6-PD, PGM
Askanian caracul	383	20.0	0.097	HB, TF, ME, LAP, ES, NP
Bison	10	20.0	0.090	AM, CP, PGM, GPI, PEP B, DP-1
Blue buck	10	16.7	0.079	6-PGD, G-6-PD, AK, LAP, FH
Domestic cattle				
Ukrainian Grey	34	26.6	0.074	TF, pTF, GC, AM, NP, AP, CA,C
Ukrainian Whiteheaded	29	26.6	0.094	TF, pTF, GC, AM, NP, AP, CA, CP
Eland	10	13.3	0.062	LAP, HK, ME, CP
European bison	40	16.7	0.053	CP, ME, ICD, PEP B, PGM
Gnu	3	10.0	0.057	G-6-PD, 6-PGD, ME
Large White pig	34	16.7	0.058	TF, 6-PGD, GPI, PGM, ADA
Mithan	7	3.3	0.042	6-PGD, G-6-PD
Saiga	10	10.0	0.043	G-6-PD, ME, PGM
Snow buck	6	16.7	0.056	TF, HB, LAP, ES, DP
			Perissodactyla	
Domestic horse				
Hutsul	23	20.0	0.088	TF, ALB, ES, GC, AlB, 6-PGD
Pony	16	16.7	0.048	TF, ES, GC, 6-PGD, GPI
Yakutian	25	20.0	0.072	TF, ALB, ES, GC, 6-PGD, GPI
Donkey	7	13.3	0.033	TF, ALB, 6-PGD, PGM
Chapman's zebra	3	13.3	0.067	TF, ALB, GC, 6-PGD
Grant's zebra	4	10.0	0.017	TF, 6-PGD, HK
Grevy's zebra	3	3.3	0.022	TF, 6-PGD
Kulan	10	20.0	0.075	TF, 6-PGD, G-6-PD, ME, AK, FH
Przewalski's Horse	31	23.3	0.040	TF, ALB, AlB, ES, 6-PGD, GPI, PGM

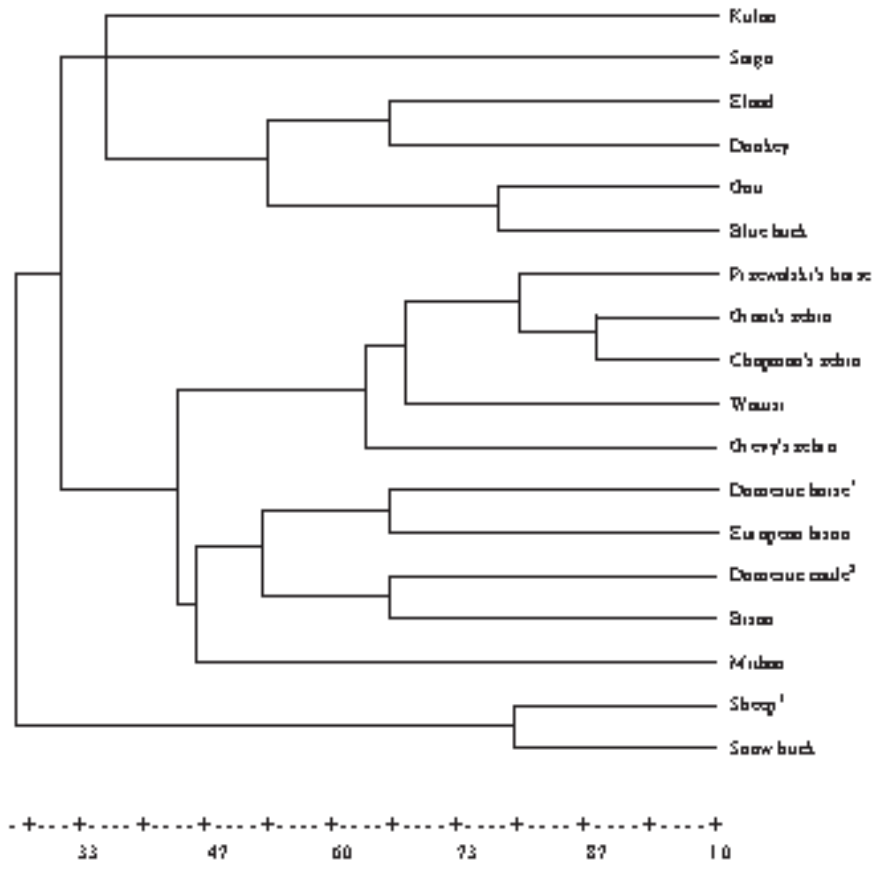

Fig. 1. Dendrogram of genetic relations between different species of *Ungulata* as calculated on the basis of polymorphic protein *loci*.
[1] Ukrainian Grey, Ukrainian Whiteheaded.
[2] Orlov's trotter, Yakutian, Hutsul.
[3] Ascanian high-prolificacy caracul.

According to genetic distance the investigated species may be grouped into two main clusters, each subdivided into two subclusters. Cluster 1 includes all investigated antelopes and kulan, domestic horse and donkey, while cluster 2 – all *Bovinae*, both *Ovis* species and four *Perissodactyla* species – Przewalski's Horse and three zebras. Cluster 1 is subdivided into two subclusters: 1a – kulan, and saiga, and 1b – antelopes (except saiga) and donkey. Cluster 2 is subdivided into subcluster 2a – Przewalski's Horse, three zebras and Watusi cattle, and subcluster 2b – *Bovinae* (except Watusi cattle), both *Ovis* species and mithan.

According to electrophoretic mobility of proteins two main groups of species may be

distinguished, the first including donkey, kulan and antelopes, and the second including the remaining *Artiodactyla* species, Przewalski's Horse and three zebras.

Evolution process in domestic and wild animal species targeted different protein systems. In domestic animals the most polymorphic appeared the transport proteins and enzymes involved in exogenous substrates metabolism, while in wild species – those of the inner cell energy metabolism (Tab. 2). Polymorphism was related to the role of the protein in cell metabolism.

Two enzymes of pentose phosphate pathway (G6PD and 6PGD) showed the widest polymorphism (figures not shown) and species investigated can be divided into five

Table 2. The contribution to polymorphism's level (part of the polymorphic *loci*) of species under natural (A) or artificial selection (B) to the variation of protein groups with different biochemical functions

Species group	Protein group		
	enzymes of intracellular energy metabolism	enzymes of exogenous substrate metabolism	transport proteins
A[1]	0.629	0.193	0.178
B[1]	0.179	0.464	0.357

[1] *Artiodactyla* wild species: 1-5 and 9-14; *Perissodactyla* wild species: 3-5 and 7.
[1] *Artiodactyla* domestic species: 6a-6c, 7 and 8; *Perissodactyla* domestic species: 1b, 1d, 1e, 2 and 6.

groups: 1 – donkey, kulan and Grevy's zebra; 2 – domestic horse, Chapman's and Grant's zebras; 3 – sheep, snow buck and mithan; 4 – domestic cattle, bison and european bison; 5 – eland, bluebuck and gnu. There were, however, two exceptions: in G6PD the similarity was found between group 1 and 5, as well as between group 3 and 4, and in 6PGD between group 1 and 3, as well as between 2 and 4.

Evolution of electrophoretic mobility pattern of enzymes from pentose phosphate pathway (G6PD and 6PGD) manifested some features, which were related to interspecies phylogenetic distance, and some other which were unrelated. Proteins of some groups were not always associated with the interspecies phylogenetic relations – proteins with the same electrophoretic mobility pattern were found in *Perissodactyla*, as well in *Artiodactyla.*

Analysis of glycolytic enzymes showed that the most slowly migrating bands of GPI appeared in majority of investigated *Artiodactyla* species (watusi, bison, cattle, sheep, snow buck, gnu), but in only one *Perissodactyla* representative – the donkey. Grevy's zebra showed the fastest migrating PGM bands, while other *Perissodactyla* species showed intermediate or slow migrating bands. Electrophoretic mobility patterns of PGM in donkey and kulan, as in the case of G6PD, appeared similar to those found in antelopes, *i.e.* eland, blue buck, and gnu. Thus, among species considered in this study, variation in glycolytic enzymes was essentially less than in enzymes of pentose phosphate pathway.

Variation in Krebs cycle enzymes (ME, MDH) was also found less than that in G6PD and 6PGD from pentose phosphate pathway. Slowly migrating ME bands appeared in cattle, bison, sheep and snow buck, and fast migrating in donkey and three antelopes, *i.e*. eland, blue buck and gnu. Simultaneously, the slowly migrating MDH bands were found in *Artiodactyla* (saiga and eland) as well as in *Perissodactyla* (all breeds of domestic horse, Grant's zebra and donkey).

Polymorphism of some transport proteins (ALB, TF, A1B) and blood plasma esterase (ES) was analysed by polyacrylamide gel electrophoresis. The fastest ALB bands were found in both *Ovis* species, while the slowest in donkey, kulan and eland. The fastest TF bands were also found in both *Ovis* species, while the slowest in saiga and blue buck as well as in mithan and domestic cattle. In other species TF showed the intermediate mobility. It is interesting that mobilities of ALB in donkey and kulan differed from those found in other *Perissodactyla*. Vitamin D receptor (GC) had similar electrophoretic mobility in all investigated *Perissodactyla* species, despite the differences appearing in ALB bands. Nevertheless, even within *Perissodactyla* the mobility pattern of ALB and GC varied independently.

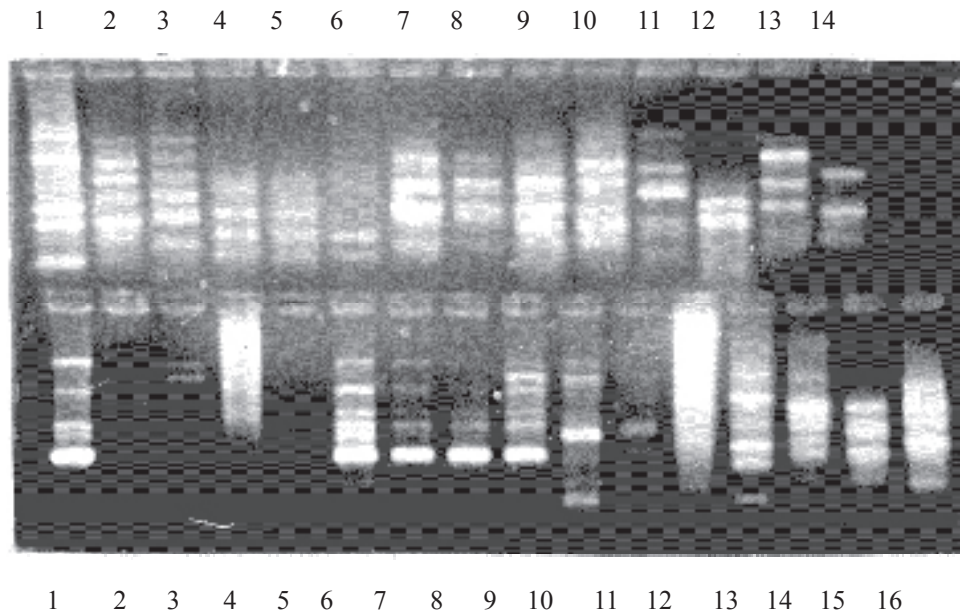

Photo 1. Amplification product patterns in *Ungulata* (RAPD-PCR), obtained using primer UBC-85 and UBC-126. Upper part – primer UBC-85: 1 – Arabian horse; 2 – Grey Ukrainian cattle; 3 – American bison; 4-6 – blue buck; 7,8 – Grevy´s zebra; 9, 10 – donkey; 11 – Large White pig; 12 – snow buck; 13 – kulan; 14 – saiga. Lower part, lines 1-12 – primer UBC-126: 1 – Arabian horse; 2 – American bison; 4, 5 – blue buck; 6, 7 – Grevy´s zebra; 8, 9 – donkey; 10, 11 – eland; 12 – gnu. Lower part, lines 13-16 – primer UBC-85: 13 – Arabian horse; 14, 15 – eland; 16 – gnu.

The second method used here to investigate phylogenetic relations was RAPD-PCR with UBC-85 and UBC-126 primers [Bailey and Lear 1994]. Size polymorphism of the amplified PCR product showed heterozygosity in length of DNA sequences flanked by inverted repeats. Fourteen species-specific different-length amplification products in RAPD-PCR spectra were revealed. Each band was marked as a *locus* (arranged with increasing molecular weight). The presence or absence of a band was marked as allelic variant A or B at this *locus*, respectively (Photo 1). Genetic distances between species were estimated using the BIOSYS-1 programme. Results are presented in Figure 2. For some traits the general pattern of species clusterization obtained by RAPD-PCR corroborates that obtained by analysis of protein polymorphism (Fig. l). Two *Perissodactyla* species were found together with some *Artiodactyla*: kulan with antelopes and Przewalski's Horse with european bison). This shows that similarities between

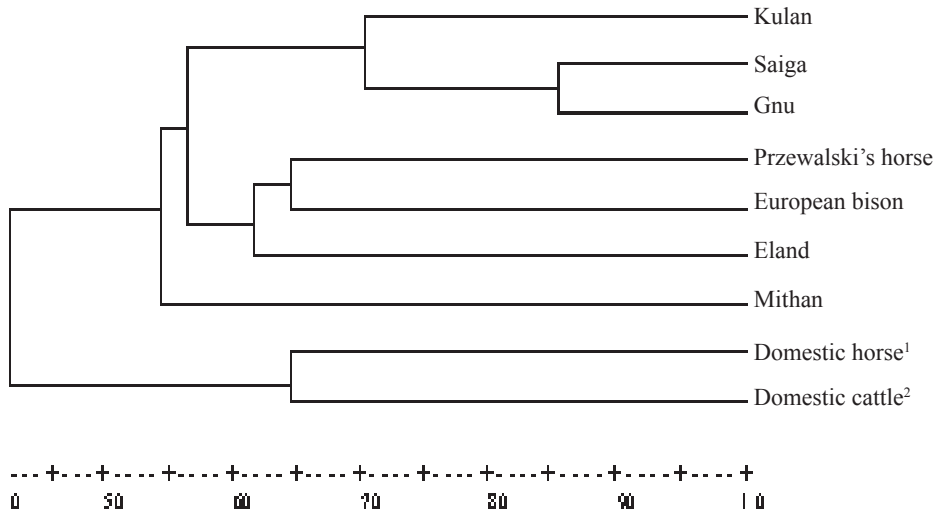

Fig. 2. Dendrogram of genetic relations between different species of *Ungulata* as calculated on the basis of PCR amplification products using UBS-85 and UBS-126 primers.
[1] Orlov's trotter, Arabian, Yakutian, Hutsul.
[2] Ukrainian Grey, Ukrainian Whiteheaded.

kulan and antelopes, or between Przewalski's Horse and some *Bovinae* exist not only on the protein level, but also on level of DNA sequences. It is interesting that, on the basis of length of amplification products, domestic cattle and domestic horse form a separate cluster. It cannot be excluded that distribution of inverted repeats of the DNA sequences in domestic species differentiates them from the wild species. Some length amplification products were found only in wild species. The 0.6 kb RAPD-PCR product appeared only in european bison, while that of 1.3 kb only in Grevy's zebra

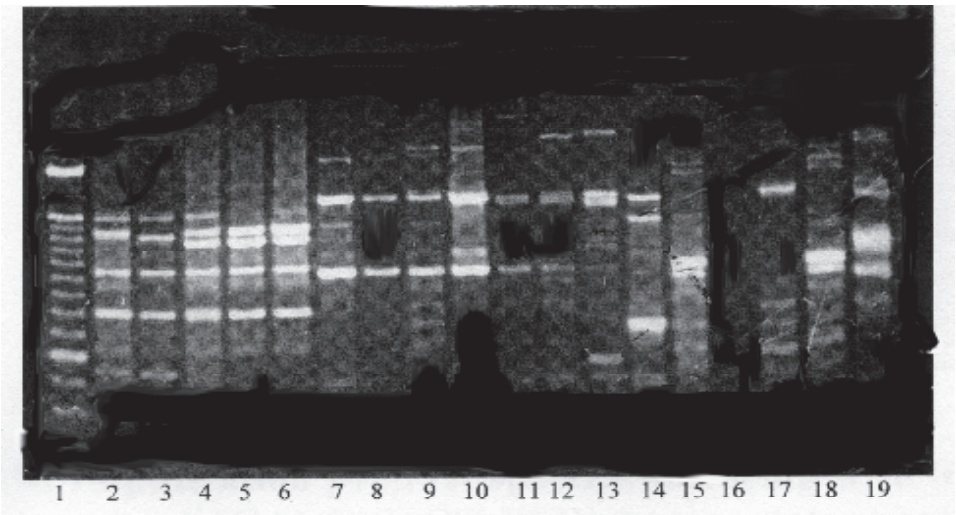

Photo 2. Amplification product patterns in *Ungulata* obtained using primer $(AG)_9C$ (ISSR-PCR). 1 – marker (100 bp Ladder, Gibco-BRL); 2 – Yakutian horse; 3 – Przewalski´s horse; 4 – kulan; 5 – Grevy´s zebra; 6 – Chapman´s zebra; 7 – Grey Ukrainian cattle; 8 – European bison; 9 – American bison; 10 – mithan; 11 – Ascanian caracul; 12 – snow buck; 13 – aoudad; 14 – saiga; 15 – eland; 16 – blue buck; 17 – gnu; 18 – black buck; 19 – Large White pig.

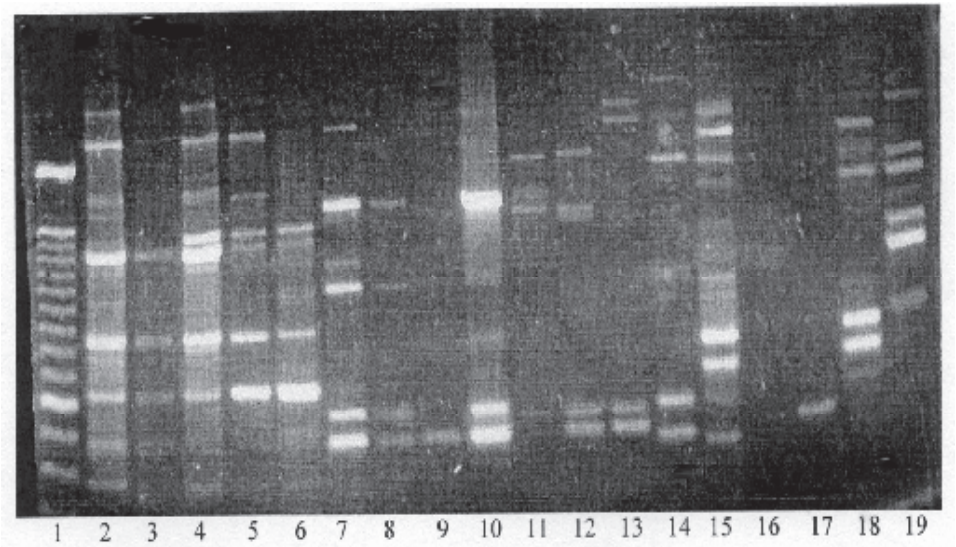

Photo 3. Amplification product patterns in *Ungulata* obtained using primer $(GA)_9C$ (ISSR-PCR). 1 – marker (100 bp Ladder, Gibco-BRL); 2 – Yakutian horse; 3 – Przewalski´s horse; 4 – kulan; 5 – Grevy´s zebra; 6 – Chapman´s zebra; 7 – Grey Ukrainian cattle; 8 – European bison; 9 – American bison; 10 – mithan; 11 – Ascanian caracul; 12 – snow buck; 13 – aoudad; 14 – saiga; 15 – eland; 16 – blue buck; 17 – gnu; 18 – black buck; 19 – Large White pig.

and american bison (UBC-85 primer). RAPD-PCR with UBC-126 primer revealed the amplification product of 0.7 kb only in Grevy's zebra, mithan and european bison, and that of 2.3 kb in Grevy's zebra, kulan, american bison and european bison.

The third method by which the phylogenetic relations were detected in this study was ISSR-PCR. Examples of the amplification product spectra are presented in Photos 2 and 3. Genetic distances between species were estimated using BIOSYS-1 programme. Results are presented in Figure 3. As in the case of RAPD-PCR, the ISSR-PCR with microsatellite primer $(AG)_9C$ showed the presence of some species-specific amplification products: 0.9 kb in american bison, 1.3 kb in kulan and Grevy's zebra and 2.2 kb in american bison and mithan. ISSR-PCR amplification products specific only for domestic species were not found. In *Perissodactyla* the mean number of amplicons (117) was higher than in *Artiodactyla* (102) – figures not tabulated – the differences being wider when estimated with ISSR-PCR than with RAPD-PCR. Long amplicons appeared more frequently in wild than in domestic species (Tab. 3).

Some features of polymorphism of three types of molecular markers appeared common for all domestic species examined. Simultaneously, they appeared different

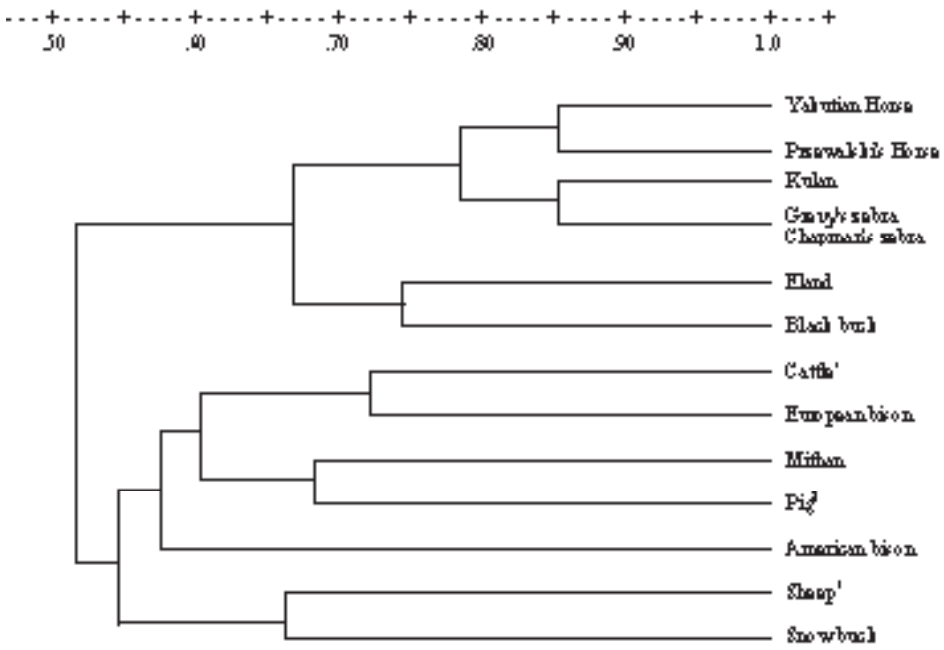

Fig 3. Dendrogram of genetic relations between different species of *Ungulata* as calculated on the basis of the length of ISSR-PCR amplification products obtained using $(AG)_9C$ and $(GA)_9C$ primers.
[1] Ukrainian Grey.
[2] Large White.
[3] Ascanian high-prolificacy caracul.

from those of wild species, closely related to them. These data suggest a "subgenome" existing in domestic animals, composed of some genes encoding important proteins

Table 3. Comparative analysis of the preferable amplicon's length in domestic and wild *Ungulata* species, obtained with the use of different primers in RAPD-PCR and ISSR-PCR

Species	Amplicon's length (%)		
	short (400-1000 bp)	medium (1100-1900 bp)	long (2000-2300 bp)
	RAPD-PCR		
Domestic[1]	36.2	30.9	12.8
Wild[2]	29.8	49.0	21.1
	ISSR-PCR		
Domestic[1]	41.9	43.0	11.8
Wild[3]	35.2	43.7	17.8

[1] *Artiodactyla*: 6a, 6b, 7 and 8; *Perissodactyla*: 1a-1c, 1e, 2 and 6.
[2] *Artiodactyla*: 1-5 and 9-14; *Perissodactyla*: 3-5 and 7.
[3] *Artiodactyla*: 6a-6c, 7 and 8; *Perissodactyla*: 1b-1e, 2 and 7.
[4] *Artiodactyla*: 2-5 and 9-13; *Perissodactyla*: 3-5 and 7.

and enzymes. Variation of a "subgenome" may be essential for domestication processes and for wide morphological diversity observed in domestic species.

Applying protein, RAPD-PCR, and ISSR-PCR markers, analyses were made of genetic interspecies relations within and between domestic *vs* wild *Artiodactyla* and *Perissodactyla.* All three methods gave similar results, leading to the conclusion that as a result of evolution, polymorphism of structural proteins is related to the species phylogenesis. Some protein features are related to domestication process rather, than to phylogenetic distances between the species investigated. Polymorphism of DNA markers is related to the phylogenetic past of the species, as well as to domestication process.

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Valerij Glazko

Próba genetycznej interpretacji procesu udomowienia zwierząt

S t r e s z c z e n i e

W obrębie 14 gatunków *Artiodactyla* i siedmiu gatunków *Perissodactyla* porównano gatunki dzikie z udomowionymi. Podstawę stanowiły dane o polimorfizmie białek krwi i wyniki uzyskane metodami RAPD-PCR i ISSR-PCR. Na podstawie różnic stwierdzonych w obrębie poszczególnych markerów między badanymi gatunkami autor wysuwa hipotezę o istnieniu „subgenomu", który zawiera określone geny kodujące fizjologicznie ważne białka i enzymy. Rozległa zmienność „subgenomu" mogła w przeszłości grać istotną rolę w procesie udomowienia, prowadząc do szerokiego morfologicznego zróżnicowania współczesnych gatunków zwierząt domowych.