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PECULIARITIES OF KARYOTYPE OF THE UKRAINIAN ABORIGINAL HUTSUL BREED OF HORSE

Abstract: Saving biodiversity and assessment of the genetic diversity of local breeds of domestic animals as priority subjects of protection in agrobiocenoses is one of the global challenges facing humanity in the 21st century. Hutsul horse is one of the oldest horse breeds in Ukraine, which according to the gene pool subject of horses in Ukraine belongs to the group “Local (mountain and ponies)” of the I category, which is already on the verge of extinction, and according to the FAO classification it is considered to be a subject of the gene pool threatened with extinction. Since the breeding chromosomal polymorphism of horses is insufficiently studied, it is timely to carry out a cytogenetic analysis of the characteristics of spontaneous mutagenesis in Hutsul horses. Karyotypic variability of Hutsul breed horses was determined using the methods of cytogenetic analysis and micronucleus test. The paper presents the results of cytogenetic analysis and micronucleus test of karyotypic variability of Hutsul breed horses. Asynchronous divergence of centromere regions of chromosomes occurs as a result of premature replication of centromere regions of heterochromatin associated with centromere activity. In the studied Hutsul horses, the magnitude of this variability corresponds to the spontaneous level for horses as a whole (2.2–9.1 %). No structural chromosomal abnormalities (chromosomal breaks) were revealed in Hutsul breed horses at Krai Neba LLC, and in the animals of the Hutsulshchina National Nature Park, the percentage of metaphase plates with chromosomal breaks was low (1.1 %), which indicates the stability of the karyotype of the studied animals. The results of the micronucleus test showed that the proportion of lymphocytes with micronuclei in the animals at the both farms was practically the same – 4.0–4.2. It can be concluded based on the data obtained that the animals under study were in ecologically clean conditions relative to the level of radionuclide contamination and were characterized by karyotype stability and reduced sensitivity to mutagenic factors of various nature.

Keywords: horses, Hutsul breed, aneuploidy, polyploidy, genomic abnormalities, karyotype stability, lymphocytes, chromosomes, chromosomal breaks, mitotic index

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ОСОБЕННОСТИ КАРИОТИПА УКРАИНСКОЙ АБОРИГЕННОЙ ГУЦУЛЬСКОЙ ПОРОДЫ ЛОШАДЕЙ

Аннотация: Сохранение биоразнообразия и оценка генетического разнообразия местных пород домашних животных как приоритетных объектов защиты в агробиоценозах – одна из глобальных задач, стоящих перед человечеством в XXI веке. Гуцульская лошадь – одна из древнейших пород лошадей в Украине, которая по объекту генофонда лошадей Украины относится к группе «Местные (горные и пони)» I категории, которая уже находится на грани исчезновения, а по классификации ФАО является объектом генофонда, находящихся под угрозой исчезновения. Поскольку породный хромосомный полиморфизм лошадей недостаточно изучен, проведение цитогенетического анализа особенностей спонтанного мутагенеза гуцульских лошадей является своевременным. Карิโอтипическую изменчивость лошадей гуцульской породы определяли с использованием методов цитогенетического анализа и микроядерного теста. В статье представлены результаты цитогенетического анализа и микроядерного теста кариотипической изменчивости лошадей гуцульской породы. Асинхронная дивергенция центромерных областей хромосом происходит в результате преждевременной репликации центромерных областей гетерохроматина, связанной с активностью центромер. У исследованных гуцульских лошадей величина этой изменчивости соответствует спонтанному уровню для лошадей в целом (2,2–9,1 %). Структурных хромосомных аномалий (хромосомных разрывов) у лошадей ООО «Край Неба» не выявлено, а у животных Национального природного парка «Гуцульщина» процент метафазных пластинок с хромосомными разрывами был низким (1,1 %), что свидетельствует о стабильности кариотипа исследуемых животных. Результаты микроядерного теста показали, что доля лимфоцитов с микроядром

у животных двух хозяйств была практически одинаковой – 4,0–4,2. Из полученных данных можно сделать вывод, что исследуемые животные находились в экологически чистых условиях относительно уровня радионуклидного загрязнения и характеризовались стабильностью кариотипа и пониженной чувствительностью к мутагенным факторам различной природы.

Ключевые слова: лошади, гуцульская порода, анеуплоидия, полиплоидия, геномные нарушения, стабильность кариотипа, лимфоциты, хромосомы, хромосомные разрывы, митотический индекс

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Introduction. To solve one of the global problems facing humanity in the XXI century - biodiversity conservation of our planet, knowledge about the structure of the gene pool and assessment of genetic diversity of local breeds of domestic animals as priority objects of protection in agrobiocenoses [1]. One of the oldest horse breeds in Ukraine is Hutsul horses. [2], which by the name of the gene pool object of horses in Ukraine belong to the group “Local (mountain and pony)” of the I-th category (gene pool object, which is already on the verge of extinction) of domestic classification and TR-cross-border regional (FAO classification) of gene pool objects by threats to existence and approaches to storage [1, 3]. One of the principles of determining the quality of Hutsul horses is genetic monitoring.

Spontaneous cytogenetic variability of horses in comparison with other species of farm animals is much less studied. In horse breeding, cytogenetic studies are mainly performed in animals in which infertility or other abnormalities are observed [4]. So far, the breed-specific chromosomal polymorphism of horses remains insufficiently studied. Therefore, the study of cytogenetic features of spontaneous mutagenesis of Hutsul horses is quite relevant today. The aim of our work was to conduct cytogenetic control of Hutsul horses to determine genetic specificity at the chromosomal level.

Materials and methods. Cytogenetic control was carried out in horses of Hutsul breed of the National Natural Park “Hutsulshchyna” (20 heads) in Kosiv and farms of LLC “Krai neba” (20 heads) in Kolomyia district. Lisna Slobidka, Ivano-Frankivsk region. Most of the studied animals are listed in the State Book of Hutsul Breeding Horses (Volume II) [2].

Cytogenetic drugs were prepared from peripheral blood lymphocytes taken from the jugular vein of the animal. Sterile vials were prepared in the laboratory for culturing blood cells; packed RPMI-1640 medium (Sigma, USA) in a sterile box of approximately 5 ml in one vial with 15-20 % - serum of cattle (preferably embryonic). Antibiotics were added to the culture at the rate of 0.001 ml of gentamicin per 1 ml of medium, 0.5 ml of whole blood, as well as mitogen - a substance that stimulates mitotic division of lymphocytes in culture. Dosage of phytohemagglutinin type P was added at a dose of 0.02 ml, type M - 0.2 ml per 10 ml of culture mixture. The mixture was cultured in a thermostat at a temperature of + 37 °C for 48-72 hours, periodically shaking the vials. Two hours before fixation, a solution of colchicine heated to 37 °C at a final concentration of 0.3-0.5 µg/ml of culture medium was introduced into the culture. For hypotension used freshly prepared 0.55 % solution of potassium chloride. Hypotension was performed for 20 min in a thermostat at a temperature of + 37 °C. After hypotension, the culture was centrifuged, the supernatant was drained, and cooled to + 4 °C fixing liquid was added to the precipitate carefully along the wall of the tube, mixing one part of glacial acetic acid with three parts of methyl (or ethyl) alcohol. After that, the precipitate was resuspended and centrifuged, repeating this operation 2-3 times. The cell suspension was applied to clean cooled slides from a height of 20-30 cm with an automatic dispenser. The glass was dried in air. The obtained preparations, after staining with the finished Giemsa dye, were analyzed for chromosomal variability under immersion magnification of the Axiostar plus microscope (Carl Zeiss, Germany) in 1000 times and microphotographed [5] (Fig. 1).

In the process of research took into account: quantitative chromosome disorders - aneuploidy (A), polyploidy (PP), cells with asynchrony of splitting of the centromeric regions of chromosomes (ARCR), structural aberrations - chromosome breaks (HR). The number of dinuclear lymphocytes (DJ) and mononuclear lymphocytes with micronuclei (MJ) and mitotic index (MI) were counted on the same drugs. The frequency of DY, MY, MI was calculated in ppm (‰), the number per 1000 cells.

Research results. The results of cytogenetic analysis of the studied horses of Hutsul breed showed that they are characterized by genomic and structural disorders of chromosomes (Table 1).

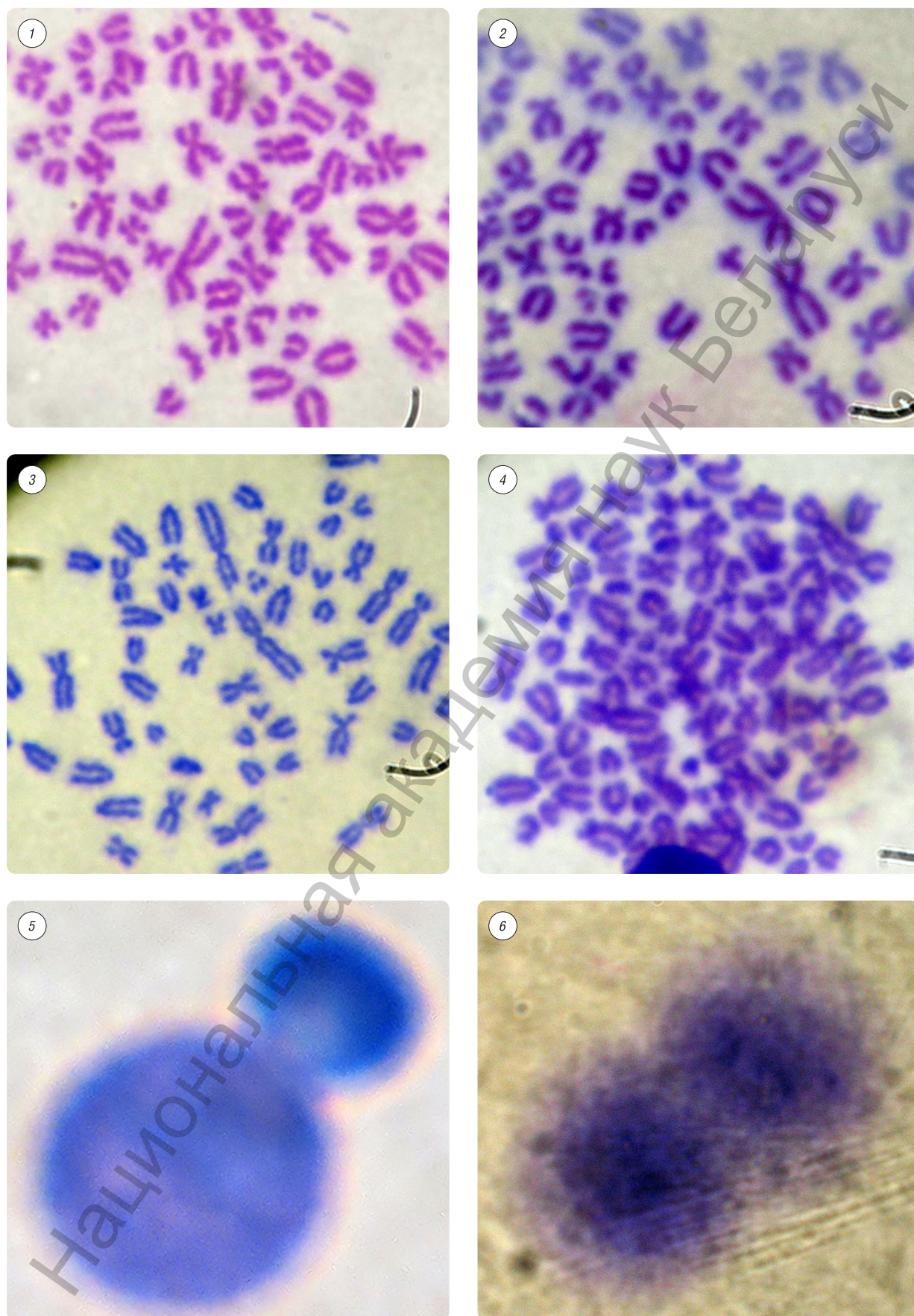


Fig.1. Features of Hutsul horse karyotype: 1 - Karyotype norm $2n = 64$; 2 - Asynchronous divergence of centromeric regions of chromosomes; 3 - Aneuploidy $2n = 58$; 4 - Polyploidy $3n = 96$; 5 - Lymphocyte with micronucleus; 6 - Dinuclear lymphocyte

Table 1. The results of the analysis of karyotypic variability of the Hutsul breed of horses in the National Nature Park “Hutsulshchyna” and LLC “Krai neba” Lisna Slobidka in Kolomyia district, %

Farm	Aneuploidy	Polyploidy	ARCRC	Chromosomal gaps
National Hutsul Nature Park	11,1±2,63	1,1±1,04	6,0±2,15	1,1±1,04
LLC “Edge of the Sky”	8,8±3,50	2,0±1,81	2,0±1,81	–
<i>M±m</i>	10,8±2,10	1,4±0,93	3,9±1,77	0,7±0,59

Genomic chromosome abnormalities, aneuploidy, were expressed mainly by hypoploid cells ($2n = 54-62$), the average of which was 10.8 %, the magnitude of the values of this variability was 8.8-11.1 % in animals of the farm LLC “Edge of Heaven” and Hutsulshchyna National Nature Park, respectively. However, the difference in the mean frequencies of aneuploid metaphases in the blood lymphocytes of horses of the two farms was statistically insignificant. According to this variability, individual polymorphism was observed in the studied animals. The correlation between age and aneuploidy in the studied Hutsul horses was weak and unreliable. The number of polyploid cells in horses of the two farms was in the range of 1.1-2.0 %, the average value of which was 1.4 % and did not exceed the species of this variability [4].

It was found that asynchronous divergence of centromeric regions of chromosomes occurs as a result of premature replication of centromeric heterochromatin regions associated with centromere activity [6]. In the Hutsul horses studied, the magnitude of this variability was 2.0-6.0 %, with an average value of 3.9 %, which corresponds to the spontaneous level for horses in general (2.2-9.1 %) [7].

Structural chromosome abnormalities (chromosomal breaks) in horses of Krai Neba LLC were not detected, and in animals of Hutsulshchyna National Nature Park the percentage of metaphase plates with chromosomal breaks was low and was 1.1 % (limit of chromosomal breaks in horses at spontaneous mutagenesis is 1.6-4.7 % [7, 8], which indicates the stability of the karyotype of the studied animals.

The results of the micronucleus test (Table 2) showed that the proportion of lymphocytes with a micronucleus for animals of the two farms was almost the same. The magnitude of this variability was 4.0-4.2 %.

Table 2. The results of the micronucleus test of the Hutsul horse breed of the National Nature Park “Hutsulshchyna” and LLC “Krai neba” Lisna Slobidka, Kolomyia district, %

Farm	Lymphocyte with micronucleus	Dinuclear lymphocyte	Mitotic index
Hutsul National Nature Park	4,2±0,76	6,7±1,01	4,7±0,70
Edge of the Sky LLC	4,0±1,15	3,8±0,79	2,8±0,59
<i>M±m</i>	4,1±1,40	6,2±0,94	4,3±0,72

To establish a connection between aneuploidy and lymphocytes with the micronucleus, a correlation analysis was performed. A direct relationship was found between cells with aneuploidy and lymphocytes with a micronucleus ($r = 0.9198$) with a significant probability of $P > 0.95$.

The number of dinuclear lymphocytes (6.2 %) exceeded the mitotic index (4.3 %). However, the difference in mean values between the frequency of dinuclear lymphocytes and the level of cell division - mitotic index, was insignificant. Studies in other animal species have shown that with increasing contamination, in particular radionuclides, increases the range of individual variability in MI, the frequency of lymphocytes with a micronucleus and decreases the number of dinuclear lymphocytes [8-10].

Therefore, from the obtained data it can be concluded that the studied animals were in environmentally friendly conditions relative to the level of radionuclide contamination and were characterized by karyotype stability and reduced sensitivity to mutagenic factors of different nature.

Conclusions. According to the results of cytogenetic analysis, the presence of genomic and structural disorders of chromosomes in the studied horses of Hutsul breed was established. It was found that genomic chromosome disorders are represented by aneuploidy, the average value of which was 10.8 %, polyploid cells with an average frequency of 1.4 %, not exceeding the species of this variability.

ity. The extent of asynchronous divergence of the centromeric regions of chromosomes (2.0-6.0 %) with an average value of 3.9 %, which corresponds to the spontaneous level for horses as a whole. The absence of chromosomal breaks in the animals of Krai Neba LLC and 1.1 % of metaphase plates with chromosomal breaks in horses of the Hutsulshchyna National Nature Park was revealed, which testifies to the stability of the karyotype of the studied animals. The proportion of lymphocytes with a micronucleus for animals of two farms was determined - 4.0-4.2 ‰. A direct relationship was found between cells with aneuploidy and lymphocytes with a micronucleus ($r = 0.9198$) with a significant probability of $P > 0.95$. It was found that the number of dinuclear lymphocytes (6.2 ‰) exceeded the mitotic index (4.3 ‰) with an insignificant difference in mean values, which indicates the stability of the karyotype of the studied animals.

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