

Original Research

Comprehensive Cytogenetic and *ISSR* Analysis in the Context of Conservation of the Local Breed of Brown Carpathian Cattle Breed

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Abstract

The article reveals the variability of the genome of cattle of the local small-numbered domestic Brown Carpathian breed using cytogenetic and molecular genetic polylocus markers, and justifies the need to integrate local breeds into the modern system of agricultural production in accordance with FAO requirements. The work aimed to study the genetic uniqueness of cows of the brown Carpathian breed using cytogenetic and molecular genetic markers. The karyotype of the animals corresponded to the species norm and was $2n = 60$ chromosomes. The level of somatic aneuploidy was 2.5%, and polyploidy and asynchronous divergence of centromeric regions of chromosomes were not detected. Chromosomal breaks were 0.56%. Various types of translocations 5;23 and 11;21, as well as centric fusion according to the Robertsonian type 1;29, were not found. *ISSR*-studies of dinucleotide $((GA)_6CC$, $(AG)_8CG$, $(AG)_8CA$, $(GA)_9C$ and $(AG)_9C$) and trinucleotide microsatellite loci $((ACC)_6G$, $(CTC)_6C$, $(GAG)_6C$) 94 amplified DNA fragments were found, of which 31 were polymorphic, which is 32.98%. The total proportion of polymorphic loci was $P = 0.218$, and the average value of the polymorphism index $PIC = 0.142$, the average per locus genetic diversity, $H_s = 0.94$, and the Shannon heterogeneity index, $I = 0.071$. The proportion of conservative loci was $K = 7.9$. Our findings demonstrated that the Brown Carpathian cattle show chromosomal stability, high genetic



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consolidation; however, a certain degree of genetic diversity is preserved and incorporated into the previous paragraph.

Keywords

Brown carpathian cattle; cytogenetic parameters; chromosome translocations; *ISSR*-studies; polymorphic loci; DNA

1. Introduction

Maintaining the biodiversity of animal species and breeds requires a systematic approach to the study of genetic processes and to the preservation of genetic resources. Genetic testing involves the analysis of the genetic structure of gene pools using DNA markers and karyotyping. When studying the gene pool of local and aboriginal breeds, special attention is paid not only to the productive qualities of animals but also to the identification of adaptation mechanisms provided by recombination and mutational variability.

The central task in the system of preserving the animal gene pool is selecting gene pool objects, establishing their genetic specificity, and studying the selection purpose. According to the Global Plan of Action, [1] adopted under the auspices of FAO, the first strategic priority for the conservation of genetic resources was a comprehensive description and cataloging of animals. Of all animal genetic resources in Ukraine as a whole, according to the international classification of FAO, it was found that about a quarter belong to local and $\frac{3}{4}$ to transboundary.

Currently, the Brown Carpathian cattle are considered to be a local, small-scale domestic breed. According to calculations, the Brown Carpathian breed is at significant risk, in accordance with the recommendations of the FAO [2]. This breed is bred in the mountainous regions of western Ukraine, where it accounts for 75% of the total cattle population.

The Carpathian Brown breed of cattle originates from the Small Carpathian Tur, which was domesticated in the Carpathian region. Then this cattle was improved through reproductive crossing of local breeds of Ryzhka and Mogan cattle. Its particular improvement occurred during the reign of the Austro-Hungarian Empire in Ukraine, when the law "On Breeding in Animal Husbandry" was issued. Then, the import of Swiss, Allgau, and other breeds of brown cattle began from the Alps. The beginnings of qualified work on improving brown cows date back to the time when Transcarpathia was under Czechoslovak rule. As a result of research, Czechoslovak scientists found that among all breeds of cattle kept on farms, the Carpathian Brown breed was the most resistant to tuberculosis. Cows were also characterized by their working qualities, they were used as draft power - for transporting cargo, plowing, and other things. Scientists from the city of Brno concluded that this is a good breed both for work and for meat and milk productivity. With the creation of collective farms, selection work continued to improve the breeding and productive qualities of cows. There were two directions for its improvement - the Schwyz breed to increase milk productivity and the Jersey breed - to increase the fat content of milk and the yield of cheese from it. It was officially registered as a breed in 1972.

This cattle is considered medium-sized, has a proportional physique, and belongs to the dairy and meat breeds. The color of the cows is from dark brown to light brown, and adult animals have

a light gray belt on their back. The nasal mirror is dark. In general, the brown Carpathian breed is divided into two types: lowland and mountain. They have different milk characteristics, differ in height, and structure. The highland cow is forced to move through the mountains; it is smaller in height and broader-chested, has a lighter, denser skeleton, and stronger hoof horn. The live weight of an adult individual is 400-450 kilograms, and milk productivity ranges from 3 to 4 thousand kg. In contrast, lowland cows are lighter in color and have a more elongated body. Their live weight is from 450 to 550 kilograms, and bulls - 816 kg, the maximum live weight is up to 1000 kg. Hopes - about 4-5 thousand kg. Both types are characterized by good meat productivity (slaughter yield 55-58%) and reproductive properties; the calf yield rate is 90-93%.

The unique characteristics of the brown cow are its adaptation to mountain conditions, especially its resistance to oxygen sickness, which is common at high altitudes. The fat content of the milk is relatively high, 3.7-4%. It is rich in calcium and protein (3.4-3.5%), saturated with lactose, so it is used to make baby food. The milk also contains a high level of casein, which is ideal for making cheese. So, although the brown Carpathian cow is inferior in milk yield to industrial cows, it is still the best among them in terms of the value and quality of its milk [3].

To preserve and develop the population of this breed, the Association of the Brown Carpathian Breed operates in Ukraine, which is part of the Federation of Brown Breeds of Europe. The association's specialists have developed a concept for preserving the breed, formed a gene pool bank, and begun genetic research on cattle. Employees of the Institute of Animal Breeding and Genetics n.d. a. M.V. Zubets NAAS, together with Transcarpathian and foreign scientists and specialists, took part in an expedition to select biological material for genetic research on brown cows. Selection and breeding work with local breeds should aim to preserve specific features and traits characteristic of local breeds [2], and should include information on the heredity of these animals and the uniqueness of their genotype. This study aims to characterize the genotype of Brown Carpathian cows using cytogenetic and molecular genetic markers.

2. Materials and Methods

2.1 Animals, Sampling, and Ethical Aspects

Biological material (peripheral blood samples) for studying the genotype specificity of Brown Carpathian cows was selected from animals (n = 30 heads) kept on private farms located in mountainous areas of western Ukraine (Figure 1).



Figure 1 Brown Carpathian cows.

2.1.1 Bioethical Norms

The study was conducted in accordance with the recommendations of the Declaration of Helsinki. It used data provided by ANABE, which did not require special animal handling practices, as they were obtained in accordance with standard protocols for control and recording in studbooks.

The Animal Care Commission at the Research Institute of Animal Breeding and Genetics n.d. a. M.V. Zubets NAAS (protocol No. 3 dated September 2, 2025) granted permission to conduct the research.

2.2 Cytogenetic Analysis

Cytogenetic preparations were prepared according to the traditional method [4]. One hundred metaphase plates were analyzed from each animal. Blood was collected from the jugular vein of the animal using sterile 5 ml plastic syringes. Sterility was maintained throughout the procedure. Blood samples were delivered to the genetic control laboratory in a thermos capable of maintaining +4°C for extended periods. Sterile vials were prepared for blood cell cultivation in the laboratory; RPMI-1640 medium (Sigma, Germany) was packed in a sterile box, approximately 5 ml per vial, with 15-20% bovine fetal blood serum (Sigma, Germany). Antibiotics were added to the culture at the rate of 0.01 ml of gentamicin per 1 ml of medium, 0.5 ml of whole blood, and PHA type P (Sigma, Germany) at a dose of 0.02 ml. The mixture was cultured in a thermostat at 37°C for 48 hours, periodically shaking the vials. Two hours before fixation, a solution of colchicine (Sigma, Germany) was warmed to 37°C and added to the culture at a final concentration of 0.3-0.5 µg/ml in culture medium.

After incubation with colchicine, the culture was shaken, transferred to centrifuge tubes, and centrifuged at 1000 rpm for 10 min. The supernatant was decanted, and the residue was resuspended in 10 ml of hypotonic solution at 37°C. A freshly prepared 0.55% potassium chloride solution was used for hypotonication. Hypotonication was carried out for 20 min, in a thermostat at +37°C. After hypotonication, the culture was centrifuged, the supernatant was decanted, and a fixing liquid cooled to +4°C was added to the precipitate. The fixing liquid consisted of 1 part glacial acetic acid with 3 parts methyl alcohol (Sigma, Germany). The tubes were transferred to a refrigerator, where they were kept at +4°C for 20 min. After that, the precipitate was resuspended and centrifuged, repeating this operation 2-3 times. The cell suspension was applied from a height of 20-30 cm onto cooled glass slides, evenly distributing it over the entire surface. The glass was dried in the air. To determine quantitative characteristics and changes in the number of chromosomes and some structural disorders, a staining method (according to Romanovsky-Giemsa) was used. The resulting preparations after staining were analyzed under an Axiostar plus microscope (Carl Zeiss, Germany) under an immersion microscope magnification of 1000 times and microphotographed.

2.3 Molecular Genetic Studies

The material for DNA studies was blood samples from 30 Brown Carpathian cows, taken during an expedition in the private sector in the western part of Ukraine.

Genomic DNA was isolated from blood samples using a standard commercial «DNA-sorb-B» reagent kit (AmpliSens, RF) according to the manufacturer's instructions. Qualitative and

quantitative assessment of the isolated DNA was performed by electrophoretic analysis by comparing the brightness of the bands of the fragments being analyzed and the standard DNA preparation (phage λ fragments). All samples were adjusted to a working concentration of 20 ng/ μ L.

The study of polymorphism of DNA fragments flanked by inverted repeats of microsatellite loci (*ISSR*-PCR markers) was performed by the standard method developed by E. Zietkiewicz [5]. Microsatellite oligonucleotides were used as primers (Table 1).

Table 1 Nucleotide structure of primers.

Primer	Nucleotide structure (5'→3')
For a trinucleotide microsatellite repeat	
(ACC) ₆ G	5'-ACCACCACCACCACCACCG-3'
(CTC) ₆ C	5'-CTCCTCCTCCTCCTCTCC-3'
(GAG) ₆ C	5'-GAGGAGGAGGAGGAGGAGC-3'
For a two-nucleotide microsatellite repeat	
(GA) ₆ CC	5'-GAGAGAGAGAGACC-3'
(AG) ₈ CG	5'-AGAGAGAGAGAGAGAGCG-3'
(AG) ₈ CA	5'-AGAGAGAGAGAGAGAGCA-3'
(GA) ₉ C	5'-GAGAGAGAGAGAGAGAGA-3'
(AG) ₉ C	5'-AGAGAGAGAGAGAGAGAGC-3'

The PCR mixture consisted of 2 μ L of DNA polymerase buffer polymerase (Fermentas, Lithuania), 1 μ L of triphosphate mixture (AmpliSens, RF), 0.8 μ L of the corresponding primer (Sigma, Germany), 0.2 μ L of DNA polymerase (Fermentas, Lithuania). Genomic DNA was added in an amount of 1.2 μ L (25 ng). The total volume of the PCR mixture was adjusted to 10 μ L of ddH₂O [5]. Amplification was performed in automatic mode on a BioRad programmable amplifier, USA. The annealing temperature, depending on the G/C composition of the primers, varied from 54°C to 64°C.

The amplification products were separated on 1.5% agarose gels at 120 V for 40 min. Ethidium bromide was used as a dye. Visualization of DNA fragments in the gel was carried out in the ultraviolet spectrum (312 nm). To determine the size of the amplified fragments, the molecular mass marker Gene Ruler 1 kb Plus DNA Ladder (Thermo Scientific™, USA) was used.

According to the research scheme, genetic and population studies were conducted to determine the genetic variability parameters of the experimental animals. Each amplicon of the spectrum was considered a single DNA locus. The polymorphism of such a locus (P) was assessed by the presence or absence of an amplicon of the corresponding length in the spectra. The calculation of the Polymorphic Information Content (PIC) index was performed according to the formula for diallel loci, for which $PIC = 2f(1 - f)$, where f is the frequency of one of the two alleles. According to Botstein (1980), loci with a PIC value >0.500 are highly polymorphic, with a PIC value between 0.250 and 0.500 are moderately polymorphic, and if $PIC < 0.250$, then the markers are lowly polymorphic.

Since the *ISSR*-PCR markers used by us, flanked by inverted microsatellite repeats, have a dominant nature of manifestation in the presence of the amplification product, f was calculated by the formula: $f = R^{1/2}$, where R is the frequency of animals among the studied ones in which a DNA fragment of a specific length was absent in the spectra of the amplification products. The

frequencies of genotypes, alleles, heterozygosity, and other biometric indicators were calculated using accepted methods [6].

2.4 Statistical Analysis

Statistical analysis was performed using the *Statistica 6.0* software package and *Excel (Microsoft Office 2007)*. Statistical data processing was performed in *Microsoft Excel* package using the integrated *Statistica XL 2.0* add-in (<http://www.statistixl.com/>) [7].

3. Results

3.1 Cytogenetic Studies

We examined 30 lowland cows. As a result of cytogenetic analysis (Table 2, Figure 2), it was found that in all examined animals the karyotype corresponded to the species norm and consisted of $2n = 60$ chromosomes, of which 58 were acrocentric autosomes, and 2 were large submetacentric X chromosomes.

Table 2 Level of somatic karyotypic variability of Brown Carpathian cows.

Number of animals (head)	Metaphases analyzed (n)	Aneuploidy, %	Chromosomal breaks, %
30	100	2.5 ± 0.45	0.56 ± 0.04

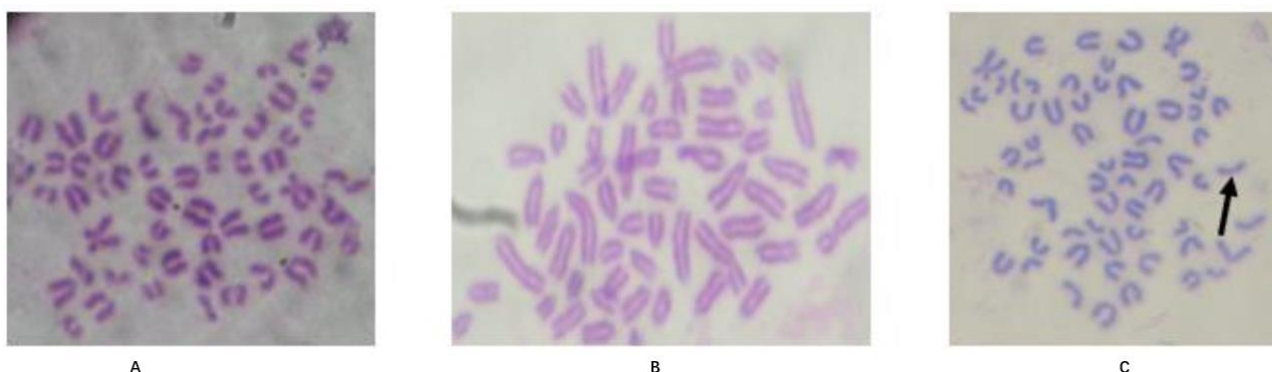


Figure 2 Karyotypic variability of Brown Carpathian cows: A - karyotype is normal, $2n = 60$; B -aneuploidy, $2n = 58$; C - chromosomal break.

The level of somatic aneuploidy in the studied animals corresponded to the average value of this variability in the breed. Cows with a sharp deviation in this indicator were not found. Quantitative chromosome abnormalities were expressed mainly as hypoploid cells, with a chromosome set of $2n = 56-58$. Numerical abnormalities of sex chromosomes were not found. Genomic chromosome abnormalities such as polyploidy and asynchronous divergence of centromeric regions of chromosomes were not found. Chromosomal breaks were 0.56% of the total number of studied cells, which does not exceed the spontaneous level of chromosome variability characteristic of this species.

In the tested cows of the Brown Carpathian breed, bred in Ukraine, structural chromosome aberrations (translocations 5;23 and 11;21), as well as centric fusion according to the Robertsonian type 1;29, were not found.

Therefore, the results of our studies show that Brown Carpathian cattle are free from chromosomal translocations, and the frequency of chromosomal variability does not exceed the spontaneous level typical of cattle, indicating good adaptive qualities established over many years of breeding. The karyotype stability of this breed's animals is shown in the histogram (Figure 3).

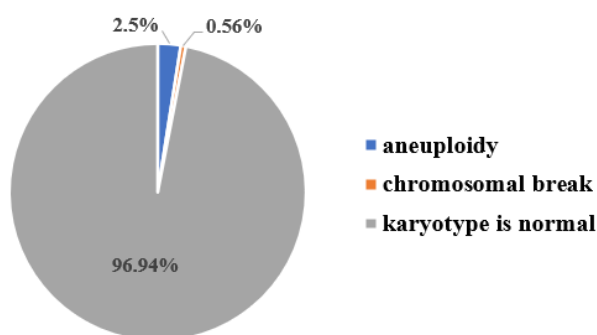


Figure 3 Genomic and structural abnormalities of the karyotype of Brown Carpathian cows.

3.2 Molecular Genetic Studies

ISSR analysis can be considered one of the simplest and most effective molecular genetic methods, used to assess the conservation and purity of different breeds, restore endangered breeds and species, and preserve their genetic diversity.

The genetic structure of the Brown Carpathian cattle breed was also studied using polymorphism assessments of DNA fragments flanked by inverted repeats of dinucleotide ((GA)₆CC, (AG)₈CG, (AG)₈CA, (GA)₉C and (AG)₉C) and trinucleotide microsatellite loci ((ACC)₆G, (CTC)₆C, (GAG)₆C, (GAG)₆C). All 8 *ISSR*-primers showed high efficiency for the study of cattle, as they detected clearly amplified DNA fragments (Figure 4). Each primer was individually analyzed by PCR with the genomic DNA of experimental cows.

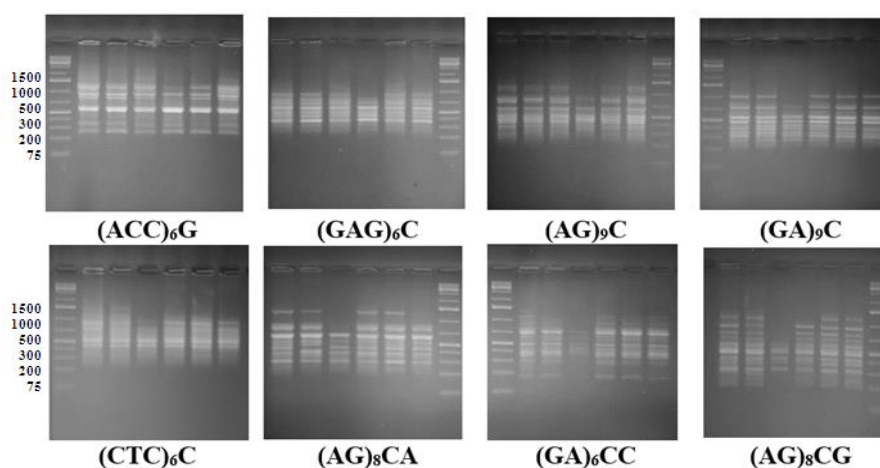


Figure 4 Spectra of amplicons obtained by *ISSR*-PCR using microsatellite primers in animals of the Brown Carpathian breed.

As a result of the analysis of the obtained DNA fragments flanked by an inverted repeat of the microsatellite locus region, which is used in PCR as one primer, spectra of amplification products of DNA sequences “unknown” in nucleotide sequence, localization in the genome, and functions were obtained. One amplicon in the spectrum was considered a single DNA locus.

When using three trinucleotide primers ($(CTC)_6C$, $(ACC)_6G$, $(GAG)_6C$) in *ISSR*-PCR spectra, from 5 to 14 amplicons were observed. The largest number of amplicons was located within the lengths of 440-930 bp. The amplicon spectrum can be conditionally divided into the following groups: “heavy” - 1240-1750 bp, “intermediate” - 1000-1240 bp, and “light” - 240-1000 bp. A different number of amplicons of these groups is detected in the studied animals, depending on the nucleotide sequence of the microsatellite locus primer. However, there is a clear predominance of light amplicons in the spectra (Table 3).

Table 3 Frequency of amplicons of different lengths when using trinucleotide microsatellite primers.

Amplicon length	%
$(CTC)_6C$	
1240-1750	20
1000-1240	0
240-1000	80
$(ACC)_6G$	
1240-1750	14
1000-1240	7
240-1000	79
$(GAG)_6C$	
1240-1750	9
1000-1240	9
240-1000	82

The amplicons spectra obtained using dinucleotide microsatellite loci primers ($(GA)_6CC$, $(AG)_8CG$, $(AG)_8CA$, $(GA)_9C$ and $(AG)_9C$) were more polymorphic (Table 4) than DNA fragments flanked by trinucleotide repeats (except for primer $(ACC)_6G$).

Table 4 Parameters of the used *ISSR*-markers.

Primer	(CTC) ₆ C	(ACC) ₆ G	(GAG) ₆ C	(GA) ₆ CC	(AG) ₈ CG	(AG) ₈ CA	(GA) ₉ C	(AG) ₉ C	Σ
Number of loci	5	14	11	14	14	10	12	14	94
Length limits	440-1550	240-1750	240-1350	160-1450	200-1290	280-1350	220-1050	240-1350	160-1750
Polymorphic loci	1	5	3	5	7	3	3	4	31

ISSR-study of the local small-numbered Brown Carpathian cattle breed of Ukraine using 8 microsatellite markers allowed to obtain reproducible and reliably detected spectra of amplification products. In total, 94 amplified DNA fragments (loci) were found, of which only 31 were polymorphic. The total number of polymorphic loci was 32.98%.

To assess the genetic diversity of the Brown Carpathian cattle breed population, a comparative analysis (*ISSR*-PCR) of amplicon spectra obtained using different primers ((*ACC*)₆G, (*CTC*)₆C, (*GAG*)₆C, (*GA*)₆CC, (*AG*)₈CG, (*AG*)₈CA, (*GA*)₉C and (*AG*)₉C) was performed: the number of amplification products (amplicons) in the spectra, the proportion of polymorphic loci, PIC-polymorphism information contents, etc. were estimated (Table 5).

Table 5 DNA polymorphism of the Brown Carpathian breed of cows by *ISSR*-markers.

No.	<i>ISSR</i> -marker	P	H _s	I	μ	h _μ	PIC	K
1	(<i>ACC</i>) ₆ G	0.2	0.96	0.096	13.47	0.04	0.146	9
2	(<i>CTC</i>) ₆ C	0.066	0.99	0.073	0.33	0.93	0.06	4
3	(<i>GAG</i>) ₆ C	0.2	0.96	0.063	6.5	0.41	0.133	8
4	(<i>GA</i>) ₆ CC	0.26	0.93	0.077	18.15	0.3	0.168	9
5	(<i>AG</i>) ₈ CG	0.4	0.84	0.086	39.44	1.82	0.243	7
6	(<i>AG</i>) ₈ CA	0.25	0.94	0.047	7.45	0.26	0.146	7
7	(<i>GA</i>) ₉ C	0.14	0.98	0.072	4.8	0.6	0.1	9
8	(<i>AG</i>) ₉ C	0.23	0.95	0.052	12.6	0.1	0.139	10
Average		0.218	0.94	0.071	12.84	0.56	0.142	7.9

Note: P – proportion of polymorphic loci, H_s – average per locus genetic diversity, I – Shannon heterogeneity index, μ – average number of alleles per locus, h_μ – proportion of rare loci, PIC – locus polymorphism index, K – number of conservative loci.

The markers differed in both the number of polymorphic loci and the number of conserved fragments. According to the results of population genetic analysis, the least polymorphic marker was (*CTC*)₆C – (PIC = 0.06). This primer revealed 4 of 5 loci as conserved. The main polymorphism was found in the zone from 1500 to 1550 bp. In general, conservative loci were detected in all *ISSR*-markers, but none of the microsatellite primers amplified only conservative fragments, i.e., all markers were polymorphic. The average value of the polymorphic information content (PIC) for the spectra of each primer varied from 0.06 to 0.243. The most variable were the amplicon spectra of the primer (*AG*)₈CG.

According to preliminary data, the amplicon 500-520 bp occurs in all studied animals of the Brown Carpathian breed according to all *ISSR*-markers; it can be characterized as breed-specific.

4. Discussion

Since the animals of the modern breed are direct descendants of the ancient Brown Carpathian cattle, which was once widespread throughout Central Europe: in the Alps it was called Alpine brown, in Switzerland - Schwyz breed, in Austria - Gorno-Inca and Montafon, and in Germany - Allgau, we conducted a retrospective analysis of the literature data on the karyotype of various brown breeds. The control of the karyotype of the Schwyz breed of cattle was carried out in Switzerland in the 70s of the last century. Testing was carried out on 224 heads of cattle and the

following results were obtained: 3 cows among the tested were homozygous for the Robertsonian translocation rob (1;29), which was 1.34% and 31 tested animals were heterozygous for this translocation and was 10.4%. All of them inherited the anomaly from a single bull (USDA-DHIA bank summary). Results of cytogenetic monitoring of Romanian Brown cattle (Romania) over the past 20 years showed that the frequency of animals carrying the Robertsonian translocation 1;29 (Rb 1;29) was 0.7% [8, 9]. The Robertsonian translocation involving chromosomes 1 and 29 — rob (1;29) — is the most common among breeds and has been identified in more than 50 cattle breeds worldwide [10]. As commented by De Lorenzi et al. [11], compared to other Robertsonian translocations, rob (1;29) is of ancient origin, as no de novo origin has ever been reported.

Our studies of brown breeds, in particular Lebedyn [12], Swiss and Brown Dairy [13], have established a wide range of individual variability in aneuploidy. Thus, in Lebedyn cows, the presence of metaphase plates with aneuploidy ranged from 0 to 15.5%; the average value of this indicator was $M \pm m = 10.2 \pm 2.10\%$. In Swiss cows, aneuploidy was 4.6%, and in Brown Dairy animals, 5.7%. The percentage of cells with aneuploidy in Lebedyn, Swiss, and Brown Dairy cows was somewhat higher than in Brown Carpathian animals, which may be due to the intensive use of cows and to individual genetic polymorphism of this variability. The presence of metaphase plates with polyploidy was detected only in the Swiss (4.0%) and Brown dairy (2.1%) breeds of cows. Chromosome breaks in brown cattle ranged from $2.0 \pm 0.22\%$ to $2.8 \pm 0.27\%$, and only in Brown Carpathian cows this indicator was 0.56%. Thus, the indicators of somatic mutagenesis in aboriginal and local breeds have wide intraspecific limits, which is due to their adaptogenic properties.

The results of *ISSR analysis* of the Brown Carpathian cattle breed indicate a limited but preserved level of genetic variability in the population. The total proportion of polymorphic loci ($P = 0.218$) and the average polymorphism index ($PIC = 0.142$) are lower than those of breeds with more numerous or selectively open populations, indicating a high degree of genetic consolidation in the studied animals. Similar results have been obtained for other local cattle populations that have been bred in isolated conditions for a long time or have a limited number [14].

The revealed structure of polymorphism between *ISSR*-markers indicates that dinucleotide primers ($(GA)_6CC$, $(AG)_8CG$, $(AG)_8CA$, $(GA)_9C$, $(AG)_9C$) provide a higher level of resolution compared to trinucleotide primers, which is consistent with data from other *ISSR*-studies in ruminants [14]. The highest level of polymorphism was observed for primer $(AG)_8CG$ ($PIC = 0.243$), which may be related to the high frequency of AG-motifs in the cattle genome.

The high number of conservative loci ($K = 7.9$) indicates a relatively homogeneous genetic background in the Brown Carpathian breed, which is a characteristic feature of aboriginal populations adapted to local conditions. This confirms the effectiveness of *ISSR*-PCR as a tool for assessing the consolidation and identity of such genetic resources. The presence of a stable amplicon of 500-520 bp in all studied individuals may indicate the breed specificity of this fragment, which requires further verification by sequencing.

The obtained indicators of genetic diversity ($H_s = 0.94$; $I = 0.071$) are consistent with the fact that the population of the Brown Carpathian breed retains a certain level of intrabreed variability, despite its small size and limited distribution. Preserving this variability is important for maintaining the adaptive potential and for restoring the breed in the future.

Thus, the results of *ISSR*-analysis confirm the feasibility of using microsatellite primers of the AG and GA types to access the genetic structure of local cattle breeds. The low level of genetic polymorphism and the high proportion of conserved loci reflect the historical stability of the gene

pool of the Brown Carpathian breed, as well as its adaptation to the conditions of the Carpathian region.

5. Conclusions

The stability of the chromosomal apparatus in brown Carpathian cattle has been established, which is due to breed adaptogenic properties.

The molecular genetic analysis conducted indicates the high efficiency of using *ISSR*-PCR markers to assess the consolidation of animals of the brown Carpathian breed using as primers microsatellite regions with bovine motifs $(ACC)_6G$, $(CTC)_6C$, $(GAG)_6C$, $(GA)_6CC$, $(AG)_8CG$, $(AG)_8CA$, $(GA)_9C$ and $(AG)_9C$.

A low proportion of polymorphic loci and high genetic consolidation are noted. Still, a certain degree of genetic diversity is preserved, which is important for the success of the restoration and preservation of the breed.

Author Contributions

Lyubov Starodub participated in the methodology of the work, cytogenetic studies, and writing the article; Nataliia Mokhnachova participated in the conceptualization, molecular genetic studies, and writing the article; Ostap Zhukorskyi reviewed and edited the article, and conducted the formal analysis.

Competing Interests

The authors have declared that no competing interests exist.

Data Availability Statement

Information on the genetic studies of Brown Carpathian cattle is stored at the Institute of Animal Breeding and Genetics named after M.V. Zubets of the National Academy of Agrarian Sciences of Ukraine and can be obtained by authorized users at the right time.

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