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Genetic Diversity of Wisent *Bison bonasus* Based on STR Loci Analyzed in a Large Set of Samples

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Abstract: Wisent *Bison bonasus* is an example of a species saved from extinction and reintroduced into nature after a few decades of captive breeding. There were only twelve founders of the Lowland–Caucasian line (LC) and even fewer (seven out of twelve) of Lowland (LB) animals. The genetic diversity in studies based on pedigree or markers is very low. In this paper, we present a summary of the long-term genetic monitoring conducted for the worldwide population of European bison. We summarized the long-term genetic monitoring studies conducted on the worldwide population of wisents to date. We genotyped 2227 wisents from two genetic lines (LC and LB) and different populations at ten microsatellite loci. We found low polymorphism, with only 2.7 alleles per locus, and much lower values of observed heterozygosity (0.380 and 0.348 in the LC and LB lines, respectively) than expected heterozygosity. The difference between the lines is only noticeable in allele proportions, so the number of markers is not enough to distinguish the two genetic lines. We also present the genetic distance among four free-roaming populations that are geographically close to each other. We found that the genetic distance of one of them is larger than that of the others, which could be the effect of genetic drift.

Keywords: microsatellites; genetic diversity; genetic distance; *Bison bonasus*; genetic drift

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1. Introduction

The wisent (*Bison bonasus*), also named European bison, is a species that passed a serious bottleneck and, for more than 30 years, was only bred in captivity and reintroduced into nature [1,2]. After WWI, there were no natural populations of this species, and the inventory performed in Europe in the year 1923 showed that there were only 54 animals (29 males and 25 females) in captivity [2]. The first reintroduction was performed in 1952 in Białowieska Forest (Poland) [3]. Since then, many free-roaming populations have been created [4–6], and currently, about 75% of the whole species is in free-roaming herds [7]. The wisent population is divided into two parts, Lowland (LB) and Lowland–Caucasian (LC) lines, which are not equally represented. At the end of 2021, there were 2288 (909 LB and 1379 LC) animals in captivity and 7266 (4049 LB and 3217 LC) in free-roaming herds [7]. In Poland, there are five free-roaming populations created more than 40 years ago, and three populations were reintroduced in the last 5 years [7]. In Belarus, the first population was established in Białowieska Forest, and later, other herds were created by moving animals from this first population [6]. Nowadays, in 10 countries, there are more than 40 free-roaming populations [8], and the global number of wisents reaches a level close to ten thousand individuals [7]. It is important to add that wisent, as the representant of European megafauna, is the umbrella species and is listed in the NT (Near Threatened) category on the IUCN Red List [8].

The genetic status of the wisent is interesting, because as a consequence of the bottleneck, the actual population is derived from only 12 founders [9,10]. Of this group, 11

founders came from the Lowland subspecies *B.b bonasus*, and only 1 animal came from the mountain subspecies *B.b caucasicus*. Therefore, the wisent population is divided into two parts, referred to as genetic lines: the Lowland line (LB), derived from 7 founders, and the Lowland–Caucasian (LC) line, which is a mixture between subspecies derived from all 12 animals [1,11]. The genetic diversity of the species is very low, and the pedigree data show that there is a high level of inbreeding and a small effective population size [12–14]. In addition, the contribution of founders is skewed, which also has an impact on genetic diversity [15]. Based on the pedigree of the released animals, the probability of unique genes in the current population was calculated to be around 0.2% [12]. In free-roaming populations, the possibility to check and evaluate the genetic variability could be based on genetic markers. Many authors have found low genetic diversity within the species [16–26].

To examine the level of genetic diversity, microsatellite loci (STRs—Short Tandem Repeats) can be used. STRs have been very widely used for the comparison of different populations, breeds, and species [27–30]. A detailed comparison of the results of such studies is very difficult due to the different sample sizes, populations, and number of loci, but a general comparison is very useful. Microsatellite loci have only been studied within the wisent species by a few authors [19–26]. In all the cited studies, the authors showed low genetic variability in the species and obtained similar results, which are briefly presented in Table 1. Generally, all studies showed low allelic richness (between 1.818 and 3.260) and rather small heterozygosity (between 0.241 and 0.506 for observed heterozygosity and between 0.245 and 0.507 for expected heterozygosity). In some studies, the number of samples was small, and most of the research was only based on genetic material from the Polish Białowieska population and Belarus (LB line) [19,20,23,25]. The Lowland–Caucasian line was mainly analyzed using material from Russian reserves and the Bieszczady (Poland) herd [23,24,26]. Only one study analyzed samples from German zoos [21]. The number of STR markers varied, so other loci were also analyzed. All the cited authors combined the analysis of 37 microsatellite loci, of which 11 markers were used in only 1 of the selected papers and only 1 locus was used in all 11 studies. However, in all cited studies, very low numbers of alleles per locus were common, with about 50% of polymorphic markers only having two alleles and very few loci with four or five alleles. Not all studies presented monomorphic loci, so the mean number of observed alleles and heterozygosity cannot be directly confronted, but in all studies, these values were low compared with those of other species (Table 1).

Table 1. Results of microsatellite locus diversity in wisent *Bison bonasus* obtained by other authors.

Source of Samples	Number of Samples	Genetic Line *	Number of Loci	Allelic Richness	Observed Heterozygosity	Expected Heterozygosity	Authors
Poland Białowieska	22	LB	21	1.905	0.241	0.245	Gralak et al., 2004 [19]
Poland Białowieska	38	LB	9	2.667	0.506	0.507	Luenser et al., 2005 [20]
Poland Białowieska	247	LB	17	2.790	0.259	0.279	Tokarska et al., 2015 [23]
Belarus	48	LB	17	2.470	0.283	0.270	Tokarska et al., 2015 [23]
Belarus	30	LB	11	2.434	0.424	0.317	Homel et al., 2020 [25]
Belarus	42	LB	11	1.818	0.281	0.277	Kostyunina et al., 2020 [26]
Russia reserves	111	LC	11	2.182	0.302	0.286	Kostyunina et al., 2020 [26]
Poland Bieszczady and Russia	56	LC	17	3.260	0.358	0.392	Tokarska et al., 2015 [23]
Russian reserves	26	LC	10	2.800	0.262	0.293	Dotsev et al., 2018 [24]
German reserves	35	LC	11	2.360	0.453	0.447	Roth et al., 2006 [21]

* LB—Lowland line; LC—Lowland–Caucasian line.

A closely related species in the same genus is the American bison, also known as buffalo (*Bison bison*), which has also been affected by a bottleneck in the past, but not to such a severe extent. In both species, microsatellite markers were applied based on those for cattle, and the chosen loci had to be polymorphic with large PIC (Polymorphism Information Content) values and widely distributed in the genome [31–34]. In work based on 725 sampled buffalos, the average number of alleles per 15 analyzed loci varied from 5 to 16 [32], and in this species, STR loci can be used for parentage testing [31]. In more recent studies where 26 loci were analyzed, the average number of alleles ranged from 3.88 to 4.92, depending on the herd [35].

The genetic structure of European bison has also been assessed using different SNPs. Authors used Illumina BovineSNP50 BeadChip [36–38] or Illumina BovineHD [38,39]. In the cited studies, the number of samples was between 10 [37] and 163 [40], and samples were mainly from the Lowland line. On the basis of 57 individuals from the LB line and 72 from the LC line, SNP markers were selected for the design of a microarray specific to wisents [38]. This dedicated microarray was used for studying a large number of samples (455 animals) [39]. Although the number of used SNPs was different, all these studies equally indicated low genetic variability within the species. Among 960 loci, the mean expected heterozygosity was between 0.31 and 0.36 [36,37] for LB animals from the Białowieska population. The values of heterozygosity differ depending on the used set of markers. For example, with 50 SNPs selected for individual identification, Ho = 0.485 and He = 0.498, with a lack of difference between genetic lines; however, with 30 SNP markers selected for line identification, those values were different between genetic lines: Ho = 0.128 and He = 0.133 for LB, but Ho = 0.423 and He = 0.445 for LC [39].

Both wisent genetic lines, Lowland (LB) and Lowland–Caucasian (LC), have seven common individuals among the founders, but five other animals founded the latter line. The aim of our study was to summarize the results of long-term genetic monitoring based on a large number of individuals from free-roaming and captive herds representing the two genetic lines of Lowland (LB) and Lowland–Caucasian (LC).

The second aim was to present, as an example of STR usefulness, the case study about the genetic structure of the free-roaming populations reintroduced at least 50 years ago in one region of Europe. For this part, four populations were chosen: three of them were from Northeast Poland (Białowieska, Borecka, and Knyszyńska), and the fourth population comprised free-roaming herds from Belarus (Figure 1). The analyzed herds were established in the 1950s (Białowieska and Belarus) [3,6], in the 1960s (Borecka) [4], and in the 1970s (Knyszyńska) [5]. For all reintroductions, the herds from Białowieża (Poland) were the main source of animals [6,41]. After release, contact between populations was not frequent, but still possible. Between Poland and Belarus, the possibility of migration existed until 1980, when a fence was built on the border [42]. The genetic distance among those herds based on the pedigree of the released animals was less than 1% [41]. In the paper [41], the groups of animals released in every forest were analyzed. Those animals came from captivity or were first-generation captures from the released herd in Bialowieska Forest. Based on pedigree, we compared the founders' contributions to the herds.

Figure 1. The four free-roaming populations of wisents: (1) Białowieska (Poland), (2) Borecka, (3) Knyszyńska, and (4) Belarus.

2. Materials and Methods

2.1. Samples and DNA Extraction

The material for genetic analyses comprised samples of biological material collected from wisents in the years 2000–2021. Samples were stored at −70 °C in Wisent Genes Bank organized by Department of Animal Genetics and Conservation according to the permit (ref. No. WPN-I.6401.90.2014.EB.1) for preserving tissues from a protected species given by Regional Director of Environmental Protection in Warsaw. The biological material was sent by owners or veterinarians and was taken from animals immobilized for other reasons and from all dead individuals. The material consisted of peripheral blood, fragments of soft tissues, and hair bulbs.

Due to the long duration of research and sampling over the years, DNA was isolated using dedicated kits from various manufacturers: DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) was used for DNA isolation from blood, soft tissue fragments, and hair bulbs; QuickGene DNA Whole Blood kit S (Kurabo, Osaka, Japan) was used for DNA isolation from blood; QuickGene DNA Tissue kit S (Kurabo, Osaka, Japan) was used for isolation from soft tissues and hair bulbs; GeneMATRIX Quick Blood DNA Purification Kit (EURx, Gdańsk, Poland) was used for isolation from blood; and GeneMATRIX Tissue DNA Purification Kit (EURx, Gdańsk, Poland) was used for isolation from soft tissues and hair bulbs. When Kurabo kits were used, a QuickGene-Mini80 (Kurabo, Osaka, Japan) nucleic acid isolation system was used for isolation. All procedures were performed following the protocols provided by the manufacturer. The isolated genetic material was subjected to spectrophotometric analysis using NanoDrop2000 (Thermo Fisher Scientific, Waltham, MA, USA), and the samples that met the requirements regarding quantity and purity (ratio parameters: A260/A280 = 1.8–2.0 and A260/A230 = 2.0–2.2) were subjected to further analysis.

In this study, we analyzed 2177 individuals divided into two genetic lines, i.e., 1220 Lowland (LB) individuals and 957 Lowland–Caucasian (LC) individuals. To our knowledge, this is the first study to cover such a large part of the wisent population. Within the Lowland (LB) line, we analyzed a set of 586 wisents in detail: a total of 252 from the Białowieska herd, 146 from the Borecka herd, 82 from the Knyszyńska herd, and 106 from Belarus. The other LB animals (634) came from Poland (523 samples from 19 herds), Germany (54 samples from 7 herds), and the Netherlands and other countries (67 samples from 6 herds). The samples from the LC line were collected from the Polish Biieszczady herd (295 samples), Germany (477 samples from 64 herds), and Sweden (106 samples from 6 herds), and 79 samples were from 23 herds from other countries.

2.2. Genotyping

Ten microsatellite markers were used for analysis. Markers were divided into two multiplex reaction panels, "55" and "60", under different PCR conditions. The forward primers of each marker were 5′-fluorescently labeled (Table 2). Polymerase chain reaction (PCR) was performed using 3.5 µL of AmpliTaq Gold™ 360 Master Mix with 0.15 µL of 360 GC Enhancer (Applied Biosystems™), 0.04 µL of each primer at a 10 µM concentration, 1 µL of DNA, and deionized PCR-grade water up to the final volume of 7 µL. The cycling conditions for the amplification of microsatellite markers in the "60" panel were as follows: a temperature of 95 °C for 9 min 30 s; followed by 19 cycles at 95 °C for 30 s, 60 °C for 1 min (touchdown of −0.1 °C per cycle), and 72 °C for 45 s; then, 14 cycles at 95 °C for 30 s, 58 °C for 1 min, and 72 °C for 45 s; final extension step at 60 °C for 30 min. The cycling conditions for the amplification of microsatellite markers in the "55" panel were as follows: a temperature of 95 °C for 9 min 30 s; followed by 29 cycles at 95 °C for 30 s, 58 °C for 1 min, and 72 °C for 1 min; final extension step at 60 °C for 30 min. All PCRs were performed using T3 Thermocycler (Biometra, Göttingen, Germany).

Table 2. Microsatellite markers with information about source data.

Locus	Forward Primer Sequence (5′–3′)	Reverse Primer Sequence (5′–3′)	Fluorescent Dye	Panel	Source
BM1824	GAGCAAGGTGTTTTTCCAATC	CATTCTCCAACTGCTTCCTTG	PET	55	[43]
HEL009	CCCATTCAGTCTTCAGAGGT	CACATCCATGTTCTCACCAC	VIC	55	[44]
ILST034	AAGGGTCTAATGCCACTGGC	GACCTGGTTTAGCAGAGAGC	6-FAM	55	[45]
INRA123	TCTAGAGGATCCCCGCTGAC	AGAGAGCAACTCCACTGTGC	NED	55	[46]
MM012	CAAGACAGGTGTTTCAATCT	ATCGACTCTGGGGATGATGT	6-FAM	55	[47]
AGLA293	GAAACTCAACCCAAGACAACTCAAG	ATGACTTTATTCTCCACCTAGCAGA	VIC	55	[31]
BM1818	AGCTGGGAATATAACCAAAGG	AGTGCTTTCAAGGTCCATGC	6-FAM	55	[48]
EBMS044	CCTTGCCACTATTTCCTCCA	CCAAATGACACATGACAGCC	NED	60	[45]
ETH010	GTTCAGGACTGGCCCTGCTAACA	CCTCCAGCCCACTTTCTCTTCTC	NED	60	[49]
ETH225	GATCACCTTGCCACTATTTCCT	ACATGACAGCCAGCTGCTACT	VIC	60	[50]

After PCR, samples were 10-fold diluted. To 1 µL of each diluted PCR product, 8.5 µL of Hi-Di™ Formamide and 0.5 µL of GeneScan™ 600 LIZ® Size Standard v2.0 were added before electrophoresis. Capillary electrophoresis was performed with 3500 Genetic Analyzer (Applied Biosystem, Foster City, CA, USA) using a 50 cm capillary array and POP-7™ Polymer. The FragmentAnalysis50_POP7 run module protocol in combination with the G5 dye set was used. The reading of the lengths of the obtained fragments was analyzed using GeneMapper® Software v4.1 (Applied Biosystems, Waltham, MA, USA).

2.3. Statistical Analysis

Allele frequencies, observed (Na) and effective numbers of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), unbiased Nei distances between analyzed populations, and F_{st}, as well as probability of identity (PI) and probability of identity between related animals (PI_{sibs}), were calculated using GenAlEx version 6.502 software [51]. To evaluate the presence of genetic divergence between populations, Principal Coordinate

Analysis (PCA) based on genetic distances was performed using GenAlEx software. PCA data visualization was performed using R studio [52] with the Scatterplot3d package [53].

The Bayesian clustering method implemented in Structure v. 2.3.4 [54] was used to illustrate the population genetic structure. Analysis was performed with the Correlated Allele Frequencies Model and the Admixture Model, with a burn-in period of 10^5 and 10^5 Markov Chain Monte Carlo (MCMC) replicates for 10 iterations of each K value (K = 1 to 5). Structure Harvester was used to assess the optimal value of K for this study (inspection of log-likelihood values according to the ΔK method developed by Evanno [55]). The raw Structure output files were combined and visualized using Clumpak v. 1.1 [56].

3. Results

3.1. Genetic Lines—LC vs. LB

The number of alleles was exactly the same at each locus for both genetic lines, from two to four. The frequency within every line was different (Figure 2), but there were no private alleles. The obtained parameters of the genetic lines are presented in Table 3.

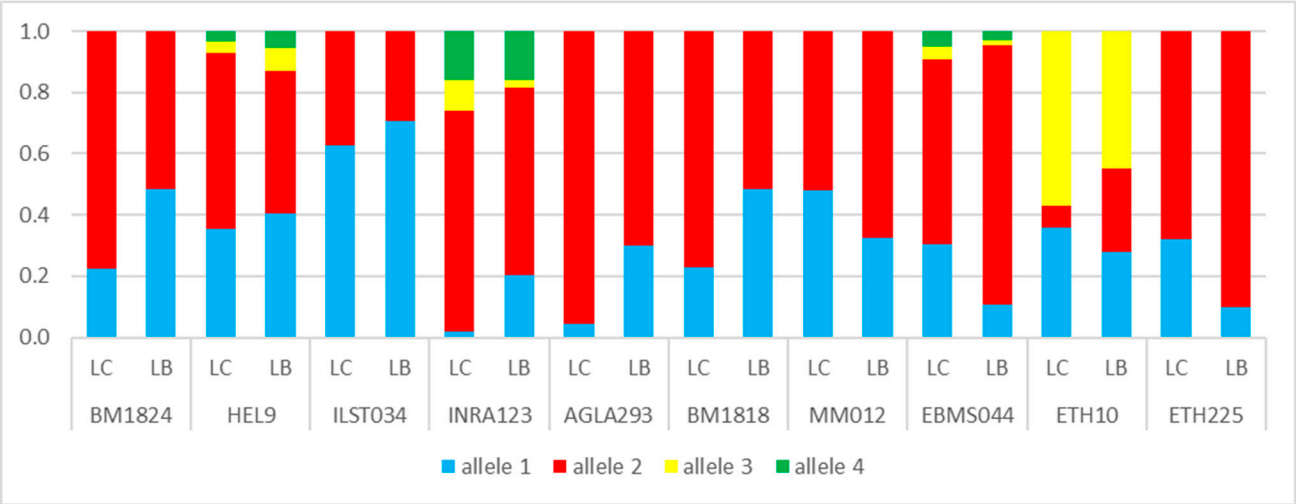

Figure 2. Frequency of alleles within all 10 loci and two genetic lines (LC and LB).

Table 3. Genetic parameters of Lowland–Caucasian (LC) and Lowland (LB) lines.

		LC Line			LB Line		
Locus	**Na**	**Ne**	**Ho**	**He**	**Ne**	**Ho**	**He**
BM1824	2	1.998	0.440	0.500	1.538	0.294	0.350
HEL009	4	2.568	0.241	0.611	2.189	0.173	0.543
ILST034	2	1.706	0.383	0.414	1.882	0.394	0.469
INRA123	4	2.257	0.601	0.557	1.786	0.521	0.440
AGLA293	2	1.719	0.389	0.418	1.090	0.080	0.083
BM1818	2	1.998	0.444	0.499	1.546	0.304	0.353
MM012	2	1.783	0.402	0.439	1.997	0.421	0.499
EBMS044	4	1.377	0.204	0.274	2.168	0.452	0.539
ETH010	3	2.833	0.576	0.647	2.185	0.476	0.542
ETH225	2	1.217	0.122	0.178	1.771	0.364	0.435
Mean	**2.7**	**1.946**	**0.380**	**0.454**	**1.815**	**0.348**	**0.425**
SE	0.3	0.159	0.049	0.046	0.111	0.044	0.044

On average, heterozygosity was slightly larger in the LC line than in the LB line, but the difference was not large. In locus AGLA293 in the LB line, the polymorphism was very small (Table 2; Figure 2). At the same time, in locus EBNS044 or ETH225, there was a reverse situation, with a smaller value for the LC line than for the LB line. Based on

10 STR loci, the difference between the two genetic lines was very low. The genetic distance between the two lines based on all 10 loci was equal to 7.1%.

When the analysis was performed on all samples without separation into genetic lines, based on 10 microsatellite loci, the probability of identity (PI) for unrelated samples was equal 4.43×10^{-5}, and PI_{sibs} was 7.44×10^{-3}. For unrelated individuals, the expected number of animals with the same multilocus genotype (calculated as respective probability $\times$ population size) was below 1 (0.096), but based on PI_{sibs}, this number was higher (16.196 individuals). The same calculation was made with the assigned genetic lines. For the Lowland line, PI was equal to 1.0×10^{-4}, and for the Lowland–Caucasian line, it equaled 4.5×10^{-5}. The PI_{sibs} parameters were 1.07×10^{-2} for LB and $7.49 - 10^{-3}$ for the LC line. Given the large number of samples (LB—1220; LC—957), the estimated numbers of unrelated individuals with the same multilocus genotype were 0.127 for LB and 0.043 for LC, while based on PI_{sibs}, in LB, 13.058 animals were expected to have the same multilocus genotype, and in LC, this number was lower (7.172 individuals).

The difference between the two genetic lines was not large, as seen in Figure 3, where the results of the PCA analysis are presented. A large part of animals belonging to different lines were genetically very similar, but a little difference was noticeable. The 3d visualization of the PCA results (file lines 3d.gif) is included in the Supplementary Materials.

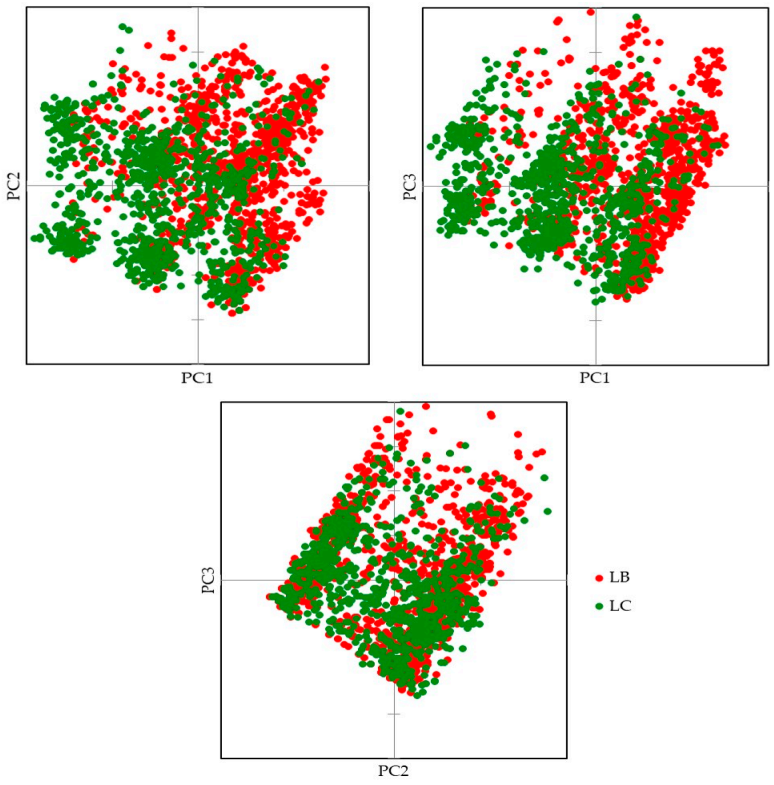

Figure 3. Principal Coordinate Analysis (PCA) of 2 genetic lines (Lowland-Caucasian LC and Lowland LB), based on 10 microsatellite markers (PC1 = 18.92%; PC2 = 14.48%; PC3 = 11.67%).

The Structure results shown in Figure 4 do not indicate a clear division into clusters differentiating genetic lines; however, it is possible to note a certain trend in the estimated proportion of membership to a given cluster. The overall proportion of "red" in the Lowland line was 0.695, and in the Lowland–Caucasian line, it was equal to 0.321. The proportions of "green" were 0.305 in LB and 0.679 in LC. As in the case of PCA, among the animals studied, we can notice those characterized by having a high percentage of belonging to a particular cluster (an extreme percentage of belonging either to the cluster of green or red color), but in both lines, there were also individuals with balanced proportions between the two clusters. However, the ΔK method supports the division into two clusters.

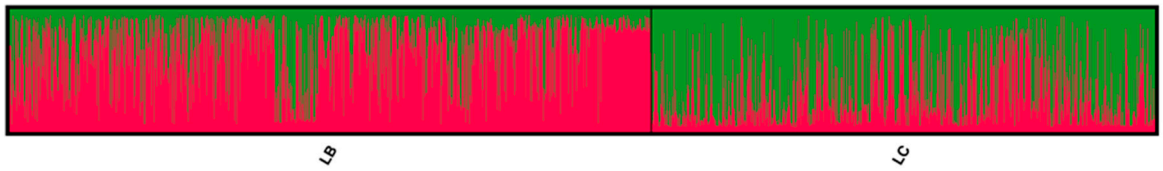

Figure 4. Results of Bayesian clustering of 2171 individuals from 2 genetic lines. The Structure plot represents the run when K = 2. Individuals are ordered by genetic line, and each of them is represented by a line proportionally divided into color segments. The black line separates individuals from different genetic lines.

3.2. Four Populations within the Lowland Line (LB)

The results of allele number, and observed and expected heterozygosity of every analyzed Lowland population are presented in Table 4, and distances among all four herds are included in Table 5.

Table 4. Heterozygosity and effective number of alleles in four populations belonging to the Lowland (LB) line.

	Białowieska			**Borecka**			**Knyszyńska**			**Belarus**		
	Ne	**Ho**	**He**	**Ne**	**Ho**	**He**	**Ne**	**Ho**	**He**	**Ne**	**Ho**	**He**
BM1824	1.63	0.36	0.39	1.37	0.27	0.27	1.84	0.29	0.46	1.75	0.43	0.43
HEL009	2.17	0.18	0.54	2.34	0.23	0.57	2.14	0.34	0.53	2.52	0.23	0.60
ILST034	1.63	0.35	0.38	1.29	0.21	0.22	1.54	0.35	0.35	1.95	0.42	0.49
INRA123	1.87	0.58	0.46	1.72	0.51	0.42	1.87	0.58	0.47	1.53	0.39	0.34
AGLA293	1.15	0.14	0.13	1.00	0	0	1.01	0.01	0.01	1.19	0.13	0.16
BM1818	1.67	0.40	0.40	1.43	0.26	0.30	1.88	0.33	0.47	1.76	0.44	0.43
MM012	1.85	0.42	0.46	1.93	0.41	0.48	1.19	0.10	0.16	1.88	0.48	0.47
EBMS044	2.42	0.50	0.59	2.20	0.49	0.55	1.74	0.19	0.43	2.44	0.63	0.59
ETH010	2.09	0.53	0.52	2.09	0.52	0.52	1.29	0.19	0.22	2.33	0.54	0.57
ETH225	1.96	0.47	0.49	1.96	0.46	0.49	1.45	0.12	0.31	1.76	0.34	0.43
Mean	**1.84**	**0.39**	**0.44**	**1.73**	**0.33**	**0.38**	**1.60**	**0.25**	**0.34**	**1.91**	**0.40**	**0.45**
s.d.	0.11	0.05	0.04	0.14	0.05	0.06	0.11	0.05	0.05	0.13	0.05	0.04

Table 5. Unbiased Nei distances among analyzed populations (below diagonal) and F_{st} values (above diagonal).

	Belarus	**Białowieska**	**Borecka**	**Knyszyńska**
Belarus	-	0.80%	2.61%	7.81%
Białowieska	1.39%	-	1.11%	8.21%
Borecka	3.14%	0.92%	-	9.85%
Knyszyńska	12.06%	12.78%	15.19%	

The Belarus population had slightly larger genetic diversity than the other herds from Poland, but compared with Białowieska, the difference was very small. In both populations, Belarus and Białowieska, the effective number of alleles and expected heterozygosity were larger than for the whole Lowland (LB) line (Tables 3 and 4). The numbers of observed alleles in the Białowieska and Knyszyńska herds were the same, as for the entire LB line. In the Belarus herd, in locus HEL009, there was one allele less than in the LB line, despite the fact that this population had the largest number of effective alleles. Locus AGLA293 in the Borecka population was monomorphic, and in two other loci, HEL009 and INRA123, the number of observed alleles was less than and equal to three. In consequence, the expected heterozygosity was the lowest in this population. In locus MM012, there was very small heterozygosity in the Knyszyńska herd in comparison with the others (Table 4).

The largest distances were found between Knyszyńska and the other populations, and there was a very small difference between populations from Belarus and Białowieska (Table 5). The Knyszyńska population was not in contact with any other population for

more than 40 years but was created by releasing animals captured from the Białowieska free-roaming herd and Białowieża reserves. It should be repeated that in the Knyszyńska population, the average heterozygosity was the lowest (Table 4).

In Figure 5, where the visualization of the results of the PCA based on genetic distances is presented, it can be seen that some individuals from the Knyszyńska population are separated from and dissimilar with respect to the rest, while the other populations are mixed together (Figures 5 and 6). The 3d visualization of the PCA results (file LB 3d.gif) is included in the Supplementary Materials.

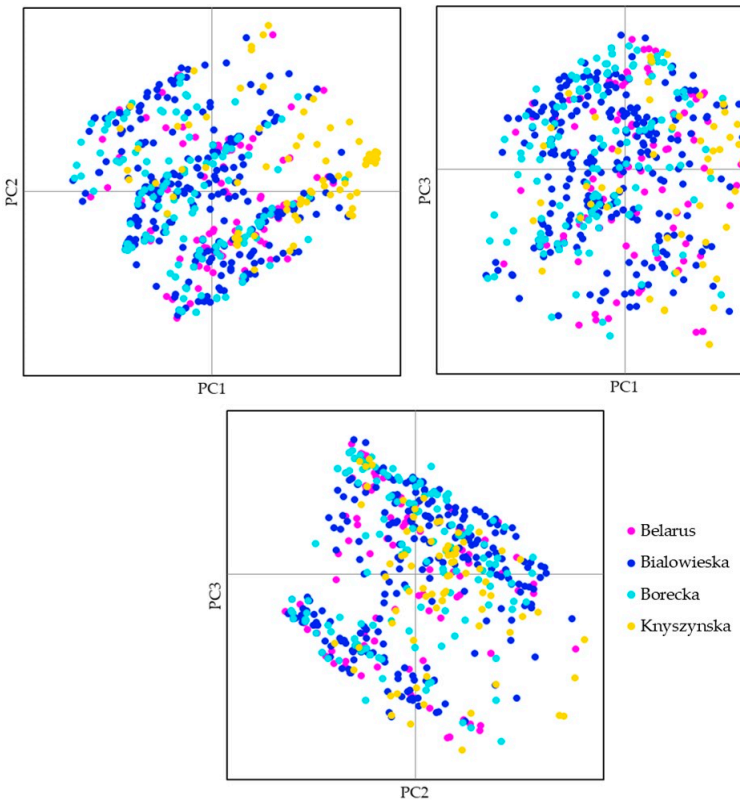

Figure 5. Principal Coordinate Analysis (PCA) of 4 populations, based on 10 microsatellite markers (PC1 = 19.41%; PC2 = 16.15%; PC3 = 13.08%).

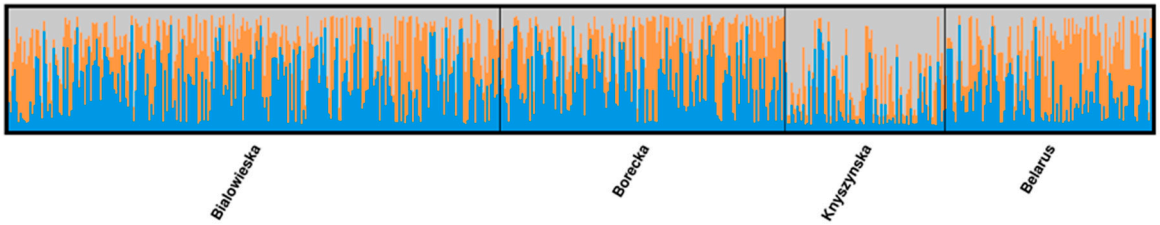

Figure 6. Structure plot that shows the genetic affiliation of LB line individuals from 4 different herds with groups computed by the program. The plot represents the run when K = 3. Individuals are ordered by origin, each represented by a line proportionally divided into color segments. Each color represents one genetic group. Black lines separate the individuals from different herds.

For a data set including four populations from the Lowland line, the ΔK value indicated three clusters. The Structure graph presented in Figure 6 shows variable participation of individuals in clusters, observed for all populations. However, the herd from Knyszyńska Forest clearly stood out, which was also evident in the PCA analysis.

4. Discussion

Our results are similar to those obtained by other authors. The average number of observed alleles was 2.7 in both lines, and this value corresponds with those reported in other studies [20,23,25] or is larger [19,26]. It is interesting that the number of alleles in the LC line is smaller than that reported in other studies [23,24] (Table 1). This cross-author comparison is difficult, and sometimes even impossible, due to the different loci analyzed, the different sources, and the size of the samples studied. However, it is possible to compare some microsatellites analyzed by different authors, and such locus is BM1824, which was used by almost all authors. In our study, we found two alleles with Ho = 0.440 and 0.294 in the LC and LB lines, respectively. In our studies, the values of the LC line were also larger than those of the LB line, which is the consequence of the larger number of founders of the LC line. Tokarska et al. [23] found three alleles in the Białowieska population and four alleles in LC animals from Russian reserves, but nobody else found more than two alleles. The heterozygosity in our study is smaller than that in other studies, which may be due to the large number of samples. In a group of 60 buffalos (*Bison bison*), the number of alleles was equal to eight, and heterozygosity was 0.83 [31], but in studies based on a much larger number of samples, those values were smaller [32]. Generally, in *Bison bison* species analyzed in many studies, the number of alleles and heterozygosity are much larger than those in wisent [57,58]. The polymorphism of buffalo is large enough to use a set of 15 microsatellites for parentage control [31,32] or for checking cattle introgression existence [34]. Our results show a small difference between the two genetic lines of wisent, and as expected, the LC line had a little more genetic variability than the LB line. The PCA graph (Figure 2) shows that both lines were not divided based on ten STR loci. The same can be noticed in Figure 4, where the samples from the two genetic lines are structured in a similar way. In that case, many more markers are needed to identify the genetic lineage. Single Nucleotide Polymorphism (SNP) analysis may be a solution [36], and detailed studies based on SNP results have shown that 30 markers with highly significant differences in allele frequency between genetic lines are sufficient for this purpose [39].

The results of comparing the four Lowland populations are interesting, and for this reason, the number of markers seems to be sufficient. The number of alleles and heterozygosity in all compared populations were similar, but the genetic distances among them show that Knyszyńska differed from the others much more (Figures 5 and 6; Table 5). This is the result of genetic drift and/or the effect of the founding group. The slightly lower number of alleles and heterozygosity values (Table 5) in this isolated population could be symptoms of genetic drift. Using pedigree data, the distance between Knyszyńska and other populations was less than 1% [36], but based on markers, the distance was between 12% and 15%. These values are larger than those obtained in cattle breeds (5% to 10%), but much lower than the distance between *Bison bison* and *Bos taurus* breeds (70–78%) [30]. Similar values of genetic distances were found between different populations of chamois (*Rupicapra* spp.) [29]. The Knyszyńska population was different from all other studied groups, but populations from Belarus and Białowieża were close to each other. This result is opposite to that obtained by [23], who concluded that the Belarusian population is no longer Lowland and is different from the Białowieska herd in Poland. This conclusion is not supported by our results, nor the results obtained in other studies [25,26]. Those two populations are at a very small geographic distance and have come into contact with each other before the border fence was built, so similarity is to be expected. As mentioned, the Knyszynska population was found to be different because of the founder effect and/or genetic drift. The impact of the released animals is unknown. Geographical isolation for more than five generations and the small size of this population could also have caused genetic drift. Such differences are important in species management and could help in preserving genetic variability in the species.

5. Conclusions

By using a set of ten microsatellites, it is not possible to recognize the genetic line of a sampled animal within the species *Bison bonasus*. Nevertheless, this number of markers could be efficient in presenting differences among herds, as for the four chosen free-roaming populations. Detailed genetic monitoring must be based on a larger set of markers. We prove that a large sample set and a small number of markers could bias the genetic structure comparison.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15030399/s1. The 3d visualization of the PCA results.

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References

1. Pucek, Z.; Belousova, I.P.; Krasińska, M.; Krasiński, Z.A.; Olech, W. *European Bison Status Survey and Conservation Action Plan*; IUCN The World Conservation Union: Gland, Switzerland, 2004; pp. 1–49.
2. Krasińska, M.; Krasiński, Z.A. *European Bison–Nature Monograph*; Springer: Berlin/Heidelberg, Germany, 2013; pp. 1–379.
3. Krasiński, Z.A. Free-living European bison. *Acta Theriol.* **1967**, *12*, 391–405. [CrossRef]
4. Krasiński, Z.A.; Krasińska, M. Free ranging European bison in Borecka Forest. *Acta Theriol.* **1992**, *37*, 301–317. [CrossRef]
5. Krasiński, Z.A.; Krasińska, M.; Leniec, H. Żubry w Puszczy Knyszyńskiej. *Park. Nar. I Rezerw. Przyr.* **1994**, *13*, 107–114. (In Polish)
6. Kozlo, P. Analysis of reintroduction and formation of European bison populations in Belarus. *Eur. Bison Conserv. Newsl.* **2013**, *6*, 21–36.
7. Raczynski, J.; Bołbot, M. *European Bison Pedigree Book 2021*; Białowieski National Park: Białowieża, Poland, 2022; pp. 1–80.
8. Olech, W.; Perzanowski, K. *European Bison (Bison bonasus) Strategic Species Status Review 2020*; IUCN SSC Bison Specialist Group and European Bison Conservation Center: Warsaw, Poland, 2022; pp. 1–148.
9. Slatis, H.M. An analysis of inbreeding in the European bison. *Genetics* **1960**, *45*, 275–287. [CrossRef] [PubMed]
10. Olech, W. The participation of ancestral genes in the existing population of European bison. *Acta Theriol.* **1989**, *34*, 397–407. [CrossRef]
11. Olech, W. The number of ancestors and their contribution to European bison (*Bison bonasus* L.) population. *Ann. Warsaw Agric. Univ. Anim. Sci.* **1999**, *35*, 111–117.
12. Olech, W. *The Influence of Individual and Maternal Inbreeding on the Survival of European Bison Calves*; Warsaw University of Life Sciences: Warsaw, Poland, 2003; pp. 1–78. (In Polish)
13. Olech, W. The analysis of European bison genetic diversity using pedigree data. In *Health Threats for the European Bison Particularly in Free-Roaming Populations in Poland*; Kita, J., Anusz, K., Eds.; The SGGW Publishers: Warszawa, Poland, 2006; pp. 205–236.
14. Olech, W.; Perzanowski, K. A genetic background for reintroduction program of the European bison in the Carpathians. *Biol. Conserv.* **2002**, *108*, 221–228. [CrossRef]
15. Olech, W. The changes of founders' numbers and their contribution to the European bison population during 80 years of species' restitution. *Eur. Bison Conserv. Newsl.* **2009**, *2*, 54–60.
16. Hartl, G.B.; Pucek, Z. Genetic depletion in the European bison (*Bison bonasus*) and the significance of electrophoretic heterozygosity for conservation. *Conserv. Biol.* **1994**, *8*, 167–174. [CrossRef]
17. Gębczyński, M.; Tomaszewska-Gruszkiewicz, K. Genetic variability in the European bison. *Biochem. Syst. Ecol.* **1987**, *15*, 285–288. [CrossRef]
18. Burzyńska, B.; Olech, W.; Topczewski, J. Phylogeny and genetic variation of the European bison *Bison bonasus* based on mitochondrial DNA D-loop sequences. *Acta Theriol.* **1999**, *44*, 253–262. [CrossRef]

19. Gralak, B.; Krasińska, M.; Niemczewski, C.; Krasiński, Z.A.; Żurkowski, M. Polymorphism of bovine microsatllite DNA sequences in the lowland European bison. *Acta Theriol.* **2004**, *49*, 449–456. [CrossRef]
20. Luenser, K.; Fickel, J.; Lehnen, A.; Speck, S.; Ludwig, A. Low level of genetic variability in European bison (*Bison bonasus*) from Bialowieża National Park in Poland. *Eur. J. Wildl. Res.* **2005**, *51*, 84–87. [CrossRef]
21. Roth, T.; Pfeiffer, I.; Weising, K.; Brenig, B. Application of bovine microsatellite markers for genetic diversity analysis of European bison (*Bison bonasus*). *J. Anim. Breed. Genet.* **2006**, *123*, 406–409. [CrossRef]
22. Łopienska, M.; Nowak, Z.; Charon, K.M.; Olech, W. A comparison of polymorphism of DQA genes in European bison belonging to two genetic lines: Lowland and Lowland-Caucasian. *Ann. Wars. Univ. Life Sci. SGGW Anim. Sci.* **2011**, *49*, 93–102.
23. Tokarska, M.; Bunevich, A.N.; Demontis, D.; Sipko, T.; Perzanowski, K.; Baryshnikov, G.; Kowalczyk, R.; Voitukhovskaya, Y.; Wójcik, J.M.; Marczuk, B.; et al. Genes of the extinct Caucasian bison still roam the Białowieża Forest and are the source of genetic discrepances between Polish and Belarusian populations of the European bison. *Bison bonasus. Biol. J. Linn. Soc.* **2015**, *114*, 752–763. [CrossRef]
24. Dotsev, A.V.; Aksenova, P.V.; Volkova, V.V.; Kharzinova, V.R.; Kostyunina, O.V.; Mnatsekanov, R.A.; Zinovieva, N.A. Study of Allele Pool and Genetic Structure of Russian Population of Lowland-Caucasian Line of European Bison (*Bison bonasus*). *Russ. J. Genet. Appl. Res.* **2018**, *8*, 31–36. [CrossRef]
25. Homel, K.V.; Sliwinska, A.A.; Valnisty, A.A.; Nikifirov, M.E. New data on the genetic diversity of European bison (*Bison bonasus*) in Belarus. *Theriol. Ukr.* **2020**, *19*, 45–53. [CrossRef]
26. Kostyunina, O.V.; Mikhailova, A.D.; Dotseva, A.V.; Zemlyanko, I.I.; Volkova, V.V.; Fornara, M.S.; Akopyana, N.A.; Kramarenko, A.S.; Okhlopkove, I.M.; Aksenova, P.V.; et al. Comparative Genetic Characteristics of the Russian and Belarusian Populations of Wisent (*Bison bonasus*), North American Bison (*Bison bison*) and Cattle (*Bos taurus*). *Cytol. Genet.* **2020**, *54*, 116–123. [CrossRef]
27. Nguye, T.T.; Genini, S.; Menetrey, F.; Malek, M.; Vogeli, P.; Goe, M.R.; Stranzinger, G. Application of bovine microsatellite markers for genetic diversity analysis of Swiss yak (*Poephagus grunniens*). *Anim. Genet.* **2005**, *36*, 484–489. [CrossRef] [PubMed]
28. Mukherjee, S.; Mukherjee, A.; Kumar, S.; Verma, H.; Bhardwaj, S.; Togla, O.; Joardar, S.N.; Longkumer, I.; Mech, M.; Khate, K.; et al. Genetic Characterization of Endangered Indian Mithun (*Bos frontalis*), Indian Bison/Wild Gaur (*Bos gaurus*) and Tho-tho Cattle (*Bos indicus*) Populations Using SSR Markers Reveals Their Diversity and Unique Phylogenetic Status. *Diversity* **2022**, *14*, 548. [CrossRef]
29. Crestanello, B.; Pechioli, E.; Vernesi, C.; Mona, S.; Martinkova, N.; Janiga, M.; Hauffe, H.C.; Bertorelle, G. The genetic impact of translocations and habitat fragmentation in chamois (*Rupicapra*) spp. *J. Hered.* **2009**, *100*, 691–708. [CrossRef]
30. Mommens, G.; Peelman, L.J.; Van Zeveren, A.; D'Ieteren, G.; Wissocq, N. Microsatellite variation between an African and five European taurine breeds results in a geographical phylogenetic tree with a bison outgroup. *J. Anim. Breed. Genet.* **1999**, *116*, 325–330. [CrossRef]
31. Mommens, G.; Zeveren, A.; Peelman, L.J. Effectiveness of bovine microsatellites in resolving paternity cases in American bison, *Bison bison* L. *Anim. Genet.* **1998**, *29*, 12–18. [CrossRef]
32. Schnabel, R.D.; Ward, T.J.; Derr, J.N. Validation of 15 microsatellites for parentage testing in North American bison, Bison bison and domestic cattle. *Anim. Genet.* **2000**, *31*, 360–366. [CrossRef]
33. Halbert, N.; Gogan, P.J.P.; Hedrick, P.W.; Wahl, J.M.; Derr, J.N. Genetic Population Substructure in Bison at Yellowstone National Park. *J. Hered.* **2012**, *103*, 360–370. [CrossRef]
34. Halbert, N.D.; Derr, J.N. A Comprehensive Evaluation of Cattle Introgression into US Federal Bison Herds. *J. Hered.* **2007**, *98*, 1–12. [CrossRef]
35. Ranglack, D.H.; Dobson, L.R.; Du Toit, J.H.; Derr, J. Genetic Analysis of the Henry Mountains Bison Herd. *PLoS ONE* **2015**, *10*, e0144239. [CrossRef]
36. Tokarska, M.; Marshall, T.; Kowalczyk, R.; Wójcik, J.M.; Pertoldi, C.; Kristensen, T.N.; Loeschecke, V.; Gregersen, V.R.; Bendixen, C. Effectiveness of microsatellite and SNP markers for parentage and identity analysis in species with low genetic diversity: The case of European bison. *Heredity* **2009**, *103*, 326–332. [CrossRef]
37. Kamiński, S.; Olech, W.; Oleński, K.; Nowak, Z.; Ruść, A. Single nucleotide polymorphisms between two lines of European bison (*Bison bonasus*) detected by the use of Illumina Bovine 50 K BeadChip. *Conserv. Genet. Resour.* **2012**, *4*, 311–314. [CrossRef]
38. Wojciechowska, M.; Nowak, Z.; Gurgul, A.; Olech, W.; Drobik, W.; Szmatoła, T. Panel of informative SNP markers for two genetic lines of European bison: Lowland and Lowland–Caucasian. *Anim. Biodivers. Conserv.* **2017**, *40*, 17–25. [CrossRef]
39. Wojciechowska, M.; Puchała, K.; Nowak-Życzyńska, Z.; Perlińska-Teresiak, M.; Kloch, M.; Drobik-Czwarno, W.; Olech, W. From Wisent to the Lab and Back Again—A Complex SNP Set for Population Management as an Effective Tool in European Bison Conservation. *Diversity* **2023**, *15*, 116. [CrossRef]
40. Oleński, K.; Kamiński, S.; Tokarska, M.; Hering, D.M. Subset of SNPs for parental identification in European bison Lowland-Białowieża line (*Bison bonasus bonasus*). *Conserv. Genet. Resour.* **2018**, *10*, 73–78. [CrossRef]
41. Grzegrzółka, B.; Olech, W.; Krasiński, Z.A. Struktura genetyczna wolnych stad żubrów nizinnych w Polsce. *Park. Nar. I Rezerw. Przyr.* **2004**, *23*, 665–677. (In Polish)
42. Krasińska, M.; Krasiński, Z.A.; Bunevich, A.N. Spatial distribution of the European bison population in Polish and Belarussian parts of Białowieża Forest. In Proceedings of the Conference European Bison Conservation, Bialowieza, Poland, 30 September–2 October 2004; p. 73.

43. Barendse, W.; Armitage, S.M.; Kossarek, L.M.; Shalom, A.; Kirkpatrick, B.W.; Ryan, A.M.; Clayton, D.; Li, L.; Neibergs, H.L.; Zhang, N.; et al. A genetic linkage map of the bovine genome. *Nat. Genet.* **1994**, *6*, 227–235. [CrossRef]
44. Kaukinen, J.; Varvio, S.L. Eight polymorphic bovine microsatellites. *Anim. Genet.* **1993**, *24*, 148. [CrossRef]
45. FAO. *Global Project for the Maintenance of Domestic Animal Genetic Diversity (MoDAD)–Draft Project Formulation Report*; FAO: Rome, Italy, 1995.
46. Vaiman, D.; Mercier, D.; Moazami-Goudarzi, K.; Eggen, A.; Ciampolini, R.; Lépingle, A.; Velmala, R.; Kaukinen, J.; Varvio, S.L.; Martin, P.; et al. A set of 99 cattle microsatellites: Characterization, synteny mapping, and polymorphism. *Mamm. Genome* **1994**, *5*, 288–297. [CrossRef]
47. Mommens, G.; Coppieters, W.; Van de Weghe, A.; Van Zeveren, A.; Bouquet, Y. Dinucleotide repeat polymorphism at the bovine MM12E6 and MM8D3 loci. *Anim. Genet.* **1994**, *25*, 368. [CrossRef] [PubMed]
48. Bishop, M.D.; Kappes, S.M.; Keele, J.W.; Stone, R.T.; Sunden, S.L.; Hawkins, G.A.; Toldo, S.S.; Fries, R.; Grosz, M.D.; Yoo, J.; et al. A genetic linkage map for cattle. *Genetics* **1994**, *136*, 619–639. [CrossRef]
49. Toldo, S.S.; Fries, R.; Steffen, P.; Neibergs, H.L.; Barendse, W.; Womack, J.E.; Hetzel, D.J.; Stranzinger, G. Physically mapped, cosmid-derived microsatellite markers as anchor loci on bovine chromosomes. *Mamm. Genome* **1993**, *4*, 720–727. [CrossRef] [PubMed]
50. Steffen, P.; Eggen, A.; Dietz, A.B.; Womack, J.E.; Stranzinger, G.; Fries, R. Isolation and mapping of polymorphic microsatellites in cattle. *Anim. Genet.* **1993**, *24*, 121–124. [CrossRef] [PubMed]
51. Peakall, R.; Smouse, P.E. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research_an update. *Bioinformatics* **2012**, *28*, 2537–2539. [CrossRef]
52. RStudio Team 2022, RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA URL. Available online: http://www.rstudio.com/ (accessed on 2 March 2022).
53. Ligges, U.; Mächler, M. Scatterplot3d–an R Package for Visualizing Multivariate Data. *J. Stat. Softw.* **2003**, *8*, 1–20. [CrossRef]
54. Evanno, G.; Regnaut, S.; Goudet, J. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [CrossRef] [PubMed]
55. Earl, D.A.; von Holdt, B.M. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. *Gen. Res.* **2012**, *4*, 359–361. [CrossRef]
56. Kopelman, N.M.; Mayzel, J.; Jakobsson, M.; Rosenberg, N.A.; Mayrose, I. CLUMPAK: A program for identifying clustering modes and packaging population structure inferences across K. *Mol. Eco. Res.* **2015**, *15*, 1179–1191. [CrossRef]
57. Cronin, M.A.; MacNeil, M.D.; Leeburg, V.; Vu, N.; Blackburn, H.D.; Derr, J. Genetic variation and differentiation of bison (Bison bison)) subspecies' and cattle (*Bos taurus*) breeds and subspecies. *J. Hered.* **2013**, *104*, 500–509. [CrossRef]
58. Wilson, G.A.; Strobeck, C. The isolation and characterization of microsatellite loci in bison, and their usefulness in other artiodactyls. *Anim. Genet.* **1999**, *30*, 226–227. [CrossRef]