Animal Science Papers and Reports vol. 40 (2022) no. 2, 147-157
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of the Polish Academy of Sciences, Jastrzębiec, Poland

Analysis of polymorphism in 11 STR markers in the European red deer (*Cervus elaphus*) population in Poland*

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(Accepted March 3, 2022)

The aim of this study was to evaluate the levels of variability in selected 11 polymorphic STR markers specific to the red deer (*Cervus elaphus*) and their potential use as a tool for identification tests. Biological material was collected from 157 animals during periodic hunting carried out in Poland. Genotyping was conducting using the PCR multiplex method within the range of 11 markers, T115, T501, T156, T193, T108, T107, T172, T507, CSSM19, HAUT14, and CSSM66, with capillary electrophoresis for product detection. The obtained values of heterozygosity (H_O >0.8), probability of exclusion (PE >0.6), power of discrimination (PD >0.96) and random match probability (RMP <0.06) facilitated assessment of the utility of the marked *loci* for purposes of identification. Based on the DNA polymorphism in biological material derived from deer, the presented set of 11 markers provides assessment of the degree of inbreeding in the studied animal population, thus combating unfair trade in game and poaching, as well as providing expertise on behalf of the administration of justice.

KEYWORDS: forensic / forensic science / genetic variability / microsatellite DNA / wild animals

*Funding: Statutory activity – maintain the research capacity, Wroclaw Medical University, SUB.A122.19.017
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Currently, analysis of population variability in animals is of great interest, but it can be challenging due to methodological problems. Routine identification and pedigree tests were carried out for many years based mostly on blood group determination. However, these analyses were limited, since no other specimen except for blood could be used as a starting biological material, which among other things meant that they were not applied in individual studies concerning wild animals such as deer. Recently, the highly dynamic development of population genetics, involving the genetic variation of humans, animals, and plants alike, has been observed in recent years [Dębska 2013], Radko 2011a].

Informative genetic markers used for identification purposes are based on microsatellite genetic polymorphism such as STR (*short tandem repeats*) due to high diversity in a population, simple Mendelian inheritance and even distribution in the genome [Bal 2013]. The STR markers are multiple repetitions of short, most often four-nucleotide sequences, which are repeated in a tandem manner. The number of repetitions is specific to a particular individual. STRs are scattered throughout the genome, mainly in the non-coding segments [Bal 2013]. Moreover, STR genotyping facilitates multiplexing the reaction of amplification of genetic material, i.e. typing from several to several dozen markers at the same time, which leads to the determination of the genetic profile.

A DNA profile is specific and unique both in humans and animals [Dębska 2013], constituting a certain 'genetic fingerprint'. The potential for its implementation is related to the development and creation of new identification systems as well as the development of genetic databases for animals. Thus, familiarity with a genetic sequence is useful in assessing genetic variation, monitoring pedigrees and comparing populations.

Here we conducted a genetic analysis of biological material derived from the red deer (*Cervus elaphus*). Among the red deer species approximately 28 subspecies and numerous ecotypes can be distinguished. The red deer is an animal native to vast forest areas spanning several continents, including Europe, Asia, the Atlas Mountains in Africa, Australia, New Zealand, and North and South America. In Poland this animal inhabits all significant forest complexes; in addition, it is possible to define the so-called regional forms, such as the Carpathian, Masurian, Greater Poland, or Lublin deer [Radko 2011a].

Conducting this type of research is significant in many areas related to animal husbandry, species protection, as well as preventing and combating related crimes. An example of the latter may include illegal shooting or trade. The development of farm breeding of deer, and thus the appearance of products from these farms on the market is synonymous with the phenomenon of 'counterfeiting' of these products, e.g. through selling farmed meat as game [Kim et al. 2020]. In addition, the skin, tissue, antlers and male sexual organs are obtained illegally from red deer. The latter are widely applied in traditional Asian medicine [Dębska 2013]. Hence, the DNA analysis can serve as an efficient tool in combating crime, especially in cases when

identification based on the morphology of the species is difficult or nearly impossible [Dębska 2013].

Such great interest in the genetic diversity of both wild and farmed deer has resulted in numerous studies concerning the genetic variability of this species based on the analysis of genetic markers. The dynamic development of molecular biology techniques, as manifested in the automation of many procedures and the availability of specialised computer software, has revolutionised all areas of biology, including population studies [Dębska 2013]. DNA analysis, which enables direct assessment of differences and changes taking place within a studied group of individuals, has become a widely-used tool to determine genetic variability of different species, breeds and populations. Since the early 1990s, a particular application for STR microsatellite markers has been found in the study of genetic diversity in humans and animals [Radko 2011a].

The main aim of this study was to assess the degree of diversification in selected STR markers and to evaluate their utility for identification studies. Moreover, another objective of this study was also to develop a system enabling individual genetic identification of animal biological traces based on genotyping using the PCR technique. The results of this study will also facilitate collection of information concerning the genetic structure of red deer as well as the use DNA polymorphism analysis data in reports required by law enforcement agencies or hunting clubs.

Material and methods

Selection of STR markers

The first stage of the study focused on the isolation of microsatellite markers in order to establish a panel of polymorphic markers of the STR type, which were then used to determine the genetic variability of the deer population in Poland. For this purpose, biological material was collected from a dozen individuals from the deer family and the Old World deer subfamily. The material was obtained in the course of periodic hunting by hunters in order to control the population of forest game in the forests of the Lower Silesia region. At this stage 11 markers of satisfactory heterozygosity were selected [Jones *et al.* 2002].

Biological material and isolation of DNA

Thanks to the courtesy and involvement of the District Board of the Polish Hunting Association in Wrocław, the study group was expanded to 157 individuals of the deer species. Samples of biological material were obtained from 57 hunting districts in Poland. The research involved representatives of the red deer species, mainly from the forests of the West Pomeranian (49 samples) and Greater Poland (40) regions, as well as the Mazovian, Lower Silesian, Pomeranian, and Łódź (between 13 and 18 individuals from each) Voivodeships. DNA was isolated using the QIAamp DNA Mini Kit (QIAGEN).

Multiplex PCR

The selection of primers was preceded by the identification of microsatellite regions characterised by sufficient polymorphism and levels of heterozygosity, based on available literature data [Jones *et al.* 2002, Radko *et al.* 2014b]. From among many microsatellite markers of the STR type, 11 *loci*, characterised by four- and two-nucleotide repetitive units, were selected. In the selection of primer pairs, particular attention was paid to ensuring that their melting points were as close as possible. In order to avoiespectivenion, the primers were verified for complementarity. Genotyping

Table 1. Characterization of 11 STR markers with the sequences of primers used for typing [Jones *et al.* 2002]

Locus name	GenBank number	Repetitive unit	Forward/Reverse PCR primers	Dye	Conc. (uM)	PLEX	Allele size / ranges (bp)
T156	AF192396	*TAGA*	forward 5' – TCTTCCTGACCTGTGTCTTG – 3' reverse 5' – GATGAATACCCAGTCTTGTCTG – 3'	TET	0.8	A	130-194
T115	AF193021	*TAGA*	forward 5' – AATGTCTGACTCTAGGTGAGTG – 3' reverse 5' – TTTGCTATCTGAGCCACTAG – 3'	FAM	4	A	160-212
T193	AF192398	*TAGA*	forward 5' – AGTCCAAGCCTGCTAAATAA – 3' reverse 5' – CTGCTGTTGTCATCATTACC – 3'	HEX	1.2	A	169-233
T501	AF442815	*TAGA*	forward 5' – CTCCTCATTATTACCCTGTGAA – 3' reverse 5' – ACATGCTTTGACCAAGACC – 3'	FAM	1.2	A	228-268
T507	AF442816	*TAGA*	forward 5' – AGGCAGATGCTTCACCATC - 3' reverse 5' – TGTGGAGCACCTCACACAT – 3'	HEX	0.8	B	139-195
T108	AF191798	*TAGA*	forward 5' – CATGTGGAGATAGGTAGACAGA – 3' reverse 5' – CCATTCTGAGTAGCTGATTCA – 3'	FAM	2	B	131-175
T172	AF192397	*TAGA*	forward 5' – AGCATCTCCCCTTTCAACA – 3' reverse 5' – CTTCCCAACCCAAGTATCG – 3'	TET	0.6	B	157-209
T107	AF193019	*TAGA*	forward 5' - ACATCCGTTCAGGTGTGA – 3' reverse 5' – CCAGAGGTAAGATAAATGGTGA – 3'	FAM	1	B	231-243
HAUT14	AF236378	*GT*	forward 5' – CCAGGGAAGATGAAGTGACC – 3' reverse 5' – TGACCTTCACTCATGTTATTAA – 3'	TET	0.8	C	104-138
CSSM19	AF232761	*GT*	forward 5' – TTGTCAGCAACTTCTTGTATCTTT – 3' reverse 5' – TGTTTTAAGCCACCCAATTATTTG – 3'	FAM	1.2	C	131-163
CSSM66	AF232764	*GT*	forward 5' – AATTTAATGCACTGAGGAGCTTGG – 3' reverse 5' – ACACAAATCCTTTCTGCCAGCTTGA – 3'	TET	1.2	C	163-195

was divided into three multiplex responses. MIX A and MIX B were tetraplex, whereas MIX C included the amplification of three markers. A QIAGEN Multiplex PCR Kit (QIAGEN), along with forward and reverse primers at appropriate concentrations, was used to prepare the PCR reaction mixture. Forward primers were labelled with fluorescent dyes (FAM, HEX, and TET) to ensure visualisation and correct reading of the results. The characteristics of selected microsatellite regions are listed in Table 1.

A multiplex amplification reaction was carried out on a single sample with a total volume of 10 µl. The reaction mixture was prepared with 1 µl of the primer mix, 5 µl of QIAGEN Multiplex PCR Master Mix, 2 µl of DNA template and 2 µl of deionised water. PCR was performed using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) according to the following thermal profile: initial denaturation for 15 min. at 95ºC, 32 cycles involving denaturation for 30 s at 94ºC, connecting primers for 90 s at 57ºC, primer extension for 60 s at 72ºC and final extension for 10 min. at 72ºC.

A negative control was included with each PCR run, ruling out potential contamination and the formation of non-specific amplification products. Moreover, the specificity of the test was verified using human genetic material.

Capillary electrophoresis

Analysis of the amplification product was conducted using capillary electrophoresis with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The GeneScan 500 TAMRA Size Standard (ThermoFisher) was applied during electrophoretic separation, enabling identification of the analysed DNA fragments within the range 35–500 bp. The lengths of fragments of individual STR markers were determined using the GeneMapper ID v 3.2.1 software (Life Technologies).

Statistical analysis

The number and frequency of alleles were determined using individual statistical analysis for each marker. The compliance of the studied population to the assumptions of the Hardy-Weinberg equilibrium was examined [Lauretto *et al.* 2009]. Based on allele frequencies, the following parameters were calculated for each analysed marker using the Microsoft Office Excel 2007 program: power of discrimination (PD), random match probability (RMP), observed (H_O) and expected (H_E) heterozygosity, the inbreeding coefficient F_{is}, the index of the degree of polymorphism (PIC *Polymorphism Information Content)* and the exclusion probability PE [Houston 1998, Nagy *et al.* 2012, Oliveira *et al.* 2006].

Results and discussion

Studies carried out on a group of 157 individuals covering 11 polymorphic STR-type markers revealed the presence of a total of 155 alleles, found at various frequencies. The frequencies of alleles are presented in Table 2. The largest number

of alleles was observed for marker T193 (19 alleles), while it was smallest for marker T107 (4 alleles). Other reports describing population studies involving red deer confirmed a low degree of polymorphism for the T107 marker, whereas the T115 system was represented by a much greater number (14) of alleles for the population in our study compared to deer in California [Meredith *et al.* 2005]. The numbers of alleles identified in the *loci* T156, T193, T501, T108, T172, and T507 are comparable to those recorded in other published reports and may determine the extent of genetic variation in the studied population [Radko *et al.* 2014b, Szabolcsi *et al.* 2014]. In the case of marker T108, 12 alleles were identified, 16 alleles in the case of T172, while for T176 it was 16 alleles. Szabolcsi et al. [2014] obtained similar numbers of alleles for these type of markers: 10, 13, and 16 for T08, T172, and T15espectivetively.

The first stage of our statistical analysis revealed the compliance between the observed and expected distributions of genotypes in the studied population, which was demonstrated using the Hardy-Weinberg equilibrium (HWE, Table 3). We observed that the HWE was maintained in the case of 5 markers: T115, T507, T108, T107, and HAUT14. In six systems the obtained probability value was below the significance level of 0.05: T156 (p = 0.0004), T193 (p = <0.0001), T501 (p = 0.0091), T172 (p = <0.0001), CSSM19 (p = <0.0001), and CSSM66 (p = <0.0001). After adjustment of the significance level for multiple testing with the Bonferroni correction (p>0.05 ÷ 11 = 0.0045), the HWE equilibrium could

Table 2. The frequencies of the alleles for the 11 analysed STR markers in the group of 157 representatives of the red deer

Locus T156			*Locus T115*			*Locus T193*			*Locus T501*			*Locus T507*			*Locus T108*			*Locus T172*			*Locus T107*			*Locus Haut14*			*Locus CSSM19*			*Locus CSSM66*		
A[1] (bp.)	n	F[2]	A[1] (bp.)	n	F[2]	A[1] (bp.)	n	F[2]	A[1] (bp.)	n	F2	A[1] (bp.)	n	F[2]	A[1] (bp.)	n	F[2]	A[1] (bp.)	n	F[2]	A[1] (bp.)	n	F[2]	A[1] (bp.)	n	F[2]	A[1] (bp.)	n	F[2]	A[1] (bp.)	n	F[2]
130	23	0.0732	160	6	0.0193	169	2	0.0063	228	13	0.0414	139	70	0.2229	131	28	0.0891	157	4	0.0127	231	8	0.0254	104	40	0.1273	131	9	0.0286	163	2	0.0063
138	31	0.0987	164	5	0.0159	173	1	0.0031	232	101	0.3216	143	84	0.2675	135	12	0.0382	161	11	0.0350	235	60	0.1910	106	36	0.1146	139	1	0.0031	165	10	0.0318
142	28	0.0891	168	24	0.0764	181	7	0.0222	236	33	0.1050	147	12	0.0382	139	27	0.0859	165	31	0.0987	239	192	0.6114	108	1	0.0031	141	2	0.0063	167	3	0.0095
146	69	0.2197	169	1	0.0031	185	11	0.0350	240	28	0.0891	151	29	0.0923	143	40	0.1273	169	1	0.0031	243	54	0.1719	112	2	0.0063	143	28	0.0891	169	5	0.0159
150	2	0.0063	172	7	0.0222	189	11	0.0350	244	69	0.2197	155	27	0.0859	147	17	0.0541	173	34	0.1082				114	8	0.0254	145	50	0.1592	171	38	0.1210
154	5	0.0159	176	35	0.1114	192	1	0.0031	248	6	0.0191	156	1	0.0031	151	123	0.3917	177	8	0.0254				116	10	0.0318	147	21	0.0668	173	27	0.0859
158	13	0.0414	180	33	0.1050	193	55	0.1751	252	12	0.0382	159	2	0.0063	155	52	0.1656	181	33	0.1050				118	9	0.0286	149	17	0.0541	175	6	0.0191
162	51	0.1624	184	26	0.0828	197	11	0.0350	256	18	0.0573	163	2	0.0063	159	1	0.0031	185	38	0.1210				120	7	0.0222	151	14	0.0445	177	29	0.0923
164	4	0.0127	188	74	0.2356	201	53	0.1687	260	29	0.0923	167	20	0.0636	163	1	0.0031	189	34	0.1082				122	26	0.0828	153	7	0.0222	179	43	0.1369
166	15	0.0477	192	10	0.0318	205	29	0.0923	264	4	0.0127	171	13	0.0414	167	9	0.0286	191	1	0.0031				124	99	0.3152	155	55	0.1751	181	41	0.1305
170	39	0.1242	196	11	0.0350	208	1	0.0031	268	1	0.0031	175	2	0.0063	171	2	0.0063	193	19	0.0605				126	17	0.0541	157	10	0.0318	183	54	0.1719
174	21	0.0668	200	57	0.1815	209	60	0.1910				179	14	0.0445	175	2	0.0063	197	52	0.1656				128	10	0.0318	159	40	0.1273	185	37	0.1178
178	10	0.0318	208	24	0.0764	213	30	0.0955				183	5	0.0159				201	13	0.0414				130	23	0.0732	161	25	0.0796	187	10	0.0318
182	1	0.0031	212	1	0.0031	217	6	0.0191				191	29	0.0923				205	19	0.0605				132	3	0.0095	163	35	0.1114	189	4	0.0127
186	1	0.0031				221	10	0.0318				195	4	0.0127				208	1	0.0031				134	13	0.0414				191	2	0.0063
194	1	0.0031				225	3	0.0095										209	15	0.0477				136	9	0.0286				193	1	0.0031
						227	1	0.0031																138	1	0.0031				195	2	0.0063
						229	16	0.0509																								
						233	6	0.0191																								

[1]Allele; [2]Frequency.

be demonstrated for marker T501 (p = 0.0091); however, the distribution observed in the remaining 5 *loci* still differed in a statistically significant manner from the expected distribution [Bland *et al.* 1995]. In the case of markers CSSM19 and CSSM66, data in the literature on the subject confirm that the Hardy-Weinberg equilibrium was not maintained in some deer populations [Zachos *et al.* 2016]. These results indicate a failure to meet the basic condition of HWE, which is a random mating of parental pairs. For red deer this may be related to the biology of the species (harems and dominant males), as well as ecological fragmentation of habitats and the resulting inbreeding. The significant limitation of the deer territory range, including fencing confinement, road construction and industrial infrastructure, may significantly contribute to the inbreeding of wild animals. Our results may implicate a necessity of changing the approach to industrial development or road construction planning, in order to provide appropriate corridors for the free and safe movement of wild animals. What is encouraging, recent planning took into account the creation of appropriate routes or corridors enabling safe movement of animals between various territories to avoid the effect of inbreeding. However, years of neglecting wildlife and ensuring its free and safe migration have significantly reduced the population's gene pool.

Based on the marked allele frequency , additional indices were calculated to evaluate usefulness of the analysed microsatellite markers in identification tests. The results of these analyses are presented in Table 3. These parameters include the degrees of expected heterozygosity (H_E) and observed heterozygosity (H_O); values close to 1 indicate the highest level of utility for genetic analyses [Rogalska-Niźnik *et al.* 2011]. The degrees of expected (H_E) and observed (H_O) heterozygosity show similar values for the markers tested in this study. For the T108 marker, the levels of expected and observed heterozygosity are nearly identical: 0.78. Observed heterozygosity H_O of the other *loci* was lower in relation to the calculated values of expected heterozygosity H_E. Their difference was greatest for the CSSM66 marker, amounting to 0.25. The largest heterozygosity was observed in the case of the HAUT14 marker (H_O = 0.828), and the lowest in the case of CSSM66 (H_O = 0.6369). Marker T107 was excluded

Table 3. Statistical parameters for all analysed *loci*

Marker STR/*locus* name	H_O	H_E	PIC	PE	F_{IS}	RMP	PD	P-value for HWE
T156	0.81	0.88	0.8652	0.6157	0.0775	0.0305	0.9694	0.0004
T115	0.81	0.87	0.8528	0.6157	0.0660	0.0358	0.9642	0.2523
T193	0.67	0.88	0.8693	0.3909	0.2336	0.0362	0.9638	0.0000
T501	0.80	0.81	0.7923	0.5920	0.0216	0.0692	0.9307	0.0061
T507	0.78	0.84	0.8281	0.5687	0.0723	0.0430	0.9569	0.4434
T108	0.78	0.78	0.7595	0.5687	-0.0015	0.0729	0.9270	0.9473
T172	0.68	0.90	0.8918	0.4003	0.2429	0.0283	0.9717	0.0000
T107	0.55	0.56	0.5072	0.2394	0.0093	0.2503	0.7496	0.5013
HAUT14	0.83	0.85	0.8371	0.6520	0.0251	0.0387	0.9613	0.2898
CSSM19	0.80	0.89	0.8791	0.5920	0.1047	0.0297	0.9703	0.0000
CSSM66	0.64	0.89	0.8765	0.3376	0.2820	0.0328	0.9671	0.0000

H_o – observed heterozygosity; H_E – expected heterozygosity; PIC – polymorphism information content; PE – probability of exclusion; F_{IS} – inbred, fixation index; RMP – random match probability; PD – power of discrimination.

from the comparison of the degree of heterozygosity, as an insufficient number of alleles represented this marker in the studied population.

The inbreeding coefficient (F_{IS}) determining the degree of inbreeding was estimated from the proportion of the degree of observed heterozygosity expected one. For most of the analysed markers, values greater than 0 were obtained for F_{IS}, demonstrating the presence of an excess of homozygotes in the studied population and possibly indicating an inbreeding effect. Only for one marker, T108, this coefficient was lower than 0.

In order to evaluate the informativeness of individual microsatellite systems, the so-called polymorphism index , for which PIC >0.5 is the recommended value, was employed [Radko 2011a]. The higher the value of the PIC coefficient for a given marker, the greater its informativeness and usefulness for genetic analyses. PIC >0.5 was found for all the analysed markers, with the lowest value observed for the T107 *locus*, for which PIC = 0.507. For the other systems, values were obtained within the range of 0.759-0.892. The analysed indices of polymorphism show very comparable values for a Hungarian red deer population and for a population of deer from south-eastern Poland [Radko *et al.* 2014b, Szabolcsi *et al.* 2014]. The PIC values smaller than the heterozygosity values may indicate that the studied population sample includes a group of unrelated individuals [Szabolcsi *et al.* 2014]. We found that only in the case of markers T501, T108, and T107 the estimated PIC values met this criterion when compared to the corresponding observed heterozygosity.

Another parameter estimated for individual markers, determining their utility for pedigree research, was provided by the probability of exclusion (PE). The PE values obtained for most of the analysed systems fell within the range of 0.338-0.652, which confirmed their utility in the verification of origin. An exception was found for marker T107, for which PE was 0.239. Based on published reports it can be stated that in terms of systems also marked in our research (i.e. T108, T172, T501, T193, T507, and T156), the PE values were found to be higher in the Hungarian red deer population than those for deer from Poland [Szabolcsi *et al.* 2014]. The combined probability of exclusion (CPE) for the analysed set of 11 loci reached a value of 0.998, which is consistent with literature data, which indicates that CPE for a set of 12 STR markers analysed in 20 cattle breeds for pedigree control reached values in the range of 0.9135–0.9961 (genotype of one of the parents was known) and 0.9935–0.9999 (referred to the determined genotype of both parents) [Radko 2011a, Rogalska-Niźnik *et al.* 2011].

An essential parameter in statistical analysis is the power of discrimination (PD), a measure which directly determines the utility of a given *locus* for the purpose of identification. For each of the investigated STR markers, the PD value was above 0.9, except for the T107 system, for which PD was 0.749. Estimated values remained at the level of PD values for systems recommended by ISAG (International Society for Animal Genetics) for the control of cattle pedigrees [Radko 2011a]. Knowing the PD values for individual markers, the so-called cumulative power of discrimination

(PDc) was calculated for the entire set of 11 STR systems; the resulting PDc was 0.9999, which means that the DNA profile evaluated using this kit will appear in the population in one individual per 4.31 × 1014.

The value of identifiers using genetic polymorphic markers is determined by the probability of random match (RMP), which indicates the chance that a randomly selected unrelated individual has the same DNA profile as that obtained from the evidence and determines the frequency of the profile in the population. In this study the smallest probability of a random match (RMP) observed for marker T172 was 0.028, while the greatest was found for the T107 marker, for which RMP = 0.25. The combined random match probability value for all the markers was 2.29×10^{-15}. For example, for a set of 10 STR markers used to identify human genetic material the total RMP value was 5.4×10^{-13}; for a set including the typing of 21 microsatellite arrays RMP was reduced to values ranging from 1.23 to 3.0×10^{-25} [Martinez-Cortéz *et al.* 2019, Rogalska-Niźnik *et al.* 2011].

The statistical analysis showed the highest rates of genetic variation for the three *loci* T156, T115, and HAUT14, including heterozygosity (H_O >0.8), power of discrimination (PD >0.96) and power of exclusion (PE >0.6), with the simultaneous maintenance of a low level of probability (approximately 0.03) for random agreement with RMP. These parameters can be indicative of the highest value of the evidence in terms of identification and population studies. Moreover, high values of heterozygosity (H_O >0.8) and power of discrimination (PD >0.9) were estimated for markers T501, T507, T108, and CSSM19. The lowest values of the index of the degree of polymorphism PIC, heterozygosity H_O and power of exclusion PE were observed in *locus* T107, as only four allelles, one of which characterised by an incidence of 0.611, were observed in this marker in the studied population. However, the presented study has some limitations, including the low number of studied individuals as well as analysed STR loci. Moreover, the distribution of some STR loci is inconsistent with the Hardy-Weinberg equilibrium. To achieve the good genetic panel with high PD some additional STR markers need to be added to the genetic panel that could be used for genetic and pedigree purposes, as well as forensic purposes.

Conclusions

Analysis of the polymorphism in a set of 11 STR markers in a Polish red deer population showed a high degree of applicability for identification tests. The obtained values for the index of degree of polymorphism , probability of exclusion , power of discrimination, and random match probability indicate the corresponding informativeness of individual *loci* and their utility for genetic and pedigree analyses, individual identification and verification of origin. The only exception is marker T107, for which the statistical parameters show their lowest values. According to ISFG (International Society for Forensic Genetics) recommendations regulating animal DNA genotyping in criminal cases, after validation tests assessing main diagnostic

parameters such as sensitivity, specificity, reproducibility, and mixed sample analysis, the set of 11 markers selected for red deer would enable the rendering of expert opinions commissioned by courts and associations dealing with the protection of animal rights [FSI: Genetics 2011, Sacks *et al.* 2016]. An additional advantage of the developed method is connected with its potential for genotyping biological traces such as tissue fragments, bloodstains, hair or other microtraces, which may contribute to the fight against crimes associated with theft or poaching.

Conflict of interest: None declared.

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