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Original article

# SNP panel for the evaluation of genetic diversity and relatedness in red deer (*Cervus elaphus*)

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## Abstract

Blood samples from 385 red deer (*Cervus elaphus*) acquired during officially approved hunting in different hunting divisions throughout Poland were used to isolate the genomic DNA. All individuals were genotyped by Bovine BeadChip (Illumina) for 54,174 Single Nucleotide Polymorphism (SNP) markers. SNPs of inappropriate clusters, with a marker call rate lower than 95% and with a Minor Allele Frequency (MAF) lower than 0.01, located on sex chromosomes and mitochondrial DNA, were removed. In total, 12,146 SNP markers were included for further analysis. Observed and expected heterozygosity amounted to 0.025 and 0.035, respectively. Among 12,146 markers, a panel of 142 SNPs were selected for relatedness analysis. The selected SNPs were unlinked and had a MAF higher than 0.2. This set of SNPs showed a probability of parentage exclusion of  $1.42 \times 10^{-6}$  and  $9.91 \times 10^{-19}$  for one and two known parents, respectively. The probability of identity was estimated at  $6.84 \times 10^{-53}$ . The probabilities obtained in this study are sufficient for the monitoring and effective management of the genetic diversity of red deer in Poland and are a cost-effective complementary tool for forensic applications.

**Keywords:** *Cervus elaphus*, red deer, genetic diversity, relatedness, SNP marker



## Introduction

Red deer is both a wild animal and half-domesticated farm-bred livestock for venison and velvet antler products and is becoming recognized as a model animal for bone, osteoporosis and regeneration research as well as for population and evolutionary studies (Bana et al. 2018). Evaluation of genetic diversity, parentage testing and animal identification are essential for the protection and efficient management of animal populations (Nicholas 2010). Moreover, assessing the relatedness between individuals is necessary to estimate the effective population size, reduce the inbreeding level and minimize mating between close relatives (Werner et al. 2004). For red deer, Short Tandem Repeats markers (STRs) were developed first by Haanes et al. (2005), then by Zsolnai et al. (2009) and finally DeerPlex I-II was developed and used for genetic diversity evaluation by Szabolcsi et al. (2014). Lower mutation and genotyping error rates, automatization of genotyping, and ease of data manipulation and calculation have caused panels of Single Nucleotide Polymorphisms (SNPs) to start displacing STRs in many domestic animals over the past 20 years (Heaton et al. 2002; Werner et al. 2004). The shift from STR to SNP markers in red deer research has been recently discussed by Perez-Gonzalez et al. (2023), who concluded that SNPs show greater precision in inferring genetic diversity and encouraged scientists and wildlife managers to prioritize their use whenever possible. This type of genetic tool for popular wild species is, however, often unavailable because of the lack of verified SNP data that is necessary for application in commercial microarrays. This problem also refers to red deer, although the genome of this species has already been assembled (mCerElal.1) (BioProject PRJEB45838). To overcome this limitation, a microarray of SNPs from related species can be used. Following this idea, a bovine SNP array was successfully used to study genetic variability in the bison (Pertoldi et al. 2010), dromedary (Bertoldini et al. 2017), alpaca (More et al. 2019) and roe deer (Oleński et al. 2023). In the current study, the authors attempted to verify the usefulness of bovine SNP markers to establish a SNP panel useful for diversity and forensic applications in red deer living in Poland.

## Materials and Methods

Blood samples from 385 red deer (*Cervus elaphus*) were collected *post-mortem* during officially approved hunting events in different hunting divisions throughout Poland between 2022 and 2025. All animals were bulls, 6-9 years old. Potential relatedness between the bulls

were not recorded in documents describing hunting events. Blood samples were kept in a freezer until the time of DNA isolation. Two hundred and fifty microliters of blood samples were used to isolate the genomic DNA using a Wizard Plus Megapreps DNA Purification System (Promega, Madison, USA). All individuals were genotyped using the Bovine BeadChip (Illumina) consisting of over 54,000 SNP markers. SNPs of inappropriate clusters were removed with a marker call rate lower than 95% and with a Minor Allele Frequency (MAF) lower than 0.01, deviating from the Hardy-Weinberg equilibrium ( $p < 0.001$ ) and located on sex chromosomes and mitochondrial DNA. Finally, 12,146 SNP markers were included for further analysis. SNPs selected for the relationship panel were located only in non-coding sequences, were not linked to each other, and their MAF was at least 0.2. For sex verification purposes, the final set of SNPs was supplemented by one SNP localized on the Y chromosome.

To convert the allelic frequencies into the probability of parentage exclusion, standard formulas were used (Jamieson and Taylor 1997):

For one parent:

$$PE_1 = 1 - 4 \sum_{i=1}^n p_i^2 + 2 \left( \sum_{i=1}^n p_i^2 \right)^2 + 4 \sum_{i=1}^n p_i^3 - 3 \sum_{i=1}^n p_i^4$$

For two parents:

$$PE_2 = 1 + 4 \sum_{i=1}^n p_i^4 - 4 \sum_{i=1}^n p_i^5 - 3 \sum_{i=1}^n p_i^6 - 8 \left( \sum_{i=1}^n p_i^2 \right)^2 + 8 \left( \sum_{i=1}^n p_i^2 \right) \left( \sum_{i=1}^n p_i^3 \right) + 2 \left( \sum_{i=1}^n p_i^3 \right)^2$$

The probabilities were calculated for each locus tested, where  $p_i$  is the frequency of allele  $i$ ,  $n$  – the number of alleles (two per SNP). The total exclusion power is calculated by combining all  $P$  values of the tested loci as follows:

$$PE = 1 - (1 - P_1)(1 - P_2)(1 - P_3) \dots (1 - P_k),$$

where:  $k$  is the number of loci used.

The probability of identity was calculated according to Waits et al. (2001):

$$PI = \prod_{i=1}^r \left( \sum_{j=1}^{n_i} p_i^4 + 4 \sum \sum p_i^2 p_j^2 \right),$$

where:  $p_i, p_j$  are the frequencies of the  $i$ -th and  $j$ -th allele,  $n$  is the number of alleles, and  $r$  is the number of the tested loci.

Both analyses were performed using the Golden Helix SVS7 analysis software v. 8.9.1 (Bozeman, MT, USA).

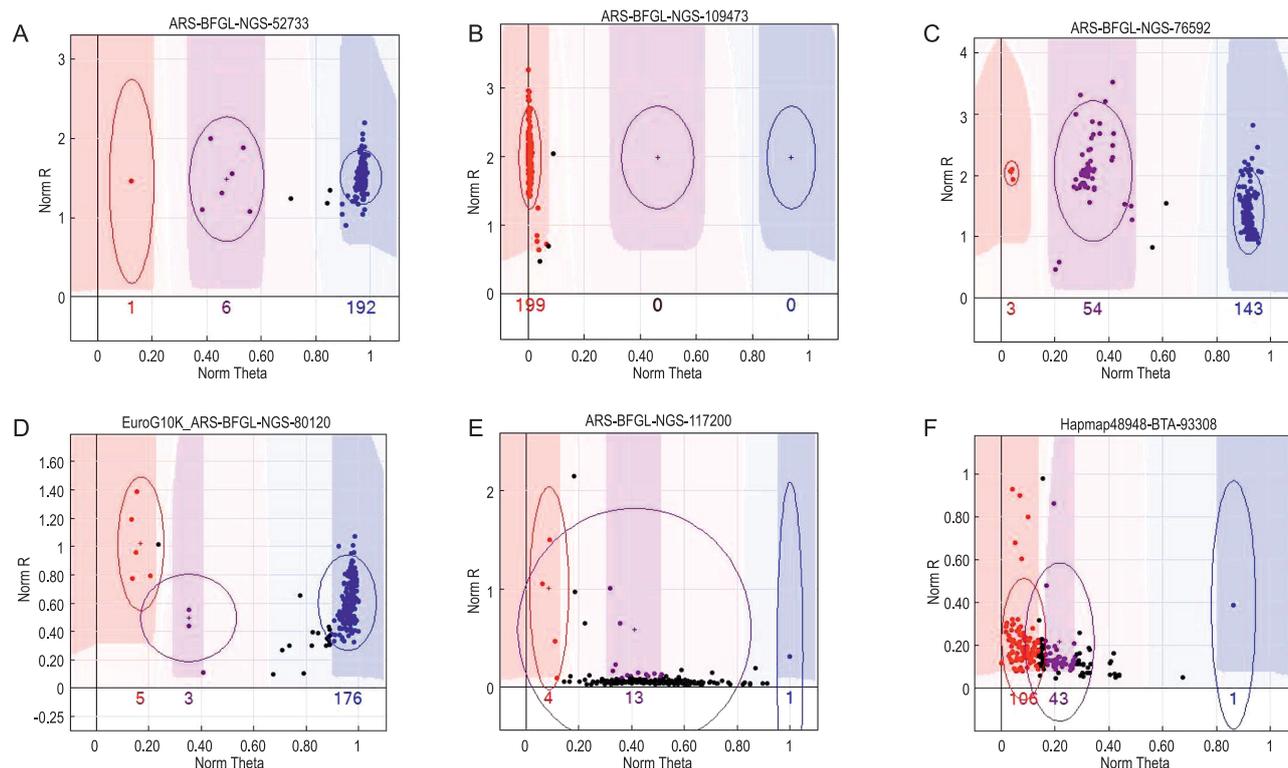


Fig. 1. Single nucleotide polymorphisms (SNPs) from the Bovine Genotyping BeadChip that cross-amplify in red deer. Genotypes are called for each sample (dot) by their signal intensity (Norm R, Y-axis) and allele frequency (Norm Theta, X-axis) relative to canonical cluster positions (dark shading) for a given SNP marker (red = AA, purple = AB, blue = BB). Dots positioned outside the shaded zone represent no call samples.

## Results

The current study enabled the extraction of 12,146 out of 54,000 SNP markers, which showed very good quality under selection criteria defined by a multi-step procedure in Illumina GenomeStudio software ensuring the correctness of the genotyping process. The quality of genotyping was verified by direct (visual) analysis of the SNP cluster image (Fig. 1). Analysis of chromosomal distribution of 12,146 SNP dataset showed that average SNP gap and SNP density were 202 kb and 204 kb, respectively. Average 404 SNPs/chromosome were evenly distributed across the whole genome (Fig. 2). Average MAF of 12,146 SNP dataset was 0.015. Observed and expected heterozygosity amounted to 0.025 and 0.022 respectively. Out of 12,146 a total of 142 SNPs were selected which presented the highest quality and a MAF equal to or higher than 0.20 (Table 1), and were proposed as a panel of SNPs useful in red deer genetic analysis. All selected SNP were checked for almost perfect alignments between the bovine and roe deer genome (data not shown) for the first 21 nucleotides flanking the variant nucleotide on either side. For 142 selected SNPs, the average MAF was 0.324.

The probability of parentage exclusion for the panel

of 142 SNPs varied from  $1.46 \times 10^{-6}$  and  $9.91 \times 10^{-19}$  for one and two known parents, respectively. The theoretical probability of identity was calculated as  $6.84 \times 10^{-53}$ .

Example of using the 142 SNP panel for relatedness analysis is shown in Table 2. Among 14 pairs of bulls, four pairs were duplicates (the same bulls genotyped twice) which showed PI value equal to 1.0. For remaining 10 pair of bulls, PI value was exact 0.5 or close to this value proving that bulls created the pair were father and son or semi-brothers (the same father, different mother). Bulls within one pair came from the same or neighbouring hunting division, which additionally confirmed their predicted relatedness. In pairwise IBD Estimates analysis of 364 pairs of brother-brother and father-son were found (data not shown), showing the potential of the SNP panel as a new tool revealing the relatedness between red deer in single hunting season and to monitor which families could be the subject of hunting more or less frequently.

## Discussion

SNP markers applied in Illumina bovine microarrays have been used worldwide in routine genotyping of cattle for over 16 years (Hayes et al. 2009).

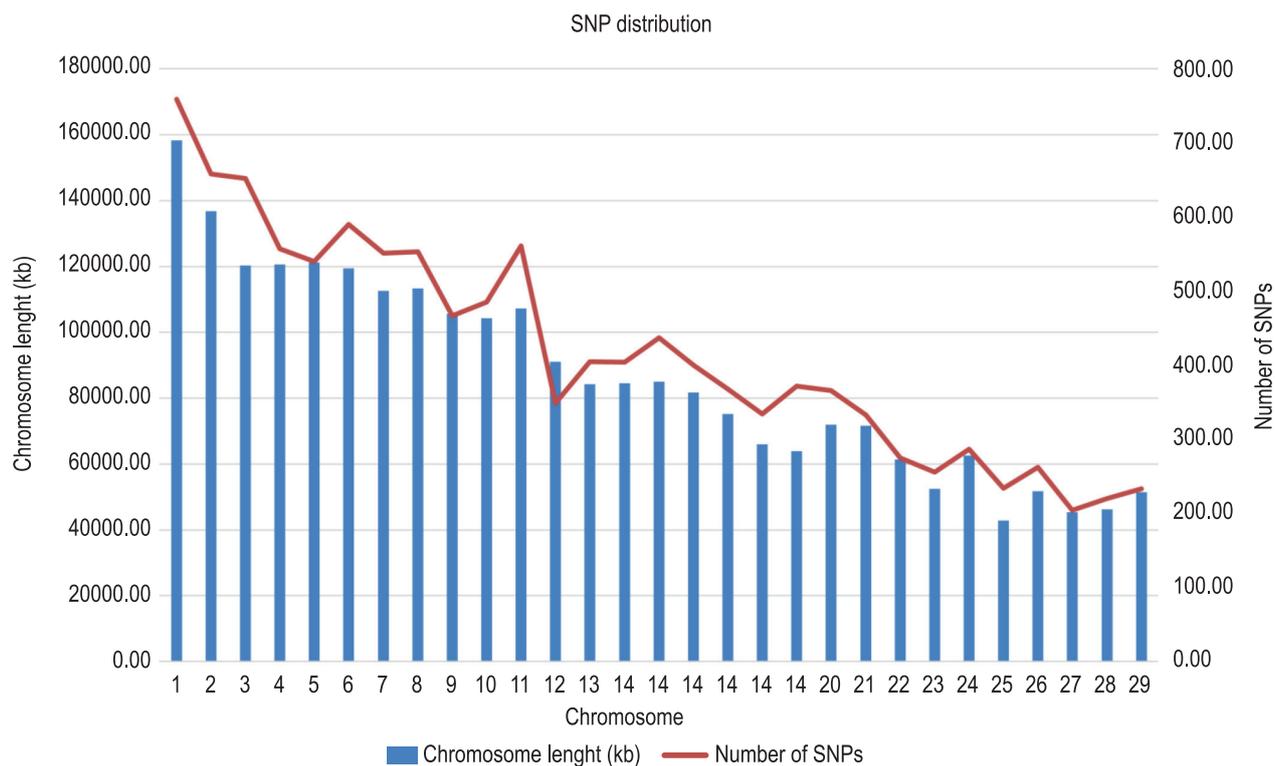


Fig. 2. Distribution of 12,146 filtered SNPs along the bovine chromosomes. They evenly represent each chromosome.

Table 1. List of 142 bovine Single Nucleotide Polymorphisms (SNPs) selected for relatedness analysis in red deer. MAF – minor allele frequency.

No	Bovine SNP Marker	MAF
1	EuroG10K_ARS-BFGL-NGS-116820	0.50
2	ARS-BFGL-NGS-119790	0.50
3	EuroG10K_Hapmap58219-rs29015952	0.50
4	ARS-BFGL-NGS-49789	0.49
5	SNP_1KG_7_16077315	0.49
6	EuroG10K_chr14_84289190	0.49
7	SNP_1KG_25_30565092	0.49
8	EuroG10K_ARS-BFGL-NGS-27367	0.49
9	ARS-BFGL-NGS-115756	0.49
10	ARS-BFGL-NGS-67542	0.49
11	BTB-01365157	0.49
12	BTB-01682090	0.48
13	BTA-98184-no-rs	0.48
14	ARS-BFGL-NGS-108037	0.48
15	BTB-00932019	0.48
16	Hapmap40539-BTA-44669	0.47
17	ARS-BFGL-NGS-68648	0.46
18	ARS-BFGL-NGS-92168	0.46
19	ARS-BFGL-NGS-41123	0.46
20	EuroG10K_ARS-BFGL-NGS-116820	0.46
21	ARS-BFGL-NGS-118826	0.45
22	Hapmap27696-BTC-045782	0.44
23	BTA-82821-no-rs	0.44
24	Hapmap49969-BTA-35641	0.43

cont. Table 1

No	Bovine SNP Marker	MAF
25	BTB-00771463	0.43
26	BTB-00774522	0.42
27	Hapmap36523-SCAFFOLD141316_865	0.42
28	BTB-01192051	0.42
29	ARS-BFGL-NGS-116036	0.42
30	ARS-BFGL-NGS-39378	0.42
31	ARS-BFGL-NGS-10880	0.42
32	BTA-122515-no-rs	0.42
33	ARS-BFGL-NGS-110400	0.41
34	ARS-BFGL-NGS-1162	0.41
35	BTB-01721539	0.41
36	Hapmap52612-rs29022803	0.40
37	ARS-BFGL-NGS-37699	0.40
38	ARS-BFGL-NGS-113507	0.40
39	BTB-00748932	0.39
40	ARS-BFGL-NGS-15927	0.39
41	Hapmap49544-BTA-24971	0.39
42	BTB-01285049	0.39
43	SNP_1KG_26_40761825	0.39
44	BTB-00998633	0.38
45	BTB-00043033	0.38
46	ARS-BFGL-NGS-62670	0.38
47	ARS-BFGL-NGS-5470	0.37
48	BTB-01084561	0.37
49	Hapmap26025-BTC-050671	0.36
50	UA-IFASA-1570	0.36
51	BTB-00213455	0.36
52	BTA-118887-no-rs	0.36
53	BTB-00501758	0.35
54	ARS-BFGL-NGS-63395	0.35
55	BTB-01224604	0.34
56	BTA-118779-no-rs	0.34
57	ARS-BFGL-NGS-100258	0.34
58	BTA-89348-no-rs	0.34
59	EuroG10K_ARS-BFGL-NGS-74920	0.34
60	ARS-BFGL-NGS-27702	0.33
61	ARS-BFGL-NGS-112168	0.33
62	BTB-01139563	0.33
63	BTB-00500486	0.33
64	BTB-01995313	0.32
65	ARS-BFGL-NGS-109852	0.32
66	BTA-110943-no-rs	0.32
67	ARS-BFGL-NGS-20442	0.32
68	UA-IFASA-1863	0.32
69	Hapmap38118-BTA-64240	0.32
70	ARS-BFGL-NGS-112919	0.32
71	EuroG10K_Hapmap53822-rs29010170	0.32

cont. Table 1

No	Bovine SNP Marker	MAF
72	ARS-BFGL-BAC-35051	0.32
73	EuroG10K_ARS-BFGL-NGS-43028	0.31
74	EuroG10K_BovineHD1500006297	0.31
75	Hapmap26077-BTC-067877	0.30
76	BTB-01518094	0.30
77	Hapmap47344-BTA-60858	0.30
78	ARS-BFGL-NGS-77933	0.29
79	ARS-BFGL-NGS-22254	0.29
80	ARS-BFGL-NGS-15033	0.29
81	ARS-BFGL-NGS-108743	0.29
82	ARS-BFGL-NGS-38536	0.29
83	ARS-BFGL-NGS-5408	0.29
84	Hapmap49521-BTA-19574	0.28
85	DB-1683-seq-rs42439709	0.28
86	EuroG10K_chr3_119037707	0.28
87	ARS-BFGL-NGS-119736	0.28
88	BTB-00848949	0.28
89	ARS-BFGL-NGS-118646	0.27
90	ARS-BFGL-NGS-107699	0.27
91	ARS-BFGL-NGS-68682	0.27
92	ARS-BFGL-NGS-115983	0.27
93	ARS-BFGL-NGS-85189	0.26
94	ARS-BFGL-NGS-23300	0.26
95	Hapmap42449-BTA-120321	0.26
96	ARS-BFGL-NGS-118532	0.26
97	BovineHD2000009506	0.26
98	ARS-BFGL-NGS-10055	0.25
99	BTA-21330-no-rs	0.25
100	ARS-BFGL-NGS-106198	0.25
101	BTB-01794883	0.25
102	ARS-BFGL-NGS-24692	0.25
103	ARS-BFGL-NGS-2382	0.25
104	Hapmap58341-rs29009898	0.25
105	Hapmap36382-SCAFFOLD210095_19074	0.24
106	ARS-BFGL-NGS-115275	0.24
107	EuroG10K_ARS-BFGL-NGS-88403	0.24
108	BTB-01249999	0.24
109	ARS-USMARC-Parent-DQ786757-rs29019900	0.24
110	BTA-74753-no-rs	0.24
111	ARS-BFGL-NGS-899	0.23
112	ARS-BFGL-NGS-9378	0.23
113	BTB-01383975	0.23
114	ARS-BFGL-NGS-116697	0.23
115	ARS-BFGL-NGS-115647	0.23
116	BTA-13329-rs29018291	0.23
117	BTB-00794279	0.23
118	ARS-USMARC-244	0.22

cont. Table 1

No	Bovine SNP Marker	MAF
119	BTB-00096498	0.22
120	BTB-00258746	0.22
121	BTB-00993221	0.22
122	ARS-BFGL-NGS-61681	0.22
123	EuroG10K_BTA-48736-no-rs	0.22
124	ARS-BFGL-NGS-68869	0.22
125	ARS-BFGL-NGS-100559	0.22
126	ARS-BFGL-NGS-20360	0.21
127	Hapmap60306-rs29023088	0.21
128	Hapmap51024-BTA-67203	0.21
129	ARS-BFGL-NGS-86536	0.21
130	Hapmap48446-BTA-70928	0.21
131	Hapmap60951-rs29020538	0.21
132	BovineHD2000006688	0.21
133	ARS-BFGL-NGS-73400	0.21
134	ARS-BFGL-NGS-23420	0.21
135	ARS-BFGL-NGS-6084	0.21
136	ARS-BFGL-BAC-2446	0.21
137	BovineHD0700007964	0.21
138	Hapmap49524-BTA-20495	0.21
139	ARS-BFGL-NGS-113043	0.20
140	ARS-BFGL-NGS-33177	0.20
141	BTB-01373902	0.20
142	Hapmap40907-BTA-121178	0.20

Table 2. Example of relatedness between 14 pair of bulls. Pair 1-4 are the same bulls genotyped twice. For pairs 5-14 the estimated PI indicate that there were pairs of fathers and sons or half-brothers. Hunting divisions in which red deer bulls were hunted are written in shortcuts.

Pair No	Bull 1	Bull 2	Estimated PI	Expected Estimated PI	$-\log_{10}$ P-value (Expected Estimated PI)	Hunting division	
						Bull 1	Bull 2
1	546B	539B	1.00	0.999	$2.93 \times 10^{-6}$	KUD	KUD
2	543B	540B	1.00	0.999	$8.81 \times 10^{-6}$	KUD	KUD
3	544B	541B	1.00	0.999	$1.46 \times 10^{-5}$	GOR	GOR
4	545B	542B	1.00	0.999	$2.05 \times 10^{-5}$	JED	JED
5	791B	799B	0.67	0.999	$6.75 \times 10^{-5}$	JED	JED
6	791B	795B	0.67	0.999	$7.34 \times 10^{-5}$	JED	JED
7	825B	610B	0.60	0.999	$9.69 \times 10^{-5}$	BAR	BAR
8	772B	774B	0.55	0.999	$1.49 \times 10^{-4}$	MIL	MIL
9	701B	595B	0.50	0.998	$7.43 \times 10^{-4}$	GOR	BAR
10	701B	608B	0.50	0.998	$7.14 \times 10^{-4}$	GOR	BAR
11	627B	667B	0.50	0.996	$1.65 \times 10^{-3}$	BAR	BAR
12	585B	670B	0.50	0.996	$1.71 \times 10^{-3}$	KUD	BAR
13	627B	670B	0.50	0.996	$1.64 \times 10^{-3}$	BAR	BAR
14	701B	832B	0.50	0.998	$8.14 \times 10^{-4}$	GOR	GOR

These markers are very well optimized and equipped with bioinformatic tools assessing the quality of genotyping (McClure et al. 2018). Cattle and red deer genomes have shown high similarity (Bana et al. 2018, Vozdova et al. 2021), especially in noncoding DNA stretches in which most of the Illumina SNP markers are located. Therefore, there is a high chance that SNP markers located in such sequences can be effectively identified in evolutionary-related species. The key factor in determining such markers is the quality of genotyping. In the current study, after applying standard protocols ensuring high quality of SNP clusters, 33.1% of bovine SNPs were evaluated as effective for reliable genotyping in red deer. Elblinger et al. (2022) achieved a slightly higher successful genotyping rate (38.6%), but they used bovine microarray with higher SNP density (777,000 SNPs). These authors selected a 63 SNP set based on genotypes obtained from 50 stags. The difference may come not only from different densities of microarrays but also from the fact that the current study used 385 stags, increasing the chance of achieving an SNP MAF higher than 0.2.

The panel of 142 selected SNPs guarantees an effective genetic analysis with a large margin because Weller et al. (2006) showed that a panel of just 25 SNP markers provides enough power for the identification of a single individual among any of five million individuals with less than a 1% chance for a match between them. Other authors indicate that panels of 40-100 SNPs with a MAF greater than 0.3 may allow accurate pedigree reconstruction (Baruch and Weller 2008, Fisher et al. 2009).

Successful use of bovine SNP markers for red deer SNP discovery has been described by Haynes and Latch (2012). They used an Illumina Bovine SNP50 BeadChip to identify polymorphic SNPs in cervids *Odocoileus hemionus* (mule deer and black-tailed deer) and *O. virginianus* (white-tailed deer). They found that 38.7% of markers could be genotyped, of which 5% ( $n=1068$ ) were polymorphic. A range of population genetic analyses have been implemented using these SNPs and a panel of 10 microsatellite loci. The three types of deer could readily be distinguished with both the SNP and microsatellite datasets.

There are other examples of using SNP microarrays in related species. Miller et al. (2011) identified 868 SNPs in bighorn (*Ovis canadensis*) and thinhorn sheep (*Ovis dalli*) using the Ovine SNP50 BeadChip developed for domestic sheep (*Ovis aries*). A further example is the study of Bertolini et al. (2017) in which the Bovine 777K SNP BeadChip and the Ovine 600K SNP BeadChip were used to extract 27,673 SNPs effective in the genotyping of the dromedary. These studies demonstrate that commercially developed SNP chips

are a viable means of SNP genotyping for non-model organisms, even when used between distantly related species.

Three microarrays with species-specific SNP markers for *Cervidae* have already been developed and used for deer breeding in New Zealand (Rowe et al. 2015), for identification of sika deer in China (Fan et al. 2021), to reveal the genomic architecture of body weight in red deer (Gauzere et al. 2023) and for evolutionary studies (Carranza et al. 2024). These SNP microarrays, however, were developed by custom design (for country-specific populations) and are not commercially available, which limits their use for other local populations in a cost-effective way.

The STR panel developed by Szablosci et al. (2014) and the SNP panel established by Elblinger et al. (2022) assessed the average probability of identity ( $PI_{ave}$ ) at the values of  $2.6736 \times 10^{-15}$  and  $4.9 \times 10^{-11}$ , respectively. The probability of identity obtained in the current study ( $1.8 \times 10^{-65}$ ) exceeds these values, showing the power of the developed SNP panel. This panel can be a complementary tool to the currently used 12 STR markers (BM1818, OarAE129, OarFCB5, OarFCB304, RM188, RT 1, RT 13, T26, T156, T193, T501, TGLA53). Conditions for simultaneous amplification (multiplex PCR) have been established and applied to evaluate the genetic structure and monitor changes in Poland's red deer population (Radko et al. 2012). Although there is no reasonable argument to suspect that the SNP panel developed in the current paper can be unreliable, we are aware that the 142 SNP panel should be validated by other method, e.g. by STR in the random blind trial. This kind of analysis would be an interesting challenge to be performed in separate work.

Validated SNPs can also be applied in linking evidence collected at a potential illegal hunting site with biological material (e.g. antlers, meat, trace blood samples) in cases of committing the offence (Poetsch et al. 2001). However, for forensic purposes, a validated DNA profiling system for red deer has been developed (Radko et al. 2012), and the SNP panel described in the current study can be used as an additional tool, especially in labs which have no facility for STR genotyping. Moreover, the overall cost of genotyping an animal by STR is much higher than by SNP since microarrays are capable of simultaneous genotyping of at least 48 animals. Therefore, the DNA profiling of red deer by bovine SNP microarray seems to be an attractive new approach for research and forensic applications in this wild species.

The panel of 142 SNPs can be used in population genetics analysis addressed to show the changes in gene pool and the fluctuations in genetic diversity within hunting division to avoid increase of inbreeding or loss

of genetic heterozygosity. The results of the current study can be applied to many fields of red deer biology since this species is an excellent model for evolutionary studies, biodiversity and social organization (Bartos and Bubenik 2011). SNP markers can also be used for the mapping of genes involved in the variation of antlers (Elblinger et al. 2022) and genes involved in meat quality and in developing traceability tests used in meat adulteration (Kaltenbrunner et al. 2018).

## Conclusion

Red deer (*Cervus elaphus*) can be genotyped using the Illumina Bovine 50K BeadChip at an acceptable call success rate and cost. After fine filtering and strict quality control, satisfactory number of SNP genotypes can be extracted for further reliable statistical analysis. The probabilities obtained for selected set of 142 SNP markers are sufficient for monitoring and effective management of the genetic diversity, relatedness, population genetics analysis and forensic applications of red deer.

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## Authors Declarations

### Ethics approval

Samples originated from the animals were collected according to 1/ Hunting Law Act of October 13, 1995 (Journal of Laws No. 147, item 713) with subsequent amendments: i.e., as of March 24, 2025 (Journal of Laws of 2025, item 539), 2/ Regulation of the Minister of the Environment of November 13, 2007, on annual hunting plans and multi-year hunting breeding plans (Journal of Laws, item 1646, 2013, item 95 and 2014, item 1900, as amended).

## Use of generative artificial intelligence

No tools for generative AI was used in the preparation of manuscript.

## Conflict of interest

The authors declare no conflicts of interest.

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