Microsatellite DNA Analysis of Genetic Diversity and Parentage Testing in the Popular Dog Breeds in Poland

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Abstract: There is growing concern that extreme breed standardization contributes to a reduction of the effective population size and high levels of inbreeding, resulting in the loss of genetic diversity in many breeds. This study examined genetic diversity among eight popular dog breeds in Poland and evaluated the effectiveness of a 21-microsatellite (STR) panel recommended by the International Society for Animal Genetics (ISAG) for parent verification. The following breeds were characterized: German Shepherd, Maltese, Irish Wolfhound, Yorkshire Terrier, Biewer Yorkshire Terrier, Golden Retriever, Labrador Retriever, and French Bulldog. STRUCTURE analysis showed breed distinctiveness among all the dog breeds under study. Reynold’s distance ranged between $\theta_w = 0.634$ and $\theta_w = 0.260$. The studied breeds showed a medium level of genetic differentiation; the mean number of alleles per locus ranged from 3.4 to 6.6, and the effective number of alleles from 2.1 to 3.5. The mean degree of heterozygosity varied from 49% to 69% and from 47% to 68% for $H_O$ and $H_E$, respectively. The population inbreeding coefficient ($F_{IS}$) indicated an absence of inbreeding in the studied breeds. The average polymorphism information content (PIC) values for most of the breeds were higher than 0.5. The cumulative power of discrimination (PD) for all the markers in all breeds reached high values (close to 1.0), while the probability of identity ($P_{ID}$) was low, ranging between $10^{-11}$ and $10^{-19}$. The cumulative exclusion probability when the genotypes of one ($P_{E1}$) and both parents ($P_{E2}$) are known showed that the parentage can be confirmed with a probability of 94.92% to 99.95% and 99.78% to 99.9999%, respectively.

Keywords: STR; domestic dog; biodiversity; individual identification; parentage

1. Introduction

The dog (*Canis familiaris*) is one of the first animals domesticated by man and has accompanied humans in their day-to-day life for thousands of years. Historically, dogs were used as working animals to herd livestock, hunt, and guard the home, and now they are also treated as companion animals [1–3]. Their extensive use is associated with the human selection of dogs for certain phenotypes. Strong selection for desirable traits often causes an irreversible loss of alleles and a reduction of genetic diversity, which may produce undesirable characteristics and health problems [3–5]. Around the world, more than 400 breeds of dogs are recognized [1]. The American Kennel Club (AKC) currently registers 197 dog breeds, and the Fédération Cynologique Internationale (FCI) officially recognizes 353 breeds officially [6,7]. Breeding to achieve specific breed standards can lead to a reduction in effective population size and result in increased levels of inbreeding within breeds, resulting in a loss of genetic diversity in many breeds [5,8]. Therefore, it is important to carry out selective breeding while maintaining breed purity and high biodiversity. To this end, the genetic structure of different dog breeds needs to be studied and genetic changes occurring in breeds have to be monitored.

Microsatellite (STR) markers are a well-known effective and powerful tool widely used to investigate the genetic structure and diversity of dog breeds [9–13]. They are also
the most important markers used for dog identification and parentage verification [9,14,15]. A panel of 21 markers recommended for parentage testing in domestic dogs by the International Society for Animal Genetics (ISAG) [16] is the panel standardized across multiple laboratories for canine genotyping and used for routine pedigree testing [14]. The analysis of polymorphism was carried out in the following breeds of dogs in Poland: Polish Hunting Dog [13] and Polish Greyhound [17] based on 21 STR recommended by ISAG, and in Borzoi and Tatra Shepherd Dog based on 18 STR [9,17]. No information is available regarding genetic variation among the most popular breeds of dogs in Poland, namely, German Shepherd, Yorkshire Terrier, Golden Retriever, Labrador Retriever, and French Bulldog [18].

In our study, the genetic analysis of these breeds was possible based on data collected as part of pedigree testing conducted at the National Research Institute of Animal Production (NRIAP). Additional analyses included Irish Wolfhound, Biewer Yorkshire Terrier, and Maltese, which are tested in fairly large numbers as part of parentage control. The objective of the study was to examine genetic variation within and among these dog breeds and to evaluate the effectiveness of an STR panel recommended for parentage testing.

2. Materials and Methods

The study was conducted based on the results of analysis of microsatellite polymorphism performed as part of canine pedigree testing at the National Research Institute of Animal Production in 2018–2020. A total of 903 samples were used from eight breeds, namely, German Shepherd (GS, n = 260), Maltese (M, n = 81), Irish Wolfhound (IW, n = 86), Biewer Yorkshire Terrier (BYT, n = 131), Yorkshire Terrier (YT, n = 77), Golden Retriever (GR, n = 48), Labrador Retriever (LR, n = 103), and French Bulldog (FB, n = 117). For all breeds, sampling was a selection of at least 70 unrelated animals (males and females) from different kennels, except for the GR breed for which 48 samples were collected.

This analysis uses the core panel of STR markers recommended by ISAG for individual identification and parentage analysis and a gender identification marker. The following microsatellite markers were used: AHTk211, CXX279, REN169O18, INU005, REN54P11, INRA21, AHT137, REN169D01, AHTh260, AHTk253, INU005, INU050, FH2848, AHT121, FH2054, REN162C04, AHTh171, REN247M23, AHTH130, REN105L03, REN64E19, and Amel locus. DNA was extracted from swabs and blood using the Sherlock AX Kit (A&A Biotechnology, Gdynia, Poland), following the manufacturer’s protocol. The extracts were quantified with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The STR loci were amplified using Phusion U Hot Start DNA Polymerase (Thermo Scientific, Wilmington, DE, USA), and the PCR reaction was performed on Veriti® Thermal Cycler amplifier (Applied Biosystems, Foster City, CA, USA), using the following thermal profile: 5 min of initial DNA denaturation at 98 °C, followed by 30 cycles of denaturation at 98 °C for 15 s, annealing at 58 °C for 75 s, elongation of starters at 72 °C for 30 s, and final elongation of starters at 72 °C for 5 min. Analysis of the obtained PCR products was performed using an ABI 3130xl capillary sequencer (Applied Biosystems, Foster City, CA, USA). The amplified DNA fragments were subjected to electrophoresis in 7% denaturing POP-7 polyacrylamide gel in the presence of a standard length of 500 Liz and a reference sample. The results of the electrophoretic separation were analyzed automatically using the GeneMapper® Software 4.0 (Applied Biosystems, Foster City, CA, USA).

Data Analysis

Population structure was analyzed using STRUCTURE software version 2.3.3 [19] considering an admixture model with correlated allele frequencies between breeds. The length of the burn in and Markov chain Monte Carlo (MCMC) simulations was 50,000 and 100,000, respectively, in 10 runs for each number of clusters (K) ranging between 4 and 10. The results were exported to STRUCTURE HARVESTER [20] for plotting the likelihood
membership coefficient (DeltaK) values so as to determine the most likely number of clusters. Genetic distance was analyzed using Reynolds’s distance—\(\theta_{w}\) [21].

\[
\theta_{w} = \sqrt{\frac{\sum_{l} \sum_{u} (X_u - Y_u)^2}{2 \sum_{l} (1 - \sum_{u} X_u - Y_u)}},
\]

where \(X_u, Y_u\) are allele frequencies from the first and second populations.

Based on this genetic distance, an unweighted pair-group method with averages (UPGMA) dendrograms were constructed to illustrate the similarities between the populations. Observed heterozygosity—\(H_O\), expected heterozygosity—\(H_E\), and inbreeding coefficient—\(F_{IS}\), were calculated according to Nei and Roychoudhury [22], and Wright [23]. The Hardy–Weinberg equilibrium (HWE) of the 21-STR loci was tested by exact test using an algorithm based on Markov Chain Monte Carlo methods [24]. The genetics parameters were calculated as follows:

1. Polymorphic information content—\(PIC\) [25],

\[
PD = 1 - \sum_{j=k}^{n} \sum_{k=1}^{n} p_{jk}^2
\]

\[
CPD = 1 - \prod_{i=1}^{n} (1 - PD_i),
\]

where \(p_{jk}\) is the allele frequency \(j,k\) for \(i\)-locus; \(CPD\) is the cumulative power of discrimination.

2. Power of discrimination—\(PD\) [26],

\[
CP_{ID} = \prod_{i=1}^{n} PD_{i},
\]

where \(p_i, p_j\) are allele frequencies \(i,j\); \(CP_{ID}\) is the cumulative probability of identity.

3. Probability of identity—\(P_{ID}\) [27],

\[
P_{ID} = \sum p_i^2 + \sum \sum (2p_ip_j)^2
\]

\[
CP_{ID} = \prod_{i=1}^{n} P_{ID},
\]

where \(p_i, p_j\) are allele frequencies \(i,j\); \(CP_{ID}\) is the cumulative probability of identity.

4. Probability of parentage exclusion for each locus, when the genotypes of one and both parents are known (\(PE_1\) and \(PE_2\)) and the cumulative probability of parentage exclusion (\(CPE\)) [28],

\[
PE_1 = \sum_{i=1}^{n} p_i^2 (1 - p_i)^2 + \sum_{i>j=1}^{n} 2p_ip_j (1 - p_i + p_j)^2
\]

\[
CPE_1 = 1 - \prod_{i=1}^{n} (1 - PE_{1i})
\]

\[
PE_2 = \sum_{i=1}^{n} P_i (1 - p)^2 - \sum_{i>j=1}^{n} (p_ip_j)^2 \left[4 - 3(p_i + p_j)\right]
\]

\[
CPE_2 = 1 - \prod_{i=1}^{n} (1 - PE_{2i}),
\]

where \(p_{jk}\) is allele frequency \(j,k\) for \(i\)-locus; \(CPE_1\), \(CPE_2\) are cumulative probabilities of identity.

The statistical analysis was performed using the IMGSTAT software, ver. 2.10.1 (2009), which supports the laboratory of the National Research Institute of Animal Production.
3. Results

A total of 185 alleles were detected at 21 STR loci across all breeds. The total number of alleles per locus ranged between 6 for AHTk211 and 12 for AHTk137 and REN169018.

3.1. Breed Relationships

The genetic population structure of each breed was determined based on the admixture level for each individual dog using the correlated allele frequencies model implemented within the STRUCTURE software. The results of Delta K indicated that the optimal number of genetic clusters representing most similar individuals for breeds was at \( K = 8 \) (Figure 1). The average proportion of assignment to the cluster above 90% was found for five breeds, i.e., GS (98%), GL (97%), IW and FB (96%), and M (93%). A lower assignment value was found in the BYT breed (88%) and the lowest in the LR and YT (85%).

![Figure 1](image_url)

**Figure 1.** Delta K values for STRUCTURE analysis of dog breeds obtained by the program Structure Harvester.

Reynold’s (1983) genetic distance between eight breeds is summarized in Table 1. The genetic distance was the greatest between GS and IW \( (\theta_w = 0.634) \), and the smallest between BYT and YT \( (\theta_w = 0.260) \). The UPGMA dendrogram revealed that the YT and BYT were grouped together, and the Irish Wolfhound breed was farthest away from others (Figure 2).

Table 1. Reynolds genetic distance \( (\theta_w) \) values of eight study dog breeds.

| Breed | FB | GR | LR | M | GS | IW | YT | BYT |
|-------|----|----|----|---|----|----|----|-----|
| BF    | 0.000 |    |    |   |    |    |    |     |
| GR    | 0.540 | 0.000 |    |   |    |    |    |     |
| LR    | 0.469 | 0.577 | 0.000 |   |    |    |    |     |
| M     | 0.409 | 0.501 | 0.459 | 0.000 |    |    |    |     |
| GS    | 0.519 | 0.596 | 0.549 | 0.459 | 0.000 |    |    |     |
| IW    | 0.574 | 0.606 | 0.588 | 0.539 | 0.634 | 0.000 |    |     |
| YT    | 0.430 | 0.503 | 0.465 | 0.364 | 0.484 | 0.500 | 0.000 |     |
| BYT   | 0.435 | 0.511 | 0.485 | 0.372 | 0.511 | 0.510 | 0.260 | 0.000 |
3.2. Diversity Analysis

The greatest number of alleles was noted for the breeds BYT (138 alleles) and M (135 alleles), and the smallest for IW (72 alleles). The mean number of alleles per locus for breeds ranged from 3.4 for IW to 6.6 for BYT, whereas the effective number from 2.1 for WI to 3.5 for M and YT (Table 2).

Table 2. The number of alleles identified per locus (N), mean number of alleles per locus (A), and mean effective number of alleles per locus (Ae) for each breed.

| Locus     | GS | M | IW | BYT | YT | GR | LR | FB | N  |
|-----------|----|---|----|-----|----|----|----|----|----|
|           | A  |    | A  | Ae  | A  | Ae | A  | Ae |    |
| AHT121    | 7  |    | 9  | 4.9 | 5  | 3.1| 9  | 4.5| 8  |
| AHT137    | 7  | 2.0| 6  | 4.5 | 2  | 1.2| 9  | 4.1| 7  |
| AHT1H171  | 7  | 2.7| 9  | 2.5 | 5  | 2.4| 5  | 1.6| 9  |
| AHT1H260  | 8  | 2.6| 6  | 4.0 | 3  | 1.6| 8  | 3.5| 6  |
| AHT1k111  | 4  | 2.8| 5  | 3.4 | 3  | 1.9| 6  | 2.9| 5  |
| AHT1k253  | 6  | 1.5| 5  | 2.5 | 3  | 1.9| 6  | 2.8| 5  |
| CXX227    | 5  | 2.8| 4  | 3.5 | 5  | 2.2| 8  | 3.5| 8  |
| FH2054     | 6  | 3.3| 8  | 5.3 | 5  | 2.4| 6  | 3.9| 6  |
| FH2848     | 6  | 2.2| 6  | 2.2 | 3  | 2.2| 7  | 3.1| 5  |
| INRA21     | 6  | 3.0| 5  | 3.8 | 4  | 2.9| 6  | 3.8| 6  |
| INU005     | 4  | 2.3| 5  | 1.5 | 3  | 2.4| 6  | 1.3| 8  |
| INU030     | 5  | 2.1| 6  | 2.6 | 2  | 1.7| 6  | 2.5| 7  |
| INU055     | 5  | 3.0| 5  | 3.8 | 4  | 1.7| 6  | 2.6| 6  |
| REN162C04  | 5  | 2.4| 6  | 1.9 | 3  | 2.1| 7  | 3.4| 7  |
| REN169D01  | 3  | 1.9| 8  | 3.7 | 2  | 1.1| 5  | 2.9| 6  |
| REN169O18  | 10 | 4.3| 5  | 2.4 | 4  | 2.1| 6  | 2.4| 5  |
| REN247M23  | 4  | 1.5| 5  | 3.3 | 3  | 2.2| 4  | 3.4| 4  |
| REN54P11   | 5  | 2.1| 8  | 5.4 | 3  | 1.9| 7  | 4.4| 6  |
| AHT1H130   | 7  | 3.8| 10 | 5.1 | 2  | 1.3| 8  | 3.5| 5  |
| REN105L03  | 8  | 2.3| 7  | 4.7 | 4  | 2.6| 7  | 3.9| 5  |
| REN64E19   | 7  | 1.2| 7  | 2.2 | 4  | 2.8| 6  | 3.2| 7  |

Out of the 185 alleles found within these breeds, 20 were breed specific (Table 3). The greatest number of private alleles was observed at the locus REN169O18 (four alleles). There were two alleles each at REN247M23 and REN105L03, and one allele each at 12
loci. No breed-specific alleles occurred at the remaining six loci—AHTk211, INU055, REN169D01, INU030, AHTh171, FH2054. The greatest number of private alleles was identified in the GS breed. Specific alleles for this breed occurred at six loci, yet in most cases with low frequencies (<0.09), only two alleles occurred with frequencies of 0.198 and 0.217 for 212 bp allele in REN162C04 and 158 bp in REN169O18, respectively. In FB, a higher frequency of 0.12 and 0.218 was noted for the 229 bp allele in REN105L03, and the 156 bp allele in REN169O18, respectively. In the YT and BYT breeds in CXX279, there was a 128 bp allele with a frequency exceeding 16% each, which did not occur in any of the other breeds. The other private alleles exhibited low frequency (less than 3%). No breed-specific alleles were found in the Irish wolfhound. The frequencies of the breed-specific alleles are presented in Table 3.

Table 3. Frequency of private alleles in the study breeds.

| Locus   | Allele (bp) | GS       | M       | BYT     | YT     | GR     | LR     | FB     |
|---------|-------------|----------|---------|---------|--------|--------|--------|--------|
| AHT121  | 92          | 0.031    |         |         |        |        |        |        |
| AHT137  | 127         |          | 0.0130  |         |        |        |        |        |
| AHT1260 | 256         |          |         | 0.0243  |        |        |        |        |
| AHTK253 | 296         | 0.0354   |         |         |        |        |        |        |
| CXX279  | 128         |          |         |        | 0.1641 | 0.1753 |        |        |
| FH2848  | 234         |          |         |         |        |        |        |        |
| INRA21  | 109         |          |         |         |        |        | 0.0185 |        |
| INU005  | 134         |          |         |         |        |        |        |        |
| REN162C04 | 212       | 0.1982   |         |         |        |        |        |        |
| REN169O18 | 156        |          |         |        |        |        |        | 0.1197 |
| REN247M23 | 274       |          |         |        |        |        |        | 0.0128 |
| REN54P11 | 240        | 0.0062   |         |         |        |        |        |        |
| AHTH130 | 117         |          |         |        |        |        |        | 0.0097 |
| REN105L03 | 229       |          |         |        |        |        |        | 0.2179 |
| REN64E19 | 159        | 0.0115   |         |         |        |        |        |        |

* Alleles common to the Biewer Yorkshire Terrier (BYT) and Yorkshire Terrier (YT) breeds only.

Estimates of within-breed genetic diversity are summarized in Table 4. The highest average heterozygosity was found for M (Ho = 0.685 and HE = 0.677), YT (Ho = 0.662 and HE = 0.698), and BYT (Ho = 0.661 and HE = 0.658). Among the eight dog breeds considered, the lowest values of heterozygosity were found for IW (Ho = 0.491 and HE = 0.474). The population inbreeding coefficient (FIS) ranged from −0.049 (GR) to 0.053 (YT). The average p-value of HWE for each breed was higher than 0.05 (Table 4).

Table 4. Mean values of genetic parameters assessed for 21 STR loci of the study breeds.

| Breed | HO   | HE   | FIS  | p-Value          | PIC  | CPD  | CP_HE | CPE1  | CPE2  |
|-------|------|------|------|------------------|------|------|-------|-------|-------|
| GS    | 0.5451 | 0.5541 | 0.0171 | 0.4840 | 0.4941 | 1 * | 1.80 × 10^-13 | 0.985991 | 0.999736 |
| M     | 0.6655 | 0.6771 | -0.0127 | 0.4907 | 0.6398 | 1 * | 4.47 × 10^-19 | 0.999443 | 0.999987 |
| IW    | 0.4911 | 0.4743 | -0.0373 | 0.3952 | 0.4139 | 1 * | 6.71 × 10^-11 | 0.949242 | 0.997768 |
| BYT   | 0.6608 | 0.6581 | -0.0041 | 0.3425 | 0.6166 | 1 * | 6.82 × 10^-18 | 0.998757 | 0.999996 |
| YT    | 0.6623 | 0.6981 | 0.0533 | 0.3150 | 0.6545 | 1 * | 2.34 × 10^-19 | 0.999495 | 0.999999 |
| GR    | 0.5922 | 0.5620 | -0.0490 | 0.4414 | 0.5135 | 1 * | 3.37 × 10^-14 | 0.990100 | 0.999875 |
| LR    | 0.5954 | 0.5886 | -0.0088 | 0.2813 | 0.5429 | 1 * | 4.38 × 10^-15 | 0.993160 | 0.999941 |
| FB    | 0.6077 | 0.6177 | 0.0173 | 0.5395 | 0.5602 | 1 * | 8.65 × 10^-16 | 0.996011 | 0.999964 |

* actual value < 1.0, equal to approximately > 0.999999.

3.3. Parentage Testing and Individual Identification

The parameters for determining the suitability of the analyzed STR for identification and parentage testing are presented in Table 4. Polymorphism exceeding 0.6 was observed only for five STR (AHT121, FH2054, AHTh171, REN54P11, and REN64E19), while a level
over 0.5 was detected for 10 markers. The lowest polymorphism below 0.4 was noted for the rest of the loci. Mean PIC values for the studied breeds varied between 0.414 (for IW) and 0.655 (YT). The mean PD values for individual markers, calculated for all the eight dog breeds together, exceeded 0.8 for AHT121, FH2054, REN54P11, and REN169O18. For the other loci, PD had mean values exceeding 0.7 or close to 0.7 (REN247M23 and INU005) (Figure 3). The power of discrimination for the whole set of 21 STR, for each of the breeds, shows the high values of 0.999999999874911 (IW) to close to 1.0 (BYT, TY).

Figure 3. Mean values of the polymorphic information content (PIC) and power of discrimination (PD) for the eight dog breeds under study.

The cumulative probability of identity for 21 STR loci resulted in values as low as $4.5 \times 10^{-19}$, $2.3 \times 10^{-19}$ and $6.8 \times 10^{-19}$ for M, YT, and BYT breeds, respectively. The higher values of $1.8 \times 10^{-13}$ and $6.7 \times 10^{-11}$ were obtained for GS and IW breeds, respectively (Table 4). The panel of 21 microsatellite markers was assessed for their power of exclusion to test parentage in dogs of eight breeds. The probabilities of exclusion were calculated for two hypothetical situations with one parental genotype available (PE$_1$) and two parental genotypes available (PE$_2$). The probability of exclusion for one parent available (PE$_1$) ranged between 0.005 (REN169DO1 in IW) and 0.467 (CXX279 in YT) and when two parents were available (PE$_2$) between 0.047 (REN169DO1 in IW) and 0.642 (REN169DO1 in YT) across different markers and breeds. The cumulative exclusion probability for PE$_1$ and PE$_2$ varied from 0.949242 (IW) to 0.999495 (YT) and from 0.997768 (IW) to 0.999999 (YT), respectively (Table 4).

4. Discussion

As a consequence of selection pressure, management in closed populations, and historical bottlenecks, many dog breeds are exposed to an increase in inbreeding and a loss of diversity [5,29]. It is therefore essential to analyze the genetic structure and evaluate the genetic variation of as many dog breeds as possible and keep track of changes in these breeds. It is also necessary to evaluate the usefulness of DNA markers used for identification and parentage testing of dogs.

With the advancement of science and technology, new genetic markers, such as single nucleotide polymorphisms, have become widely used; however, due to the cost and time of analysis, STR typing is still used in biodiversity and kinship analysis [8–15,17].

We used 21 STRs to determine the genetic population structure of eight canine breeds chosen for this study. Relationships of breeds were analyzed by two approaches—the Reynolds genetic distance, which provides the highest sensitivity for highly divergent populations [21,30], and model-based clustering [19]. The results of STRUCTURE confirmed that the eight dog breeds analyzed could be subdivided into eight genetic clusters. The results for K = 7, for Biewer Yorkshire Terrier and Yorkshire Terrier breeds, showed one cluster (Figure 4).
Figure 4. STRUCTURE analysis of 21 STR genotypes from all study dogs (903 samples). The samples were grouped by the eight breeds (K = 8). In the case of K = 7, YT and BYT breeds were grouped in one cluster.

The two clusters formed by BYT and YT are suggestive of genetic sub-structuring that resulted from using animals belonging to divergent selection lines. The Biewer breed was founded in Germany in 1984 as a result of the selection for white hair genes to produce tricolor dogs. The Biewer Yorkshire Terriers were ultimately recognized as a distinct breed in 1989 by the Allgemeiner Club der Hundefreunde Deutschland (ACH). The Biewer Terrier became the first breed proven to be distinct and unique using genetic testing [31]. This breed became recognized in 2021 by the AKC as the organization’s 197th breed [5]. The estimated genetic distance confirmed the genetic similarity between YT and BYT (θw = 0.26). Both breeds showed genetic closeness to Maltese (θw < 0.4), which is considered, alongside the Black and Tan Terrier and Skye Terrier breeds, as the ancestor of the Yorkshire Terrier. Similar genetic proximity (θw = 0.41) was observed between Maltese and French Bulldog. The genetic distance between the other breeds was θw > 0.50. The differences between GR and LR, which belong to the same group of retrievers and flushing dogs (pointer type), may be due to their different origins. Golden Retriever is a breed developed in the 19th century in Great Britain, while Labrador Retriever dates back from the 18th century and comes from Newfoundland. Labrador Retriever shows similar genetic distance (θw = 0.55) with German Shepherd. Phylogenetically the most distant breed is Irish Wolfhound which originated in Ireland and was used to hunt and protect against wolves. The breed became extinct as wolf numbers decreased and post-19th century it was presumably recreated by dog fanciers [32].

The highest within-breed diversity was characteristic of the M, BYT, and YT breeds, in which the largest number of alleles was identified. In Maltese, 4 out of 135 identified alleles were specific for this breed, but their frequency was low (less than 3%). In contrast, a 128 bp allele at the CXX279 locus, common only to BYT and YT, was present in both breeds with a frequency of more than 16%. For these breeds, the highest degree of heterozygosity (over 65%) was also noted. For Yorkshire Terrier, Ho = 0.662, which is similar to the studies in the UK [8] and the US [33], where Ho for this breed was 0.66 and 0.789, respectively, indicating good levels of diversity in the YT breed. For the other breeds (GS, GR, LR, and FB), the degree of heterozygosity exceeded 50%. The genetic analysis of GS, which was performed based on the same STR panel and using a different 15-STR panel, showed similar values as in our study: Ho = 0.56 and Ho = 0.54, for dogs from the UK and Italian, respectively [8,9]. Tahir et al. [34], who used a 15-STR panel for GS breed in Pakistan, showed H0 to be 0.742. In the same study, higher values were also observed for the Labrador Retriever breed (H0 = 0.675) than in our study. A degree of heterozygosity below 50% was observed only for IW. This breed was characterized by the lowest total number of identified alleles. Furthermore, only two alleles each were determined in as many as four loci, among which 131 bp (AHT130) and 216 bp alleles (REN169D01) had a frequency of 0.912 and 0.945, respectively. The mean effective number of alleles per locus was only 2.1. Limited genetic variation may be the result of the origin of this breed from a common founder population involving four founder lines [35]. Preliminary studies for Irish Wolfhound reported by UC Davis Veterinary Genetics Laboratory showed that H0 = 0.502 for 33 STR, and when a 21-STR panel was used, H0 = 0.483, and this value was similar to that obtained in our study (H0 = 0.491) [36]. In the breeds under study, the mean H0 and H values were similar
to one another. The inbreeding coefficient for five breeds was negative, but the mean $F_{IS}$ had low negative values (from $-0.004$ to $-0.049$), which suggests no inbreeding in these breeds. The lack of observed genetic bottlenecks in any breed, despite a breeding system, may be due to multiple pedigree lines used. The lack of inbreeding was also reported in Bracco Italiano ($F_{IS} = 0.061$), Tatra Shepherd ($F_{IS} = -0.005$), six livestock guard dog breeds (average $F_{IS}$ value $= 0.024$), and in Polish Hunting Dog ($F_{IS} = -0.01$) [9,11,13,36].

The usefulness of the studied markers for individual identification of dogs was evaluated based on standard PIC, PD, and $P_{ID}$ genetic parameters. As reported by Botstein et al. [25], markers with PIC values greater than 0.5 are considered to be informative, values between 0.25 and 0.50 are fairly informative, and values lower than 0.25 are not very informative. In our study, the mean PIC values were greater than 0.5 for most of the studied breeds, similar to many other breeds of dogs investigated [11,13,34,37–40]. Only in the GS and IW breeds were slightly lower PIC values (0.49 and 0.41, respectively) obtained (Table 4). An immediate measure of the usefulness of the analyzed STR panels for individual identification is the power of discrimination. The higher the power of discrimination of a given STR panel is, the greater the chance that it can be used for individual identification [41]. The PD for the panel of 16 microsatellite markers for Shiba Inu breed was more than 0.999999 [8], while cumulative PD for the 21 markers in all breeds in our study was close to 1.0. The 21-STR panel used for the human gave a similar value of $0.99999999999999999971$ [42]. The probability of identity was calculated to assess the suitability of tested panels for individual identification. $P_{ID}$ shows the probability with which two unrelated, randomly selected individuals in the population will have the same genotype. It is accepted that $CP_{ID}$ values ranging between $10^{-3}$ and $10^{-4}$ are sufficiently low for the identification of individuals in natural animal populations [43], whereas $CP_{ID}$ value estimated only for 15 STR markers in canine amounted to $10^{-8}$ [44]. In our study, $CP_{ID}$ ranged between $10^{-11}$ for IW and $10^{-19}$ for M and YT. For all breeds, the obtained low $CP_{ID}$ values should be sufficient to distinguish individual dogs. The usefulness of the investigated panel of markers for parentage verification was determined by calculating the probability of exclusion (PE). For a previously used commercial panel of 10 STR, CPE was 0.994 [45]. The use of 17 or 18 STR markers gave a CPE of 0.99998%, and 0.99996%, respectively [11,46]. For all breeds, the recommended panel of 21 STR, used in this study, achieved a CPE of 0.9995 and CPE of 0.999, except for GS (CPE of 0.986) and IW breeds (CPE of 0.949, CPE of 0.998). The values higher than CPE of 0.999 and CPE of 0.999 were obtained for YT, M, and BYT. The highest CPE of 0.999999 was observed for YT.

5. Conclusions

Analysis of the microsatellite DNA markers provides valuable information about canine diversity, and a 21-STR panel is an effective tool for individual identification and parentage testing. Our study showed the lowest PIC (0.414), PE (0.949), and $P_{ID}$ (0.998) with the highest $P_{ID}$ ($6.71 \times 10^{-11}$) for the Irish Wolfhound breed, illustrating the lower effectiveness of the STRs panel. In contrast, Yorkshire Terrier and Maltese breeds had the highest PIC (0.655 and 0.640), PE (0.9995 and 0.9994), and PE (0.999999 and 0.9999987) with the lowest $P_{ID}$ ($2.34 \times 10^{-19}$ and $4.47 \times 10^{-19}$). The results suggest the popular breeds in Poland have sufficient diversity relative to other populations that have been studied. The research here provides baseline data for monitoring and managing the breeds.

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References

1. Wayne, R.K.; vonHoldt, B.M. Evolutionary genomics of dog domestication. *Mamm. Genome* **2012**, *23*, 3–18. [CrossRef] [PubMed]
2. Larson, G.; Karlsson, E.K.; Perri, A.; Webster, M.T.; Ho, S.Y.W.; Peters, J.; Stahl, P.W.; Piper, P.J.; Lingaas, F.; Fredholm, M.; et al. Rethinking dog domestication by integrating genetics, archeology, and biogeography. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 8878–8883. [CrossRef] [PubMed]
3. Pedersen, N.C.; Liu, H.; Leonard, A.; Griffioen, L. A search for genetic diversity among Italian Greyhounds from Continental Europe and the USA and the effect of inbreeding on susceptibility to autoimmune disease. *Canine Genet. Epidemiol.* **2015**, *2*, 17. [CrossRef] [PubMed]
4. Keijser, S.F.A.; Fieten, H.; Bos-Looijens, M.; Pieck, C.J.; Anderson, H.; Donner, J.; Scholten, J.; Nielen, M.; Hesselink, J.W.; Van Steenbeek, F.G. Heterozygosity testing and multiplex DNA panel screening as a potential tool to monitor health and inbreeding in a small, closed dog population. *Canine Genet. Epidemiol.* **2018**, *5*, 12. [CrossRef] [PubMed]
5. Lampi, S.; Donner, J.; Anderson, H.; Pohjoismäki, J. Variation in breeding practices and geographic isolation drive subdivision differentiation, contributing to the loss of genetic diversity within dog breed lineages. *Canine Med. Genet.* **2020**, *7*, 5. [CrossRef] [PubMed]
6. American Kennel Club. Available online: https://www.akc.org/press-center/articles-resources/facts-and-stats/breeds-year-recognized (accessed on 1 January 2021).
7. The Fédération Cynologique Internationale. Available online: http://www.fci.be/en/Presentation-of-our-organisation-4.html (accessed on 27 April 2020).
8. Mellanby, R.J.; Ogden, R.; Clements, D.N.; French, A.T.; Powel, R.; Corcoran, B.; Schoeman, J.P.; Summers, K.M. Population structure and genetic heterogeneity in popular dog breeds in the UK. *Vet. J.* **2013**, *196*, 92–97. [CrossRef]
9. Bigi, D.; Marelli, S.P.; Randi, E.; Polli, M. Genetic characterization of four native Italian Shepherd dog breeds and analysis of their relationship to cosmopolitan dog breeds using microsatellite markers. *Animal* **2015**, *9*, 1921–1928. [CrossRef] [PubMed]
10. Arata, S.; Asahi, A.; Takeuchi, Y.; Mori, Y. Microsatellite loci analysis for individual identification in Shiba Inu. *J. Vet. Med. Sci.* **2016**, *78*, 439–441. [CrossRef] [PubMed]
11. Radko, A.; Rubis, D.; Szumiec, A. Analysis of microsatellite DNA polymorphism in the Tatra Shepherd Dog. *J. Appl. Anim. Res.* **2017**, *46*, 254–256. [CrossRef]
12. Turcsán, B.; Tatrai, K.; Petró, E.; Topál, J.; Balogh, L.; Egyed, B.; Kubinyi, E. Comparison of Behavior and Genetic Structure in Populations of Family and Kennelbred Beagles. *Front. Vet. Sci.* **2020**, *7*, 183. [CrossRef] [PubMed]
13. Goleman, M.; Balicki, I.; Radko, A.; Jakubczak, A.; Formal, A. Genetic diversity of the Polish Hunting Dog population based on pedigree analyses and molecular studies. *Livest. Sci.* **2019**, *229*, 114–117. [CrossRef]
14. Van Asch, B.; Alves, C.; Gusmão, L.; Pereira, V.; Pereira, F.; Amorim, A. A new autosomal STR nineplex for canine iden-tification and parentage testing. *Electrophoresis* **2009**, *30*, 417–423. [CrossRef] [PubMed]
15. Dimitrijevic, V.; Stevanovic, J.; Savic, M.; Petrujicic, B.; Simeunovic, P.; Milesevic, I.; Stanimirovic, Z. Validation of 10 microsatellite loci for their use in parentage verification and individual identification in the Yugoslavian Shepherd Dog Sharplanina. *Ann. Anim. Sci.* **2013**, *13*, 715–722. [CrossRef]
16. ISAG Panel DOG. 2005. Available online: www.isag.us/Docs/2005ISAGPanelDOG.pdf (accessed on 1 July 2005).
17. Goleman, M.; Balicki, I.; Radko, A.; Rozempolska-Rucinska, I.; Żęba, G. Pedigree and Molecular Analyses in the Assessment of Genetic Variability of the Polish Greyhound. *Animals* **2021**, *11*, 353. [CrossRef] [PubMed]
18. Dog Way. Available online: https://dogway.pl/blog/p/najpopularniejsze-rasy-psow-w-polsce/ (accessed on 7 May 2019).
19. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* **2000**, *155*, 945–959. [CrossRef]
20. Earl, D.A.; vonHoldt, B.M. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Res.* **2012**, *4*, 359–361. [CrossRef] [PubMed]
21. Reynolds, J.; Weir, B.S.; Cockerham, C.C. Estimation of the coancestry coefficient: Basis for a short-term genetic distance. *Genetics* **1983**, *105*, 767–779. [CrossRef] [PubMed]
22. Nei, M.; Roychoudhury, A.K. Sampling variances of heterozygosity and genetic distance. *Genetics* **1974**, *76*, 379–390. [CrossRef]
23. Wright, S. *Evolution and the Genetics of Populations*; University of Chicago Press: Chicago, IL, USA, 1978.
24. Guo, S.W.; Thompson, E.A. Performing the Exact Test of Hardy-Weinberg Proportion for Multiple Alleles. *Biometrics* **1992**, *48*, 361. [CrossRef] [PubMed]
25. Botstein, D.; White, R.L.; Skolnick, M.; Davis, R.W. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* **1980**, *32*, 314–331. [PubMed]
26. Kimberly, A.H. Statistical analysis of STR data. *Profiles DNA* **1998**, *1*, 14–15.
27. Paetkau, D.; Strobeck, C. Microsatellite analysis of genetic variation in black bear population. *Mol. Ecol.* **1994**, *3*, 189–195. [CrossRef] [PubMed]
28. Jamieson, A.; Taylor, S.C.S. Comparisons of three probability formulæ for parentage exclusion. *Anim. Genet.* **1997**, *28*, 397–400. [CrossRef] [PubMed]
29. Leroy, G. Genetic diversity, inbreeding and breeding practices in dogs: Results from pedigree analyses. *Veter. J.* 2011, 189, 177–182. [CrossRef] [PubMed]

30. Libiger, O.; Nievergelt, C.M.; Schork, N.J. Comparison of Genetic Distance Measures Using Human SNP Genotype Data. *Hum. Biol.* 2009, 81, 389–406. [CrossRef] [PubMed]

31. Veterinary Genetics Laboratory, UC Davis, in Collaboration with Dr. Niels C. Pedersen and Staff. Genetic Diversity Testing for Biewer. Available online: https://vgl.ucdavis.edu/canine-genetic-diversity/biewer (accessed on 1 January 2019).

32. Bulldog Francuski. Available online: http://www.piesporadnik.pl/title,pid,45,oid,47,-%20cid,176.html (accessed on 1 January 2020).

33. Wictum, E.; Kun, T.; Lindquist, C.; Malvick, J.; Vankan, D.; Sacks, B. Developmental validation of DogFiler, a novel multiplex for canine DNA profiling in forensic casework. *Forensic Sci. Int. Genet.* 2013, 7, 82–91. [CrossRef]

34. Tahir, M.S.; Hussain, T.; Babar, M.E.; Nadeem, A.; Naseer, M.; Ullah, Z.; Intizar, M.; Hussain, S.M. A panel of microsatellite markers for genetic diversity and parentage analysis of dog breeds in Pakistan. *J. Anim. Plant Sci.* 2015, 25, 351–356.

35. Veterinary Genetics Laboratory, UC Davis, in Collaboration with Dr. Niels C. Pedersen and Staff. Davice Genetic Diversity Testing for Irish Wolfhounds. Available online: https://vgl.ucdavis.edu/canine-genetic-diversity/irish-wolfhound (accessed on 19 August 2019).

36. Veterinary Genetics Laboratory, UC Davis. Statistics and Breed-Wide Allele Frequency—Irish Wolfhound. Report Issued August 19. Available online: https://vgl.ucdavis.edu/canine-genetic-diversity/irish-wolfhound/stats (accessed on 19 August 2019).

37. Radko, A.; Slota, E. Application of 19 microsatellite DNA markers for parentage control in Borzoi dogs. *Pol. J. Vet. Sci.* 2009, 12, 113–117. [PubMed]

38. Ciampolini, R.; Cecchi, F.; Bramante, A.; Casetti, F.; Presciuttini, S. Genetic variability of the Bracco Italiano dog breed based on microsatellite polymorphism. *Ital. J. Anim. Sci.* 2011, 10, 267–270. [CrossRef]

39. Ichikawa, Y.; Takagi, K.; Tsumagari, S.; Ishihama, K.; Morita, M. Test in based on microsatellite polymorphisms. *J. Vet. Med. Sci.* 2001, 63, 1209–1213. [PubMed]

40. Kang, B.-T.; Kim, K.-S.; Min, M.-S.; Chae, Y.-J.; Kang, J.-W.; Yoon, J.; Choi, J.; Seong, J.-K.; Park, H.-C.; An, J.; et al. Microsatellite loci analysis for the genetic variability and the parentage test of five dog breeds in South Korea. *Genes Genet. Syst.* 2009, 84, 245–251. [CrossRef] [PubMed]

41. Eichmann, C.; Berger, B.; Parson, W. Relevant aspects for forensic STR analysis of canine DNA: Repeat-based nomenclature and sensitive PCR multiplexes. *Int. Congr. Ser.* 2006, 1288, 813–815. [CrossRef]

42. Boonderm, N.; Suriyanratakorn, D.; Sangpueng, S.; Onthong, N.; Nettakul, A.; Waiyawuth, W. Population genetic data of 21 STR markers in Thais of southern border provinces of Thailand. *For. Sci. Int. Genet.* 2017, 6, 523–525. [CrossRef]

43. Waits, L.P.; Luikart, G.; Taberlet, P. Estimating the probability of identity among genotypes in natural populations: Cautions and guidelines. *Mol. Ecol.* 2001, 10, 249–256. [CrossRef] [PubMed]

44. Eichmann, C.; Berger, B.; Steinlechner, M.; Parson, W. Estimating the probability of identity in a random dog population using 15 highly polymorphic canine STR markers. *Forensic Sci. Int.* 2005, 151, 37–44. [CrossRef] [PubMed]

45. Radko, A.; Slota, E.; Kościelnny, M. Polymorphism of 10 microsatellites and their usefulness for paternity control in dogs. In *Biotechnology, Agriculture and the Food Industry*; Zaikov, G.E., Ed.; Nova Scie. Publishers: New York, NY, USA, 2006; pp. 141–144.

46. Dodd, J.N.; Morris, B.G.; Oliveira, D.A.A.; Bernoco, D. DNA testing for parentage verification and individual identification in seven breeds of dogs. *Rev. Bras. Reprod. Anim.* 2001, 25, 35–41.