Analysis of microsatellite DNA polymorphism in the Tatra Shepherd Dog

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ABSTRACT
The Tatra Shepherd Dog is one of five Polish native breeds of dogs originating from the Polish Tatra Mountains. The objective of the study was to determine genetic variation in a population of 60 dogs of this breed, based on polymorphism of 18 microsatellite (STR) markers, recommended by ISAG for canine parentage testing. The analysis showed considerable genetic variability in the studied loci. The 100 alleles identified in the test material were used to determine the polymorphism of the discussed markers. The highest polymorphism was found in the locus AHT171, in which 8 alleles were identified and PIC and H_{E} values exceeded 0.8. The lowest polymorphism was detected for AHTk211, in which 3 alleles were determined, and PIC and H_{O} values were 0.233 and 0.281, respectively. Average F_{S} had a low negative value, which suggests zero inbreeding of the studied breed. The probability of parentage exclusion estimated for the 18 markers totalled 99.996%.

1. Introduction
In addition to the Polish Lowland Sheepdog, the Polish Hound, the Polish Hunting Dog and the Polish Greyhound, the Tatra Shepherd Dog is one of the five breeds of dogs native to Poland. Tatra Shepherd Dog is associated with the Tatra Mountains because it originates from the Podhale region (the south of Poland). It is believed that the breed traces back to white guardian dogs descended from the Tibetan mastiff, which migrated with pastoral nomads from Asia, along the Carpathians, to Europe. Originally the Tatra Shepherd Dog was used as a herder and watchdog, but today the overwhelming majority of Tatra Shepherd Dogs are guardian dogs (watching over a specific area) as well as family and companion dogs. They are found not only in Podhale but all over Poland. Apart from dogs registered in the Polish Kennel Club, many animals are of unknown origin and unregistered. The number of dogs registered in the Polish Kennel Club ranged from 383 in 2012 to 473 in 2011. Every year, between 271 and 311 pups are born in 50–55 litters.

Developments in breeding over the last few years in Poland have brought some positive trends: bitches are mated to different sires, also abroad; dogs bred in other countries are imported and, after getting breeding rights, they are used by Polish breeders; typical dogs of unknown origin are searched and registered in the open stud book to expand the gene pool of the population in Poland. Increase in effective population size will reduce the degree of relatedness and preserve genetic variation, thus preventing increase in homozygosity and consequent inbreeding depression. Breeding success is accomplished by maintaining the population’s genetic variation because the mating of genetically similar individuals increases homozygosity in the population, which may result, for example, in lethal genes.

Genetic variation is commonly assessed using neutral microsatellite DNA markers, which became also an internationally recognized genetic tool for individual identification and for parentage testing in different species of animals, including dogs (Koskinen & Bredbacka 2000; Denise et al. 2004; Irion et al. 2005; Asch et al. 2009; Radko & Słota 2009; Dimitrijevic et al. 2013).

The first available commercial panel of 10 microsatellite DNA markers (PEZ1, PEZ3, PEZ5, PEZ6, PEZ8, PEZ11, PE12, PEZ18, UCB2054 and UCB2079), used for parentage verification in dogs, was not always effective in parentage identification possibly due to a rigorous artificial selection for specific dog traits and consequent reduced genetic variation in many breeds. Rigorous selection for different traits preferred in dog breeding reduced genetic variation and increased inbreeding in many breeds. Limited genetic variation is expressed in the limited number of alleles at some microsatellite loci, which makes it difficult to choose a marker panel that is informative and could be used for parentage verification in different breeds of dogs. Consequently, Society for Animal Genetics (ISAG) recommends a new set of 18 polymorphic STR markers for parentage verification in dogs, which were used also in this study, and additional three markers (REN105L03, AHT130 and REN64E19) were proposed recently. The objective of the study was to determine genetic variation in Tatra Shepherd Dogs based on the polymorphism of microsatellite DNA markers recommended by ISAG for parentage testing in dogs, and to evaluate the usefulness of the investigated panel of markers for parentage verification in this breed.

2. Material and methods
A total of 60 Tatra Shepherd dogs bred in Poland were collected by Polish Kennel Club and Tatra Shepherd dog breeders. The study population included 22 males and 38 females, forming...
5 complete and 10 incomplete families. The other samples used for analysis included 31 unrelated individuals from 21 breeders. All selected animals we had confirmed their pedigree data provided by the owners.

The blood samples were collected with EDTA as anticoagulant. All samples were stored until analysis at −20°C.

The panel of 18 markers recommended by ISAG was analysed in the studied animals: AHTk211, CXX279, REN169O18, INU005, REN54P11, INRA21, AHT137, REN169D01, AHTh260, AHTk253, INU005, INU030. FH2848, AHT121, FH2054, REN162C04, AHTh171 and REN247M23. These markers are included in the commercial Canine Genotypes 1.1 kit (Thermo Fisher Scientific).

Genomic DNA was isolated from blood using a commercial DNA isolation kit (Wizard® Genomic DNA Purification Kit from Promega). The DNA samples were stored at −20°C until PCR analysis. Multiplex polymerase chain reaction (PCR) was carried out according to the protocols described in the kit manual. PCR reactions were performed on an ABI GeneAmp PCR System 9700. Using the following thermal profile: 3 min. of initial DNA denaturation at 98°C, followed by 30 cycles of denaturation at 98°C for 15 s, annealing at 60°C for 75 s, elongation of primers at 72°C for 30 s and final elongation of primers at 72°C for 5 min.

The PCR products were analysed using an ABI 3130xl capillary sequencer (Life Technology). The amplified DNA fragments of different length were electrophoresed on a 7% denaturing polyacrylamide gel (POP-7), using the 500 LIZ size standard and a reference sample. The results of electrophoretic separation were analysed automatically using GeneMapper software.

The frequency of alleles detected was used to calculate the following parameters: observed heterozygosity – HO and expected heterozygosity – HE (Nei & Roychoudhury 1974), inbreeding coefficient – FIS (Wright 1978), polymorphic information content – PIC (Botstein et al. 1980), the probability of parentage exclusion for each locus, when the genotypes of one and both parents are known (PE1 and PE2) and the combined probability of parentage exclusion (PEC) for all 18 loci together (Jamieson & Taylor 1997).

3. Results and discussion

The analysis showed considerable genetic variation in the microsatellite markers. The analysis of the same set of markers in dog breeds in Poland have only been reported by previous studies of Borzoi dogs (Radko & Słota 2009).

In the present study we identified a total number of 100 alleles with 5.5 alleles per locus on average. The identified alleles were used to determine the polymorphism of the markers.

The highest polymorphism was characteristic of the locus AHT171, which had 8 alleles, with PIC and HO values exceeding 0.8. An equally high degree of polymorphism for this marker was observed in a study of Borzoi dogs (Radko & Słota 2009). High variation was also detected for the loci AHT121, AHTH260. FH2054, INU005 and INU055, in which 7 alleles were determined, and PIC and HO values ranged from 0.578 and 0.509 (AHT121) to 0.732 and 0.754 (FH2054), respectively. The lowest polymorphism showed locus AHTk211, which had only 3 alleles, with one of them (91 bp) being highly predominant (frequency = 0.85). PIC and HO values for this locus were 0.233 and 0.281, respectively. The other markers exhibited a similar polymorphism (Table 1), with the number of alleles in the range of 4–6, and PIC and HO values ranging from 0.452 and 0.544 (INRA21) to 0.749 and 0.807 (REN169D01).

The ratio of observed to expected heterozygosity was used to calculate the coefficient of inbreeding (FIS), which expresses the degree of inbreeding in a population. The estimated FIS values were generally similar to HO values. The mean FIS

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**Table 1. Polymorphism of 18 microsatellite DNA markers in 60 tatra shepherd dogs.**

| Marker       | No. of alleles | Range of alleles | PIC   | HO   | HE   | FIS   | PE1  | PE2  |
|--------------|----------------|------------------|-------|------|------|-------|------|------|
| AHT121       | 7              | 98–102           | 0.578 | 0.509| 0.610| 0.1662| 0.220| 0.400|
| AHT137       | 4              | 131–153          | 0.372 | 0.456| 0.429| −0.064| 0.093| 0.208|
| AHT171       | 8              | 219–237          | 0.827 | 0.877| 0.845| −0.037| 0.522| 0.689|
| AHTH260      | 7              | 240–258          | 0.609 | 0.632| 0.639| 0.012 | 0.248| 0.432|
| AHTR211      | 3              | 87–91            | 0.233 | 0.261| 0.258| −0.086| 0.033| 0.122|
| AHTK253      | 4              | 284–292          | 0.548 | 0.632| 0.622| −0.016| 0.198| 0.341|
| CXX279       | 6              | 116–130          | 0.543 | 0.632| 0.574| −0.101| 0.190| 0.366|
| FH2054       | 7              | 152–176          | 0.732 | 0.754| 0.761| 0.0089| 0.380| 0.562|
| FH2848       | 6              | 232–244          | 0.693 | 0.702| 0.732| 0.0412| 0.327| 0.507|
| INRA21       | 4              | 99–101           | 0.529 | 0.544| 0.582| 0.0658| 0.178| 0.335|
| INU005       | 7              | 122–134          | 0.672 | 0.677| 0.722| −0.215| 0.304| 0.475|
| INU030       | 4              | 144–154          | 0.452 | 0.544| 0.535| −0.017| 0.146| 0.261|
| INU055       | 7              | 208–224          | 0.711 | 0.719| 0.753| 0.0444| 0.345| 0.522|
| REN162C04    | 5              | 200–208          | 0.547 | 0.579| 0.619| 0.0651| 0.197| 0.340|
| REN169D01    | 5              | 202–220          | 0.749 | 0.807| 0.784| −0.029| 0.392| 0.571|
| REN169O18    | 5              | 160–170          | 0.622 | 0.526| 0.677| 0.2222| 0.257| 0.423|
| REN247M23    | 6              | 268–278          | 0.680 | 0.825| 0.729| −0.131| 0.310| 0.483|
| REN54P11     | 5              | 226–242          | 0.664 | 0.719| 0.711| −0.012| 0.293| 0.467|
| Total        |                |                  | 0.598 | 0.645| 0.643| −0.0046|     |      |

PEc1 (%) = 99.62
PEc2 (%) = 99.96

Notes: PIC – polymorphic information content.
HO – observed (HO) and expected heterozygosity (HE).
FIS – coefficient of inbreeding.
PE1 – probability of exclusion when the genotype of one parent is known (PE1) and (PE2) when the genotypes of both parents are known.
PEC – combined probability of exclusion when the genotype of one parent is known (PEC1) and when the genotypes of both parents are known (PEC2).
value was negative and close to zero (−0.0046), which suggests zero inbreeding of the breed. Greater disproportion between the $H_O$ and $H_E$ values, being indicative of heterozygosity deficiency, was only found for REN169O18, where $F_{IS}$ equalled 0.222. The observed increase in homozgyosity at this locus may be due to many factors, including selection for specific productive traits, but also the possible occurrence of null alleles. However, this supposition needs to be supported by more extensive research.

The usefulness of the investigated panel of markers for parentage verification was determined by calculating the probability of exclusion (PE). Our study showed that a single microsatellite marker gives PE values of 3.3–52.2% when the genotype of one parent is known and of 12.2–68.9% when the genotypes of both parents are known (Table 1). The use of the largest possible number of polymorphic markers increases the probability of correct parentage assignment. Based on nine microsatellite markers, PE estimated in six breeds of dogs ranged from 99.46% to 99.96% (Klukowska et al. 2001). For a previously used commercial panel of 10 STR, it was 99.4% (Radko et al. 2006). The use of 17 loci resulted in a PE of 99.998% (Dodd et al. 2001), while in our study, the use of 18 markers gave a PE of 99.996% when the genotypes of both parents were known.

The high level of polymorphism in the analysed sequences and the high PE indicate that they could be used for routine parentage testing of Tatra Shepherd Dogs.

Disclosure statement
No potential conflict of interest was reported by the authors.

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