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CHARACTERIZATION OF THE WILMS-TF MICROSATELLITE MARKER IN HUNGARIAN DOG POPULATIONS

SHORT COMMUNICATION

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Demand for correct and cost-effective genetic-based identification and parentage control has increasing importance in domestic animals, including dogs. In our study the applicability of a canine hyperpolymorphic microsatellite marker – which localized in the WILMS-TF (tumor factor) gene – was examined in mixed breed and purebred canine populations. The redesigned and shortened amplicons were genotyped using an allelic ladder which was constructed from sequence verified fragments. The nomenclature for allele calling based on repetition structures is suitable for international comparisons. Our study justified the potential use and efficiency of the marker D18S12 in parentage control.

Keywords: Canine – STR – polymorphism – parentage control

The canine genome has been intensively investigated from several aspects [6]. In the context of genetic research, the microsatellites are widely used for genetic mapping to explore background of diseases as well as phylogenetic research or paternity testing. The polymorphic $(GAAA)_n$ microsatellite in canine WILMS tumor 1 (WT1) gene intron is previously described [8]. In case of individual identification the importance of polymorphic STR loci is widely known, but the degree of polymorphism is specially emphasized. Taking international demands as a basis, the individualization of dogs is realizable in a standard and compatible way through the use of uniform allele nomenclature [5]. The request for parentage control arises when the parents are not precisely known [7], i.e. in case of multiple putative sires or artificial fertilization.

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Table 1
Main population statistical data of mixed and Labrador retriever populations

	$H_{(exp)}$	$H_{(obs)}$	PE	PD	PIC	F
Mixed	0.915	0.769	0.812	0.968	0.899	
Labrador retriever	0.802	0.646	0.604	0.914	0.766	0.195

Saliva and hair samples from 48 genetically independent Labrador retriever and 52 individual from different pure breeds were collected. As a casework study Labrador retriever samples (bitch, two alleged sires and two puppies) were examined. The DNA isolation was previously described [1]. The locus was amplified with redesigned shortened primers and PCR conditions [2.5 µl 10× Buffer II, 2.5 µl $MgCl_2$ (25 mM), 2,5 µl dNTPs (10 mM), 0.5 µl Taq, 0.2–1 ng DNA, 2.5 µl primers (10 pM) 5'-CCCAATCTCCAGAGATTTTCC, 5'-CCCACTGTTCTGTGGTTTGC, GeneAmp PCR System 2400: 95 °C-10'', 32× (94 °C-1', 58 °C-1', 72 °C-1'), 72 °C-15']. Electrophoresis and data collection were performed on ABI PRISM 310 Genetic Analyzer. The PCR products were classified by size, and one or more representatives of classified fragments were sequenced. The allele designation was developed based on repetition [3] and the reference allelic ladder was created from the sequenced fragments. Basic population-statistical analysis was performed on both – Labrador retriever and mixed breed population – groups.

The observed numbers of alleles in both populations – 19 different alleles in the mixed and 10 different alleles in the Labrador retriever population (Fig. 1) – are comparable to other studies [4], in which 24 different allele types were published from 131 randomly selected dogs. From these 24 alleles we managed to reveal 18 alleles altogether, and also one allele D18S12(9), which has not been published yet (Fig. 1). The observed heterozygosity (H_{obs} = 0.65) in the Labrador group comparing to other

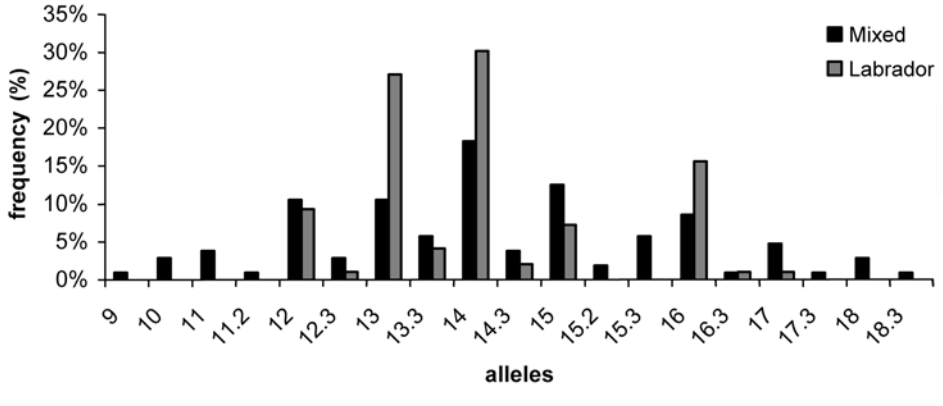

Fig. 1. Observed allele frequency in mixed and in Labrador retriever population

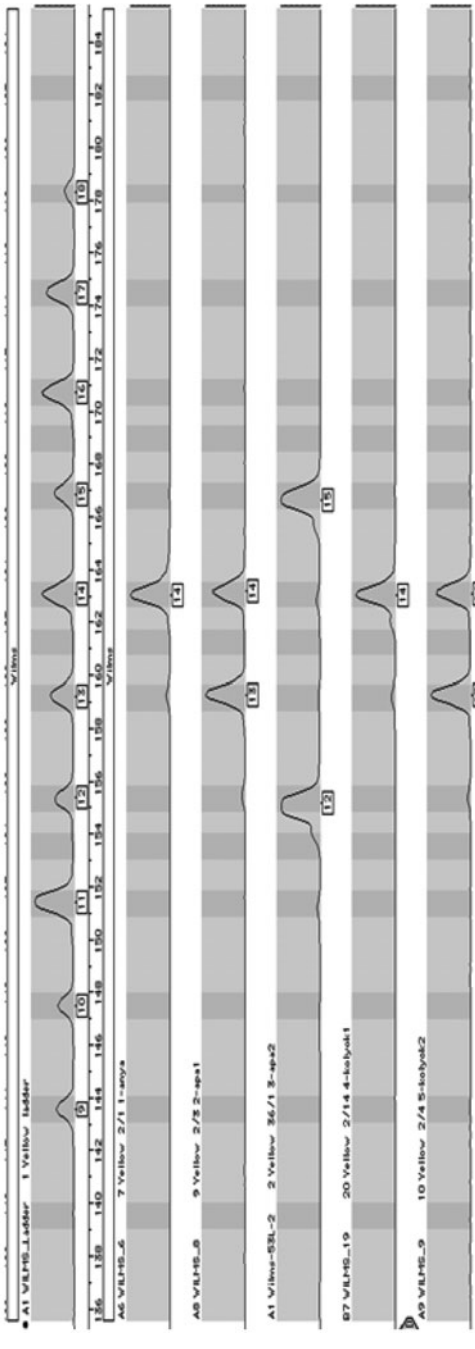

Fig. 2. Parentage control in Labrador retriever stock – from above: Wilms allelic ladder, hair samples of the mother, the two possible sires and the two puppies

polymorphic loci in Hungarian dog populations (H_{obs} = 0.58 in average) [9] proved to be fairly enough. Differences of the observed and expected heterozygosity (H_{obs} and H_{exp}) values between the mixed breed- and the purebred population (Table 1) may be associated with the relatively low number of samples and/or the level of inbreeding [9]. Due to the putatively direct and limited propagation relatively high inbreeding coefficient (F = 0.195) manifested in the Labrador group. The genetic diversity based on the polymorphism information content (PIC) values was acceptably high at this locus (Table 1) and showed similarity to non-Hungarian dog populations (between 0.56 and 0.95) [4]. This is related to the structural complexity of the marker, the numerous intermediate alleles and their relative distribution. Power of exclusion (PE) and power of discrimination (PD) values in both populations are fairly high (Table 1) compared to the human individualization (PE = 0.42–0.76; PD = 0.84–0.96) [2]. From a technical viewpoint, due to the redesigned amplicons (less than 200 base pairs) the sensitivity (200 pg template DNA) is sufficient for successful genotyping even though the origin of sample cells were the hair bulbs. The casework study supported an alternative application of WILMS (D18S12) locus for parentage control even in the strongly inbred Labrador stock (Fig. 2) but for a certain statement it should be completed with other polymorphic – e.g. PEZ6 (D27S4), PEZ8 (D17S1) – markers.

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