J Forensic Sci, March 2006, Vol. 51, No. 2
doi:10.1111/j.1556-4029.2006.00049.x
Available online at: www.blackwell-synergy.com

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A Proposal for Standardization in Forensic Canine DNA Typing: Allele Nomenclature of Six Canine-Specific STR Loci

ABSTRACT: In this study a proposal for the allele nomenclature of six polymorphic short tandem repeat (STR) loci (PEZ3, PEZ6, PEZ8, PEZ10, FHC2161, and FHC2328) for canine genotyping (*Canis lupus familiaris*) is presented. The nomenclature is based on the sequence data of the polymorphic region of the microsatellite markers as recommended by the DNA commission of the International Society of Forensic Haemogenetics (ISFH) in 1994 for human DNA typing. To cover commonly and rarely occurring alleles, a selection of homozygous and heterozygous animals were analyzed and subjected to sequence studies. The alleles consisted of simple tri- and tetra-nucleotide repeat patterns as well as compound and highly complex repeat patterns. Several alleles revealing the same fragment size but different repeat structures were found. The allele designation described here was adopted to the number of repeats, including all variable regions within the amplified fragment. In a second step the most commonly occurring alleles were added to an allelic ladder for each marker allowing a reliable typing of all alleles differing in size. A total number of 142 unrelated dogs from surrounding municipal animal homes, private households, and canines in police duty were analyzed. The data were added to a population database providing allele frequencies for each marker.

KEYWORDS: forensic science, DNA typing, *Canis familiaris*, short tandem repeats (STR), sequence data, allele nomenclature, allelic ladder, population data

DNA typing of biological stain material of nonhuman origin has become a powerful and well-accepted tool in wildlife and forensic investigation (1–7) focused on species identification and individualization. Therefore, the advantages of PCR amplification of species-specific polymorphic microsatellite markers could be used in animal forensics where mostly quality and quantity of DNA are the limiting factors of analysis caused by the minute amount of trace material or degradation. Especially stains deriving from animals living in close proximity to humans such as cats and dogs are of increasing interest by prosecuting attorneys and forensic scientists. In cases where a cat or dog is sharing someone's home, it was demonstrated that the transfer of animal hairs—as well as human hairs and fibers—cannot be avoided. They are easily transferred and attached to various surfaces (8,9), and cases in which dog hairs were submitted as evidence would be most common. Trace evidence deriving from animals linked to crime scenes have been described in several cases, such as sexual abuse or fatal dog biting attacks (10–13). Some of them are well known in the forensic community, e.g., the "Snowball case," in which a feline hair helped to solve a homicide case in 1994 by DNA investigations (14).

A large number of canine-specific STR markers and appropriate primer sequences are described (15–18) and widely used in the field of kinship analysis for breeding purposes and in forensics. Nevertheless, there is little information about sequence data and no internationally accepted allele nomenclature is available. Thus, data examined by different laboratories using a variety of analytical equipment can neither be compared, nor can the population data be shared by the community. Additionally, a large number of population statistics were presented throughout the previous years (19–22). Therein allele frequencies, expected and observed heterozygosities, and values for polymorphism information content (PIC) in several breeds were demonstrated. Unfortunately, no information was given about the allele structure, its DNA sequence, and the appearance of intermediate alleles.

A first approach toward a repeat-based nomenclature on the basis of sequenced alleles was performed in the course of a casework example for the canine-specific STR marker PEZ20 (12). Recently, for 15 canine-specific STR loci (FH2010, FH2054, FH2079, FH2087Us, FH2087Ul, FH2132, FH2611, PEZ2, PEZ6, PEZ12, PEZ15, VWFX, WILMS-TF, ZuBeCa4, and ZuBeCa6) a comprehensive sequencing study was presented, forming the first basis for a repeat-based allele nomenclature according to the ISFH guidelines for human STR markers (23). In the current study, a further set of the canine-specific STR loci PEZ3, PEZ8, PEZ10, FHC2161, and FHC2328 is described, including fragment size, sequence data, and a nomenclature based on the number of repeats according to the ISFH recommendations (24). Population data of randomly selected dogs out of a total number of 142 unrelated individuals are presented. For PEZ6, an alternative nomenclature to the previously published one (23) is introduced, including just the variable regions leading to integer numbers for the most commonly appearing alleles.

To select appropriate alleles for sequencing reactions covering main alleles as well as intermediate alleles, it was essential to distinguish PCR products followed by electrophoretic separation in 1 bp steps. Therefore several alleles of the STR loci PEZ6, PEZ8, and PEZ10 had to be displayed in one plot to determine

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Received 14 May 2005; and in revised form 9 Sept. 2005; accepted 3 Oct. 2005; published 9 Feb. 2006.

whether they resemble equal or different fragment lengths. This process was simplified by the use of an allelic ladder consisting of the most commonly occurring alleles as well as some rare intermediate ones. The ladder was used for the interpretation of analyzed results serving as size standard for each locus investigated.

Methods

DNA Extraction and PCR Conditions

Saliva samples from 142 dogs of 36 different pure breeds and several cross breeds were collected on cotton swabs. DNA was isolated using the ReadyAmp™ Genomic DNA Purification System (Promega, Mannheim, Germany) according to the manufacturer's protocol. Singleplex amplification of the canine STR loci PEZ3, PEZ6, PEZ8, PEZ10, FHC2161, and FHC2328 was carried out in a total reaction volume of 25 µL consisting of 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.5 µM of each primer, 1.25 U AmpliTaq® Gold DNA Polymerase (Applied Biosystems, Darmstadt, Germany), and 1–5 µL saliva DNA extract. Primer sequences are listed in Table 1. The amplification was performed in a Gene Amp PCR System 9700 thermal cycler (Applied Biosystems) for 30 cycles, with an annealing temperature of 54°C for all markers. Amplicons were separated and detected by capillary electrophoresis on an ABI Prism 310 Genetic Analyzer (Applied Biosystems) using POP4 and a 36-cm capillary array. Apparent DNA fragment size was analyzed with the internal size standard Genescan® 400HD [ROX] and GeneScan Analysis Version 3.7 software (Applied Biosystems).

Sequencing Reaction

The alleles included in the ladder and additional alleles were selected and sequenced from homozygous and heterozygous animals, respectively, following the amplification procedure as described above using the unlabeled primer pairs. Amplicons of homozygous alleles were purified from the PCR mixture with QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced with the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) in a total volume of 10 µL with 10 pmol primer for 25 cycles as described (23). Heterozygous alleles were separated prior to the sequencing reaction by a Spreadex EL 600 Minigel run on a SEA 2000 Electrophoresis System (both Elchrom Scientific, Cham, Switzerland) as described (25), stained by SybrGold (Invitrogen, Karlsruhe, Germany) and visualized on a Dark Reader transilluminator (Clare Chemical Research, Dolores, CO). Samples were desalted with Microcon-30 devices (Millipore, Eschborn, Germany) prior to electrophoresis. Allele bands were picked from the gel with a BandPick™ device (Elchrom Scientific) and directly transferred to the sequencing reaction mixture without any further purification, whereby each gel slice revealed a volume of approximately 5 µL. The removal of residual dye terminators was carried out with Centri-Sep columns (Princeton Separations, Adelphia, NJ) according to the manufacturer's protocol. Electrophoresis was performed on an ABI Prism 310 Genetic Analyzer using POP6 and a 61-cm capillary array. Data were analyzed with the Sequencing Analysis 3.7 software and edited by SeqEd v1.0.3 software (all Applied Biosystems).

Preliminary Allelic Ladder

Most commonly occurring alleles were selected for ladder design. Therefore, saliva of each corresponding homozygous animal was amplified in a total volume of 50 µL under conditions as described and PCR products were purified by Microcon-30 devices (Millipore) to remove the excess of primer. Equal volumes of purified products were mixed to form the preliminary ladder, separated electrophoretically, and analyzed with the internal size standard Genescan® 400HD [ROX] as described.

Nomenclature

The system of the allele nomenclature proposed in this paper is based on the recommendations reported for human DNA typing (24,26) including complex STR markers (27). The nomenclature presented reflects the number of tandem repeat motifs in the case of simple and compound repeat sequences. In the case of complex repeat motifs, including variable single base stretches (e.g., polyA) and di-, tri-, tetra-, and oligomeric repeats, the whole variable sequence was used for nomenclature assuming the published general repeat motif.

TABLE 1—*Primer sequences and PCR product size.*

STR Locus Name/*Locus Symbol*	Primer Sequence (5′–3′)	Reference	Size Range (bp)	Number of Alleles
PEZ3/*D19S8*	CAATATGTCAACTATACTTC	(16)	95–143	11
	*CACTTCTCATACCCAGACTC	(16)		
PEZ6/*D27S4*	*ACACAATTGCATTGTCAAAC	(16)	168–205	18
	ATGAGCACTGGGTGTTATAC	(16)		
PEZ8/*D17S1*	†TATCGACTTTATCACTGTGG	(16)	219–247	16
	ATGGAGCCTCATGTCTCATC	(16)		
PEZ10/*D14S4*	‡gTTCATTGAAGTATCTATCC	(16)	266–339	19
	†CCTGCCTTTGTAAATGTAAG	(16)		
FHC2161/*D21S1*	*TCAGCAAGAAACCCTCCAGT	(15)	234–270	10
	CATTCCCAACGGAGGACTCT	(15)		
FHC2328/*D29S5*	†ACCAGGTAGTTTTCAGAAATGC	(32)	177–217	10
	AGTTATGGGACTTGAGGCTG	(32)		

*HEX-labeled strand.
†FAM-labeled strand.
‡g unlabeled primer was tailed by a single G nucleotide neighboring TT at 5′ affecting an addition of a single A nucleotide to the labeled amplicon at 3′ by the *Taq* polymerase (28).
Locus symbol was available on the Internet at the DogMap-Database (http://www.dogmap.ch).
The number of alleles observed in this study is indicated.

Population Statistics

Saliva samples from a different number of unrelated dogs (n) independent of breed origin were subjected to DNA typing for the STR loci PEZ3 ($n = 108$), PEZ6 ($n = 127$), PEZ8 ($n = 93$), PEZ10 ($n = 114$), FHC2161 ($n = 64$), and FHC2328 ($n = 120$) applying the preliminary allelic ladder and the proposed nomenclature to the interpretation of results.

Results and Discussion

STR Analysis and Sequencing

Saliva samples collected from the dogs contained DNA in an appropriate amount and quality to perform all PCR reactions in singleplex amplification steps for the canine-specific STR loci PEZ3, PEZ6, PEZ8, PEZ10, FHC2161, and FHC2328. Inhibitory effects have not been observed. A modification of the 5′ primer sequence was introduced for the PEZ10 nonlabeled primer affecting the insertion of an additional A nucleotide to the 3′ labeled PCR product by the *Taq* polymerase facilitating genotyping (28,29). In some cases, extra peaks have been observed consisting of stutter peaks one repeat unit shorter than the true allele as well as true length allele peaks (n) or peaks formed by the addition of an extra base by the *Taq* polymerase ($n+1$) during the elongation step of PCR amplification as described for human STR loci (30,31). These extra peaks never reached 15% of the major peak height and did not lead to misinterpretation of results. All amplified PCR fragments were recorded and grouped according to their apparent size by 1 bp steps. Provided that all grouped fragments correspond to one allele, PCR products of appropriate animals were subjected to sequencing reactions as described. Best results were achieved from heterozygous animals whereas amplified alleles were separated by Spreadex EL 600 gel (Elchrom Scientific, Cham, Switzerland) runs prior to sequencing reactions. Using this electrophoresis platform, heterozygous alleles differing merely by 4 bp in size could be cut off the gel and sequenced separately without any further purification steps. For most common alleles, more than one representative fragment was sequenced. If sequence alterations were detected for fragments of the same length originating from different animals, additional alleles would have been investigated. Sequence information was used to assign a proposal to the nomenclature of alleles.

Allele Assignment and Nomenclature

Tetranucleotide repeat sequences are most commonly used for forensic purposes as they produce PCR artifacts described as stutter bands or slippage artifacts to a lesser extent than dinucleotide repeats (30). All STR loci presented in this study have been described as revealing tetranucleotide repeat units (15,16,32), with the exception of PEZ3 described in contradictory reports as tetranucleotide (16) or trinucleotide (22) repeat units, respectively. More detailed loci information are available on the Internet at the DogMap-Database (http://www.dogmap.ch), including locus symbol, chromosomal localization, primer sequences, observed number of alleles, and bibliographic information. The proposed nomenclature for the STR loci PEZ3, PEZ6, PEZ8, PEZ10, FHC2161, and FHC2328 presented in this study is adopted from the recommendations of the International Society of Forensic Genetics (formerly the International Society of Forensic Heamogenetics) (24) based on the number of repeat units within the amplified fragment. In all cases investigated, the variable repeat structure was obvious and could be used to group loci into three classes as recently described (23) for 15 polymorphic canine-specific STR loci (simple, compound, and complex STRs).

PEZ3

A total number of 11 different amplified fragment groups could be detected by the typing of 108 unrelated dogs. Representatives of nine groups were sequenced, and they revealed the sequence as listed in Fig. 1. The repeat motif consists of a compound trimeric repeat structure of (AAA), (GAA), and (GCA) neighboring repeats. An ambiguous nucleotide N (A to G transition) was detected 3′ of the forward primer-binding site without any influences on the fragment length and nomenclature. The numeric nomenclature of alleles only represents the number of repeats observed within the amplified fragment. Several different repeat motifs with the same total number of repeats were found for single fragment groups not differing in size: e.g., the fragments consisting of 116 bp revealed the repeat sequences $\ldots(AAA)_1(GAA)_{16}(GCA)_5\ldots$ as well as $\ldots(GAA)_{15}(GCA)_7\ldots$ both with the total number of 22 repeats, both assigned as allele 22. The occurrence of allele variants sharing electrophoretic mobility, revealing the same sequence size but different repeat patterns, is well known and is described for canine (23) as well as human genotyping (27,33,34). According to the considerations from the European DNA profiling group (EDNAP), this phenomenon does not affect interpretation (26). Observed alleles consisted of 22–31 total number of repeats. One further rare allele with 15 repeats was typed in our study. The previously described tetrameric repeat structure (16) for PEZ3 could not be confirmed; rather a trimeric repeat structure was obvious. Allele 27 was most abundant ($f > 0.25$) followed by alleles 23, 24, 25, 26 ($f > 0.1$).

PEZ6

Sequence data and a proposed nomenclature for the STR locus PEZ6 were described recently (23) based on the total region consisting of $G(A)_n$ pattern, including invariant portions of the repeat and repeat subunits, as determined within the sample of 131 dogs. The approach leads to an arbitrary tetrameric repeat structure resembling intermediate repeat numbers designated by a suffix (x.3) for the most commonly occurring alleles. The sequences obtained in the current study are consistent with those published (23). Here, we present a new proposal to the allele nomenclature based exclusively on the variable sequence (Fig. 2). A total number of 18 different amplified fragment groups were detected by the typing of 127 unrelated dogs. Two different types of alleles could be distinguished, revealing a constant region (CR) and an 11 bp block (α11) 5′ of CR or a 13 bp block (β13) 3′ of CR, respectively. Both types share an A stretch with a variable number of nucleotides at 5′ and a (GAAA) tetrameric repeat motif varying in the number of repeats at 3′. In single cases, an incomplete repeat of three nucleotides (GAA) was observed leading to intermediate alleles (x.3). The most commonly occurring alleles contained the α11 sequence leading to integer numbers, whereas more rare alleles contained the β13 sequence leading to intermediate numbers (x.1 and x.2) in the nomenclature. The alleles 17, 18, and 19 were most abundant ($f > 0.15$). This new nomenclature can also be adapted to the 131 PEZ6 genotypes obtained in (23). Furthermore, the allele frequency distributions between the two-studied populations are congruent as far as the abundant alleles are concerned.

PEZ8

A total number of 16 different amplified fragment groups could be detected by the typing of 93 unrelated dogs. Representatives of seven groups were sequenced, and they revealed the sequence as listed in Fig. 3. The variable region consists of a simple tetrameric

*CAATATGTCAACTATACTTC*N*ATAT**(AAA)$_m$(GAA)$_n$(GCA)$_o$(GAA)$_p$(GCA)$_q$**GCGAG*GAGTCTGGGTATGAGAAGTG*

bp[1]	N*	(AAA)$_m$	(GAA)$_n$	(GCA)$_o$	(GAA)$_p$	(GCA)$_q$	allele
116	A	1	16	5			**22**
116	G		15	7			**22**
119	A	1	17	5			**23**
122	A	1	18	5			**24**
122	G		17	7			**24**
125	G		18	7			**25**
125	A	1	19	5			**25**
128	G		19	7			**26**
131	G		20	7			**27**
134	G		22	6			**28**
137	A	1	18	5	1	4	**29**
140	A	1	18	6	1	4	**30**

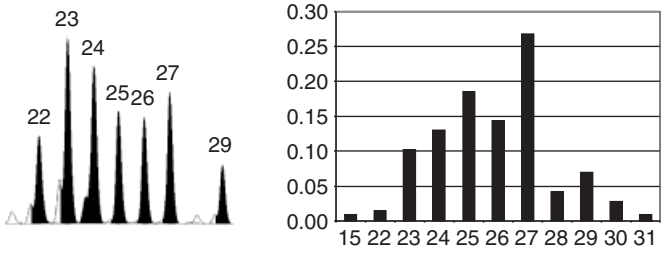

FIG. 1—*PEZ3 sequence data, allele frequencies and preliminary allelic ladder, N* A/G transition, underlined primer sequences, bold polymorphic sequence, bp[1] fragment length of the sequenced allele. Example for allele nomenclature based on a trimeric repeat motif: allele 23 depends on 1 (AAA) [1 repeat]+17 (GAA) [17 repeats]+5 (GCA) [5 repeats] = 1+17+5 = 23.*

repeat structure of (GAAA) and an A stretch with a variable number of nucleotides at the 5′ end. Single A–G transitions were found to produce a (GAGA) repeat not influencing fragment length and nomenclature. The numeric nomenclature of alleles represents the number of repeats observed within the amplified fragment, including the A-stretch region (4 or 5 bp). Occurring

*ATGAGCACTGGGTGTTATAC*TATATGTTGGCAAATCGAACTTCAATAAA**(A)$_m$(GAAGAAAGAAA)$_{\alpha 11}$**GAAGAAAG
AAGGAAAGAGAAAGAAAAA**(GAAAGAGAAAGAA)$_{\beta 13}$(GAAA)$_{nop}$**ACCTTTCAAACTTCTA*GTTTGACAATGCAATT
GTGT*

bp[1]	(A)$_m$	α11/β13	(GAAA)$_n$	(GAA)$_o$	(GAAA)$_p$	allele
174	5	β	11			**15.2**
176	5	α	12			**16**
180	5	α	13			**17**
184	5	α	14			**18**
186	5	β	14			**18.2**
187	5	α	10	1	4	**18.3**
188	5	α	15			**19**
189	4	β	15			**19.1**
193	4	β	16			**20.1**
197	4	β	17			**21.1**
201	4	β	18			**22.1**
205	4	β	19			**23.1**

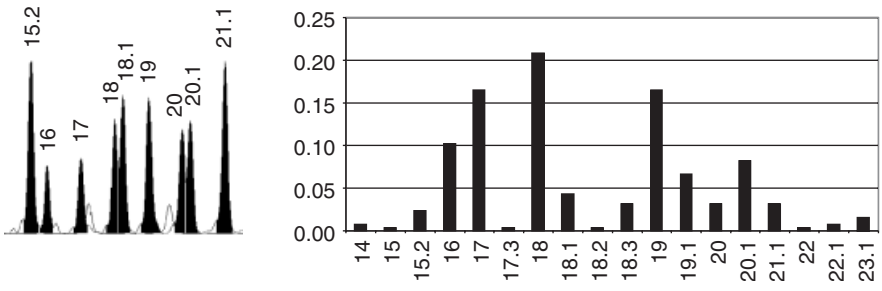

FIG. 2—*PEZ6 sequence data, allele frequencies and preliminary allelic ladder, underlined primer sequences, bold polymorphic and variable sequences, bp[1] fragment length of the sequenced allele. Example for allele nomenclature based on a tetrameric repeat motif: allele 16 depends on 12 (GAAA) [12 repeats]+5 (A) [1.1 repeats]+α11 [2.3 repeats] = 12+1.1+2.3 = 16.*

*ATGGAGCCTCATGTCTCATC*AGGCTCCCTGCTCACCATGGAGTCTACTTCTCCCTCTCAGTCTGCCCCTCCCTC
CACTCTCTTTCTCTCAAATAAATAAACAAAATCTAAAAAAAAG**(A)$_m$(GAAA)$_n$(GAGA)$_o$(GAAA)$_p$**GGAGAAAG
ACAAAGTACACCTCCAATTTAGCCTC*CCACAGTGATAAAGTCGATA*

bp[1]	(A)$_m$	(GAAA)$_n$	(GAGA)$_o$	(GAAA)$_p$	allele
223	4	12			**13**
224	5	12			**13.1**
227	4	13			**14**
232	5	14			**15.1**
235	4	10	1	4	**16**
236	5	15			**16.1**
239	4	11	1	4	**17**

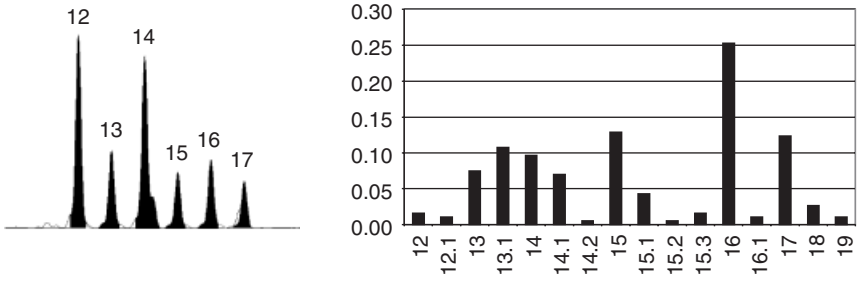

FIG. 3—*PEZ8 sequence data, allele frequencies and preliminary allelic ladder, underlined primer sequences, bold polymorphic sequence, bp[1] fragment length of the sequenced allele. Example for allele nomenclature based on a tetrameric repeat motif: allele 13.1 depends on 5 (A) [1.1 repeats]+12 (GAAA) [12 repeats] = 1.1+12 = 13.1.*

alleles consisted of 12–19 total number of repeats and several intermediate alleles (*x*.1, *x*.2, and *x*.3). Allele 16 was most abundant ($f > 0.25$).

PEZ10

A total number of 19 different amplified fragment groups could be detected by the typing of 114 unrelated dogs. Representatives of 15 groups were sequenced and revealed the sequence as listed in Fig. 4. Additional representatives of two rare fragment groups (274 bp and 289 bp; not added to the population data shown in this work) deriving from an American population of dogs were sequenced providing more detailed data of intermediate alleles. The G-tail at the 5′ unlabeled primer remained unconsidered in nomenclature and sequence data presented. The amplified fragments consist of a complex hypervariable region mainly based on tetrameric motifs of (AAAA), (GAAA), and (GAGA) repeats as well as a pentameric motif of (GAAAA) repeat. One allele revealed an additional dimeric (AA) motif in between the (GAAA) repeats. A final hexamer motif GAAAAA sequence common to all alleles investigated was added to the variable region to obtain integer numbers for the majority of alleles in the nomenclature. A G–T transition was found for small alleles (alleles 16.3, 17.3, 18.3) within the 3′ flanking region not considered by the nomenclature. The nomenclature was based on assuming a general tetrameric repeat structure. Typing of alleles consisting of more than two (AAAA) repeat motifs (alleles 16.3, 17.3, 18.3, and 22.2) revealed slippage artifacts caused by the *Taq* polymerase in the resulting electropherograms, and should be taken into consideration in interpretation (data not shown). Alleles 21–28 were most abundant ($0.08 < f > 0.15$), whereas intermediate alleles appeared very rarely.

FHC2161

A total number of 10 different amplified fragment groups could be detected by the typing of 64 unrelated dogs. Representatives of seven groups were sequenced, and they revealed the sequence as listed in Fig. 5. The variable region consists of a simple tetrameric repeat structure of (GAAA) with a variable number of repeats ranging from 12–21. Intermediate alleles have not been detected yet. Alleles 14–17 were most abundant ($0.15 < f > 0.3$)

FHC2328

A total number of 10 different amplified fragment groups could be detected by the typing of 120 unrelated dogs. Representatives of nine groups were sequenced and revealed the sequence as listed in Fig. 6. The variable region consists of a simple tetrameric repeat structure of (GAAA) with the insertion of a 4 bp sequence starting with an ambiguous nucleotide N (A–G transition) followed by AGA treated as a tetrameric repeat in the nomenclature. Occurring alleles consisted of 11–21 total number of repeats. Intermediate alleles have not been detected yet. Allele 14 was most abundant ($f > 0.25$)

Preliminary Allelic Ladders

The use of an allelic ladder as a control supplemented by a consensus nomenclature delivering correct designation of alleles in samples is strongly recommended, especially if laboratories are to compare data (26) such as population statistics. Their advantage in cases in which STR loci consist of a large number of allelic variants with the same fragment length but different repeat motifs is described (27) and therefore essential for a correct typing of the STR locus PEZ3. Allelic ladders (Figs. 1–6) were composed containing all commonly occurring alleles as well as some rare intermediate alleles (e.g., for the STR locus PEZ6). The rungs in the allelic ladder should be related to the repeat motif size of the locus itself. As not all alleles have been sequenced, we used each allelic ladder merely as a preliminary internal standard facilitating the interpretation of results for the recording of population data presented in this study.

gTTCATTGAAGTATCTATCCAAGACAAGC(AAAA)$_m$(GAAA)$_n$(AA)$_o$(GAAA)$_p$(GAGA)$_q$(GAAAA)$_r$(GAAAAA)$_s$**GAA
AAAAN***AAAAATTTTAATGTTTTAAAAACACTGAAATGCACAAAAAGTGTATATGTGTATATTCCCATAATCCTACA
CTTTTAAAATCATATAAAATAAGTTTCTTCTCCATCCATTCATCCAAAGTATATACCCAATATTTTCTTACATTTAC
AAAGGCAGG

bp[1]	(AAAA)$_m$	(GAAA)$_n$	(AA)$_o$	(GAAA)$_p$	(GAGA)$_q$	(GAAAA)$_r$	(GAAAAA)$_s$	N*	allele
266	3	11				1	1	T	**16.3**
270	3	12				1	1	T	**17.3**
274	3	13				1	1	T	**18.3**
275	2	13				2	1	G	**19**
279	1	14			1	2	1	G	**20**
283	1	15			1	2	1	G	**21**
287	1	16			1	2	1	G	**22**
289	3	2	1	12	1	2	1	G	**22.2**
291	2	16			1	2	1	G	**23**
295	1	18			1	2	1	G	**24**
299	1	19			1	2	1	G	**25**
303	1	20			1	2	1	G	**26**
307	1	21			1	2	1	G	**27**
308	1	20			1	3	1	G	**27.1**
311	1	22			1	2	1	G	**28**
315	1	23			1	2	1	G	**29**
319	1	24			1	2	1	G	**30**

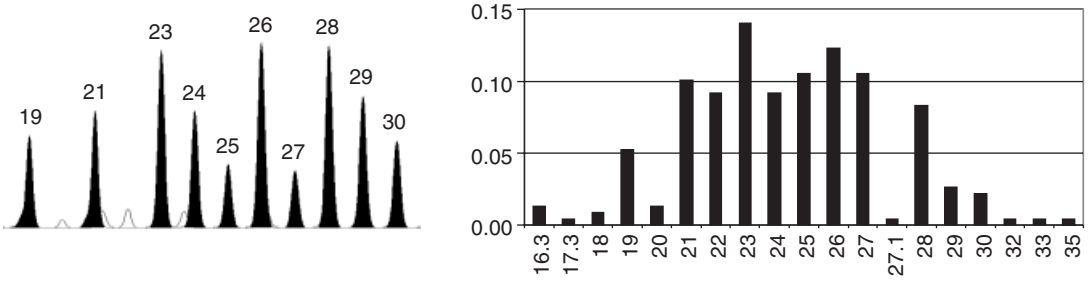

FIG. 4—*PEZ10 sequence data, allele frequencies and preliminary allelic ladder, N* T/G transition, **g unlabelled primer was tailed by a single G nucleotide, underlined primer sequences, bold polymorphic sequence, bp[1] fragment length of the sequenced allele. Example for allele nomenclature based on a tetrameric repeat motif: allele 16.3 depends on 3 (AAAA) [3 repeats]+11 (GAAA) [11 repeats]+(GAAAA) [1.1 repeats]+(GAAAAA) [1.2 repeats] = 3+11+1.1+1.2 = 16.3.*

TCAGCAAGAAACCCTCCAGTTACATCCACATTAAAGTAAATGGTAGGTATATATCCTTTCTGATGGCTGAGTAA
TGTTCCAATGTTTTTTTTTTCTTTTTTTTCTAGCAGAAGAAAAAGAAAAA(**GAAA**)$_n$GAAGAAAACAACCAAGGA
AAGAGAAAAAAGGAAAAAGAGCGAGAGAGTCCTCCGTTGGGAATG

bp[1]	(GAAA)$_n$	allele
238	13	**13**
242	14	**14**
246	15	**15**
250	16	**16**
254	17	**17**
258	18	**18**
262	19	**19**

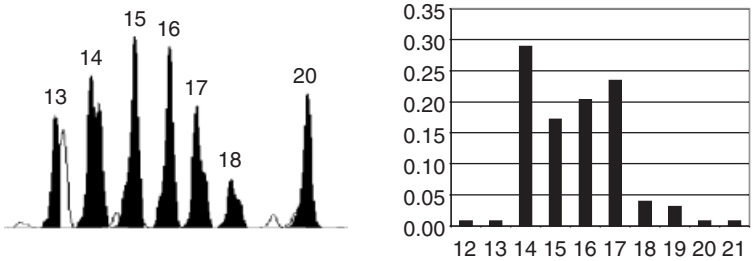

FIG. 5—*FHC2161 sequence data, allele frequencies and preliminary allelic ladder, underlined primer sequences, bold polymorphic sequence, bp[1] fragment length of the sequenced allele.*

*ACCAGGTAGTTTTCAGAAATGC*TGTTGTATTTAATACTTATTACTGTATCATATGTCAAAAAAATAAAGGAAAG
AAAAAAAGAAAGAAAGAAAGAAAGAAGAAAGA**(GAAA)$_n$N***AGA**(GAAA)$_o$**GAAAAAG*CAGCCTCAAGTCCCAT
AACT*

bp[1]	(GAAA)$_n$	N*AGA	(GAAA)$_o$	allele
181	7	G	4	**12**
189	9	G	4	**14**
193	10	G	4	**15**
197	11	G	4	**16**
201	12	A	4	**17**
205	13	A	4	**18**
209	14	A	4	**19**
213	15	A	4	**20**
217	16	A	4	**21**

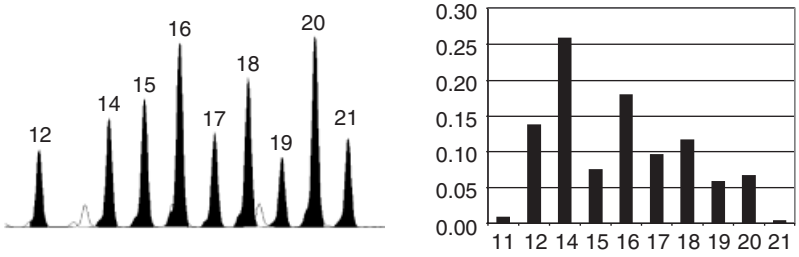

FIG. 6—*FHC2328 sequence data, allele frequencies and preliminary allelic ladder, underlined primer sequences, N* A/G transition, bold polymorphic sequence, bp[1] fragment length of the sequenced allele. Example for allele nomenclature based on a tetrameric repeat motif: allele 12 depends on 7 (GAAA) [7 repeats]+1 (GAGA) [1 repeat]+4 (GAAA) [4 repeats] = 7+1+4 = 12.*

Summary

In addition to recently published results (12,23) and with the effort of standardization presented in this study, a total number of 21 precisely described canine-specific STR markers is now available. Further steps concerning the determination of a standard set of loci sufficient for canine individualization in forensics and the development of allelic ladders for each marker must be taken into consideration. The use of a standard DNA sample generally available (e.g., DNA from a ubiquitous canine cell line) as a control could be useful and should be introduced. Such a sample can help to adapt canine typing results in different laboratories to an internationally accepted allele assignment based on the repeat pattern of polymorphic microsatellites as recommended by the ISFH. Thereby, the exchange of data between single laboratories will be feasible, and published population data can be used by the whole forensic community.

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