Mammalian Genome 6, 11–18 (1995).

Mammalian Genome

Variation of short tandem repeats within and between species belonging to the *Canidae* family

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Received: 17 May 1994 / Accepted: 16 August 1994

Abstract. Frequency distribution and allele size in 20 canine microsatellite loci were analyzed in 33 flat-coated retrievers, 32 dachshunds, 10 red foxes, and 10 Arctic foxes. Overall, the major difference between the two dog breeds was the relative allele frequencies rather than the size ranges of alleles at the individual locus. The average heterozygosity within the two dog breeds was not significantly different. Since the average heterozygosity at several polymorphic loci is a relative measure of heterogeneity within the population, analysis of heterozygosity within microsatellite loci is suggested as a measure for the diversity of populations. Eighty percent (16 of 20) of the canine microsatellite primer pairs amplified corresponding loci in the two fox species. This reflects a very high sequence conservation within the *Canidae* family relative to findings in, for instance, the *Muridae* family. This indicates that it will be possible to utilize the well-characterized fox karyotype instead of the dog karyotype as a step towards physical mapping of the dog genome. Analysis of exclusion power and probabilities of genetic identity between unrelated animals by use of the seven most informative loci demonstrated that it will be possible to assemble a panel of microsatellite loci that is effective for parentage analysis in all breeds.

Introduction

Repetitive sequences can be found scattered throughout the genomes of higher organisms. A subclass of these sequences consists of iterations of 1–5 base pairs commonly referred to as simple sequence repeats or microsatellites (Hamada et al. 1982; Litt and Luty 1989; Weber and May 1989). The repeat motif most widely found in eucaryotic genomes is $(dC\text{-}dA)_n$–$(dG\text{-}dT)_n$ (Stallings et al. 1991; Hamada et al. 1982). These microsatellite loci have been shown to be highly polymorphic, because of variation in the number of repeat units (Litt and Luty 1989; Weber and May 1989). They can be amplified faithfully with the polymerase chain reaction (PCR), enabling precise allele designation both in pedigree and population surveys. This makes them applicable both in gene mapping, population genetics, and for individual identification.

Although the chromosomal distribution of $(dC\text{-}dA)_n$–$(dG\text{-}dT)_n$ microsatellites have been found to be highly conserved in mammalian genomes, there does not appear to be extensive conservation of dinucleotide repeat positions in evolutionary distant species (Stallings et al. 1991). However, microsatellite loci are often conserved among related species of mammals. Between closely related species, as for instance cattle and sheep, it has been shown that roughly 60% heterologous PCR primers can be used (Moore et al. 1991), whereas in the case of mouse and rat only 12–16% heterologous primers are successful (Kondo et al. 1993).

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The ease of isolation and utility of microsatellites have made it possible to investigate and apply them to a wide range of different species in which extensive basic genetic analysis has not previously been feasible. Currently efforts are made towards a characterization of the canine genome, based on the use of microsatellites (Ostrander et al. 1993; Holmes et al. 1993). We have isolated microsatellites from two canine partial genomic plasmid libraries in order to contribute towards the construction of a linkage map. As the knowledge about the frequency distribution of microsatellite alleles within and between canine species currently is sparse, we have investigated two different dog breeds and two different fox species with 20 canine microsatellite primer pairs to generate data on similarities and divergence of populations within the *Canidae* family. Furthermore, the allele frequencies in the two dog breeds were used to evaluate the efficiency of the microsatellites with regard to parentage control.

Materials and methods

Samples

EDTA-stabilized blood was collected from 32 unrelated dachshunds (7 wire-haired standard, 2 wire-haired dwarf, 14 short-haired standard, 6 long-haired standard, and 3 long-haired dwarf), 33 randomly chosen flat-coated retrievers, 10 unrelated farmed red foxes (*Vulpes vulpes*), and 10 Arctic foxes (*Alopex lagopus*) randomly sampled in Greenland. Pedigrees of 5–12 animals of varying dog breeds were used for analysis of Mendelian segregation. DNA was prepared by using a modification of the salting out procedure described by Miller et al. (1988; Fredholm et al. 1993).

Cloning and sequencing of microsatellites

High-molecular-weight DNA was isolated from a golden retriever male as described above. The DNA was partially digested with *RsaI*/*AluI*/*Hae*III (library 1) or *RsaI*/*AluI*/*Dra*I (library 2). Fragments ranging in size from 300 to 600 bp were purified by preparative agarose gel electrophoresis (Bio 101) and blunt-end ligated into *Eco*RV-digested plasmid vector pBluescript KS + II (Stratagene). Transformation into high-efficiency, competent *E. coli* Sure^R cells (Stratagene) was performed according to standard procedures (Maniatis et al. 1982).

Nitrocellulose colony lifts were screened by hybridization to synthetic $(dG\text{-}dT)_9$ and $(dC\text{-}dA\text{-}dG)_6$ oligonucleotide, which had been end labeled to a specific activity of 5×10^9 cpm/μg DNA with γ^{32}P-dATP. Prehybridizations were performed in 6 × SSC, 1 × Denhardts, 0.5% SDS, 100 μg/ml sheared and denatured herring sperm, 0.05% $Na_4P_2O_7$ at 37°C for 2 h. Hybridizations were carried out in 6 × SSC, 1 × Denhardts, 20 μg/ml tRNA (Sigma) and 0.05% $Na_4P_2O_7$ at 37°C. Filters were washed at 37°C in 6 × SSC, 0.05% $Na_4P_2O_7$ for 1 h and exposed to Kodac X-omat™ AR film for 20 h at −70°C with a Cronex Lightning Plus intensifying screen.

DNA sequencing was performed on alkaline denatured plasmid DNA by the dideoxynucleotide chain termination method (Sanger et al. 1977) with modified T7 polymerase (USB). PCR products were sequenced directly after treatment with ExonucleaseI/shrimp alkaline phosphotase by

use of the Sequenase PCR product sequencing kit supplied by Amersham™.

Detection of microsatellite polymorphism

Flanking primers for the amplification of the individual microsatellites were designed with OLIGO™ version 3.4 software according to principles described previously (Fredholm et al. 1993). Primers were initially tested in a standard PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 0.1% gelatine) with 1.5 mM $MgCl_2$, 200 µmol of each dNTP, 3 pmol of each primer (the reverse primer was 5′ end labeled with γ^{32}P-dATP by use of T4 polynucleotide kinase (Promega), 50 ng genomic DNA, and 0.625 U SuperTaq (Stratagene). PCR was carried out on a Hybaid Thermal cycler with the following cycles (Touch Down): 1 × 94°C, 3 min; 2 × T_m + 15°C, 1 min, 72°C, 30 s, 94°C 30 s; 2 × T_m + 14°C, 1 min, 72°C, 30 s, 94°C 30 s; 2 × T_m + 13°C, 1 min, 72°C, 30 s, 94°C 30 s; 2 × T_m + 12°C, 1 min, 72°C, 30 s, 94°C 30 s; 2 × T_m + 11°C, 1 min, 72°C, 30 s, 94°C 30 s; 25 × T_m + 10°C, 1 min, 72°C, 30 s, 94°C 30 s; 1 × T_m + 10°C, 1 min, 72°C, 5 min. Five µl of the amplification product was mixed with formamide dye and separated on a 6% denaturing polyacrylamide gel. Optimal conditions for each primer pair were established by adjusting the $MgCl_2$ concentration and the annealing temperature.

Allele identification and allele sizes were determined by comparison with an adjacent DNA sequencing ladder, and by loading a common set of amplified DNA fragments on all gels.

Computation and statistics

The results obtained from genotyping of the unrelated animals were analyzed according to the Freq and Means procedures of the SAS program (SAS Institute Inc., North Carolina).

Exclusion power of the microsatellites and probability for genotypic identity was calculated according to formulas adapted from Jamieson (1966) where there are n alleles at each locus with frequencies $p_i \ldots p_n$. Exclusion power for each microsatellite when both parents are available was calculated as: $P = \Sigma p(1-p)^2 - \Sigma(p_i \times p_j)^2 \times [4 - 3(p_i + p_j)]$. Exclusion power for each microsatellite when only one of the parents is available was calculated as: $P = \Sigma 2p_ip_j(1-p_i-p_j)^2 + \Sigma p_i^2(1-p_i)^2$. The combined exclusion probability was calculated as P = 1 – (1 – P for locus 1)(1 – P for locus 2) . . . (1 – P for locus n). Genetic identity by chance at a given microsatellite locus was calculated as $P_{ID} = \Sigma p_i^4 + \Sigma 4p_i^2p_j^2$, and the combined genetic identity as $P_{comb.ID}$ = (P_{ID} for locus 1) (P_{ID} for locus 2) . . . (P_{ID} for locus n).

Results

The microsatellite loci analyzed in the present investigation were selected from a total of 73 sequenced loci. Thirty-nine of the clones representing these loci were isolated from library 1 constructed from *Rsa*I/*Alu*I/*Hae*III-digested genomic DNA, while 34 clones were isolated from library 2 constructed from *Rsa*I/*Alu*I/*Dra*I-digested DNA. The overall distribution among the three categories perfect, imperfect, and compound repeat sequences (Weber 1990) was in accordance with the distributions reported in, for instance, human (Weber 1990) and pig (Winterø et al. 1992); that is 68%, 21%, and 11% respectively in the three categories. However, when only the clones from library 2 are taken into consideration, the distribution corresponded to the findings obtained from analysis of canine microsatellites (Ostrander et al. 1993), that is, 81% perfect repeat sequences, 28% imperfect repeat sequences, and 8% compound repeat sequences.

In total, 20 primer pairs designed for analyzing canine microsatellites were used for amplification of homologous sequences in two different dog breeds and two fox species. All microsatellites investigated were demonstrated to be inherited in a Mendelian fashion by the use of pedigrees of varying dog breeds (results not shown). Table 1 gives the laboratory designation of the 20 microsatellite loci, the primer sequences, and the annealing temperature and Mg^{2+} concentration established by optimization of each set of primers.

Allele sizes and frequency of alleles in each microsatellite based on typing of 33 flat-coated retrievers, 32 dachshunds, 10 red foxes, and 10 Arctic foxes are shown in Table 2. Comparison of the microsatellite loci between the two dog breeds revealed that all microsatellite loci except *CPH4* are polymorphic in both breeds, with the number of alleles varying from 2 to 14. The average number of alleles is 4.5 and 5.6 in flat-coated retrievers and dachshunds respectively. In general, the overall size range of alleles detected for each locus is similar for the two dog breeds investigated. Nonetheless, breed-specific alleles are observed in all loci except *CPH15*. Although the number of alleles observed in the two breeds is very similar, the frequency distribution of these alleles varies significantly. The most obvious feature of the frequency distribution is that in all but 5 microsatellites (*CPH1, CPH4, CPH18, CPH19, CPH20*) the frequency of the commonest allele deviates between the two breeds.

The degree of heterozygosity, reflecting the fraction of individuals with two different alleles, differs substantially between breeds within the individual microsatellite. However, the average heterozygosity within the two dog breeds is not significantly different. In flat-coated retrievers it is 52%, while it is 55% in dachshunds. Allele non-amplification, or silent alleles, as reported by others (Koorey et al. 1993), was not observed in the family analysis performed. They could not be uncovered by the population investigation since the sample sizes are too small to allow the data to be analyzed for conformity to Hardy-Weinberg expectations relative to the number of alleles in each microsatellite. Consequently, the results obtained on average heterozygosity might be underestimated. Nevertheless, because of the large number of loci investigated, the average heterozygosity can be used as an indicator of the genetic diversity within the breeds.

Sixteen of the 20 loci investigated were amplified in both the red fox and the Arctic fox with the primers designed on the basis of the canine sequences (Table 2). Thirteen of these are polymorphic, while three are monomorphic in each species. Monomorphism can most likely be explained by the loss of repeat units (relative to the length of the dog sequence). To investigate this further, we sequenced PCR products from the CPH12 locus in 1 flat-coated retriever, 1 dachshund, 1 red fox, and 1 Arctic fox representing the 206, 202, 194, and 194 alleles respectively. The corresponding repeat blocks turned out to be: $(AC)_{16}$ in the 206 allele in the flat-coated retriever, $(AC)_{14}$ in the 202 allele in the dachshund, $(AC)_7$ in the 194 allele in the red fox, and $(AC)_4AG(AC)_2$ in the 194 allele in the Arctic fox. Thus, monomorphism in the Arctic fox can be explained by the C-to-G transversion, interrupting the stretch of perfect repeats.

In the foxes the overall numbers of alleles in the polymorphic loci are comparable to the numbers found in the two dog breeds. This is, however, probably not illustrative for the populations because of the small sample sizes. Nevertheless, it is apparent from Table 2 that both allele frequency distribution and distribution of allele sizes in the foxes are quite different from the findings in dogs. Furthermore, the most common allele deviates between the Arctic fox and the red fox, and the diversity between the two species is further accentuated by a tendency towards a difference in size range in a number of the microsatellites (*CPH1, CPH5, CPH10, CPH11, CPH12, CPH16, CPH18*).

Since microsatellite alleles can be assigned to specific loci and, therefore, allele frequencies can be ascertained, conventional parentage indices and probabilities for individual identification can be calculated. To determine the relative utility of the microsatellites in parentage determinations, we calculated exclusion power on the basis of the seven most informative loci in each of the dog breeds (*CPH2, CPH3, CPH6, CPH9, CPH11, CPH14,* and *CPH16* in flat-coated retrievers; *CPH3, CPH6, CPH8, CPH10, CPH11,*

Table 1. Microsatellite primer sequences and PCR amplification conditions.

Microsatellite locus	Primers	PCR conditions: mM $MgCl_2$; °C
CPH1	5′GCCTAGCCCAGTGAAAGTTAAC3′ 5′TTCCAATGCCTGATAACTGAGA3′	2.5; 58
CPH2	5′TTCTGTTGTTATCGGCACCA3′ 5′TTCTTGAGAACAGTGTCCTTCG3′	3.0; 57
CPH3	5′CAGGTTCAAATGATGTTTTCAG3′ 5′TTGACTGAAGGAGATGTGGTAA3′	2.0; 58
CPH4	5′ACTGGAGATGAAAACTGAAGATTATA3′ 5′TTACAGGGGAAAGCCTCATT3′	2.0; 59
CPH5	5′TCCATAACAAGACCCCAAAC3′ 5′GGAGGTAGGGGTCAAAAGTT3′	2.0; 57
CPH6	5′CATTGGCTGTTTGACTCTAGG3′ 5′ACTGATGTGGGTGTCTCTGC3′	2.0; 58
CPH7	5′ACACAACTTTCCATAATACTTCCCA3′ 5′ATCAATGCTCTCCTCCCCAG3′	1.5; 62
CPH8	5′AGGCTCACAATCCCTCTCATA3′ 5′TAGATTTGATACCTCCCTGAGTCC3′	3.0; 58
CPH9	5′CAGAGACTGCCACTTTAAACACAC3′ 5′AAAGTTCTCAAATACCATTGTGTTACA3′	3.0; 59
CPH10	5′TGCAAGACATGATATGTGTTTATG3′ 5′GTGAGAGGCAAAAATGACCA3′	3.0; 58
CPH11	5′TTAATGTTTCTCCGATGTTTACAT3′ 5′GAAAGCCAAGCATGACTAGG3′	3.0; 58
CPH12	5′GGCATTACTTGGAGGGAGGAA3′ 5′GATGATTCCTATGCTTCTTTGAG3′	3.0; 58
CPH13	5′AGGGGTCTTGAACTATGTTCTAGAC3′ 5′GAAGAGGCTTTGAGTTTCAGTTG3′	1.5; 62
CPH14	5′GAAAGACAATCCCTGAAATGC3′ 5′ACCCCATTTATGAGAATCATGT3′	1.5; 62
CPH15	5′GCCTATATAAAATGCATCTGAGC3′ 5′CCGTGACTCCTGTCTTCTGAC3′	3.0; 58
CPH16	5′CTACACCAGTTAGGGAATCTAGC3′ 5′CAGATTCAAATCCACTCTCAGAC3′	2.0; 60
CPH17	5′GAGAACAAAAGTCCCATGCAC3′ 5′GCATTGATGCTAATGCAAATG3′	1.5; 60
CPH18	5′CAGAGATACGTCTTGACACTAGCAGA3′ 5′AGCAGACAGTGGGCCATGTT3′	1.5; 62
CPH19	5′AGTCCTATTGTGAAATTCAGCC3′ 5′CGTATTTTAGGCAATGGCAC3′	3.0; 59
CPH20	5′GGTTCCTGGGCCCAATTTCA3′ 5′TGGTGTGGTGAATCGCTTGTTG3′	3.0; 59

CPH13, and *CPH16* in dachshunds). When both parents are available, the exclusion power is 99.3% and 99.9% for flat-coated retrievers and dachshunds respectively. Corresponding figures are 93.7% and 98.9% when only one of the parents is available for testing (Table 3). The probability of two unrelated animals sharing the microsatellite alleles in all seven systems is 7×10^{-7} and 2×10^{-9} in the two breeds respectively.

Discussion

$(GT)_n$ repeats are ubiquitously distributed throughout eukaryotic genomes. The three categories of microsatellites described by Weber (1990)—perfect, imperfect, and compound repeat sequences—have been identified in a wide variety of species, albeit of varying abundance. The discrepancy between numbers in each category from the two libraries screened in the present investigation indicates that small deviations in hybridization stringency and/or the enzymes used for digestion of DNA for the libraries can influence the range of clones obtained for sequencing. Although not in accordance with the findings of Ostrander and coworkers (1993), the nature of the $(GT)_n$ blocks in dogs most likely resembles the nature of the human $(GT)_n$ blocks.

Despite the fact that the criteria for selection of loci for further analysis suggested by Hudson and co-workers (1992) were used in this investigation (that is, more than 13 successive repeats), a somewhat lower heterozygosity (only 60% of the microsatellite loci with heterozygosity higher than 0.50 compared with 80% in the human) was found (Table 2). This finding is, however, comparable to findings in the domestic pig (Fredholm et al. 1993), and it can be ascribed to the fact that in these species the populations have limited gene pools and nonrandom mating. The degree of heterozygosity is not significantly different between flat-coated retrievers and dachshunds. The slightly higher diversity in the dachshunds, which also is reflected in a better exclusion power and a smaller chance of genetic identity of unrelated animals, could be ascribed to the fact that this population is less homogeneous. Comparison of heterozygosity, or diversity, between dogs and foxes is not relevant, first because of the small fox population samples, and second because most likely a bias has been introduced by the initial selection of clones from the dog library. The genetic diversity based on average heterozygosity in the two dog breeds is very similar (52% and 55% respectively). Additional analysis and comparisons between breeds and populations could facilitate the utility of diversity as an indication of the gene pools underlying different populations.

Whereas the size range of alleles is similar for the two dog breeds investigated, heterozygosity and frequency distribution of the alleles within the 20 microsatellite loci varies considerably. Thus, in accordance with a similar investigation of human populations (Wall et al. 1993), the major difference between the breeds is not the size range of alleles at the individual loci but their relative frequencies. Overall, there are groups of alleles, at one or more loci, whose frequencies are different among the dog breeds. This is further emphasized by breed-specific alleles. Consequently, appropriate investigations of allele frequencies in the individual breeds would make it conceivable to differentiate between them. This could in rare cases be of interest in forensic investigations. The same general features apply to the allele distribution in the two

Table 2. Allele sizes and allele frequencies of canine microsatellites.

Microsatellite locus	Core sequence	Allele	Flat-coated retriever[a]	Dachshund[b]	Arctic fox[c]	Red fox[d]
CPH1	$(TGG)_3$ TAG$(TGG)_{11}$	125			1.00	
		132				0.30
		135		0.14		
		138				0.70
		141	0.83	0.56		
		144	0.17	0.30		
H^e			**0.21**	**0.56**	**0.00**	**0.40**
CPH2	$(AC)_{15}$	95	0.20			
		97	0.36	0.36		
		99	0.20	0.06		
		103		0.06		
		107		0.52		
		109	0.24			
H^e			**0.94**	**0.47**		
CPH3	$(GA)_2TA(GA)_{17}$	154			0.15	0.05
		158			0.10	0.25
		162	0.29		0.45	0.30
		164			0.15	0.40
		166			0.15	
		168	0.09	0.28		
		172	0.02	0.03		
		174		0.02		
		176	0.32	0.28		
		178	0.20	0.20		
		180	0.02	0.14		
		182	0.08	0.05		
H^e			**0.67**	**0.63**	**0.80**	**0.90**
CPH4	$(TG)_{17}$	118			1.00	1.00
		140		0.05		
		142		0.03		
		144	1.00	0.59		
		146		0.28		
		150		0.05		
H^e			**0.00**	**0.63**	**0.00**	**0.00**
CPH5	$(TG)_{17}$	111		0.03		
		113	0.42	0.28		
		115	0.12	0.06	0.05	
		117	0.06	0.48		
		119	0.39	0.14	0.05	
		127			0.10	
		129			0.10	
		131			0.30	
		133			0.10	
		135			0.05	
		136				1.00
		137			0.10	
		139			0.10	
		141			0.05	
H^e			**0.79**	**0.50**	**0.80**	**0.00**
CPH6	$(CA)_{19}$	107			0.30	
		117				0.10
		119			0.10	
		121				0.05
		122	0.26			
		123			0.15	
		124	0.15	0.09		
		125			0.05	0.35
		126		0.34		
		127			0.15	0.40
		128	0.17	0.18		
		129			0.10	0.10
		130	0.09	0.08		
		131			0.05	
		132	0.30	0.14		
		134	0.03	0.11		
		135			0.10	
		136		0.05		
H^e			**0.73**	**0.63**	**0.70**	**0.80**
CPH7	$(TG)_{16}$	159			0.83	
		163			0.17	0.25
		165		0.03		0.40
		167	0.92	0.03		0.05
		169		0.08		0.30
		171	0.02	0.05		
		173	0.06	0.81		
H^e			**0.15**	**0.25**	**0.33**	**0.50**
CPH8	$(GT)_{18}$	186	0.46	0.17	0.13	0.15
		188	0.02			
		190		0.17		

Continued on next page

Table 2. *Continued.*

		192		0.14		
		194		0.20	0.06	0.40
		196	0.02	0.19		0.05
		198	0.15	0.13	0.13	0.15
		200	0.36		0.56	0.15
		202			0.06	0.10
		204			0.06	
H[e]			**0.58**	**0.56**	**0.38**	**0.70**
CPH9	$(GT)_{18}$	139	0.20			
		141	0.38	0.27		0.10
		143		0.05		
		145		0.03		
		149	0.06	0.59	0.45	
		151	0.05		0.10	0.10
		153	0.32	0.06	0.35	0.40
		155			0.10	0.40
H[e]			**0.79**	**0.63**	**0.90**	**0.70**
CPH10	$(GT)_{17}$	148	0.20	0.30		1.00
		152		0.08		
		154	0.11	0.14	0.90	
		156		0.17		
		158	0.70	0.13	0.10	
		160		0.05		
		162		0.02		
		164		0.13		
H[e]			**0.52**	**0.81**	**0.20**	**0.00**
CPH11	$(TA)_{26}(CA)_{15}$	120				0.15
		122				0.25
		124				0.50
		126				0.10
		144			0.06	
		146	0.02			
		148	0.20	0.13		
		150		0.10	0.33	
		152	0.06	0.03	0.11	
		154	0.53	0.03	0.16	
		156	0.10		0.06	
		158		0.02	0.17	
		160		0.13	0.11	
		162	0.02	0.07		
		164	0.03	0.02		
		166	0.02	0.11		
		170		0.11		
		172		0.07		
		174		0.16		
		176	0.03	0.02		
		178		0.02		
H[e]			**0.64**	**0.67**	**0.44**	**0.70**
CPH12	$(GT)_{16}$	196				0.40
		194			1.00	0.60
		202	0.18	0.12		
		204		0.44		
		206	0.80			
		212	0.02	0.02		
		214		0.11		
		216		0.31		
H[e]			**0.39**	**0.56**	**0.00**	**0.60**
CPH13	$(AC)_5GA(AC)_{16}$	147		0.12		
		151	0.02	0.02		
		153	0.11			
		155		0.43		
		157		0.05		
		159	0.47	0.10		
		161	0.34	0.15		
		163	0.05	0.03		
		165		0.03		
		167	0.02	0.06		
H[e]			**0.65**	**0.53**		
CPH14	$(CCA)_{14}$	182	0.15			
		185	0.03			
		191	0.29	0.11		
		194		0.31		
		197		0.58		
		200	0.30			
		203	0.23			
H[e]			**0.70**	**0.44**		
CPH15	$(AC)_{18}$	147			0.05	
		149			0.05	0.30
		151			0.60	0.35

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Table 2. *Continued.*

		153			0.15	0.35
		155	01.2	0.16	0.15	
		165	0.35	0.59		
		167	0.53	0.25		
H[e]			**0.45**	**0.47**	**0.60**	**0.90**
CPH16	$(AC)_{17}$	149	0.52	0.22		
		155			0.22	0.05
		159			0.05	
		161			0.44	
		163			0.28	
		165		0.27		
		169				0.05
		171	0.18	0.14		
		173	0.13	0.27		
		175	0.02			
		177	0.13	0.11		
		179	0.04			
		181				0.10
		183				0.40
		187				0.40
H[e]			**0.75**	**0.84**	**0.44**	**0.60**
CPH17	$(TG)_{15}$	229	0.15	0.58		
		237	0.02	0.05		
		247		0.03		
		249	0.42	0.18		
		251	0.41	0.16		
H[e]			**0.24**	**0.39**		
CPH18	$(TG)_{12}$	212			0.05	
		216				0.22
		218		0.11		
		220	0.85	0.75	0.25	0.06
		222	0.12	0.14	0.05	
		224	0.03		0.10	
		226			0.30	0.33
		228			0.10	
		232			0.15	
		234				0.28
		236				0.06
		238				0.06
H[e]			**0.24**	**0.47**	**0.50**	**0.56**
CPH19	$(CA)_{21}$	152			0.17	
		156	0.68	0.38	0.56	0.60
		158	0.02			0.30
		160	0.09	0.13		
		162	0.03	0.19	0.28	0.10
		164		0.30		
		166	0.18	0.02		
H[e]			**0.52**	**0.72**	**0.56**	**0.40**
CPH_{20}	$(GT)_{13}$	86			0.05	
		88			0.05	
		90			0.10	
		92			0.45	
		94			0.10	0.90
		96			0.20	
		98	0.42	0.14		
		100		0.03		
		102	0.58	0.83	0.05	
		104				0.10
H[e]			**0.42**	**0.28**	**0.70**	**0.20**

[a] Based on investigation of 33 animals.
[b] Based on investigation of 32 animals.
[c] Based on investigation of 10 animals.
[d] Based on investigation of 10 animals.
[e] Heterozygosity.

fox species. Therefore, as demonstrated in humans (Bowcock et al. 1994), evolutionary relationships can also be established in the *Canidae* family with appropriate microsatellite loci.

The domestic dog belongs to the *Canidae* family together with the wolf and fox. The dog is a very close relative of the gray wolf with almost identical chromosome morphology (Wayne and O'Brien 1987) and differing from it by at most 0.2% of mtDNA sequence (Wayne and Jenks 1991). In contrast, the diploid chromosome number ranges from 78 in dogs (all acrocentric) to 50 in the Arctic fox and 36 in the red fox (most metacentric) (Wayne and O'Brien 1987; Wayne 1993). In spite of the fact that the chromosome morphology is very different between dog and fox, the observed primer success rate (16 of 20) indicates that dog/fox is diverged by no more than 2–4% at nonselected sites. In comparison, in rats and mice, which both belong to the *Muridae* family, the following observations have been made: 16% of 153 rat primer pairs have been shown to amplify specific DNA segments in mice, while 12% of 166 mouse primer pairs amplify specific segments from the rat genome (Kondo et al. 1993). On the basis of cytological studies and linkage analyses, extensive chromosomal homology has been found between mouse and rat (Levan et al. 1991; Serikawa et al. 1992). The discrepancy in PCR primer success rate within the *Canidae* and the *Muridae* family is in accordance with the respective divergence times of the respective families, and

Table 3. Exclusion power with selected microsatellites.

(A) Flat-coated retrievers:

Microsatellite	Exclusion power (2 parents)	Sum	Exclusion power (1 parent)	Sum
CPH2	0.485	0.485	0.310	0.310
CPH3	0.554	0.771	0.381	0.573
CPH6	0.574	0.902	0.395	0.742
CPH9	0.464	0.948	0.295	0.818
CPH11	0.460	0.972	0.278	0.869
CPH14	0.515	0.986	0.336	0.913
CPH16	0.452	0.993	0.274	0.937

(B) Dachshunds:

Microsatellite	Exclusion power (2 parents)	Sum	Exclusion power (1 parent)	Sum
CPH3	0.571	0.571	0.393	0.393
CPH6	0.664	0.856	0.435	0.657
CPH8	0.654	0.950	0.479	0.821
CPH10	0.664	0.983	0.496	0.917
CPH11	0.803	0.996	0.675	0.973
CPH13	0.568	0.998	0.382	0.983
CPH16	0.564	0.999	0.387	0.989

variability in chromosome morphology is not indicative of divergence. The low divergence between dog and fox is an important observation because of the implications it has for mapping of the dog genome, that is, it will be possible to use the well-characterized fox karyotypes instead of the very difficult dog karyotype as a step towards physical mapping of the dog genome.

The choice of loci for parentage control has to be made with care. It is of great importance to eliminate loci with silent alleles and to avoid loci with high mutation rates. None of these factors have been taken into consideration in the present study. Nevertheless, in spite of its shortcomings, the present investigation illustrates that microsatellites are powerful tools for parentage analysis in dogs. It demonstrates that every breed will need a database of allele frequencies for cases that rely on inclusion. The fact that four of the seven most informative loci are common for the two dog breeds, that is, *CPH3, CPH6, CPH11,* and *CPH16* (Table 3), indicates that it will be possible to assemble a panel of microsatellite loci that are informative in most breeds if a larger number of microsatellites is investigated. It is, however, important to mention that experience from parentage cases in different dog breeds (data not presented) has indicated that the variation in the microsatellite loci is very limited in some breeds.

In conclusion, the present investigation testifies that there are pronounced differences between the profiles of microsatellite allele frequencies in different dog breeds and that there is a very high sequence conservation in the *Canidae* family. Thus, canine microsatellites are powerful tools both in regard to gene mapping, population genetic analysis, and for individual identification.

Acknowledgment. The authors thank Mette Sørensen for excellent technical assistance and Knud Christensen for statistical advice. We thank Christian Moliin Kapel, Division of Parasitology, RVAU, for making the Arctic fox samples available and Outi Lohi, Department for Fur animals, Research Center Foulum for providing the red fox samples. DVM Olav Nørgaard is gratefully acknowledged for establishing the dachshund panel, and Dr. Gaudenz Dolf, Institute of Animal Breeding, University of Bern, is acknowledged for designing the primers for CPH20. This work was funded by the Biotechnology Research Center for Animals and Fish, and The Danish Dog Breeders Association.

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