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An STR Forensic Typing System for Genetic Individualization of Domestic Cat (*Felis catus*) Samples*

ABSTRACT: A forensic genotyping panel of 11 tetranucleotide STR loci from the domestic cat was characterized and evaluated for genetic individualization of cat tissues. We first examined 49 candidate STR loci and their frequency assessment in domestic cat populations. The STR loci (3–4 base pair repeat motifs), mapped in the cat genome relative to 579 coding loci and 255 STR loci, are well distributed across the 18 feline autosomes. All loci exhibit Mendelian inheritance in a multi-generation pedigree. Eleven loci that were unlinked and were highly heterozygous in cat breeds were selected for a forensic panel. Heterozygosity values obtained for the independent loci, ranged from 0.60–0.82, while the average cat breed heterozygosity obtained for the 11 locus panel was 0.71 (range of 0.57–0.83). A small sample set of outbred domestic cats displayed a heterozygosity of 0.86 for the 11 locus panel. The power of discrimination of the panel is moderate to high in the cat breeds examined, with an average P_m of 3.7E-06. The panel shows good potential for genetic individualization within outbred domestic cats with a P_m of 5.31E-08. A multiplex protocol, designed for the co-amplification of the 11 loci and a gender-identifying locus, is species specific and robust, generating a product profile with as little as 0.125 nanograms of genomic DNA.

KEYWORDS: forensic science, domestic cat, STR, forensic typing system, multiplex amplification, cat breeds

The use of DNA markers to identify sources of crime scene evidence has revolutionized forensic science. Since the application of variable number of tandem repeat loci (VNTR) (1) and the more robust short-tandem repeat (STR) loci to forensic analysis (2–5), DNA genotyping for human genetic identification at scenes of crime, war and human disaster has become routinely used in hundreds of laboratories throughout the world. Until recently, the genetic individualization of biological samples of non-human origin, an alternative biological target of forensic investigation, has been limited due to the lack of species-specific hypervariable locus probes and population databases required to compute match likelihoods. However, with isolation of polymorphic STR markers across eukaryotic genomes (2,6), and their incorporation into genetic linkage maps of domestic animal gene maps (7–15), the tools for forensic identification of animals that are integral to our daily lives are now available.

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Previously, we reported on the genetic individualization of a domestic cat hair associated with a human crime scene, which was part of the physical evidence introduced to court contributing to a second-degree murder conviction (16,17). This legal precedent for introducing animal genetic individualization in a homicide trial stimulated an interest from a number of forensic laboratories for feline testing and demonstrated the need for a formalized STR forensic typing system for the precise genetic individualization of domestic cat specimens. The current test for human identification utilizes tetranucleotide repeat STRs that minimize stutter band products generated during PCR amplification (18) which facilitates the interpretation of genotypes from mixed DNA samples. Cat hairs will likely be the most common felid forensic sample. Whereas cat hairs are discreet and would not intuitively suggest the possibility of a mixed sample, grooming practices of cats can involve the licking of one another, introducing the saliva of one cat to another. As a first step in the development of a formalized feline STR typing system, we report on initial polymorphism screenings of candidate tri and tetra-nucleotide STR loci in outbred domestic cats, an in-depth characterization of 22 highly polymorphic tetranucleotide STR in 28 cat breeds, and the selection of 11 loci as a forensic panel for the genetic individualization of domestic cat samples. Additionally, we present a protocol for the multiplex amplification of the 11-member panel and a gender-identifying sequence tagged site (STS) from the domestic cat Y-chromosome SRY gene.

Materials And Methods

Cat Breed Sample Collection

Blood and buccal swab samples of domestic cats, representing 28 breeds recognized by the Cat Fanciers' Association (CFA) (<http://www.cfainc.org/>) and/or The International Cat Association (TICA) (<http://www.tica.org/> cat registries), were donated by

private breeders (under request for anonymity). Samples included: 10 Abyssinian, 7 American Shorthair, 5 Bengal, 9 Birman, 9 Bombay, 5 British Shorthair, 8 Burmese, 5 Chartreux, 4 Colorpoint Shorthair, 10 Cornish Rex, 10 Devon Rex, 10 Egyptian Mau, 10 Exotic, 9 Havana Brown, 3 Japanese Bobtail, 5 Korat, 8 Maine Coon, 8 Manx, 7 Norwegian Forest Cat, 5 Oriental Shorthair, 8 Persian, 8 Ragdoll, 9 Russian Blue, 9 Scottish Fold, 9 Selkirk Rex, 6 Siamese, 10 Sphynx, 7 Tonkinese. Blood samples from outbred domestic cat samples (6) were obtained from the NIH cat colony, which originated from Liberty Labs (Waverly, NY).

DNA Extraction

DNA was extracted from blood and buccal samples using Qiagen Qiam[®] DNA Blood Midi and Mini Extraction kits following the suggested protocols of the manufacturer.

Pedigree

Mendelian inheritance testing was performed for the new candidate STRs in DNA samples from a multi-generation pedigree (267 individuals) of outbred domestic cats maintained by the Nestlé-Purina PetCare Company which the Laboratory of Genomic Diversity is utilizing to generate a full genome genetic linkage map of microsatellites in the domestic cat.

Amplification of Individual STR Loci Used in Initial (Table 1) Screening Studies

PCR amplification of individual STR loci using fluorescently labeled primers (Invitrogen) was performed as described (17,19), with the exception that a "touchdown" amplification protocol was used as follows: one cycle at 95°C for 10 min; two cycles each of the following set of conditions with the annealing temperature reduced by 2°C in each subsequent cycle (range 60°C–50°C): 95°C for 15 s, annealing temperature (60°C–50°C) for 30 s, 72°C for 45 s; 28 cycles with an annealing temperature of 50°C; one cycle of 72°C for 30 min; hold at 4°C.

Conditions and Amplification of Cat STR-Multiplex:

PCR amplifications were performed in 20 µL reaction volumes containing 1X PCR Gold buffer (Applied Biosystems) with final reaction concentrations: 1.5 mM magnesium chloride (MgCl₂), 200 µM of four deoxyribonucleoside 5'-triphosphates (dATP, dCTP, dGTP, and dUTP), 2.0 Units *AmpliTaq* gold DNA polymerase (Applied Biosystems), 0.16 mg/mL bovine serum albumin (BSA, fraction V; Sigma), 4 µL cat STR primer mix- see final concentrations Table 6. Optimal DNA quantities with the standard 28 cycle PCR conditions are 1–5 ng genomic DNA (Coomber et al., In preparation). Thermal cycling conditions were performed with the GeneAmp 9700 (Applied Biosystems) using the following conditions in 9600-emulation mode (i.e. ramp speeds of 1°C/s). Full adenylation of all peaks should be observed under these PCR conditions. Cycling conditions: 1 cycle 90°C for 10 min, 28 cycles: 94°C for 1 min, 59°C for 1 min, 72°C for 1 min; 1 cycle of 60°C for 45 min; 25°C hold.

Allele Detection and Analysis

PCR products were diluted appropriately and 3 µL of each amplified product was combined with 0.22 µL GS500 LIZ (Applied Biosystems) and 8.78 µL Hi-Di[™] formamide (Applied Biosystems). The samples were electrophoresed on an ABI 3100 Genetic Analyzer using the following parameters; Dye Set: G5, Run

Module: GeneScan36_POP4DefaultModule using the 3100 POP[™] 4 sieving polymer (Applied Biosystems), 1X Genetic Analyzer Buffer with EDTA and a 36 cm capillary array. Data were collected using the ABI 3100 Data Collection Software Version 1.0.1 and analyzed using GeneScan Version 3.7 Analysis Software and GenoTyper[™] Version 2.5 Software.

Population Genetic Analyses

Expected heterozygosities and allele frequencies were calculated using CERVUS (20) and Microsat ToolKit (21) where heterozygosity is computed as the unbiased expected heterozygosity according to equation 8.4 in Nei (22). Match probabilities were estimated using the software Powerstats (23) following Jones (24) without adjustment for sample size, given the size of the sample sets. Observed heterozygosity was computed as in Hedrick (25).

Results

We examined a total of 49 tri and tetra-nucleotide STR loci as candidates for a forensic panel for genetic individualization of domestic cat samples (Table 1). All loci have been mapped in either a radiation-hybrid (RH) (11) or genetic linkage map of the cat (10) relative to 579 Type I (coding genes) and 255 Type II (STR) loci, with the exception of locus FCA1058 which exhibited low retention frequency in the RH panel and has not been mapped in the genetic linkage map. All loci have demonstrated Mendelian inheritance patterns from genotype profiles exhibited in a family of domestic cats of several generations, 267 individuals and 483 potential informative meioses (data not shown) (Coomber et al., In preparation).

Table 1 summarizes the results of two sets of heterozygosity screenings: (1) An initial set of screenings was performed on all 49 loci in a set of outbred domestic cats ($n = 10$) (Seventeen of these 49 loci had been reported in an earlier study (10)). (2) Twenty-two loci which demonstrated high heterozygosity in outbred cats were then selected as candidates for screening in cat breeds, in order to select markers with the highest discriminating power for forensic analysis in cat breeds. (Heterozygosity values will be reported in this manuscript as expected heterozygosity, unless otherwise stated.) The data from both of these screenings are reported in Table 1. The 22 loci (Table 1, asterisk) were genotyped in a sample set of 28 cat breeds (3–10 animals/breed, $n = 213$), and a small sample set of outbred domestic cats ($n = 6$). Genotypes for the entire sample set are available at http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp. In subsequent tables we present measures of genetic diversity for the STR loci as analyzed in (1) the *entire* sample collection of individuals of recognized breed ($n = 213$) (2) the *individual* cat breeds, and (3) outbred domestic cats. Outbred domestic cats are individuals, which are not of a recognized breed. Allele frequencies, locus-specific allele sizes and distributions as observed in the individual breeds and across the complete set of breed individuals ($n = 213$) are presented in a supplementary table ([Http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp](http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp)).

The average expected heterozygosity that was observed across the complete set of individuals of recognized breed ($n = 213$) for the 22-locus set was relatively high at 0.80 (Table 2). Individual cat breeds demonstrated a range of heterozygosities for the 22 locus set, ranging from 0.51 to 0.80 (Table 2), while the average heterozygosity obtained for the sample set of outbred domestic cats for the 22-locus set was 0.81 (Table 2). Allelic diversity was high across the complete set of breed animals ($n = 213$), with an average

TABLE 1—Forty nine cat tri and tetranucleotide repeat STR loci screened for inclusion in a DNA typing panel.

STR*	STR Repeat	Chromosomal Assignment	Mapping Information†	Range (bp)	Heterozygosity [‡] (He)	Forward Primer [§]	Reverse Primer
FCA 721	(GATA)9	A1	RH	176–192	0.71 (9)	CAATTTTAAATTGGGTTGGTGT	CACTGCCGATAGATAAAGA
FCA 722	(CA)8(CAA)10	A1	RH	199–211	0.69 (10)	GCTTTTGTGATGGTGGCTA	ACAAATGGTGTGCCCTACAA
FCA 723*	(GGAA)8 G (GAAAA)15	A1	RH	194–264	0.90 (211)	TGAAAGGCTAAGGCACGATAGA	CGGAAAGATACAGGAGGGTGA
FCA 724	(GATA)8	A1	RH	202–218	0.65 (10)	GGTGATPAAACGTCAAAGGAGTTC	GGTGTCTGCGCAGAGTTGTGA
FCA 725*	(GATA)12	A1	RH	148–235	0.65 (211)	TGAATCACTATGTTGTACACCTGAAA	GAGGCTCAATCTCATACCAAG
FCA 726	(GATA)11	A2	RH	229–245	0.66 (10)	GCACAGAGGATCCCCATAA	GCCCCTGTTTGTGTGTACT
FCA 727	(GTT)11	A2	RH	203–221	0.44 (8)	GCCATTTCTCAGCCATTGTT	TAGGAACACCGCTTGAGTGC
FCA 728	(GGAA)11	A2	RH	108–140	0.66 (10)	TTGAGCTTTTCTCTCTGACAA	CCTGCTGTATTTCCCTACAA
FCA 729	(CTAT)11/(CATC)11	A3	RH	158–194	0.77 (7)	AGCTCTCCAGTGGCTTCTAGA	TTGTCCTTCTCCCTCCACTAC
FCA 730	(GATA)10	B1	RH	149–175	0.71 (6)	ATTGGAAATTGTAGCCAAAGG	CTCCAAGTGATGGAGCAATT
FCA 731*	(CCAT)8/(CCAT)11	B1	RH	324–344	0.78 (211)	GATCCGCTATCCCATCTGTCC	ATGCTCCCTGAAGCTGTAA
FCA 732*	(ATCT)7	B2	RH	230–262	0.67 (209)	CCTGTGGGTGGCTTCTACTT	AGAATTGCAAGGAGGCCACT
FCA 733*	(GATA)11	B2	RH	182–230	0.89 (195)	GAAGATGTGGCGATAGATACACAA	TGTTTCAGAGGGCAAAATTTCA
FCA 734	(GAAA)11	B3	RH	134–190	0.85 (9)	TGCTAGCTTCTCTCTTGACA	ACTGGAGGGGTGAGGACTTT
FCA 735	(CCAT)6/(AC)15	B4	RH	101–109	0.62 (6)	TCAAGGCCAATTGTAGAGCA	TTCCATTTCTATGGAAATAGTCAGT
FCA 736*	(ATAC)10 (CA)3 (ATAG)14	B4	RH	196–280	0.89 (204)	ACCGAGCTCTGTTCTGGGTA	CAGACTGCACCACTGCCTAA
FCA 737	(GATA)10	C1	RH	197–205	0.64 (10)	TCTCCACTCTTCCCTCACTCA	CCACACCTTTGGTCTCAGT
FCA 738*	(AAC)13	C1	RH	189–210	0.67 (210)	TCTTCACTGCTTCTGCGCTCA	GTGGCTGAGATGCTCTGAT
FCA 739	(GATC)9	C1	RH	191–235	0.85 (10)	GTGCTGATTTTGTATCTGTATCTGT	AAAGGAAAGTGACCACTGGA
FCA 740*	(GATA)11	C1	RH	199–223	0.75 (209)	CACITTTTGAAGGAGCAGCA	AAACAAATGGAGTTTGTGG
FCA 741	(GAT)12	D1	RH	151–166	0.65 (7)	GCATGGAGAGGGTTTATAGCC	TTCCGTCAGTCCATGATAATG
FCA 742*	(CTTT)11	D4	RH	123–175	0.87 (207)	TC AATGTCTTGACAAGCATAA	AGGATGCAAGCACAGGAAC
FCA 743	(GATA)10	D4	RH	204–220	0.50 (10)	CGACTCACACTCGCTGCTC	CAITTTGGTGGAGTTTGTGT
FCA 744	(GATA)9	D4	RH	152–172	0.84 (10)	CATTGGCCTACAGCCTACT	TCAACACCTCACACCAATG
FCA 746	(GATA)10	D4	RH	178–194	0.66 (8)	GCCTCCAGGATTAITTTCAA	TGCGTAAAGTGTITTTCAAAG
FCA 747	(GATA)10	D4	RH	134–144	0.83 (9)	GCCTTTTGGCAACCATTAG	TCTTGGAAATTACTCTGGTAAACA
FCA 748	(GATA)7/(GGAA)18	F2	RH	246–294	0.87 (10)	CCACAATACCATAATGCTTCA	TIAGAGACTTTCTTTGGTCTTTG
FCA 749*	(GATA)10/(GATA)6	F2	RH	219–263	0.83 (208)	CTGGGGAGGAGGCTTACTTA	GATTTGAAAGAGGCCAGCAG
FCA 750*	(GATA)11/(GGAT)8	F2	RH	355–411	0.90 (127)	TTGGCCATGAGTTC AAAGGTT	TTCTGTTTAAAGGCAAGTGTTC
FCA 1058	(GTTT)8	not mapped	low retention in RH	185–212	0.53 (6)	AACAGCCTGTTCAAAGAAA	GAGTTC AAGCCCTGTGCTG
FCA 1059	(GAAA)9	A1	RH	121–141	0.74 (9)	TGAAAAGCATATGCAAAAAGTTGA	TCTCCAAATTCCTATCTCACAAAC
F115	(GAA)15	B2	GL	193–217	0.77 (10)		
F124*	(GAAA)15	E1	BOTH	211–265	0.89 (212)		
F141*†	(GAAA)13	A1	RH	278–336	0.89 (206)	GC AATGAGACTAGATGGAAGGA	GGCTCCAGACTCCTTGTAGCA
F146	(GTT)9	A1	GL	145–158	0.73 (10)		
F164	(AAC)9	C2	BOTH	146–166	0.65 (10)		
F27	(GAAA)14	B1	GL	97–129	0.71 (10)		
F37*	(TTTA)11	C1	BOTH	221–243	0.83 (164)		
F41*	(A)13(AAAG)11(AAAG)16	D2	BOTH	158–208	0.93 (211)		
F42*	(GAAA)13	A1	BOTH	202–238	0.80 (211)		
F49*	(TTG)8	D1	GL	110–122	0.57 (159)		
F53*	(GAAA)8	A1	GL	154–190	0.88 (211)		
F85*	(CTTT)10(CT)10(T)4(CTTT)15	B1	BOTH	216–316	0.94 (209)		
F98	(CAA)12	B4	BOTH	171–183	0.53 (10)		
FCA 391*	(GATA)10	B3	BOTH	240–268	0.62 (181)		
FCA 424	(GATA)11	C2	BOTH	171–187	0.68 (10)		
FCA 441*	(GATA)9	D3	BOTH	152–172	0.78 (210)		
FCA 453	(GATA)7	A1	BOTH	186–198	0.62 (10)		
FCA 559*	(GAAA)8 complex	B1	BOTH	134–202	0.76 (185)		

* 22 Loci selected for amplification in breed panel of 213 domestic cats; the 11 STRs in bold were tentatively selected for the DNA typing panel. DNA sequences for loci with FCA numbers above 700 and all F loci were submitted to GenBank and were assigned accession numbers. All other loci were previously submitted.

† Used published primers (10) for genotyping and used primers indicated for mapping in radiation hybrid panel. Unable to map first published primers in radiation hybrid panel due to amplification of product in hamster control.

‡ RH-mapped in radiation hybrid panel (11). GL-mapped in radiation hybrid panel and genetic linkage map.

§ Heterozygosity (He)-Unbiased gene diversity (22); (), number of animals used for determination of He. ¶ Absent primers were previously published (10).

TABLE 2—Population statistics for 22 STR loci in cat breeds and mixed breed domestic cats.

Breed/Species	n	Loci	He*	Ho†	Av No Alleles/locus
Abyssinian	10	22	0.53	0.48	3.5
American Shorthair	7	22	0.70	0.61	4.5
Bengal	5	21	0.78	0.59	5.0
Birman	9	22	0.54	0.51	3.4
Bombay	9	22	0.58	0.51	3.4
British Shorthair	5	22	0.56	0.50	3.4
Burmese	8	22	0.65	0.59	4.2
Chartreux	5	22	0.67	0.55	3.9
Colorpoint Shorthair	4	21	0.70	0.54	3.5
Cornish Rex	10	22	0.61	0.54	4.1
Devon Rex	10	22	0.63	0.56	5.0
Egyptian Mau	10	22	0.54	0.46	3.6
Exotic	10	22	0.61	0.61	4.5
Havana Brown	9	22	0.51	0.48	3.6
Japanese Bobtail	3	22	0.65	0.55	3.0
Korat	5	22	0.63	0.64	3.5
Maine Coon	8	22	0.71	0.63	4.4
Manx	8	22	0.77	0.75	5.4
Norwegian Forest Cat	7	22	0.80	0.73	6.0
Oriental Shorthair	5	21	0.74	0.49	4.4
Persian	8	22	0.66	0.50	4.3
Ragdoll	8	22	0.68	0.57	4.5
Russian Blue	9	22	0.65	0.47	4.4
Scottish Fold	9	22	0.72	0.64	5.7
Selkirk Rex	9	22	0.70	0.69	5.3
Siamese	6	22	0.66	0.48	4.0
Sphynx	10	22	0.69	0.61	5.5
Tonkinese	7	22	0.67	0.55	4.4
Average	7.6	22	0.65	0.57	4.3
Complete breed set‡	213	22	0.80	0.57	14.3
Outbred domestic cats	6	22	0.81	0.67	5.6

* He-Unbiased gene diversity (22).

† Ho-Observed heterozygosity.

‡ Complete breed set‡ = entire number of breed individuals analyzed as a unit.

of 14.3 alleles observed per locus (Table 2) (http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). The average number of alleles/locus observed within breeds was 4.3 alleles per locus, as compared to 5.6 alleles per locus in the sample set of outbred domestic cats (Table 2) (http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). The expanded allelic diversity in the complete set of breed individuals is likely a reflection of both the much larger sample size in this set ($n = 213$) relative to sampling size in the individual cat breeds (average = 7.6 cats/breed), and what appear to be reduced allelic ranges in individual breeds (http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). Though there were examples of distinctive allele sizes within some breeds (http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp), a larger sample set will be necessary to confirm this observation.

Table 3 presents expected heterozygosities for the independent loci in (1) the complete set of breed individuals ($n = 213$), (2) the 28 individual cat breeds and (3) outbred domestic cats are presented. Locus-specific heterozygosities for the 22 markers across the complete set of breed individuals ($n = 213$) ranged from 0.57–0.95 (Table 3). Within the individual 28 cat breeds, locus specific heterozygosities were highly variable (Table 3). Additionally, there were marked differences among breeds in the distribution of alleles, size range and frequency of alleles for the 22 loci (http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). Three loci (FCA738, FCA742, F49) exhibited fixed allele sizes in a cat breed(s) (for sample sizes greater than 4 individuals). FCA738 was fixed in Birman, Chartreux and Selkirk Rex breeds, with the latter two breeds displaying a common fixed allele size (204 bp)

(http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). Locus F49, which demonstrated the lowest average expected heterozygosity across the sample set (0.45), displayed a common fixed allele size in the Devon Rex, Tonkinese, Abyssinian and Korat breeds (118 bp) (http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). Locus FCA742 displayed a single allele size in the Havana Brown (127 bp) (http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). Individual breeds often exhibited reduced or compressed allelic size ranges relative to the complete range observed in the complete set of breed individuals (http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp). The outbred domestic cat set of individuals, despite the small sample size, exhibited neither a fixed allele for any locus nor compressed allelic size ranges relative to the complete set of breed individuals. This suggests that the STR composite set will have high potential for genetic individualization in outbred domestic cats. Though there were examples of distinctive allele sizes within some breeds (http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp), a larger sample set will be necessary to confirm this observation.

Selection of a Forensic Typing Panel of 11 STR Loci

A set of eleven highly polymorphic loci was selected as a forensic typing panel (Table I) from the 22 loci genotyped in cat breeds to optimize the potential to generate discriminating composite profiles. Selection criteria were based on identifying a panel of loci that were *both* unlinked and demonstrated high heterozygosity across multiple cat breeds. (Therefore, the loci with the 11 *highest* heterozygosities were not selected.) All loci selected are tetranucleotide repeat loci: six loci are simple tetranucleotide repeat loci (F53, FCA441, FCA733, FCA740, FCA742, F124; three are compound loci (FCA723, FCA731, FCA749), and two loci are complex (FCA736, F85) (Table 1) (GenBank Accession numbers AY988109–AY988153). The loci are well distributed across the cat's 18 autosomes, with 7 loci mapping to independent chromosomes (B2, B4, C1, D3, D4, E1 and F2), and four loci located on separate arms of the largest chromosomes A1 and B1 (26). Cat chromosomes, as opposed to being sequentially numbered like human chromosomes, are designated first by a letter which identifies chromosomes with similar centromere positions (27,28). We know from reference of closely mapped markers positioned in both the RH and genetic linkage maps (26) that the pairs of markers on chromosomes A1 and B1 are not genetically linked (i.e. recombination between them is greater than or equal to 50 percent).

Within individual cat breeds, average heterozygosities for the entire 11 locus set were moderately high, ranging from 0.57 (Abyssinian, Birman, Havana Brown) to 0.83 (Norwegian Forest Cat, Bengal) (Table 4). An overall average heterozygosity of 0.71 was obtained for the entire 11 locus set in the 28 breeds, as compared to an average heterozygosity of 0.86 obtained in the sample of outbred domestic cats (Table 4). Locus specific heterozygosities for the 11 independent loci ranged from 0.75 (FCA740) to 0.95 (locus F85) (Table 5). Within the entire set of breed individuals ($n = 213$), an average locus heterozygosity of 0.86 was obtained for the entire panel of STR loci. The number of alleles/locus observed for the 11 independent loci in the entire set of 213 animals averaged 15.6 alleles/locus (Table 5), ranging from 6 alleles in locus FCA731 to 32 alleles in the complex locus F85 (http://home.ncifcrf.gov/ccr/lgd/forensics/str_forensic.asp).

Match Probabilities

The probability of match (P_m) of each locus was calculated for the individual breeds according to the formula of Jones (24), essentially

TABLE 3—Locus specific heterozygosities observed in cat breeds/species.

Breed/Species	N	Locus Name											
		FCA391	FCA441*	FCA559	FCA723*	FCA725	FCA731*	FCA732	FCA733*	FCA736*	FCA738	FCA740*	
Abyssinian	10	ABY	0.47	0.60	0.10	0.42	0.19	0.44	0.62	0.60	0.28	0.51	0.68
American Shorthair	7	ASH	0.65	0.50	0.78	0.79	0.74	0.76	0.65	0.68	0.71	0.40	0.69
Bengal	5	BEN	0.78	0.89	0.53	0.93	0.71	0.80	0.53	1.00	1.00	0.80	0.38
Birman	9	BIR	0.68	0.50	0.59	0.29	0.11	0.43	0.47	0.58	0.84	0.00	0.50
Bombay	9	BOM	0.61	0.66	0.37	0.48	0.50	0.47	0.50	0.61	0.51	0.50	0.61
Brithish Shorthair	5	BSH	0.46	0.20	0.00	0.62	0.53	0.78	0.25	0.75	0.62	0.64	0.71
Burmese	8	BUR	0.68	0.70	0.26	0.75	0.33	0.23	0.77	0.68	0.70	0.40	0.58
Chartreux	5	CHA	0.61	0.73	0.43	0.93	0.71	0.87	0.53	0.61	0.64	0.00	0.69
Colorpoint Shorthair	4	CSH	0.46	0.82	0.61	0.46	0.73	0.71	0.61	0.83	0.93	0.54	0.25
Cornish Rex	10	CRE	0.58	0.80	0.62	0.83	0.53	0.68	0.42	0.67	0.66	0.44	0.61
Devon Rex	10	DRE	0.39	0.50	0.81	0.69	0.10	0.74	0.36	0.36	0.90	0.76	0.70
Egyptian Mau	10	MAU	0.27	0.69	0.77	0.60	0.31	0.10	0.40	0.57	0.68	0.10	0.43
Exotic	10	EXO	0.31	0.62	0.46	0.82	0.42	0.57	0.20	0.39	0.55	0.56	0.70
Havana Brown	9	HAV	0.46	0.64	0.59	0.81	0.11	0.21	0.14	0.76	0.77	0.31	0.34
Japanese Bobtail	3	BOB	0.53	0.00	0.60	0.87	0.73	0.93	0.33	0.53	0.60	0.53	0.80
Korat	5	KOR	0.38	0.80	0.78	0.73	0.73	0.51	0.56	0.73	0.84	0.71	0.20
Maine Coon	8	MCC	0.63	0.76	0.49	0.73	0.68	0.78	0.62	0.91	0.67	0.43	0.80
Manx	8	MAX	0.60	0.77	0.53	0.75	0.63	0.67	0.71	0.82	0.89	0.68	0.78
Norwegian Forest Cat	7	WEG	0.71	0.74	0.64	0.89	0.82	0.86	0.51	0.86	0.91	0.67	0.66
Oriental Shorthair	5	OSH	0.50	0.60	0.71	0.89	0.62	0.82	0.80	0.82	0.96	0.61	0.47
Persian	8	PER	0.14	0.70	0.60	0.88	0.57	0.50	0.49	0.68	0.54	0.58	0.78
Ragdoll	8	RAG	0.60	0.78	0.17	0.83	0.34	0.82	0.77	0.74	0.92	0.59	0.58
Russian Blue	9	RUS	0.44	0.75	0.26	0.87	0.66	0.75	0.47	0.75	0.67	0.67	0.66
Scottish Fold	9	SFO	0.35	0.62	0.68	0.75	0.78	0.59	0.45	0.82	0.78	0.48	0.78
Selkirk Rex	9	SRE	0.44	0.62	0.71	0.67	0.70	0.72	0.61	0.86	0.58	0.00	0.78
Siamese	6	SIA	0.64	0.53	0.76	0.68	0.71	0.30	0.59	0.88	0.82	0.62	0.17
Sphynx	10	SPH	0.20	0.75	0.77	0.88	0.47	0.66	0.62	0.81	0.82	0.44	0.84
Tonkinese	7	TOK	0.82	0.74	0.62	0.66	0.53	0.53	0.47	0.87	0.89	0.44	0.62
Average	7.6		0.51	0.64	0.54	0.73	0.54	0.62	0.52	0.72	0.74	0.48	0.60
Complete breed set [‡]	213	Hetz.	0.62	0.78	0.76	0.90	0.65	0.78	0.67	0.89	0.89	0.67	0.75
Outbred domestic cats	6	FCA	0.67	0.71	0.91	0.88	0.83	0.84	0.44	0.96	0.91	0.85	0.83

Breed/Species	N	Locus Name											
		FCA742*	FCA749*	FCA750	F37	F41	F42	F49	F53*	F85*	F124*	F141	
Abyssinian	10		0.70	0.70	0.71	0.71	0.83	0.68	0.00	0.54	0.73	0.63	0.43
American Shorthair	7		0.89	0.82	0.46	0.53	0.81	0.73	0.53	0.89	0.77	0.85	0.85
Bengal	5		0.87	0.51	NA [†]	0.79	0.80	0.67	0.73	0.93	0.93	0.93	0.96
Birman	9		0.46	0.61	0.44	0.59	0.78	0.65	0.47	0.63	0.68	0.75	0.82
Bombay	9		0.78	0.74	0.61	0.78	0.78	0.58	0.20	0.58	0.79	0.56	0.58
Brithish Shorthair	5		0.62	0.84	0.82	0.33	0.89	0.36	0.33	0.64	0.71	0.51	0.76
Burmese	8		0.78	0.88	0.87	0.75	0.78	0.68	0.25	0.67	0.80	0.87	0.89
Chartreux	5		0.82	0.73	0.87	0.20	0.80	0.86	0.73	0.64	0.87	0.76	0.78
Colorpoint Shorthair	4		0.79	0.75	NA	0.61	0.93	0.75	0.60	0.75	0.86	0.75	0.86
Cornish Rex	10		0.77	0.52	0.47	0.57	0.86	0.62	0.27	0.65	0.85	0.78	0.19
Devon Rex	10		0.74	0.55	0.79	0.80	0.89	0.76	0.00	0.77	0.81	0.60	0.76
Egyptian Mau	10		0.85	0.61	0.87	0.77	0.51	0.66	0.50	0.67	0.78	0.55	0.11
Exotic	10		0.68	0.70	0.56	0.61	0.75	0.50	0.71	0.85	0.84	0.76	0.83
Havana Brown	9		0.00	0.40	0.41	0.00	0.81	0.69	0.67	0.78	0.84	0.76	0.71
Japanese Bobtail	3		0.87	0.60	1.00	0.53	0.87	0.53	0.00	0.80	0.87	0.80	0.87
Korat	5		0.56	0.20	0.82	0.73	0.71	0.78	0.00	0.78	0.80	0.73	0.78
Maine Coon	8		0.76	0.34	1.00	0.59	0.83	0.83	0.62	0.67	0.83	0.81	0.77
Manx	8		0.77	0.88	0.89	0.76	0.85	0.77	0.67	0.83	0.94	0.88	0.84
Norwegian Forest Cat	7		0.86	0.74	0.89	0.84	0.88	0.84	0.77	0.75	0.96	0.93	0.90
Oriental Shorthair	5		0.93	0.89	NA	0.43	0.84	0.89	0.51	0.82	0.84	0.84	0.80
Persian	8		0.71	0.68	0.50	0.54	0.90	0.79	0.77	0.62	0.93	0.83	0.83
Ragdoll	8		0.68	0.60	0.83	0.68	0.92	0.80	0.41	0.82	0.62	0.73	0.82
Russian Blue	9		0.68	0.80	0.53	0.41	0.65	0.61	0.54	0.78	0.76	0.80	0.69
Scottish Fold	9		0.86	0.80	0.88	0.62	0.87	0.69	0.66	0.75	0.93	0.85	0.78
Selkirk Rex	9		0.82	0.84	0.79	0.64	0.90	0.66	0.65	0.81	0.88	0.86	0.86
Siamese	6		0.71	0.88	0.80	0.20	0.88	0.74	0.43	0.71	0.80	0.82	0.89
Sphynx	10		0.62	0.83	0.82	0.79	0.86	0.44	0.49	0.85	0.66	0.84	0.80
Tonkinese	7		0.60	0.96	0.68	0.68	0.84	0.62	0.00	0.54	0.82	0.89	0.84
Average	7.6		0.72	0.69	0.73	0.59	0.82	0.68	0.45	0.73	0.82	0.77	0.75
Complete breed set [‡]	213		0.87	0.83	0.91	0.83	0.93	0.80	0.57	0.88	0.95	0.89	0.89
Outbred domestic cats	6		0.80	0.89	0.83	0.80	0.88	0.87	0.44	0.82	0.94	0.87	0.88

* The 11 loci selected for the DNA typing panel.

† NA-no amplification; loci which failed to amplify were not included in calculations of average heterozygosity.

‡ Complete breed set-the entire 213 individuals analyzed as a single unit.

