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Research paper

Establishing a database of Canadian feline mitotypes for forensic use

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ABSTRACT

Hair shed by pet animals is often found and collected as evidence from crime scenes. Due to limitations such as small amount and low quality, mitochondrial DNA (mtDNA) is often the only type of DNA that can be used for linking the hair to a potential contributor. mtDNA has lower discriminatory power than nuclear DNA because multiple, unrelated individuals within a population can have the same mtDNA sequence, or mitotype. Therefore, to determine the evidentiary value of a match between crime scene evidence and a suspected contributor, the frequency of the mitotype must be known within the regional population. While mitotype frequencies have been determined for the United States' cat population, the frequencies are unknown for the Canadian cat population. Given the countries' close proximity and similar human settlement patterns, these populations may be homogenous, meaning a single, regional database may be used for estimating cat population mitotype frequencies. Here we determined the mitotype frequencies of the Canadian cat population and compared them to the United States' cat population. The two cat populations are statistically homogenous, however mitotype B6 was found in high frequency in Canada and extremely low frequency in the United States, meaning a single database would not be appropriate for North America. Furthermore, this work calls attention to these local spikes in frequency of otherwise rare mitotypes, instances of which exist around the world and have the potential to misrepresent the evidentiary value of matches compared to a regional database.

1. Introduction

The domestic cat is one of the most popular pets in North America [1,2]. An inevitable byproduct of cat ownership is the accumulation of shed cat hairs. Fastidious groomers such as cats can shed hundreds of thousands of hairs each year [3]. Given that avoiding pet hair transfer during criminal activity is virtually impossible [4], hair from these animals can be a valuable piece of forensic evidence and has already been used in criminal proceedings to link, for example, perpetrators to crimes [5,6].

Shed hair recovered from a crime scene often contains no nuclear DNA, but mitochondrial DNA (mtDNA) can usually be recovered from the shafts of these telogen hair samples [7–9]. Since mtDNA inheritance is strictly maternal and the genome does not undergo recombination, the only source of variation within mtDNA is random mutation [10]. As a result, mtDNA has lower exclusionary power than nuclear DNA. However, studies of variation within an approximately 400 base pair (bp) non-coding region of the cat mitochondrial control region (mtCR) have revealed that mtDNA has an exclusion capacity that can be forensically useful [11,12].

As is true for other species, because of the low levels of variation in mtDNA, multiple cats can carry the same mtDNA sequence even within the highly variable control region. The frequency of specific mtDNA control region sequences, or mitotypes, within a regional population must be determined in order to understand the forensic exclusionary power of mtDNA for a particular geographic region. Analyses of cat populations across the world have shown that some mitotype frequencies vary by region [11–13]. Globally, 4 common mitotypes, namely A, B, C, and D, have been described as well as 30 subtypes. An extreme case of mitotype variance was found within the cat population of Dubai (N = 10), where only one common mitotype (D) was found in the cats sampled, and 40% of the cats sampled were found to carry subtype D3, a mitotype observed nowhere else in the world [12].

Of domestic cats residing in North America, mitotype frequencies have only been estimated for United States' populations. These studies have shown that United States' cats are a generally homogenous population and a single national mtDNA forensic database can be used to accurately represent the mitotype

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frequencies of this region [11,12]. Canada shares nearly an entire border with the United States, with only a political barrier impeding movement of cats between the two countries. Despite similar human settlement patterns by Western Europeans into Canada and the United States, differences in the cat populations between the two countries may exist [14]. Here we have assessed the mitotype diversity of cats from three Canadian regions and compared these populations to previously published samples from the United States to determine the genetic diversity within and between these populations and expand upon the application of cat mtDNA in North America.

2. Materials and methods

2.1. Sample collection

Blood samples of 96 cats were obtained from Ottawa, Ontario; Winnipeg, Manitoba; and Vancouver, British Columbia and represented the domestic cat population of each location. The EDTA anti-coagulated whole blood were the remains of complete blood count samples collected by private practitioners in the three regions. DNA was isolated from whole blood using the Qiagen DNeasy Tissue extraction kit following manufacturer's specifications (Qiagen Inc, Valencia CA). The relatedness of the random bred cats is unknown. However, during collection, only one individual from an obvious litter was sampled and the cats were from different owners.

2.2. DNA amplification and sequencing

A segment of the mtCR was amplified using previously published primers Jh_mt_F3, 5′-gatagtgcttaatcgtgc-3′ and Jh_mt_R3, 5′-gtcctgtggaacaatagg-3′ [11]. These primers targeted a 472 bp sequence of the mtCR, from bp 16,759 to bp 240 of the published feline mitochondrial genome [15] (Genbank no. NC_001700.1). Final reagent concentrations for the polymerase chain reaction (PCR) were: 1–4 ng/μL DNA, 1× Invitrogen PCR Buffer, 4 mM Invitrogen $MgCl_2$, 0.2 mM Invitrogen deoxynucleotide triphosphates (dNTPs), 0.25 μM forward primer, 0.25 μM reverse primer, and 0.04 U/μL Taq DNA Polymerase recombinant (Invitrogen, Grand Island, NY). Each reaction was brought to a final volume of 25 μL with molecular biology grade water. Thermal cycler conditions for PCR were based on Grahn et al. [12]: 94 °C for a 3 min initial denaturation, followed by 35 cycles of 94 °C for 45 s, 60 °C for 30 s, and 72 °C for 45 s, with a final extension for 10 min at 72 °C. PCR products were stored at 4 °C.

PCR products were size separated on a 1% agarose gel stained with a 1:10,000 dilution of SYBR Green I (Life Technologies, Grand Island, NY) for 45 min at 70 V in an electrophoresis chamber with 11.5 centimeter separating the electrodes. Products were compared to Low DNA Mass Ladder (Life Technologies, Grand Island, NY) to assess the size and concentration of each amplicon. Products of the expected size and appropriate concentration were prepared for sequencing using ExoSAP-IT exonuclease clean-up (Affymetrix, Santa Clara, CA) as per the manufacturer's instructions and sent to Eurofins MWG Operon (Louisville, KY) for bi-directional sequencing using the same primers as used in PCR.

Resultant sequences were edited and aligned using Sequencher 5.1™ software (Gene Codes Corporation, Ann Arbor, MI). For each sample, the forward and reverse sequences were assembled to create a single consensus sequence. Using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) each consensus sequence was then aligned against the entire cat mitochondrial genome to confirm that the target locus plus flanking sequence was amplified [15] (Genbank no. NC_001700.1).

2.3. Multiple alignments

Sequencher 5.1™ software (Gene Codes Corporation, Ann Arbor, MI) was used to create various multiple alignments. To assign mitotype designations to the newly collected sequences, all newly sequenced Canadian samples and all previously published mitotypes from Grahn et al. [12] were aligned. Population analyses were carried out using several additional multiple alignments including one for each of the Ottawa, Winnipeg, and Vancouver subpopulations, the total Canadian population, individual United States' subpopulations, and the total United States' population. Alignments of the sequences found in each United States' population were created by using mitotype frequency data from Grahn et al., Table 1 [12], the previously described mitotypes from Grahn et al., Table 3 [12], and the unique sequences obtained from R. A. Grahn (personal communication). Multiple alignments were also created for all possible pairwise comparisons between the aforementioned populations, excluding comparisons between United States' subpopulations. The Sylvester reference sequence (SRS) (bp 16813 to bp 206 of the published feline mitochondrial genome [15]) was used as a reference sequence for each alignment and was removed from each contig before the FASTA file was exported [11].

2.4. Mitotype designation

The alignment of all newly sequenced Canadian samples and all previously published mitotypes from Grahn et al. [12] was uploaded to DNA collapser (http://users-birc.au.dk/biopv/php/fabox/dnacollapser.php), which groups sequences that have identical nucleotide compositions. Sequences that grouped with previously published mitotypes were assigned that previously published designation. For those sequences not matching any of the previously defined mitotypes of Grahn et al. [12], a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) against the entire NCBI nucleotide database (http://www.ncbi.nlm.nih.gov/nucleotide) was performed to determine if these sequences matched any mitotypes that had been previously published outside of Grahn et al. [12]. Sequences that did not group with any other sequence in the dataset or the nucleotide database were considered unique sequences and were deposited in the GenBank sequence database. Since mitotype designations are defined by their single nucleotide polymorphisms with respect to SRS, mitotype designations were also checked manually by identifying the SNPs in every sample using Sequencher 5.1™'s "select next ambiguous base" tool and then checking these SNPs against the mitotype definitions in Grahn et al. [12]. A Table of SNPs was compiled for all mitotypes in the Canadian sample set.

2.5. Dataset analysis

Mitotype and nucleotide diversities as well as pairwise differences were calculated for each Canadian subpopulation and the total sampled Canadian population using Arlequin 3.5.1.2 [16]. MEGA 5.2.2 [17] was used to calculate the maximum number of pairwise differences within each population. The exclusion capacity for each population was calculated as:

$$1 - \sum_{i=1}^{n} f_i^2$$

where f is the frequency of the i-th mitotype in a given population and n is the total number of mitotypes in that population [18]. For comparisons of each Canadian subpopulation to the other Canadian subpopulations and to the United States' populations,

Table 1
Mitotype Frequencies by Region.

	Ottawa		Winnipeg		Vancouver		Canada		United States[a]	
	n = 28		n = 53		n = 15		N = 96		N = 493	
Mitotype	Number	Frequency	Number	Frequency	Number	Frequency	Number	Frequency	Number	Frequency
A	3	0.1071	16	0.3019	3	0.2000	22	0.2292	111	0.2252
A1	0	0.0000	0	0.0000	0	0.0000	0	0.0000	1	0.0020
A1a	0	0.0000	0	0.0000	0	0.0000	0	0.0000	2	0.0041
A6a	0	0.0000	0	0.0000	0	0.0000	0	0.0000	8	0.0162
A6b	1	0.0357	0	0.0000	0	0.0000	1	0.0104	0	0.0000
A9	0	0.0000	0	0.0000	0	0.0000	0	0.0000	1	0.0020
B	3	0.1071	5	0.0943	6	0.4000	14	0.1458	138	0.2799
B1	0	0.0000	1	0.0189	0	0.0000	1	0.0104	2	0.0041
B4	0	0.0000	0	0.0000	0	0.0000	0	0.0000	1	0.0020
B6	8	0.2857	7	0.1321	0	0.0000	15	0.1563	2	0.0041
C	8	0.2857	15	0.2830	4	0.2667	27	0.2813	90	0.1826
C1	0	0.0000	0	0.0000	0	0.0000	0	0.0000	7	0.0142
C4	0	0.0000	0	0.0000	0	0.0000	0	0.0000	1	0.0020
D	0	0.0000	0	0.0000	0	0.0000	0	0.0000	24	0.0487
E	0	0.0000	0	0.0000	0	0.0000	0	0.0000	10	0.0203
F	2	0.0714	2	0.0377	0	0.0000	4	0.0417	15	0.0304
G	2	0.0714	0	0.0000	0	0.0000	2	0.0208	11	0.0223
H	0	0.0000	2	0.0377	1	0.0667	3	0.0313	16	0.0325
I	0	0.0000	1	0.0189	0	0.0000	1	0.0104	4	0.0081
K	0	0.0000	0	0.0000	0	0.0000	0	0.0000	18	0.0365
L	0	0.0000	0	0.0000	0	0.0000	0	0.0000	4	0.0081
OL1	0	0.0000	2	0.0377	0	0.0000	2	0.0208	0	0.0000
OL2	0	0.0000	0	0.0000	0	0.0000	0	0.0000	3	0.0061
U	1	0.0357	2	0.0377	1	0.0667	4	0.0417	24	0.0487

Row U represents the number of unique mitotypes, or those of which are found in only one individual, in the population data set.
[a] Sequences collected by Grahn et al. [12] [see Methods].

Arlequin 3.5.1.2 was used to calculate the fixation index (Fst) and perform an analysis of molecular variance (AMOVA) within each population and between all pairs of populations. Following the methods of Smalling et al. [19], a modified Fisher's exact test for homogeneity was performed with a null hypothesis of "no difference" in mitotypes between regions to ensure that analysis of possible population substructuring was complete for all population comparisons, excluding any comparisons between two United States' subpopulations.

4. Results

4.1. Overview of newly collected Canadian samples

A 472 bp region of the mtCR was successfully amplified and sequenced from 96 domestic cat samples collected from populations residing in Ottawa, Ontario (n = 28); Winnipeg, Manitoba (n = 53); and Vancouver, British Columbia (n = 15). All sequences were trimmed to 402 bp, which corresponded to the Sylvester Reference Sequence and ensured that only high quality sequence data was used for analysis. When compared to the SRS, 25 variant sites were identified across the three Canadian populations. No heteroplasmy was detected in any of the sequences. Fifteen mitotypes were identified within the 96 cat sample set, comprised of 11 previously described mitotypes and 4 unique mitotypes (Table 1; Table 2). The four unique mitotype sequences have been submitted to GenBank (GenBank no. KT344778–KT344781).

4.2. Canadian cat mitotype distributions

For the Canadian cat population as a whole, 76.0% (n = 73) of the cats were found to carry one of the 7 universal mitotypes A-C and F-I. An additional 17.7% (n = 17) of the cats carried one of 3 subtypes of the universal mitotypes A and B, namely A6b (n = 1), B1 (n = 1) and B6 (n = 15). Another 2.1% (n = 2) of the cats carried OL1, and 4.2% (n = 4) of the cats had unique mitotypes (Table 1). The four most common mitotypes in the Canadian cat population, A, B, B6, and C, were found in 81.25% (n = 78) of all sampled Canadian cats. Mitotype C was the most common mitotype found in the Canadian population, with a consistently high prevalence in every subpopulation (Table 1). The frequency of mitotype B6 varied most by

Table 2
Polymorphisms defining unique mitotypes.

SRS	Position	11	46	54	86	160	172	173	175	184	255	259	326	355	356	365	369
	NTP	A	C	C	T	T	A	T	C	G	C	T	T	T	C	T	G
Fcat	17587	G	.	T	.	.	.	.	.	.	.	A	.	C	.	.	.
	18144	G	T	.	.	.	G	.	.	.	T	A	.	C	T	.	.
	18743	.	.	.	.	.	.	C	T	A	.	.	C	.	.	.	A
	18738	.	T	.	C	A	G	.	.	.	T	.	.	.	.	C	.

The second column from the left lists the sample number of each cat with a unique mitotype followed by the single nucleotide polymorphisms (SNP) defining each type. The top row contains the coordinate of each SNP relative to the Sylvester reference sequence (SRS) with the SRS base calls at each position in the second row. Dots (.) represent positions that matched the SRS.

Table 3
Mitotype Statistics In Canadian and United States' Populations.

Populations	Mitotype Diversity (# Mitotypes)	Exclusion Capacity	Mean No. Pairwise Differences ± SD (Max Pairwise Difference)	Nucleotide Diversity ± SD
Vancouver (n = 15)	0.7714 ± 0.0720 (5)	0.7200	4.1143 ± 2.1701 (9)	0.0102 ± 0.0061
Ottawa (n = 28)	0.8307 ± 0.0429 (8)	0.8010	3.7751 ± 1.9608 (11)	0.0094 ± 0.0054
Winnipeg (n = 53)	0.8512 ± 0.0290 (11)	0.7967	5.0167 ± 2.4766 (13)	0.0124 ± 0.0068
Total Canadian Pop. (N = 96)	0.8625 ± 0.0153 (15)	0.8183	4.6160 ± 2.2852 (13)	0.0115 ± 0.0063
United States[a] (N = 493)	0.8319 ± 0.0094 (45)	0.8302	3.3986 ± 1.7427 (32)	0.0083 ± 0.0047

SD—Standard Deviation.
[a] Sequences collected by Grahn et al. [12] [see Methods].

subpopulation, ranging from 28.6% in Ottawa to 0% in Vancouver (Table 1).

4.3. Genetic differentiation within canadian subpopulations

The maximum number of pairwise differences within each subpopulation were within two base pairs of Ottawa's maximum pairwise difference (Table 3). The mean number of pairwise differences for each Canadian subpopulation were also highly similar, differing by at most just ~1.3 base pairs (Table 3). Mitotype diversities across subpopulations were similar with the probability that two samples randomly chosen from a subpopulation differed ranging from 0.7714–0.8512 (Table 3). The relatively low mitotype diversity in Vancouver contributed to a lower exclusion capacity for this subpopulation when compared to Ottawa or Winnipeg (Table 3). Finally, nucleotide diversities for all subpopulations differed by less than 0.003 from one another (Table 3). Significant genetic variation was detected between the Ottawa and Winnipeg subpopulations with 5.475% of the variation found among the subpopulations ($p = 0.02$) (Table 4). Pairwise Fst values also supported significant genetic differentiation from the random expectation at the 0.05 level when comparing the Ottawa and Winnipeg subpopulations (Table 5A). No significant genetic variation was detected between the other Canadian subpopulations.

4.4. Genetic differentiation within and between canadian and United States' populations

Mitotype frequencies across Canada and the United States were generally consistent. The mitotype diversities of the Canadian and United States' populations were within 0.0306 of each other with exclusion capacities differing by just 0.012 (Table 3). When considering all subtypes of and including the universal mitotypes A and B, frequencies for these mitotypes were consistent across both populations (Table 1). However upon closer inspection, the frequency of mitotype B6 was again found to vary greatly between the populations (Table 1). Despite the variable frequency of B6, both the AMOVA and pairwise Fst calculations failed to detect significant population structure between the Canadian and United States' cat populations (Table 5A).

As regional variation has been demonstrated in the United States' domestic dog population, the North American cat subpopulations were assessed for local variation in this study [19,20]. Significant genetic variation was detected between Ottawa and two United States' subpopulations: Hawaii and New York (Table 4). This population structure was also supported by the pairwise Fst values, which differed from the random expectation at the 0.05 level for the Ottawa vs Hawaii and the Ottawa vs New York comparisons (Table 5B). Finally, pairwise Fst values supported significant genetic differentiation between the Canadian population and Hawaiian subpopulation (Table 5B).

5. Discussion

Mitochondrial DNA extracted for pet hair has the potential to link victims, suspects, and accomplices to the scene of a crime. The evidentiary value of mtDNA evidence stems directly from the mitotype distribution within the local population. According to a 2014Canadian Animal Health Institute survey, the domestic cat is the most popular pet in Canada, with 7 million cats considered

Table 4
Populations Exhibiting Significant Genetic Variation As Assessed by Analysis of Molecular Variance (AMOVA).

Dataset		Degrees of Freedom	Percent Variation
Ottawa and Winnipeg	Among Population	1	5.48
	Within Population	79	94.52
	Total	80	
	Fst Value:	0.05475 *p*-value: 0.01857	
Ottawa and Hawaii[a]	Among Population	1	8.18
	Within Population	85	91.82
	Total	86	
	Fst Value:	0.08179 *p*-value: 0.01857	
Ottawa and New York[a]	Among Population	1	4.92
	Within Population	126	95.08
	Total	127	
	Fst Value:	0.04922 *p*-value: 0.02639	

Fst values between 0.05–0.15 correspond to moderate genetic differentiation.
The 34 pairwise comparisons between the remaining Canadian and United States' subpopulations yielded Fst values <0.05 indicating little genetic differentiation and/or insignificant genetic differentiation between populations ($p > 0.05$).
[a] Sequences collected by Grahn et al. [12] [see Methods].

Table 5
Population average pairwise Fst and population average pairwise differences for Canadian and United States datasets.

A.

	United States[a]	Canada	Ottawa	Winnipeg	Vancouver
United States[a]	3.39856 (0)	3.27151	3.00616	3.46894	3.06924
Canada	0.01576 (0.00433)	3.11294 (0)	4.23921	4.81447	4.321042
Ottawa	0.09524 (0.02299)	0.04364 (0.00738)	2.42328 (0)	4.67251	3.82619
Winnipeg	0.01676 (0.00516)	−0.00188 (0)	0.2766 (0.05475*)	3.50581 (0)	4.69937
Vancouver	0.01282 (0)	−0.05473 (0)	−0.11852 (0)	0.13388 (0.02268)	2.71429 (0)

Values above the diagonal are the average number of pairwise differences between populations (PiXY). Values on the diagonal (shaded in gray) are the average number of pairwise differences within population (PiX). Values below the diagonal are the corrected average pairwise difference (PiXY−(PiX + PiY)/2). Values in parentheses are population average pairwise Fst values, with asterisks (*) denoting values significantly different from the random expectation at the 0.05 level.

B.

	Canada	Ottawa	Winnipeg	Vancouver
Texas[a]	0.09234 (0.02514)	0.16818 (0.03683)	0.11266 (0.02353)	0.00291 (0)
New York[a]	0.06746 (0.01408)	0.25163 (0.04922*)	0.04901 (0.01010)	0.11668 (0.01996)
Missouri[a]	0.00650 (0.00408)	0.32275 (0.08962)	0.04320 (0.00927)	−0.02719 (0)
North California[a]	0.00731(0.00179)	0.08301 (0.01781)	0.00851 (0.00222)	−0.01758 (0)
South California[a]	−0.0011 (0)	0.02828 (0.00555)	0.03756 (0.01023)	−0.0686 (0)
Hawaii[a]	0.10767 (0.02569*)	0.33682 (0.08179*)	0.05743 (0.01362)	0.21353 (0.05086)
Florida[a]	−0.00919 (0)	−0.00888 (0)	0.05446 (0.01329)	−0.12582 (0)

Values are the corrected average pairwise difference (PiXY−(PiX + PiY)/2). Values in parentheses are population average pairwise Fst values, with asterisks (*) denoting values significantly different from the random expectation at the 0.05 level.

[a] Sequences collected by Grahn et al. [12] [see Methods].

household pets, however there has yet to be a survey of Canadian cat mitotypes.

In this study, mitotypes were determined for three populations of cats from cities on an East to West transect spanning Southern Canada. Each population was from a city less than 100 kilometers of the United States' border and together are a representative sampling of the majority of the Canadian cat population as approximately 75% of the Canadian human population lives within 100 kilometers of the United States' border (http://travel.nationalgeographic.com/travel/countries/canada-facts). Although the Canadian sample size (N = 96) was considerably smaller than a previous survey of the United States' domestic cat population that included close to 500 individuals, it has been shown that randomly sampling 50–150 unrelated local cats is sufficient to achieve 95% mitotype saturation within a surveyed population [22]. Given these statistics, our mitotype distributions are representative of the Canadian population and further sampling is unlikely to uncover a significant number of new mitotypes.

Despite only sampling 15 cats from the Vancouver subpopulation, the most common mitotypes in Canada (excluding mitotype B6) are present in this local population, and these mitotypes are also the most common mitotypes within Vancouver. Mitotype B6 is unlikely to be a common mitotype in Vancouver given that the mitotype was not found in the 15 cats sampled but is present in approximately 1 in 3.5 (8/28) and 1 in 7.5 (7/53) cats sampled from Ottawa and Winnipeg, respectively. Since mitotype B6 is found nowhere else in the world besides Ottawa, Winnipeg, and at a frequency of 1.0% in both Southern California and New York, this normally rare mitotype is likely also rare in Vancouver.

The exclusion capacity and mitotype diversity of the Canadian domestic cat population are within the ranges of those estimated for other cat populations world-wide (Table 1; [12]). The AMOVA and pairwise Fst values indicate no significant genetic variation between the Canadian and United States' cat populations as a whole. The only significant genetic variation detected between the total Canadian population and a United States' subpopulation, Hawaii, can partly be attributed to Hawaii's high frequency of unique mitotypes (13.6%) relative to the unique mitotype frequency in Canada (4.2%). In this case, the unique mitotype frequency across the United States (4.9%) corresponds more closely to the Canadian population than the Hawaiian subpopulation, implying that the Canadian and United States' cat populations are more homogenous and the Hawaiian subpopulation is the outlier [12] (Table 1). These unique types in the Hawaiian subpopulation are also the likely source of population structure between Hawaii and Ottawa (Table 4).

The disparate frequencies of mitotype B6 across the Canadian populations and between the Canadian and United States' populations prevents the North American cat population from being regarded as homogenous. Going from east to west across Canada, the frequency of subtype B6 decreased from 28.6% to 13.2% to 0.0%. The B6 subtype is found at a frequency of just 0.4% in the United States' population. The extremely high frequency of subtype B6 in Ottawa (28.6%) relative to the Winnipeg and New York subpopulations (13.12% and 1.0%, respectively) contributed in part to statistically distinct population structure between these regions. To test this theory, the B6 mitotype sequences were removed from the datasets and AMOVA and modified Fisher's exact test for homogeneity were recalculated and showed no significant genetic structure between either pair of populations.

6. Conclusion

A forensic database of cat mitotype distributions must be representative of the population from which mtDNA evidence is being compared or else the database risks misrepresenting the exclusionary power of certain mitotypes in the region. In this study, we have estimated the mitotype frequencies of the cat populations in Canada and compared them to those of the United States. The frequency of mitotype B6 prevents us from recommending that a single database be used to represent the mitotype frequencies of the North American cat population. A match between crime scene evidence and a suspected contributor found in Canada with a B6 mitotype will have low exclusionary power, as mitotype B6 is carried by nearly 16% of cats in the population. That same match will have a greatly increased exclusionary power if found in the United States where that mitotype exists in the population at a frequency of just 0.4%. While populations may not

be statistically different, not accounting for special cases between populations, such as the B6 mitotype, has the potential to misrepresent the power of evidence in these instances.

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References

[1] American Veterinary Medical Association, U.S. Pet Ownership & Demographics Sourcebook, American Veterinary Medical Association, Schaumburg, IL, 2012.
[2] Canadian Federation of Humane Societies, Cats in Canada: A Comprehensive Report on the Cat Overpopulation Crisis, Canadian Federation of Humane Societies, Ottawa, Ontario, Canada, 2012.
[3] W.H. Hendriks, M.F. Tarttelin, P.J. Moughan, Seasonal hair loss in adult domestic cats (Felis catus), J. Anim. Physiol. Anim. Nutr. 79 (1–5) (1998) 92–101.
[4] F. D'Andrea, F. Fridez, R. Coquoz, Preliminary experiments on the transfer of animal hair during simulated criminal behavior, J. Forensic Sci. 43 (6) (1998) 1257–1258.
[5] M.A. Menotti-Raymond, V.A. David, S.J. O'Brien, Pet cat hair implicates murder suspect, Nature 6627 (1997) 774.
[7] K. Takayanagi, K. Tsukada, M. Ota, S. Saito, H. Fukushima, Investigation of DNA extraction from hair shafts, International Congress Series 1239 (2003) 759–764.
[8] K.A. Roberts, C. Calloway, Mitochondrial DNA amplification success rate as a function of hair morphology, J. Forensic Sci. 1 (2007) 40–47.
[9] E.A.M. Graham, DNA reviews: hair, Forensic Sci. Med. Pathol. 3 (2) (2007) 133–137.
[10] M.M. Holland, T.J. Parsons, Mitochondrial DNA sequence analysis—validation and use for forensic casework, Forensic Sci. Rev. 11 (1) (1999) 21–50.
[11] C.R. Tarditi, R.A. Grahn, J.J. Evans, J.D. Kurushima, L.A. Lyons, Mitochondrial DNA sequencing of cat hair: an informative forensic tool, J. Forensic Sci. 56 (1) (2011) S36–S46.
[12] R.A. Grahn, J.D. Kurushima, N.C. Billings, J.C. Grahn, J.L. Halverson, E. Hammer, C.K. Ho, T.J. Kun, J.K. Levy, M.J. Lipinski, J.M. Mwenda, H. Ozpinar, R.K. Schuster, S.J. Shoorijeh, C.R. Tarditi, N.E. Waly, E.J. Wictum, L.A. Lyons, Feline non-Repetitive mitochondrial DNA control region database for forensic evidence, Forensic Sci. Int. Genet. 5 (1) (2011) 33–42.
[13] M. Wesselink, L. Bergwerff, D. Hoogmed, A.D. Kloosterman, I. Kuiper, Forensic utility of the feline mitochondrial control Region—a dutch perspective, Forensic Sci. Int. Genet. 17 (2015) 25–32.
[14] M.J. Lipinski, L. Froenicke, K.C. Baysac, N.C. Billings, C.M. Leutenegger, A.M. Levy, M. Longeri, T. Niini, H. Ozpinar, M.R. Slater, N.C. Pedersen, L.A. Lyons, The ascent of cat breeds: genetic evaluations of breeds and worldwide random-bred populations, Genomics 91 (1) (2008) 12–21.
[15] J.V. Lopez, S. Cevario, S.J. O'Brien, Complete nucleotide sequences of the domestic cat (Felis catus) mitochondrial genome and a transposed mtDNA tandem repeat (Numt) in the nuclear genome, Genomics 33 (2) (1996) 229–246.
[16] L. Excoffier, H.E.L. Lischer, Arlequin suite ver 3. 5: a new series of programs to perform population genetics analyses under linux and windows, Mol. Ecol. Resour. 10 (3) (2010) 564–567.
[17] K. Tamura, J. Dudley, M. Nei, S. Kumar, MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4. 0, Mol. Biol. Evol. 24 (8) (2007) 1596–1599.
[18] M. Stoneking, D. Hedgecock, R.G. Higuchi, L. Vigilant, H.A. Erlich, Population variation of human mtDNA control region sequences detected by enzymatic amplification and sequence-Specific oligonucleotide probe, Am. J. Hum. Genet. 48 (2) (1991) 370–382.
[19] B.B. Smalling, J.A. Satkoski, B.K. Tom, W.Y. Szeto, B.J. Erickson, T.F. Spear, D.G. Smith, B. Budowle, K.M. Webb, M.W. Allard, S. Kanthaswamy, Geographic differences in mitochondrial DNA (mtDNA) distribution among United States (US) domestic dog populations, Open Forensic Sci. J. 3 (2) (2010) 22–32.
[20] A. Spadaro, K. Ream, C. Braham, K.M. Webb, Local mitochondrial DNA haplotype databases needed for domestic dog populations that have experienced founder effect, Forensic Sci. Int. 248 (2015) 113–118.
[22] R.A. Grahn, H. Alhaddad, P.C. Alves, E. Randi, N.E. Waly, L.A. Lyons, Feline mitochondrial DNA sampling for forensic analysis: when enough is enough!, Forensic Sci. Int. Genet. 16 (2015) 52–57.