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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.

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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,

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