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Feline mitochondrial DNA sampling for forensic analysis: When enough is enough!

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ABSTRACT

Pet hair has a demonstrated value in resolving legal issues. Cat hair is chronically shed and it is difficult to leave a home with cats without some level of secondary transfer. The power of cat hair as an evidentiary resource may be underused because representative genetic databases are not available for exclusionary purposes. Mitochondrial control region databases are highly valuable for hair analyses and have been developed for the cat. In a representative worldwide data set, 83% of domestic cat mitotypes belong to one of twelve major types. Of the remaining 17%, 7.5% are unique within the published 1394 sample database. The current research evaluates the sample size necessary to establish a representative population for forensic comparison of the mitochondrial control region for the domestic cat. For most worldwide populations, randomly sampling 50 unrelated local individuals will achieve saturation at 95%. The 99% saturation is achieved by randomly sampling 60–170 cats, depending on the numbers of mitotypes available in the population at large. Likely due to the recent domestication of the cat and minimal localized population substructure, fewer cats are needed to meet mitochondria DNA control region database practical saturation than for humans or dogs. Coupled with the available worldwide feline control region database of nearly 1400 cats, minimal local sampling will be required to establish an appropriate comparative representative database and achieve significant exclusionary power.
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1. Introduction

While domestic cats may exhibit increased seasonal shedding, each individual cat hair has its own growth cycle and therefore may be shed at different times [1]. D'Andrea et al. [2] demonstrated that during criminal activity, it is nearly impossible for a perpetrator to leave a pet owner's home without some pet hair transfer to their clothing. Although nearly 40 million homes in the United States contain cats [3,4], feline forensic evidence has experienced limited use in the resolution of legal issues [5,6]. As most shed hairs are derived from the undercoat, diagnostic phenotypic characteristics are limited in microscopic analyses, which focus on the guard hairs. With respect to genetic analyses, natural degradation of genomic DNA occurs as hairs mature, making genetic STR marker analysis difficult. However, success rates are increasing with improved isolation and PCR amplification techniques, as well as the targeted reduction in amplicon size [7–9]. Because the copy number is increased and there is a slightly more protective double membrane system in the mitochondria, degradation in hair shafts is less severe for mtDNA [10]. Thus, mtDNA analysis afford criminalists the greatest likelihood of success with respect to diagnostic data generation from shed hairs [11]. However, in the absence of adequate reference databases, the informative value derived from shed hairs is significantly reduced [12,13]. The value of any genetic evidence is at least

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partially dependent upon the exclusionary power of that evidence.

The complete domestic cat mitochondrial DNA (mtDNA) sequence has been published [14] and examined for various forensic, species identification, and evolutionary studies. Previous studies have examined the cat mtDNA control region (CR), which has a conserved core flanked by repeat segments RS2 and RS3 that have high rates of heteroplasmy [15–17]. The 402 bp region examined in these previous cat mtDNA CR forensic studies represents the region between the two repeat regions, thus avoiding the highly heteroplasmic region in RS2. Additionally, analysis in this region avoids the 7.9 kb feline *Numt* (position 529–8454) mitochondrial region integrated into the nuclear genome [18]. Grahn et al. [19] established a cat mtDNA CR database sequencing the 402 bp region that included global samples of random and fancy breed cats. One hundred-forty-nine mitotypes were identified in 1394 cats. Twelve major mitotypes represented 83% of the cats and 66% of the cats had four major mitotypes. Approximately 7.5% ($n = 104$) of the mitotypes were unique. However, these percentages were not constant across populations and frequencies for specific geographical regions may need to be determined to sufficiently represent mitotype structure and frequency within a specific dataset.

Determining when sufficient samples have been ascertained to adequately represent a population depends upon the population size and the mitotype diversity observed within a population. An ideal sample set would be considered saturated when sampling additional individuals from the population does not further increase the absolute number of observed types. With the high mutation rate of the mtDNA CR and the vast population of cats, this point would not be realized unless every individual were sampled: an unrealistic proposition at best for any population of most any species. Pereira et al. [20] first defined practical saturation of human mtDNA CR mitotypes as the point at which increasing sample sets by 100 individuals does not increase the number of observed mitotypes by 5%. This value is achieved at 900 individuals when evaluating the less polymorphic right domain of the human mtDNA HVRI. If a more stringent 1% cutoff is used, 1200 samples are required. However, as suggested by Webb and Allard [21] for canine mtDNA, reduced exclusionary power resulting from fewer potential mitotypes compared to humans may prove advantageous when attempting to reach sample dataset saturation. Domestic felines, like dogs, have far fewer mitotypes than humans [19,22], thus achieving a representative population would require far fewer individuals. In fact, 50% less dogs were required ($N = 450$) as compared to the similar mtDNA control region domain in humans ($N = 900$) to achieve the 5% saturation cutoff. For the 1% saturation cut-off, the same number of domestic dogs are required ($N = 450$) as compared to a required sampling of 1200 humans [21].

An extensive feline mtDNA database was established for forensic analysis represented by nearly 1400 samples from 25 distinct geographic populations and 26 different breeds [19]. However, the regional distribution and frequencies of mitotypes are not equally represented across all populations. The current research evaluates the numbers of feline samples needed to adequately represent the mitotype diversity for any given population of domestic cats. Practical saturation threshold sampling points of 5% and 1% were determined for all pedigreed cats, all worldwide random bred (RB) cats representing the geographically distinct populations, fourteen subsets of the RB cats including both geographical and genetic partitioning based upon Lipinski et al. [23], breeds based upon genetic partitioning and breeds plus RB cats combined based upon genetic affinity. As feline mitotypes are globally distributed, combining locally obtained types with the established database will provide maximal exclusionary power.

2. Materials and methods

2.1. Cat dataset

The 402 bp mtDNA CR mitotype database of Grahn et al. [19] was used for random sampling in this study. The cat mtDNA CR region represented positions 16814–206 of the Sylvester Reference Sequence [14,22]. The published database ($N = 1394$) was increased by additional cats from Spain, Portugal, Italy and Slovenia producing a dataset ($N = 1408$) for analysis of 1076 random bred (RB) cats and 332 breed cats. The mtDNA CR mitotypes were generated and defined as previously described [19,22]. Concerted efforts were made to collect unrelated cats when constructing this database. RB cats, which represented non-pedigreed, non-mixed breed, street, household and feral populations, were collected opportunistically as part of studies to examine the overall genetic diversity of cats [22,23]. Although RB cats cannot be definitely proven to be unrelated, to maximize the likelihood of unrelatedness among samples, only one cat from an obvious litter for RB cats was selected and adult cats from shelters were sampled. Sampling was conducted at veterinary clinics as owners randomly brought their cats into the clinics for veterinary care. In addition, some sampling was conducted by luring cats from market places and streets by offering food, providing the food after the buccal swab collection. Breed cats were selected at cat shows, mainly in the USA, and did not share grand-parents as defined by their breeder submitted pedigrees. The overall RB dataset was also parsed into thirteen groupings based upon geographic and genetic criteria with an additional seven analyses including breeds (Table 1). The Eastern USA RB ($n = 151$) are represented by cats from New York ($n = 101$) and Florida ($n = 50$). The Western USA RB population ($n = 299$) is represented by cats from CA ($n = 247$), TX ($n = 28$) and MI ($n = 24$). The USA RB include the Eastern and Western USA combined with the Hawaii USA RB ($n = 64$). The remainder of the RB samples were analyzed together, non-USA RB ($n = 562$), and partitioned based upon geography and genetics. Geographic European RB samples ($n = 99$) are from Portugal and Spain ($n = 10$), Germany ($n = 21$), Italy ($n = 29$) and Slovenia ($n = 39$). Geographic Mediterranean RB samples ($n = 275$) include representatives from Egypt ($n = 131$), Israel ($n = 41$) Turkey ($n = 55$),

Table 1
Cat populations and mitotypes for mtDNA database saturation resampling.

Population	Sample size	No. mitotypes	5% cutoff	1% cutoff
World-wide RB	1076	113	80	160
USA RB	514	47	50	110
Breeds	332	55	70	130
Eastern USA RB	151	21	50	100
Western USA RB	299	31	50	90
Hawaii USA RB	64	15	50	90
Non-USA RB	562	82	70	170
Geographic European RB	99	49	80	140
Genetic European RB	633	73	60	100
European breed	180	36	70	100
Genetic European RB + breed	813	96	90	150
Geographic Mediterranean RB	275	30	50	100
Genetic Mediterranean RB	263	45	60	100
Genetic Eastern Mediterranean RB	132	36	60	110
Mediterranean breed	27	9	60	140
Genetic Mediterranean RB + breed	290	46	60	90
Geographic Central Asian RB	135	19	<40	70
Genetic Asian RB	35	9	<40	40
Asian breed	121	24	60	80
Genetic Asian RB + breed	156	29	60	100
Egyptian RB	131	21	50	80
Iranian RB	99	16	50	60
Indian RB	53	8	<40	40

RB implies random bred.

Tunisia ($n = 14$), and Kenya ($n = 34$). Geographic Central Asian RB ($n = 135$) samples are from Iran ($n = 65$), Iraq ($n = 7$), Sri Lanka ($n = 24$), Dubai ($n = 10$) and India ($n = 29$). The 26 cat breeds ($n = 332$) presented in previous studies [19,23] were grouped as one population and were also partitioned based upon genetic affinity (European, Asian and Mediterranean) and analyzed with and without the progenitor populations. Genetic European RB ($n = 633$) includes Portugal, Spain, Germany, Slovenia, Kenya, and the US populations. Genetic Mediterranean RB ($n = 263$) includes Italy, Egypt, Israel, Turkey, and Tunisia. Genetic Eastern Mediterranean RB ($n = 132$) includes Italy, Israel, Turkey, and Tunisia. Genetic Asian RB ($n = 35$) includes China ($n = 12$), Taiwan ($n = 12$) and Vietnam ($n = 11$). Lastly, the Indian population ($n = 53$) (India and Sri Lanka), Iranian samples ($n = 99$) (Iran, Iraq, Lamu ($n = 11$), Pate ($n = 6$), Dubai) and Egyptian samples were each analyzed as independent populations.

2.2. In silico random sampling and unique mitotype analysis

A script in R (http://www.r-project.org/) was created to randomly sample, with replacement, a given number of individuals from each of the 23 defined populations. The sampling was repeated 100 times for each sampling data point. A mean number of mitotypes was then calculated for each sampling. This process was repeated until 1000 individuals were randomly sampled with replacement for each population. Script design is as follows: let (S_i) be a subpopulation of population (Pop_j) where the Pop includes breeds, Western US, Eastern US, etc. Let (n_i) be the number of individuals sampled with replacement from Pop_j (where i is the subpopulation number and j is the population number). The number of sampled individuals of the first subpopulation (n_1) is 30 and the number of additionally sampled individuals for each of the remaining subpopulations (n_2, n_3, n_4, ..., n_{97}) is 10. The subpopulation size of the first subpopulation (S_1) = n_1 and the size of subpopulations (S_2, S_3, S_4, ..., S_{97}) is depicted by the following formula $S_i = S_{i-1} + n_i$ (for i from 2 to 97). The resulting subpopulation size is (30, 40, 50, ..., 1000). This sampling process described above was replicated ($r = 100$) times which resulted in subpopulations ($S_{i \cdot r}$).

The number of mitotypes (m_i) was determined for each subpopulation (S_i) in each of the 100 replicas, independently. The mean of the number of unique mitotypes in each subpopulation class (i.e. $m_{1 \cdot 1}$, $m_{1 \cdot 2}$, $m_{1 \cdot 3}$, ..., $m_{1 \cdot 100}$) is the number of unique mitotypes for subpopulation S_1 in each of the 100 replicas. The means of the subpopulations ($n = 97$) for all replicates ($r = 100$) were calculated. A t-test analysis was conducted on the differences in the means of unique mitotypes between each two adjacent subpopulations (t-test (m_i, m_{i+1}), where i is from 1 to 96 and 97 is the total number of subpopulations. The t-test was conducted for (1) the presence of a 5% increase in the mean number of mitotypes of (m_i) in (m_{i+1}) and (2) the presence of a 1% increase in the mean number of mitotypes of (m_i) in (m_{i+1}). The significant p-value chosen was 0.05 for the two scenarios.

The procedure was repeated to determine the effect of sample acquisition increase increments. The above script was modified from a starting population of 30 to increase by 20, 50 and 100 sampled individuals. As before, 100 replicates were performed and averaged for each data point.

3. Results

3.1. Sampling saturation

Sixteen RB, four breed and three mixes of breed and RB populations were evaluated for sampling saturation effects (Table 1). RB cats were distributed into non-intersecting geographic subpopulations and as genetic subpopulations. For example, the USA RB grouping was divided into the eastern, western and Hawaiian USA RB samples. Similarly, based upon previous work by Lipinski et al. [23], RB cats were subdivided into established populations based upon structure analysis of STR markers. The breeds were analyzed together and subdivided based upon their genetic affinity with the same set of markers. Populations varied in numbers of samples, available mitotypes and mitotype frequencies for each grouping. The fewest mitotypes available ($n = 8$) were in the Indian RB population while the most, 96, were found in the genetic European RB plus associated breeds.

Starting with a subset of 30 individuals from a given population, the sample size of the population was incrementally increased by 10 individuals, up to 1000 total individuals for each population. This process was repeated 100 times. These data were graphed and mean values for numbers of mitotypes sampled were generated for each population (Fig. 1, Supplementary Figs. 1–6). The point at which a 5% and 1% increase in the numbers of observed mitotypes was observed is indicated. Table 1 indicates the 5% and 1% cutoff point for each population. Minimal 5% cutoff values were as low as <40 for the geographic central Asian, genetic Asian and Indian RB cats to as many as 90 cats in the genetic European RB plus breeds. When considering a $<1\%$ observed increase in mitotypes sampled, as few as 40 cats in the genetic Asian and Indian RBs as many as 170 cats in the non-USA RB populations were required.

3.2. Sampling increment increase effects on saturation

Three data sets were examined to determine the effect of sampling size increments on the numbers of mitotypes extracted. The mean of 100 replicates of sampling with replacement of 10, 20, 50 and 100 individuals is presented in Table 2. For each increase, regardless of population, both the 5% and 1% practical saturation point for the number of cats required increases by a factor of 1.75–2.4.

4. Discussion

The power of forensic genetic evidence is directly correlated to the exclusionary value generated by the dataset available. In the absence of adequate and appropriate databases, exclusionary value is significantly diminished and can compromise the value of DNA evidence during adjudication. An extensive database was created for feline mtDNA mitotype comparison that includes cats from 18 countries and 26 breeds from the USA [19,22]. Twelve universal mitotypes with a frequency of $>1\%$ are defined in cats; however, most of the universal types are likely derived from mitotypes A and D as they have three or fewer defining mutations. Cat CR mitotypes are defined by SNPs and one indel at position 62. Almost all cat mitotypes are identified in different regional populations in the USA, but not in all world populations. Cat breeds in the USA generally had the mitotype that was most common to their feral population (race) of origin, but were not defined by any one mitotype.

Cats have a different and unique history of domestication and breed development as compared to dogs and other agricultural species. Cats likely somewhat self-domesticated as humans transitioned from hunter – gathers and nomads to more sedentary farmers who developed villages and civilizations, approximately 8000–10,000 years ago [24]. Regional wildcat subspecies (*Felis silvestris*) closed in on village grain stores and refuse piles to obtain meals of mice, rats and other vermin. Once further tamed, these “domesticated” cats then spread around the world with commerce and trade as Old World humans expanded, conquered, and colonized other worldwide territories [25]. Thus, feral, RB populations of street, house, and barn cats became established around the globe,

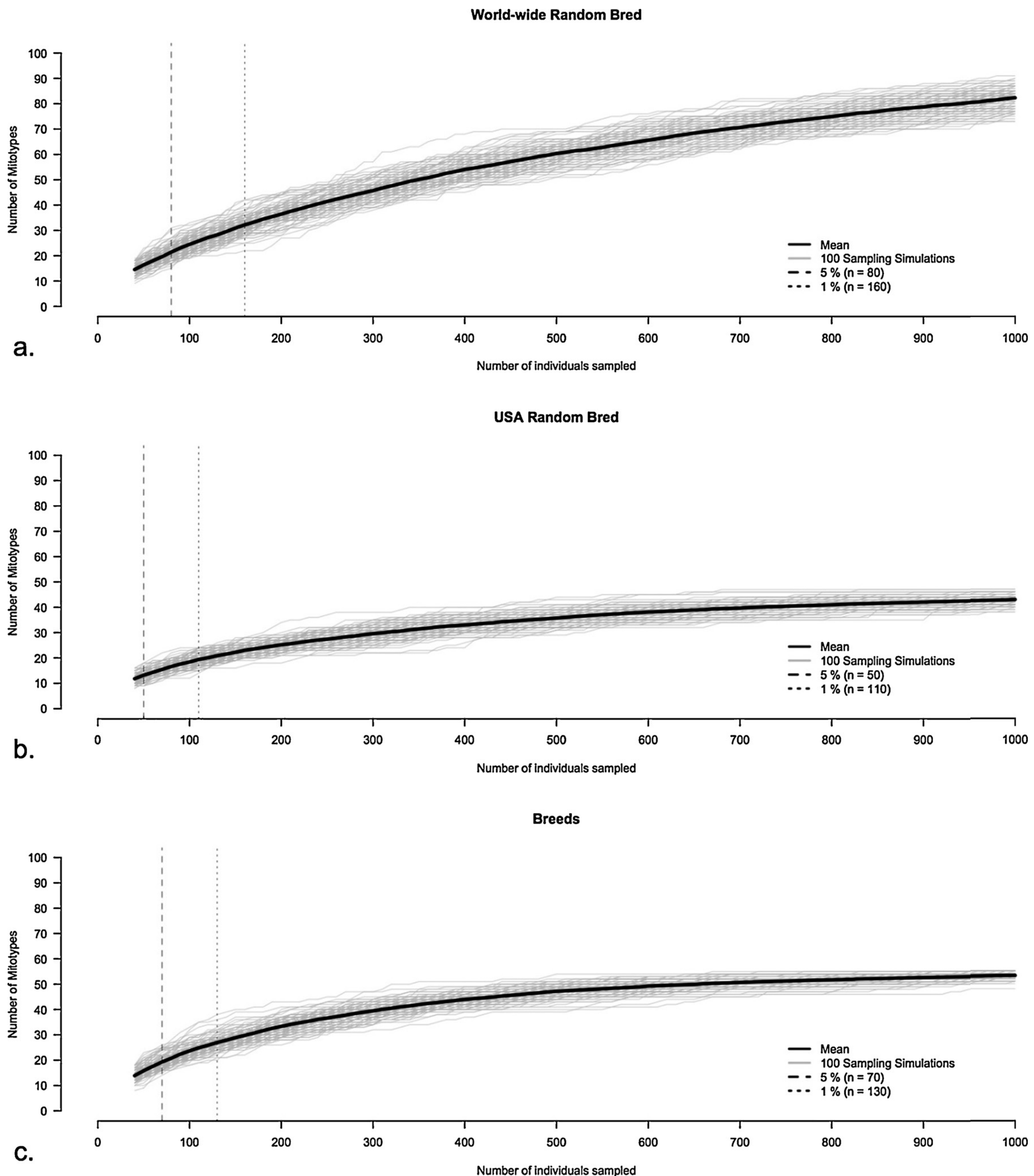

Fig. 1. Incremental random mitotype sampling – 100 replicates. Three cat groupings are compared: (a) all random bred cats combined worldwide, (b) random bred cats from the USA only, and (c) all pedigreed breed cats combined. Each gray line indicates one of 100 replicates for a random sample increase of 10 starting from 30 individuals. Solid black line indicates the mean of 100 replicates. The large-block broken vertical line represents the point at which a 5% increase in the mean number of mitotypes was no longer observed. The small-block broken vertical line represents the point at which a 1% increase in the mean number of mitotypes was no longer observed.

thousands of years before the development of any breeds and have performed the same role and function that is required by humans until this day – vermin control and companionship. Once cats spread into new territories and niches around the world, populations became well established and only geographical and potentially political barriers caused migration isolations. Based on STRs and SNPs, genetic studies have defined approximately eight genetically distinct populations of cats, "cat races", including cats from Western European (including the Americas), Eastern Mediterranean, Egyptian, Middle Eastern, West Indian Ocean, Indian, Southeast Asian and East Asian races [23,26]. No domestic cats are indigenous to the New World and Australia. Thus, cat populations in those regions likely represent the haplotypes of cats from the colonizing home countries. Admittedly, some of the sample sets may lack sufficient samples to be truly representative of a population. The genetic Asian RB population was represented by only 35 samples with nine mitotypes and the Indian RB with 53 samples and eight mitotypes. Additional sampling from these regions and areas not yet evaluated may reveal additional types and relationships.

Cat breeding was established in the United Kingdom approximately 150 years ago. In contrast, some breeds of dogs and other agricultural species have existed for many centuries. Cats were not specific "breeds" at that time, but only coat color and fur type variants. For example, cats from the "Orient" were noted as a different breed and presented the Siamese color pattern. The first approximately five cat breeds grew slowly for the first few decades

Table 2
Resampling size effects on saturation points for cat mtDNA CR database.

	5% cutoff	1% cutoff
World-wide random bred		
10 sample increments	80	160
20 sample increments	150	350
50 sample increments	330	830
100 sample increments	630	1030
USA random bred		
10 sample increments	50	110
20 sample increments	110	230
50 sample increments	230	480
100 sample increments	430	930
Non-USA random bred		
10 sample increments	70	150
20 sample increments	130	350
50 sample increments	280	680
100 sample increments	530	1030

of the 20th century, with a majority of breeds developing in the past 50 years, after the world wars. Cat enthusiasts will split their breeds based solely on a color variant or fur type. For example, Persians, Exotics, Selkirk Rex and Scottish Folds are all basically Persian cats and also cluster strongly genetically [23,27,28]. For this project, the cat breeds that have been shown to be genetically distinct by other genetic studies were selected for mtDNA CR sequencing and analysis with the expectation to potentially identify diverse or unique mitotypes.

The sample size required to effectively represent a local population is a product of allele frequencies and the mitotypes represented by those frequencies. However, the dilemma remains of what is an appropriate number of individuals to sample, especially when the true size of the population may be unknown. This study evaluated the power of the cat mtDNA database in a comparable fashion to previous studies that have focused on the human and dog mtDNA databases [20,21]. Webb and Allard [21] established that saturation occurs with 450 individuals when examining the less polymorphic right domain and 650 individuals for the left domain of the variable regions of the dog mtDNA. These numbers are significantly greater than determined for the cat regardless of data parsing based on geography or genetic history. An increased number of replicates ($n = 100$) were performed to lower variance for the cat estimates of saturation points. Approximately 5% of cats in the USA have a unique mtDNA CR mitotype [19]. The average for the entire dataset is 7.4%. Expectedly, geographic regions with fewer mitotypes require a smaller sampling of individuals to effectively saturate the dataset, while populations with more mitotypes require greater sampling. Likewise increasing the point of saturation from a 5% to a 1% increase in new mitotypes requires almost doubling the sampling of individuals to achieve saturation. Most USA RB cat populations require a sampling of approximately 50 individuals for sufficient representation at the 5% saturation cut-off, while 80 or less are required for the rest of the worldwide groupings. The USA samples require approximately 110 cats to be sampled for adequate representation at the 1% saturation point, whereas the non-USA RB cats, which represent very diverse world locations, requires 170 cats, requiring 55% more sampling. Evaluation of the USA RB population with their likely genetic progenitors, the European RBs, requires an additional 10 samples to reach the 5% practical saturation point but 10 fewer for the 1%.

Of note is the impact frequencies of represented types have on practical saturation values. For example, 113 types exist in the world wide RB population. Practical saturation is achieved at 80 samples but this only represented 21 mitotypes. This is a product of frequency of alleles in a random population. Within the 1076 samples, 81 are unique, 101 are rare – and represent 20 types occurring in 2–10 individuals. The remaining 894 are relatively common. Assuming the least common of the rare types and none of the unique types had been chosen, with 21 types established, the ratio of identified vs. novel mitotypes in the pool is nearly 10:1. Thus, the odds of picking a cat with a type not previously chosen, are low.

While differences may exist between the cat and the dog resulting from age of domestication [29] and selective breeding strategies [30], the difference may be explained by statistical factors as well. In the canine study, sampling increases (1) were set at 50 individual increments to determine saturation, (2) were performed by randomly sampling without replacement, and (3) relative frequencies of the mitotypes were unknown. The right mtDNA domain had 12 possible mitotypes while the left domain had 64 mitotypes. Thus, in each sampling, one and three new mitotypes, respectively, needed to be selected to remain above the 5% saturation point, while the pool of potential selections decreased. The saturation point was not achieved for the more variable, left side domain, for the entire data set, therefore the saturation values were estimated from the slope of the curve. For the feline dataset, specific mitotypes were not necessarily found as frequently as the numbers of different mitotypes. Thus, if one sampling found an average of 20 types, the next incremental sampling would need to identify >21 to remain above the 5% saturation point. When evaluated with the same incremental increase as the canine study ($N = 50$) with the same number of possible mitotypes (compared to the dog left domain-64 types) and similar sample numbers, the cat was saturated at 280 samples. However, sampling this number of individuals is unnecessary because (1) the population in question would have to be unique with respect to the remainder of domestic cats of the world, and (2) a basal analysis of global population frequencies has been established [19]. Due to the recent domestication of cats, the likelihood of populations existing that are so genetically distinct due to isolation that they warrant a novel database seems remote. Additionally the establishment of a nearly 1400 cat global representative database renders the necessity of sampling 280 local cats moot.

Sampling was performed with replacement to mimic random sampling of a population. Since the parsed datasets have a fixed number of individuals, removal of any one type without replacement would change the odds of randomly extracting any other type. Sampling with replacement maintains the probability of sampling a mitotype at the frequency in which it is represented in the population. Regardless, sampling without replacement produced similar sample numbers (±10) for both the 95 and 99% saturation points across the data sets (data not shown).

The previous canine mtDNA saturation studies evaluated saturation values of polymorphic sites [20,21]. For any given dataset, the number of sites is fixed by the length of the sequence examined. Thus, the number of sites that may be polymorphic is limited. A mitotype may be defined by more than one polymorphic site. If one of these diagnostic sites has mutated back to the ancestral state, a new mitotype is created, but the number of polymorphic sites has not changed. The net effect is a devaluation of reversion mutations. Since each mitotype is defined by a suite of characters inherited as a unit, focusing on the polymorphic content is less meaningful in judicial proceedings compared to establishing population structure. Two previous cat mtDNA CR studies evaluated the random match probabilities of non-overlapping segments of the cat mtDNA CR. The 1394 database considered in this study had a genetic diversity value of 0.8813 ± 0.0046 with a random match probability (RMP) of 12.0% compared to 0.8767 ± 0.0277 for a smaller Japanese feline dataset with a 14.1% RMP [17]. The similarities of the diversity and RMP suggested that the regions are comparable in forensic applications. The present study suggests that the current cat mtDNA CR datasets may

be sufficient as their sizes are larger than the 5% practical saturation levels and most of the 1% levels.

The marked difference between the saturation values of cats and humans likely results from the short evolutionary history of the domestic cat and number of types available. The most common human European mitotype is H. H1, a subclade of H, is subdivided into 253 distinct phylogenetic groupings [31]. There are over 90 basal subclades for mitotype H alone, all may be further divided. To date, only 149 different mitotypes have been identified for the region under analysis in the domestic cat. Thus, the potential number of mitotypes to be identified in any human population is significantly greater than in cats. As such, sampling requirements for establishing a local population data set, based on geography or genetics, for local mitotype frequency is expected to be significantly decreased.

5. Conclusions

The current research demonstrates that by sampling 50–150 cats from a region, a local representative dataset can be achieved for the domestic cat in regards to the mtDNA CR. Fewer samples are required to achieve exclusionary power relative to humans and dogs owing to the recent evolutionary history, high allelic frequencies and limited numbers of global mitotypes in the cat. Coupled with the established global database of mtDNA CR mitotypes from over 1400 cats, this dataset should provide sufficient exclusionary power for most global forensic applications regarding feline mtDNA CR analysis. Caution should be taken with cat populations however, as they may not disperse as widely as other species, and, parent offspring relationships may be common in a given area. Thus, mtDNA data should be used primarily as an exclusionary tool.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fsigen.2014.11.017.

References

[1] M.L. Ryder, Seasonal changes in the coat of the cat, Res. Vet. Sci. 21 (1976) 280–283.
[2] F. D'Andrea, F. Fridez, R. Coquoz, Preliminary experiments on the transfer of animal hair during simulated criminal behavior, J. Forensic Sci. 43 (1998) 1257–1258.
[3] APPMA, National Pet Owner's Survey, American Pet Product Manufacturing Association, Greenwich, CT, 2006.
[4] AVMA, US Pet Ownership and Demographics Sourcebook, American Veterinary Medical Association, Schaumburg, IL, 2007.
[5] M.A. Menotti-Raymond, V.A. David, S.J. O'Brien, Pet cat hair implicates murder suspect, Nature 386 (1997) 774.
[6] L.A. Lyons, R.A. Grahn, T.J. Kun, L.R. Netzel, E.E. Wictum, J.L. Halverson, Acceptance of domestic cat mitochondrial DNA in a criminal proceeding, Forensic Sci. Int. Genet. 13 (2014) 61–67.
[7] K. Muller, C. Brugger, R. Klein, E. Miltner, F. Reuther, P. Wiegand, STR typing of hairs from domestic cats, Forensic Sci. Int.: Genet. Suppl. 1 (2008) 3.
[8] P. Grubwieser, R. Muhlmann, B. Berger, H. Niederstatter, M. Pavlic, W. Parson, A new miniSTR-multiplex displaying reduced amplicon lengths for the analysis of degraded DNA, Int. J. Legal Med. 120 (2006) 115–120.
[9] J.M. Butler, Y. Shen, B.R. McCord, The development of reduced size STR amplicons as tools for analysis of degraded DNA, J. Forensic Sci. 48 (2003) 1054–1064.
[10] C.A. Linch, Degeneration of nuclei and mitochondria in human hairs, J. Forensic Sci. 54 (2009) 346–349.
[11] B. Budowle, M.W. Allard, M.R. Wilson, R. Chakraborty, Forensics and mitochondrial DNA: applications, debates, and foundations, Annu. Rev. Genomics Hum. Genet. 4 (2003) 119–141.
[12] M.M. Holland, Y.J. Parsons, Mitochondrial DNA sequence analysis-validation and use for forensic casework, Forensic Sci. Rev. 11 (1999) 30.
[13] B. Budowle, M.R. Wilson, J.A. DiZinno, C. Stauffer, M.A. Fasano, M.M. Holland, K.L. Monson, Mitochondrial DNA regions HVI and HVII population data, Forensic Sci. Int. 103 (1999) 23–35.
[14] 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 (1996) 229–246.
[15] J.L. Halverson, C. Basten, Forensic DNA identification of animal-derived trace evidence: tools for linking victims and suspects, Croat. Med. J. 46 (2005) 598–605.
[16] J.L. Halverson, L.A. Lyons, Forensic DNA identification of feline hairs: casework and a mitochondrial database, Proc. Am. Acad. Forensic Sci. X (2004) B150.
[17] T. Tamada, N. Kurose, R. Masuda, Genetic diversity in domestic cats Felis catus of the Tsushima Islands, based on mitochondrial DNA cytochrome b and control region nucleotide sequences, Zool. Sci. 22 (2005) 627–633.
[18] J.V. Lopez, M. Culver, J.C. Stephens, W.E. Johnson, S.J. O'Brien, Rates of nuclear and cytoplasmic mitochondrial DNA sequence divergence in mammals, Mol. Biol. Evol. 14 (1997) 277–286.
[19] 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 (2011) 33–42.
[20] L. Pereira, C. Cunha, A. Amorim, Predicting sampling saturation of mtDNA haplotypes: an application to an enlarged Portuguese database, Int. J. Legal Med. 118 (2004) 132–136.
[21] K. Webb, M. Allard, Assessment of minimum sample sizes required to adequately represent diversity reveals inadequacies in datasets of domestic dog mitochondrial DNA, Mitochondrial DNA 21 (2010) 19–31.
[22] 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 (Suppl. 1) (2011) S36–S46.
[23] 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, The ascent of cat breeds: genetic evaluations of breeds and worldwide random-bred populations, Genomics 91 (2008) 12–21.
[24] J. Clutton-Brock, A Natural History of Domesticated Mammals, Cambridge University Press, British Museum, London, 1987.
[25] N.B. Todd, Cats and commerce, Sci. Am. 237 (1977) 100–107.
[26] L.A. Lyons, J.D. Kurushima, A Short Natural History of the Cat and its Relationship with Humans, Elsevier Saunders, St. Louis, MO, 2012.
[27] S. Filler, H. Alhaddad, B. Gandolfi, J.D. Kurushima, A. Cortes, C. Veit, L.A. Lyons, G. Brem, Selkirk Rex: morphological and genetic characterization of a new cat breed, J. Hered. 103 (2012) 727–733.
[28] J.D. Kurushima, M.J. Lipinski, B. Gandolfi, L. Froenicke, J.C. Grahn, R.A. Grahn, L.A. Lyons, Variation of cats under domestication: genetic assignment of domestic cats to breeds and worldwide random-bred populations, Anim. Genet. 44 (2013) 311–324.
[29] S. Bjornerfeldt, M.T. Webster, C. Vila, Relaxation of selective constraint on dog mitochondrial DNA following domestication, Genome Res. 16 (2006) 990–994.
[30] M.M. Gray, J.M. Granka, C.D. Bustamante, N.B. Sutter, A.R. Boyko, L. Zhu, E.A. Ostrander, R.K. Wayne, Linkage disequilibrium and demographic history of wild and domestic canids, Genetics 181 (2009) 1493–1505.
[31] M. van Oven, M. Kayser, Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation, Hum. Mutat. 30 (2009) E386–E394.