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Research paper

Local populations and inaccuracies: Determining the relevant mitochondrial haplotype distributions for North West European cats

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ABSTRACT

Typing of different portions of the feline mitochondrial control region has illustrated pronounced differences in haplotype distributions between cats from the Netherlands and other parts of the world. To gain a better understanding of the haplotype distribution of North West Continental Europe, 605 bp of mitochondrial DNA was typed from randomly selected cats from the Netherlands (N = 146), Belgium (N = 64) and South West Germany (N = 128). The genetic differences between these randomly sampled European populations correlate to the geographical distances, with the Dutch and the South West German populations furthest apart and the Belgian population as an intermediate (Fst values 0.01–0.03).

Comparison of North West European mainland distributions to published feline mitochondrial haplotype distributions illustrated moderate to large genetic differentiation (Fst values 0.01–0.32). In this comparison, the correlation between geographical and genetic distance was absent, leading to founder effects and human impact on cat population structure and dispersion being considered as important parameters.

When an accurate estimation of feline haplotype distribution is required in forensics, care should be taken when deciding whether extrapolating the frequency data from a certain source to a larger area (country/continent) is justified or whether additional typing of local populations is necessary. This may differ from case to case as local frequencies can be relevant, but can also be deceitful. To improve the applicability of forensic feline mitochondrial DNA studies, documentation and publishing of sampling strategies is advised, as is the implementation of measures to help eliminate potentially erroneous haplotypes.

1. Introduction

The domestic cat is an extremely popular household pet throughout many parts of the world including Europe. In the North West European countries Germany, Belgium and the Netherlands 20%, 26% and 24% of households respectively are estimated to have at least one cat. In these countries, the cat population is estimated to be approximately one sixth of the human population [1]. An inevitable by-product of living in the proximity of cats, is the accumulation of shed cat hairs on humans and their belongings. As the transfer of such hairs, both in friendly contact and during crimes, is unavoidable [2], cat hairs have played a part in the investigation of a variety of human crimes [3–5].

Due to the DNA content of shed hairs, mitochondrial DNA (mtDNA) is most often used to investigate hair despite the fact that it is less informative than nuclear DNA due to its maternal inheritance and lack of recombination [6,7]. To reliably use mtDNA from hairs as trace evidence, a robust technique, with sufficient discriminating capacity and knowledge of the haplotype distribution of relevant populations is a prerequisite. Different portions of the feline mitochondrial control region (CR) have been studied in cats sampled in different parts of the world [8–10]. Most of the available public data is limited to the 402 bp conserved core region located between tandem repeat structures RS2 and RS3 in the feline CR designated 'Sylvester Reference Sequence' (SRS) [10,11]. So far the SRS region has been typed for European cats from Germany (n = 21) and Italy (n = 25) [11], Poland (N = 181) [12] and the United Kingdom (N = 152) [13]. A larger portion of the feline control region, 605 bp encompassing the SRS region, was typed for 113 random bred cats and 136 fancy breed cats from the

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Netherlands [14]. All these studies observed pronounced differences between the genetic composition of the mainland European cats and cats sampled in other parts of the world, mainly in the proportion of singleton haplotypes [11] or the abundance of dominant haplotypes [12–14].

To apply feline mtDNA in forensic case work in different European countries, a better understanding of the European cat population is required. Biological and reproduction factors that are related to human and cat populations as well as sterilisation and breeding practices are thought to be comparable between densely populated North West European countries. As the Netherlands shares land borders with Belgium and Germany and a sea border with the United Kingdom, these countries were chosen to determine whether the previously established haplotype distribution for the Netherlands is specific for that country or if it could be representative of North-West Europe. As movement of cats between the United Kingdom and Continental Europe occurs by human facilitation (both coincidental and intentional), the movement of cats between mainland European countries is thought to occur more frequently. Therefore this study focusses on cats from the mainland European countries of the Netherlands, Belgium and Germany. To evaluate the presence of micropopulations and the influence of sampling strategies on haplotype distribution estimates, cats were sampled randomly throughout the Dutch, Belgian and German populations and from two micropopulations: a single municipality in Belgium and a city in Germany.

2. Material and methods

2.1. Sample collection

Random sampling of the Dutch population was performed through buccal sampling of cats living in close contact with (friends or relatives of) personnel of the Netherlands Forensic Institute (The Hague, the Netherlands) throughout the country (sampling locations provided as Supplementary material S1). Breeds and maternal relatedness were assigned by the owners. If maternal relatedness was indicated, only one individual from each maternal group was included. The previously described 129 randomly sampled cats [14] were supplemented giving a total of 146 cats. The majority of these samples were collected in the areas of the country most densely populated by humans. In total swabs were obtained from 125 random bred cats, 6 mixed breed cats, 3 British Shorthairs, 3 Maine Coons, 1 Persian, 1 Siamese/Oriental, 1 Bengal, 1 Birman, 3 Siberian Forest Cats and 2 Norwegian Forest Cats.

Sampling of the Belgian cat population was performed as described for the Dutch population, by (friends or relatives of) personnel of the National Institute of Criminalistics and Criminology (Brussels, Belgium) (sampling locations as Supplementary material S1). One hair sample and 63 buccal swabs were collected from cats living in close contact with humans throughout Belgium. Of these samples, 60 originated from random bred cats, 2 from mixed breed cats, 1 from a British Shorthair and 1 from an Egyptian Mau.

Sampling of the south west German feline population consisted of two sampling strategy efforts. Random sampling of cats living in close contact with humans from the feline micropopulation around the city of Wiesbaden, in the south west of Germany was performed by (friends or relatives of) personnel of the Bundeskriminalamt, Forensic Science Institute (Wiesbaden, Germany). Buccal swabs from 79 cats from the Wiesbaden area were collected. Swabs were obtained from 61 random bred cats, 5 mixed breed cats, 2 British Shorthairs, 3 Maine Coons, 3 Persians, 1 Siamese/Oriental, 1 Birman and 3 Chartreux. Additionally samples were obtained from 49 not further specified cats, sent to the Institute of Veterinary Pathology, Justus-Liebig-Universität Giessen (Giessen, Germany) for post mortem examination.

A stray cat micropopulation in Putte, Belgium was sampled. Buccal swabs were collected from 19 cats caught in Putte Municipality, that had been brought to a veterinarian in a trap-neuter-release program. The familial relationship of these individuals is unknown.

Sequences published by others and used for comparison, originated from a random selection matching human population density in the United Kingdom (UK population, n = 120), supplemented with samples from a city in the south of the United Kingdom (UK micropopulation, n = 32, sum N = 152) [13], from random bred cats from seven different areas of the United States (total N = 493, four micropopulations, Hawaii, New York and Texas not specified) [11], from three Canadian micropopulations (total N = 96) [15] and from eight Polish micropopulations (total N = 181) [12].

2.2. Laboratory procedures

DNA extraction of the majority (n = 129) of the Dutch samples has been described previously [14]. The additional Dutch and Belgian samples were processed following this protocol. DNA extraction of the German samples was performed as described elsewhere [16]. Primer pairs FCB-Z [8] & F16483-M13R [17] and M13F-JHmtF & JHmtR3 [10] were used for duplex amplification of the control region positions 16065–16483 and 16756–17009/0-223 (numbers corresponding to Genbank U20753/NC_001700 [18]) as described in [17]. Evaluation of PCR success through gel-electrophoresis, purification of PCR products and sequencing was performed as described in [14] using FCB-Z, JHmtR3 and AMBeR M13 sequencing primers.

2.3. Sequence analyses

All sequences were manually evaluated and forward and reverse sequences were assembled in Geneious 4.7.6 [19]. Following assembly, sequences were aligned and trimmed to nucleotide positions (NP) 16315–16483 and 16780–17009/0-206, resulting in sequences of approximately 605 bp (Supplementary material S1). A phylogeographic network of haplotypes was constructed with the median joining algorithm implement in Network 5.0 (Fluxus Technology Lt.) [20]. Arlequin 3.5.2.2 [21] was used to calculate population statistics including gene diversity, nucleotide diversity, AMOVA, fixation indices (Fst) and population pairwise differences (within population, between population and corrected values/Nei's distance). Pairwise Fst p values were adjusted by the sequential Bonferroni correction [22]. Sequences of all haplotypes were aligned with the SRS, and previously described SRS haplotypes [10–13,15]. Following trimming of all sequences to SRS length (NP 16814–17009/0-206), the unique sequences were retained and population statistics recalculated using Arlequin as described for the 605 bp sequences.

The corrected population pairwise differences (Nei's distances) were visualized using the Neighbor Joining (NJ) method [23] implemented MEGA5 [24], after which the phylogram was unrooted to only present the degree of kinship (opposed to an evolutionary path), and edited in MrEnt 2.5 [25].

3. Results

3.1. Haplotype designation

Sequences of on average 605 bp were obtained from all 338 randomly selected cats living in contact with humans from the