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Forensic utility of the feline mitochondrial control region – A Dutch perspective

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ABSTRACT

Different portions of the feline mitochondrial DNA control region (CR) were evaluated for their informative value in forensic investigations. The 402 bp region located between RS2 and RS3 described most extensively in the past is not efficient for distinguishing between the majority of Dutch cats, illustrated by a random match probability (RMP) of 41%. Typing of the whole region between RS2 and RS3, and additional typing of the 5'portion of the feline CR decreases the RMP to 29%, increasing the applicability of such analyses for forensic investigations.

The haplotype distribution in Dutch random bred cats ($N = 113$) differs greatly from the distributions reported for other countries, with a single haplotype NL-A1 present in 54% of the population. The three investigated breeds showed haplotype distributions differing from each other and the random bred cats with haplotype NL-A1 accounting for 4%, 29% and 32% of Maine Coon, Norwegian forest cats and Siamese & Oriental cats. These results indicate the necessity of validating haplotype frequencies within continents and regions prior to reporting the value a mtDNA match. In cases where known purebred cats are involved, further investigation of the breed may be valuable.

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1. Introduction

In the Netherlands, a country with almost 17 million human inhabitants, the number of cats is estimated to fluctuate around 3 million [1,2]. As in other parts of the world, approximately 95% of these domestic cats are random bred, also referred to as European shorthair or non-breed, the remaining 5% consisting of fancy breed cats [1,3]. In the densely inhabited parts of the Netherlands, some cats have an indoor life (mainly fancy breed cats), others pass time outdoor (mainly random bred cats). Few cats live independently of humans, due to human control of feral cat populations [4,5]. Due to this lifestyle and the continuous, year round shedding of hairs, cat hairs are easily spread to the belongings of people living in proximity of cats.

When these people become involved in a crime, whether as a victim, witness or perpetrator, cat hairs can be transferred. Like hairs from other species, in this manner cat hairs can be silent witnesses of a crime, when for example they are transferred from a suspect's clothes to tape used to restrain a victim. If nuclear STR markers can be amplified from such trace evidence, the link between the trace evidence (a hair) and the donor (the cat) can be extremely informative [6]. In our experience, as also pointed out by others [7,8], amplifying nuclear DNA (nDNA) markers from animal hairs encountered in case work is generally unsuccessful. The amplification of mitochondrial DNA (mtDNA) from shed hairs has significantly higher success rates, mainly due to the high copy number and greater stability of the mtDNA molecule and the distribution of mtDNA in hairs [9]. This makes mtDNA the DNA molecule of choice for forensic investigation of cat hairs, despite the fact that it is less informative than nDNA due to its maternal inheritance and lack of recombination [10,11].

To use the mtDNA from hairs as trace evidence, a reliable and robust technique is necessary with which a maximum of information can be obtained from such small traces containing only a limited amount of mtDNA. The mtDNA control region (CR), also referred to as displacement loop, D-loop and hypervariable regions (HV), has proven to be the most informative part of the mtDNA in many species including humans [12], dogs [13] and cats [7,14]. In humans and dogs specific portions of the CR have been

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recognized that have diverse characteristics and therefore are of different value for forensic purposes. The extent to which the applicability of these regions has been investigated varies.

The tandem repeat structures present in the feline CR (RS2 and RS3) have been shown to be too variable for forensic applications, as the number of repeats may vary within an individual, often prohibiting complete sequencing of both strands of DNA [15]. Due to the presence of a numt and the possibility of amplifying nDNA instead of mtDNA [16], the information content of the 3′ portion of the feline CR has only been investigated briefly [17]. The remaining two portions of the feline CR, the 5′ portion located before RS2 (also referred to as HVI and HV1a) and the middle portion located between RS2 and RS3 (also referred to as HVII and HV1b) have both been described as forensically informative regions, but their informative value has not yet been compared.

One of the factors in determining the value of a portion of the mtDNA CR may be the availability of relevant reference samples for which this region has been typed [8]. Geographical origin, random bred vs fancy breed cats and human factors all influence whether publicly available data can be used. Several studies have focused on specific parts of the mtDNA in specific breeds, random bred cats or wild cats in different regions. Portions of the mtDNA of cats from islands (Japan and the United Kingdom) have been described [18,19], and from several mainland European countries (Italy, Spain and Poland) [17,20], however as different regions of the mtDNA were used the data from these studies cannot be compared. Analysis of large number of cats from different regions of the United States resulted in comparable haplotype distributions, but differed from a relatively small sampling of non-US samples [21] and analyses of non-US cats was advised.

To investigate whether cat breeds could be distinguished by their mtDNA, several individuals (4–25) of different breeds mainly from the US were typed, however no breed specific haplotypes were identified [21]. This fits the observation that the development of cat breeds is a relatively recent process with most breeds originating within the last 50–100 years and the fact that crossbreeding of cats from different breeds is permitted within breed standards as some are defined by coat pattern, hair length or color mutations alone. The genetic relationship and admixture between several breeds has been clearly shown by autosomal DNA studies [22–25].

To be able to apply feline mtDNA in forensic case work in The Netherlands a better understanding of random bred cats and fancy breed cats in The Netherlands and North West Europe is essential. Therefore we evaluated the value of the different portions of the feline mitochondrial control region for nucleotide diversity and random match probability within random bred cats from the Netherlands. We additionally explored substructuring of the domestic cat population in the Netherlands to establish a framework for interpretation of mtDNA matches in Dutch casework.

2. Material and methods

2.1. Sample collection

A random sampling of Dutch random bred cats was obtained by collecting buccal swabs of cats owned by (relatives of) personnel of the Netherlands Forensic Institute living throughout the Netherlands. In cases where swabs of multiple littermates or parents and offspring had been collected, only one sample of each known maternal group was included in this study. This random sampling resulted in the collection of 113 random bred cats (RBC, 88%), 4 mixed breed cats, 3 British Shorthairs, 2 Maine Coons, 2 Persians, 2 Siamese, 1 Bengal, 1 Birman, and 1 Siberian Forest cat, the majority collected in the most densely inhabited part of the country (distribution of RBC samples in Fig. 1).

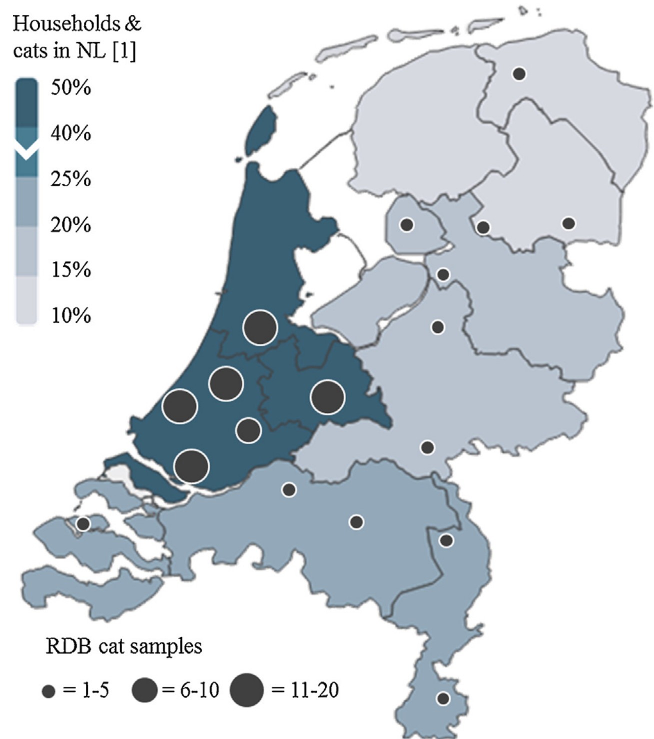

Fig. 1. Distribution of households, cats and RBC samples. Map generated with LocalFocus.

Based on their prevalence in the Netherlands [1] and described relatedness [22,23], three groups of fancy breeds were selected for comparisons within and between breeds. Maine Coon cats (MC) and Norwegian Forest cats (NF) were chosen as distinct, frequently occurring closely related breeds. Siamese and Oriental Shorthair (S–O) were chosen as a third group, relatively distinct from MC and NF. Catteries breeding MC, NF and S–O cats were requested to support this study by providing buccal swabs of their cats and pedigrees of swabbed individuals. In cases where pedigrees indicated a maternal relationship in the first to third degree, only one sample of each maternal group was included in this study, leaving 51 MC, 41 NF and 44 S–O samples. Due to the transportation of both queens and kittens the exact location of sample collection was considered irrelevant.

2.2. Laboratory procedures

DNA was extracted from buccal swabs using the DNeasy Blood and Tissue kit (Qiagen GmbH, Germany) following the manufacturer's protocol. Total DNA concentration was estimated through spectrophotometry with a NanoDrop ND-1000 (NanoDrop Technologies, DE, USA).

A portion of the feline mtDNA CR was amplified using forward primer FCB-Z [18] and reverse primer JHmtR3 [26] (Fig. 2). PCR reactions were performed in 25 μl containing 1× PCR Buffer (containing 1.5 mM $MgCl_2$), 0.5 mM $MgCl_2$, 0.75 unit of HotStarTaq 0.1 mM each dNTP (all Qiagen), 5 pmol of each primer and 0.5–25 ng of template DNA. PCRs were performed on MyCycler (BioRad Laboratories) or GeneAmp 9700 (Applied Biosystems) thermocyclers using the following cycling parameters: 15 min at 95 °C; 35 cycles of 94 °C for 45 s, 58 °C for 30 s, and 72 °C for 1 min; 10 min at 72 °C.

PCR success was determined by gel-electrophoresis followed by ethidium bromide staining and UV detection. Positive and negative controls were used throughout. ExoSAP-IT® (Affymetrix) was used following the manufacturer's protocol for removal of unincorporated

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