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Research paper

# Application of a mitochondrial DNA control region frequency database for UK domestic cats

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ABSTRACT

DNA variation in 402 bp of the mitochondrial control region flanked by repeat sequences RS2 and RS3 was evaluated by Sanger sequencing in 152 English domestic cats, in order to determine the significance of matching DNA sequences between hairs found with a victim's body and the suspect's pet cat. Whilst 95% of English cats possessed one of the twelve globally widespread mitotypes, four new variants were observed, the most common of which (2% frequency) was shared with the evidential samples. No significant difference in mitotype frequency was seen between 32 individuals from the locality of the crime and 120 additional cats from the rest of England, suggesting a lack of local population structure. However, significant differences were observed in comparison with frequencies in other countries, including the closely neighbouring Netherlands, highlighting the importance of appropriate genetic databases when determining the evidential significance of mitochondrial DNA evidence.

## 1. Introduction

As all pet owners will appreciate, their pet's fur is frequently found on clothing and home furnishings. Consequently the recovery of pet hairs whilst taping evidential items for fibres can help establish the likelihood that transfer occurred through contact with the clothing of a person or at a place with which a particular animal is closely associated. Although even an individual pet's coat hairs may vary in colour and morphology these characteristics can be invaluable in selecting hairs for DNA analysis which, via the shared possession of a rare genetic variant, can provide stronger evidence of origin from a common source than appearance alone. Indeed there have been instances when nuclear short tandem repeat (STR) [1] or mitochondrial DNA (mtDNA) matches [2] with cat hairs have provided vital evidence in forensic casework. The majority of transferred cat hairs have been naturally shed, and lack the large root bulb required for reliable high-discrimination STR profiling [3]. However, even hair shafts typically contain enough mtDNA for sequencing, which is still capable of excluding many potential sources despite lower discriminating power than nuclear DNA [4].

For both marker types it is necessary to create databases to assess the frequency of alleles or haplotypes within a relevant population [5]. For haploid markers such as mtDNA, where profiles are expected to be shared by many matrilineally-related individuals, the strength of the evidence is determined not only by the variability of the sequence but also the size of the geographically relevant genetic database [6], which should be large enough to accurately reflect the local diversity.

In the United Kingdom (UK) an estimated 10.2 million cats live in 26% of households [7], and regularly 'tag' the furnishings and clothes of their owners with their shed hairs. Consequently it is inevitable that cases will occur in which cat hairs could provide critical evidence. In July 2012 a dismembered human torso was washed up on a beach in Southsea, a seaside resort in Portsmouth, England (Fig. 1a). The torso was wrapped in a shower curtain and sealed within a black plastic bin liner. The victim was identified as a local resident, and one of his associates became the prime suspect after voluntarily presenting himself at a police station in a

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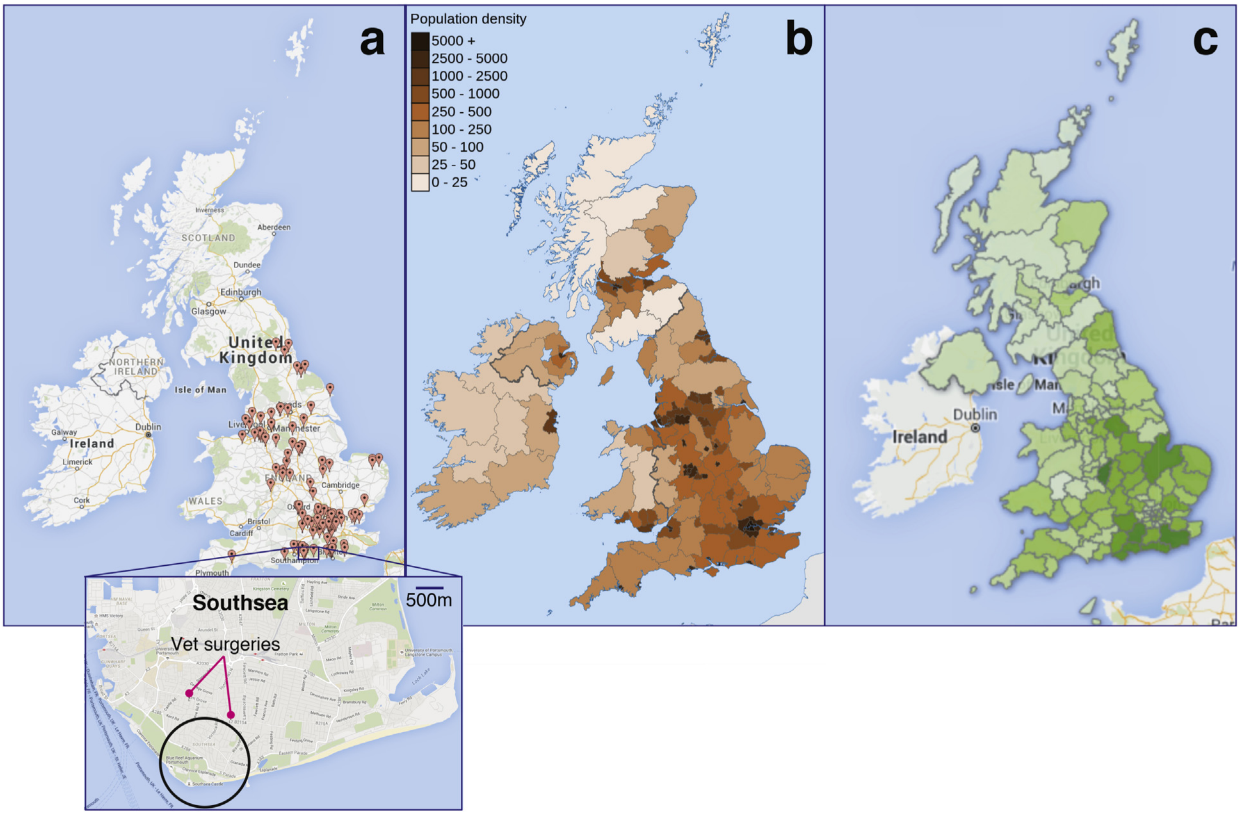

**Fig. 1.** Geographical distribution of cat samples, crime-scene location, and human and feline population densities.
a) Map of British Isles showing the distribution of English veterinary surgeries contributing samples. Inset below is an enlarged map of Southsea showing local vets: suspect's and victim's addresses, and the location of the victim's remains, all lie within the black circle. Map data © copyright Google. b) Human population density in the British Isles (contains Ordnance Survey data © Crown copyright and database right; sourced from en.wikipedia.org/wiki/British_Isles#/media/File:British_Isles_population_density_2011_NUTS3.svg). c) UK distribution of microchipped cats based on data from Petlog (www.petlog.org.uk) as a proxy for feline population density. Note that microchipping of cats is not obligatory and that the practice may vary by socio-economic status and geography.

confused and intoxicated condition stating that he had done something "really bad" and thought that he may have killed someone, but was unable to remember any details. Whilst no human traces other than those of the victim were discovered associated with the body, eight cat hairs were found amongst a population of fine animal hairs recovered on tapes from the outside of the shower curtain. Both suspect and victim were cat owners, and the hairs matched the colour and morphology of those of the suspect's cat, but were dissimilar to those of the victim's cat (D. Hopwood pers. comm.).

The reporting officer of the forensic service provider (LGC) carrying out the examination invited the Veterinary Genetics Laboratory (VGL) at UC Davis to compare DNA recovered from the hairs on the shower curtain with the DNA of the cats belonging to suspect and victim. The hairs from the curtain yielded insufficient DNA for STR profiling [8], but a 402-bp fragment of the mtDNA control region flanked by repeat sequences RS2 and RS3 was successfully amplified and Sanger sequenced. The resultant sequence did not match any of the 149 mtDNA haplotypes (mitotypes) seen within a database of 1394 cats from 25 distinct populations in the USA, Asia and Europe [9]. The victim's cat possessed a different mitotype and was excluded as a potential source, but the suspect's cat was a match for this globally rare sequence.

Hampshire Constabulary, the investigating police force, required an opinion on the relevance of the database used by VGL for determining the evidential significance. Given that there was significant variation in the frequencies of the 12 common mitotypes between countries it was clear that a database relevant to the UK and possibly the immediate area of the crime scene was needed, in order for the evidence to be evaluated appropriately. We describe here the creation of a UK domestic cat mtDNA database spanning the 402-bp region of the control region, its application in the investigation of this case, and its implications for future uses of domestic cat hair profiling within the UK and beyond.

## 2. Materials and methods

Surplus blood collected during routine examinations of 152 cats at 105 veterinary surgeries throughout England was provided by IDEXX, the largest national veterinary diagnostic company. With only six weeks to build and analyse the database before the court submission date, 32 samples were accumulated over 22 days from seven surgeries in the Portsmouth postal code area, including 23 samples from two surgeries within a mile of the homes of both suspect and victim and the location where the body was recovered (Fig. 1a). These were supplemented with 120 samples collected from male cats over two days from elsewhere in England with a mean of 1.25 samples per surgery.

The ages of 93% of donor cats were known and there was only one case where two samples from the same location had the same age and could thus represent duplicate samples from the same individual. With an average age at sampling of 12.2 years, close to the average life expectancy of cats, and a mean number of cats per cat-owning household of 1.66 [7] there is a very low probability that maternally related individuals were sampled. Cat geographic origins matched closely with human population density (Fig. 1b) and also the density of cats (Fig. 1c), which in the UK are