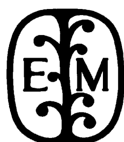

International Congress Series 1239 (2003) 841–845

Cytochrome *b* and HVI sequences of mitochondrial DNA to identify domestic animal hair in forensic casework

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Keywords: Cytochrome *b*; HVI sequence; DNA; Animal hair

1. Introduction

Biological traces that appear at the scene of a crime or on the body of the victim may be of human, animal and/or vegetable origin. Among those of animal origin, household pets are a common source, with pet hair being one of the most frequent traces found. Consequently, it is necessary to have laboratory methods capable of identifying traces from pets and domesticated animals in general.

Savolainen et al. [1] have developed a basic method of sequencing the HVI region of mitochondrial DNA from *Canis familiaris* using single hairs as template, with a discrimination capacity of 1 in 10 individuals.

The species from which a biological trace has come can be identified by analyzing a short fragment of the cytochrome *b* (cyt*b*) gene sequence of the mitochondrial genome. This gene contains species-specific information and has been used in phylogenetic as well as in forensic investigations in a number of studies. Parson et al. [2] have confirmed the usefulness of cyt*b* analysis in identifying the biological origin of casework specimens.

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PII: S0531-5131(02)00592-7

Due to the homology between certain segments of the hypervariable region in a number of animal species, the aim of the present study is to show that in order to identify an animal hair, it is first necessary to determine the species of origin by cyt*b* analysis, before proceeding to identification through analysis of the HVI region.

2. Materials and methods

A number of specimens of canine, feline and bovine origin among others, were analyzed. The samples analyzed were saliva and hairs from control animals and traces of hair from forensic casework. Cell lysis was performed using proteinase K and SDS. The DNA was purified using the phenol–chloroform method. Lysis of the hair samples lasted as long as necessary to dissolve the hair, adding proteinase K every 24 h.

PCR amplification of 358 pb of cyt*b* was done under the conditions described by Parson et al. [2]. PCR amplification of the HVI region was performed according to the method described by Savolainen et al. [1].

All the samples were sequenced using the dRhodamine Terminator kit (Applied Biosystems) method in an automatic ABI Prism 310 DNA sequencer. The sequences were aligned using the Clustal W (1.5) program and they were also used to identify the biological origin of the samples by aligning to the cyt*b* gene sequence entries using the program BLAST.

3. Results

3.1. Analysis of fragment 15561–15708 of C. familiaris (Genbank accession U96639)

Hairs coming from *C. familiaris* are the animal vestige found most frequently in homes, clothes and vehicles of individuals implied in criminal cases. The analysis of the D-loop of mitochondrial DNA has been demonstrated to be useful to carry out animal identification and for this reason, we have made the analysis of the mitochondrial DNA in saliva and hair from different dogs.

Samples of eight different dogs have been analyzed. The samples of the six first animals were saliva; the samples of dog 7 were saliva and hair; the samples of dog 8 were 12 hairs. Also 12 hairs gathered by a vacuum from a vehicle were analyzed.

The amount of extracted DNA of shed hairs and hairs gathered by vacuum is variable and very scarce. Therefore, the amplification of long fragments of DNA does not usually give result. For this reason, we have restricted the analysis to a small region of 125 bp. To get enough amplified product, a first PCR reaction of 145 bp was carried out. Then, the amplified product was subjected to a second semi-nested PCR, using the primer D7 located internally.

Electropherograms of the sequences of DNA show smaller noise in the saliva and shed hair than in hairs gathered by vacuum. This can be because the DNA of hairs gathered by vacuum is very degraded by microscopic organisms and also because it presents contamination with DNA from human vestiges.

The DNA sequences were aligned with the U96639 sequence of canine mitochondrial DNA of GenBank. Table 1 shows the results of the nucleotide substitutions observed in the eight *C. familiaris* studied. This small region of ADNmt of *C. familiaris* is highly polymorphic, its probability of random coincidence being 15.6%. The presence of heteroplasmy of substitution in canine hair has been observed. Fig. 1 shows this heteroplasmy in position 15635. Heteroplasmy is frequent in hair so that lack of coincidence of some base between different hairs does not discard that they belong to the same animal.

Five of the twelve hairs gathered by vacuum gave results. All showed the same sequence, and equal to the one of dogs 2 and 8, two other hairs had a mixture of DNA sequences, giving a reading of bases with multiple indeterminations. The remaining samples did not give amplification product. The lack of product of PCR can be due because: (a) the DNA is excessively degraded, (b) inhibitors of the reaction of PCR exist or (c) the species of the vestige is different to the species of used primers.

3.2. Analysis of cytochrome b

With the purpose of beginning in the technique of identification of species by means of the cyt*b*, we have analyzed human DNA, DNA of dog and cat and DNA of cow milk. In all the cases, the cytochrome *b* sequences were compared with GenBank data bases using the program BLAST. Each sequence was coincident with its species.

Table 1
Mitochondrial HVI sequences of eight animals of the species *C. familiaris* (s: saliva, h: hair)

Gbank	ACATAA-TATTATATCCTTACATAGGACATATTAACTCAATCTCATAATTCACTGATCTTTCAACA-GTAATCGAATGCATATCACTTAGTCCAATAAGGG
1s	-ACATAA-TATTATATCCTTACATAGGACATAT**C**AACTCAATCTCATAATTCA**T**TGATCT**G**TCA**G**C-AGTAATC**A**AATGCATATCACTTAGTCCAATAAGGG
2s	-ACATAA-TATTATAT**T**CTTACATAGGACATAT**C**AACTCAATCTCATAATTCA**T**TGATCT**G**TCA**G**C-AGTAATC**A**AATGCATATCACTTAGTCCAATAAGGG
3s	-ACATAA-TATTATATCCTTACATAGGACATAT**C**AACTCAATCTCATAATTCA**T**TGATCT**G**TCA**G**C-AGTAATC**A**AATGCATATCACTTAGTCCAATAAGGG
4s	-ACATAA-TATTATATCCTTACATAGGACATATTAACTCAATCTCATAATTCACTGATCT**A**TCA**A**C-AGTAATCGAATGCATATCACTTAGTCCAATAAGGG
5s	-ACATAA-TATTATATCCTTACATAGGACATATTAACTAATCTCATAATTCACTGATCTTTCAACAG-TAATCGAATGCATATCACTTAGTCCAATAAGGG
6s	ACATAAATATTATATCCTTAC**G**TAGGACATATTAACTCAATCTCATA**G**TTCACTGATCT**A**TCAAC-AGTAATC**A**AATGCATATCACTTAGTCCAATAAGGG
7s	-ACATAA-TATTATATCCTTACATAGGACATAT**C**AACTCAATCTCATAATTCA**T**TGATCT**G**TCA**G**C-AGTAATC**A**AATGCATATCACTTAGTCCAATAAGGG
8ph	-ACATAA-TATTATAT**T**CTTACATAGGACATAT**C**AACTCAATCTCATAATTCA**T**TGATCT**G**TCA**G**C-AGTAATC**A**AATGCATATCACTTAGTCCAATAAGGG

The nucleotide substitutions are shown in bolded characters. These subtitutions are located in polymorphic sites of the canine HVI region. The insertion of A is also observed in the sequence of dog 6 (Alaskan malamute). Dogs 2 and 8 (Boxer and Crossbreed) show the same sequence in the 80 analyzed positions.

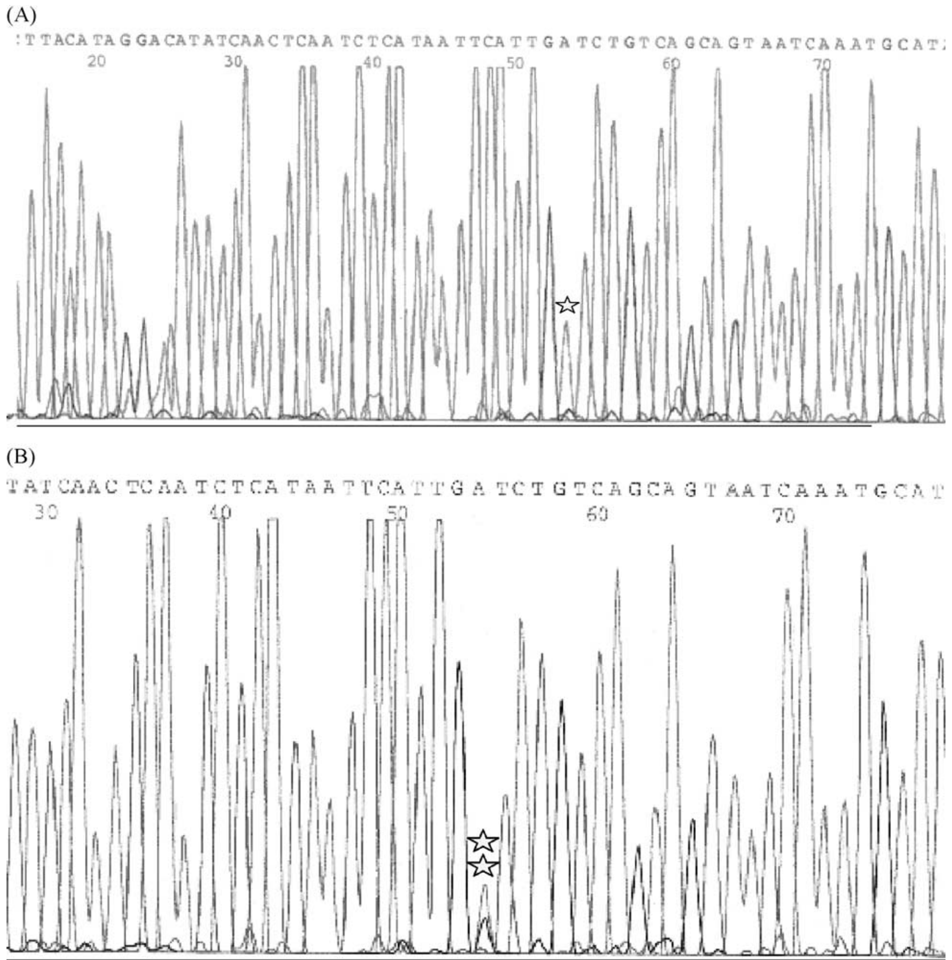

Fig. 1. Example of heteroplasmy in canine hair: (A) the position 15635 is adenine in the sample of saliva; (B) adenine and guanine bases were found in a hair of the same animal.

Then, we analyze the 12 hairs gathered by vacuum whose region HVI had been previously studied with canine HVI primers. The obtained results were:

- Two of these hairs showed a human sequence cyt*b*. None of these hairs had been able to be amplified using canine HVI primers.
- The 10 remaining hairs did not produce enough product of PCR to be sequenced.

None of the five hairs that had given result in the analysis of 145 bp of the segment HVI using canine primers, gave result in the analysis of the cyt*b*.

The fact that these DNAs had been previously amplified it indicates that they do not have inhibitors of the PCR reaction.

The negative amplification of cytochrome *b* in canine hair can be explained by the difference in the number of bases to amplify. The analyzed HVI segment has 145 bp, while the segment of cyt*b* has 358 bp. Furthermore, the PCR product obtained by using HVI primers can be improved by means of semi-nested reactions, while cytochrome *b* can only be reamplified.

In consequence, the long size of cyt*b* segment makes the analysis of the scarce DNA very difficult. This is the case when canine hair is short or very thin or highly degraded.

Although cyt*b* is an extremely valuable tool in the species identification, its long size prevents good results to be obtained in highly degraded forensic vestiges. For this reason, it would be of interest the design of new primers to allow the analysis of small fragments of cyt*b* to obtain results in any type of vestige of forensic interest.

In summary, our results indicate that sequences of human DNA can be obtained when canine DNA is amplified with human HVI primers, and it is also possible to obtain sequences of canine DNA when human DNA is amplified with canine HVI primers in procedures that require several PCR reactions. cyt*b* fragments and mitochondrial HVI should be analyzed in every sample in order to obtain accurate information whether the nucleotide differences found indicate merely that the specimens are from different sources of the same species, or whether the samples are from different species. Because a great number of forensic samples are highly degraded, the amplification of cyt*b* in smaller fragments than 300 bp would facilitate forensic species identification.

Acknowledgements

This work was suported by two grants from Universidad del País Vasco (1998) and Gobierno Vasco (Aquitania-Euskadi 2000).

References

[1] P. Savolainen, B. Rosén, A. Holmberg, T. Leitner, M. Uhlén, J. Lundeberg, J. Forensic Sci. 42 (4) (1997) 593–600.

[2] W. Parson, K. Pegoraro, H. Niederstätter, M. Föger, M. Steinlechner, Int. J. Leg. Med. 114 (2000) 23–28.