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Short Communication

Comparison of *Lagostomus maximus* amelogenin gene (*AMELX*) with other mammals

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The aim of this study was to compare the *Lagostomus maximus AMEL* sequence to assess their similarity with the *AMEL* genes from other mammals. Previously, a sequence of the *AMEL* gene of *L. maximus* which corresponds to the intron 3 of the human sequence for *AMELX* (*AMEL* on the X chromosome) was reported. The comparisons made, by the use of the algorithm provided by the NCBI (Basic Local Alignment Search Tool: BLAST) allowed the determination of the possible similitude between the sequence corresponding to the *AMEL* gene of some species of mammals with the *AMEL* sequence of *L. maximus*. The reported sequences for this gene should be studied further.

Key words: Amelogenin gene, *Lagostomus maximus*, intron 3 sequence.

INTRODUCTION

The *Lagostomus maximus* is a wild rodent whose females have a different ovary from the rest of the mammals with a high ovulation rate caused by the down regulation of the apoptotic pathway (Jensen et al., 2008).

Amelogenin is the major protein in tooth enamel (Delgado et al., 2005). The amelogenin gene, functional in Eutherians and Marsupials, is located in a pseudoautosomal ancient boundary (PAB) of the X and Y chromosomes where recombination is prevented. The importance and evolutionary meaning of *AMEL* gene was reported by Toyosawa et al. (1998), Iwase et al. (2003) and Wang et al. (2012) among others. The *AMEL* sequence is useful as sex determining method in forensic and anthropological sciences because the human *AMELX* (*AMEL* on the X chromosome) is shorter than the human *AMELX* gives a 106 base pairs amplification product while *AMELY* gives a 112 base pairs amplicon. The difference is due to a deletion of 6 bases in intron 1 of *AMELX*. Human males DNA (XY) show two bands on an electrophoresis agarose gel while human females (XX) show only one band. Thus, by PCR sex determination is possible with small samples of anthropological remains. This method is also used for sex determination in cetacean and artiodactyla (Macé and Crouau-Roy, 2008) as well as for bovids (Chen et al., 1999; Tsai et al., 2011). Detailed information about the size of PCR products generated on the X- and Y-homolo-gues can be found in Haas-Rochholz and Weiler (1997).

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TTAGTAATTTTAGCCATGATTTGAGTTAAAGATATCCTAG
CATATGTAAAGAGTGGTATATTGATTATTCAGCAAGATG
TTTCTCAAATGGTCACTTTTTTTCAGTTCCCACCAGCAGCT
TTAAGCCCCGAGTGATGGCCTCAAGCCTGCATTTCCAAGC
GCCCTCCTTCCTGGTCACTCTGACTCAGCCTCTACTTTAAA
CCTAGT

Figure 1. Nucleotide sequence of intron 3 of AMELX from Lagostomus maximus.

METHODS

The materials and methods were described in an earlier work (Fernández Feijóo et al., 2010). Briefly, genomic DNA from a male *L. maximus* was the template for PCR amplification of the *AMEL* genomic region. The primers sequence for the PCR assays was identical to the sequence of codons from the 3´end of the human *AMELX* and *AMELY* (primer forward: AGCTACCACCTCATCCT, 17 bases and primer reverse: TGCCCTATCATGGAGCCT, 18 bases). A previously reported sequence of the *AMEL* gene of *L. maximus* (which corresponds to the intron 3 of the human sequence for *AMELX*) (Fernández Feijóo et al., 2010) was compared with sixteen sequences of the *AMEL* gene reported for fourteen species from five taxonomic groups of mammals (Primates, Rodentia, Artiodactyla, Perissodactyla and Carnivora). The comparisons were done using the BLAST algorhitm (Altschul et al., 1990).

The *L. maximus AMEL* gene sequence has many striking similarities with both the amelogenin Y isoform precursor and the *AMELX* from rodents (*Crisetulus griseus*, *Rattus sp*. and *Mus sp*.) and from human and primates (*Pan troglodytes*). Also similarities with the amelogenin of *Bos Taurus* were observed.

RESULTS

The DNA sequence obtained by PCR using a male *L. maximus* DNA template (Genebank: FJ001811.1) corresponds to *L. maximus* amelogenin gene (*AMELX*) intron 3 and the base sequence is shown in Figure 1.

Sequences corresponding to the *AMEL* gene, which are in the Nucleotide Database from the National Center for Biotechnology Information (NCBI on line library), were used for comparisons. Sequences, belonging to *Cavia porcellus* and *Peromyscus maniculatus bairdii* did not show any similarity with the *Lagostomus maximus*' intron 3 sequence. The other sequences, belonging to humans (*Homo sapiens*), chimpanzees (*Pan troglodytes*), rhesus macaque (*Macaca mulata*), squirrel monkey (*Saimiri boliviensis*), hamster (*Cricetulus griseus*), mice (*Mus musculus*), rats (*Rattus norvegicus*), azara's grass mouse (*Akodon azarae)*, cows (*Bos taurus*), pigs (*Sus scrofa*), horse (*Equus ferus caballus*) and dogs (*Canis lupus*), showed a similarity ranging from 70 to 92% with the *L. maximus*' intron 3 amelogenin gene. The comparisons were made by using BLAST algorithm (available on line at http://blast.ncbi.nlm.nih.gov/Blast.cgi) between the sequence (207 nucleotides) from *L. maximus* and the nucleotide database for the amelogenin gene from the species shown in Table 1.

DISCUSSION

The similarity between the *Lagostomus maximus´* intron 3 and the *AMEL* gene sequence from fourteen species ranged from 70 to 92%. This fact indicates the presence of AMEL, sequences with high similarity, between Primates, Rodentia, Artiodactyla, Perissodactyla and Carnivora. Interestingly, the highest similarity was found between the intron 3 from Akodon azarae and the intron 3 from Lagostomus maximus (92%). This may be explained by the technique employed, both sequences (from *Akodon* sp. and *Lagostomus* sp.) were obtained with the same experimental conditions. The Amel gene (Amelogenin) it is an important genomic region. Amelx is being studied in *Mesocricetus auratus*: The golden hamster (GeneBanK). The complete sequencing of the *Lagostomus maximus* AMEL gene should be performed to determine if it can be used as tool for sex determination as is done with the human AMEL.

Conflict of Interests

The authors have not declared any conflict of interests.

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DISCLOSURES AND ETHICS

The animals were treated according to the guidelines

Table 1. Sequence similarity between the *Lagostomus maximus*' intron 3 and the *AMEL* gene from fourteen mammalian species.

Taxa	*Identities (%)	NCBI reference accession number
Primates		
Homo sapiens	73	AY 040206.1
Pan troglodytes	73	AY 694820.1
Macaca mulatta	74	NC_007878.1
Saimiri boliviensis	73	U88981.1
Rodentia		
Akodon azarae	92	FJ001810
Crisetulus griseus	89	NW_003613948.1
Mus musculus	86	D 83067.2
Rattus norvegicus	85	NW_001091916.1
Peromyscus sp.	NSSF	JQ069029.1
Cavia porcellus	NSSF	NT_176303.1
Artiodactyla		
Bos Taurus	72	AB091789.1
Sus scrofa	70	AM 903326.1//AM884152.2//AM884151.2
Perissodactyla		
Equus caballus	73	AB091793.1
Carnivora		
Canis lupus	71	NC_006621.3

NSSF = No significant similarity found. U88981.1 = Corresponds to the intron 3 complete sequence, and JQ069029.1 = corresponds to a Amelx RNAm (the BLAST was performed using the corresponding DNA sequence).

from the Canadian Council on Animal Care. The protocol involving animals was reviewed and authorised by the Institutional Committee on the Use and Care of Experimental Animals (CICUAE, Universidad Maimonides).

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