

Paternal inheritance of mitochondrial DNA in the sheep (*Ovine aries*)

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Abstract Paternal inheritance of mitochondria DNA in sheep was discovered by examination of 152 sheep from 38 hybrid families for mtDNA D-loop polymorphisms using PCR-RFLP, amplification of repeated sequence somain, and PCR-SSCP of the D-loop 5' end region of a 253 bp fragment. Our findings have provided the first evidence of paternal inheritance of mtDNA in sheep and possible mechanisms of paternal inheritance were discussed.

Keywords: sheep, mitochondria DNA, paternal inheritance.

Mitochondria DNA (mtDNA) is the only extranuclear genome in the cytoplasm, and exists as multiple copies with high mutant rate. Mammalian mtDNAs encode 37 genes, including 13 peptides genes, 22 tRNA genes and 2 rRNA genes, all of which encode essential components of oxidative phosphorylation (OXPHOS) in mitochondria inner membrane, generating cellular energy in the main form of adenosine triphosphate (ATP). In recent years, studies on the structure and function of mtDNAs become highlights in the research area of molecular evolution, classification, population genetic analysis, relative identification, forensic judgement, aging, disease diagnose, apoptosis and quantitative traits loci (QTL)^[1-4].

Animal mtDNA was once thought to be strictly maternal inheritance. The typical experiment was the mtDNA RFLP of the reciprocal hybrid lines between horse (*Equus caballus*) and donkey (*Equus asinus*). The mules' mtDNA patterns were identical to those of horse, and the hinnies exhibited the identical pattern to donkey^[5]. However, evidence for incidental paternal inheritance was reported in fruit fly, human, mouse and some other animals. In *Drosophila*, paternal mtDNAs were detected in 14 of 16 lines^[6]. The estimated ratio of paternal mode in humans was 0.1%—1.5%^[7]. Mice paternal inheritance frequency of mtDNAs was 10⁻⁴^[8]. Interspecific hybrids of mice showed leaked paternal mtDNA at a frequency of 1/10 in F1 generation, and it cannot be detected any more in F2 progeny^[9]. Analysis of two-cell to blastocyst stage embryos in cloned cattle revealed mixing and co-existence of paternal mtDNA^[10]. Paternal mtDNA of *Lepidopteran* insects was detected at the ratio of about 1 part per 500^[11]. In honeybee, male contribution represented up

to 27% of the total mtDNA in the fertilized eggs, but in subsequent developmental stages the portion of paternal mtDNA gradually decreased until hatching of the larvae when only traces could be detected^[12]. Paternal inheritance in the marine mussel mtDNA was proven to be more extensive. It has been confirmed that mussels have two types of mitochondrial DNA: one is transmitted from the mother to both female and male offspring (F type), and the other is transmitted from the father to sons only (the M type). Both male and female embryos receive M mtDNA through the sperm. Within 24 h after fertilization the M mtDNA is eliminated or is drastically reduced in female embryos but maintained in male embryos^[13].

The completed mtDNA sequence has been determined in sheep^[14], which will be favorable to further study on ovine mtDNA variations. The displacement loop region (D-loop) is the control region of mtDNA, and within the D-loop exists a tandemly repeated sequence region with motif of 75 bp in length, sited between Pro-tRNA gene and conserved sequence block 1 (CSB 1); the repeated number is usually four^[15]. In this work, we designed primers using the gene sequence of Pro-tRNA and Phe-tRNA to amplify the D-loop region and its subsections of 5' terminal region, 3' terminal region and repeated sequence region by polymerase chain reaction (PCR) method. The primer sequences and yielded products are listed in table 1. The primers location in mtDNA D-loop is illustrated in fig 1.

Table 1 The primer sequences and yielded products

Primer names	Primer sequences	Yielded products	Fragment length of PCR products/bp
P1F	5'-CAACACCCAAAGCTGAAGTTC-3'	D-loop	963+75 <i>n</i>
P4R	5'-CTAGGCATTTTCAGTGCCTTGC-3'		
P1F	5'-CAACACCCAAAGCTGAAGTTC-3'	5' terminal of D-loop	253
P2R	5'-CCCGTTTGCATGTTTAAGATAG-3'		
P3F	5'-GTTTCACTGAAGCATGTAGGG-3'	3' terminal of D-loop	700
P4R	5'-CTAGGCATTTTCAGTGCCTTGC-3'		
P2F	5'-GTCTATCTTAAACATGCAAACGG-3'	repeated sequence region	40+75 <i>n</i>
P3R	5'-CCCTACATGCTTCAGTGAAAC-3'		

n repeats number.

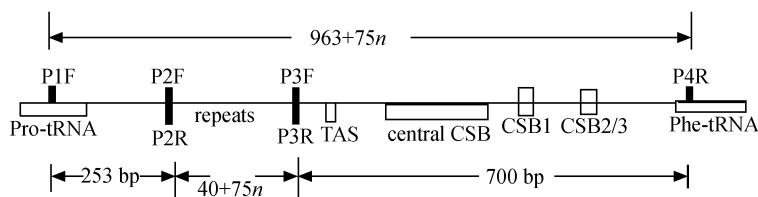


Fig. 1. Structure of sheep mtDNA D-loop, primers and yielded PCR products.

1 Materials and methods

1.1 Sheep

120 sheep came from four different breeds, i.e. Small Tail Han sheep, Wuzhumuqin, Hu-yang and Sharolais, with 30 sheep randomly selected from each breed. In order to identify the paternal mtDNA inheritance, 11 hybrid families of Dorset (♂), Small Tail Han sheep (♀) and the F1