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Ovine mitochondrial DNA: mapping and sequencing data in comparison with bovine mtDNA

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INTRODUCTION

Mitochondrial DNA (mtDNA) is maternally inherited (Gyllensten *et al*, 1985) and evolves 5–10 times faster than single-copy nuclear DNA in mammals (Brown *et al*, 1979). These properties make mtDNA an excellent tool for analyzing evolutionary relationships (Wilson *et al*, 1985).

We studied the mtDNA structure of *Ovis aries* and compared it to the *Bos taurus* (Anderson *et al*, 1982) sequence.

MATERIALS AND METHODS

mtDNA was prepared from the liver of a single Merinolandschaf, using the method described by Hecht *et al* (1988). Restriction endonucleases (*Eco*RI, *Eco*RV, *Bam*HI, *Bgl*II, *Hin*dIII, *Hinc*II, *Kpn*I, *Pst*I and *Xba*I) were used according to the manufacturers' recommendations. DNA fragments were separated in 1% agarose gels, stained with ethidium bromide, photographed and transferred onto Hybond-N-Nylon membranes (Amersham product information). Hybridization analysis was performed using a [^{32}P]dCTP-labeled (Sambrook *et al*, 1989) 2.5 kb *Hin*dIII fragment of porcine mtDNA, encompassing parts of the ATPase6 and ND4 genes and the complete COIII, ND3 and ND4L genes. Two 2.6 and 3.4 kb *Eco*RI fragments of ovine mtDNA were isolated on diethylaminoethyl (DEAE) membrane filters, cloned into puc13 and the ligation products used to tranform *E coli* (JM83) (Sambrook *et al*, 1989). Plasmid DNA for dideoxysequencing was prepared as described in the Diagen Application Protocol (1988). ^{35}S-Sequencing reactions were performed with the T_7-sequencing kit supplied by Pharmacia according to the manufacturers' instructions. Cronex X-ray film (Dupont) was used for autoradiographs.

RESULTS AND DISCUSSION

Total ovine mtDNA as well as cloned fragments were used to map 28 cleavage sites produced by *Eco*RI, *Eco*RV, *Bam*HI, *Bgl*II, *Hin*dIII, *Hin*cII and *Xba*I by the double-digestion method. Hybridization analysis with a fragment of porcine mtDNA of known gene content along with sequencing data was used for orientation of the map along the bovine sequence (Anderson *et al*, 1982). The 9 enzymes (*Pst*I and *Kpn*I apparently do not cut ovine mtDNA) used, have a total of 31 recognition sites in bovine mtDNA (there is no *Eco*RV site in bovine mtDNA). Of the 28 restriction sites observed in the ovine sequence, only 11 are assumed to be present in the bovine sequence (fig 1). The proportion of sites shared is expected to decline as the organisms' DNA sequences diverge. The application of equations 10 and 8 from Nei and Li (1979) to these data yields an estimated sequence divergence of 16.4% between the two species.

Sequencing analysis of the terminals of 2 cloned fragments yielded 1.105 kb of sequence information from ovine mtDNA. Genes partially sequenced were ND5, Cyt.b, ATPase6 and COIII. The comparison of nucleotide and predicted amino acid sequences of the 2 species revealed a clustering of replacement nucleotide substitutions within parts of the sequenced regions of the ND5 (fig 2), Cyt.b and COIII genes (data not shown). Silent substitutions on the other hand seem to be distributed evenly across the sequences. Gene-specific and overall sequence differences are given in table I. The 4 genes showed almost the same rate of nucleotide substitutions, although the COIII (76–79%) and Cyt.b genes (73–74%) were more conserved than the ND5 gene (65–71%), for example, when compared between mouse, cow and human (Anderson *et al*, 1981, 1982; Bibb *et al*, 1981). This was probably a result of partial analysis of the genes. Amino acid replacements were highest in the ND5 gene. The lack of any amino acid replacements in the short stretch of ATPase6 DNA was probably due to the high conservation of this region within the group of species mentioned above.

Table I. Sequence differences between ovine and bovine mtDNAs at the nucleotide and predicted amino acid levels.

Gene	*Nucleotides sequenced*	*% of gene*	*Nucleotide substitutions*		*Amino acid substitutions*	
			n	*%*	*n*	*%*
ATPase6	74	10.9	11	14.9	0	0
COIII	193	24.7	30	15.5	5	7.8
Cyt.b	279	24.6	43	15.4	6	6.5
ND5	559	30.8	86	15.4	16	8.6
total	1105	6.7[a]	170	15.4	27	7.3

[a] % of mt genome.

More than 80% of all nucleotide replacements were transitions. This is in accordance with results obtained by Wilson *et al* (1985) in primates.

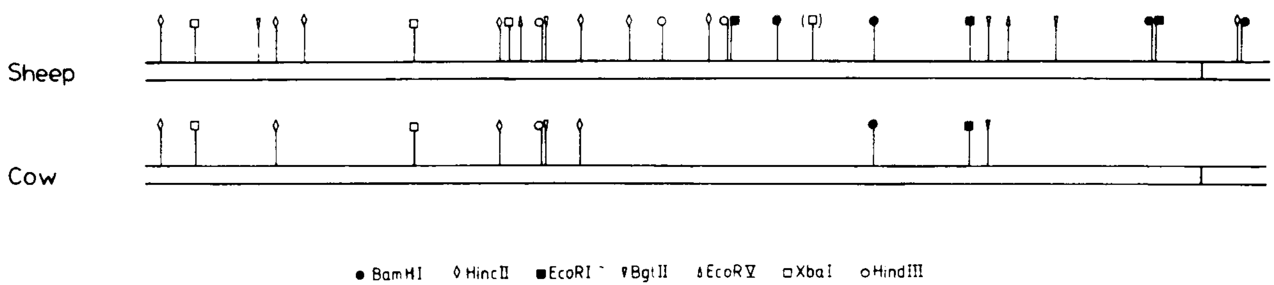

Fig 1. Comparison of the ovine and bovine restriction maps. In the bovine map only sites shared with the ovine map are shown.

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       ND 5
       <-->
         A  I  N           H     F  T                          T        C
SHEEP  AGCAATCAATTTTAACACCCATAAATTCACCAATTATCCACTCTATGTAAAAACAACCATTTCATGCGCTTTCATCACTAGCATAATCCCC
        **   * **         * *   ****    *  *        *         * *  *    *   *     *  *        *
COW    AATAATAAGCTTTAACACCTACAAACCTTCCAACTACCCACTCTACGTAAAAACAGCTATCTCATACGCCTTCATTACCAGCATAATTCCC
         M  M  S           Y     P  S                          A        Y

                          T           M
       ACAATAATATTCATCCACACAGGACAAGAAATAATTATCTCAAACTGACACTGACTAACCATCCAAACCCTCAAACTATCACTCAGCTTCA
                  *      *    *      *       *                             *  *   *    *        *
       ACAATAATATTTATCCACTCAGGCCAAGAACTAATTATTTCAAACTGACACTGACTAACCATCCAAACTCTTAAATTATCCCTCAGCTTTA
                          S           L

                F                 V
       AAATAGACTTCTTCTCAATAATATTCGTTCCAGTAGCACTATTCGTTACATGATCTATTATA
                **              ** *                 *
       AAATAGACTATTTCTCAATAATATTTATCCCAGTAGCACTATTCGTCACATGATCTATTATA
                Y                 I
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Fig 2. Sequence comparison of ovine mtDNA (ND5 gene region) with the corresponding bovine sequence (Anderson *et al*, 1982; nucleotide positions 12.167–12.410, L-strand). Nucleotide substitutions are denoted by asterisks. Differences in the predicted amino acid sequences are shown.

The overall sequence difference of 15.4% at the nucleotide level corresponds well with 16.4% sequence divergence calculated from restriction map comparison. Using the data reported by Wilson *et al* (1985), sheep and cow would have evolved from a common ancestor 7.7–16.4 $\times$ 10^6 years ago.

An example illustrating the limitations inherent in the comparison of restriction mapping data is a *Bgl*II site, assumed to be identical in the ovine and bovine maps, which corresponds to bovine nucleotide no 12 706 according to Anderson *et al* (1982). The recognition site in the bovine sequence is actually not identical to the ovine site as shown in figure 1, but 5 nucleotides downstream from it. Taking this into account, the calculated sequence divergence is 18% according to Nei and Li (1979), which results in 9.0–18.0 $\times 10^6$ years as the time of divergence between the two species.

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