Analysis of Mitochondrial DNA Indicates That Domestic Sheep Are Derived From Two Different Ancestral Maternal Sources: No Evidence for Contributions From Urial and Argali Sheep

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To investigate the origins and phylogenetic relationships of domestic sheep, mitochondrial DNA (mtDNA) from 243 sheep of five European, one African, and four Asian breeds and several mouflon (*Ovis musimon*), urial (*O. vignei bochariensis*), and argali (*O. ammon nigrimontana, O. a. collium*) were assayed for restriction fragment length polymorphisms (RFLP). Twenty haplotypes were identified which occurred in three major phylogenetic groups: urial/argali, mouflon/domestic, and domestic sheep. From the branches that contain mouflon and domestic sheep, two major domestic sheep lineages are apparent. One lineage, termed European lineage, contains the majority of haplotypes detected among European domestic sheep. These mtDNAs resemble mouflon haplotypes. The other lineage, termed Asian lineage, consists of haplotypes found in central Asian and some European domestic sheep. The mean sequence difference between these two lineages (0.72%) is of similar magnitude as that between two argali subspecies. To accurately estimate sequence differences between the European and Asian mtDNA types, the mitochondrial control region of one animal from each lineage and of one mouflon and urial were completely sequenced. Sequence comparisons show that Asian and European domestic sheep lineages differ by 4.43%. The mouflon sequence diverges from the Asian type by 4.52%, but by only 1.36% from the European type. Our data supports the hypothesis that some modern domestic sheep and European mouflon derive from a common ancestor and provide evidence of an additional wild ancestor, other than the urial and argali groups, which has yet to be identified.

The origin of the modern domestic sheep (*Ovis aries*) remains uncertain. Several wild sheep species or subspecies, the taxonomy of which is confused, have been proposed as ancestors of modern domestic sheep (Ryder 1984) or are thought to have contributed to specific breeds.

According to Zeuner (1963) the urial (*O. vignei*) was first domesticated in the Aralo-Caspian basin and these domestic forms subsequently spread throughout the Middle East and into Europe. Another line of domesticated sheep derived from mouflon (*O. musimon* or *O. orientalis*) stock that was brought into Europe and mixed with the urial derivatives. According to this view, the domestic sheep of Southeast Asia are derived from urial, however, argali (*O. ammon*) alleles have been introduced repeatedly into these lines.

Extensive cytogenetic studies conducted by Nadler et al. (1971) and Woronzow et al. (1972) of the wild sheep populations of Iran, Turkmenia, Tadschikistan, and Kazakhstan established the chromosome number of several mouflon ($2n = 54$), urial ($2n = 58$), and argali ($2n = 56$) populations. The authors concluded that their chromosome data did not agree with ideas regarding the urial as the source of most domestic breeds, because European and Central Asian breeds of domestic sheep have $2n = 54$. This suggests the mouflon group as the ancestral stock from which domestic strains were derived. However, wild sheep populations with different chromosome number ($2n = 54$ and $2n = 58$) hybridize and give rise to animals with $2n = 55$, 56, or 57, which may have normal fertility (Nadler et al. 1971). Argali × mouflon hybrid ewes with $2n = 55$ produce ova with 27 chromosomes. This suggests prezygotic selection toward a lower chromosome number and shows that the 54 chromosomes of modern domestic sheep need not have come solely from the mouflon (Ryder 1984). A modern example of introgression of alleles from sheep with more than 54 chromosomes into domestic stock is the crossbreeding experiment be-

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Table 1. Mitochondrial DNA fragment patterns detected with 14 restriction enzymes in argali, urial, mouflon, and domestic sheep

Haplotype no.	*Ovis* ssp.	Restriction enzyme (4-base, 6-base) 4 *Alu*I	6 *Bam*HI	6 *Bgl*II	6 *Eco*RI	6 *Eco*RV	4 *Hae*III	6 *Hin*dIII	4 *Hin*fI	4 *Mbo*I	4 *Msp*I	4 *Rsa*I	4 *Taq*I	6 *Stu*I	6 *Xba*I
1	*O. ammon nig.*	D	B	A	C	B	C	A	J	F	A	E	C	A	A
2	*O. ammon col.*	E	B	A	C	A	C	A	I	G	A	E	D	C	A
3	*O. vignei boc.*	F	C	A	D	A	F	A	H	H	C	C	E	D	A
4	*O. musimon* a	A	A	A	A	A	B	A	A	A	A	D	A	A	A
5	*O. musimon* b	A	A	A	A	A	B	A	A	A	A	A	A	A	A
6	*O. musimon* c	A	A	A	A	A	B	A	C	E	A	A	A	A	A
7	*O. aries* j	B	B	A	A	A	A	A	D	A	A	B	A	A	A
8	*O. aries* n	A	A	A	A	A	A	A	E	D	A	F	A	A	A
9	*O. aries* k	B	B	A	A	A	B	A	D	D	A	B	A	A	A
10	*O. aries* d	A	A	A	A	A	D	A	A	A	A	D	A	A	A
11	*O. aries* e	A	A	A	A	A	A	A	F	A	A	A	A	A	A
12	*O. aries* l	B	B	B	A	A	B	A	D	C	A	B	A	A	A
13	*O. aries* m	B	B	A	A	A	B	A	G	D	A	B	A	B	A
14	*O. aries* i	C	A	A	A	A	B	A	A	A	A	A	A	A	B
15	*O. aries* a	A	A	A	A	A	E	A	A	A	A	A	A	A	A
16	*O. aries* g	A	A	A	A	A	A	A	A	A	B	A	A	A	A
17	*O. aries* c	A	A	A	B	A	B	A	A	A	A	A	A	A	A
18	*O. aries* h	A	A	A	A	A	C	A	C	A	A	A	B	A	A
19	*O. aries* f	A	A	A	A	A	A	A	A	B	A	A	A	A	A
20	*O. aries* b	A	A	A	A	A	B	A	B	A	A	A	A	A	A

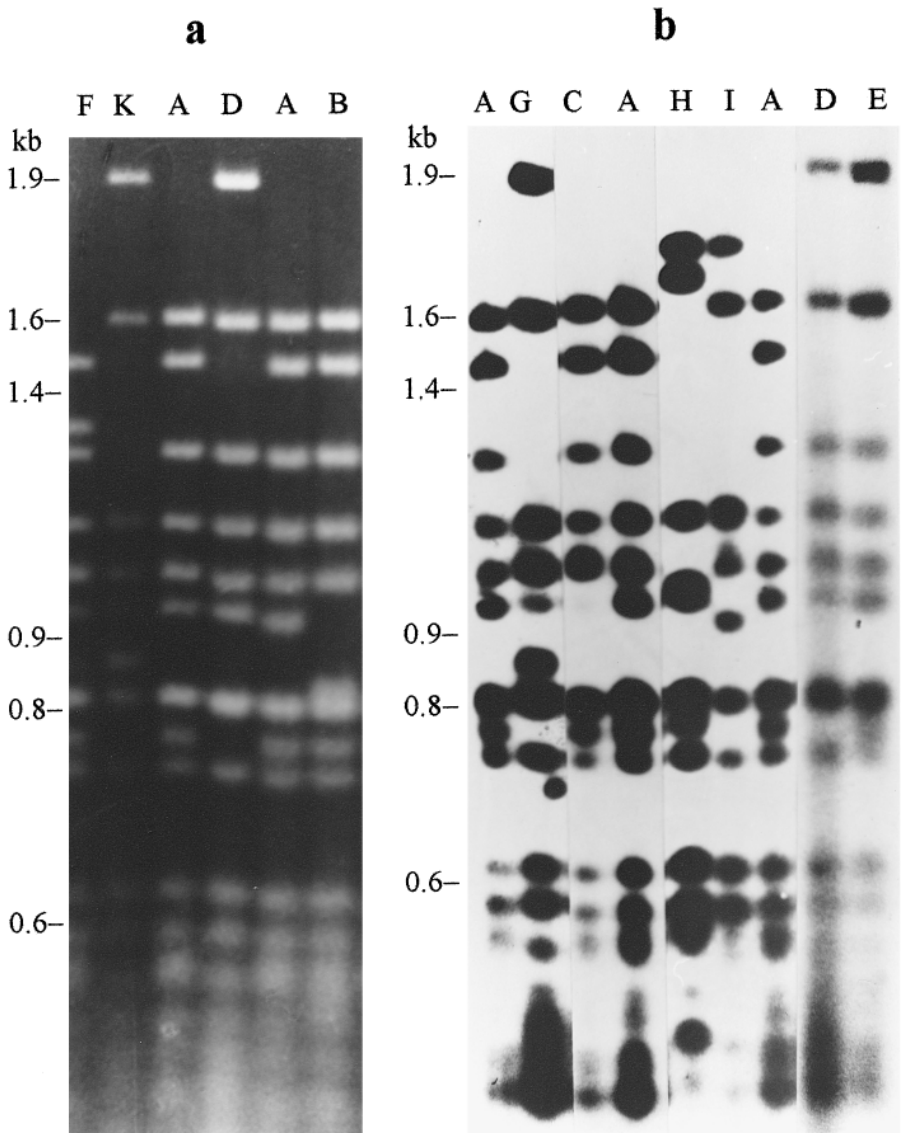

Figure 1. Variable fragment patterns in wild and domestic sheep for the restriction enzyme *Hin*fI: **(a)** agarose gel; **(b)** autoradiograph. Patterns are shown along the top of each gel as described in Table 1. Approximate fragment sizes (kb) are indicated.

tween argali and merino sheep in Kazakhstan which has established a new breed, the arkharo-merino with $2n = 54$ (Woronzow et al. 1972).

Mitochondrial DNA (mtDNA) is predominantly or exclusively clonally and maternally inherited via oocyte cytoplasm (Gyllensten et al. 1985, 1991) and evolves 5 to 10 times more rapidly than nuclear DNA (Brown 1985). Since mtDNA commonly shows haplotype diversity within species, it is a useful tool in which to establish phylogenetic relationships at or below the species level (Avise et al. 1987). We conducted a phylogenetic analysis of mtDNA haplotypes of both wild and domestic sheep in order to investigate the hypothesis that different forms of wild sheep have contributed to modern breeds.

Materials and Methods

Sample Collection

Blood or liver samples were collected from Merinolandschaf ($n = 80$), East Friesian Milk sheep ($n = 111$), Rhoenschaf ($n = 42$), Schwarzkoepfiges Fleischschaf ($n = 2$), Skudde ($n = 1$), Awassi ($n = 1$), Camerun ($n = 3$), Edilbey ($n = 1$), Gizarr ($n = 1$), and Astrachan ($n = 1$) breeds. The Edilbey sample was obtained in the Alma-Ata (Kazakhstan) region, Astrachan was collected near Tschimkent (Kazakhstan), and the Gizarr liver sample was from the Moscow State University. Awassi and Camerun samples were provided by the Veterinary Department of the Justus-Liebig University, Giessen. The other domestic sheep samples were obtained from the herds of the "Oberer Hardthof" and other

Table 2. Percent sequence divergence between mtDNA haplotypes (Table 1) estimated according to Nei and Miller (1990)

Haplotype no.	*Ovis* ssp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	*O. ammon nig.*																			
2	*O. ammon col.*	0.865																		
3	*O. vignei boc.*	2.482	2.207																	
4	*O. musimon* a	2.324	1.937	2.640																
5	*O. musimon* b	2.275	1.889	2.588	0.027															
6	*O. musimon* c	2.354	1.967	2.670	0.138	0.109														
7	*O. aries* j	2.023	1.787	2.779	0.688	0.595	0.595													
8	*O. aries* n	2.434	1.967	2.752	0.483	0.451	0.451	0.305												
9	*O. aries* k	2.023	1.787	2.695	0.688	0.595	0.595	0.165	0.305											
10	*O. aries* d	2.455	2.067	2.777	0.139	0.166	0.166	0.660	0.280	0.846										
11	*O. aries* e	2.324	1.937	2.640	0.166	0.138	0.138	0.568	0.424	0.749	0.139									
12	*O. aries* l	2.147	2.281	2.842	0.803	0.710	0.710	0.273	0.477	0.163	0.961	0.864								
13	*O. aries* m	2.168	2.132	2.695	0.870	0.775	0.775	0.332	0.479	0.164	1.032	0.933	0.330							
14	*O. aries* i	2.201	2.030	2.865	0.255	0.227	0.227	0.838	0.687	0.838	0.397	0.367	0.959	1.027						
15	*O. aries* a	2.275	1.889	2.744	0.082	0.054	0.054	0.595	0.451	0.655	0.166	0.138	0.770	0.836	0.282					
16	*O. aries* g	2.434	2.047	2.752	0.194	0.165	0.165	0.595	0.451	0.777	0.166	0.138	0.891	0.961	0.395	0.165				
17	*O. aries* c	2.153	1.986	2.619	0.112	0.085	0.085	0.682	0.536	0.682	0.251	0.222	0.800	0.865	0.317	0.139	0.249			
18	*O. aries* h	2.245	1.781	2.640	0.223	0.194	0.194	0.749	0.603	0.811	0.310	0.338	0.926	0.933	0.425	0.194	0.307	0.278		
19	*O. aries* f	2.324	1.937	2.723	0.166	0.138	0.138	0.568	0.424	0.749	0.139	0.110	0.864	0.933	0.367	0.138	0.138	0.222	0.280	
20	*O. aries* b	2.383	1.998	2.618	0.109	0.081	0.081	0.682	0.538	0.682	0.250	0.221	0.797	0.803	0.309	0.136	0.248	0.166	0.221	0.221

farms in Germany or at a local slaughterhouse.

Argali tissue was obtained from *O. ammon nigrimontana* (Kazakhstan, Karatau Mountains, $n = 2$) and *O. a. collium* (Kazakhstan, Karaganda area, $n = 1$). Urial samples were collected from *O. vignei bochariensis* (Turkmenia, $n = 2$). Mouflon (*O. musimon*) blood samples ($n = 6$) were obtained in game preserves in Germany. We have used the detailed wild sheep nomenclature provided by Woronzow et al. (1972) in this study.

DNA Extraction

Mitochondrial DNA was purified from fresh or frozen liver samples as described by Hiendleder et al. (1991). Total genomic DNA was isolated from blood samples by the method of Montgomery and Sise (1990). We extracted DNA from liver samples stored in ethanol according to the following procedure: Approximately 1 g tissue was soaked in TE buffer for 30 min, ground to a fine dust under liquid nitrogen, and suspended in 40 ml buffer (10 mM Tris, 100 mM EDTA, pH 8.0, 0.5% SDS, 100 μg/ml proteinase K). The suspension was incubated at 50°C for 3 h, extracted with phenol/chloroform, precipitated, washed, and dissolved in 500 μl TE buffer.

Restriction Enzyme Analysis of mtDNA

We digested purified mtDNA (100–400 ng) or genomic DNA (1 μg) with a total of seven restriction enzymes which recognize 6-base sequences, and seven enzymes which recognize 4-base sequences (Table 1). Digested DNA fragments were analyzed by gel electrophoresis on 0.8–1.0% (6-base enzymes) or 1.2–1.4% (4-base enzymes) Tris-borate-EDTA agarose gels, stained in ethidium bromide (Sambrook et al. 1989), and photographed under ultraviolet light. Southern blot analysis of gels containing genomic DNA was carried out by alkaline transfer to Hybond N^+ nylon membranes (Amersham) according to the manufacturers instructions. Filters were prehybridized at 67°C in 10 ml of prehybridization solution (6× SSC, 5× Denhardt's, 10% dextran sulfate, 0.5% SDS, 20 μg/ml denatured salmon sperm DNA) for 8 h. A ^{32}P-labeled probe (Prime-IT kit, Stratagene), obtained from two *Eco*-RV fragments comprising the mtDNA of a domestic sheep (Hiendleder et al. 1991) was added to the prehybridization solution and hybridization continued for 18 h. After hybridization, filters were washed at room temperature with 2× SSC/0.5% SDS for 5 min and with 2× SSC/0.1% SDS for 15 min. This was followed by two 30 min washes at 67°C with 1× SSC/0.1% SDS and 0.2× SSC/0.1% SDS. Filters were air dried, covered with plastic wrap, and exposed to Hyperfilm MP (Amersham) for 48 to 120 h.

Cloning and Sequencing of mtDNA

Either pure mtDNA from liver or PCR fragments were cloned and sequenced. We purified mtDNA from liver samples of individuals with *O. aries* haplotypes a (European type) and m (Asian type) as described. The DNA samples were digested with the restriction enzymes *Eco*RI/*Xba*I and 2.1 kb fragments containing the mitochondrial control region (Hiendleder et al. 1991) were isolated from a 1% agarose gel with the Quiaex gel extraction kit (Quiagen). The fragments were cloned into pUC18 and subsequently subcloned as *Eco*RI/*Bam*HI and *Xba*I/*Bam*HI fragments into the plasmid Bluescript II KS. To determine the complete sequence of the control region, ^{35}S sequencing reactions were performed with the Sequenase 2.0 sequencing kit (Amersham) and T3 and T7 primers (Stratagene). In addition, we used the $tRNA^{Phe}$ and $tRNA^{Pro}$ primers mtDA (5′-CGGAATTCTCATCTAGGCATTTTCAGTG-3′) and mtDB (5′-GCTCTAGACTCACCATCAACCCCCAAAGC-3′) derived from the bovine sequence (Anderson et al. 1982) for sequencing. The flanking tRNA primers with artificial *Eco*RI/*Xba*I sites were also used to amplify mouflon (*O. musimon)* haplotype b and urial (*O. vignei bochariensis*) mitochondrial control regions from total genomic DNA under the following PCR conditions: 95°C, 30 s; 50°C, 60 s; and 72°C, 90 s; 40 cycles. Cloning, subcloning, and sequencing of the PCR products was performed as described above.

Data Analysis

We estimated the pairwise nucleotide sequence divergence between haplotypes from the restriction fragment data as described by Nei and Miller (1990). From this distance data, we constructed an mtDNA phylogenetic tree with the program NJTREE of RESTSITE version 1.2 (Miller 1990). Parsimony analysis was performed using the PHYLIP version 3.5c (Felsenstein 1993) with the programs SEQBOOT, MIX (Wagner option), and CONCENSE. Restriction fragment data and mtDNA samples can be made available to researchers interested in work on the phylogeny of wild and domestic sheep.

Results

MtDNA Haplotypes of Wild and Domestic Sheep

All of the restriction enzymes used in this study, with the exception of *Hin*dIII, detected polymorphisms (Figure 1). The 13 informative enzymes collectively accounted for 61 different fragment patterns (Table 1). A total of 20 mtDNA haplotypes could be distinguished among the 254 wild and domestic sheep analyzed. Among these, 14 were in domestic sheep, 3 in mouflon, 2 in argali (*O. a. nigrimontana, O. a. collium*), and 1 in urial. Two of the mouflon mtDNAs were identical (haplotype b) and two others were identical to *O. aries* haplotype a, which was found in Merinolandschaf and Rhoenschaf individuals. Haplotypes from African domestic sheep were identical to two haplotypes (*O. aries* i and g) detected among European domestic sheep. The two individuals of *O. ammon nigrimontana* and *O. vignei* did not reveal intraspecific differences.

Genetic Distances Between mtDNA Haplotypes

The restriction fragment data suggest sequence differences between *O. aries* haplotypes range from 0.110 to 1.032% ($\bar{x}$ = 0.492%) while differences among mouflon haplotypes range from 0.027 to 0.138% ($\bar{x}$ = 0.091%) (Table 2). The two argali subspecies haplotypes diverge by 0.865%. The sequence divergence between urial and domestic sheep ranges from 2.618 to 2.865% ($\bar{x}$ = 2.724%), between argali and domestic sheep from 1.781 to 2.455% ($\bar{x}$ = 2.115%), and between mouflon and domestic sheep from 0.054 to 0.870% ($\bar{x}$ = 0.465%). Differences between urial and mouflon, and between argali and mouflon are of the same magnitude as the differences observed between urial or argali and domestic sheep.

Both the distance analysis and the parsimony analysis suggested that the domestic breeds derive from two different maternal sources. The neighbor-joining tree (Figure 2a) constructed with the data described above reveals three major branches, which consist of urial/argali, mouflon/domestic, and domestic sheep haplotypes.

From the different branches that contain domestic sheep mtDNAs, two distinct lineages are apparent. One, the European lineage, consists of the majority of European *O. aries* (*O. aries* a–i), and the other, the Asian lineage, contains some European *O. aries* (*O. aries* l and m) and central Asian *O. aries* haplotypes which were derived from Astrachan (*O. aries* k), Edilbey (*O. aries* n), and Gizarr (*O. aries* j) breeds. The mean estimated sequence divergence between these two lineages is 0.716%.

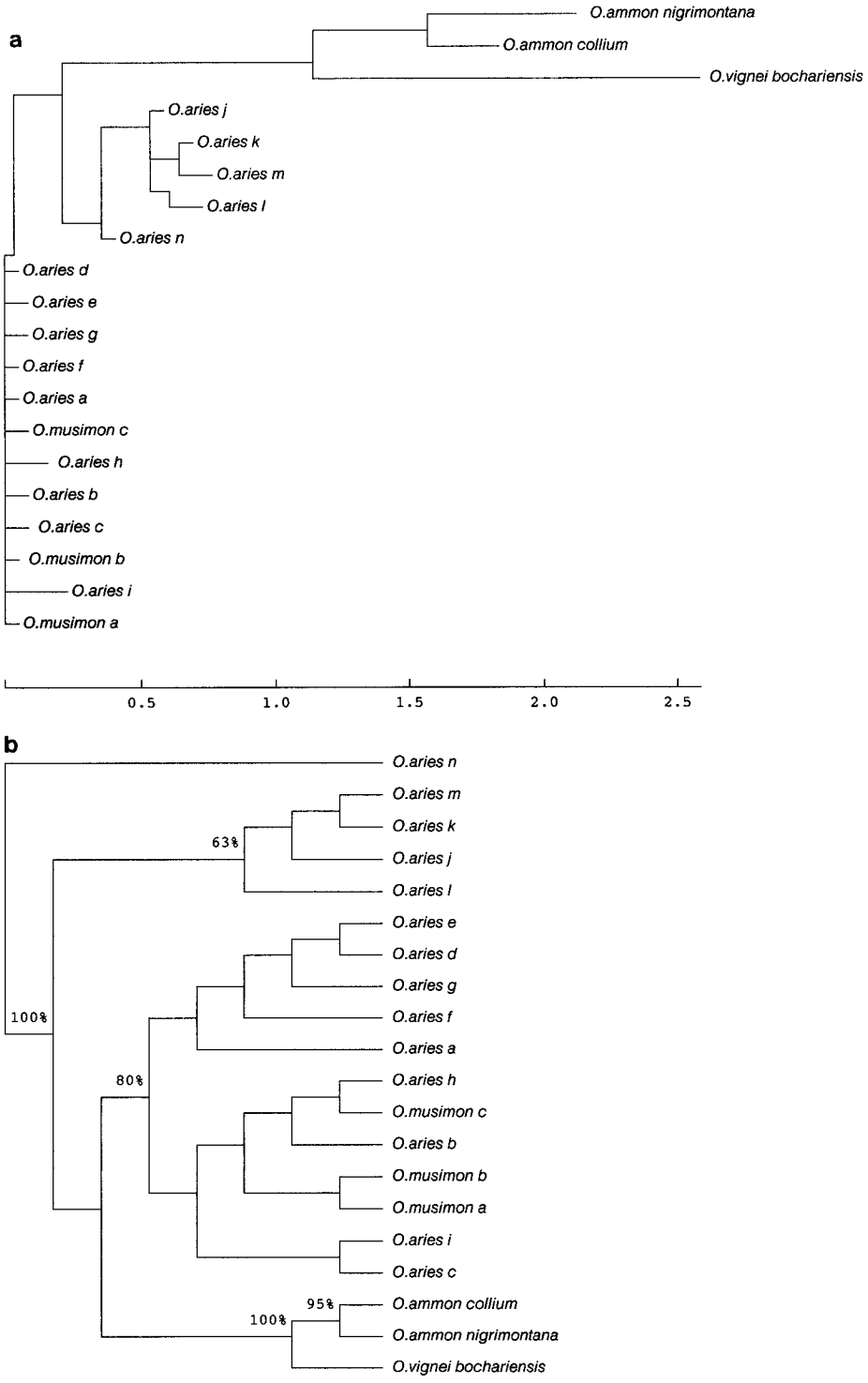

Figure 2. Phylogenetic trees of mtDNA haplotypes of wild and domestic sheep: **(a)** unrooted neighbor-joining tree with argali (*O. ammon nigrimontana, O. ammon collium*), urial (*O. vignei bochariensis*), mouflon (*O. musimon*) and domestic sheep of the Asian (*O. aries* j–n), and European type (*O. aries* a–i); **(b)** unrooted consensus tree derived from the parsimony analysis of the same data. Only bootstrap values above 50% are indicated.

As in the distance analysis, parsimony analysis (Figure 2b) placed urial/argali, mouflon/European *O. aries,* and Asian *O. aries* haplotypes in distinct branches. The relationship of the urial/argali to the mou-

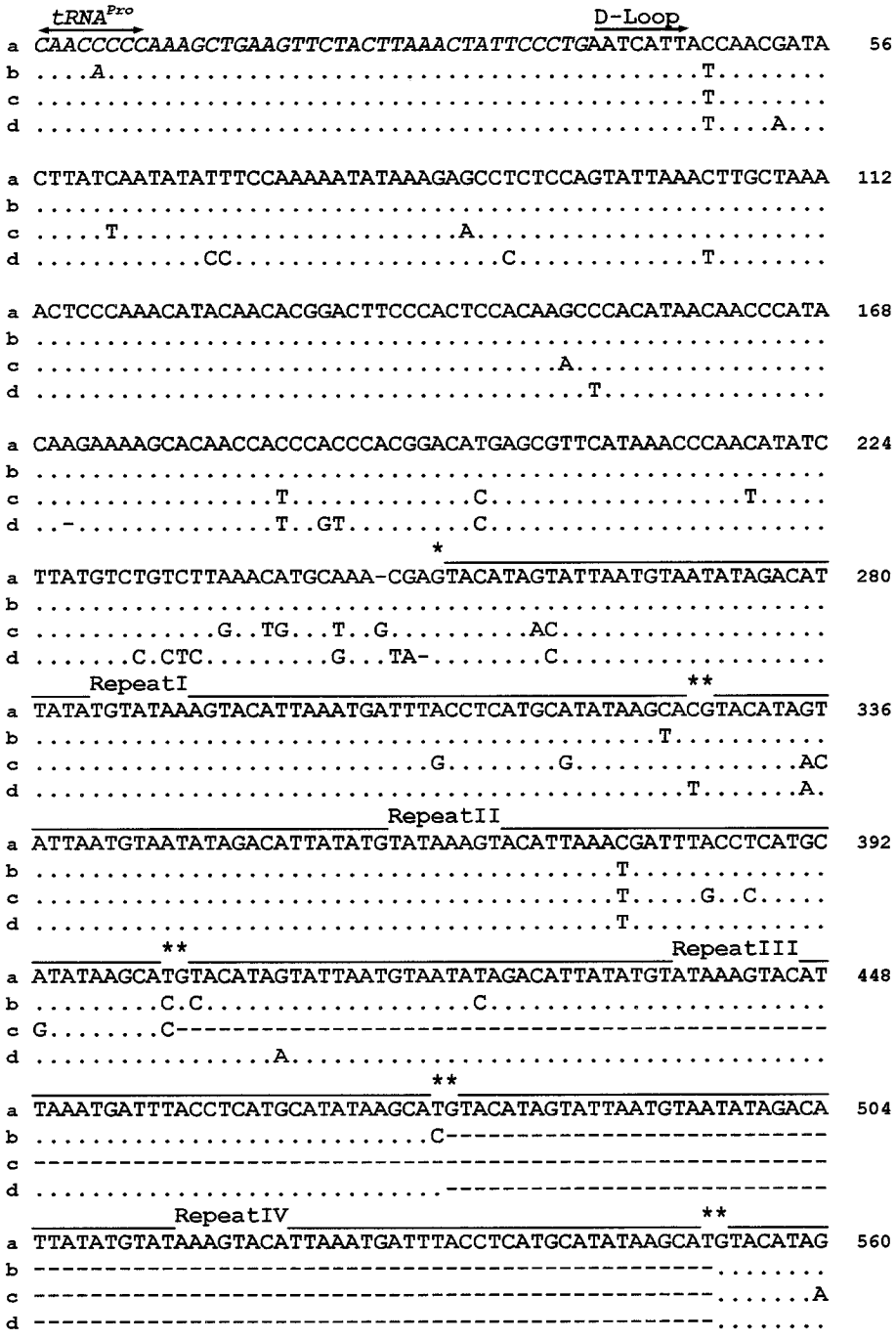

Figure 3. Nucleotide sequence comparison of the mitochondrial control regions of *O. musimon* b **(a)**, *O. aries* a **(b)**, *O. aries* m **(c)**, and *O. vignei bochariensis* **(d)** haplotypes of Figure 2 (GenBank accession no. AF039577-80). *O. aries* a is of the European type while *O. aries* m is of the Asian type. Dots indicate homology with sequence a, dashes represent insertions or deletions. Partial sequences of the proline and phenylalanine tRNAs are given in italics. The tandem repeat elements are overlined (repeats I–V). Conserved sequence boxes (CSB1, CSB2+3), light- and heavy-strand promoters (LSP, HSP), and the origin of heavy-strand replication (OH), as deduced from comparisons with the bovine sequence (Ghivizzani et al. 1993), are indicated.

flon/*O. aries* haplotypes differs from that in the neighbor-joining tree. In the parsimony tree, urial/argali are combined with mouflon/European *O. aries* instead of Asian *O. aries* haplotypes. However, this branch is only supported by a bootstrap value of 50%.

Sequence Analysis of the Mitochondrial Control Region

To accurately estimate sequence divergence within and between the two lineages which contain domestic sheep, we determined the complete nucleotide sequence of the mitochondrial control region of one sheep from each lineage (haplotypes a and m of Figure 2) and one mouflon (*O. musimon* b). The control region of *O. vignei bochariensis* was also sequenced as an outgroup (Figure 3). All four sequences show considerable variability in length. The mouflon sequence is the longest with 1254 nucleotides, followed by *O. aries* a with 1180 nucleotides, and urial with 1177 nucleotides. The *O. aries* m sequence is shortest with only 1106 nucleotides. Apart from minor insertions/deletions, the observed length variations among domestic and wild sheep are caused by different copy numbers of a 75 bp motif which is tandemly repeated in the tRNA^{Pro} half of the control region. This repeat motif shows only minor variation within and between mouflon and domestic sheep, and is well conserved in the urial (Figure 4a,b). When length variations due to the tandem repeats are neglected, the domestic sheep lineages differ by 4.43% while the mouflon sequence diverges from the European mtDNA type (*O. aries* a) by only 1.36%, but by 4.52% from the Asian type (*O. aries* m). As expected from the restriction data, the urial control region shows less sequence similarity to the other sheep, with a divergence of 5.59 to 6.97% from the other three sequences.

Discussion

The prevailing opinion on the origins of domestic sheep considers the Aralo-Caspian basin as a major center of domestication where Urial (*O. vignei*) were domesticated and subsequently spread throughout the Middle East and Europe. Another line of domesticated sheep is assumed to have derived from mouflon (*O. musimon* or *O. orientalis*) stock which was brought to Europe and mixed with the urial derivatives. According to this view, the sheep of Southeast Asia derived from urial, but argali (*O. ammon*) alleles have been introduced repeatedly into these lines (Zeuner 1963). Our results clearly show that neither urial (*O. vignei bochariensis*) nor argali (*O. ammon nigrimontana, O. a. collium*) mtDNA is closely related to any of the 14 mtDNA haplotypes found among the 243 domestic sheep of European, African, and central Asian origin. Rather our phylogenetic analysis shows urial, argali, and mouflon/domestic sheep in distinct lineages. The urial was more diverged from domestic sheep than the argali, and showed 5.59 to 6.97% sequence divergence from mouflon (*O. musimon*) and domestic sheep in the mitochondrial control

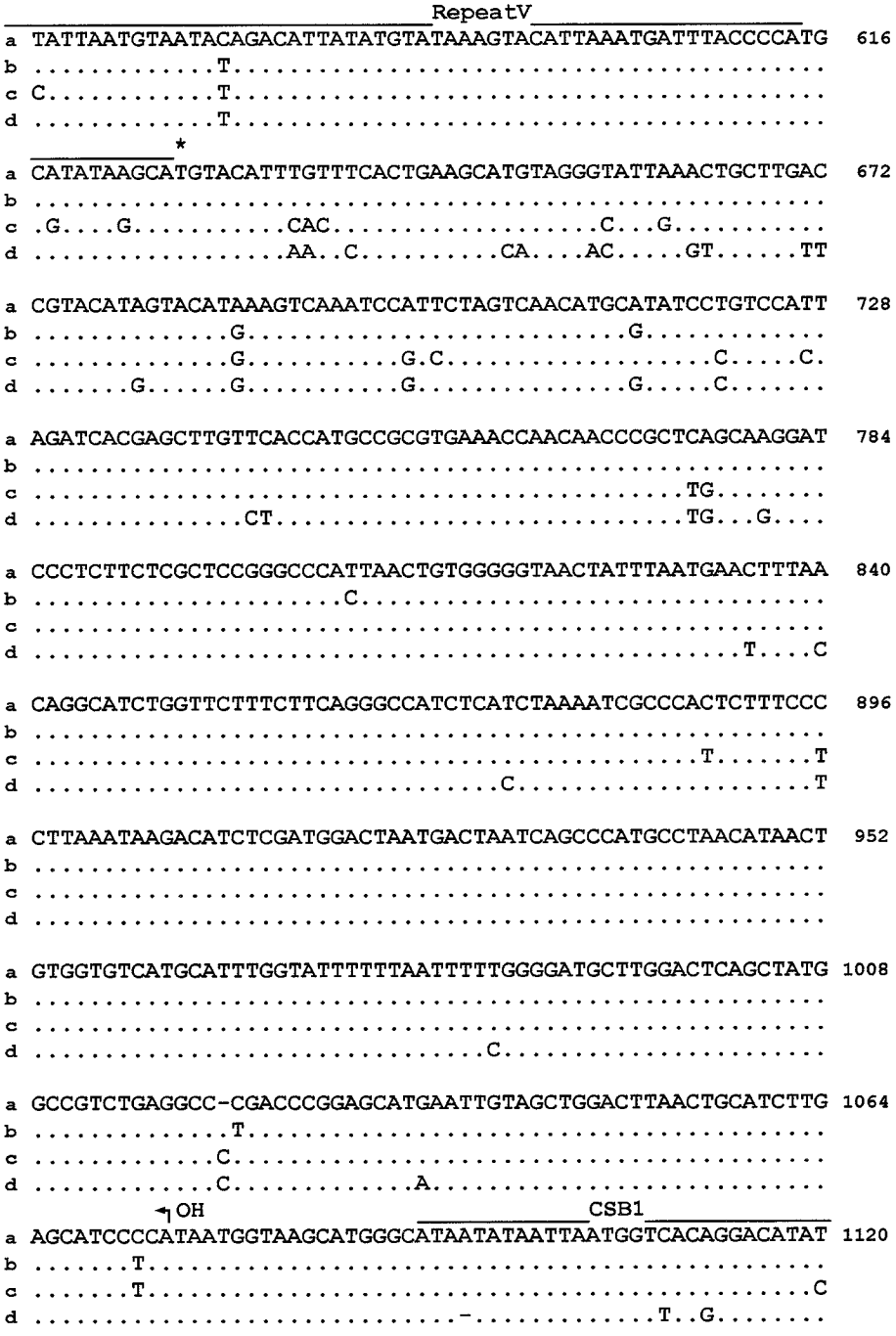

Figure 3. Continued.

region. These results are in agreement with the available data on chromosomes and some data on transferrins of wild and domestic sheep. Based on the knowledge that all breeds of domestic sheep display $2n = 54$, Nadler et al. (1971) postulated that domestic breeds of European origin probably arose from ancestral wild stock occupying a range west of central Iran, where sheep referrable to the *orientalis* group of mouflon display $2n = 54$ and not from the Aralo-Caspian basin where wild sheep of the *vignei* group are characterized by $2n = 58$. This assumption was substantiated by apparent similarities between transferrin allele frequencies of the *O. orientalis* group and European domestic sheep, which were distinct from the *O. vignei* group (Lay et al. 1971). However, Bunch and Foote (1976) found no correlation of transferrin frequencies between Iranian domestic and western wild sheep populations to substantiate common ancestry. However, these results are not necessarily contradictory, since nuclear allelic introgression of eastern wild sheep beyond chromosomal (and mtDNA) detection into eastern breeds could have occurred. Evidence for allelic introgression between *O. vignei* and *O. orientalis* beyond chromosomal detection was observed by Lay et al. (1971) in an *orientalis* × *vignei* hybrid zone. Furthermore, separation techniques available today have identified more alleles than those described by Bunch and Foote (1976) and Lay et al. (1971), so their results may be biased.

Our analyses have identified two major domestic sheep mtDNA lineages, termed European and Asian lineages, within branches that contain European mouflon (*O. musimon*) and domestic sheep or domestic sheep alone. Mouflon were placed in the same branch as most of the European domestic sheep. Furthermore, of the six mouflon mtDNAs analyzed, two were identical to haplotypes found in European domestic breeds which depicted the European mtDNA type, and the remaining three mouflon haplotypes were closely related to other mtDNAs of this type. These findings are in agreement with the low degree of sequence divergence (0.09–0.27%) that was previously detected in four mitochondrial genes of two European domestic sheep and one mouflon (Hiendleder et al. 1991). According to one theory, based on paleontological data, European mouflon originated from feral neolithic sheep (Poplin 1979). These assumptions are supported by hemoglobin β-chain allele distributions reported by Bunch et al. (1978). According to this study, the *Hb* A allele occurs only in domestic sheep and Corsican mouflon, but not in Asiatic and Middle Eastern mouflon. However, this allele has not been detected in mouflon populations of Czechoslovakia (Stratil and Bobak 1988) and Italy (Naitana et al. 1990), so that the A allele could have been introduced into some populations by interbreeding with domestic sheep. In addition, the possibility remains, that the *Hb* A alleles described in domestic sheep and mouflon are not identical (Montgelard et al. 1994). The *Hb* B alleles of mouflon and domestic sheep have already been shown to be different, although they appear identical in starch-gel electrophoresis (Naitana et al. 1990; Stratil and Bobak 1988). The latter assumptions would not exclude the possibility that modern sheep breeds were derived from European mouflon. Our

Figure 3. Continued.

findings can be interpreted in both ways. The low degree of sequence variation between mouflon and domestic sheep mtDNAs of the European lineage could stem from a common ancestor of mouflon and domestic sheep or it could reflect the derivation of mouflon from early domestic stock. The position of mouflon haplotypes in the phylogenetic trees tends to support the latter hypothesis. However, if this assumption is correct, the question about the identity of the common ancestor of these European type mtDNAs still remains.

Our study demonstrates two distinct lineages of mtDNA in a survey of 243 individual European, African, and Asian domestic sheep. Haplotypes of the two lineages diverge by up to 1.032%, with a mean sequence divergence of 0.72%. This is of the same magnitude as the RFLP-derived mtDNA sequence divergence reported for European (*Bos taurus*) and Zebu (*B. indicus*) cattle (Kikkawa et al. 1995), which are considered as subspecies by many authors (Epstein and Mason 1984). Similarly the estimated sequence difference between the two sheep mtDNA lineages is of the same magnitude as the difference between the two argali subspecies included in this study. This suggests, that European and Asian mtDNA haplotypes derive from two different wild ancestors with subspecific taxonomic status. The available chromosomal data and our results point to populations of the mouflon group of wild sheep as likely candidates. Direct sequence analysis of the control region of selected European- and Asian-type mtDNAs and mouflon mtDNA confirm the existence of two distinct mitochondrial sheep lineages. As expected from the restriction data, there is a high degree of sequence similarity between European type domestic sheep and mouflon haplotypes, but a considerable difference (4.4%) between European- and Asian-type domestic sheep mtDNA. While this article was processed, Wood and Phua (1996) published extensive sequence data on mitochondrial control regions from domestic sheep of New Zealand. Their study also identified two major domestic sheep mtDNA lineages with sequence differences that are of the same magnitude as the nucleotide divergence between the Asian and European haplotypes presented in this study. The observed differences in control region length, which are caused by different copy numbers of a tandemly repeated sequence, are probably not a characteristic of these distinct sheep mtDNAs, as we have observed heteroplasmy for length variants in several domestic (Hiendleder 1994) and wild sheep (Hiendleder S, unpublished data). However, the extent of sequence divergence between European and Asian mtDNA control regions lends further credibility to the concept of a dual maternal origin of domestic sheep, as sequence differences between *B. indicus* and *B. taurus* control regions are of the same magnitude (Loftus et al. 1994a). The average rate of sequence divergence of the whole mtDNA molecule in mammals is estimated to be 2–4% per million years (Brown et al. 1979). Extrapolating from this value, we estimate that the mtDNA haplotypes of domestic sheep with European- and Asian-type mtDNA diverged approximately 375,000 to 750,000 years ago. This is much longer than the 12,000 years of domestication history suggested by archaeological data (Ryder 1984). One explanation for this is the domestication of different wild populations of ancestral sheep as outlined above. Another possibility is that lineage sorting, for example, by genetic drift, has lead to the present-day haplotype structure. However, this seems unlikely, when the extent of sequence divergence in the control region of Asian and European haplotypes in comparison to *B. taurus* and *B. indicus* is considered. Unlike in cattle, wild populations of presumptive ancestors of domestic sheep still exist. An evaluation of their mtDNA genetic diversity could help clarify this question. In addition, the construction of multiple gene trees with mitochondrial and nuclear genes could lend support to the hypothesis of a dual maternal origin of modern domestic sheep.

The presence of two major mitochondrial lineages in cattle (Kikkawa et al. 1995; Loftus et al. 1994a,b) has led to the hypothesis of two independent domestications from two different subspecies of *B. primigenius* which gave rise to *B. taurus* and *B. indicus* cattle (Loftus et al. 1994a). Studies of Asian and European pig breeds have also identified two very distinct mtDNA lineages which refer to the dual maternal origin (*Sus vitattus* and *S. scrofa)* of modern breeds (Watanabe et al. 1986). Our results provide evidence for a dual maternal origin in an additional major farm animal species. The mtDNA polymorphisms and control region sequences of wild and domestic sheep described in this investigation provide a basis for further studies with an expanded number of individuals from domestic and wild sheep of Western Europe, Turkey, Iran, and central Asia. Such an investigation could provide sufficient genetic data to trace the origins of modern sheep breeds and allow the identification of wild ancestors. Finally, additional mitochondrial control region sequences from wild sheep populations could be used to clarify the systematic

a

Ovis musimon

```
Repeat
  I   GTACATAGTATTAATGTAATATAGACATTATATGTATAAAGTACATTAAATGATTTACCTCATGCATATAAGCAC
  II  ..................................................C.....................T
  III ..................................................C.....................T
  IV  ..................................................C.....................T
  V   ..................C.................................C.....................T
```

Ovis aries (European type)

```
Repeat
  I   GTACATAGTATTAATGTAATATAGACATTATATGTATAAAGTACATTAAATGATTTACCTCATGCATATAAGTAC
  II  ..................................................C............C..
  III .C................C.................................................C..
  IV  ..................................................C............C.T
```

Ovis aries (Asian type)

```
Repeat
  I   GTACATAACATTAATGTAATATAGACATTATATGTATAAAGTACATTAAATGATTTGCCTCATGCGTATAAGCAC
  II  ..................................................C..............
  III ..................................................A............G...T
```

Ovis vignei bochariensis

```
Repeat
  I   GTACATAGCATTAATGTAATATAGACATTATATGTATAAAGTACATTAAATGATTTACCTCATGCATATAAGCAT
  II  .......AT..........................................................
  III .......AT..........................................................
  IV  ........T..........................................C...............
```

b

```
a GTACATAGTATTAATGTAATACAGACATTATATGTATAAAGTACATTAAATGATTTACCCCATGCATATAAGCAT
b .....................T.....................................................
c .......AC............T.....................................T.....G....G....
d .....................T.....................................................
```

Figure 4. Comparison of the tandem repeat regions of *O. musimon, O. aries* (European type), *O. aries* (Asian type), and *O. vignei bochariensis*: **(a)** sequence comparison of the different repeats within individual mitochondrial control regions; **(b)** sequence comparison of the last repeat of each of the four different control regions. a: *O. musimon,* b: *O. aries* (European type), c: *O. aries* (Asian type), and d: *O. vignei bochariensis.*

status and nomenclature of the genus *Ovis.*

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