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Hybrid origin of the Pliocene ancestor of wild goats

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Abstract

Recent theories on speciation suggest that interspecific hybridization is an important mechanism for explaining adaptive radiation. According to this view, hybridization can promote the rapid transfer of adaptations between different species; the hybrid population thus invades new habitats and diversifies into a variety of new species. Although hybridization is well accepted as a fairly common mechanism for diversification in plants, its role in the evolution of animals is more controversial, because reduced fitness would typically condemn animal hybrids to an evolutionary dead-end. Here, we examine DNA sequences of four mitochondrial and four nuclear genes selected for resolving phylogenetic relationships between goats, sheep, and their allies. Our analyses provide evidence of strong discordance for the position of *Capra* between mitochondrial and nuclear phylogenies. We suggest that the common ancestor of wild goats arose from interspecific hybridization, and that the mitochondrial genome of a species better adapted to life at high altitudes was transferred via this route into the common ancestor of *Capra*. We propose that the acquisition of more efficient mitochondria has conferred a selective advantage on goats, allowing their rapid adaptive radiation during the Plio–Pleistocene epoch. Our study therefore agrees with theories that predict an important role for interspecific hybridization in the evolution and diversification of animal species.

Keywords: Hybridization; Introgression; Gene tree; Adaptation; Radiation; *Capra*; Pliocene

1. Introduction

Adaptive radiation occurs when a single ancestor diverges rapidly into an array of species inhabiting a variety of environments and using various morphological, physiological, and behavioral traits to exploit these environments (Schluter, 2000). Several factors may facilitate adaptive radiation, including release from competition in an underutilized environment (e.g., new island, new lake) and key evolutionary innovations opening up access to an entirely new range of resources (e.g., bird wings, tetrapod lungs) (Schluter, 2000; Seehausen, 2004). Recent theories on speciation suggest that interspecific hybridization is an important mechanism for explaining adaptive radiation (Seehausen, 2004). According to this view, hybridization can promote the rapid transfer of adaptations between different species; the hybrid population thus invades new habitats and diversifies into a variety of new species. Although hybridization is well accepted as a fairly common mechanism for diversification in plants (Rieseberg et al., 2003; Arnold, 2004), its role in the evolution of animals is more controversial. Whereas some authors consider that hybridization may cause rapid genetic variation likely to promote adaptive evolution and speciation (Arnold, 1997; Seehausen, 2004), others argue against such a significant role, because reduced fitness would typically condemn hybrids to an evolutionary dead-end (Arnold, 1997; Burke and Arnold, 2001). Our study suggests, however, that hybridization played a crucial role in the origin and diversification of wild goats.

The genus *Capra* includes the domesticated goat (*C. hircus*) and eight species of wild goats, which inhabit most

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mountains of Eurasia, North Africa and Arabia (Shackleton, 1997) (Fig. 1). The earliest remains of *Capra* have been found in the middle Pleistocene of Europe, but paleontologists consider that the genus originates from the Pliocene of Asia (Crégut-Bonnoure, 1992; Fedosenko and Blank, 2001). All previous molecular studies were exclusively or mainly based on mitochondrial sequence analyses (Gatesy et al., 1997; Hassanin et al., 1998a; Ludwig and Fischer, 1998; Manceau et al., 1999; Ropiquet and Hassanin, 2005a,b). They have indicated close affinities between goats and the Himalayan tahr (*Hemitragus jemlahicus*), and have suggested that species of *Capra* rapidly radiated during the Plio–Pleistocene epoch.

Here, we analyze DNA sequences of eight molecular markers, yielding a total of 5912 characters, and including four mitochondrial genes (*12S, CO2*, *Cyb*, and *ND1*) and four nuclear gene segments (*κCas*, *PRKCI*, *SPTBN1*, and *TG*). Our phylogenetic analyses reveal a conflicting position for *Capra* between the mitochondrial and nuclear trees. Mitochondrial genes indicate that *Capra* and *Hemitragus* are closely related, confirming previous molecular investigations (Gatesy et al., 1997; Hassanin et al., 1998a; Ludwig and Fischer, 1998; Manceau et al., 1999; Ropiquet and Hassanin, 2005a,b). By contrast, nuclear genes show that *Capra* is the sister group of a clade containing two biogeographical groups: the first one includes *Hemitragus* (Himalayan tahr) and *Pseudois* (bharals), two genera found in the high mountains of Central Asia, and the second unites *Ammotragus* (aoudad) and *Arabitragus* (Arabian tahr), two genera distributed in the arid mountains of North Africa and Arabia, respectively. Our interpretation is that the analyses of mitochondrial sequences result in a misleading phylogenetic pattern, as the mitochondria of proto-*Hemitragus* have been transferred into the common ancestor of *Capra*. We suggest that the acquisition of more efficient mitochondria has conferred a selective advantage on goats, allowing their rapid adaptive radiation during the Plio–Pleistocene epoch.

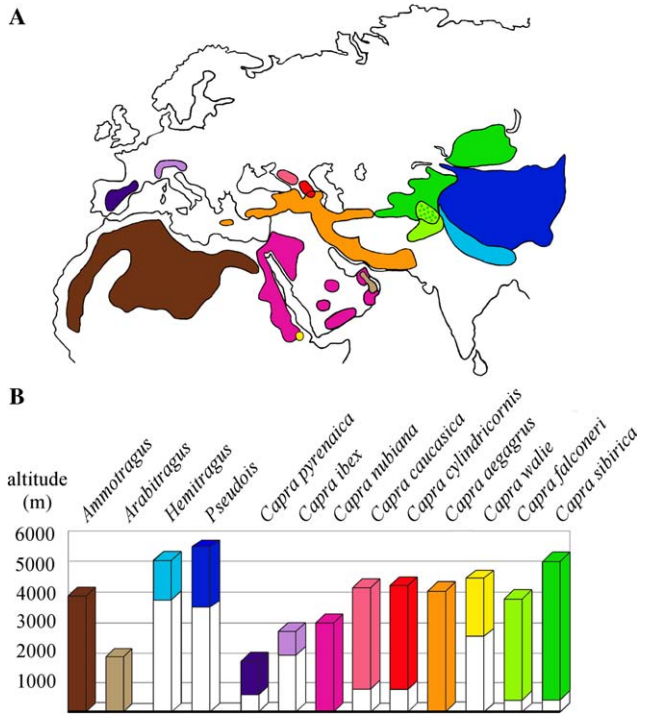

Fig. 1. Geographic (A) and altitudinal (B) distributions of *Capra*, *Ammotragus*, *Arabitragus*, *Hemitragus*, and *Pseudois*. All species of wild goats are indicated in the figure: from west to east, *C. pyrenaica* (Spanish ibex), *C. ibex* (Alpine ibex), *C. nubiana* (Nubian ibex), *C. caucasica* (West Caucasian tur), *C. cylindricornis* (East Caucasian tur), *C. aegagrus* (Bezoar goat), *C. walie* (Walia ibex), *C. falconeri* (Markhor), and *C. sibirica* (Siberian ibex).

2. Materials and methods

2.1. Taxonomic sample

Previous molecular studies have shown that all caprine species can be classed in the tribe Caprini *sensu lato*, a monophyletic group containing 13 genera (Hassanin et al., 1998a; Ropiquet and Hassanin, 2005a,b). In this study, the taxonomic sample includes 18 caprine species, with at least one member for each of the 13 genera (Table 1). The goats are represented by four species here, covering most of the geographic distribution of the genus *Capra* (Shackleton, 1997) (Fig. 1A): *C. ibex* (Alpine ibex) in Western Europe, *C. nubiana* (Nubian ibex) in North Africa, *C. falconeri* (Markhor) in the South-West Asia, and *C. sibirica* (Siberian ibex) in Central Asia. The outgroup genera include *Muntiacus* (Cervidae), *Bos* (Bovidae, Bovinae), and three members of the subfamily Antilopinae—*Aepyceros* (Aepycerotini), *Damaliscus* (Alcelaphini), and *Hippotragus* (Hippotragini) (Ropiquet and Hassanin, 2005a,b).

2.2. DNA sequences

Eight molecular markers were analyzed, including four mitochondrial genes—*12S rRNA* (958 nt in *Ovis aries*), subunit II of the cytochrome oxidase (*CO2*, 582 nt), cytochrome *b* (*Cyb*, 1140 nt), and subunit I of the NADH deshydrogenase (*ND1*, 1008 nt)—and four nuclear gene segments—exon 4 of the κ-casein (*κCas*, 406 nt in *O. aries*), intron 1 of the protein kinase C iota gene (*PRKCI*, 513 nt in *O. aries*), intron 1 of the β-spectrin nonerythrocytic 1 gene (*SPTBN1*, 576 nt in *O. aries*), and intron and exon regions of the thyroglobulin gene (*TG*, 814 nt in *O. aries*). The *κCas* and *PRKCI* nuclear markers were chosen because most sequences were already available in the nucleotide databases (Table 1). The two other nuclear markers, i.e., *SPTBN1* and *TG*, were chosen because the sequences produced by Matthee et al. (2001) for *Capra hircus*, *Ovibos moschatus*, and *O. aries*, have revealed potentially interesting indels (insertions and deletions). The four nuclear gene segments are located on different human chromosomes: 4 for *κCas*, 3 for *PRKCI*, 2 for *SPTBN1*, and 8 for *TG*. The genomic data available for the family Bovidae indicate that at least three of these four genes are also located in different chromosomes in *Bos taurus* and/or *C. hircus*: *κCas* is found in the chromosome 6 (6q32 for both *Bos* and

Table 1
Origin of the sequences

Species	Collection reference	12*S*	*CO2*	*Cyb*	*ND1*	*κCas*	*SPTBN1*	*PRKCI*	*TG*
Muntiacus reevesi		NC_004069[1]	NC_004069[1]	NC_004069[1]	NC_004069[1]	U37509[12]	AF165678[11]	AF165677[11]	AF165682[11]
Bos indicus		NC_005971[2]	NC_005971[2]	NC_005971[2]	NC_005971[2]	AY367769[13]	AF165718[11]	AF165717[11]	AF165722[11]
Aepyceros melampus	PhC 20, SSM, MNHN	M86496[9]	AY689194[3]	AF036289[5]	DQ236320*	AY121998[14]	AF165782[11]	AF165781[11]	AF165786[11]
Damaliscus pygargus	DDV1, F. Claro, Vincennes Zoo, MNHN	M86499[9]	AY689195[3]	AF036287[5]	DQ236321*	AY122002[14]	DQ236280*	AY846794[4]	AF165778[11]
Hippotragus niger	HNV1, F. Claro, Vincennes Zoo, MNHN	AY670653[7]	AY846771[4]	AF036285[5]	DQ236322*	AY122001[14]	DQ236281*	AY846795[4]	AF165746[11]
Ammotragus lervia	ZA 0034, Vincennes Zoo, MNHN	AY670654[7]	AY846772[4]	AF034731[6]	DQ236323*	AY670670[7]	DQ236282*	AY846803[4]	DQ236302*
Buborcas taxicolor	CG 1902-409, MNHN	AY670655[7]	AY846773[4]	AY669320[7]	DQ236324*	AY670671[7]	DQ236283*	AY846811[4]	DQ236303*
Capra falconeri	Cyto 01-214, MNHN	AY670656[7]	AY846774[4]	AF034736[6]	DQ236325*	AY670672[7]	DQ236284*	AY846797[4]	DQ236304*
Capra ibex	Cyto 02-037, MNHN	AY846815[4]	AY846775[4]	AF034735[6]	DQ236326*	AF525023[15]	DQ236285*	AY846798[4]	DQ236305*
Capra nubiana	J.L. Berthier, Ménagerie, MNHN	AY670657[7]	AY846776[4]	AF034740[6]	DQ236327*	AY670673[7]	DQ236286*	AY846799[4]	DQ236306*
Capra sibirica	J.L. Berthier, Ménagerie, MNHN	AY670658[7]	AY846777[4]	AF034734[6]	DQ236328*	AY670674[7]	DQ236287*	AY846800[4]	DQ236307*
Hemitragus jemlahicus	J.L. Berthier, Ménagerie, MNHN	AY670659[7]	AY846780[4]	AF034733[6]	DQ236329*	AY670675[7]	DQ236288*	AY846801[4]	DQ236308*
Arabitragus jayakari	J.M. Mwanzia, HH Sheikh Zayed – Private Department - UAE	AY846816[4]	AY846779[4]	AY846791[4]	DQ236330*	DQ236300*	DQ236289*	AY846804[4]	DQ236309*
Nilgiritragus hylocrius	CG 1935-402, MNHN	AY846817[4]	AY846778[4]	AY846792[4]	DQ236331*	DQ236301*	DQ236290*	AY846808[4]	DQ236310*
Naemorhedus sumatraensis	CG 1993-4240, MNHN	AY670660[7]	AY846781[4]	AY669321[7]	DQ236332*°	AY670676[7]	DQ236291*	AY846812[4]	DQ236311*
Oreamnos americanus	Cyto 02-547, MNHN	AY670661[7]	AY846782[4]	AF190632[8]	DQ236333*	AY670677[7]	DQ236292*	AY846814[4]	DQ236312*
Ovibos moschatus	M98105, P.S. Barboza - Alaska	AY670662[7]	AY846783[4]	AY669322[7]	DQ236334*	AY670678[7]	DQ236293*	AY846813[4]	DQ236313*
Ovis aries	JCT1, J.C. Thibault – Corsica, France	AY670663[7]	AY846785[4]	AF034730[6]	DQ236335*	AY670679[7]	DQ236294*	AY846806[4]	DQ236314*
Ovis dalli	CG 1938-124, MNHN	AY670664[7]	AY846786[4]	AF034728[6]	DQ236336*	AY670680[7]	DQ236295*	AY846807[4]	DQ236315*
Pantholops hodgsonii	CG 1993-4237, MNHN	AF400659[10]	AY846787[4]	AF034724[6]	DQ236340*	AY670681[7]	DQ236299*	AY846796[4]	DQ236319*
Pseudois nayaur	M9407, JL Berthier, Ménagerie, MNHN	AY670665[7]	AY846788[4]	AF034732[6]	DQ236337*	AY670682[7]	DQ236296*	AY846802[4]	DQ236316*
Rupicapra pyrenaica	ONF, B. Guffond, Pyrénées, France	AY846818[4]	AY846789[4]	AF034726[6]	DQ236338*	DQ236341*	DQ236297*	AY846810[4]	DQ236318*
Rupicapra rupicapra	Cyto 01-175, MNHN	AY670666[7]	AY846790[4]	AF034725[6]	DQ236339*	D32182[16]	DQ236298*	AY846809[4]	DQ236317*

*Present study; [1]Zhang et al. Unpublished; [2]Miretti et al. Unpublished; [3]Hassanin and Ropiquet (2004); [4]Ropiquet and Hassanin (2005b); [5]Hassanin and Douzery (1999a); [6]Hassanin et al. (1998a); [7]Ropiquet and Hassanin (2005a); [8]Hassanin and Douzery (2000); [9]Allard et al. (1992); [10]Kuznetsova and Kholodova (2002); [11]Matthee et al. (2001); [12]Cronin et al. (1996); [13]Aravindakshan and James Unpublished; [14]Hassanin and Douzery (2003); [15]Jann et al. (2004); [16]Chikuni et al. (1995); °*Naemorhedus crispus* Cyto 01-154.

Capra); *PRKCI* in the chromosome 1 of *Bos* (1q34-q36); and *TG* in the chromosome 14 (14q13dist for *Bos*, and 14q15 for *Capra*). In bovids, the location of *SPTBN1* is unknown. According to these data, we can therefore assume that at least three of the four nuclear gene segments used for this study are unlinked phylogenetic markers.

All DNA samples were extracted as indicated in previous studies (Hassanin et al., 1998a; Hassanin and Douzery, 1999a,b; Hassanin and Douzery, 2003; Hassanin and Ropiquet, 2004; Ropiquet and Hassanin, 2005a,b). The standard PCR conditions were as follows: 3 min at 94 °C; 30 cycles of denaturation/annealing/extension with 1 min at 94 °C for denaturation, 1 min at 55 °C for annealing, and 1 min at 72 °C for extension; and 7 min at 72 °C. The new sequences of *κCas* were obtained using primers previously published by Ropiquet and Hassanin (2005a). By contrast, new sets of primers were used for amplifying and sequencing the three following markers: *ND1* (5′-GTG-GCA-GAG-CCC-GGT-AAT-TG-3′ and 5′-TTA-CTC-TAT-CAA-AGT-AAC-TC-3′), *SPTBN1* (5′-AGT-GCA-GCC-TTG-AAA-GGT-AC-3′ and 5′-GGC-AAA-GTC-TTG-GTA-ACA-GA-3′) and *TG* (5′-GAG-CCC-AAG-AAA-TGT-GAG-TC-3′ and 5′-CCA-GCA-CTG-TTC-TGA-GCC-TC-3′). The sequences were obtained by double-strand DNA cycle sequencing with a CEQ2000 Dye terminator cycle Sequencing Quick Start kit in a CEQ2000 Beckman (v4.3.9) sequencer. The resulting output was edited using Sequencher 4.5 (Gene Codes, Ann Arbor, Michigan). Sequences generated for this study are available from the GenBank/EMBL/DDBJ databases under Accession Nos. DQ236280-DQ236341 (Table 1).

2.3. Phylogenetic analyses

DNA alignments were performed with Sequence Alignment Editor Version 2.0 alpha 11 (Andrew Rambaut, software available at http://evolve.zoo.ox.ac.uk/). The regions with ambiguity in the position of the gaps were excluded from the analyses to avoid erroneous hypotheses of primary homology. Unambiguous indels for DNA alignment were coded as binary characters. Phylogenetic analyses were performed on the mitochondrial and nuclear datasets, and on each of the eight markers separately, by using Maximum Parsimony (MP) and Bayesian methods.

The MP analyses were conducted on PAUP 3.1.1 (Swofford, 1993) with differential weighting of the character-state transformations using the product CIex. S (CIex: consistency index excluding uninformative characters, S: slope of saturation) as detailed in Hassanin et al. (1998a,b): for each substitution-type of each marker (i.e., A-G, C-T, A-C, A-T, C-G, G-T, and indels), the amount of homoplasy was measured through the CIex, and the saturation was assessed graphically by plotting the pairwise number of observed differences against the corresponding pairwise number of inferred substitutions calculated by PAUP (the slope of the linear regression [S] was used to evaluate the level of saturation). Bootstrap percentages (BP_{MP}) were computed after 1000 replicates.

Bayesian inferences used MrBayes 3.1 (Huelsenbeck and Ronquist, 2001) by applying the model of sequence evolution selected by MrModeltest 2.2 (Nylander, 2004). These models are GTR+I+Γ for 12*S* and *Cyb*, GTR+I for *κCas*, HKY+I+Γ for *CO2* and *ND1*, HKY+Γ for *PRKCI*, K80+Γ for *SPTBN1* and *TG*, and GTR+I+Γ for the concatenated mt and nuclear datasets. Unambiguous indels were coded as binary characters, and analyzed using the parsimony options. Five Markov chains were run for 1,000,000 generations and sampled every 100 generations after an initial burn-in period of 10,000 cycles. The node robustness was estimated, first, by the Bayesian posterior probabilities (PP), and second, by the Bayesian Bootstrap percentages (BP_B). For the Bayesian bootstrap analysis, 100 pseudoreplicates of the matrix were first created using SEQBOOT 3.5c (Felsenstein, 2004), and the values were obtained by constructing the consensus of the 100 Bayesian trees with CONSENSE 3.5c (Felsenstein, 2004).

2.4. Molecular dating

Divergence times were calculated using the relaxed Bayesian molecular clock method implemented in Multidivtime (Thorne and Kishino, 2002). The expected number *a priori* of time units between tip and root (rttm) was set at 30 MYA, with a standard deviation of 15 MYA. The Markov chains were sampled 10,000 times every 100 generations, and the burn-in period was set at 100,000 generations. Three calibration points were used for the analyses: the first corresponds to the emergence of the family Bovidae in the fossil record, i.e., between 18 and 20 MYA (Vrba and Schaller, 2000), the second is the oldest fossil attributed to the genus *Ovis*, i.e., between 2 and 3 MYA (Mead and Taylor, 2005) and the third refers to the first appearance of the genus *Rupicapra*, i.e., between 0.35 and 1.5 MYA (Masini and Lovari, 1988).

3. Results

3.1. Discordant positions for Capra between the mitochondrial and nuclear trees

The four mitochondrial genes (*12S, CO2*, *Cyb*, and *ND1*) were analyzed separately or in combination (Table 2 and Fig. 2A). The mitochondrial tree agrees with the monophyly of the tribe Caprini *sensu lato* (Hassanin and Douzery, 1999a), the basal divergence of *Pantholops* (Tibetan antelope), the monophyly of the genera *Rupicapra* (chamois and isard) and *Ovis* (domestic and Dall sheep), the sister-group relationships between *Ovis* and *Nilgiritragus*, and the association of *Naemorhedus* (serow) with *Ovibos* (muskox). All these nodes are strongly supported by the Bayesian posterior probability (PP = 1) and by the Bootstrap percentages obtained with either Bayesian method ($BP_B \geqslant 99$) or Maximum Parsimony ($BP_{MP} \geqslant 98$) (see details in Table 2). In addition, they are also strongly supported by the analyses based on the combination of the four nuclear gene

Table 2
Robustness of the nodes estimated with the Bayesian posterior probability (PP) and Bootstrap proportion (BP)

	Mitochondrial DNA											Nuclear DNA									
	Combined			*12S*		*CO2*		*Cyb*		*ND1*		Combined			*κCas*		*PRKCI*		*SPTBN1*		*TG*	
Nodes	PP	BP_B	BP_{MP}	PP	BP_{MP}	PP	BP_{MP}	PP	BP_{MP}	PP	BP_{MP}	PP	BP_B	BP_{MP}	PP	BP_{MP}	PP	BP_{MP}	PP	BP_{MP}	PP	BP_{MP}
Caprini *sensu lato*	1.00	100	100	0.95	69	1.00	65	1.00	84	1.00	81	1.00	95	96	0.87	60	0.96	73	-	-	0.50	-
R.pyrenaica… C.nubiana	1.00	99	98	0.98	81	0.88	37	-	-	1.00	82	1.00	97	98	0.90	50	0.92	52	1.00	97	-	-
Rupicapra	1.00	100	100	1.00	100	1.00	100	1.00	100	1.00	100	1.00	100	100	1.00	94	1.00	95	0.98	73	1.00	95
Naemorhedus + Ovibos	1.00	100	100	1.00	98	1.00	100	1.00	97	0.98	99	1.00	86	96	-	-	-	-	1.00	94	0.97	43
Nilgiritragus + Ovis	1.00	100	100	1.00	99	1.00	77	1.00	99	1.00	91	1.00	100	100	0.93	59	0.84	57	1.00	97	0.77	60
Ovis	1.00	99	100	1.00	79	1.00	87	1.00	99	0.48	91	1.00	94	93	0.87	-	0.91	67	-	-	0.99	86
Ammotragus + Arabitragus + Capra + Hemitragus + Pseudois	-	-	25	0.25	-	-	-	-	-	#	-	1.00	78	85	-	-	0.99	80	0.94	63	-	-
Ammotragus + Arabitragus	1.00	99	98	0.91	41	1.00	72	0.33	33	0.99	83	0.79	72	63	-	-	0.40	48	-	-	-	-
Capra	-	51	-	0.96	62	-	57	-	-	0.84	45	1.00	91	88	0.30	32	-	-	-	-	0.30	-
C.ibex… C.nubiana	1.00	100	99	1.00	86	-	35	1.00	90	0.98	80	0.36	39	25	-	-	-	-	-	-	-	-
C falconeri + C nubiana	-	-	-	-	-	-	-	0.79	51	-	-	0.87	63	78	1.00	87	-	-	-	-	-	-
Capra + Hemitragus + Pseudois	**1.00**	**96**	**93**	**0.42**	**-**	**-**	**-**	**0.89**	**54**	**-**	**53**	#	#	#	#	#	**-**	**-**	#	#	#	#
Capra + Hemitragus	**1.00**	**100**	**100**	**0.99**	**67**	**0.84**	**70**	**1.00**	**88**	**1.00**	**83**	#	#	#	#	#	**-**	**-**	#	#	#	#
Ammotragus + Arabitragus + Hemitragus + Pseudois	#	#	#	#	-	-	#	#	#	#	#	**1.00**	**100**	**100**	**1.00**	**84**	**-**	**-**	**1.00**	**74**	**1.00**	**76**
Hemitragus + Pseudois	#	#	#	#	-	-	#	#	#	#	#	**0.99**	**93**	**86**	**-**	**-**	**-**	**-**	**0.93**	**64**	**0.85**	**60**

BP_B: Bootstrap proportion obtained from the Bayesian analysis. BP_{MP}: Bootstrap proportion obtained from the Maximum Parsimony analysis. The shading lines highlight the discordance between mitochondrial and nuclear trees. #: the node is not found, and an alternative hypothesis is strongly supported (PP > 0.95 and/or BP > 70). —: the node is not found, but no other hypothesis is strongly supported.

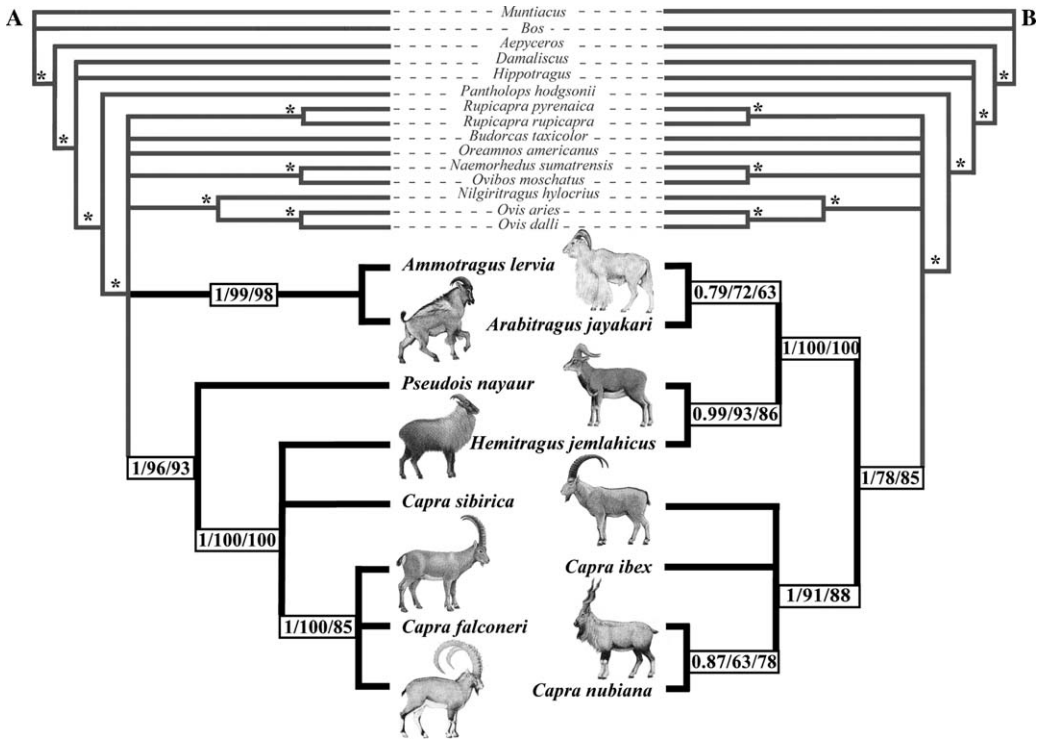

Fig. 2. Phylogenetic relationships between caprine species inferred from the combined analyses of mitochondrial (A) and nuclear (B) genes. For each node, the three values on the branch indicate, from left to right, (1) the Bayesian posterior probability (PP), (2) the Bayesian Bootstrap percentage (BP_B), and (3) the Bootstrap percentage obtained with the Maximum Parsimony method (BP_{MP}). The nodes that were not supported by BP_B and BP_{MP} values superior to 50 are not shown in the figure. Asterisks indicate that the nodes are supported by PP = 1, $BP_B \geqslant 86$ and $BP_{MP} \geqslant 90$ (see Table 2 for details).

segments (*κCas*, *PRKCI*, *SPTBN1*, and *TG*) (Fig. 2B; PP = 1; $BP_B \geqslant 86$; $BP_{MP} \geqslant 93$). The genus *Capra* is found monophyletic in the combined analysis of nuclear genes (Fig. 2B; PP = 1; $BP_B = 91$; $BP_{MP} = 88$), and in some analyses of the mitochondrial genes (12*S*: PP = 0.96; $BP_{MP} = 62$; *CO2*: $BP_{MP} = 57$, ND1: PP = 0.84; $BP_{MP} = 45$; and Bayesian combined analysis: $BP_B = 51$). As previously observed by Hassanin et al. (1998a), the genus *Hemitragus* is grouped with *Capra sibirica* in the analyses of *Cyb* sequences (PP = 0.71; $BP_{MP} = 62$).

Our phylogenetic inferences reveal conflicting positions for *Capra* between the mitochondrial and nuclear trees (Fig. 2). Mitochondrial genes indicate that *Capra* and *Hemitragus* are closely related, confirming previous molecular investigations (Gatesy et al., 1997; Hassanin et al., 1998a; Hassanin and Douzery, 1999a; Ludwig and Fischer, 1998; Manceau et al., 1999; Ropiquet and Hassanin, 2005a,b). This association is strongly supported in the combined analyses of the mitochondrial markers (PP = 1; $BP_{B/MP} = 100$). In addition, it is recovered independently with all mitochondrial genes (*12S*/*CO2*/*Cyb*/*ND1*: PP = 0.99/0.84/1/1; BP_{MP} = 67/70/88/83). By contrast, nuclear genes show that *Capra* is the sister-group of a clade containing the four genera *Ammotragus*, *Arabitragus*, *Hemitragus*, and *Pseudois*. These four latter genera are robustly enclosed together in the combined analyses (PP = 1; $BP_{B/MP} = 100$) as well as in the separate analyses of three nuclear markers (*κCas*/*SPTBN1*/*TG*: PP = 1/1/1; $BP_{MP} = 84/74/76$). In addition, they share a unique deletion of seven nucleotides in the *SPTBN1* gene.

3.2. Paralogy, incomplete lineage sorting, or interspecific hybridization?

In theory, three main biological processes can produce discordant gene trees: paralogy, incomplete lineage sorting, and hybridization (Sang and Zhong, 2000; Funk and Omland, 2003; Hudson and Turelli, 2003) (Fig. 3).

Phylogenetic analyses using paralogous sequences may be misinterpreted if the orthology of the nuclear alleles is erroneously assumed. Orthologous genes derive from the same locus, whereas paralogous genes derive from different loci that originated by a gene duplication event (Funk and Omland, 2003). In Fig. 3A, we illustrate how the occurrence of two nuclear paralogous sequences in *Capra* may explain the discordance evidenced between nuclear and mitochondrial phylogenies. Obviously, this hypothesis can be ruled out, because it would imply that the same complex evolutionary scenario, involving one ancestral gene duplication event followed by four gene deletions, has occurred at least three times independently, i.e., in the three unlinked nuclear gene segments that do not agree with the mtDNA topology (*κCas*, *SPTBN1*, and *TG*).

Nuclear mitochondrial pseudogenes or 'Numts' are segments of mtDNA translocated to the nuclear genome. As these paralogous sequences are commonly found in animal genomes, the use of PCR without prior purification of mtDNA can lead to accidental amplification of Numts (Lopez et al., 1997; Bensasson et al., 2001). The undetected presence of Numts in the analyses can result in erroneous interpretations of phylogenetic relationships, because Numts

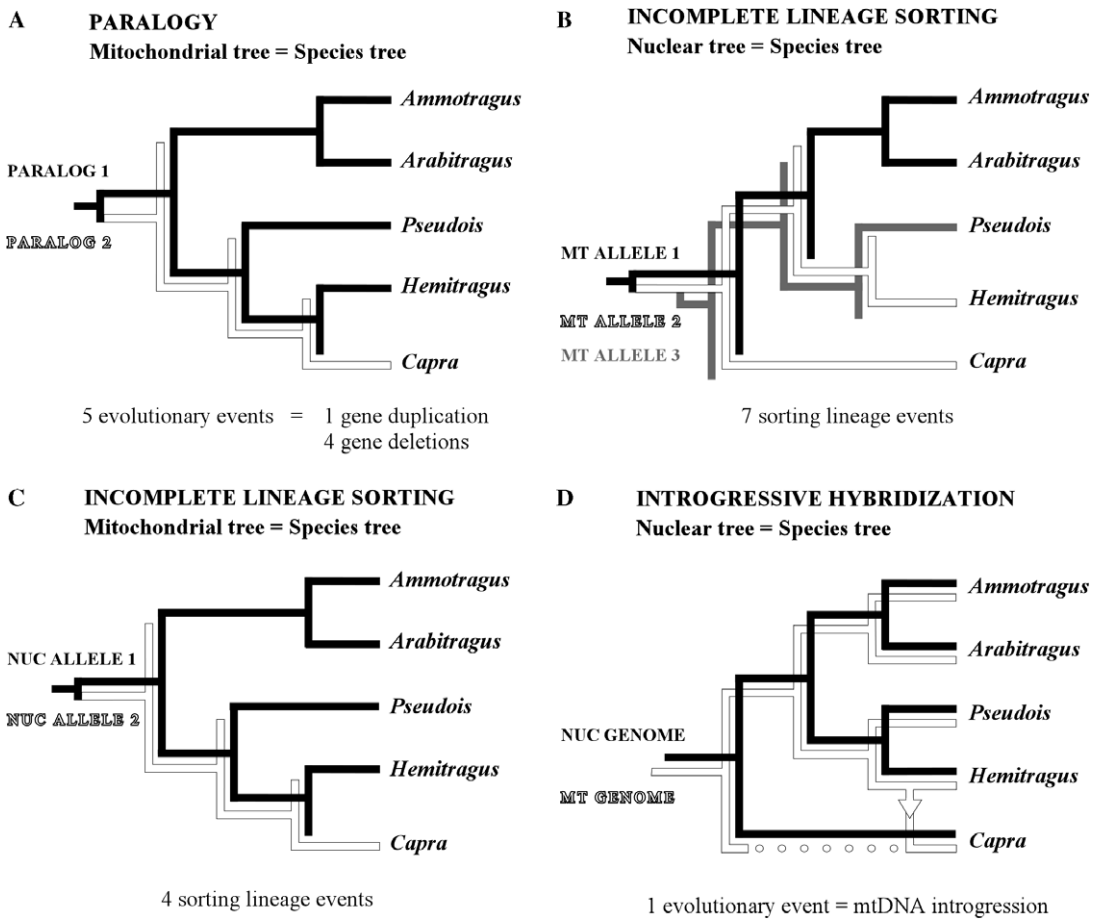

Fig. 3. Four hypotheses explaining the mitonuclear discordance for the position *Capra*. (A) Paralogy; (B) Incomplete lineage sorting of mitochondrial alleles; (C) Incomplete lineage sorting of nuclear alleles; (D) Introgressive hybridization between *Hemitragus* and the common ancestor of *Capra*.

evolve under constraints that are different than those of mtDNA: the nuclear and mtDNA genomes have different rates and patterns of mutations; and, because Numts are not functional, they do not evolve under purifying selection, explaining why their substitution rates are equal with respect to codon position, and why they readily accumulate stop codon and frameshift mutations. Three main arguments suggest that the association of *Hemitragus* and *Capra* with mt sequences is not due to the presence of Numts: (1) while all the four mt makers, i.e., 12*S*, *CO2*, *Cyb*, and *ND1*, were amplified and sequenced independently, they have produced similar topologies (Table 2), where *Hemitragus* and *Capra* are closely related; (2) all the three mt protein-coding genes (*CO2*, *Cyb*, and *ND1*) do not exhibit stop codon or frameshift mutations; and (3) our *Cyb* and 12*S* sequences of *Ammotragus*, *Capra*, *Hemitragus*, and *Pseudois*, are very similar to those published elsewhere and generated with other primers (Groves and Shields, 1996; Ludwig and Fischer, 1998; Manceau et al., 1999; Kuznetsova and Kholodova, 2002; Cao et al., 2004).

The incomplete sorting of ancestrally polymorphic allelic lineages represents another potential source of incongruence between mitochondrial and nuclear trees (Sang and Zhong, 2000; Funk and Omland, 2003; Hudson and Turelli, 2003). When two or more populations become separated and gene flow ceases, stochastic extinction of alleles will occur in the daughter populations, until all but one parental allele become extinct. The amount of time required for a neutral allele to become fixed in a daughter population (i.e., for lineage sorting to go to completion) depends on the effective population size (Ne). After 4Ne generations of population isolation, it is highly probable that lineage sorting of neutral nuclear alleles will have gone to completion and the populations will be reciprocally monophyletic (Funk and Omland, 2003; Ballard and Whitlock, 2004).

If lineage sorting of mtDNA may be the cause of the incongruence between our mitochondrial and nuclear trees, we would need to assume a very unlikely scenario, involving that three mt alleles coexisted in the common ancestor of the five goat-like genera (*Capra*, *Ammotragus*, *Arabitragus*, *Pseudois*, and *Hemitragus*), and that seven allelic lineages have been lost independently (Fig. 3B). A second argument for excluding this hypothesis is that incomplete lineage sorting is less of a concern for mitochondrial than for nuclear loci. Indeed, as the mitochondrial genome is haploid and maternally inherited, the Ne is generally smaller than that of nuclear loci, and stochastic lineage sorting is expected to progress more rapidly for mitochondrial alleles (Ballard and Whitlock, 2004).

Alternatively, lineage sorting of nuclear alleles is another possibility for explaining the discordant positions for *Capra*

between mitochondrial and nuclear trees (Fig. 3C). Assuming that the mitochondrial tree represents the species tree, this hypothesis would imply that two nuclear alleles arose in the common ancestor of the five goat-like genera, and that, subsequently, one allele has been lost in the common ancestor of *Capra*, whereas the other allele has been lost independently in the three branches leading to *Hemitragus*, *Pseudois*, and the common ancestor of *Ammotragus* and *Arabitragus*. Although this scenario could be inferred for one nuclear gene only, it seems really improbable that it may have occurred identically and independently in the three unlinked nuclear segments *κCas*, *SPTBN1*, and *TG*. For this reason, we consider that this hypothesis cannot be retained.

Interspecific hybridization is the most likely hypothesis for explaining the conflicting positions for *Capra* between the mitochondrial and nuclear gene trees.

The most common result of interspecific hybridization is "introgression", the transfer of foreign genetic material between hybridizing taxa via backcrossing (Arnold, 1997). In our analyses, the hypothesis of introgressive hybridization is highly supported by the fact that it involves only one evolutionary event corresponding to the transfer of the mitochondrial genome of proto-*Hemitragus* into the common ancestor of *Capra* (Fig. 3D). This scenario is also corroborated by the fact that the mtDNA is known to be particularly susceptible to the effects of introgression (Ballard and Whitlock, 2004). This hypothesis implies that a misleading phylogenetic pattern is given by the mitochondrial genes, and that the species tree is in fact given by the nuclear genes, which show that *Capra* is the sister-group of a clade containing two biogeographical groups (Fig. 1B): the first one includes *Hemitragus* and *Pseudois*, two genera found at high elevations in the rugged mountains of Central Asia, and the second unites *Ammotragus* and *Arabitragus*, two genera distributed in the rocky, arid mountains of North Africa and Arabia, respectively.

4. Discussion

4.1. Ancient mtDNA introgression in the common ancestor of wild goats

Here, the mitonuclear discordance for the position of *Capra* reveals that the mitochondrial genome of proto-*Hemitragus* was transferred into the common ancestor of wild goats. Other cases of mtDNA introgression have been previously reported in mammals, but they involved closely related species or subspecies, and were of recent origin. For instance, analyses of mitochondrial and nuclear markers in African elephants (Roca et al., 2005) and macaques (Tosi et al., 2003) have produced convincing evidence of mtDNA introgression. Compared with previous cases of introgression, the introgressive hybridization evidenced in this study is exceptional for three reasons. First, it implicates two divergent genera, *Capra* and *Hemitragus*, which show important morphological, ethological, biogeographical, molecular, and cytogenetic differences. In particular, all species of *Capra* have $2n = 60$ chromosomes, while *Hemitragus* has only $2n = 48$ chromosomes (Bunch and Nadler, 1980). Second, the evolutionary event of hybridization is ancient, as it occurred in the common ancestor of wild goats, that is, during the Pliocene epoch according to our molecular date estimates (between 2.1 and 6.6 MYA with nuclear data, and between 2.6 and 4.5 MYA with mtDNA; Table 3). Third, the introgression was followed by the diversification of *Capra* into an array of species during the Plio–Pleistocene.

Table 3
Divergence times estimates (expressed in million years ago)

Nodes	Mitochondrial DNA	Nuclear DNA
Caprini sensu lato	7.85–11.17	7.14–12.92
Capra + *Hemitragus* + *Pseudois* + *Ammotragus* + *Arabitragus*	5.10–7.75	3.71–8.51
Ammotragus + *Arabitragus*	3.59–5.90	1.58–5.58
Capra + *Hemitragus* + *Pseudois*	4.02–6.52	
Capra + *Hemitragus*	2.60–4.48	
Hemitragus + *Pseudois*		1.21–4.97
Hemitragus + *Pseudois* + *Ammotragus* + *Arabitragus*		2.00–6.21
Capra		2.08–6.62

4.2. Sex-biased gene flow from Hemitragus to Capra

The mitochondrial introgression was not accompanied by apparent nuclear introgression because none of the four nuclear genes analyzed for this study agrees to group *Hemitragus* with *Capra*. Although undetected nuclear alleles of *Hemitragus* may have persisted in the goat genomes, these data confirm that the maternally inherited mtDNA introgresses between species more rapidly than nuclear genes (Chan and Levin, 2005; Llopart et al., 2005). The reasons for more rapid mtDNA introgression are not clearly understood (Ballard and Whitlock, 2004; Chan and Levin, 2005; Llopart et al., 2005), but a sex-biased gene flow from proto-*Hemitragus* to proto-*Capra* can explain the observed pattern. In fact, we suggest that hybridization was unidirectional and sexually asymmetric, and took place as follows: the first generation of cross-mating occurred between proto-*Hemitragus* females and proto-*Capra* males, and produced fertile hybrid females and sterile hybrid males; each subsequent backcrossing of hybrid females with proto-*Capra* males may have diluted the proportion of tahr nuclear alleles by half, until the populations had overwhelmingly goat nuclear alleles whilst retaining the maternally-inherited mtDNA genome of proto-*Hemitragus*. This hypothesis is supported by the fact that hybrid males had potentially lower fitness than hybrid females. Genetic studies on natural and experimental populations have indeed shown that hybrids of the heterogametic sex (males XY, in the case of mammals) are more frequently affected by inviability or sterility (Haldane's rule) (Coyne and Orr, 2004). Moreover, sex differences in social and reproductive behavior may have also contributed to the failure of tahr or hybrid males to reproduce successfully with female goats.

In goats and tahrs, the reproductive success of males is directly correlated with body strength and size of horns, as males fight for gaining access to females in estrus (Schaller, 1977). Therefore, tahr and hybrid males may have been easily out-competed by male goats, which have much longer horns.

4.3. Positive selection for mtDNA introgression

Under the oxidative phosphorylation (OXPHOS) process, the mitochondria oxidize metabolic substrates including carbohydrates and fats in order to generate energy and water, with O_2 acting as the terminal acceptor for the electron transport chains (Ballard and Whitlock, 2004). In homeotherms like mammals, the mitochondria play an essential dual role, as the energy released is used to synthesize ATP and maintain body temperature (Wallace, 2005). As a toxic by-product of OXPHOS, the mitochondria generate most of the reactive oxygen species (ROS or oxygen radicals), which are known to damage proteins, lipids, and nucleic acids (Ballard and Whitlock, 2004; Wallace, 2005).

At high altitude, oxygen limitations decrease mitochondrial capacity for OXPHOS, resulting in increased ROS production by the mitochondrial electron transport system (Hoppeler et al., 2003; Gelfi et al., 2004). In addition, it has been shown that low temperatures enhance the production of ROS, as hypothermia disrupts the function of the mitochondrial enzyme-complexes involved in the electron transport system and reduces enzyme-scavenging efficiency (Camara et al., 2004). Consequently, both hypoxia and hypothermia must have imposed considerable selective pressures on mitochondria to maximize their functionality in species evolving at high altitude. In agreement with that, the data available for humans suggest that populations living at high altitude and/or in cold temperatures have developed specific mitochondrial adaptations (Hoppeler et al., 2003; Mishmar et al., 2003; Gelfi et al., 2004; Ruiz-Pesini et al., 2004).

The two genera *Hemitragus* and *Pseudois* are found in the highest mountains of the world, in the Himalayas and on the Tibetan Plateau, respectively (Fig. 1A), where they usually live at altitudes of between 3500 and 5500 m (Fig. 1B). As our nuclear analyses indicate that *Hemitragus* and *Pseudois* are sister-genera (Fig. 2B), it can be inferred that their common ancestor was already adapted for physical activity at high altitude, and that it already lived in the mountains of Central Asia. An evolutionary history of such length in the most extreme environment of the terrestrial world implies that their mitochondria must have been strongly selected for maximizing the OXPHOS efficiency and for minimizing the production of ROS. We suggest, therefore, that the fixation of the mtDNA haplotype of proto-*Hemitragus* into the ancestral population of *Capra* is the result of positive selection, as the acquisition of mitochondria better adapted to life at high altitude has conferred a great selective advantage on goats. As the Himalayan tahr and Siberian ibex (*C. sibirica*) currently share sympatric areas in the western Himalayan region of India (Shackleton, 1997) (Fig. 1A), it is likely that the hybridization between proto-*Hemitragus* and proto-*Capra* took place in this zone. The lack of recombination in the mitochondrial genome and its uniparental (maternal) inheritance may have favored the rapid adaptive selection of the new advantageous mtDNA haplotype. Although the possession of much longer horns in male goats may have been an important morphological advantage for interspecific competition, this characteristic alone does not, however, explain why *Capra* succeeded in colonizing all the mountains in the Palearctic region. Our data suggest that the rapid adaptive radiation of *Capra* benefited from the transfer of "foreign" mitochondria perfectly adapted to physical activity under conditions of hypoxia and hypothermia, as the Plio–Pleistocene epoch was associated with the global change towards cooler, drier and more variable climates, and with the onset of Northern Hemisphere glaciations.

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