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The Genetic Impact of Translocations and Habitat Fragmentation in Chamois (*Rupicapra*) spp.

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Abstract

The chamois is a useful species with which to investigate the combined genetic impact of habitat fragmentation, over hunting, and translocations. Genetic variation within and between chamois (genus *Rupicapra*) populations was analyzed in 259 individuals from 16 sampling sites located in Italy, Spain, Slovakia, and the Czech Republic. Two mitochondrial DNA markers (control region and cytochrome *b*) and 11 nuclear microsatellites were typed. The principal results of this study can be summarized as follows: 1) high and significant differentiation between almost all chamois populations is observed even on a microgeographical scale, probably caused by the patchy distribution of this species, sharp geographical barriers to gene flow, and drift effects related to recent bottlenecks; 2) historical translocation events have left a clear genetic signature, including interspecific hybridization in some Alpine localities; 3) the Apennine subspecies of chamois, *Rupicapra pyrenaica ornata*, shows a high and similar level of divergence (about 1.5 My) from the Pyrenean (*Rupicapra pyrenaica pyrenaica*) and the Alpine (*Rupicapra rupicapra*) chamois; therefore, the specific status of these taxa should be revised. These results confirm the potential of population genetic analyses to dissect and interpret complex patterns of diversity in order to define factors important to conservation and management.

Key words: *conservation*, *hybridization*, *management*, *microsatellites*, *mtDNA*, *taxonomy*

Introduction

In a large-scale landscape, mountains represent islands with specific environmental conditions isolated by various migration filters (Lomolino and Davis 1997; Brown 2001). They usually possess a higher number of nonvolant mammal species than the surrounding lowland and are often considered biodiversity hot spots (Mitchell-Jones et al. 1999). The level of population isolation in alpine areas increases with elevation because valleys separating mountain peaks and lowlands separating mountain ranges represent strong migration barriers. In addition, nonvolant mammal species adapted to alpine habitats are often unable to disperse across large swaths of dense forest (Lomolino and Davis 1997). Isolation may result in low rates of colonization and reduced gene flow between populations of the same species (Brown 2001). These fragmented and fragile habitats are heavily impacted by human activities and are considered to be one of the most sensitive to climate change (Beniston 2006). The chamois is a typical and conspicuous resident of the boreal alpine habitat and therefore was chosen as an appropriate species with which to test the combined impact of anthropic and natural factors affecting the genetic pattern of a medium-sized alpine ungulate.

The chamois (genus *Rupicapra*) is distributed throughout the mountainous areas of Southern Europe, the Balkans, and the Near East, with rocky outcrops and alpine pastures from 1000 to 2500 m above sea level (a.s.l.) representing its typical habitat. This species occasionally descends to lower altitudes (400–500 m a.s.l.) but only into areas characterized by stony ground, ecological heterogeneity, and steep slopes (Tosi and Perco 1981). Low valleys tend to separate populations, although they do not represent absolute

barriers to chamois movements (Loison et al. 1999). However, females show a high level of philopatry to geographical units; consequently, colonization of new areas by this species is rare. Increasingly, human activities along valley floors, especially in the Alps, further reduce natural corridors between suitable habitats.

Chamois have traditionally been classified as a single species, *Rupicapra rupicapra* (family Bovidae, subfamily Caprinae), subdivided into 10 subspecies on the basis of geographical distribution (Lydekker 1913; Couturier 1938; Dolan 1963). More recently, morphological, behavioral, and molecular evidence have indicated that chamois are more appropriately classified as 2 species: the Alpine chamois, *R. rupicapra* (with the following geographically isolated subspecies: *cartusiana*, *rupicapra*, *tatrica*, *carpatica*, *balcanica*, *asiatica*, and *caucasica*), present over a large part of the mountainous regions from the European Alps to the Caucasus and Turkey, and the Pyrenean chamois, *Rupicapra pyrenaica* (with geographically isolated subspecies: *parva*, *pyrenaica*, and *ornata*), that has a discontinuous distribution in southwestern Europe including the Pyrenees, the Cantabrian Mountains, and the central Apennines of Italy (Lovari and Scala 1980; Nascetti et al. 1985; Masini and Lovari 1988; Hammer et al. 1995; Pérez et al. 2002). However, controversy surrounds the taxonomy of this group. Some authors have suggested that *Rupicapra pyrenaica ornata* from Apennines of Central Italy should be considered a third species (Camerano 1914; Pérez et al. 2002), whereas a recent paper by Rodríguez et al. (2009) claims that postglacial recolonization was accompanied by hybridization between the *pyrenaica* and *rupicapra* clades, and conclude, as in the earlier studies, that only one *Rupicapra* species exists.

Several chamois subspecies are included in the IUCN Red list (IUCN 2008). In fact, in the last 2 centuries, over hunting, habitat destruction, urbanization, epidemics, and restocking/reintroduction practices have strongly affected the size and geographic distribution of chamois populations (Roucher 1999; Sfougaris et al. 1999; Jurdíková 2000). As far as genetic variation is concerned, we can reasonably assume that the effects of human activities have been stronger in the chamois compared with other more homogenously distributed species because living in semi-isolated mountain peaks is expected to produce low variation within groups and significant divergence between them as well as highly selected local adaptations (Forbes and Hogg 1999; Maudet et al. 2002; Worley et al. 2004). The possibility of identifying the impact of past reintroduction activities on genetic variability and its geographical distribution are of primary interest when implementing conservation and management strategies (Caughley and Gunn 1996; King and Burke 2000).

In this study, we analyzed the pattern of genetic variation at 2 different mitochondrial DNA (mtDNA) markers and 11 microsatellite loci in 16 chamois populations from across Europe with various demographic and management histories, addressing the following questions. 1) How and to what extent does a patchy distribution and low, male-biased migration affect genetic structure and variation at micro- compared with macrogeographical scales? 2) Are the effects of past translocation programs (see Table 1) genetically detectable? 3) Which taxonomic hypothesis is supported by the genetic information? Answers to these questions have important implications for management policies because taxonomic controversies and lack of detailed genetic information for European populations of *Rupicapra* seriously jeopardize conservation efforts to protect evolutionarily significant units and prevent genetic erosion within this genus.

Materials and Methods

Sampling Sites

Tissue samples (skeletal muscle, blood, or hair) were collected between 2001 and 2004 from 239 alpine chamois (*R. rupicapra*) from 14 different populations belonging to 2 different subspecies (*rupicapra* and *tatrica*). The geographic location and the sizes of each sample are reported in Figure 1 and Table 1, respectively. Three major geographical regions were sampled: the Western Alps (2 populations, named WA1 and WA2), the Eastern Alps (7 populations, EA1–EA7), and a central European region (4 populations from Slovakia and 1 from the Czech Republic, CE1 to CE5). In addition, tissue samples were collected between 2001 and 2003 from 2 putative subspecies of the Pyrenean chamois: *Rupicapra pyrenaica pyrenaica* from several valleys of the eastern Pyrenees (RPP) and *R. p. ornata* from a breeding center in the Abruzzo National Park in the Apennines of Central Italy (RPO).

All samples were classified taxonomically on the basis of their geographic origin. For each population, a brief history of recent translocation events is reported in Table 1. Historical records were used to define "native," "translocated," and "introduced" populations: native populations are those never subjected to documented human-mediated restocking or reintroductions or those that naturally exchange individuals with adjacent populations; translocated populations are those that at some time in the recent past have been restocked with individuals transported by humans from one or more different geographical areas; and introduced populations are those established entirely artificially in areas where chamois were not present previously.

Molecular Analyses

Total genomic DNA was extracted from frozen (–80 °C) or alcohol-preserved (95% ethanol) tissues using the QIAGEN DNeasy Tissue Kit (QIAGEN Inc., Hilden, Germany) following the manufacturer's protocols. Two mtDNA fragments and 11 nuclear microsatellites were then genotyped.

A 1179-bp fragment of the mtDNA (hereafter referred to as the "control region"), including tRNA-Thr (69 bp), tRNA-Pro (66 bp), and the entire control region (1044 bp), was amplified by the polymerase chain reaction (PCR) in 259 samples using the primer MF (Mannen et al. 2001) and Hphe (Douzery and Randi 1997). A volume of 2 μl (~100 ng) of

DNA was used as template for amplification in a 20 μl reaction mix containing: *Taq* buffer 1× (Polymed, Florence, Italy), 3 mM $MgCl_2$, 100 μM dNTPs, 25 μM of each primer, and 1 unit of *Taq* (Polymed). The thermocycling regime consisted of incubation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 15 s, 60 °C for 1 min, and 72 °C for 1 min, with a final extension of 72 °C for 5 min.

In a subset of 67 individuals, chosen to represent major clades of the control region tree (see Results), we also analyzed a 500-bp fragment of the cytochrome *b* (cytb) gene (corresponding to positions 459–958 in *R. pyrenaica* cytb gene; GenBank accession number AF034726) using the primer pair Cytb1/Cytb3 (Kirstein and Gray 1996). In this case, the PCR amplification was carried out in a 20 μl reaction mix containing: 2 μl template DNA, "AmpliTaq Gold" DNA polymerase buffer 1× (Applied Biosystems, Foster City, CA), 3 mM $MgCl_2$, 100 μM dNTPs, 10 μM of each primer, and 1 unit of AmpliTaq Gold. The thermocycling regime consisted of incubation at 94 °C for 10 min, followed by 29 cycles of 94 °C for 1 min, 55 °C for 2 min, and 72 °C for 1.5 min, with a final extension of 72 °C for 5 min.

For all amplifications, contamination was rigorously excluded by means of blank extractions and PCR-negative controls. Before sequencing, the excess primers and dNTPs were removed using ExoSAP-IT (USB Corporation, Cleveland, OH). The sequence of the control region was determined using 3 different primers: MF and 2 internal primers, H493 and L362 (Douzery and Randi 1997), whereas that of cytb using the primer Cytb1, following the ABI Prism Big-Dye Terminator Kit v.1.1 (Applied Biosystems) standard protocol. The sequencing reaction products were run on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). The resulting sequences were edited with Chromas Version 1.45 (http://www.technelysium.com.au/chromas.html), aligned using Clustal X (Thompson et al. 1997), and checked by eye. All sequences were deposited in GenBank (accession numbers for the cytochrome *b*: GQ246873-GQ246939; accession numbers for the control region: GQ291323-GQ291586).

Eleven dinucleotide microsatellite loci, originally designed for various domestic ungulates, were amplified: SR3, SR1 (Arevalo et al. 1994), ILSTS28 (Kemp et al. 1995), INRA36, INRA11 (Vaiman et al. 1992, 1994), ETH10 (Solinas-Toldo and Fries 1993), ETH225 (Steffen et al. 1993), SR11 (Kogi et al. 1995), SR8, BOVIRBP (Moore et al. 1991), and TGLA40 (Barendse et al. 1994). For each locus, the forward primer was fluorescently labeled with FAM, HEX, or NED (Applied Biosystems). In total, 245 samples were examined from 14 different populations (Table 1). Contamination was rigorously excluded by means of negative controls. PCR products were run on an ABI310 automatic DNA sequencer with commercially prepared size standard markers (Genescan Rox350) and 2 reference samples were inserted into each run in order to avoid errors due to diverse electrophoretic conditions. Microsatellite data were collected, analyzed, and genotyped using ABI commercial software Genescan version 3.1 and Genotyper version 3.7 (Applied Biosystems).

Because it was not possible to amplify many of the poor-quality samples (serum) of the RPO population, we prudently excluded this population from further statistical analyses.

Statistical Analysis

Control Region

Phylogenetic relationships among control region haplotypes were reconstructed using neighbor joining (NJ; Saitou and Nei 1987) and maximum likelihood (ML; Felsenstein 1981) methods as implemented in PAUP* 4.0 (Swofford 2000). The model of sequence evolution, selected by Modeltest 3.7 (Posada and Crandall 1998) under the Akaike information criterion (Akaike 1974), was the Hasegawa–Kishino–Yano (HKY; Hasegawa et al. 1985) model with gamma distributed rates and a significant proportion of invariable sites (HKY + G + I; proportion of invariable sites, I = 0.69; gamma distribution shape parameter, $\alpha = 0.52$; transition/transversion ratio, ti/tv = 47.57; and base frequencies A = 0.30, C = 0.26, G = 0.17, and T = 0.27).

For ML analysis, we employed heuristic searches with a branch-swapping algorithm with 150 random additions under the tree bisection and reconnection option (Felsenstein 2004). The robustness of these analyses was assessed using 1000 and 250 bootstrap replications (Felsenstein 1985) for NJ and ML, respectively.

The pattern of sequence evolution was also represented using a median-joining network (Bandelt et al. 1999) generated with the program Network 4.112 (http://www.fluxus-engineering.com).

Standard diversity indices (number of haplotypes, *K*; number of polymorphic sites, *S*; haplotype diversity, *H*; and nucleotide diversity, π_n) were calculated for each chamois population using Arlequin 3.1 (Schneider et al. 2000). Allelic richness (AR) was also estimated using the rarefaction method described by El Mousadik and Petit (1996) using the program FSTAT.

The analysis of molecular variance (AMOVA; Excoffier et al. 1992), as implemented in Arlequin 3.1, was performed to estimate the average level of differentiation between populations. The genetic diversity at different hierarchical levels was then analyzed by grouping populations according to prior information on taxonomy, geographical origin, translocation history, or the results of the phylogenetic analyses presented here. Genetic distances were corrected for multiple hits by the method of Tamura and Nei (1993) assuming a predetermined gamma shape parameter $\alpha = 0.52$ (alternative models such as HKY are not available in Arlequin). The significance of the different variance and Φ components (molecular equivalents of Wright's *F* statistics; Wright 1951) was obtained by a random permutations procedure (10 000 permutations; Excoffier et al. 1992).

Cytb

The phylogenetic analysis of cytb sequences was performed on a joint data set that included the 67 sequences generated by us, GenBank sequences from *Rupicapra rupicapra rupicapra*,

Table 1. Locations, sample sizes and brief descriptions of the different chamois populations sampled for each subspecies

Species	Subspecies	Population sampled (area, park, valley or hunting jurisdiction; mountain range; country)	Pop. Abbrev.	Sample Size	Category	Brief Description of Populations
R. rupicapra	*rupicapra*	Gran Paradiso National Park; Western Alps; Italy	WA1	22[a,b]	Native	No substantial demographic changes in recent history. Commonly used source population for reintroduction and restocking throughout the Alps (e.g., EA7). ACS*: 10000 (Bassano B, personal communication).
		Alpi Marittime Natural Park; Western Alps; Italy	WA2	6[b,c]	Native	No substantial demographic changes in recent history. Population size never less than 1000 individuals (Dematteis A, personal communication). Commonly used source population for reintroduction and restocking throughout the Alps (e.g., EA7). ACS in 2001: 4700 (Maritime Alps Natural Park-Archives).
		Breguzzo; Eastern Alps;Italy	EA1	28[b,d]	Native	No substantial demographic changes in recent history. ACS in 2003: 2800 (Forests and Wildlife Service, Autonomous Province of Trento).
		Paganella; Eastern Alps; Italy	EA2	30[b,d]	Native	Population underwent bottlenecks during both World Wars. ACS in 2003: 700 (Forests and Wildlife Service, Autonomous Province of Trento).
		Alta Val di Non; Eastern Alps; Italy	EA3	19[b,d]	Native	Population underwent bottlenecks during both World Wars; extensive natural colonisation from neighboring regions. ACS in 2003: 640 (Forests and Wildlife Service, Autonomous Province of Trento).
		Val di Fiemme e Fassa; Eastern Alps; Italy	EA4	27[b,d]	Native	Population underwent bottlenecks during both World Wars. ACS in 2003: 2120 (Forests and Wildlife Service, Autonomous Province of Trento).
		Primiero; Eastern Alps; Italy	EA5	22[b,d]	Native	Population underwent bottlenecks during both World Wars. ACS in 2003: 680 (Forests and Wildlife Service, Autonomous Province of Trento).
		Caldonazzo; Eastern Alps; Italy	EA6	18[b,d]	Traslocated	Extinct by 1970. Reintroduction of more than 20 individuals from different mountain ranges in Trentino (1976–1992) and natural recolonisation from EA7 (Brugnoli A, personal communication). ACS in 2003: 150 (Forests and Wildlife Service, Autonomous Province of Trento).
		Velo; Eastern Alps; Italy	EA7	32[b,d]	Traslocated	Population underwent severe bottlenecks during both World Wars . Restocking with >40 individuals from different mountain ranges of the Western Alps (WA1 and WA2) in 1972–1973. ACS 2008: 170 (Toniolo L, personal communication).
		Great Fatra Mountains; Slovakia (central Europe)	CE1	6[a,e]	Introduced	Translocation of 21 animals from CE3 in 1960. ACS: 60 (no official censuses, Bodova M, personal communication).

Table 1. Continued

Species	Subspecies	Population sampled (area, park, valley or hunting jurisdiction; mountain range; country)	Pop. Abbrev.	Sample Size	Category	Brief Description of Populations
		Slovensky Raj Mountains; Slovakia (central Europe)	CE2	5[a,e]	Introduced	6 individuals introduced from CE3 in 1963. ACS: 90 (Martínková N, personal communication).
		Jeseniky Mountains; Czech Republic (central Europe)	CE3	7[a,b]	Introduced	Introduced from Muersteg reserve near Vienna, Austria. Source population for animals introduced to CE1 and CE2 (Slovakia). ACS: 140 (no official census size available, Vlcek M, personal communication).
	tatrica	High Tatras Mountains; Slovakia (central Europe)	CE4	7[a,b]	Native	Population underwent bottlenecks after both World Wars; a demographic decline was observed about 10 years ago, recently followed by a population expansion. Source population for animals introduced to CE5. ACS in November 2008: 770 (Martínková N, personal communication). This subspecies is listed as "Critically Endangered" (IUCN 2008).
		Low Tatras Mountains; Slovakia (central Europe)	CE5	10[a,b]	Introduced	Translocation of 30 animals from CE4 (1969–1976). ACS in 2006: 100 (Martínková N, personal communication).
R. pyrenaica	*ornata*	Abruzzo; Apennines; Italy	RPO	11[b,e]	—	Captive herd lacking natural population dynamics. This subspecies underwent severe bottlenecks during each World War. Since 1945, protection programs have resulted in a steady increase in population size. ACS: 1100. This subspecies is considered "Vulnerable" (IUCN 2008).
	pyrenaica	Eastern Pyrenees; Pyrenees; Spain	RPP	9[a,b,c]	—	Pooled sample from various Eastern Pyrenean valleys not representative of a natural population. Individuals of this subspecies are still fairly numerous; however, its former range was much wider. Threats include habitat fragmentation and competition with livestock.

[a] Various tissue samples collected from carcasses of wild individuals.

[b] Samples typed for both the control region and microsatellite loci.

[c] Blood samples collected during monitoring programs.

[d] Muscle tissue collected from hunted individuals.

[e] Samples typed at the control region.

[*] Approximate Census Size.

Rupicapra rupicapra tatrica, and *R. pyrenaica* (accession number AF034725, AB050506, and AF034726, respectively), and 3 other species from the same tribe but different genera *Naemorhedus caudatus*, *Capricornis crispus*, and *Oreamnos americanus* (accession number U17861, D32191, AF190632, respectively). Sequences of *Capra hircus* and *Ovis aries* (accession number AB110597 and DQ097410, respectively) were used as out-groups.

Several methods were employed to reconstruct cytb phylogeny. ML (Felsenstein 1981) and NJ (Saitou and Nei 1987) were initially applied as for the control region sequences (see above), then because profound differences between the 2 topologies were observed, we also analyzed the data using maximum parsimony (MP; Fitch 1971) and Bayesian (BI; Huelsenbeck et al. 2001) methods. Heuristic MP searches were performed using PAUP* 4.0 with 1000 bootstrap replicates (using both random stepwise addition and the fast heuristic approaches). For BI, we used the variant of the Markov Chain Monte Carlo algorithm implemented in MrBayes 3.064 (Huelsenbeck and Ronquist

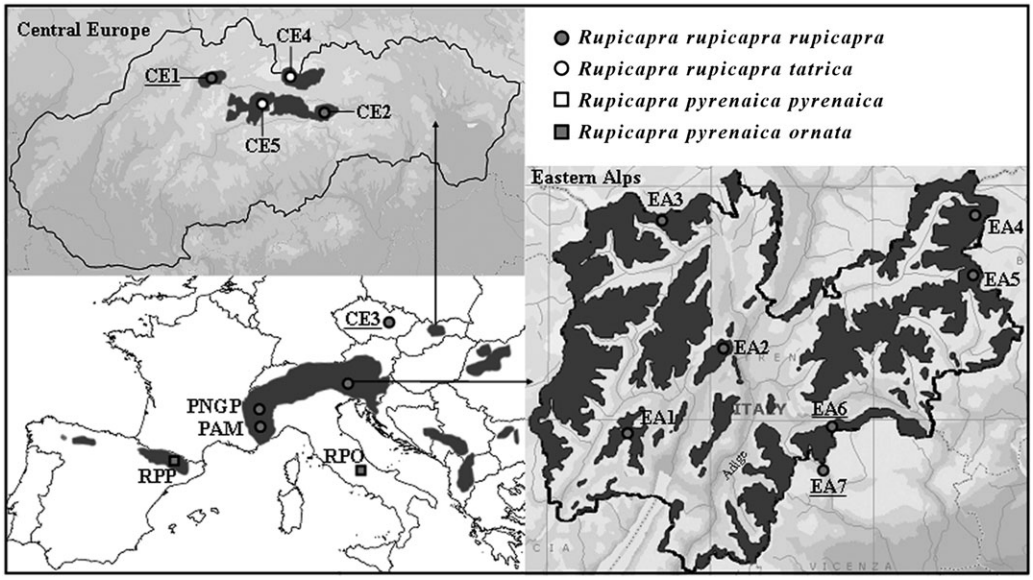

Figure 1. Maps of sites where chamois were sampled for this study. The range of the various species and subspecies, and the presence of this species within single areas, are indicated as dark areas. Gray scales indicate altitude with lighter gray corresponding to higher elevations. Major river basins are also indicated. The classification of the samples is based on geographical and morphological evidence (for abbreviations and definitions, see Table 1). Underlined abbreviations indicate translocated populations. Copyright of Eastern Alps and Slovakia maps: Microsoft Encarta Encyclopedia Plus. ©1993-2002 Microsoft Corporation.

2001). The resulting 50% majority rule tree was constructed from 15 000 trees sampled from the posterior distribution, once the first 5000 trees had been excluded as "burn-in." Statistical support for nodes was estimated by their Bayesian posterior probability. All phylogenetic analyses were conducted under the setting for the best nucleotide substitution model selected by Modeltest on the complete data set (HKY with gamma distribution rate heterogeneity: HKY + G; α = 0.13; ti/tv = 16.80; estimated base frequencies A = 0.30, C = 0.35, G = 0.12, and T = 0.23). A median-joining network (Bandelt et al. 1999) was also reconstructed as for the control region.

Divergence times between principal taxonomic groups of chamois were estimated by calibrating the cytb clock with the divergence time between *Capra ibex* and *C. hircus*. A normal distribution with a mean of 1.5 My and a standard deviation of 0.2 My was used as a prior distribution for the *C. ibex–C. hircus* divergence (Lalueza-Fox et al. 2005). The software Beast was then used to estimate the posterior distribution of the pairwise divergence times between the 3 major chamois groups (*R. p. pyrenaica*, *R. p. ornata*, and *R. rupicapra*), using the Yule pure birth process as a prior for the topology and the branch lengths (Rannala and Yang 1996). The analysis was run 3 times, each time for 1×10^7 generations with a 10% burn-in, assuming the nucleotide substitution model selected by Modeltest as in the previous analyses.

Using the cytb sequences, the hybridization process between *R. rupicapra* and *R. pyrenaica* was investigated in an Approximate Bayesian Computation (ABC) framework (Beaumont et al. 2002). In particular, 2 alternative demographic models were compared: model 1 (M1) which assumes that recent gene flow from *R. pyrenaica* to *R. rupicapra* is due to translocations and model 2 (M2) which assumes that *R. pyrenaica* and *R. rupicapra* have occasionally exchanged migrants since the end of the last glaciation (see Rodríguez et al. 2009). This analysis employed a demographic scenario with 5 parameters (prior distributions are provided as Supplementary Material): 3 for the effective population sizes of *R. pyrenaica*, *R. rupicapra*, and their common ancestral population; 1 for the divergence time between the 2 groups; and 1 for the unidirectional migration rate from *R. pyrenaica* to *R. rupicapra*. As the haplotype distribution shows (see Results), a natural migration or artificial translocation event resulting in gene flow from *R. rupicapra* to *R. pyrenaica* populations can probably be excluded, thus reducing the number of parameters to be estimated from 6 to 5. Under M1, the "translocation model," migration was specified to occur in the last 24 generations or last 150 years (1 generation equals 6.24 year in chamois; Gaillard 1992), when the exchange of game animals between European royalty (and hence, between several of our study populations) was common. Instead, for M2, the "postglacial model," migration was assumed to have occurred anytime during the last 2500 generations (about 15 000 years). Nine summary statistics were used under the ABC approach: for each species (*R. rupicapra* and *R. pyrenaica*), the number of segregating sites, the number of haplotypes, the mean pairwise difference, and the Tajima's *D*, and the F_{ST} statistics to summarize the genetic distance between the 2 groups. Using the Bayesian version of Serial SimCoal (Anderson et al. 2005), we performed 1 000 000 simulations

for each model, with posterior distributions of the parameters approximated using the best 5000 simulations and the R function "makepd4" (R Development Core Team 2008). The posterior probabilities of the 2 models were approximated by fitting a logistic regression (Beaumont 2008) with the R function "calmod." Both makepd4 and calmod are written by M. A. Beaumont (available at http://www.rubic.rdg.ac.uk/~mab/stuff/).

Microsatellites

The genetic diversity within populations was evaluated by estimating mean number of alleles (*A*), AR, observed heterozygosity (H_O) and expected heterozygosity (H_E) using Genetix 4.02 (Belkhir et al. 1999) and FSTAT 2.9.3.2 (Goudet 2001). Each locus in every population was tested for deviations from the Hardy–Weinberg equilibrium (HWE) using the permutation test included in Arlequin 3.1 (Guo and Thompson 1992). The same program was used to test linkage disequilibrium (LD) between pairs of loci using a likelihood-ratio test (Excoffier and Slatkin 1998). The possible influence of null alleles (Chakraborty et al. 1992; Brookfield 1996) was analyzed using Micro-checker (Van Oosterhout et al. 2004).

Population differentiation was investigated with the classical F_{ST} (Weir and Cockerham 1984; Wright 1951) or the molecular R_{ST} (Slatkin 1995) approach, using Arlequin. The genetic variation was partitioned into different hierarchical levels using an AMOVA, as for the control region sequences. The relationships between populations and single individuals were also represented graphically by factorial correspondence analysis (FCA), using Genetix 4.02.

Finally, we applied the Bayesian clustering method implemented in Structure v.2.0 (Pritchard et al. 2000) to detect cryptic population structure or hybridization between differentiated stocks. We initially tested each value of *K* between 2 and the number of sampled populations plus 3 (i.e., between 2 and 16), with 3 independent simulations based on a Markov chain with 10^5 iterations, following a burn-in period of 10 000 iterations. For the most likely value of *K* and the 4 surrounding values (from K − 2 to K + 2), we repeated 3 independent analyses based on a Markov chain with 10^6 iterations, following a burn-in period of 50 000 iterations.

The significance of each of the above analyses was adjusted using a sequential Bonferroni type correction in the case of multiple simultaneous tests (Holm 1979).

Results

Control Region

A 41-bp deletion was detected in all *R. p. ornata* and 2 *R. p. pyrenaica* control region sequences, and a repetition polymorphism (1–4 copies of the 8-bp repeat unit AAACCCAC) was found in 11 *R. r. rupicapra* individuals. These mutations involving multiple bases were not considered in subsequent analyses; therefore, the length of the consensus sequence is 1091 bp. Of 259 individuals, 54 haplotypes can be identified with a total of 169 polymorphic sites (15.5%, including 164 transitions and 9 transversions), 143 of which are parsimony informative. Sequence divergence among *Rupicapra* haplotypes varies from a minimum of 0.092% (1 substitution) between haplotypes from the same subspecies in the same or adjacent locations to a maximum of 7.7% between haplotypes from 2 different species.

ML, NJ, and median-joining algorithms produce a very similar picture of the phylogenetic relationships among haplotypes (Figures 2 and 3): 3 principal groups separated by >50 substitutions are well supported by bootstrap values and represent *R. rupicapra*, *R. p. pyrenaica*, and *R. p. ornata*. The only important exception to this pattern is that the haplotype HRD1 (see Figures 2 and 3), found in the Western and Eastern Alps (59%, 22%, and 17% of individuals in WA1, EA7, and EA6, respectively), falls within the *R. p. pyrenaica* clade.

Within *R. rupicapra*, 4 distinct and geographically or historically meaningful clades of haplotypes can be identified (Figure 2): clade A, which includes only *R. r. tatrica* haplotypes (bootstrap values of 97% for ML and 100% for NJ analyses); clade B, grouping genetically similar haplotypes (HRD8 and HRD 10 to HRD15) found both in the Eastern Alps (EA1, EA2, and EA6) and in most individuals (13/18) from the introduced populations in central Europe (CE1, CE2 and CE3); clade C, typical of individuals from the Western Alps (WA1 and WA2); and clade D, including only individuals in the Eastern Alps (EA1-EA7), with a single exception represented by HRD36 observed in the introduced Great Fatra Mountains populations (CE1).

The network shown in Figure 3 is characterized by many missing internal haplotypes. In addition, most of the haplotypes are found on the tips of the network and, in general, are strongly differentiated from one another, even on a restricted geographical scale. Only 3 haplotypes (HRD16, 30, and 43) have multiple connections to other haplotypes, but none can be considered central within the network.

The majority of *R. rupicapra* haplotypes (78%; $N = 46$) are limited to single populations. Pairs of populations sharing the same haplotypes are always adjacent to one another (e.g., EA6–EA7; EA4–EA5) or are known to have exchanged individuals during restocking or introductions (e.g., WA1–EA7; EA1/EA2/EA3–EA6; CE1–CE3; CE4–CE5; see also Table 1). Even within the same limited geographic area, however, haplotype sharing is very uncommon. For example, the native populations situated on the western side of the Adige Valley in the Eastern Alps (EA1, EA2, and EA3) do not share any haplotypes with those on the eastern side (EA4, EA5) (see Figures 1 and 3).

The large divergence between various *R. rupicapra* populations is confirmed by the AMOVA, with 61% of the molecular variation attributed to differences between populations ($P < 0.0001$). Only 17.6% (16/91) of pairwise Φ_{ST} values are not significant (after sequential Bonferroni correction; $P < 0.0036$; Table 2). On average, native populations from the *R. r. tatrica* are separated by Φ_{ST} =

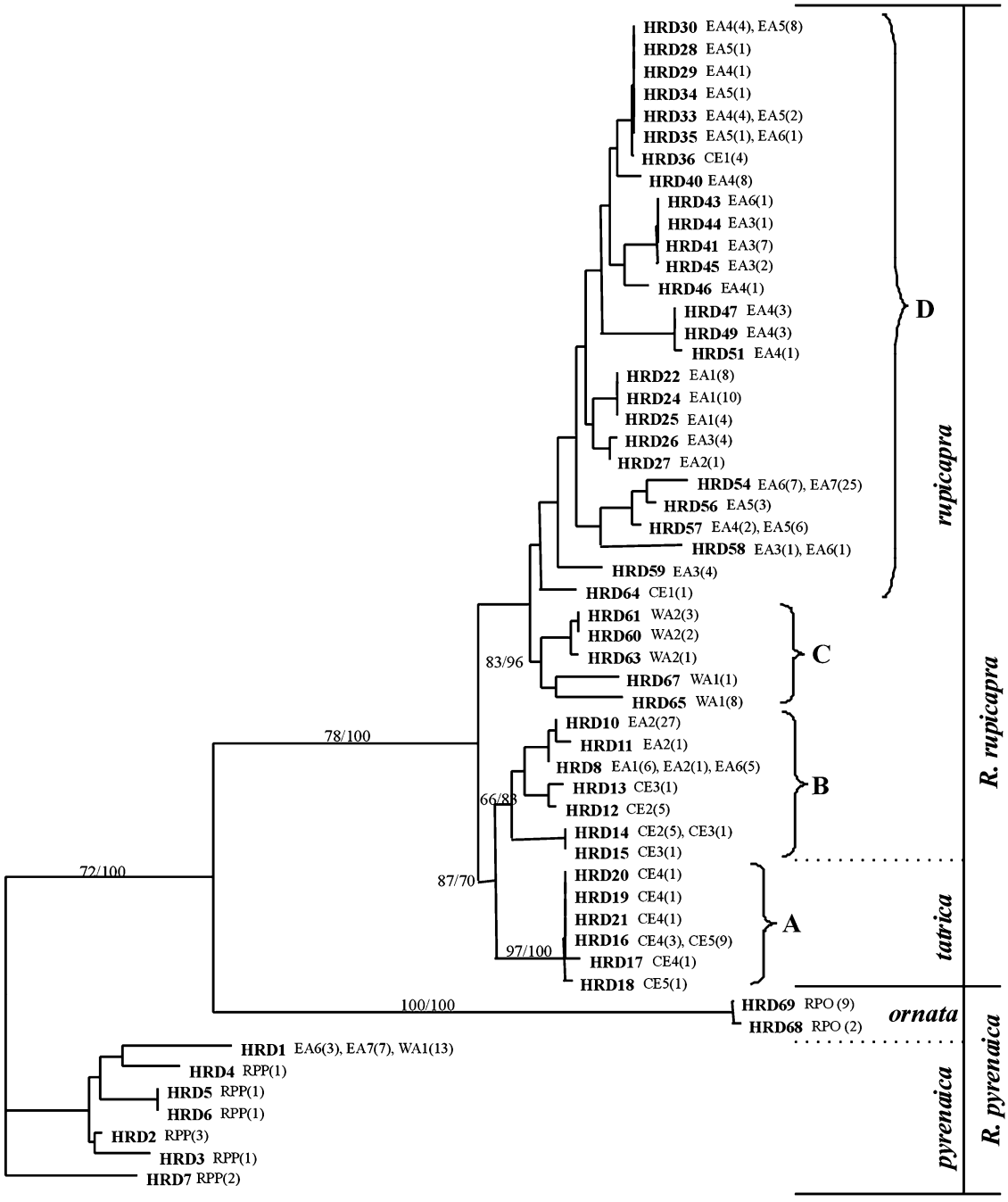

Figure 2. Phylogenetic relationships between 54 chamois control region haplotypes based on ML analyses. The NJ algorithm produced an identical topology for the main clades (data not shown). Bootstrap values for these clades are indicated for both ML and NJ analyses (out of 1000 replications). Populations of origin and absolute frequencies are indicated in parentheses next to each haplotype (see Table 1 for abbreviations). The principal clades within the species *R. rupicapra* are labeled from A to D.

0.69 from those of the *R. r. rupicapra* subspecies and populations of *R. r. rupicapra* from Eastern and Western Alps by $\Phi_{ST} = 0.62$. Average Φ_{ST} values between pairs of native populations within the Western and the Eastern Alps are 0.48 and 0.53, respectively.

Levels of genetic variation within populations are extremely variable (Table 3), which may reflect the different history of each group and/or the small sample sizes rather than differences in current population sizes. For example, considering only the populations with at least 10 individuals, AR, heterozygosity, and nucleotide diversity are much lower in EA2, CE5, and RPO, which are characterized by documented bottlenecks, recent origin by translocation, or captive conditions, respectively. Similarly, the highly

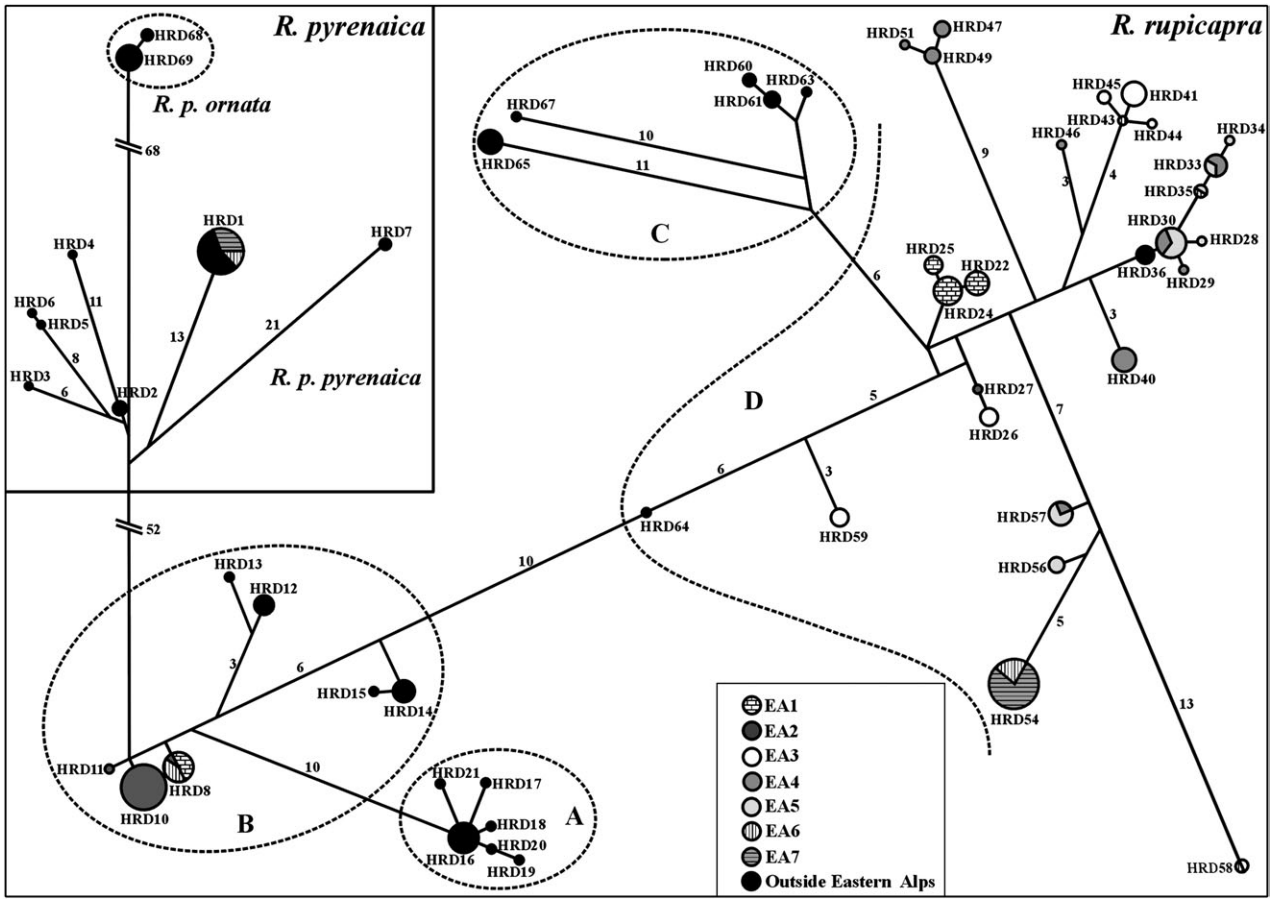

Figure 3. Median-joining network showing the relationships between the 54 distinct *Rupicapra* control region haplotypes. Circles are proportional to the number of individuals having a particular haplotype. Branch lengths are not scaled. Differences greater than 1 between neighboring haplotypes are reported as a number on the branch connecting them. The 4 major *Rupicapra rupicapra* clades identified in the ML and NJ analyses (A, B, C, and D; see Figure 2) are enclosed by dotted lines.

divergent HRD1 haplotype in EA6, EA7, and WA1 (see Figure 3) significantly increases the nucleotide diversity observed in these samples. Overall, the nucleotide diversity within *R. rupicapra* and *R. p. pyrenaica* is 2.4% and 1.7%, respectively and close to 0 in the captive *R. p. ornata* sample.

Cytb

For the 67 individuals analyzed, 14 different cytb haplotypes were identified with a total of 43 polymorphic sites out of 500 bp (8.6%; 40 transitions and 4 transversions).

The network analysis of the cytb gene (data not shown) confirms the existence of 3 main groups of sequences separated from each other by at least 20 mutations. The first clade includes 8 *R. rupicapra*-specific haplotypes, one of them shared by all *R. r. tatrica* and 4 *R. r. rupicapra* individuals. The second clade includes only 1 haplotype observed in all *R. p. ornata* animals. The third clade includes 4 *R. p. pyrenaica*-specific haplotypes and 1 haplotype (HRC1) found in the same Alpine individuals that carry the control region haplotype HRD1.

The phylogenetic trees reconstructed by different methods (NJ, ML, MP, and BI) also confirm that the genus *Rupicapra* is a strongly supported monophyletic assemblage relative to the 3 other genera of the tribe *Rupicaprini* (Figure 4). On the other hand, contrasting divergence patterns are obtained within *Rupicapra*. The NJ and MP analyses (Figure 4a) support the current taxonomy (i.e., *R. rupicapra*, *R. p. pyrenaica*, and *R. p. ornata*). In contrast, the BI and ML analyses (Figure 4b) suggest that there was a more recent split between the chamois in Central Italy (*R. p. ornata*) and Alpine chamois (*R. rupicapra*). However, the bootstrap and posterior probability values for the branch leading to the clade *R. rupicapra* + *R. p. ornata* or *R. p. pyrenaica* + *R. p. ornata* are low (Figure 4a,b). The resolution of this topology is problematic because the single *R. p. ornata* haplotype is almost equally separated from *R. p. pyrenaica* (20–26 mutations) and *R. rupicapra* haplotypes (21–27 mutations), whereas *R. p. pyrenaica* and *R. rupicapra* are slightly more differentiated (28–39 mutations).

The pairwise divergence times between 3 major taxonomic units are similar, with largely overlapping

Table 2. Pairwise divergence between *Rupicapra rupicapra* populations

	EA1	EA2	EA3	EA4	EA5	EA6	EA7	WA1	WA2	CE1	CE2	CE3	CE4	CE5
EA1	—	**0.08**	**0.03**	**0.10**	**0.08**	**0.09**	**0.18**	**0.07**	**0.10**	**0.22**	na	na	**0.30**	**0.26**
EA2	**0.73**	—	**0.08**	**0.13**	**0.11**	**0.09**	**0.16**	**0.09**	**0.13**	**0.24**	na	na	**0.30**	**0.29**
EA3	**0.43**	**0.82**	—	**0.09**	**0.08**	**0.08**	**0.17**	**0.06**	**0.07**	**0.21**	na	na	**0.31**	**0.27**
EA4	**0.43**	**0.79**	**0.35**	—	**0.02**	**0.11**	**0.18**	**0.11**	**0.12**	**0.21**	na	na	**0.28**	**0.24**
EA5	**0.46**	**0.82**	**0.37**	0.13	—	**0.10**	**0.19**	**0.10**	**0.12**	**0.25**	na	na	**0.32**	**0.28**
EA6	**0.30**	**0.41**	**0.28**	**0.28**	**0.26**	—	**0.02**	**0.07**	**0.09**	**0.18**	na	na	**0.31**	**0.28**
EA7	**0.45**	**0.59**	**0.40**	**0.39**	**0.34**	0.05	—	**0.13**	**0.16**	**0.21**	na	na	**0.36**	**0.34**
WA1	**0.58**	**0.63**	**0.54**	**0.60**	**0.59**	**0.28**	**0.36**	—	**0.06**	**0.22**	na	na	**0.29**	**0.27**
WA2	**0.58**	**0.93**	**0.53**	**0.58**	**0.61**	0.27	0.40	0.48	—	**0.25**	na	na	**0.38**	**0.31**
CE1	0.36	**0.85**	**0.28**	0.13	0.15	0.17	0.34	**0.47**	**0.63**	—	na	na	**0.53**	**0.44**
CE2	**0.65**	**0.84**	**0.74**	**0.71**	**0.76**	0.25	0.46	**0.46**	**0.97**	0.74	—	na	na	na
CE3	**0.57**	**0.82**	**0.68**	**0.66**	**0.70**	0.26	**0.45**	**0.49**	**0.87**	0.60	**0.78**	—	na	na
CE4	**0.73**	**0.88**	**0.75**	**0.72**	**0.77**	**0.33**	**0.48**	**0.50**	**0.93**	**0.77**	**0.93**	**0.84**	—	0.03
CE5	**0.76**	**0.91**	**0.79**	**0.75**	**0.80**	**0.37**	**0.51**	**0.54**	**0.98**	**0.83**	**0.99**	**0.90**	0.09	—

Below the diagonal: Φst (control region); above the diagonal: F_{ST} (microsatellites); in bold: significant values ($P < 0.0036$ after a Bonferroni correction). na, not available.

confidence intervals. The posterior distribution of the divergence between *R. rupicapra* and *R. p. ornata*, *R. p. pyrenaica* and *R. p. ornata*, and *R. p. pyrenaica* and *R. rupicapra* has a mean of 1.4, 1.8, and 1.9 My (95% limits: 0.6–2.5, 0.8–3.1, and 0.9–3.1), respectively. This result supports the *R. rupicapra–R. p. ornata* clustering suggested earlier by the BI and the ML trees. However, the large confidence intervals indicate that the difference between the 3 estimated divergence times is not significant.

The ABC analysis provided unequivocal support for M1, the translocation model, over M2, the postglacial model. In fact, the estimated posterior probabilities of these models were 0.98 and 0.02, respectively. In addition, varying the size of the migration window around the fixed values assumed for M1 or M2 did not alter this conclusion. For example, allowing a migration process under M1 in the last 50, and not 24, generations resulted in probabilities for M1 and M2 of 0.95 and 0.05, respectively. The posterior distributions of all the demographic parameters estimated under M1 are reported as Supplementary Material. It is interesting to note that the support interval for the migration rate *m* (per generation) from *R. pyrenaica* to *R. rupicapra* in the last 24

Table 3. Genetic variation within chamois populations of the mtDNA dloop and 11 microsatellite loci

		mtDNA-control region							STR						
Subspecies	Population	*N*	*K* (PH)	AR	*H* (SD)	*S*	π_n% (SD)		*N*	*A* (SD)	H_E (SD)	H_O (SD)	AR	PA	HWE
Rupicapra rupicapra															
rupicapra	Eastern Alps														
	EA1	28	4 (3)	3.29	0.75 (0.04)	22	0.76 (0.40)		28	5.27 (2.41)	0.54 (0.24)	0.57 (0.29)	3.34	5	—
	EA2	30	4 (3)	1.60	0.19 (0.09)	22	0.15 (0.10)		30	5.00 (2.61)	0.59 (0.19)	0.60 (0.19)	3.45	—	1
	EA3	19	6 (5)	3.77	0.80 (0.06)	28	0.88 (0.47)		19	4.82 (1.99)	0.55 (0.25)	0.54 (0.28)	3.50	—	1
	EA4	27	9 (6)	4.39	0.87 (0.04)	29	0.88 (0.46)		27	4.63 (2.62)	0.54 (0.24)	0.52 (0.23)	3.24	1	—
	EA5	22	7 (3)	3.79	0.80 (0.06)	20	0.80 (0.43)		22	4.36 (2.77)	0.49 (0.25)	0.47 (0.23)	3.10	—	1
	EA6	18	6 (1)	3.61	0.78 (0.07)	90	3.26 (1.67)		18	5.27 (2.28)	0.61 (0.22)	0.58 (0.19)	3.65	—	1
	EA7	32	2 (0)	1.80	0.35 (0.08)	67	2.65 (1.32)		32	4.82 (1.72)	0.57 (0.16)	0.58 (0.15)	3.20	1	1
	Western Alps														
	WA1	22	3 (2)	2.23	0.54 (0.07)	74	3.74 (1.88)		22	5.09 (2.26)	0.58 (0.24)	0.59 (0.24)	3.46	1	1
	WA2	6	3 (3)	3.00	0.73 (0.16)	3	0.11 (0.09)		6	3.45 (1.35)	0.48 (0.29)	0.55 (0.36)	3.28	—	—
	Central Europe														
	CE1	6	3 (2)	2.67	0.60 (0.21)	25	1.06 (0.65)		6	2.18 (1.08)	0.28 (0.27)	0.27 (0.90)	2.09	—	1
	CE2	5	1 (1)	1.00	0	0	0		—	na	na	na	na	na	na
	CE3	7	3 (2)	2.43	0.52 (0.21)	14	0.38 (0.25)		—	na	na	na	na	na	na
tatrica	CE4	7	5 (4)	4.43	0.86 (0.14)	6	0.18 (0.13)		6	2.18 (1.25)	0.33 (0.28)	0.42 (0.39)	2.15	1	—
	CE5	10	2 (1)	1.60	0.20 (0.15)	1	0.02 (0.03)		9	2.82 (0.98)	0.41 (0.22)	0.40 (0.25)	2.51	—	1
Rupicapra pyrenaica															
ornata	RPO	11	2 (2)	1.82	0.33 (0.15)	1	0.03 (0.04)		—	na	na	na	na	na	na
pyrenaica	RPP	9	6 (6)	4.57	0.89 (0.09)	47	1.69 (0.94)		9	4.00 (1.41)	0.54 (0.23)	0.57 (0.27)	3.43	9	—

N, sample size; *K*, haplotype number; PH, private haplotype; *H*, gene diversity; SD, standard deviation; *S*, number of polymorphic sites; π_n, nucleotide diversity; *A*, mean number of alleles; PA, number of private alleles; HWE: number of loci deviating significantly from the HWE at $P < 0.05$; na, not available.

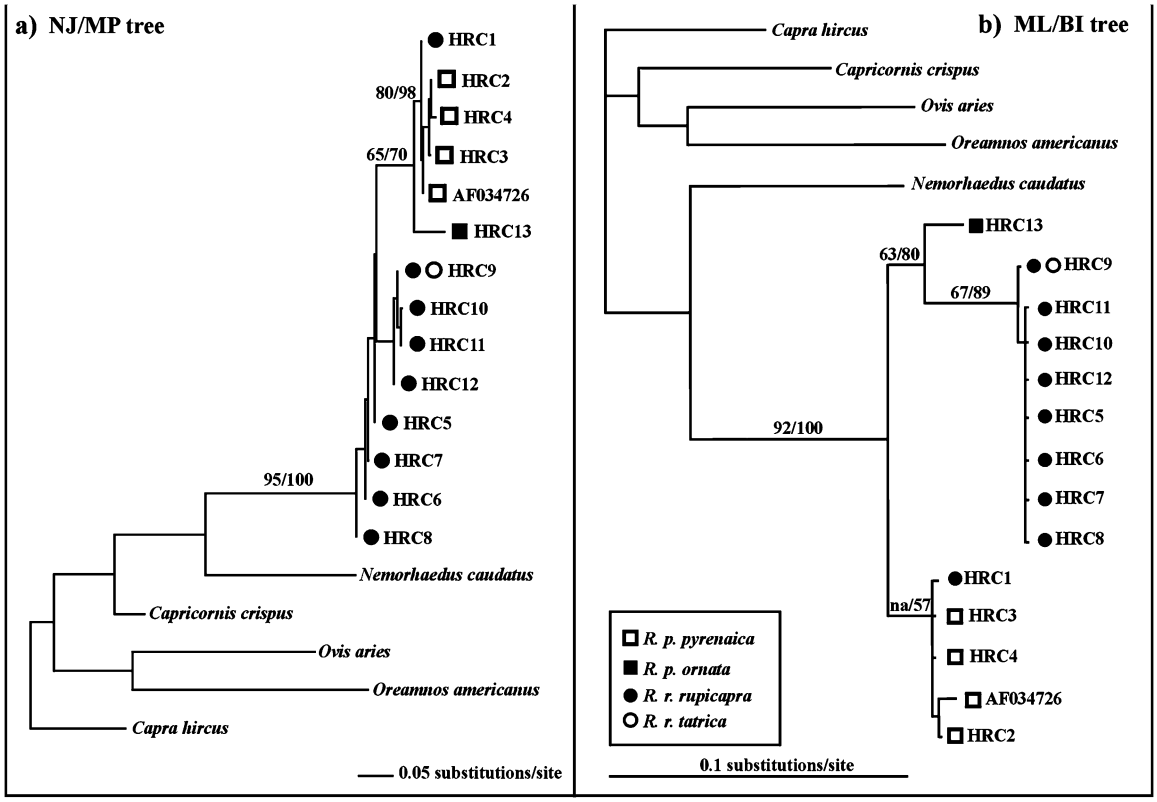

Figure 4. Phylogenetic trees of cytb haplotypes reconstructed using different methods: (a) ML and BI; (b) NJ and MP. Note that haplotype HRC1 found in *R. r. rupicapra* individuals clusters with *Rupicapra pyrenaica* haplotypes. Bootstrap values >50% are reported.

generations (150 years) was 0.009–0.035. Assuming that local *R. rupicapra* genotypes reduce by a fraction $m = 0.009$–0.035 every generation, this continuous gene flow is expected to produce in 24 generations a populations with a fraction of 20–57% of allochthonous genotypes ($1 - (1 - m)^{24}$). This value is similar to the observed fraction of *R. pyrenaica* haplotypes observed in some Alpine populations.

Microsatellites

All 11 microsatellite loci are polymorphic, presenting a total of 100 alleles (per locus: range 2–16; mean 9.1; $H_E = 0.30$–0.88). No loci show a systematic deviation from HWE, and only 8 of 143 tests are significant with $P = 0.05$. This number is fully compatible with the number of significant tests expected by chance. Evidence for the presence of null alleles was not found. In total, 68 alleles are species specific and 22 of them are also population specific (Table 3). Interestingly, comparing the 12 *R. rupicapra* populations, the number of private alleles is either 0 or 1, with the exception of EA1 where 5 specific alleles are observed. However, no locus can be considered diagnostic for a single species or population.

Overall, the levels of expected heterozygosity in species and subspecies range from 0.51 to 0.64 (*R. rupicapra*: 0.64; *R. p. pyrenaica*: 0.57; *R. r. rupicapra*: 0.63; and *R. r. tatrica*: 0.51). The values for each population, averaged over 11 loci, suggest that populations in the Alps and the Pyrenees have similar levels of variation (between 0.48 and 0.61; Table 3). On the other hand, the central European populations analyzed at these markers (CE1, CE4, and CE5) have much lower values (between 0.28 and 0.41). Within the Alps, the highest values are observed in EA6, EA7, and WA1 (0.61, 0.57, and 0.58, respectively), where highly divergent control region and cytb haplotypes are also found, but also in EA2 (0.59) where low control region variation was detected.

Nuclear genetic divergence between populations is significant, with 17–18% of variation due to differences between populations depending on the use of F_{ST} or R_{ST} approaches (AMOVA; $P < 0.001$ in both cases). The analysis of the pairwise distances (Table 2) or the hierarchical partition of the genetic variation (data not shown) suggests, as for the control region analysis, that genetic distances are correlated with taxonomy, geographic distances, and documented translocation history. For example, on average, the F_{ST} value between *R. pyrenaica* and *R. rupicapra* is 0.37, between *R. r. tatrica* and Alpine *R. r. rupicapra* is 0.30, and between *R. r. rupicapra* populations from the Eastern and Western Alps is 0.10. Within the Alps, the smallest values are observed for the geographically neighboring pairs EA4–EA5, EA1–EA3, EA6–EA7, and WA1–WA2 ($F_{ST} = 0.02$, 0.03, 0.02, and 0.06, respectively).

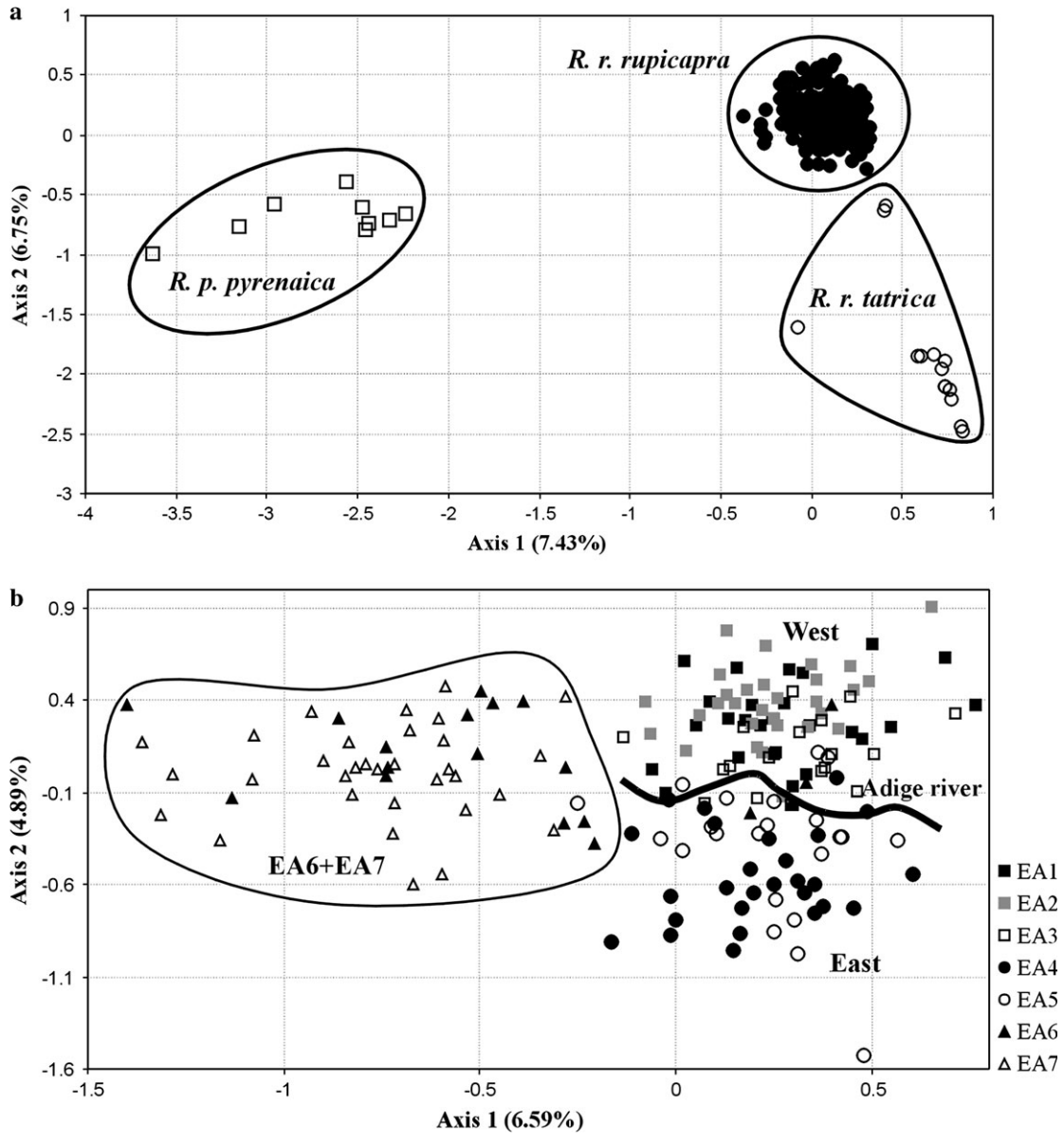

Figure 5. FCA of the individual microsatellites genotypes: (a) all sampled individuals; (b) only individuals from the Eastern Alps.

A low value (0.03) is also observed between CE4 and CE5 in central Europe. Instead, the mean F_{ST} values between CE1 and the Eastern Alps populations (EA1–EA7) is 0.22, less than half of the value observed when CE1 is compared with native central European populations (CE4 and CE5; 0.49). Finally, EA7, 1 of the 3 Alpine populations with the HRD1 haplotype, is highly divergent from the other Alpine populations (EA1–EA6, WA1, and WA2; mean F_{ST} = 0.15). All pairwise F_{ST} values were significant after Bonferroni correction, with exception of the CE4–CE5 comparison (Table 2).

The results of FCA confirm the analysis of F_{ST} values, supporting the taxonomical relationships inferred for *R. p. pyrenaica* and the 2 subspecies *R. r. rupicapra* and *R. r. tatrica* (Figure 5a). In contrast with the control region results, however, none of the *R. rupicapra* individuals cluster closely with those of *R. pyrenaica*. When FCA is repeated including only the Eastern Alps populations (EA1–EA7), 3 clusters emerge (Figure 5b): 1 including populations from mountain ranges east of the Adige Valley (EA4, EA5), 1 including populations from mountain ranges on the west side of the same valley (EA1, EA2, and EA3), and 1 including the 2 Eastern Alps groups (EA6 and EA7) where the HRD1 haplotype was found.

Bayesian analysis suggests that K = 9 genetically homogeneous groups can be inferred from our original 13 populations (Table 4). Four inferred groups (1, 2, 3, and 9) include 4 different pairs of populations with large contributions (EA4–EA5, EA6–EA7, WA1–WA2, and CE4–CE5), the same pairs of homogenous groups identified in other

Table 4. The estimated genetic contribution of the 9 inferred groups to the sampled chamois populations based on Structure analysis

	Inferred populations								
Sampled populations	1	2	3	4	5	6	7	8	9
EA1	0.033	0.054	0.019	0.005	0.03	0.031	0.082	**0.732**	0.013
EA2	0.027	0.017	0.014	0.008	0.019	**0.786**	0.036	0.088	0.005
EA3	0.025	0.021	0.019	0.005	0.041	0.079	**0.683**	0.121	0.006
EA4	**0.774**	0.029	0.020	0.007	0.046	0.029	0.048	0.039	0.007
EA5	**0.701**	0.025	0.022	0.007	0.018	0.063	0.051	0.105	0.010
EA6	0.069	0.045	**0.616**	0.007	0.040	0.092	0.082	0.043	0.006
EA7	0.014	0.031	**0.882**	0.006	0.020	0.013	0.014	0.015	0.004
WA1	0.019	**0.769**	0.068	0.006	0.014	0.015	0.077	0.023	0.010
WA2	0.050	**0.726**	0.021	0.007	0.016	0.024	0.121	0.029	0.005
CE1	0.006	0.006	0.009	0.003	**0.946**	0.007	0.007	0.014	0.003
CE4	0.005	0.006	0.003	0.042	0.029	0.007	0.004	0.005	**0.898**
CE5	0.009	0.005	0.003	0.003	0.131	0.005	0.014	0.007	**0.823**
RPP	0.004	0.006	0.004	**0.967**	0.004	0.003	0.004	0.003	0.005

The boxed number may indicate ongoing admixture (see text for more details). Numbers in bold indicate the inferred group making the largest contribution to each sampled population.

analyses. The remaining 5 inferred groups suggest a large contribution from a single sampled population and very small contributions from almost all the others. Again, this suggests that the major inferred groups are genetically distinct. The populations EA6, EA7, or WA1, where several individuals with the HRD1 haplotype were found, do not show any specific affinities with *R. pyrenaica* populations, and this is also true when the genetic composition of single individuals from these populations is considered (results not shown). Interestingly, the gene pool of some individuals in EA6 appears to be mixed, with a consistent contribution from the other populations in the Eastern Alps (EA1–EA5). The individual analysis also identifies 3 individuals from CE5 with a significant contribution from CE1; this result is summarized by the 13% contribution of inferred group 5 (dominated by the contribution from CE1) to CE5 in Table 4 (boxed number).

Discussion

The genetic information we obtained in this study, based on 259 individuals and 2 mitochondrial and 11 nuclear markers, allows us to investigate several aspects of geographic structure, divergence, and taxonomy in the chamois as well as the effects of past management practices.

Genetic Impact of a Patchy Distribution

This study documents extensive genetic variation in chamois at different geographical scales, revealing the impact of natural and artificial habitat fragmentation and low dispersal rates on patterns of genetic diversity. Most of the chamois populations studied here show relatively high levels of expected heterozygosity at microsatellite loci and control region nucleotide diversity (range: 0.28–0.61 and 0.02–3.74, respectively; Table 3), similar to those reported in other alpine ungulates such as thinhorn sheep (*Ovis dalli*) and bighorn sheep (*Ovis canadensis*; range: H_E = 0.36–0.67, π_n (%) = 0.4–2.7; Gutiérrez-Espeleta et al. 2000; Worley et al. 2004; Loehr et al. 2006) and greater than that reported for endangered populations of ibex (*Capra ibex ibex*; H_E = 0.29–0.45; Maudet et al. 2002). Similar levels of variation have also been detected in other large populations of ungulates such as African buffalo (*Syncerus caffer*; Simonsen et al. 1998), hartebeest (*Alcelaphus buselaphus*), topi (*Damaliscus lunatus*), wildebeest (*Connochaetes taurinus*; Arctander et al. 1999), impala (*Aepyceros melampus melampus*; Lorenzen and Siegismund 2004), and moose (*Alces alces*; Hundertmark et al. 2002). None of the populations we analyzed appear to have suffered a severe loss of genetic variation, but the consequences of bottleneck events are still recognizable is some groups. In fact, the lowest values of genetic variability are found in those populations that were reportedly subject to strong founder effects and/or long-term reduction in population size (e.g., those from central Europe: CE1, CE2, CE3, CE4, CE5, and RPO from the Apennines). On the contrary, higher-than-average values of genetic variation in translocated populations (EA6, EA7) can instead be attributed, as discussed below, to admixture and hybridization between individuals of different geographical origins and/or taxa.

Significant patterns of genetic structure were found among most of the chamois populations studied. The genetic distance between native *R. rupicapra* populations is high (F_{ST} for microsatellites: 0.11; Φ_{ST} for the control region: 0.61) and similar to the values reported in other mountains ungulates (thinhorn sheep F_{ST} = 0.16, Worley et al. 2004; Φ_{ST} = 0.58, Loehr et al. 2006). This finding supports the marked genetic structuring found in chamois in other mountain ranges (Pérez et al. 2002; Schaschl et al. 2003). Strong genetic structure was even found for population pairs separated by short geographical distances (e.g., for populations in the Eastern Alps: F_{ST} = 0.09; Φ_{ST} = 0.61), indicating that gene flow is low even on a microgeographic scale. At this very local scale, we also note that genetic differentiation is largely concordant with the geographical features of the sampled areas. Two results

support this conclusion. First, the 2 major genetic groups correspond to native populations from either side of the Adige Valley (east side: EA4–EA5; west side: EA1–EA2–EA3; Figure 5b), which is (and probably also was even before urbanization) the principal geographic barrier to chamois dispersal in this region. Second, the Paganella population (EA2), which is located on a small mountain range surrounded by deep valleys, shows levels of differentiation and genetic variation which are among the highest and the lowest observed in our study, respectively.

Finally, for all pairs of populations, differentiation is always higher for the control region than for microsatellites (Table 2). This result is expected given the low effective population size of mtDNA, and consequently, the higher sensitivity of this locus to genetic drift. Sex-biased migration rates, with males dispersing more than females, could also contribute to this pattern; however, although a higher dispersal rate in males compared to females was observed in 2 game reserve populations (Loison et al. 1999), the same pattern was not documented in an undisturbed population in the Gran Paradiso National Park (Lovari et al. 2006). Therefore, the effective role of female phylopatry in explaining the differences between mtDNA and microsatellite geographic structure still needs to be clarified.

Genetic Impact of Translocations and Hybridizations

Recent studies have shown that past translocations almost always leave a genetic signature, which in turn can be used to reconstruct illegal or undocumented events. The genetic impact of restocking and reintroductions has been noted not only in ungulates such as the white-tailed deer (DeYoung et al. 2003), roe deer (Vernesi et al. 2002; Randi et al. 2004), and wild boar (Vernesi et al. 2003) but also in many other non-ungulate species (for a review, see Bertorelle et al. 2009). Our results confirm the capacity of genetic markers to identify and reconstruct documented or unrecorded translocation events and to determine their effects on the local gene pool. This process, which is only made possible by the simultaneous analysis of more than one type of genetic marker, is important for identifying hybrid populations, where the original genetic composition is compromised or at risk, and to distinguish them from populations with naturally high levels of genetic variability.

In several *R. r. rupicapra* individuals from EA6, EA7, and WA1, we find a mtDNA haplotype (named HRD1 for the control region and HRC1 for cytb) that falls within the *R. p. pyrenaica* cluster (Figures 2 and 3). Although this haplotype could represent a polymorphism shared by the 2 species since their divergence, it could also be the result of a natural or human-mediated migration. It seems very unlikely that HRD1/HRC1 represents a shared polymorphism, given the large genetic divergence between this single haplotype and all the other *R. rupicapra* haplotypes. In addition, the large contrast observed in EA6, EA7, and WA1 between nucleotide diversity (very high) and number of alleles (similar to other populations) suggests that heterogeneity among mtDNA sequences in these populations is much higher than would be expected for a population in equilibrium (see Table 3) and supports the view that these populations have been subject to, and affected by, recent introductions/migration.

If we can exclude the shared polymorphism hypothesis, when and how was HRD1 introduced into some Alpine populations? Rodríguez et al. (2009), who recently found 2 additional *pyrenaica* cytb haplotypes in Val di Susa, a valley in the Western Alps less than 50 km from the Grand Paradiso National Park (WA1, where we found several individuals with *pyrenaica* haplotypes), suggest that "West" and "East" clades began to hybridize after the last glaciation creating a suture zone. This hypothesis, which implies that only one species of chamois should be recognized (Rodríguez et al. 2009), appears unlikely considering that 1) the distribution of *pyrenaica* haplotypes is scattered across a few populations throughout the western and central Alps (Val di Susa, WA1, EA6, and EA7), only 2 of which are on the western edge of the *R. rupicapra* range and 2) in this species, postglaciation warming probably favored a retreat to higher altitudes and not migrations across mountain ranges. Instead, a more likely explanation for the patchy presence of a *pyrenaica* haplotypes in 3 Alpine populations (WA1, EA6, and EA7) is an undocumented restocking or reintroduction from the Pyrenees to the Western Alps (WA1) within the last 150 years followed by a documented introduction of descendents of these animals from the Western to the Eastern Alps (EA6, EA7) in the early 1970s (about 5 generations ago; Table 1). This hypothesis is plausible, given that the Gran Paradiso National Park (WA1) was a royal hunting reserve from 1856 to 1922 and exchange of animals between kingdoms was a common practice throughout Europe at that time. To formally address this point, we estimated the posterior probability of the translocation (M1) and the postglacial (M2) models using a Bayesian approach. M1 obtained a probability close to 1; in other words, individuals with *pyrenaica* genomes probably arrived in the Western and the Eastern Alps a few generations ago, initially creating a distinct gene pool in these areas, with subsequent backcrossing and recombination events now preventing the identification of hybrid individuals at nuclear markers.

Our results provide strong evidence of the dramatic effect that reintroductions and restocking can have on the genetic composition of a population. For example, the 3 populations where the *pyrenaica* haplotype was observed (EA6, EA7, and WA1) show a much higher variation compared with other groups, and the unexpected relationships between different measures of variation (e.g., low heterozygosity and large nucleotide diversity in the same sample; see Table 3 and Figure 5b) clearly indicate that the origin of this "artificial" pattern of variation is related to an admixture event.

The genetic impact of translocations, and the possibility of identifying such management practices using a small number of genetic markers, is also clear from the analysis of the central European populations of chamois. In fact, all the introduced populations in that area (CE1, CE2, and CE3) have a genetic composition very similar to that observed in the populations from the Eastern Alps sampled

geographically close to their areas of origin (Figure 2). In contrast to the reintroduction events that occurred in the Alps described above, in the case of central Europe, entire populations of *R. r. rupicapra* were artificially established within the geographical range of a different subspecies, *R. r. tatrica* (Blahout 1972). This recent assemblage of 2 genetically differentiated taxa could constitute a significant threat to the persistence of the Tatra chamois because hybridization between these 2 pairs of populations is possible (Jurdíková 2000), and our results suggest that in some areas, hybridization is indeed occurring (Table 4).

Did the Genus *Rupicapra* Rapidly Diverge into 3 Species?

Almost one hundred years ago, mainly on the basis of skull and horn morphometrics, Camerano (1914) classified the chamois in the Apennines of Central Italy as a distinct species, *Rupicapra ornata*. This conclusion was implicitly supported 65 years later again on the basis of divergent phenotypic traits "hardly understandable on the basis of environmental factors alone" (Lovari and Scala 1980). However, although the commonly accepted taxonomy for the genus, supported by fossil, morphological, behavioral, and available genetic evidence, recognizes 2 species, *R. rupicapra* and *R. pyrenaica*, none of these studies has been able to resolve the status of the Apennine chamois (Lovari and Scala 1980; Nascetti et al. 1985; Masini and Lovari 1988; Hammer et al. 1995; Pérez et al. 2002). The single *ornata* individual typed at 23 microsatellite markers by Pérez et al. (2002) deviates from both *R. rupicapra* and *R. pyrenaica* individuals, but the authors concluded that a bottleneck effect could explain this result. More recently, Rodríguez et al. (2009) suggested that *R. rupicapra* and *R. pyrenaica* are polyphyletic and only 1 chamois species actually exists.

Our results from the sequencing of 2 mtDNA markers appear to support Camerano's (1914) original hypothesis, whereas refuting that of Rodríguez et al. (2009). In fact, we found that 1) *R. rupicapra* and *R. pyrenaica* polyphyly should be regarded as a very recent human-mediated process with a geographically restricted relevance; 2) similar levels of control region and cytb divergence separate each pair in the *ornata*, *pyrenaica*, and *rupicapra* comparisons (mean pairwise difference between *R. rupicapra–R. p. pyrenaica*, *R. rupicapra–R. p. ornata*, and *R. p. pyrenaica–R. p. ornata* is 68, 77, and 73 substitutions, respectively); 3) these levels of divergence are much higher than that observed between other *Rupicapra* subspecies (*tatrica* and *rupicapra*; mean pairwise differences = 24); and 4) the cytb divergence between *R. rupicapra* and *R. pyrenaica* (mean pairwise differences = 30) is comparable, and in some cases, even higher than the values observed for other pairs of species of the same genus within the Caprinae subfamily (Lalueza-Fox et al. 2005). In light of these results, we would suggest a revision of the specific status of the Apennine chamois, including a more complete genetic analysis with a larger number of samples and additional mitochondrial and nuclear markers.

All our phylogenetic analyses appear to infer that the most ancient differentiation within the genus *Rupicapra* occurred between the species presently recognized as *R. rupicapra* and *R. pyrenaica* but disagree as to origin of the Apennine chamois (*R. p. ornata*). For example, the Bayesian analysis (Figure 4b) supports a closer relationship between *R. p. ornata* and *R. rupicapra*, but the pairwise divergence times estimated between these 3 groups have large and overlapping confidence intervals with means between 1.4 and 1.9 Ma. This poorly resolved relationship could be evidence for a trichotomy in the tree; that is, a speciation event in which 3 species evolved almost simultaneously. Although the limited length of the cytb sequences (500 bp) may hamper the resolution of this relationship, the approach is important because it implicates a similar divergence age of the 3 principal taxonomic groups (*rupicapra*, *pyrenaica*, and *ornata*). Therefore, our data appear to support the hypothesis proposing a single colonization event of western Europe, followed by expansions and contractions that alternately isolated and brought into contact peripheral populations, resulting in the 3 principal taxonomic groups and numerous subspecies. Moreover, we note that this hypothesis is also supported by the close association of genetic and geographic distances, both within and between species, compatible with a single colonization without subsequent major migrations, as documented by Pérez et al. (2002).

Conclusions

This study has shown that both the control region and microsatellites are useful for understanding not only the natural but also the human-induced displacements in the chamois and for investigating the genetic consequences of these events. Translocations and subsequent hybridization between individuals from different populations/taxa have had a significant genetic impact in this species. Unfortunately, the translocation of individuals from geographically (and therefore genetically) distant populations continues to be a common management strategy in the chamois. Our results suggest that conservation units for the various taxa of *Rupicapra* urgently need to be defined so that management strategies, which may or may not include translocations with individuals carrying the appropriate genetic background, can be developed. In the meantime, the intraspecific hybridization occurring in some Alpine populations should be regarded as an ongoing and unplanned experiment and the evolutionary consequences of this process should be carefully monitored.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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