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The complete mitochondrial DNA sequence of the Montecristo goat

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ABSTRACT

The remote island of Montecristo is renowned for a resident goat population, whose origins are unclear. We describe the complete mtDNA sequence of a male specimen captured on the island. The sequence turned out to belong to haplogroup (Hg) A. Our results exclude that the sequence belongs to a lineage more closely related to bezoars than domestic goats. The lineages most similar to the Montecristo sequence are currently found in Western Europe, favouring partial feralization for the Montecristo isolate. Positioning of the Montecristo mitogenome in the emerging phylogenetic tree for domestic goats reveals a new lineage with multiple derived nucleotide states shared with lineages so far described in Europe as well as in North Asian breeds. This reveals the presence, within Hg A, of lineages with a palearctic distribution, whose descendants are now grown from Eastern Asia to Western Europe.

1. Introduction

The remote island of Montecristo (42°20′N; 10°19′E) has an area of 10 km^2 and is located in the Thyrrenian sea, midway between the mainland Italian coast and the French island of Corsica. Among other aspects, the island is renowned for its resident goat population, currently numbering to 200–250 heads. However, the origins of this population are unclear. While some scholars maintain that goats were introduced as a food resource for a monk community settled in the island from the 7th to the 16th century C.E., others favour a more ancient introduction (Masseti, 2009). Partial additions to the extant population in the late 19th century are documented, when the island served as a game reserve. Today the island is a protected area and is subjected to actions aimed at the eradication of some invasive species (http://www.montecristo2010.it/index.asp).

The goat population of Montecristo is interesting for several inter-related aspects. On one hand, the animals exhibit phenotypic traits that resemble domestic goats, such as a relatively small size, the body length/height at the withers ratio, and a wide variability of coat colors. On the other hand, other traits are reminiscent of the bezoar (*Capra aegagrus*) and other semi-wild goat populations of the Mediterranean area, such as the Cretan Agrimi goat (*C. aegagrus cretica*). The horns have a marked variability in shape (more prominent in males), with two basic types: a scimitar-shaped type, that bends backward with a modest basal angle, width at tip, and a narrow cross-sectional shape on which irregular longitudinal lumps develop with age, like the horns of the Agrimi goat (Ciani and Masseti, 1991), and a second type with more pronounced lateral growth and width at tip, similar to the horns of some domestic breeds (Spagnesi et al., 1986). These features then raise questions on the source population from which the founders of the Montecristo isolate were drawn, the timing of the first introduction(s) being also a potentially relevant information. In fact, possible observations favouring antiquity of the population would bear to the understanding of human maritime movements across the Mediterranean, which brought portable farm animals from the early domestication centres of the Middle East westward, to many large islands with stable human settlements.

In this work, we describe the complete mtDNA sequence of a male specimen captured on the island and translocated to the Rome zoo. We provide evidence that the presence of this sequence in Montecristo is the result of feralization from a pool of domestic matrilines. In addition, positioning of this sequence in the emerging phylogenetic tree for domestic goats (Luikart et al., 2001; Piras et al., 2012; Doro et al., 2014; Colli et al., 2015) reveals the spread of a lineage with multiple derived nucleotide states shared with lineages so far described also in North Asian breeds (Nomura

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et al., 2013).

2. Materials and methods

2.1. The specimen

A male goat currently kept at the Rome zoo with inventory n. 39648 was sampled. The animal was transferred from Montecristo in the frame of the E. U. project LIFE +08 NAT/IT/00353 Montecristo 2010.

The blood aliquot used for DNA preparation was part of a sample routinely collected during animal health surveys carried out by expert veterinary personnel, and its collection did not cause any additional burden to the animal.

For this study specific approval by a review board was not necessary, as none of the procedures used here met the criteria to define them experiments as defined in the EC Directive 86/609/EEC concerning ethical guidelines for care and use of animals for research.

2.2. DNA sequencing

The entire mtDNA was subdivided into 33 partially overlapping amplicons, amplified separately, as described (Doro et al., 2014). Hassanin et al. (2010) reported the existence of a numt similar to mtDNA positions 12,006–13,886. In order to re-check variants in the region, we replicated the amplification of the segment between positions 12,712 and 13,071 (in the reference sequence GU068049) with primers 27c-F (5′-CCT ACC TAG CAT TCC TCC AC-3′) and 27c-R (5′-TGA TGA GAG TTG GGA ATC GG-3′).

2.3. Data analysis

The complete sequence was aligned to the reference sequence GU068049 with Clustal (Thompson et al., 1997), as implemented in MEGA5 (Tamura et al., 2011). Throughout the text positions are numbered according to GU068049.

Assignment to the major mtDNA haplogroups (Hg) was obtained by using the entire Montecristo sequence as query in BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), limiting the search to the taxon Capra (taxid:9922).

A discrimination between domestic and wild goat sequences was obtained in an alignment including the overlapping 481 D-loop positions (15,709 to 16,190) of the 28 domestic sequences (Doro et al., 2014) and the 29 bezoar sequences affiliated with Hg A (Naderi et al., 2008). A neighbour joining tree was obtained with MEGA, by using the same parameters of these latter authors. The sequence KJ192236, derived from an Hg C domestic individual, served as an outgroup.

Assignment of the Montecristo sequence to Hg A subclades was obtained by considering the described allele arrangements (Piras et al., 2012), after renumbering the positions according to GU068049 (Supplemental Table 1).

Comparisons with the sequences reported by Nomura et al. (2013) was by visual inspection. Inference of amino acid substitutions in the Montecristo sequence was obtained by conceptual translation performed with MEGA. The complete sequence here obtained was submitted to GenBank and received Accession n. KR349363.

3. Results

Upon BLAST search, the entire Montecristo sequence turned out to display the maximum similarity with the complete sequence KR059181, obtained from a domestic animal (Colli et al., 2015), confirming its assignment to the common haplogroup A.

3.1. Comparisons involving the D-loop

We then wanted to assay whether the Montecristo sequence was more closely related to Hg A sequences obtained from domestic vs wild goats. To this aim we compared our sequence with those from bezoars (Naderi et al., 2008), obtaining a maximum identity of only 98.5%. The 29 sequences from Hg A bezoars scored at the top of the list. We then constructed a neighbour joining tree based on 481 positions of the D-loop (Fig. 1). In this tree the 29 Hg A bezoar and the 28 domestic sequences with firm assignment to one of the Hg A clades, formed distinct clusters. The Montecristo sequence fell in a quite deep branch, forming a clade with three domestic sequences. In particular, two variants distinguished the Montecristo sequence from all others in this alignment, i.e. 15756 (C) and 16048(G) (Table 1). On the other hand, the Montecristo sequence could be clearly assigned to the group of sequences defined by Piras et al. (2012) as clade A5, as it had the distinctive alleles at all four defining positions [15749(G)-15811(C)-15833(C)-16043(T)]. The Montecristo clade in Fig. 1 indeed included the remaining A5 sequences in the alignment.

In order to infer the most likely source population(s) for the Montecristo lineage, we took advantage of the large dataset assembled by Naderi et al. (2007), which also includes information on the geographical origin of each specimen. The 100 most similar sequences displayed an identity $>98.75\%$ with the Montecristo sequence, were all were affiliated with Hg A and 94 of them with clade A5. Ninety-eight of the 100 most similar sequences turned out to be sampled in Europe, the most represented countries being Portugal (25), Italy (17), Switzerland (16) and France (11). By contrast, the same search performed with the reference sequence GU068049 (derived from a White Cashmere animal) returned Asian sampling locations in 90/100 cases.

When compared with three mitochondrial segments obtained from Cretean Agrimi goats (Bar-Gal et al., 2002), the corresponding segments in the Montecristo sequence turned out to be identical to those obtained from five individuals (Hai-Bar and Agrimi 8, 10, 17, 21). This identity, however, was uninformative as far as all three segments display little variation in Hg A; it was therefore not possible to infer the affiliation of the Agrimi goats to any of the Hg A clades.

3.2. Variation outside the D-loop

Outside the D-loop, the Montecristo sequence displayed variants which distinguished it from GU068049 and all of the nearly-complete sequences reported by Doro et al. (2014). Among these, none of the 11 variants previously reported to be private for GU068049 was found in the Montecristo sequence. Additional 8 variants are listed in Table 1. A T/A transversion never described so far occurs in the gene for 16S RNA. The remaining 7 are protein-coding variants, of which 2 non-synonymous.

When searching in additional sequences, the 12,851 C/T variant was found in four sequences (GU229278, GU229279, GU229280 and GU229281) previously affiliated with HG's A, B, C and D). This position resides in a region of large divergence between the same four sequences and both GU068049 and NC_005044, attributable to the spurious presence of a numt sequence in the former ones (Hassanin et al., 2010). This prompted us to re-test the 12,851C/T variant in a PCR product obtained with novel primers (see Materials and Methods). This confirmed the presence of the T variant (Supplemental Fig. 1) but not many other variants, supporting 12,851(T) as a genuine mitochondrial variant of the Montecristo sequence.