

The first mitochondrial survey on the current population of the Maltese cattle breed testifies a strong and significant founder effect and a maternal influence from Northern Europe

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ABSTRACT

Local breeds represent an important and often unique pool of endangered sources of genetic variability, particularly when confined to an isolated geographic area. The Maltese breed of cattle is considered to be of ancient origin. Late Pleistocene Oxen skeletal remains and Neolithic representations of primitive cattle have been suggested as proof of a possible local domestication. The objective of the present study is to explore the current mitochondrial DNA (mtDNA) diversity of this breed in order to reconstruct its maternal origins and to identify any residual genetic variants to be preserved.

A mtDNA control-region analysis performed on the entire Maltese cattle population identified only two different mtDNAs (out of a total amount of 19 samples), one encompasses about 90% of the current population and confirms a strong founder effect on the mitochondrial gene pool; the remaining 10% seems to testify for the importation of British cattle, documented in historical records since 1809. The complete mtDNA has defined two novel clades T3c and T3d, both dated to ~9.5 thousand years ago, encompassing the Maltese breed and only a few other breeds of Northern European ancestry.

This new piece of information does not support the hypothesis of a local domestication in Malta since the Maltese cattle mtDNAs are nested within the known domestic founding lineage T3, but confirms a strong maternal influence from Northern Europe rather than from the African coastline.

BACKGROUND

The Maltese cattle: an ancient breed that might be traced back to the prehistoric periods

The skeletal evidence excavated from Late Pleistocene deposits and cave-paintings, suggests the presence of horned bovine in the Malta Islands (Figure 1) linked to the hunter Paleolithic populations (Anati & Anati 1988). Thus, it has been suggested that primitive cattle were not introduced into the Islands by the Neolithic populations, but rather they could have been already present in the wild and subsequently domesticated. Although the phenotypic traits resemble those of the modern Maltese breed, there is no scientific proof about their relationships.

Peculiar phenotypic traits

There is limited information on the Maltese breed of cattle, also known as "Il-Gendus Malti" (Figure 2). Historical sources have remarked that this indigenous breed is little known beyond the shores of Malta (Borg 1915). It has been described as phenotypically characterized by a large size and a coat of short reddish hair, with underlines slightly lighter (MacGill 1839), and kept exclusively for agricultural purposes and draft, but not as a dairy animal. Since only enough males were kept to propagate the breed, the level of inbreeding had to be significant. Indeed the genetic pool of the local breed quickly collapsed and therefore the use of other breeds in mating, slowly adulterated the original genetic pool resulting in a steady erosion of the original genetic profile. Since 1809, milk cows were imported by the British (Galt 1812; MacGill 1839). The origins of the breed are also shrouded by mystery: based on their phenotypic traits, Stockdale (1934) claims that the true Maltese cattle resemble the Spanish stock; more recently the Maltese breed was linked to the Egyptian Baladi cattle. In the early nineties a focused breeding program with the Chianina cattle and back breeding between offspring was used to increase numbers within the herd. The current population size is made up of 2 herds consisting of 12 males and 19 females.

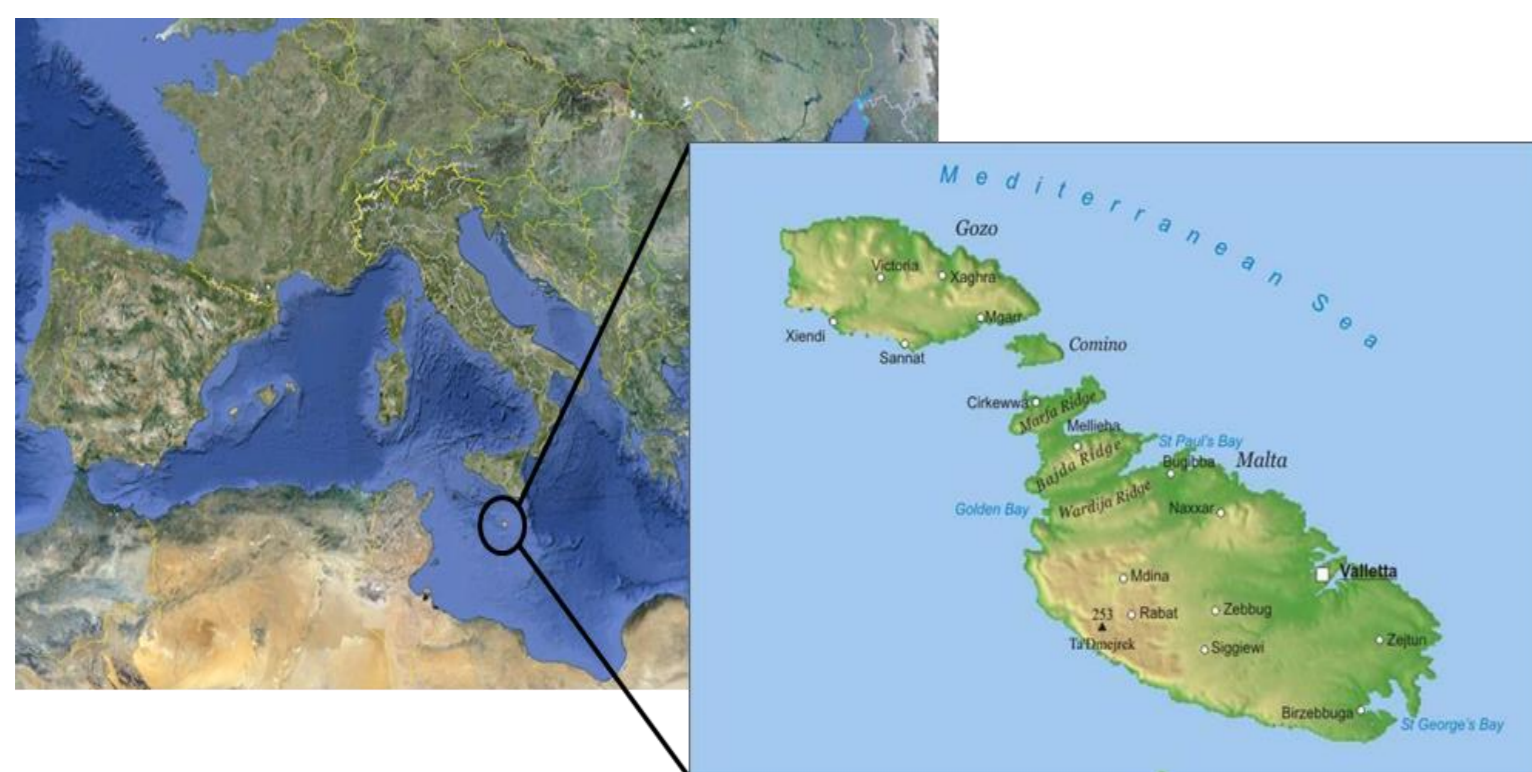


Figure 1. Geographical localization of Maltese Islands.



Figure 2. The photo shows a typical Maltese cow.

MATERIALS & METHODS

Sample collection: blood samples were collected from 19 healthy Maltese cattle, which represented most of the entire population.

Control region analysis: the first step in the mtDNA molecular analyses consisted of DNA extraction followed by PCR amplification and sequencing of the mtDNA control region. Electropherograms were aligned, assembled and compared to the bovine reference sequence (BRS, GenBank, accession number V00654) using the software Sequencher™ 5.1 (www.genecodes.com). The mutational differences relative to the BRS were registered.

Complete mtDNA sequencing: following published protocols (Achilli et al. 2008), the entire mtDNA was first amplified in 11 overlapping PCR fragments using a set of primers with matching annealing temperatures. After PCR, the fragments were purified using the EXOSAP enzymatic system. Sequencing reactions were performed by an external service using a set of nested specifically designed oligonucleotides.

Phylogeographic analysis: both control-region and complete mitogenome sequences were compared to the available mtDNA sequences in the GenBank database. Evolutionary relationships of the complete mitogenome sequences were evaluated through a tree construction performed by the Molecular Evolutionary Genetics Analysis Integrated tool (MEGA 6.06; www.megasoftware.net).

RESULTS & DISCUSSION

Initially, mtDNA control-region profiles of all 19 samples were determined by sequencing 731 base pairs (bps) between nucleotide positions (nps) 15823 and 215, thus including most of the D-loop segment (nps 15792-363). Data analyses identified only two mtDNA haplotypes: the first (HT1) differed from BRS by only two transitions; the second (HT2) specified by five different transitions. Both haplotypes could be classified as T3 (Achilli et al. 2009).

Only 2 samples were characterized by HT1, while 89.5% of samples showed the HT2 haplotype, confirming a strong founder effect on the mitochondrial gene pool. When compared to the other control region sequences deposited in GenBank, HT1 turned out to be identical to a sequence derived from the Ayrshire breed (AY378143), thus seems to testify for the importation of British cattle, documented in historical records since 1809. No perfect match was found for HT2. Complete sequencing of HT1 and HT2 mitogenomes confirmed their classification into haplogroup T3, which represents 88.6% of western Eurasian taurine mtDNAs (Bonfiglio et al. 2010). Maltese cattle haplotypes stood uniquely in the currently known cattle mitogenome phylogeny (Figure 3), creating two novel sub-clades, here named T3c and T3d (inset of Figure 3), both dated to ~9.5 thousand years ago, together with few closely related mitogenomes all of North European ancestry (White Park Cattle, Red Mountain Cattle and Angus).

Because T3 was one of the few founder lineages directly involved in the domestication event that occurred 10-11 ky ago in the Near East (Troy et al. 2001; Achilli et al. 2008), our results do not support the hypothesis of a local domestication in Malta and confirm a strong maternal influence from Northern Europe rather than from the surrounding Mediterranean areas.

This study highlights the importance of genetic surveys as a tool to reassess morphological inferences and supports the importance of monitoring geographically isolated groups that could conceal unique genetic variants. These variants might be targeted by focused conservation programs in order to avoid the dramatic and unrecoverable loss of biodiversity that is often triggered by the strong economic emphasis.

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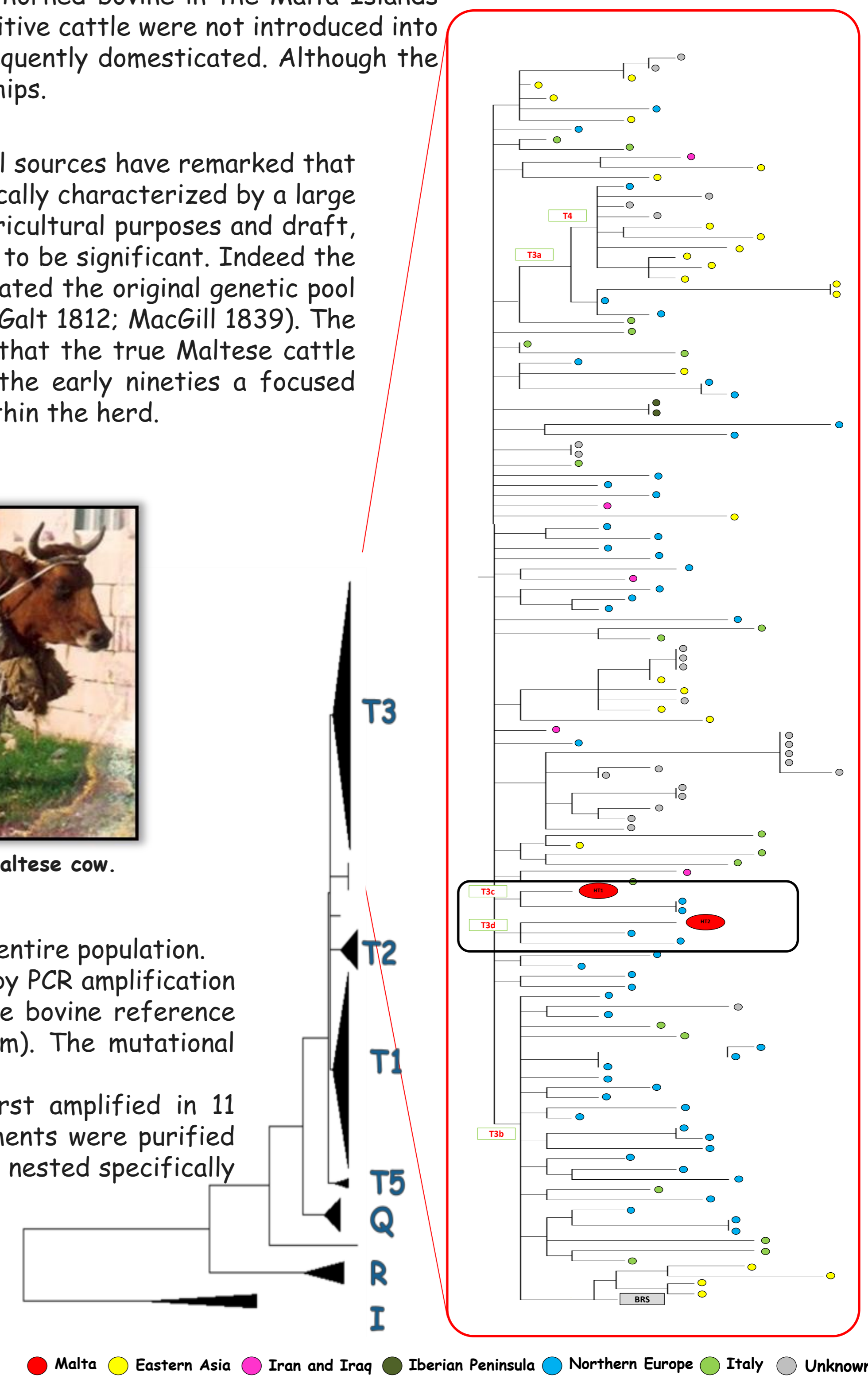


Figure 3. Schematic phylogeny of complete mtDNA sequences and detailed representation of the haplogroup T3. Samples are marked with different colors to identify their geographical origin. The two novel clades T3c and T3d are highlighted in the box.