



An overview of Maltese breed of cattle through mitochondrial DNA

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BACKGROUND

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

The skeletal evidence excavated from various Neolithic sites, suggest that the bull of Bos species that inhabited the Malta island (Figure 1) during that period was characterized by long horns and a large hump on the back over the shoulder, similar to that found in the various modern breeds of zebu (Bos indicus). Since oxen skeletal remains were found in Late Pleistocene deposits in Malta, it has been suggested that the Neolithic Ox was not introduced into the Island by the Neolithic man, but rather it could have been already present in a wild state and was subsequently domesticated. Although the phenotypic traits resemble those of the modern Maltese breed, there is no scientific proof about their relationship.

Peculiar phenotypic traits

There is limited information describing the Maltese breed of cattle, also known as the GENDUS (Figure 2). MacGill (1839) describes the Maltese breed as being "fine; the true beast of Malta is a stately animal and feeds to a great weight; they are kept for agricultural purposes and for breeding. Borg (1915), remarks that the breed is little known beyond the shores of Malta and is described as being well shaped of large size, generally having a reddish coat of short hair with underlines slightly lighter. Since only enough males were kept to propagate the breed, the level of inbreeding had to be significant. The references above seem to indicate that during the period, the genetic pool of the local breed was greatly reduced and therefore use of other breeds in mating, slowly adulterated the original genetic pool resulting in a steady erosion of the original genetic profile. Galt (1812) and MacGill (1839) asserted that the first milk cows were introduced by the British in 1809. The origins of the breed are also shrouded by mystery: 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 last decade a breeding programme on Chianina cattle with 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.

MATERIALS & METHODS

Sample Collection: blood samples were collected from 19 healthy Maltese cattle, from both available herds.

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. Elettropherograms were aligned, assembled and compared to the mtDNA complete 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 the same scheme previously published (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 (GE Healthcare). 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 GenBank database. Evolutionary relationships of complete mitogenome sequences were evaluated through a tree construction performed by the Molecular Evolutionary Genetics Analysis Integrated tool (MEGA 6.06; www.megasoftware.net).



Figure 1. The Maltese Islands: a strip of landmasses in the heart of Mediterranean Sea.

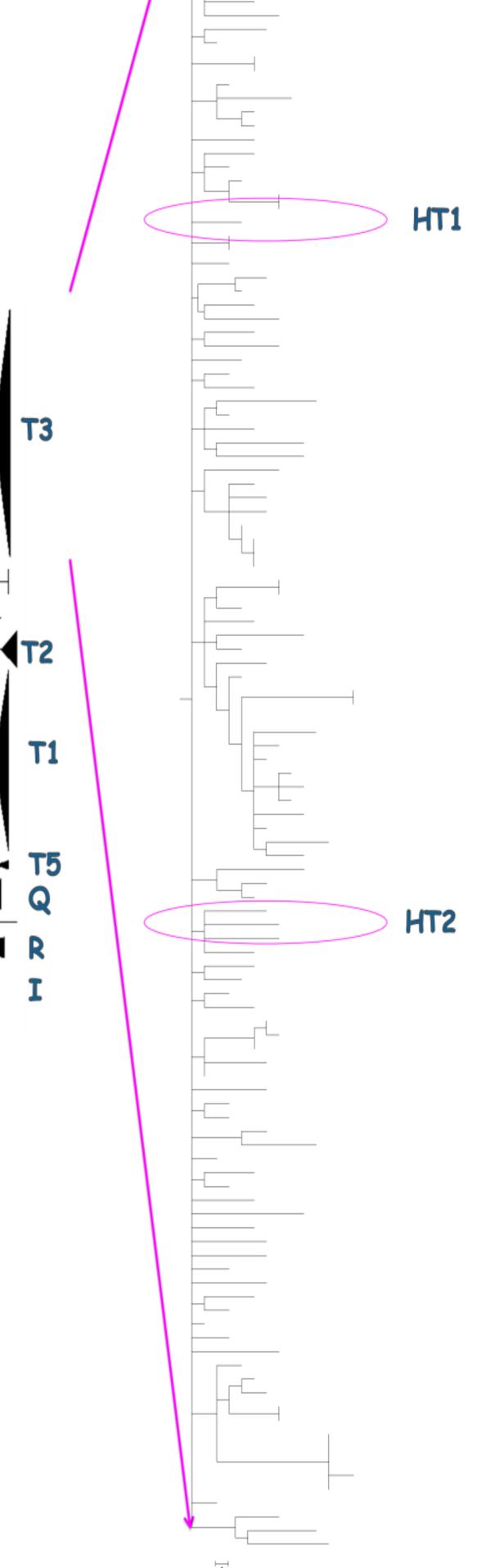


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Figure 2. Maltese cattle.

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 (at nps 24 and 169); the second (HT2) specified by five transitions at nps 16022, 16231, 169, 174 and 190 (see table below).



Original ID	Breed	Control region haplotype	from	to	Haplotype
MLT04	Maltese Cattle	24, 169	15823	215	HT1
MLT10	Maltese Cattle	16022, 16231, 169, 174, 190	15823	215	HT2

Only 2 samples were characterized by HT1, while 89.5% of samples showed the HT2 haplotype. According to the control region mutational diagnostic motifs (Achilli et al., 2009), both HT1 and HT2 could be classified as haplogroup T3. When compared to the other control region sequences deposited in GenBank, HT2 resulted a unique mtDNA haplotype belonging to haplogroup T3, while HT1 turned out to be identical to the GenBank sequence registered in 2003 as AY378143 (Xuebin et al., direct submission) and belonging to the Ayrshire cattle, a breed of dairy cattle originating from Ayrshire in south west Scotland. Such an identity confirms that approximately 10% of Maltese Cattle population matches with the Ayrshire breed, thus genetically confirming the well documented post-war importation of English cattle.

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).

These haplotypes have no perfect matches in the currently known cattle mitogenome phylogeny (Figure 3). HT1 directly diverges from the root of T3, also considering that the previously mentioned GenBank record AY378143 has no coding-region sequence available. On the other side, HT2 mitogenome clusters with three samples (EU177816, KJ709686 and AY676873) from Chianina, Red Mountain Cattle and Angus-X breeds. It is well known that Chianina and Red Mountain Cattle are ancient breeds typical of southern Europe, while Angus was largely used to improve the genetic quality of various cattle herds. The HT2 clustering might indicate a southern European origin and support its primitive origin from ancient taurine breeds. Only thanks to the refinement of the mtDNA phylogeny with all Mediterranean cattle breeds, including North African regions, could improve the knowledge about Maltese cattle.

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Figure 3. Schematic phylogeny of complete mtDNA sequences and detailed representation of the haplogroup T3. The two Maltese haplotypes are indicated by circles.

Concluding remarks

The phenotypic peculiarities of Maltese cattle inspired the present genetic analysis, which aimed to reconstruct the maternal origin of this geographically isolated breed, strongly affected over time by anthropogenic activities. Our findings confirmed a strong founder effect, with a unique haplotype representing about 90% of the current population, which might be phylogenetically linked to ancient Mediterranean breeds, such as Chianina and Red Mountain Cattle.

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