Forensic Science International 245 (2014) 51–57

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

An old dog and new tricks: Genetic analysis of a Tudor dog recovered from the *Mary Rose* wreck

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ARTICLE INFO

Article history:
Received 22 April 2014
Received in revised form 23 August 2014
Accepted 4 October 2014
Available online 14 October 2014

Keywords:
Ancient DNA
Mary Rose
Terrier breed
Canine coat colour
SLC2A9

ABSTRACT

The Tudor warship the *Mary Rose* sank in the Solent waters between Portsmouth and the Isle of Wight on the 19th of July 1545, whilst engaging a French invasion fleet. The ship was rediscovered in 1971 and between 1979 and 1982 the entire contents of the ship were excavated resulting in the recovery of over 25,000 objects, including the skeleton of a small to medium sized dog referred to as the *Mary Rose Dog* (MRD). Here we report the extraction and analysis of both mitochondrial and genomic DNA from a tooth of this animal. Our results show that the MRD was a young male of a terrier type most closely related to modern Jack Russell Terriers with a light to dark brown coat colour. Interestingly, given the antiquity of the sample, the dog was heterozygotic for the SLC2A9 gene variant that leads to hyperuricosuria when found in modern homozygotic animals. These findings help shed light on a notable historical artefact from an important period in the development of modern dog breeds.

1. Introduction

1.1. Historical context and rediscovery

At 700 tonnes and provisioned with 91 guns the *Mary Rose* was one of the largest of Henry VIII's warships. Listed with a crew of 185 soldiers, 200 mariners and 30 gunners, she was a state of the art fighting machine. She sank in the Solent whilst engaging a French invasion fleet on the 19th of July 1545. The ship sank on her starboard side to a depth of 12 m. Tidal action carried silts in suspension across the wreck site, these sediments produced anoxic conditions resulting in excellent preservation conditions. The ship was rediscovered in 1971 and between 1979 and 1982 the entire contents of the ship were excavated and the wreck raised [1]. The raised wreck included the hold of the ship and the starboard side with portions of four decks surviving. The hull was opened to the public in 1983 and subsequently a museum containing recovered artefacts opened in 1984. A new museum built around the hull opened in early summer 2013.

1.2. Location and recovery of the sample

Between the 12th July and 14th October 1981, the nearly complete remains of a small dog were recovered as four separate samples (MR81S0215, MR81S0264, MR81S0328 and MR81S0444). Each sample contained animal bones together with associated sediment, so that any articulated bones remained at least closely associated, the remains were found within a 2 m radius of each other in the stern (Fig. 1a). Three of the samples were found on the main deck of the ship, and the fourth (MR81S0328) directly below on the orlop deck. Two of the three samples from the main deck (MR81S0215, MR81S0264) were found within gaps created by the edges of five chests which had slid across the main deck as the ship heeled to starboard during the sinking. These chests came to rest against a partition, later identified as one of the walls of the carpenter's cabin (Fig. 1b).

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http://dx.doi.org/10.1016/j.forsciint.2014.10.001
0379-0738/

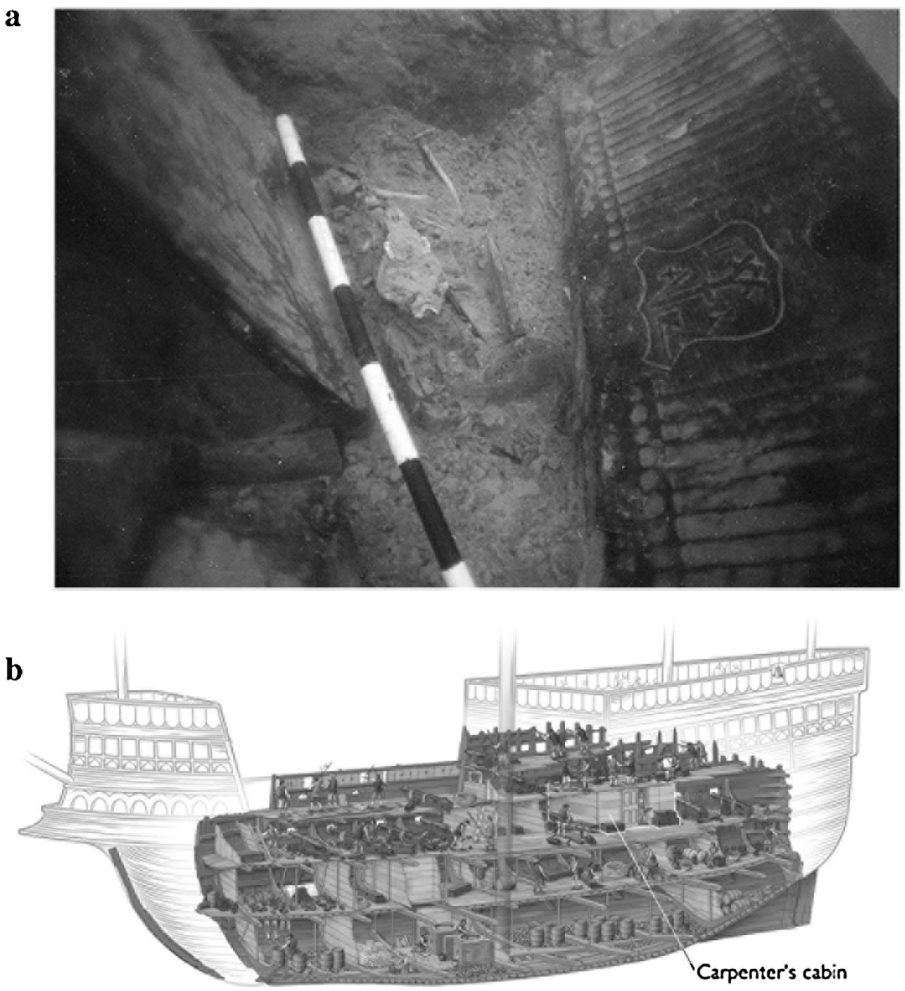

Fig. 1. Location of the remains of the *Mary Rose Dog*. (a) Photograph of the location of the recovery site and (b) schematic showing the location of the carpenters cabin in the intact ship.

The third sample from the main deck (MR81S0444) was found inside the cabin, directly in line with a sliding door which was found partly open and into which the edge of one chest had penetrated. As the distribution of the bones was haphazard it was not easy to determine whether the dog drowned whilst inside or outside the cabin, although the position of the skull suggested the most likely position of the dog was just outside the cabin. The remains were assigned as Feature 69, later becoming known as the *Mary Rose Dog* (MRD). Here we describe genetic analysis of the both mitochondrial and genomic DNA extracted from the tooth of the MRD.

2. Materials and methods

2.1. DNA extraction and PCR amplification

The teeth were washed initially with Decon (5% solution), subsequently washed briefly with 1% bleach, rinsed with distilled water and dried in a clean UV laminar flow overnight. The outer layer was sandblasted with sterile sandblaster dish and pulverised with a flamed drill. The powder was collected in sterile plastic containers and stored in Lo-Bind DNA free tubes (Eppendorf) at 20 °C. Glassware was soaked overnight in 1 M HCl, rinsed with double distilled water, autoclaved at 135 °C, baked at 100 °C for 12 h and were exposed to UV for 24 h. Disposable plastic ware was manufacturer irradiated and autoclaved, solutions were prepared fresh and exposed to UV 2–3 h before use. DNA was extracted with an adapted spin column isolation technique [2] or the method of Binladen et al. [3]. All DNA isolation and PCR setup were conducted in dedicated ancient DNA Laboratory facilities at the University of Portsmouth and Durham University. Strict contamination controls were exercised throughout all steps according to commonly accepted recommendations [4,5]. No contamination was detected in the negative controls of DNA extraction or PCR amplification.

2.2. Confirmation of selected amplifications

The use of species-specific primers excluded the possibility of amplification of human DNA [6]. To check for the possibility of other contamination, mitochondrial analysis of the samples were independently validated at a separate ancient DNA laboratory, located in the Department of Archaeology at Durham University, UK. Mitochondrial PCR was successful and upon sequencing the DNA aligned with the sequences produced at Portsmouth.

2.3. Mitochondrial PCR analysis

Mitochondrial HVI DNA sequences were amplified by two overlapping amplicons [7,8]. Resulting HVI sequences were aligned using the programme of DNA alignment (www.fluxus-engineering.com). After removing primer sequences and accommodating previously published sequences [9], sequence lengths