



Ritual complexity in a past community revealed by ancient DNA analysis of pre-colonial terracotta items from Northern Ghana

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ABSTRACT

The pre-colonial 6th–14th century terracotta forms of Koma Land, Northern Ghana, contain cavities which may have been intended to hold liquids. These have been linked to traditional African libation, but the specific nature of their contents is unclear. We used generic polymerase chain reactions that would amplify DNA from a range of plant and fungal species in order to identify remains of libations applied to fourteen terracotta items. We anticipated difficulties in distinguishing genuine ancient DNA sequences from those resulting from contaminating material, and therefore also carried out a series of control experiments to assess the extent to which the samples had become contaminated with exogenous DNA during burial, excavation and downstream analysis. Taking account of the results of the control experiments, as well as the difficulties in assigning matches between ancient DNA sequences and database entries, we provide evidence for the use of three different types of plant – plantain/banana, pine and grasses – in libations associated with the terracotta items. We also identified DNA from *Coniochaeta* yeast within the mouth cavity of one figurine, suggesting that this structure was burnt prior to deposition.

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1. Introduction

The striking terracottas from Koma Land, Northern Ghana, are a compelling record of a 6th–14th century AD community for which there is no written history or oral tradition (Kröger, 1988). These anthropomorphic, zoomorphic and conical pottery items have drawn considerable attention since anthropological studies and excavation of four mounds in the region in the 1980s (Anquandah, 1987, 1998, 2003; Kröger, 1988), and the excavation of two further mounds in 2007–2011 (Kankpeyeng and Nkumbaan, 2008, 2009; Kröger and Saibu, 2010; Kankpeyeng et al., 2011, 2013; Insoll et al., 2012, 2013).

Koma Land, in Northern Ghana, covers an area of approximately

100 km² (Anquandah, 1998; Kankpeyeng et al., 2013) and features hundreds of mounds 4–35 m in diameter, with a prominent mound cluster in the village of Yikpabongo. Some of the mounds contain human remains (Anquandah, 1987), and it has been proposed that others functioned for medicinal rituals or shrine worship (Kankpeyeng et al., 2011), perhaps as places where ritually powerful materials, including the figurines, were deposited (Insoll et al., 2012). The mounds were initially dated by thermoluminescence techniques to the 15th–17th centuries AD, although this chronology was subsequently extended to c.1200–1800 AD (Anquandah, 1998).

An early anthropological survey of Yikpabongo and nearby villages equated the locally spoken Mampruli language with the modern Koma language Konni, and concluded that the society which produced the pottery items was continuous with the modern population (Rattray, 1932). However, a subsequent survey recognized Konni as having developed separately (Naden, 1986), and it is now widely accepted that the modern Koma population migrated to this region, most likely arriving after 1880 (Kröger and

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Saibu, 2010). Local oral traditions recount the discovery of ruins at the site, for which it was named Yikpabongo ('forest ruins') (Dagan, 1989). It is suggested that the earlier pottery-producing society may have abandoned their location due to pressures introduced by disease, famine or the slave trade (Insoll et al., 2012; Kankpeyeng et al., 2013). This community is thought to have occupied Yikpabongo between the 6th/7th and 13th/14th centuries AD (Kankpeyeng and Nkumbaan, 2008, 2009; Kankpeyeng et al., 2011, 2013).

Many of the terracotta structures recovered from the mounds feature cavities up to 20 mm in depth, as visualized by tube current modulation computer tomography (Insoll et al., 2012, in press). In figurines, these cavities typically originate from the top of the head, ear, nostril or mouth, and in cones the cavities extend downwards from the bowl. It has been suggested that these cavities were reservoirs for libation (Insoll et al., 2012), the liquid ceremonial offerings that can form a component of indigenous African religious practice (Kilson, 1969; Mbiti, 1990; Essien, 2014; Essel, 2014).

Pre-colonial West African libation is documented as having been offered with palm wine (Baum, 1999; Essel, 2014) and cola nut infusions (Kröger and Saibu, 2010), sorghum or millet beer (Mbiti, 1990; Mulemi, 2004; Kröger and Saibu, 2010), water (Mbiti, 1990; Baum, 1999; Kröger and Saibu, 2010; Essel, 2014), and in some instances, milk (Mbiti, 1990) or animal blood (Baum, 1999). The present Yikpabongo population cultivates pearl millet, sorghum, African rice, yam and cassava (Kröger and Saibu, 2010), all of which can be fermented (Jespersen, 2003; Mulemi, 2004; Mukisa et al., 2012; Lentz, 2013). Additionally, bark/leaf infusions or pastes of 'magic' and medicinal plants (Myren, 2011; van Andel et al., 2012) may have been applied to the items, given the suggested disease and scapegoat themes in some of the figurine designs (Kankpeyeng et al., 2011; Insoll, 2015).

Analysis of ancient DNA (aDNA) from high temperature environments is usually considered inadvisable (Murray et al., 2012; Campana et al., 2013), as heat accelerates DNA hydrolysis and oxidation, reducing the likelihood of DNA sequence recovery (Pääbo and Wilson, 1991; Burger et al., 1999; Krings et al., 1999; Reed et al., 2003; Willerslev and Cooper, 2005; Lorenzen and Willerslev, 2010). Ancient DNA sequences have only infrequently been recovered from warmer latitudes, typically from sites with cool microclimates (Poinar et al., 2003; Gutiérrez-García et al., 2014), such as caves, which were the source of the first ancient African human genome (Gallego Llorente et al., 2015). One notable exception is the recovery of aDNA from 17th century human remains from the tropical Caribbean island of St. Martin (Schroeder et al., 2015). In North Africa, DNA has been shown to degrade to fragments <100 bp in length within 2000 years (Marota et al., 2002; Hekkala et al., 2011; Khairat et al., 2013). However, only a third of Africa shares the 'hot desert' climate of the North (Kottek et al., 2006), and aDNA sequences up to 160 bp have successfully been recovered from 2000-year-old desert cattle bones from Mali in West Africa and Eritrea in the East (Edwards et al., 2004; Asuncion et al., 2007). The possible contribution of aDNA studies in Sub-Saharan and central temperate and sub-tropical Africa should therefore not be overlooked, especially in view of the archaeological richness of these regions (e.g. McIntosh, 1994, 2005; Yellen, 1998; Insoll, 2003; Stahl, 2004).

Non-destructive, forensic-inspired swab recovery methods can efficiently recover aDNA from pottery, enabling systematic studies of large pottery assemblages without the typical risks associated with sampling delicate artefacts (Foley et al., 2012). Here we report the use of these methods to assess the usage of various terracotta items excavated in Koma Land.

2. Materials and methods

2.1. Terracotta items

Samples were taken from nine terracotta figurines, two cones and three circular disc/horn stoppers from mound YK07 (Kankpeyeng and Nkumbaan, 2008, 2009) and mound YK10-3/YK11 (Kankpeyeng et al., 2011, 2013; Insoll et al., 2012), both in Yikpabongo village, Koma Land (Fig. 1, Fig. 2, Table 1). The items collectively span the five Koma Land pottery forms (Kröger, 1988): cones with heads (category I), anthropomorphic figures (II), Janiform figures, (III) animal forms (IV), and other uninterpreted forms (V). These items were excavated over four seasons (Kankpeyeng and Nkumbaan, 2009; Insoll et al., 2012), including two seasons during which excavators wore gloves and implemented packing procedures in anticipation of downstream aDNA analysis (Insoll et al., 2012). Thermal age estimates (Smith et al., 2003) for the excavation series ranged from 18,567 to 28,929 10^3 °C thermal years, with predicted mean fragment lengths of 13–19 bp, based on the degradation pattern of bone aDNA (Table 2), placing the terracotta items close to the likely threshold for aDNA recovery. Samples were taken with permission of the Ghana National Museum, Accra.

2.2. Ancient DNA authentication regime

Sampling was carried out under clean conditions at the Manchester Museum on work surfaces covered with two layers of aluminium foil. Personnel wore protective clothing including forensic suits, face masks, hair nets, goggles and two pairs of sterile gloves at all times, and all utensils and equipment were treated with DNA-Away (Thermo Scientific) before and after use.

Ancient DNA analyses were performed in the aDNA laboratories of the University of Manchester in a suite of independent, physically isolated laboratories, each with an ultrafiltered air supply maintaining positive displacement pressure and a managed access system. All surfaces within the laboratories were periodically sterilized by UV irradiation and cleaned with 5% bleach and 70% ethanol, and all utensils and equipment were treated with DNA-Away before and after use. Plasticware such as test tubes were UV irradiated (254 nm, 120,000 $\mu\text{J cm}^{-2}$ for 2×5 min, with 180° rotation between the two exposures) before use. Aqueous solutions were similarly irradiated for 15 min. Personnel wore protective clothing as described above at all times. DNA extractions were carried out in a Class II biological safety cabinet in one laboratory within the facility, and PCRs were set up in a laminar flow cabinet in a second, physically-isolated laboratory. All DNA extractions were accompanied by at least two sample blanks (normal extraction but without sample), and every set of eight PCRs was accompanied by at least one PCR blank (set up with water rather than DNA extract). A positive PCR (i.e. product of correct size and meaningful sequence) was only considered authentic after replication with a second PCR of the same extract.

2.3. Sampling and DNA analyses

Samples were taken from various cavities and surfaces from the terracotta items. Following removal of soil with a sterile pick, each cavity was sampled with a sterile rayon swab soaked in Tween 80 + lecithin buffered to pH 7.4 with sodium thioglycolate (Scientific Laboratory Supplies) or, for smaller cavities, with sterile glass wool swabs prepared in the aDNA laboratory, dipped in lysis buffer A (Promega Wizard Magnetic DNA Purification System for Food) at the point of sampling. Tubes of lysis buffer A were also opened on the workbench prior to the sampling of each item to provide the sample blanks.

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