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Tracking the origin of worked elephant ivory of a medieval chess piece from Belgium through analysis of ancient DNA

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Abstract

The morphological identification of raw biological material used to produce archaeological artefacts is sometimes difficult or even impossible. In recent years, newly developed biochemical techniques have allowed more reliable identification of exploited animal species, even for otherwise taxonomically undiagnostic fragments, and thus can help pinpoint the geographical origin of the raw material. However, in addition to being costly, these techniques involve destructive sampling. This explains why they are rarely applied to archaeological artefacts, especially those made of precious, imported raw material or those representing intact works of art. Here, we analysed the ancient DNA (aDNA) of a medieval chess piece made of ivory of unknown origin, recovered from a medieval settlement in Jambes (Namur), Belgium. This chess piece was broken during excavation. We took this unfortunate event as an opportunity to perform aDNA extraction, to try to answer three questions: 1) What Proboscidean species does the ivory come from?; 2) Can we establish the geographic origin of the ivory more precisely?; and 3) Does doing so help our understanding of (part of) the trade route followed by the ivory? We sequenced two short fragments of the mitochondrial genome and compared them with publicly available DNA data. This enabled the identification of the raw material as an African elephant (genus *Loxodonta*). Although the results cannot exclude that the ivory comes from a forest elephant, the recovered DNA sequence is currently found only among savanna elephant DNA records. The ivory likely originates from an eastern or southern African country and was therefore probably transported along the African trade route passing through the Swahili Corridor. However, the precise itinerary followed by this ivory from the African shore of the Mediterranean Sea to Europe, and then to the archaeological site from which it was recovered, remains unknown. Such identification contributes to documenting past trade networks and long-distance exchange.

1. Introduction

The use of Proboscidean ivory for figurative carving has a long tradition in Europe, dating back to the early Upper Palaeolithic (Conard, 2003). With the contraction of the range of the woolly mammoth (*Mammuthus primigenius*), which became restricted to Siberia from around 12,000 BC onward (Stuart et al., 2002), ivory was no longer available as a local resource, except as fossilised material. However, between the disappearance of the mammoth and the use of fossil mammoth ivory recovered from the Siberian permafrost, mostly exploited for crafts from as late as the 19th century AD onward (Raubenheimer et al., 1990; Rijkelijkhuizen, 2008), Proboscidean ivory was nevertheless used for carving in Europe, as for more than two millennia, elephants were the prime source of ivory. Other mammals were also exploited, mainly marine mammals, such as sperm whale (*Physeter macrocephalus*), walrus (*Odobenus rosmarus*) and narwal (*Monodon monoceros*), in a European context, or hippo (*Hippopotamus amphibius*), around the Mediterranean Sea (MacGregor, 1985). Whatever its geographic origin, from the Holocene onwards, ivory exploited in western Europe had to have been imported, either from northern regions, in the case of marine mammals, or from eastern or southern climes, in the case of elephant ivory. As a luxury product tied to long-distance exchange, ivory long remained an exclusive product reserved for the upper classes. In this context, the import of ivory, either as raw material or as a finished object, constitutes powerful evidence for tracing ancient, long-distance trade routes.

Extant elephants are divided into two genera, encompassing the African elephants (genus *Loxodonta*) and the Asian elephant (genus *Elephas*). Modern DNA analyses, including more comprehensive genomic analyses, support the distinction of two African species, which were formerly considered subspecies of the same species: the African forest elephant (*Loxodonta cyclotis*) and the African savanna elephant (*Loxodonta africana*) (Rohland et al., 2007; Shetty & Vidya, 2011). Nowadays, both species inhabit territories south of the Sahara (Figure 1), with the African savanna elephant mostly found in the eastern and southern parts of Africa and the African forest elephant mostly found in the western and central parts (Wittemyer, 2011). However, the current distribution does not necessarily reflect historical distributions. In addition, African savanna elephants have been reported from Central and West Africa, in transitional environments between forest and savanna where the two species can overlap and potentially interbreed (Mondol et al., 2015). Potential hybrids ($P > 0.95$; Figure 1) have been detected by means of genetics in the Garamba region of the north-eastern part of the Democratic Republic of Congo; along the border between Uganda and the Democratic Republic of Congo; in the northern part of the Central African Republic; and along the border between Benin and Burkina Faso (Mondol et al., 2015).

Although the African elephant was once present in North Africa (Figure 1), it went extinct as a consequence of Roman overexploitation, probably well before 600 AD (see Guérin, 2013, for a summary) and perhaps starting as early as the 2nd century AD (Ogata, 2017). The taxonomic status of this extinct North African elephant, frequently named *Loxodonta africana pharaoensis*, remains unclear.

The Asian elephant (*Elephas maximus*) ranges west to east from India to southern China, and south into Indonesia, where it mostly occurs as isolated populations. Formerly, it has been proposed that the natural range of the Asian elephant extended even farther westward, into Mesopotamia, i.e., modern Iraq, Syria and southern Turkey (e.g., Olivier, 1978), where it would have formed a relict population described as the Syrian elephant (*Elephas maximus asurus*; Deraniyagala, 1955). However, this hypothesis of a natural occurrence of the Asian elephant in Mesopotamia has been questioned by several scholars, from both a biological and an archaeological standpoint (Caubet & Poplin, 2010; Lister et al., 2013; Vila, 2014).

Recently, Çakırlar & Ikram (2016) reviewed the available evidence and proposed that this local population of the Asian elephant, or Syrian elephant, was not endemic but was instead introduced to Mesopotamia by humans; captive animals eventually returned to the wild to form naturally breeding populations, between approximately 1800 and 800/700 BC.

Macroscopic traits generally enable the distinction between ivory from Proboscidean and other animal groups because Proboscidean ivory displays a characteristic 'chevron' pattern visible in cross-section. This pattern is created by intersecting lines called Schreger lines (Espinoza & Mann, 1992). Measuring the angles formed by these lines generally allows the separation of mammoth (*Mammuthus primigenius*) from elephant (*Loxodonta* sp. and *Elephas maximus*) ivory, as Schreger lines form acute angles in mammoth and obtuse angles in living species, although some overlap exists between 90° and 115° (Espinoza & Mann, 1992). More research is needed to assess the potential of Schreger angles for distinguishing between African elephants *lato sensu* and Asian elephant (Trapani & Fisher, 2003). Inner Schreger angles, which are those formed close to the pulp cavity, are poorly visible and should be avoided as diagnostic features; taxonomic distinction should be made using the angles of the outer Schreger lines only (Espinoza & Mann, 1992). During study of these artefacts, these characteristics are sometimes difficult to assess, in particular in small or thin objects, depending on how the piece was extracted from the tusk.

As it is not possible to attribute elephant ivory to species using morphologic characteristics, identification tools employing spectrometry (which is also non-destructive) and biochemical analysis (which is destructive) have been tested. Among these non-destructive techniques, X-ray fluorescence has been shown to be effective in differentiating between African elephant and Asian elephant (Buddhachat et al., 2016), and near-infrared Fourier transform Raman spectroscopy (NIR FT-Raman), in distinguishing between ivory from mammoth and ivory from elephant, but also between ivory from African forest elephant tusks, sometimes called 'hard ivory', and that from African savanna elephant tusks, termed 'soft ivory' (Shimoyama et al., 1997; Shimoyama et al., 2003). Destructive biomolecular techniques, including those involving the study of isotopes, proteins, and DNA, can allow the identification of the taxon from which the ivory derives and thus help pinpoint the geographic origin of the ivory more precisely. These destructive biomolecular techniques were primarily used to help fight elephant poaching, through the identification of ivory sold on the international market in recent times. Both large mitochondrial DNA fragments and nuclear loci (microsatellite data) were used to investigate the geographic origin of ivory seizures (Comstock et al., 2003; Ishida et al., 2013; Mailand & Wasser, 2007; Wasser et al., 2015; Wasser et al., 2007). Today, the ivory trade is tightly controlled.

Biomolecular techniques have also been applied to archaeological ivory material. Isotopic analyses were successfully conducted on hippo and elephant ivory from the 14th-century BC shipwreck at Uluburun, Turkey (Lafrenz, 2004); on elephant ivory from a 17–18th century AD site in Amsterdam, the Netherlands (Rijkelijkhuizen et al., 2015); and on elephant ivory from a 19th century AD site in East Africa (Coutu, Lee-Thorp, et al., 2016). Combined analyses of isotopes and peptides have also proved successful, on 7th–10th-century AD elephant ivory from KwaZulu-Natal, South Africa (Coutu, Whitelaw, et al., 2016). More recently, broad genomic data collected from a post-medieval unmodified ivory tooth found in Portugal, as well as from the raw ivory of the Portuguese *Bom Jesus* shipwreck, provided evidence for historical ivory trade between West Africa and Portugal (de Flamingh et al., 2021; Psonis et al., 2020). However, aDNA analysis is seldom applied to archaeological artefacts to track raw material sources.

Researchers are inhibited from applying such destructive techniques to intact archaeological objects made of ivory for two main reasons. These are rare finds in the archaeological record, frequently carved into works of art that are precious testimonies of past human cultures. This pitfall was noted by François Poplin (2012), who nevertheless recognised at the time that aDNA studies are key to future research into raw materials. In addition, the proteins and ancient DNA are ofttimes degraded, and the success of the analysis is therefore not guaranteed. Since then, a combination of short mitochondrial and nuclear DNA sequences has been shown to be useful for identifying elephant species from old tissue samples (Cappellini et al., 2014; Ngatia et al., 2019). There are few reports of DNA analysis on worked ivory in the scientific literature, especially of archaeological worked ivory. A few communications by wildlife forensic investigators (e.g., the U.S. Fish & Wildlife Service Forensics Laboratory) used DNA extracted from recent ivory carvings or idols to identify elephant species (Gupta et al., 2011). Recently, Ewart et al. (2020) optimised DNA extraction protocols on both raw and worked elephant ivory and demonstrated that powdering the ivory directly with a drill, as well as short decalcification times (2 hours), produces results comparable to sample powdering in liquid nitrogen and a longer decalcification time (3 days). In addition, sampling the cementum and using a total demineralisation DNA extraction method contribute to minimising the amount of elephant ivory that is necessary in order to recover DNA (Winters et al., 2018). These improvements may encourage more frequent DNA analysis, to better complement the other biomolecular approaches that are already available for worked archaeological ivory.

We had the opportunity to study a finished chess piece made of ivory dating to before the 13th century AD, found in a medieval context during an archaeological rescue excavation conducted in Jambes (Namur, Belgium). The piece was slightly damaged during excavation, which allowed us to sample small fragments of the inner part of the object that could not be refit during the restoration process. They were tested for the presence of aDNA in the hope of identifying the Proboscidean species from which the ivory originates and, additionally, to try to establish the geographical origin of the ivory, as this could help reconstruct medieval trade routes.

2. Material and methods

Archaeological excavation performed in 2017 along the Meuse River in Jambes, part of the modern-day city of Namur (Belgium; Figure 1), brought to light destruction layers of a medieval building that are associated with a fire event. This building is interpreted as a habitation, possibly including horse stables on the ground floor. Several remarkable finds, such as a coffer key made of copper alloy and the ivory chess piece investigated here, underline the aristocratic status of the inhabitants, who likely belonged to the Namur elite. If the keeping of horses were proven, this would further underline their status (Vanmechelen et al., 2018b).

The ivory chess piece (inventory number NR.17.JAMA.01045.0002; Figure 2) is the first such item to be discovered from a secure archaeological context in Wallonia (the southern half of Belgium). The fire event that destroyed the building where the chess piece was discovered is dated to the beginning of the 13th century AD based on the associated material culture (Vanmechelen et al., 2018a, 2018b). This gives a *terminus ante quem* for the dating of this chess piece. Its schematic, or abstract, shape corresponds to set A of the two style sets established by Anna Contadini (1995) for the collection of the Ashmolean Museum (Oxford). Style set B comprises pieces that represent figures in a more naturalistic way. With the documentation currently available, it is not possible to establish a chronology for these two styles or to know precisely when chess pieces became abstract, since no chess piece older

than the 9th century AD has yet been discovered; but it is likely that both styles co-existed before the Islamic era (Contadini, 1995). Pieces related to style set A are the oldest known, appearing as early as the 9th century AD, but are in use until the 15th century AD. In contrast, the scarcer pieces of style set B appear later (Contadini, 1995). The protrusion at the front of the Jambes chess piece symbolises the horse protome, which identifies the piece as a knight (Contadini, 1995; Grandet & Goret, 2012). Similar though not identical knights have been recovered from other countries in north-western Europe, such as France, at the castles of Rougemont-le-Château (prior to the 13th century AD), Crèvecoeur-en-Auge (11th–12th century AD) and Châtenois (11th century AD) (Grandet & Goret, 2012). However, the Jambes knight appears more abstract in shape than these examples.

For the purpose of analysing the DNA, a total of 96 mg of ivory powder was collected from a small ivory fragment from the inner part of the chess piece that was accidentally detached during excavation. DNA extraction was performed in a DNA lab dedicated to ancient DNA, equipped with UV lamps, under positive air pressure to avoid contamination, and where Elephantidae had never before been analysed. Best practices recommended for working with ancient DNA were applied (Gilbert et al., 2005; Willerslev & Cooper, 2005). In particular, UV disinfection was applied before and after each experiment. Clean lab coats, masks, shoe covers, and hair caps were worn for each experiment. Gloves were changed after each tube opening. Contacts with other DNA labs were banned (only sterile material was used, and access to other labs was not permitted before or during the ancient DNA analysis). Extraction negatives (samples treated like all others but without any ivory powder inside) were included in all experiments. For DNA extraction, first the outer layer of the ivory was removed, by scraping off its surface using a structured tooth tungsten carbide cutter attached to a hand rotary tool (8100 8v Max Rotary Tool). After 10 minutes of exposure to UV, ivory powder was collected by drilling inside the ivory fragment, using the hand rotary tool at 5000 rpm, with an engraving cutter (1.6 mm). DNA was extracted from the ivory powder following the protocol of Dabney et al. (2013) and was eluted twice in 45 µl of Tris-EDTA buffer with Tween-20. From this extracted DNA, we attempted to amplify six short DNA fragments (four mitochondrial and two nuclear fragments) by polymerase chain reaction (PCR). The short mitochondrial DNA fragments were selected because they contain diagnostic positions to discriminate between the Asian elephant (genus *Elephas*) and the African elephants (genus *Loxodonta*) and may provide some information on the geographic origin of the ivory (Cappellini et al., 2014). In addition, we tried to amplify two nuclear fragments that contain nucleotide sites that are diagnostic for the two African elephant species, which cannot be identified using mitochondrial data. Indeed, due to hybridisation, some savanna elephants carry the mitochondrial DNA originally found in forest elephants (Ishida, Demeke, et al., 2011; Ishida et al., 2013). Two mitochondrial fragments of 331 and 33 base pairs (bp) of the D-loop region were targeted with the primer pairs AFDL3/AFDL4 (Eggert et al., 2002; Lee et al., 2013) and Ele-CytbF1/Ele-CytbR1 (Cappellini et al., 2014), respectively. One additional mitochondrial fragment of 116 bp of the cytochrome *b* gene was targeted with the primer pair L15123/H15240 (Ngatia et al., 2019). One last mitochondrial fragment of 53 bp of the ND5 gene, coding for the NADH dehydrogenase 5 protein, was amplified using the primers Ele-ND5-F3/Ele-ND5-R3 (Cappellini et al., 2014). The nuclear fragments, consisting of 4 and 26 bp of the PHK (phosphorylase kinase) and the BGN (biglycan) genes, respectively, were tested using the primer pairs PHK-s1F/PHK-s1R and BGN-s1F2/BGN-s1R2 (Ishida, Demeke, et al., 2011; Ishida, Oleksyk, et al., 2011). Each PCR consisted of a mix of 25 µl with 3 µl of DNA template, 1.5 mM of Mg^{2+}, 0.2 mM of each dNTP, 0.5 µM of each primer, 0.2 µg/µl of bovine serum albumin, 0.03 units/µl of Platinum Taq DNA Polymerase and 1× PCR buffer (Invitrogen, ThermoFisher). The PCR profiles for the mitochondrial markers

consisted of a first step at 94 °C for 3 min; a second step of 40 cycles at 94 °C for 30 s, primer annealing at 51, 52, 52 and 57 °C (for the primer pairs L15123/H15240, Ele-CytbF1/Ele-CytbR1, Ele-ND5-F3 and Ele-ND5-R3 and AFDL3/AFDL4, respectively) for 30 s and 72 °C for 15 s; and a final step at 72 °C for 7 min. For the nuclear markers, a touchdown PCR was applied, with 45 cycles at annealing temperatures of 60, 58, 56, 54, 52 and 50 °C for 3, 5, 5, 5, 5 and 22 cycles, respectively. PCR reactions were repeated with the two elutions obtained from the DNA extraction. PCR products were purified using ExoSAP-IT (ThermoFisher) and then sequenced in both directions using the BigDye Terminator v.1.1 Cycle Sequencing Kit (Life Technologies) and an ABI 3130xl Genetic Analyser (LifeTechnologies). Chromatograms were visualised using CodonCode Aligner v. 8.0.2 (CodonCode Corporation). Base calling and consensus sequence were double checked manually and independently, by two researchers. The Basic Local Alignment Search Tool (BLAST) (Zhang et al., 2000), available on the website of the National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov), was used to align the DNA sequence obtained here with the public nucleotide collection and to retrieve the most similar sequences (allowing a maximum of 5000 output sequences). In parallel, we aligned the fragments that were successfully sequenced here with elephantid DNA sequences downloaded from GenBank using the tool ClustalW (Thompson et al., 1994), implemented in Mega 7.0.26 (Kumar et al., 2016). We computed all pairwise distances in Mega 7.0.26 (Kumar et al., 2016). Finally, we constructed a haplotype network (or reticulated graph) for the DNA marker showing the most variations in order to visualise the relationships among the short DNA sequences available for the elephant genus to which the previous analysis could assign our sample based on our ancient DNA fragment. This analysis was performed in PopART (http://popart.otago.ac.nz), using the median joining network method (Bandelt et al., 1999).

3. Results and discussion

A macroscopic examination verified that the raw material used to shape the medieval chess piece from Jambes is Proboscidean ivory (Goffette & Pigière, 2017). The piece was carved in the axis of a tusk, and its base, cut transversally to the axis, shows a polished cross-section, allowing observation of the characteristic Schreger lines (Espinoza & Mann, 1992; Locke, 2008; Raubenheimer et al., 1990). However, these lines and the angles they form are difficult to distinguish on the Jambes piece, even with the aid of a photocopier machine, which usually yields a good reading of these lines. The angles measured fall within the overlap between Asian and African elephant, but also within that between elephants and mammoth (mean of 103°, measured on 10 angles), which precludes reliable identification of the species involved based on morphological features.

DNA sequences were obtained from the ivory powder for two of the six fragments targeted: 33 bp and 53 bp of the mitochondrial D-loop and the ND5 gene, respectively (GenBank accession numbers MZ374757 and MW557501). They correspond to the two smallest mitochondrial fragments of our experiments, suggesting that the DNA in our sample was degraded to a point that prevented the amplification of nuclear fragments or of mitochondrial fragments longer than ca. 100 bp. This observation contributes to authenticating the ancient origin of the DNA fragments sequenced here. Sequences obtained from independent PCRs with the two elutions of the DNA extraction as template and using the same primer pairs were identical. The BLAST search retrieved public nucleotide sequences that matched exactly (100% coverage and 100% identity) the sequences retrieved here for D-loop and ND5. For D-loop, the 5000 exact matches that were displayed were from *Loxodonta* and other species (but

not *Elephas maximus*; Data S1) while for the ND5 gene (Data S2), the exact matches were DNA sequences that are exclusively from African savanna elephants (*Loxodonta africana*), all of which have been identified to the species level using nuclear data (microsatellites or nuclear markers) (Ishida et al., 2013; Lei et al., 2008; Murata et al., 2009). The alignment obtained here for the 33 bp D-loop fragment (our sequence and published D-loop sequences of *Loxodonta*; Data S3) showed only two variable sites. Two of the three most frequent haplotypes (representing 92% of the sequences and including the haplotype obtained here; Data S4) were from both *Loxodonta africana* and *Loxodonta cyclotis*. Compared with other elephantids, the ND5 sequence obtained here showed zero to six nucleotide differences with other sequences from *Loxodonta africana* (African savanna elephant), six to seven differences with *Loxodonta cyclotis* (African forest elephant) and six to twelve differences with *Elephas maximus* (Asian elephant). The alignment based on African elephants comprised only 11 variable sites and 11 different haplotypes (Data S5 and S6). This 53 bp DNA sequence was found in the sequences occurring exclusively in the savanna elephant (Figure 3A) and, more precisely, in the African 'savanna-wide' subclade (belonging to 'S' clade in Ishida et al., 2013), which is distributed in the savanna belts immediately south of the Sahara, as well as across eastern and southern Africa. It is also found in Cameroon (Figure 1), in a single area that is disconnected from the remanent of the known distribution of the subclade. We have to note that the geographical distribution of all elephants sequenced so far represents a partial view of their current and past distribution. For example, individuals from the 'savanna-wide' subclade may exist but may not yet have been reported in the geographic region connecting the spot in Cameroon to the remainder of its distribution. Based on current knowledge, the 'savanna-wide' subclade represents the vast majority of the haplotypes found in Kenya and Namibia (Ishida et al., 2013). Among all ND5 sequences covering the 53 bp fragment analysed here, available for contemporary African elephants with a known geographic origin, 281 of 689 matched our ancient DNA sequence. They were represented in most specimens originating from Namibia (98%), Kenya (79%) and Tanzania (56%) (cf. haplotype 'I' in Figures 3A and 4, respectively). These 281 records were from six African countries: 124 from Kenya, 59 from Namibia, 46 from Tanzania, 28 from Botswana, 16 from South Africa, seven from Zimbabwe and one from Cameroon (Figure 3B). Since hybridisation has been demonstrated to occur between savanna and forest elephants (Ishida, Demeke, et al., 2011; Ishida, Oleksyk, et al., 2011), we cannot exclude that our ivory comes from a forest elephant carrying the mitochondrial DNA of a savanna elephant. However, based on the data currently available, the 'savanna-wide' subclade is carried only by savanna elephants. As for the geographic origin of the ivory, there is only one report of the 'savanna-wide' subclade outside of southern and eastern Africa. Again, based on the data currently available, which comes from 21st-century samples, the vast majority of the elephants showing the ND5 DNA sequence recovered from our ancient ivory live in southern or eastern Africa. Therefore, the ivory analysed here seems likely to stem from those regions.

The African origin of the ivory used to produce the Jambes chess piece fits the general pattern of people exploiting African elephant instead of Asian elephant for ivory craftwork in Europe and around the Mediterranean Sea. African elephant ivory was more desired than Asian elephant ivory for craftwork, at least for the production of large artefacts, since the Asian elephant grows smaller tusks (Guérin, 2013). In addition, only male Asian elephants have tusks, which reduces the number of tusks produced compared with African elephants, in which both males and females bear tusks. Therefore, in China and India, where indigenous ivory was available and exploited, there was constant demand for imported African elephant tusks during the Middle Ages (Guérin, 2010, 2013). The related export to China and India continued beyond the medieval period, as illustrated by the shipwreck of the *Bom Jesus*, lost

in 1533, while sailing on the India route with more than 100 African forest elephant tusks from West Africa aboard (de Flamingh et al., 2021). African ivory was exported not only to Asia, but also around the Mediterranean basin and farther north in Europe. The hard ivory of the African forest elephant has sometimes been preferred for carving to the softer ivory of the African savanna elephant, such as in post-medieval Japan; however, in China, carvers were not selective (Nishihara, 2012).

From the beginning of the Roman period to the 6th century AD, elephant ivory reaching Europe originated from North Africa and from Aksum, in the Ethiopian Highlands (Horton, 1987). At the end of the 6th century AD, the elephant ivory supply to Europe suffered from the decline in Mediterranean trade that resulted from the economic instability that followed the fall of the Western Roman Empire. Between the 6th and the 10th century AD, elephant ivory working around the Mediterranean Sea and in Europe apparently strongly diminished, although existing ivory artefacts may have been re-worked (Horton & Middleton, 2000). Elephant ivory was replaced by other materials, such as walrus ivory (Horton & Middleton, 2000). Between the 9th and the 13th century AD, the walrus ivory trade flourished and tusks were imported from the northeast Atlantic and Greenland into Europe (Star et al., 2018), to the point of creating competition with elephant ivory (Dectot, 2018). However, from the 10th century AD onward, elephant ivory was again imported in large quantities in Europe, suggesting that a new source became available, with raw material likely originating in sub-Saharan Africa and passing through the Red Sea (Horton & Middleton, 2000). During the 10th century AD, the African ivory trade was controlled by people known today as the Swahili, who occupied 2400 km of the eastern coast of Africa, including the shores of modern-day Somalia, Kenya, Tanzania and Mozambique (Horton & Middleton, 2000). Their ancestors traded tusks with inland, Bantu-speaking people, who obtained elephant tusks from two major sources. The first and most accessible was located in modern-day Kenya, while the second was farther from the coast, in modern-day Zimbabwe, southern Mozambique and South Africa (Guérin, 2010, 2013). The tusks travelled along the East African coast, via the Swahili Corridor, to the Red Sea, before reaching Egypt, which was part of the Fatimid empire starting in 969 AD (Guérin, 2010, 2013; Horton, 1987). In Egypt, tusks were carried to the Mediterranean ports partly through the desert and along the River Nile, to Cairo and to Alexandria, from where they were shipped to Christian Europe. This period, spanning the 10th and 11th centuries AD, saw a significant development of ivory carving in Egypt, given its central position in the ivory trade, as well as in parts of Europe where tusks were imported, from Byzantium to Al-Andalus (southern Spain) (Guérin, 2010). During the late 11th to 12th centuries AD, the number of tusks traded around the Mediterranean Sea declined because of political instability in Egypt, resulting in a dearth of elephant ivory in northern Europe (Guérin, 2010).

At the same time, an alternative trade route was in use that brought ivory from another African source. This trans-Saharan commercial route connected the Mediterranean world with sub-Saharan Africa via a network of caravan routes across the Sahara Desert, led by Amazigh merchants. Although primarily used to transport gold from the rich mines located in present-day Mali and Guinea, then ruled by the Ghana Empire, the trans-Saharan caravans also transported ivory of West African elephants (Guérin, 2013). From 909 AD onward, the part of the Mediterranean coast of North Africa called Ifrīqiya, which covered present-day Algeria, Tunisia and Libya, was under the control of the Fatimid dynasty, which took advantage of the pre-existing trans-Saharan route network (Guérin, 2013). After reaching the Mediterranean shore, ivory was shipped from Ifrīqiyan ports to Europe, the closest being those located in southern Italy, such as in Sicily, Salerno or Amalfi (Guérin, 2013). It is therefore no surprise that Italy is identified as the most likely entry point of chess into

Europe, together with Al-Andalus, where the game was imported by Islamic traders shortly before 1000 AD (Murray, 1913). The game of chess then spreads rapidly to central and northern Europe, reaching Germany and France within a few decades, whereas it took about two centuries to reach northern Scotland and Scandinavia (Bourgeois, 2012, 2015; Murray, 1913).

Although the Jambes chess piece is small and did not require a large tusk for its production, it fits in the general pattern of exploiting African rather than Asian elephant ivory for craft purposes. The benefit of our results lies in suggesting the source of the ivory within Africa.

Having established the origin of the ivory of the Jambes chess piece, we can contribute to determining the route that was followed at the time to distribute African ivory to Europe. The ivory tusks transported via the trans-Saharan route was mainly of West African origin (Guérin, 2013). In contrast, the ivory tusks transported along the Swahili Corridor mostly originated from South and East Africa, within the current boundaries of Kenya, Mozambique, Zimbabwe and South Africa (Guérin, 2013; Horton, 1987). The 'savanna-wide' haplotype detected in the aDNA of the ivory of the Jambes chess piece has so far not been detected in contemporary elephant populations living to the west of Cameroon (Ishida et al., 2013), seemingly excluding a West African origin for the Jambes ivory. Instead, the fact that the 'savanna-wide' haplotype is widespread in eastern and southern African countries (Ishida et al., 2013) indicates that the ivory used to make the chess piece from Jambes was likely transported to Egypt via the Swahili Corridor and the Red Sea, from where it entered Europe. Guérin (2013) suggests that before the conquest of Egypt by the Fatimids in 969 AD, 'ivory came from West Africa and not through the Swahili coast'. On this basis, given that the ivory used to produce the Jambes chess piece likely transited through the Swahili Corridor, we argue that this chess piece was probably crafted between the end of the 10th century and the beginning of the 13th century AD, which is the *terminus post quem* based on the dating of the archaeological context from which the chess piece was recovered.

It remains unknown how this chess piece reached Jambes, or through which port it entered Europe and in what state, that is, carved or uncarved. Indeed, the place where the chess piece was carved is unknown. Workshops in Mediterranean Europe mostly produced figurative ivory chess pieces (style set B) comparable to various finds from northern Europe, such as the so-called Charlemagne chess set, produced in southern Italy. Comparable figurative pieces made of red deer (*Cervus elaphus*) antler were recovered in France from the castle of Loisy (10th–11th century AD) (Grandet & Goret, 2012). In contrast, the schematic style of the Jambes knight (style set A) was most popular in the Islamic world. However, this chess piece need not necessarily have been produced in, or even close to, the Islamic world, as schematic representations had a strong influence on the types of medieval chess pieces that were produced in Europe (Contadini, 1995). This is illustrated, for example, by the abstract chess pieces made of cervid antler recovered from the castle of Châtenois (11th century AD) (Grandet & Goret, 2012). Both styles co-existed in the productions of north-western Europe, where intermediate, partly figurative pieces were also carved (Bourgeois, 2012). Given the diversity of shapes in the pieces produced in north-western Europe, the Jambes chess piece may be a European imitation of an Islamic form.

4. Conclusion

This study successfully demonstrates the effectiveness of aDNA analysis as a tool to identify the source of ancient ivory. We used very small fragments of the object recovered during an archaeological excavation, which were unusable for the purpose of restoration, to extract and analyse aDNA to identify the species. Although macroscopically it is obviously Proboscidean

ivory, the morphometric criteria observed on this chess piece preclude an accurate taxonomic identification, as they fall within the overlap between extant African (*lato sensu*) and Asian elephants and the now-extinct woolly mammoth. The recovered DNA identifies an African elephant (genus *Loxodonta*) as the source of the raw ivory. The finding that the ivory from which this chess piece was made likely comes from an African savanna elephant from a region where the 'savanna-wide' haplotype is represented suggests that this ivory reached Europe via the Swahili Corridor, the Red Sea, and Egypt.

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Supporting information

Supporting information is available online (in the Supporting Information section at the end of the article), including the best-matching public DNA sequences retrieved from the BLAST searches (Data S1 and S2) and the alignments and the haplotype compositions of the D-loop (Data S3 and S4) and ND5 (Data S5 and S6) DNA fragments analysed here.

Conflict of interest

The authors have no conflict of interest to declare.

[figure captions]

Figure 1. Map showing the location of Jambes (Namur, Belgium) and illustrating the past distribution of African savanna elephant (*Loxodonta africana*) and African forest elephant (*Loxodonta cyclotis*) (Grubb et al., 2000; Furstenburg, 2010). The distribution of the 'savanna-wide' haplotype is based on Ishida et al. (2013), and the records of potential hybrids ($P > 0.95$) detected from DNA samples are taken from Mondol et al. (2015). The Swahili Corridor and the main trans-Saharan routes are represented based on Horton (1987), Guérin (2010) and Smith (2015), respectively. Map modified from http://d-maps.com

Figure 2. Four views of the ivory chess piece from Jambes (inventory number NR.17.JAMA.01045.0002; photographs R. Gilles © AWaP)

Figure 3. Haplotype networks constructed with the median joining method and representing the ND5 DNA sequences (53 bp) available for African elephants (*Loxodonta* spp) and obtained here from the chess piece. All identical sequences (forming a haplotype) are

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Figure 4. Geographical distribution of the ND5 DNA sequences (53 bp) available for African elephants (*Loxodonta* spp) and analysed here (Data S2 and S6). Colours in the pie charts represent the haplotypic compositions for each country. *: Haplotype I (white) was obtained from the chess piece. The size of the 13 pie charts is proportional to the total number of sequences from each country

References

Bandelt, H., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution, 16*(1), 37-48. doi:10.1093/oxfordjournals.molbev.a026036

Bourgeois, L. (2012). Introduction et mutations du jeu d'échecs en Occident (Xe-XIIIe siècles). In J.-F. Goret & M. Grandet (Eds.), *Échecs et trictrac. Fabrication et usages des jeux de tables au Moyen Âge. Catalogue de l'exposition présentée du 23 juin au 18 novembre 2012 au Musée du château de Mayenne* (pp. 23-32). Paris: Errance.

Bourgeois, L. (2015). Les échecs médiévaux, jeu des élites, jeux de couleurs. In S. Joye, L. Jégou, T. Lienhard, & J. Schneider (Eds.), *Faire lien. Aristocratie, réseaux et échanges compétitifs. Mélanges d'histoire médiévale offerts à Régine Le Jan* (pp. 269-277). Paris.

Buddhachat, K., Thitaram, C., Brown, J. L., Klinhom, S., Bansiddhi, P., Penchart, K., Ouitavon, K., Sriaksorn, K., Pa-in, C., Kanchanasaka, B., Somgird, C., & Nganvongpanit, K. (2016). Use of handheld X-ray fluorescence as a non-invasive method to distinguish between Asian and African elephant tusks. *Scientific Reports, 6*(1), 24845. doi:10.1038/srep24845

Çakırlar, C., & Ikram, S. (2016). 'When elephants battle, the grass suffers.' Power, ivory and the Syrian elephant. *Levant, 48*(2), 167-183. doi:10.1080/00758914.2016.1198068

Cappellini, E., Gentry, A., Palkopoulou, E., Ishida, Y., Cram, D., Roos, A.-M., Watson, M., Johansson, U. S., Fernholm, B., Agnelli, P., Barbagli, F., Littlewood, D. T. J., Kelstrup, C. D., Olsen, J. V., Lister, A. M., Roca, A. L., Dalén, L., & Gilbert, M. T. P. (2014). Resolution of the type material of the Asian elephant, *Elephas maximus* Linnaeus, 1758 (Proboscidea, Elephantidae). *Zoological Journal of the Linnean Society, 170*(1), 222-232. doi:10.1111/zoj.12084

Caubet, A., & Poplin, F. (2010). Réflexions sur la question de l'éléphant syrien. In H. Kühne (Ed.), *Dur-Katlimmu 2008 and Beyond* (pp. 1-10). Wiesbaden: Harrassowitz Verlag.

Comstock, K., Ostrander, E., & Wasser, S. (2003). Amplifying nuclear and mitochondrial DNA from African elephant ivory: a tool for monitoring the ivory trade. *Conservation Biology, 17*, 1840-1843.

Conard, N. J. (2003). Palaeolithic ivory sculptures from southwestern Germany and the origins of figurative art. *Nature, 426*(6968), 830-832. doi:10.1038/nature02186

Contadini, A. (1995). Islamic Ivory Chess Pieces, Draughtsmen and Dice in the Ashmolean Museum. In J. Allan (Ed.), *Islamic Art in the Ashmolean Museum* (pp. 30-51). Oxford: Oxford University Press.

Coutu, A. N., Lee-Thorp, J., Collins, M. J., & Lane, P. J. (2016). Mapping the elephants of the 19th century East African ivory trade with a multi-isotope approach. *PLoS ONE, 11*(10), e0163606. doi:10.1371/journal.pone.0163606

Coutu, A. N., Whitelaw, G., le Roux, P., & Sealy, J. (2016). Earliest evidence for the ivory trade in southern Africa: isotopic and ZooMS analysis of seventh–tenth century AD ivory from KwaZulu-Natal. *African Archaeological Review, 33*(4), 411-435. doi:10.1007/s10437-016-9232-0

Dabney, J., Knapp, M., Glocke, I., Gansauge, M.-T., Weihmann, A., Nickel, B., Valdiosera, C., García, N., Pääbo, S., Arsuaga, J.-L., & Meyer, M. (2013). Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proceedings of the National Academy of Sciences, 110*(39), 15758-15763. doi:10.1073/pnas.1314445110

de Flamingh, A., Coutu, A., Sealy, J., Chirikure, S., Bastos, A. D. S., Libanda-Mubusisi, N. M., Malhi, R. S., & Roca, A. L. (2021). Sourcing elephant ivory from a sixteenth-century Portuguese shipwreck. *Current Biology, 31*(3), 621-628.e624. doi:10.1016/j.cub.2020.10.086

Dectot, X. (2018). When ivory came from the seas. On some traits of the trade of raw and carved sea-mammal ivories in the Middle Ages. *Anthropozoologica, 53*(1), 159-174. doi:10.5252/anthropozoologica2018v53a14

Deraniyagala, P. E. P. (1955). *Some extinct elephants, their relatives and the two living species*. Ceylon: Government Press.

Eggert, L. S., Rasner, C. A., & Woodruff, D. S. (2002). The evolution and phylogeography of the African Elephant inferred from mitochondrial DNA sequence and nuclear microsatellite markers. *Proceedings: Biological Sciences, 269*(1504), 1993-2006.

Espinoza, E. O., & Mann, M.-J. (1992). *Identification guide for ivory and substitute*. Baltimore: World Wildlife Fund Publications.

Ewart, K. M., Lightson, A. L., Sitam, F. T., Rovie-Ryan, J. J., Mather, N., & McEwing, R. (2020). Expediting the sampling, decalcification, and forensic DNA analysis of large elephant ivory seizures to aid investigations and prosecutions. *Forensic Science International: Genetics, 44*, 102187. doi:10.1016/j.fsigen.2019.102187

Furstenburg, D. (2010). Focus on the African Elephant (*Loxodonta africana*). *South African Hunter, 05040*, 46-49.

Gilbert, M. T. P., Bandelt, H.-J., Hofreiter, M., & Barnes, I. (2005). Assessing ancient DNA studies. *Trends in Ecology & Evolution, 20*(10), 541-544. doi:10.1016/j.tree.2005.07.005

Goffette, Q., & Pigière, F. (2017). *Identification de la matière première d'un objet (n° inv. NR17JAMA/01.045/0002). Report of the Royal Belgian Institute of Natural Sciences for the Heritage Service of the Walloon Region*. Brussels.

Grandet, M., & Goret, J.-F. (2012). *Échecs et trictrac. Fabrication et usages des jeux de tables au Moyen Âge. Catalogue de l'exposition présentée du 23 juin au 18 novembre 2012 au musée du château de Mayenne*. Paris: Errance.

Grubb, P., Groves, C. P., Dudley, J. P., & Shoshani, J. (2000). Living African elephants belong to two species: *Loxodonta africana* (Blumenbach, 1797) and *Loxodonta cyclotis* (Matschie, 1900). *Elephant, 2*(4), 1-4. doi:10.22237/elephant/1521732169

Guérin, S. M. (2010). *Avorio d'ogni ragione*: the supply of elephant ivory to northern Europe in the Gothic era. *Journal of Medieval History, 36*(2), 156-174. doi:10.1016/j.jmedhist.2010.03.003

Guérin, S. M. (2013). Forgotten Routes? Italy, Ifrīqiya and the Trans-Saharan Ivory Trade. *Al-Masāq, 25*(1), 70-91. doi:10.1080/09503110.2013.767012

Gupta, S. K., Thangaraj, K., & Singh, L. (2011). Identification of the source of ivory idol by DNA analysis. *Journal of Forensic Sciences, 56*(5), 1343-1345. doi:10.1111/j.1556-4029.2011.01750.x

Horton, M. (1987). The Swahili Corridor. *Scientific American, 257*(3), 86-93.

Horton, M., & Middleton, J. (2000). *The Swahili*. Oxford-Malden: Blackwell Publishers.

Ishida, Y., Demeke, Y., van Coeverden de Groot, P. J., Georgiadis, N. J., Leggett, K. E. A., Fox, V. E., & Roca, A. L. (2011). Distinguishing forest and savanna African elephants using short nuclear DNA sequences. *Journal of Heredity, 102*(5), 610-616. doi:10.1093/jhered/esr073

Ishida, Y., Georgiadis, N. J., Hondo, T., & Roca, A. L. (2013). Triangulating the provenance of African elephants using mitochondrial DNA. *Evolutionary Applications, 6*(2), 253-265. doi:10.1111/j.1752-4571.2012.00286.x

Ishida, Y., Oleksyk, T. K., Georgiadis, N. J., David, V. A., Zhao, K., Stephens, R. M., Kolokotronis, S.-O., & Roca, A. L. (2011). Reconciling apparent conflicts between mitochondrial and nuclear phylogenies in African elephants. *PLoS ONE, 6*(6), e20642. doi:10.1371/journal.pone.0020642

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution, 33*(7), 1870-1874. doi:10.1093/molbev/msw054

Lafrenz, K. A. (2004). *Tracing the source of the elephant and hippopotamus ivory from the 14th century B.C. Uluburun shipwreck: The archaeological, historical, and isotopic evidence.* University of South Florida, Tampa.

Lee, E.-j., Lee, Y.-h., Moon, S.-h., Kim, N.-y., Kim, S.-h., Yang, M.-s., Choi, D.-h., & Han, M.-s. (2013). The identification of elephant ivory evidences of illegal trade with mitochondrial cytochrome b gene and hypervariable D-loop region. *Journal of Forensic and Legal Medicine, 20*(3), 174-178. doi:10.1016/j.jflm.2012.06.014

Lei, R., Brenneman, R. A., & Louis Jr., E. E. (2008). Genetic diversity in the North American captive African elephant collection. *Journal of Zoology, 275*(3), 252-267. doi:10.1111/j.1469-7998.2008.00437.x

Lister, A. M., Dirks, W., Assaf, A., Chazan, M., Goldberg, P., Applbaum, Y. H., Greenbaum, N., & Horwitz, L. K. (2013). New fossil remains of *Elephas* from the southern Levant: Implications for the evolutionary history of the Asian elephant. *Palaeogeography, Palaeoclimatology, Palaeoecology, 386*, 119-130. doi:10.1016/j.palaeo.2013.05.013

Locke, M. (2008). Structure of ivory. *Journal of Morphology, 269*(4), 423-450. doi:10.1002/jmor.10585

MacGregor, A. (1985). *Bone, antler, ivory and horn. The technology of skeletal materials since the Roman Period*. London.

Mailand, C., & Wasser, S. K. (2007). Isolation of DNA from small amounts of elephant ivory. *Nature Protocols, 2*(9), 2228-2232. doi:10.1038/nprot.2007.318

Mondol, S., Moltke, I., Hart, J., Keigwin, M., Brown, L., Stephens, M., & Wasser, S. K. (2015). New evidence for hybrid zones of forest and savanna elephants in Central and West Africa. *Molecular Ecology, 24*(24), 6134-6147. doi:10.1111/mec.13472

Murata, Y., Yonezawa, T., Kihara, I., Kashiwamura, T., Sugihara, Y., Nikaido, M., Okada, N., Endo, H., & Hasegawa, M. (2009). Chronology of the extant African elephant species and case study of the species identification of the small African elephant with the molecular phylogenetic method. *Gene, 441*(1), 176-186. doi:10.1016/j.gene.2009.01.014

Murray, H. J. R. (1913). *A history of chess*. Oxford: Clarendon Press.

Ngatia, J. N., Lan, T. M., Ma, Y., Dinh, T. D., Wang, Z., Dahmer, T. D., & Chun Xu, Y. (2019). Distinguishing extant elephants ivory from mammoth ivory using a short sequence of cytochrome b gene. *Scientific Reports, 9*(1), 18863. doi:10.1038/s41598-019-55094-x

Nishihara, T. (2012). Demand for forest elephant ivory in Japan. *Pachyderm, 52*, 55-65.

Ogata, K. (2017). *Elephant in Antiquity and the Middle Ages.* (PhD thesis), Université Libre de Bruxelles, Brussels.

Olivier, R. (1978). Distribution and status of the Asian Elephant. *Oryx, 14*(4), 379-424. doi:10.1017/s003060530001601x

Poplin, F. (2012). Préface. In M. Grandet & J.-F. Goret (Eds.), *Échecs et trictrac. Fabrication et usages des jeux de tables au Moyen Âge. Catalogue de l'exposition présentée du 23 juin au 18 novembre 2012 au musée du château de Mayenne* (pp. 12-13). Paris: Errance.

Psonis, N., de Carvalho, C. N., Figueiredo, S., Tabakaki, E., Vassou, D., Poulakakis, N., & Kafetzopoulos, D. (2020). Molecular identification and geographic origin of a post-Medieval elephant finding from southwestern Portugal using high-throughput sequencing. *Scientific Reports, 10*(1), 19252. doi:10.1038/s41598-020-75323-y

Raubenheimer, E. J., Dauth, J., Dreyer, M. J., Smith, P. D., & Turner, M. L. (1990). Structure and composition of ivory of the African elephant (*Loxondonta africana*). *South African Journal of Science, 86*, 192–193.

Rijkelijkhuizen, M. J. (2008). *Handleiding voor de determinatie van harde dierlijke materialen : bot, gewei, ivoor, hoorn, schildpad, balein, hoef*. Amsterdam: University Press.

Rijkelijkhuizen, M. J., Kootker, L. M., & Davies, G. R. (2015). Multi-isotope analysis of elephant ivory artefacts from Amsterdam: a preliminary provenance study. *World Archaeology, 47*, 504-524.

Rohland, N., Malaspinas, A.-S., Pollack, J. L., Slatkin, M., Matheus, P., & Hofreiter, M. (2007). Proboscidean mitogenomics: chronology and mode of elephant evolution using mastodon as outgroup. *PLOS Biology, 5*(8), e207. doi:10.1371/journal.pbio.0050207

Shetty, N., & Vidya, T. N. C. (2011). To split or not to split: The case of the African elephant. *Current Science, 100*, 810-812.

Shimoyama, M., Maeda, H., Sato, H., Ninomiya, T., & Ozaki, Y. (1997). Nondestructive discrimination of biological materials by near-infrared Fourier transform Raman spectroscopy and chemometrics: discrimination among hard and soft ivories of African elephants and mammoth tusks and prediction of specific gravity of the ivories. *Applied Spectroscopy, 51*(8), 1154-1158. doi:10.1366/0003702971941674

Shimoyama, M., Ninomiya, T., & Ozaki, Y. (2003). Nondestructive discrimination of ivories and prediction of their specific gravity by Fourier-transform Raman spectroscopy and chemometrics. *Analyst, 128*(7), 950-953. doi:10.1039/b301239e

Smith, R. (2015). Trade and commerce across Afro-Eurasia. In B. Z. Kedar & M. E. Wiesner-Hanks (Eds.), *The Cambridge World History: Volume 5: Expanding webs of exchange and conflict, 500CE–1500CE* (Vol. 5, pp. 233-256). Cambridge: Cambridge University Press.

Stuart, A. J., Sulerzhitsky, L. D., Orlova, L. A., Kuzmin, Y. V., & Lister, A. M. (2002). The latest woolly mammoths (*Mammuthus primigenius* Blumenbach) in Europe and Asia: a review of the current evidence. *Quaternary Science Reviews, 21*(14), 1559-1569. doi:10.1016/S0277-3791(02)00026-4

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting,

position-specific gap penalties and weight matrix choice. *Nucleic acids research, 22*(22), 4673-4680. doi:10.1093/nar/22.22.4673

Trapani, J., & Fisher, D. C. (2003). Discriminating Proboscidean taxa using features of the Schreger pattern in tusk dentin. *Journal of Archaeological Science, 30*(4), 429-438. doi:10.1006/jasc.2002.0852

Vanmechelen, R., Timmermans, J., & Devillers, C. (2018a). Bâtiment médiéval et pion d'échecs à Jambes, rue Mazy (Nr). *Archaeologia Mediaevalis, 41*, 223-230.

Vanmechelen, R., Timmermans, J., & Devillers, C. (2018b). Namur/Jambes : bâtiment médiéval et pion d'échecs, rue Mazy. *Chronique de l'Archéologie Wallonne, 26*, 176-181.

Vila, E. (2014). The 'Syrian Elephant' revisited – Preliminary analysis of the elephant bones at Michrife/Qatna in Late Bronze Age Syria. In P. Pfälzner (Ed.), *Qatna and the Networks of Bronze Age Globalism* (pp. 37-46). Stuttgart: Harrasowitz Verlag.

Wasser, S. K., Brown, L., Mailand, C., Mondol, S., Clark, W., Laurie, C., & Weir, B. S. (2015). Genetic assignment of large seizures of elephant ivory reveals Africa's major poaching hotspots. *Science, 349*(6243), 84-87. doi:10.1126/science.aaa2457

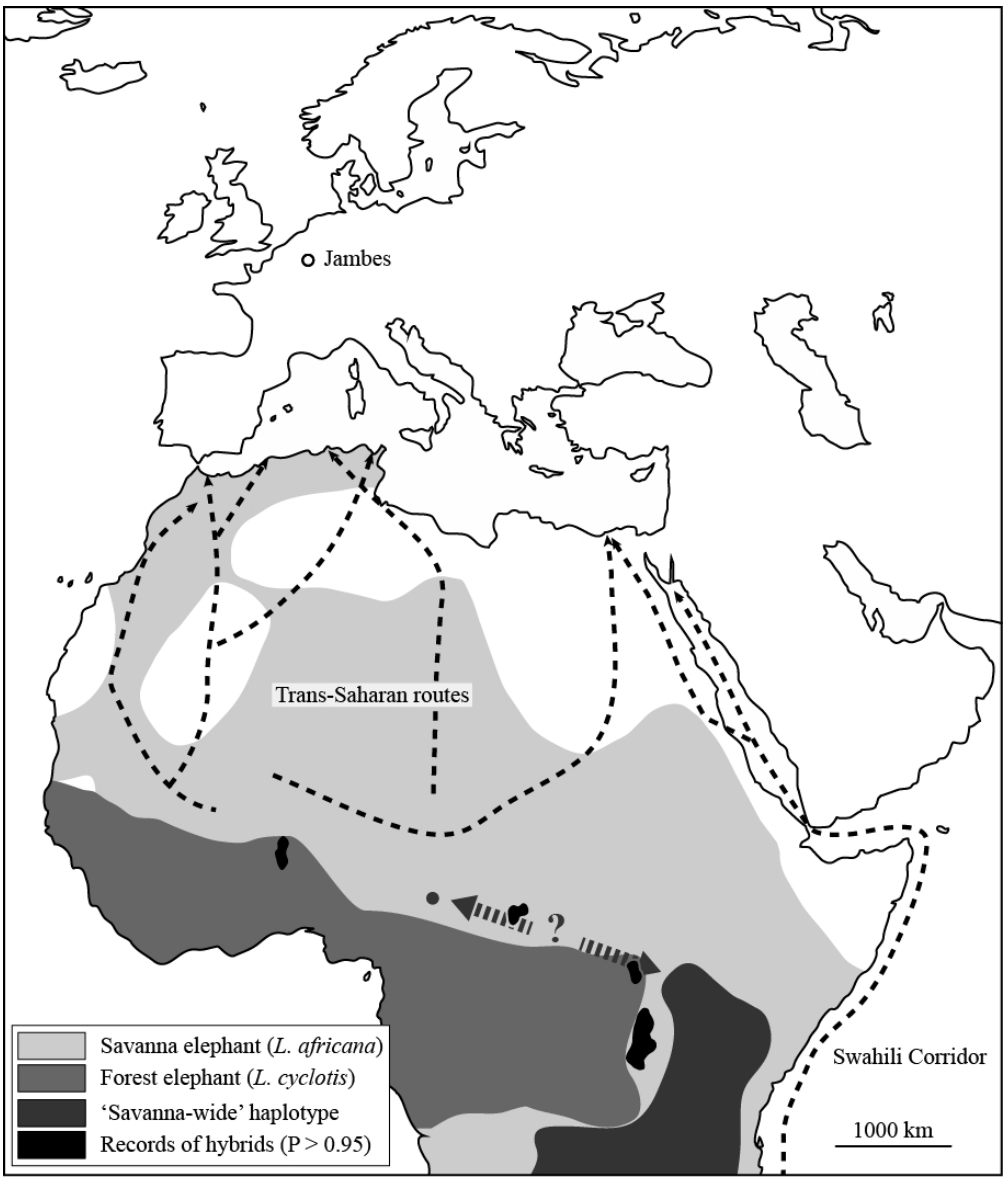
Wasser, S. K., Mailand, C., Booth, R., Mutayoba, B., Kisamo, E., Clark, B., & Stephens, M. (2007). Using DNA to track the origin of the largest ivory seizure since the 1989 trade ban. *Proceedings of the National Academy of Sciences, 104*(10), 4228-4233. doi:10.1073/pnas.0609714104

Willerslev, E., & Cooper, A. (2005). Review Paper. Ancient DNA. *Proceedings of the Royal Society B: Biological Sciences, 272*(1558), 3-16. doi:10.1098/rspb.2004.2813

Winters, M., Torkelson, A., Booth, R., Mailand, C., Hoareau, Y., Tucker, S., & Wasser, S. K. (2018). Isolation of DNA from small amounts of elephant ivory: Sampling the cementum with total demineralization extraction. *Forensic Science International, 288*, 131-139. doi:10.1016/j.forsciint.2018.04.036

Wittemyer, G. (2011). Family Elephantidae (Elephants). In D. E. Wilson & R. A. Mittermeier (Eds.), *Handbook of the Mammals of the World. Volume 2: Hoofed Mammals*. Barcelona: Lynx Edicions.

Zhang, Z., Schwartz, S., Wagner, L., & Miller, W. (2000). Greedy algorithm for aligning DNA sequences. *Journal of computational biology, 7*, 203-214. doi:10.1089/10665270050081478



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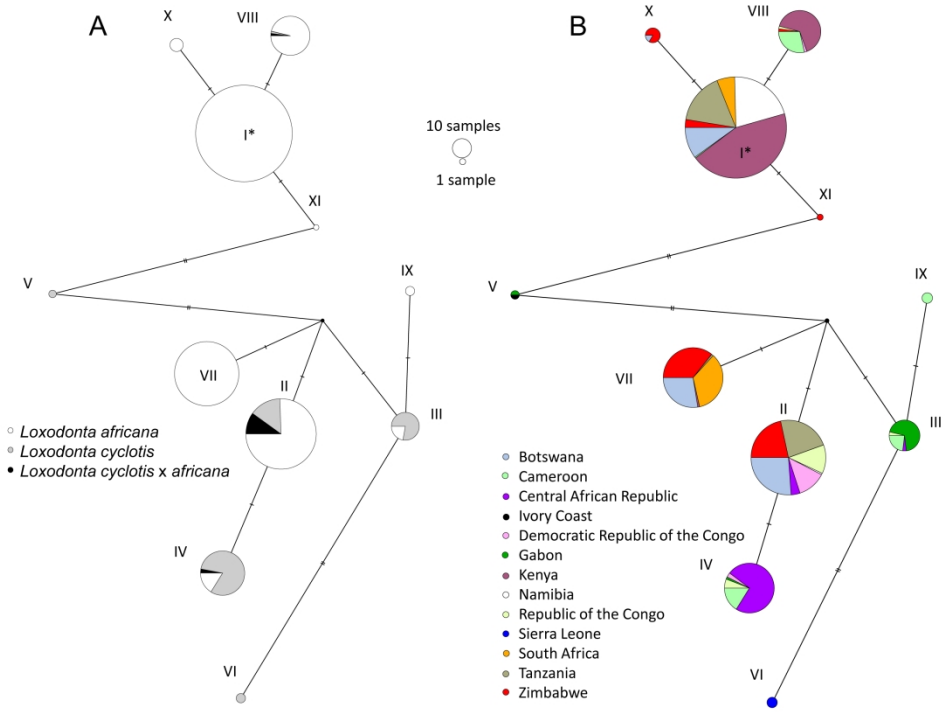

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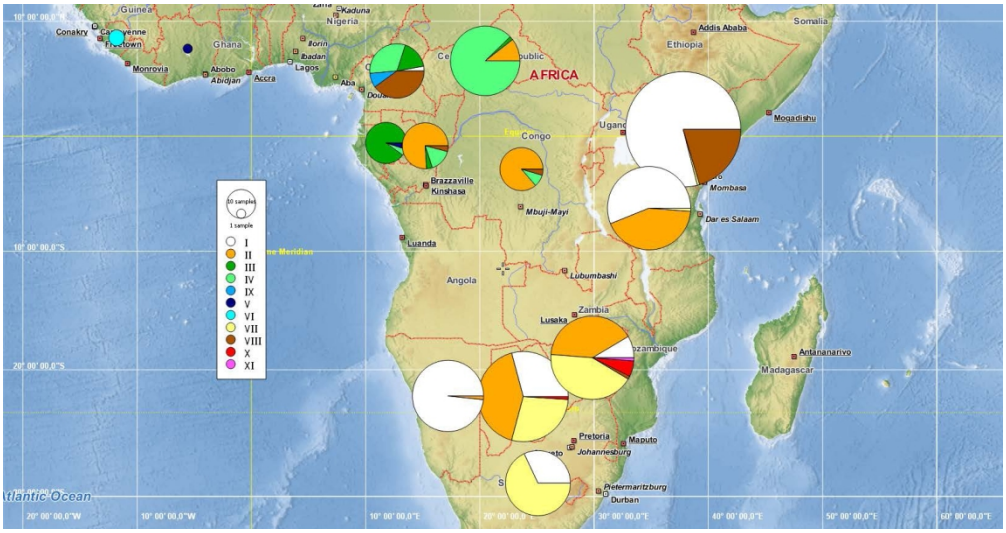

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