

Elephant Species Identification from Ivory through Polymerase Chain Reaction and Sequencing Analysis for Application in Works of Art



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Introduction

- Ivory is a tooth that protrudes out of an animal's mouth. It is the hard, white material that makes up the tusks of elephants and other animals. It has been used for centuries in medicines, weapons, and art. In China today, ivory is seen as a status symbol and is in high demand despite how lethal ivory harvesting is to animals.
- Ivory has primarily come from elephants. There are three main species: African Bush Elephant (*Loxodonta africana*), African Forest Elephant (*Loxodonta cyclotis*), and Asian Elephant (*Elephas maximus*).



Flora, African Elephant (Vulnerable) Shirley, Asian Elephant (Endangered)
Elephant Sanctuary in Tennessee

- There is no known way to differentiate ivory from African and Asian elephant species morphologically.
- Ivory species identification is currently important for many reasons:
 - Art exhibitions are required to identify the ivory source's species in order to get through customs. CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) laws have been more strictly enforced, necessitating species identification.



Left:
A Florentine Lady
(c. 1892, The Walters Art Museum, Baltimore)



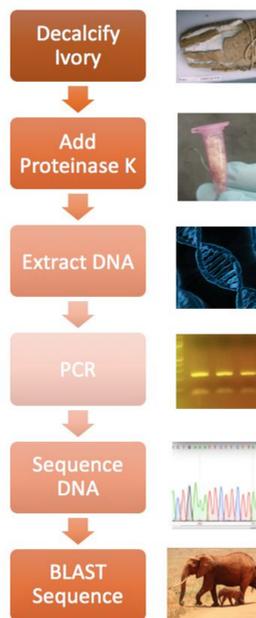
Right:
Crozier with the Virgin and Child, and the Crucifixion
(1350-1360, The Walters Art Museum, Baltimore)

- Knowledge of the species will provide information that places the ivory object in physical, historical, and cultural context.
- Elephants are being poached for their ivory and species testing assists in the investigations of illegal trade, helping endangered species survive.
- Mitochondrial DNA (mtDNA) is DNA contained in the mitochondria apart from genomic DNA. Using known single nucleotide polymorphisms (SNPs) it is possible to determine the species origin of an ivory sample.¹
- In order for this technique to be used by art conservators, the sample size must be deemed small enough to not be greatly destructive to the artwork.

Hypothesis

- Using known SNPs in mtDNA, the minimum sample size for determining the species of elephant an ivory sample came from, can be found.
- We wish to test if current genetic methods of ivory identification are suitable to art conservation science studies, given the small sample size requirement.

Methods



PCR Results

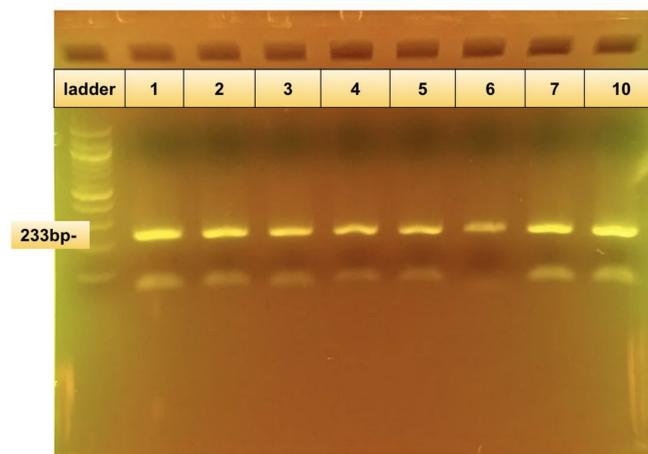
Bands containing amplified mtDNA fragments confirm success of PCR with bands appearing at ~200bp. The fragment of mtDNA is 233bp.

Sample	Weight	Description
1	0.0008g	Two thin short splinters.
2	0.0006g	One Thin short splinters.
3	0.0000g	Speck the size of a pen tip.
4	0.0019g	Powder.
5	0.0082g	Powder.
6	0.0152g	Powder.
7	0.0242g	Powder.
10	-----	Control. Elephant Blood DNA.

References:

- Kitpipit, Thitika, et. al. "Mini-SNaPshot Multiplex Assays Authenticate Elephant Ivory and Simultaneously Identify the Species Origin." *Forensic Science International: Genetics* 27 (2017): 106-15.
- Flora. Digital image. *The Elephant Sanctuary in Tennessee*. Web. <https://elephants.com/elephants/flora>
- Shirley. Digital image. *The Elephant Sanctuary in Tennessee*. Web. <https://elephants.com/elephants/flora>

Successful Nested PCR Amplification of Ivory mtDNA, samples 1-7 and positive control .



Sequencing Results

Loxodonta cyclotis isolate DS1511 mitochondrion, complete genome
Sequence ID: KY616976.1 Length: 16010 Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
302 bits (163)	3e-78	170/174 (98%)	1/174 (0%)	Plus/Plus

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    Range 1: 15362 to 15535  GenBank  Graphics  Next Match  Previous Match
    Query 24  AGGTCACCTTACCTCGCTATCAATACCCAAAACGAAATCTTCTTAAACTATTCCTCCG 83
    Sbjct 15362 AGGTCACCTTACCTCGCTATCAATACCCAAAACGAAATCTTCTTAAACTATTCCTCCG 15421
    Query 84  AAGCAAAACAACCCGCTATGATATATCGTGCATTAATGCTGTCGCCAATGATGATA 143
    Sbjct 15422 AAGCAAAACAACCCGCTATGATATATCGTGCATTAATGCTGTCGCCAATGATGATA 15481
    Query 144 TATATTACTAATACTTAATCTTACATAGACCATACTATGN-TNATCGTCCA 196
    Sbjct 15482 TATATTACTAATACTTAATCTTACATAGACCATACTATGN-TNATCGTCCA 15535
  
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Ivory mtDNA Can Determine Ivory Origin. BLAST alignments of ivory-extracted mtDNA determining the ivory to be of African origin. Alignments 1-3 were performed with Primer Set 4, confirming *Loxodonta* genus. Alignment 4 used Primer Set 1, giving the *L. cyclotis* species.

Conclusions

Many variables are involved in the availability of mtDNA in ivory. The level of degradation of mtDNA varies due to the environment the ivory was in, and how long it has existed. There are also portions of the tusk that may not contain an abundance of DNA, and often there is no means to determine from what part of the tusk the object was carved. The minimum sample size may vary based on how old the ivory is and how the ivory was kept.

We believe that while results are promising, identification of species through DNA analysis needs to be explored more before deciding whether it is a worthwhile option for use in works of art.

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