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Genetic Analysis of Elephant Species in Guinea Conakry

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Determining Taxonomic Origin of Elephant Populations Using Fecal DNA

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ABSTRACT

Poaching is a major cause of elephant population decline and molecular techniques are crucial elements for conservation. There are two species of elephants in Africa, African savanna elephant (*Loxodonta africana*) and African forest elephant (*Loxodonta cyclotis*). Knowing the taxonomy of elephants in understudied populations such as Guinea-Conakry is imperative for sound management practices. Using non-invasively collected fecal samples and diagnostic single nucleotide polymorphisms we examined the taxonomy of the elephants in these populations. Understanding the extent of hybridization will contribute to a database for triangulating the provenance of confiscated illegal ivory.

INTRODUCTION

- African savanna elephant (*Loxodonta africana)* and African forest elephant (*Loxodonta cyclotis)* populations are threatened by poaching and habitat destruction
- Effective conservation efforts would benefit from knowledge of hybridization within unique populations of African elephants where the range of each species overlap
- The Ziama Forest Reserve is one of the few areas with suitable elephant habitat within Guinea-Conakry. (Figure 1)
- Dung samples collected from Botswana are strictly located in the savanna and are a beneficial example as a positive control
- Diagnostic single nucleotide polymorphisms can be amplified from these elephants to determine their taxonomic origins

Figure 1. Map of West Africa with protected and unprotected elephant ranges in green (Blanc et al. 2007). The Ziama Forest Reserve is circled in blue.

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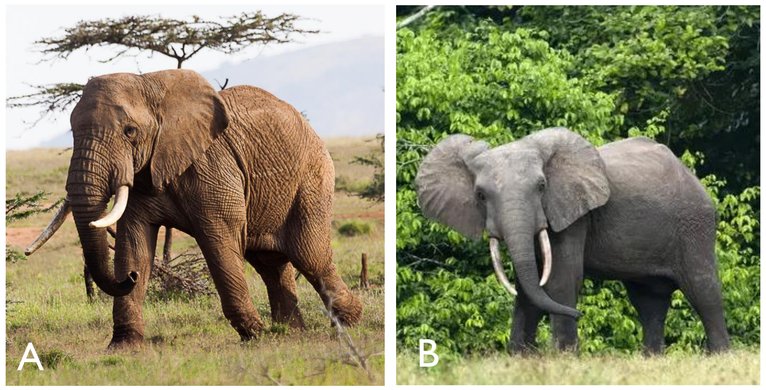

Figure 2. (A) African savanna elephant (Loxodonta africana) (B) African forest elephant (Loxodonta cyclotis).

OBJECTIVES

- Identify the taxonomic origin of the elephants in Guinea-Conakry.
- Determine presence or absence of hybridization among sampled elephants.
- Contribute to a public genetic database for use in triangulating the provenance of confiscated ivory.

METHODS

- 25 dung samples were collected from the Ziama Forest reserve in Guinea-Conakry and stored in a salt saturated DMSO buffer solution.
- DNA was isolated from dung using Qiagen DNA Stool Kit with modifications:
  - A larger starting sample of 400µl
  - 20 min incubation at 70 °C
  - Two 30 minute extended elution of DNA
  - A secondary clean up with the Zymo One Step PCR Inhibitor Removal Kit
- Short regions of the *PHKA2* and *PLP* genes regions were amplified by PCR
  - Modifications included increasing the amount of magnesium chloride and primer concentration for efficient amplification
- M13 tagged primers were utilized to elongate the primer region and aid in amplification of short nuclear fragments.
- PCR samples were enzymatically cleaned up with Exonuclease I and Shrimp alkaline phosphatase
- Samples were sequenced using Big Dye Terminator Sequence
- Sequences were resolved by capillary electrophoresis at the Core DNA Sequencing Facility at the University of Illinois, Urbana-Champaign
- SEQUENCHER (version 5.4.6) was used to trim and align sequences, and identify polymorphic regions.

Results

Table 1. Species-diagnostic Single Nucleotide Polymorphic Sites

	PHK		*PLP*		
	39	71	319	345	361
L. *cyclotis*	**C**	A	R (G/A)	T	**G**
L. *africana*	**T**	A	G	T	**A**
CH0001	**T**	A			
CH0002			G	T	**A**
CH0003	**T**	A			
CH0005	**T**	?	R	T	**?**
CH0006	**?**	?	G	T	**A**
CH0007	**T**	?			
CH0008	**T**	A			
ZF0001		A	G	T	**A**
ZF0002		A	G	T	-
ZF0005		A	G	T	**A**
ZF0007			G	T	**A**
ZF0022			G	T	**A**
ZF0004	**C**				
ZF0008		A			
ZF0011			G	T	**A**
ZF0006			G	T	**A**
ZF0013			G	T	**A**
ZF0002			G	C?	**G**

- Two loci successfully amplified in 7 elephants from Chobe National Park (Botswana) and 11 elephants from the Ziama Forest (Guinea-Conakry)
- SNP positions that differentiate savanna (*L. africana*) from forest (*L. cyclotis*) elephants (*Ishida et al. 2011*) are indicated in boldface; at variable sites both bases are shown (Table 1).

CONCLUSIONS

Preliminary evidence supports Savanna typical SNPs with the an unconfirmed hybrid (ZF0004). These SNPs can be found on base 361 of *PLP* and base 39 of *PHK*. However, further work must be done, re-sequencing failed samples and better aligning sequences in Sequencher, to confirm these SNPs. *BGN*, which has not yet been sequenced, must also be sequenced to provide further evidence of this identification.