**Conserving Thai Elephants: Insights from Ivory-Related Genetic Markers**

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**Abstract** : ~~Asian elephants (Elephas maximus) hold immense cultural and economic significance in Thailand as a national symbol. They are a keystone species, shaping the land and supporting the ecosystem, playing a vital role in maintaining biodiversity. Habitat loss and the severe poaching of their ivory have led to a critical threat for elephants in the realm of conservation biology. Artificial selection imposed by selective poaching of male elephants with long ivory tusks, leading the elephants to evolve without tusks and, consequently, resulting in a loss of genetic diversity. In this study, blood samples were collected from 60 captive elephants, consisting of 20 males with short ivory tusks, 20 males with long ivory tusks, and 20 females. Genome-wide SNP DArtseq analysis was conducted to identify genetic markers associated with ivory traits. The candidate loci will be mapped to a reference genome constructed using hybrid assemblies of high-coverage Illumina sequencing, long-read PacBio, long-read Oxford Nanopore sequencing, and Hi-C. Furthermore, these ivory-related genetic markers offer the potential to accelerate complete genome sequencing efforts and may provide valuable insights into the linkage with the sex chromosome. Findings from this study will aid in both in situ and ex situ conservation management and breeding strategies. Understanding their diversity related to ivory will inform a conservation action plan and drive genomic research innovations. These results will bolster~~ sustainable tourism and conservation efforts, elevating Thailand as a global center for elephant research.

## Keywords : ~~Asian elephants; conservation biology; ivory-related genetic markers; genome sequencing; elephant diversity~~

## Introduction

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## Material and Method

The elephant sampled in this study were in the Asian … elephant (Elephas maximus ~~maximus, Linnaeus, …~~), which are actually present in the … part of Thailand. In this study, 33 adult and healthy individuals are selected for sample collection. Among them X are male and X are female. As the studied aims to investigate the SNPs ~~responsible~~ of the development of Ivory, the presence of tusk are … visually and then recorded (Table SX). With a collaboration with the … farm, …, Thailand, the blood samples were collected by … X ml of blood for each individual. All … of the animals were conducted with respect the guidelines provided by the Institutional Animals Ethics Committee (IAEC). Once the biological samples were collected, they are stored at -X° C until genomic DNA extraction.

The DNA extraction were isolated using the salting out method (Miller et al. …). Polymerase chain reaction were performed according to the following parameter : … . The amplified DNA were … at a concentration of … . Gel electrophoresis were then performed to check … of the obtained DNA using a … % agarose gel. The ddRAD sequencing library was prepared to selectively target a … target site on the genome by using the … and the … enzyme and the … adapter. The DNA sequencing were performed following the … instructions which provided fastq sequence file that will be used in the next step.

Sequence quality parameters were performed using FastQC (ref), and only the reads having length greather than … bp were selected using the … software (ref). The sequences were trimmed using the Trimmomatic version … (ref), and then aligned to a reference genome i.e. *Elephas maximums* (Linnaeus …) (ref) available in NCBI (AN …, accessed on …) and alignment index were built using BWA (ref). The obtained map file were in the S… (SAM) (ref) format, which were converted to … (BAM) (ref) format using Samtools version … (ref), and ultimatelly sorted.

The obtained map file were used to call SNPs using the STACKS pipeline version 1.9 (ref). The ref\_map.pl utility was used to create the … of SNP loci. ~~First, sequences aligned to the same genomic location were stacked together and merged to form loci. Loci with a sequencing depth of three or more reads per individual were retained and catalogues have been created. SNPs at each locus were selected using a maximum likelihood frame- work.~~ Then the populations program was called from the “…” toolkit using the following parameter : 0.70 for the minimum samples per population, 0.05 for the minimum m… allele frequencies, 0.7 for the max observed heterozygosity. The STACKS pipeline returned a variant calling file (VCF) as an output and further SNP ~~statistical~~ analysis were done with those files. The “gen” utility from Plink version … (ref) were used to generate the SNP … summary data from the VCF file. By editing the “…” file provided by the “gen” utility, the trait data of each individual were … to the PLINK pipeline. The … test were performed using the “fisher” … test from the PLINK toolkit. The fisher … summary data were used to plot the Manhattan plot highlighting the trait-associated loci.

By using the VCF Tools version … (ref), all SNPS present on the X, Y chromosomes and mitochondrial DNA were removed and were not studied in the further step. Minor allele frequency (MAF < 0.05), missing genotypes (0.8) and HWE deviation (P < 0.001) filtering was done using PLINK version … to only … high-quality SNPs for … .

## Results

The sequencing … outcomes a total of … ddRAD sequences, having a average of … bp (Figure X), by taking into account the forward and reverse … an average of X bp per sample were extracted. By filtering by length, X % of the obtained sequences pass the quality check (X bp), which remains a total of X sequences. The SNPs calling pipeline revealed a total of X SNPs in the elephant genome. Maximum number of SNPs were on chromosome 1 (as it is the largest chromosome in the *Elephas maximus* genome), and the chromosome X have the less number of SNPs. A total of X sites were polymorphic, which held X % of the elephant genome.

Trait-association study revealed that X SNPs are related to the development of tusk in the elephant. Having an haplotype diversity between X and X. The Benferroni significance … revealed a … threshold … which includes a total of X SNPs (Table).

## Discussion

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## Conclusion

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