

**DNA typing the Asian elephant in Sri Lanka:
An assessment of the genetic structure of the population
&
Establishment of paternity**

HIMESHA VANDEBONA

Thesis submitted in fulfillment of the requirements for the Degree of
Doctor of Philosophy
of the
University of Colombo, Sri Lanka.

August 2001

503674



ABSTRACT

The Asian elephant, *Elephas maximus*, is a globally threatened species. The elephant in Sri Lanka, *Elephas maximus maximus*, is a nominated subspecies of the Asian elephant. The elephant population in Sri Lanka is currently fragmented and restricted to a few isolated small jungle habitats. Maintenance of a high degree of genetic diversity within the population is essential for its future survival.

There were two main objectives in this study. Firstly, as a prerequisite for future conservation and management measures, this study aimed at assessing the genetic population structure of the elephant in Sri Lanka with regard to geographic population structure and levels of genetic variability. The Second objective was, the development and use of DNA fingerprinting technology for the accurate assignment of paternity of elephants born in captivity. The establishment of paternity of progeny with certainty is also of considerable importance since the captive breeding programmes that are implemented for conservation of elephants depend critically on maintenance of genetic diversity within captive populations.

Blood samples were collected for DNA extraction from one hundred and twenty seven Sri Lankan elephants (captive 120; wild 7), one elephant from Thailand and two African elephants. The genetic variability was analyzed in two mitochondrial genes i.e. cytochrome b (Cyt b) (n=70) and NADH dehydrogenase subunit 5 (ND5) (n=67) by Polymerase Chain Reaction (PCR) based amplification of elephant DNA using universal primers and analysis of DNA sequence data of their PCR products. The sequence divergence in the above two genes in the Asian elephant in three different geographical regions (Northern, Mahaweli and Southern) of Sri Lanka, was analyzed in this study.

Analysis of sequence polymorphisms identified 12 polymorphic sites within the Cyt b sequence (410 bp) giving rise to 5 mtDNA haplotypes. ND5 sequence data (550 bp) revealed 9 polymorphic sites and 5 mtDNA haplotypes. A total of 67 elephant samples were analysed for sequence divergence in both genes, yielding seven distinct ND5-Cyt b mtDNA haplotypes. Phylogenetic analysis showed that one haplotype (T3M6) distinctly

separated out from the other haplotypes (T1M5, T2M2, T2M5, T2M8, T4M1 and T5M2) with a mean sequence divergence of 1.61%. However, the study did not provide evidence to include T3M6 as a separate subspecies of the Asian elephant. T2M2, T3M6, T2M5 and T1M5 were common haplotypes, whereas the other haplotypes were very rare. Within Sri Lanka, the sequence divergence in the two mitochondrial genes among the three geographical elephant populations analyzed was very small. However, significant geographic substructure was observed with regard to haplotype frequencies, indicating limited genetic exchange among the three geographic elephant populations at present. Genetic variability was high in all three analyzed regions. The inferred limitation of present long-distance genetic exchange could reflect an effect of the fragmentation of the former continuous distribution. Further, the study did not support the classification of the elephant in Sri Lanka as a separate subspecies of the Asian elephant.

DNA fingerprinting technology was used for the establishment of paternity of elephants born in captivity. First, the multilocus probe 33.15 (Jeffrey's) was tested and used successfully to obtain a unique discrete banding pattern for each elephant DNA sample. Three calves born in captivity at the Pinnawela Elephant Orphanage and their dams (n=2) and three putative sires were DNA fingerprinted by the procedure developed in this study. A total of 57 variable banding positions were observed and the mean number of bands per individual was 19 ± 2.14 . The sire of each calf was identified unambiguously by a comparative analysis of the fingerprints of the calf and its dam with each of the putative sires. Pairwise comparison of band sharing probabilities confirmed these assignments.

The sequence data of part of the mitochondrial ND 5 gene of both the Asian and the African elephant, and the assessment of the genetic structure of the Asian elephant population in Sri Lanka by combined analysis of sequence data derived from two mitochondrial genes, are reported first in this thesis.