UNIVERSITI PUTRA MALAYSIA

CYTOGENETIC AND RANDOM AMPLIFIED POLYMORPHISM DNA ANALYSIS OF SUBSPECIES OF ASIAN ELEPHANTS (ELEPHAS MAXIMUS)

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By

SAMSUL BARIAH SHARUDIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Master of Science

April 2005

DEDICATION

Dedicated to……..

My beloved husband and daughter

My Parents, My sisters and Brother

And all my beloved friends……..

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman : Rosnina Hj. Yusoff, PhD

Faculty : Veterinary Medicine

A study was carried out to investigate the chromosome constitution of 3 subspecies of elephants from cultured lymphocytes; karyotypes were constructed according to standard procedure. Lymphocytes were cultured in RPMI 1640 medium supplemented with a mitogen (phytohemagglutinin-PHA or pokeweed-PWM), penicillin- streptomycin and bovine calf serum. The karyotype showed that the Asian elephant has a diploid number (2n) of 56 and fundamental number (NF) of 66 in both male and female. The autosomes comprised 6 pairs of submetacentric, 10 pairs of large acrocentric and the remaining 11 pairs are characterized as small acrocentric. On the other hand, the sex chromosomes consisted of the largest submetacentric X chromosome while the Y chromosome is a small acrocentric.

G-, C- and NORs bandings were carried out to assist the conventional banding. G-banded karyotypes of the three subspecies were identical as well as for the C- banded karyotypes. NORs banding revealed active nucleolar organizer regions chromosomes 2, 4, 13 and 17. These results were the first banded karyotypes established for the Asian elephants.

Investigations of DNA polymorphisms using RAPD technique provided identical information between the three subspecies. Blood samples from three subspecies of the Asian elephants were employed in the study. Based on band sharing frequency value of pair-wise comparison within subspecies, the genetic relationship between Malayan and Indian elephants was determined to be low and likewise between the Indian and Myanmar elephants. Interestingly, the band sharing frequency value between Malayan elephant and Myanmar elephant are genetically closely similar to the Malayan elephants compared with the Indian elephants. With regards to the similarities in chromosome morphology as well as at the DNA level, they are able to breed amongst themselves without any complication related to parental chromosomal incompatibility.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

ANALISIS SITOGENETIK DAN RAPD SUBSPESIES GAJAH ASIA *(ELEPHAS MAXIMUS)*

Oleh

SAMSUL BARIAH SHARUDIN

April 2005

Pengerusi : Rosnina Hj. Yusoff, PhD

Fakulti : Perubatan Veterinar

Pada permulaan perkembangan metodologi, penyelidikan untuk mengkaji kromosom bagi 3 subspecies gajah Asia; Gajah Malaya (*Elephas maximus sumatranus*), Gajah Myanmar *(Elephas maximus indicus)* dan Gajah India (*Elephas maximus maximus)* dengan menggunakan teknik pengkulturan dan kariotip disediakan mengikut prosedur standard. Kombinasi kultura yang terdiri daripada media kultur (80 %) RPMI 1640, phytohemagglutinin (PHA) atau Pokeweed (PWM), streptomycin (20%) dan serum fetus anak lembu.

Hasil daripada analisis yang dijalankan didapati gajah mempunyai kromosom komplemen iaitu 2n = 56 dan nombor fundanental (NF) bagi gajah jantan dan gajah betina adalah 66. Kariotip tersebut terdiri daripada 6 pasang submetasentrik, 10 pasang

akrosentrik besar dan selebihnya adalah 11 pasang akrosentrik kecil. Kromosom seks terdiri daripada kromosom X adalah submetasentrik yang paling besar dan kromosom Y adalah akrosentrik yang kecil.

Teknik penwarnaan G-, C- dan NORs dijalankan untuk menyokong teknik penwarnaan konvensional. Bagi teknik penwarnaan G- kariotip bagi ketiga-tiga subspesis gajah Asia ini adalah serupa, begitu juga bagi kariotip daripada teknik penwarnaan C-. Daripada teknik penwarnaan NORs, didapati NORs yang aktif adalah pada kromosom nombor 2, 4, 13 dan 17. Keputusan ujikaji ini merupakan kariotip penwarnaan yang pertama dihasilkan bagi gajah Asia.

Hasil penyelidikan polimorfasi DNA dengan menggunakan teknik RAPD menunjukkan maklumat yang sama bagi ketiga-tiga subspesis gajah Asia. Sampel darah daripada ketiga-tiga subspesis gajah Asia digunakan dalam penyelidikan ini. Berasaskan kepada nilai kekerapan perkongsian jalur terhadap perbandingan pasangan jalur yang sama di antara subspesis gajah, perhubungan genetic di antara gajah Malaya dan India telah ditentukanrendah, begitu juga dengan hubungan genetic di antara gajah India dan Myanmar. Apa yang menarik ialah nilai kekerapan perkongsian jalur di antara gajah Malaya dan gajah Myanmar adalah sangat tinggi. Ini menunjukkan bahawa gajah Myanmar adalah amat rapat secara genetic berbanding dengan gajah India.

Dengan merujuk kepada persamaan kromosom dan DNA, dengan demikian mereka boleh diperbaikbiak antara satu sama lain tanpa kecacatan berkaitan dengan ketidaksepadanan kromosom induk.

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
$AgNO_3$	Silver/ Argentum nitrate
$Ba(OH)_2$	Barium hydroxide
Bp	Base pairs
dH_2O	Distilled water
DNA	Deoxybonucleic acid
DNTP	Deoxynucleoside triphosphate
EDTA	Dissodium ethylene diamine tetraacetate
H_2O_2	Hydrogen peroxide
ISCNDA	International System for Cytogenetic Nomenclature of Domestic Animals
IUCN	International Union for the Conservation of Nature
KCL	Potassium chloride
NaCl	Sodium chloride
NORs	Nucleolar Organizer Regions
mM	milimolar
ng	nanogram
OD	Optical density
PBS	Phosphate buffer saline
PCR	Polymerase Chain Reaction
PHA	Phytohemagglutinin A

PWM	Pokeweed mitogen
RAPD	Random Amplified Polymorphism DNA
RE	Restriction enzymes
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic acid
Rpm	Rotor per minute
RPMI	Roswell Park Memorial Institute
RRNA	Ribosomal RNA
SSC	Standard saline citrate (sodium chloride and nitrate solution)
TAE	Tris Acetate EDTA
ul	microliter
V	volt

CHAPTER I

INTRODUCTION

Throughout history, the elephant has played a crucial role in both the animal and the plant ecosystems. Elephants modify the habitat by converting woodlands to grasslands, providing water for other species by digging water holes in dry riverbeds, acting as seeds dispersers through fecal matter and creating fire-breakers and rain water conduits through their paths. They also influence the economy, religion and culture of mankind. Therefore, they are considered as keystone species (Wilson, 1993; Shoshani, 1998) whereby the existence of a large number of other species in the ecosystem depends on the elephants such as deer, wild cattle and other small mammals like porcupine and rabbits. If these elephants are extirpated from a system, the species they supported and species that are dependent on the elephants also will disappear. Shrinking off their habitats will lower the biodiversity and affect all 'players' in a food web (Wilson, 1993).

The elephant is classified under the order, Proboscidea, family Elephantidae, subfamilies Stegotetra belontidae and Elephantinae, with a total of six genera and 26 species. However, there are only 2 genera existing in this world today. The two species of elephants that exist today are the African elephant (*Loxodonta africana)* and the Asian elephant *(Elephas maximus*), which are the end results of over fifty million years of evolution. The other 4 genera are extinct and the elephants in these 4 genera are thought to posses a well-developed trunk (Burton, 1980). Criteria used to distinguish between the 2 genera of elephants are their physical appearance, size, morphology, habitat and

also their gestation period or physiology. Although the African and Asian elephants descended from the same prehistoric elephant family, Mammuthus (the huge hairy mammoth), the hairy skin of the Asian elephant indicates that it is closely related to the mammoth than the African elephant (Ozawa *et al.*, 1997; Noro *et al.*, 1998; Greenwood *et al.*, 1999). Recently, a study on two species of African elephants, (*Loxodonta africana africana* and *Loxodonta africana cyclotis*) revealed that the phylogenetic distinction between the *L. africana africana* and *L. africana cyclotis* is most likely similar of *L.africana cyclotis* and *E.maximus*. The two species of African elephants have been proven to be two separate species within the family of *Loxodonta* (Roca *et al.*, 2001).

The immense size, strength and stature of this largest living land animal have intrigued people of many cultures for hundreds of years. Elephants have served as beasts of burden, entertainer in circuses and festivals around the world. Considered easier to handle and train, the Asian elephant is widely used as a draught animal in India and Southeast Asia. The range of Asian elephants distribution in the past extended from the Tigris-Euphrates river systems in west Asia, eastward through Persia into the Indian sub-continent, South and Southeast Asia and north into China. Today, it is found in only a small fraction of its past distribution and with continuous fragmentation, will further isolate the existing populations. Currently, the Asian elephants are found in 13 countries: Nepal, Bangladesh, Bhutan, Thailand, India, Sri Lanka, China, Myanmar, Cambodia, Laos, Vietnam, Indonesia and Malaysia. Populations are scattered, isolated and many of them have low probability of viability (Santosh, 1999).

Many subspecies of the Asian elephants have been described in the past but few have been accepted as valid by elephant biologists (Deraniyagala, 1955; McKay, 1973; Shoshani and Eisenberg, 1982 and Shoshani, 1991). There are three subspecies of the *Elephas maximus, E. maximus sumatranus*, that is found in Malaysia and Sumatra; *E. indicus,* which inhabits the jungle of Indochina and China and *E. maximus*, which roams the vegetation of Sri Lanka and Southern India (Shoshani 1991; Cavendish 1993). It is quite difficult to differentiate them from each other because the three subspecies vary only slightly in body size, skin color, the size as well as shape of their ears. The physical characteristics among these three forms of the Asian elephants change gradually from one extreme to another, beginning at Sri Lanka in the western part of their distribution and ending with Sumatra at the eastern limit. *E. maximus* are the largest and the darkest of the three elephants having largest ears and patches of depigmentation on the ears, face, trunk and belly. *E. sumatranus* is the smallest and has the lightest skin color with least depigmentation, while the *E. indicus* has the combination of features from the other two subspecies. They are also distinguished by physical traits related to their geographic location. For example, *E. maximus* elephants tend to have larger ears which are useful for regulating body temperature in the hotter climate of Sri Lanka (Cavendish, 1993).

The Asian elephant, *Elephas maximus* is classified as an endangered species by the International Union for the Conservation of Nature (IUCN) Species Survival Commission' s Asian Elephant Specialist Group in the Red Data Book (Baillie and Groombridge, 1996). To date, there are only between 38000 and 51000 wild Asian elephants in comparison to their distant cousin, the African elephants with over 600,000

heads. Fragmentation and loss of existing habitat to agriculture and developmental activities pose as main threats to the survival of this population. For centuries, the elephant' s massive tusks have been prized for their ivory (Eltringham, 1991). Poaching also causes long-term damage to the demography as only male Asian elephants have tusks and selective poaching of one sex has resulted in highly skewed sex ratios (Santosh, 1999).

Facing the problem of extinction, the survival of Asian elephants in the future depends on their ability to survive and reproduce. Their genetic background is the most important information that could be used in breeding programs and to identify specific genetic markers as to verify the animals in forensic cases as well as enforcement of law regarding ruthless killing of this endangered species (Deacon and Lah, 1989). In the long term, the conservation program of Asian elephants should be a major importance if the species is to survive in this millennium.

Documentation on Asian elephant's karyotype is very scarce. Available literature on the cytogenetics of Asian elephants described the conventional karyotype only. In our literature search, there have been very few reports on the cytogenetic and molecular aspects of the Asian elephants. Moreover, the molecular studies concentrated only on the demonstration of phylogenetic position among African elephants, Asian elephants and Mammoths.