UNIVERSITI PUTRA MALAYSIA

GENETIC CHARACTERIZATION OF THREE DEER SPECIES IN MALAYSIA

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By

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

2005

DEDICATION

TO MY SMALL FAMILY

MY HUSBAND AND MY DAUGHTERS

Sara, Shaima and Asma

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

GENETIC CHARACTERIZATION OF THREE DEER SPECIES IN MALAYSIA

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HABIBA ALI A. ELJAAFARI

October 2005

Chairman: Associate Professor Jothi Malar Panandam, PhD

Faculty: Agriculture

In recent years, there has been much interest in the domestication and farming of deer in Malaysia for velvet, skin and meat production. Various deer species and subspecies have been imported into the country and this has resulted in the introduction of new germplasm and the risk of mixture of these, making it necessary to evaluate the genetic background of the various species before they are indiscriminately diluted or altered. This study was carried out to characterize three deer species in Malaysia, namely rusa (*Cervus timorensis*), sambar (*Cervus unicolor*) and sika (*Cervus nippon*), by using the karyotyping, biochemical polymorphisms and randomly amplified polymorphic DNA (RAPD) techniques. The rusa herd at the Deer Breeding Unit of University Research Park, Universiti Putra Malaysia, the sambar herd at Pusat Permbiakan Ternakan Sabray, Kenungu, Sabah and the sika herd at Pusat Ternakan Haiwan, Batu Arang were used as the research populations.

Conventional, G-banded and C-banded karyotypes were generated for three male and three female deer of each species. The chromosome number was derived from 100 good metaphase spreads per animal. The morphology of the chromosomes was based on their relative lengths and position of the centromeres. Rusa, sambar and sika displayed a total of 60, 62 and 66 chromosomes in the majority of the cell spreads, respectively. The rusa deer had five pairs of metacentric/submetacentric and 24 pairs of acrocentric autosomes, and a pair of sex chromosomes. The X chromosome was characterised as the largest acrocentric chromosome, while the Y chromosome was a small acrocentric chromosome. The sambar deer had four pairs of metacentric/submetacentric and 26 pairs of acrocentric autosomes. The sex chromosomes were similar to that of rusa deer. In the sika deer there were two pairs of metacentric/submetacentric and 30 pairs of acrocentric autosomes. The pair of sex chromosomes was similar to those of the rusa and the sambar. The homologous chromosomes were paired with respect to their sizes, shapes and banding patterns generated from C-banding and G-banding. The nucleolar organizer regions (NORs) for the three deer species were located on different chromosomes. The male rusa displayed NORs on the telomeric regions both homologues of Chromosomes 1 and 6, but the female displayed NORs on only one homologue of Chromosome 1, and both homologues of Chromosome 6. Male and female sambar deer both had three telomeric NORs located on the homologous pair of Chromosome 6 and a single homologue of Chromosome 7. Female and male sika deer displayed four NORs which were on the acrocentric Chromosomes 1 and 2.

Cellulose acetate and starch gel electrophoresis were used to study enzyme/protein polymorphisms. Blood samples from 38 rusa, 9 sambar and 34 sika deer were analysed for 15 biochemical markers, however, only six markers generated results. Lactate dehydrogenase (LDH), Albumin (ALB), Transferrin and X-protein were monomorphic. Haemoglobin (HB) was polymorphic with three phenotypes for the three species, which could be attributed to two codominant alleles, HB^A and HB^B. The frequency of HB^A was highest in the rusa population, 0.553, while HBB was predominant in sambar, 0.611, and sika, 0.574. The rusa and sika populations showed significant ($P<0.05$) deviations from HWE for this locus. Glucose-6-phosphate dehydrogenase (G6PD), although polymorphic, was monomorphic within each species. Rusa and sambar showed the same phenotype, but this was different from that of the sika. The genetic distance between rusa and sambar based on the above biochemical markers was 0.001, between rusa and sika it was 0.144, and between sambar and sika it was 0.141.

The animals used in the biochemical study were also analyzed for RAPD markers using 10 arbitrary primers. The primers amplified a total of 164 makers, of which 59 were shared by all three species. The overall percent polymorphism was 99.39%, with rusa showing 128 polymorphic markers (97.71%), sambar showing 66 (68.04%) and sika showing 118 (95.16%) polymorphic markers. The 62B-1 (800 bp) band was a common monomorphic marker for the three species. Sambar had five exclusive monomorphic markers, while sika had one and rusa none. The genetic distance based on Dice and Jaccard similarity indices showed sambar and sika to be the most closely related, followed by rusa and sambar.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENCIRIAN GENETIK TIGA SPESIS RUSA DI MALAYSIA

Oleh

HABIBA ALI A. EL-JAAFARI

Oktober 2005

Pengerusi : Profesor Madya Jothi Malar Panandam, PhD

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Pada tahun kebelakangan ini, terdapat banyak minat dalam perjinakkan dan perladangan rusa di Malaysia untuk produksi beledu, kulit dan daging. Pelbagai spesis dan subspesis rusa telah diimport ke dalam negara dan ini telah mengenalkan germplasma baru dan risiko pencampuran ini, menjadikannya penting untuk menilai latarbelakang genetik pelbagai spesis tersebut sebelum mereka dicairkan atau diubah secara tidak diskriminasi. Kajian ini dijalankan untuk mencirikan tiga spesis rusa di Malaysia, iaitu rusa (*Cervus timorensis*), sambar (*Cervus unicolor*) dan sika (*Cervus nippon*), dengan menggunakan teknik-teknik kariotip, polimorphisme biokimia dan 'randomly amplified polymorphic DNA' (RAPD). Gerompok rusa di Unit Pembiakan Rusa di Taman Penyelidikan Universiti, Universiti Putra Malaysia, sambar di Pusat Pembiakan Ternakan Sabray, Kenungu, Sabah dan sika di Pusat Ternakan Haiwan, Batu Arang digunakan sebagai populasi penyelidikan.

Kariotip konvensi, jalur-G dan jalur -C dihasilkan untuk tiga ekor rusa jantan dan tiga ekor rusa betina untuk setiap spesis. Bilangan kromosom diperoleh daripada 100 sebaran metafasa yang baik per haiwan. Morfologi kromosom didasarkan pada

sebaran metafasa yang baik per haiwan. Morfologi kromosom didasarkan pada panjang relative mereka dan kedudukan sentromere. Rusa, sambar dan sika menunjukkan sejumlah 60, 62 dan 66 kromosom dalam kebanyakan sebaran sel, masing-masing. Rusa mempunyai lima pasang autosom metasentrik/submetasentrik dan 24 pasang autosom akrosentrik, dan sepasang kromosom jantina. Kromosom X dicirikan sebagai kromosom akrosentrik yang terbesar, manakala kromosom Y adalah kromosom akrosentrik yang kecil. Sambar mempunyai empat pasang autosom metasentrik/submetasentrik dan 26 pasang autosom akrosentrik. Pasangan kromosom jantina adalah sama seperti pada rusa. Pada sika terdapat dua pasang autosom metasentrik/submetasentrik dan 30 pasang autosom akrosentrik. Pasangan kromosom jantina adalah sama seperti pada rusa dan sambar. Kromosom homologus dipasangkan berdasarkan saiz, bentuk dan corak jalur yang dihasilkan daripada penjaluran-C dan penjaluran-G. 'Nucleolar organizer regions' (NORs) untuk ketiga-tiga spesis rusa ditempatkan pada kromosom yang berlainan. Rusa jantan menunjukkan NORs pada bahagian telomerik kedua-dua homolog Kromosom 1 dan 6, tetapi rusa betina menunjukkan NORs hanya pada satu homolog Kromosom 1 dan padfa kedua-dua homolog Kromosom 6. Kedua-dua rusa jantan dan betina sambar mempunyai tiga NORs telomerik yang bertempat pada pasangan homologos Kromosom 6 dan satu homolog Kromosom 7. Sika betina dan jantan menunjukkan empat NORs yang terdapat pada akrometrik Kromosom 1 dan 2.

Elektroforesis selulosa asetat dan gel kanji digunakan untuk mengaji polimorfisme enzim/protein. Sampel darah daripada 38 ekor rusa, 9 ekor sambar dan 34 ekor sika telah dianalisa untuk 15 penanda biokimia, walaubagaimanapun, hanya enam penanda menghasilkan keputusan. Lactate dehydrogenase (LDH), albumin (ALB), transferring

dan X-protein adalah monomorfik. Hemoglobin (HB) adalah polimorfik dengan tiga fenotip untuk ketiga-tiga spesis tersebut, yang boleh dikaitkan kepada dua alel kodominan, HB^A dan HB^B. Frekuensi HB^A adalah tertinggi dalam populasi rusa, 0.553, manakala HB^B adalah predominan dalam sambar, 0.611, dan sika, 0.574. Populasi rusa dan sika menunjukkan penyimpangan ketara ($P<0.05$) daripada HWE untuk lokus ini. Glucose-6-phosphate dehydrogenase (G6PD), walaupun polimorfik, adalah monomorfik untuk setiap spesis. Rusa dan sambar menunjukkan fenotip yang sama, tetapi berbeza dengan sika. Jarak genetik di antara rusa dan sambar berdasarkan penanda biokimia di atas adalah 0.001, antara rusa dan sika ia adalah 0.144, dan antara sambar dan sika ia adalah 0.141.

Haiwan yang digunakan untuk kajian biokimia juga dianalisa untuk tanda RAPD dengan menggunakan 10 primer arbitari. Primer-primer tersebut menghasilkan sejumlah 164 penanda, antara mana 59 dikongsi oleh ketiga-tiga spesis. Peratus polimorfisme keseluruhan adalah 99.39%, manakala rusa menunjukkan 128 penanda polimorfik (97.71%), sambar mempunyai 66 (68.04%) dan sika mempunyai 118 (95.16%) penanda polimorfik. Sambar mempunyai lima penanda monomorfik yang eksklusif, manakala sika mempunyai satu dan rusa tiada langsung. Jaraket berdasarkar indeks persamaan Dice dan Jaccard genetik menunjukkan pertalian sambar dan sika adalah yang paling rapat, diikuti dengan rusa dan sambar.

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I certify that an Examination Committee has met on 6th October 2005 to conduct the final examination of Habiba, A.A.Eljaafari on her Doctor of Philosophy thesis entitled ''Genetic Characterization of Three Deer Species in Malaysia'' in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

Habiba

HABIBA ALI A. EL JAAFARI

Date: 20/10/05

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LIST OF ABBREVATIONS

ACP	Acid phophatase
AFLP	Amplified Fragment Length Polymerase
$AgNO_3$	Silver nitrate
AK	Adenilate kinase
ALB	Albumin
$Ba(OH)_2$	Barium hydroxide
CA	Cellulose acetate
CAE	Cellulose acetate electrophoresis
C-banding	Constitutive heterochromatin banding
CAT	Catalase
DIA	Diaphorase
dNTP	Dinucleotide triphosphate
EDTA	Ethylene Diamine Tera acetic Acid
ES	Arylesterase
FN	Fundamental number
G-banding	Giemsa banding
GDH	Glucose dehydrogenase
GPI	Glucose phosphate isomerase
GPD	Glucose phosphate dehydrogenase
αGLU	Slow-α-glucose
G6PD	Fundamental number
GOT	Glutamate Oxaloacetate Transaminase
IDH	Isocitrate dehydrogenase

G6PD	Gluose -6-phosphate dehydrogenase
HB	Haemoglobin
HCl	Hydrochloric acid
H_e	Heterozygosity
H_2O_2	Hydrogen peroxide
HWE	Hardy-Weinberg equilibrium
KH_2PO_4	Potasium dihydrogen phosphate
LDH	Lactate dehydrogenase
µl	microliter
mM	millimole
ME	Malic enzyme
MDH	Malate dehydrogenase
$MgCl_2$	Magnesium Chloride
mtDNA	mitochondrial DNA
MTT	Methylthiazolyl blue
MPI	Mannose Phosphate Isomerase
Na_2HPO_4	Disodium hydrogen phosphate
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
2n	Diploid chromosome number
ng	nanogram
NP	Nucleoside phosphorylase
NORs	Nucleolar organizer regions
PCR	Polymerase chain reaction
PEP	Peptidase

PGD	Phosphogluconate Dehydrogenase
PGM	phosphoglucomutase
PLG	Plasminogen
PBS	Potassium buffer saline
PMS	Phenazine MethoSulphate
PI2	Protease inhibitor
PTF	Post transferrin
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restricted Fragment Length Polymorphism
RPMI	Roswell Park Memoral Institute
rRNA	Ribosomal RNA
SOD	Superoxide dimutase
SSC	Standard sodium and citrate solution
Tf	Transferrin
UV	ultraviolet
VNTRs	Variable Number of Tandem Repeats
1X	One time
XP	X-protein

CHAPTER 1

INTRODUCTION

Populations of game animals, such as deer, are often exposed to extensive loss of genetic variability. The main reasons are the increasing isolation of wildlife populations by alteration of the landscape and deer being constantly slaughtered by men for their venison and hides, and being hunted for sports or for their antlers. In recent years, there has been much interest in the domestications and farming of deer under varying degrees of intensification. The establishment of artificial populations in enclosures with consequent annual reductions of population size by culling is also a contributory factor to loss in genetic variation. The number of farmed deer in the world is difficult to estimate because the deer industry is expanding at 20 percent per annum. However, in 1993 the international herd stood at over five million (Chardonnet, 1993).

Variability in animal populations was for long time studied at the morphological level. Subsequently, variants in chromosomal structure and biochemical genetic methods were used to investigate the genetic constitution of populations. Over the past four decades, molecular characterization of animal populations has become popular. Polymorphism at biochemical and molecular levels are good indicators of inherited genetic variations and serve as useful tools to evaluate the available genetic resources, population dynamics, inbreeding within populations, and to monitor changes in populations over time as a result of human activities.