UNIVERSITI PUTRA MALAYSIA

CYTOGENETIC STUDIES OF MALAYAN GAUR (*Bos gaurus hubbacki*), SAHIWAL-FRIESIAN CATTLE AND THEIR HYBRID BACKCROSSES

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By

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October 2009

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In early April 1983, a stranded Malaysian gaur (*Bos gaurus hubbacki*) mated with a group of Sahiwal-Friesian dairy heifers and this resulted in the birth of an inter-specific hybrid calf named SELEMBU. In this study, chromosome analysis of four Malayan gaur (*Bos gaurus hubbacki*), ten Sahiwal-Friesian cattle and seven hybrid backcrosses was undertaken to determine their karyotype status and differences.

The gaur and cattle has chromosome complements of 2n = 56 and 2n = 60, respectively. Two types of chromosomal arrangements of the backcrosses were observed; namely an intermediate to the parental species (2n = 58) and a cattle type (2n = 60). The backcrosses with 2n = 60 shared similar chromosomal arrangement and banding characteristics of the cattle. Backcrosses with 2n = 58 exhibited the non-homology of two submetacentric

and two acrocentric autosomes, where the variations were inherited from the parental species that possessed different karyotypes.

A comparison of the gaur with cattle, as the model of the ancestors of the modern bovids, showed structural and characteristic differences in their karyotypes. The gaur exhibited two pairs of submetacentric chromosomes and lacked two chromosome pairs, which had resulted from Robertsonian translocations during their karyotype evolution. Banded karyotypes revealed extensive similarities of chromosomes 1 and 2 of the Malayan gaur to the homologous acrocentric chromosomes of cattle. For the first time, chromosome 1 of the gaur was identified to contain an ancient origin inverted segment compared to cattle, which was homozygous in all the studied gaur samples. The intensity of the C-bands on chromosomes 1 and 2 suggested that the translocations occurred at different periods of time during the karyotype evolution.

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

KAJIAN SITOGENETIK SELADANG (*Bos gaurus hubbacki*), LEMBU SAHIWAL-FRIESIAN DAN KACUKAN SELEMBU

Oleh

MAMAT HAMIDI KAMALLUDIN

Oktober 2009

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Pada awal bulan April 1983, seekor seladang (*Bos gaurus hubbacki)* yang terperangkap telah mengawan dengan sekumpulan lembu tenusu Sahiwal-Friesian dan menghasilkan anak kacukan yang dinamakan SELEMBU. Analisis kromosom empat ekor seladang, sepuluh ekor lembu Sahiwal-Friesian dan tujuh ekor hibrid kacukan balik telah dijalankan untuk mengenalpasti status kariotip dan perbezaan di antara spesies ini.

Seladang dan lembu masing-masing mempunyai pasangan kromosom 2n = 56 dan 2n = 60. Dua jenis penyusunan kromosom kacukan balik telah didapati iaitu pertengahan di antara spesies induk (2n = 58) dan jenis lembu (2n = 60). Kacukan balik dengan 2n = 60 mempamerkan penyusunan kromosom dan sifat penjaluran yang sama dengan lembu. Kacukan balik dengan 2n = 58 mempamerkan kromosom yang tidak sepadan iaitu dua submetasentrik dan dua akrosentrik, yang mana variasi tersebut diwarisi dari spesies induk yang mempunyai karotip yang berbeza.

Perbandingan antara seladang dan lembu sebagai model keturunan bovid moden, menunjukkan perbezaan struktur dan ciri-ciri dalam karotip. Seladang mempamerkan dua pasang kromosom submetasentrik dan kekurangan dua pasangan lain akibat translokasi 'Robertsonian' semasa evolusi kariotip. Kariotip berjalur membuktikan persamaan yang tinggi antara kromosom 1 dan 2 seladang dengan kromosom akrosentrik yang separa pada lembu. Buat pertama kali, kromosom 1 pada seladang dikenalpasti mempunyai segmen terbalik berbanding lembu pada setiap seladang yang dikaji. Intensiti jalur-C pada kromosom 1 dan 2 menandakan kemungkinan translokasi telah berlaku pada jangkamasa yang berbeza semasa evolusi kariotip.

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I certify that an Examination Committee has met on 29th October 2009 to conduct the final examination of Mamat Hamidi Kamalludin on his Master of Science thesis entitled " Cytogenetic Studies of Malayan Gaur (*Bos gaurus Hubbacki*), Sahiwal-Friesian Cattle and Their Hybrid Backcrosses" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Requlations 1981. The Committee recommends that the student be awarded the Masters of Science.

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Date: 11th February 2010

DECLARATION

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

MAMAT HAMIDI KAMALLUDIN

Date: 18 December 2009

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BrdU	-	Bromodeoxyuridine (5-bromo-2-deoxyuridine)
C-bands	-	Constitutive Heterochromatin bands
DVS	-	Department of Veterinary Services
DWNP	-	Department of Wildlife and National Park
F1 Hybrids	-	Gaur x cattle hybrids
G-band	-	Giemsa bands
ISCN	-	International System for Human Cytogenetic Nomenclature
ISCNDA	-	International System for Cytogenetic Nomenclature of Domestic Animals
ISCNDB	-	International System for Chromosome Nomenclature of Domestic Bovids
IUCN	-	International Union for Conservation of Nature and Natural Resources
R-band	-	Reverse bands
RPMI 1640	-	Rosewell Park Memorial Institute 1640
SF	-	Sahiwal-Friesian

CHAPTER 1

INTRODUCTION

In early April 1983 at one of the Department of Veterinary Services (DVS) farm, Pusat Ternakan Haiwan Padang Hijau, Kluang, a stranded Malaysian gaur (*Bos gaurus hubbacki*), locally known as seladang mated with a group of Sahiwal-Friesian dairy heifers. This chance mating resulted, for the first time in Malaysian history, the birth of an inter-specific hybrid calf, which was named as SELEMBU (coined from two Malay words seladang meaning gaur and lembu meaning cattle). Soon after a few more hybrids were born until it was observed that the hybrid calves outgrew the dairy calves (Nor Azman, 1989), which indicated the potential of the animals as meat producer.

Malaysia was estimated to be at 121% and 114% self-sufficiency in poultry meat and eggs, respectively, in 2007. However, the country was only at 23.0% and 9.0% self-sufficiency in beef and mutton, respectively. In 2008, local production of beef was only 25.0% provisional of the domestic demand (DVS, 2009). Realizing that Malaysia was not self-sufficient in beef, cattle farming was emphasized so that more beef could be produce and to reduce the national import bill, thus saving the nation's foreign exchange.

Crossbreeding between *Bos indicus* for its adaptability and *Bos taurus* for its productivity is quite common in Malaysia. In 1982, the Malaysian government embarked on an importation program of Sahiwal-Friesian cattle to be used as dual-purpose animal *i.e*. for dairy and beef production. Sahiwal-Friesian bulls are animals with body weight of about 700kg and the females of about 400kg (Nor Azman, 1989). The colour of Sahiwal-Friesians is a blend of brown, yellow, light brown, white and black. The cattle genome is composed of 29 autosome pairs and two sex chromosomes. All autosomes are acrocentrics with small size differences (ISCNDB, 2000).

Hybridization between species is often arranged through human intervention and rarely in a natural manner. However, it may occur if closely related species share an overlapping habitat. Natural interspecies hybridization is considered as an important evolutionary process (Barton, 2001; Dowling and Secor, 1997). Uncontrolled hybridization as it occurs in nature may have had a significant impact on the formation of domestic breeds, but can also affect the genetic integrity of domestic and wild species.

Selembu or the interspecific hybrid of cattle and gaur has phenotypic features intermediate to both of the parents. However, it had been observed that the hybrids at Padang Hijau farm inherited most of the characteristic feature of the sire (gaur) particularly in the coat colour and the gradual changes of coat colour (Nor Azman, 1989). They were reported to have an

intermediate number of chromosomes (2n=58) compared to both parents while gaur and cattle chromosome complements are 2n=56 and 2n=60, respectively. The hybrid karyotype consists of 54 acrocentric autosomes, 2 submetacentric chromosomes and two sex chromosomes (Bongso *et al.*, 1988).

However, the fertility status of the hybrids was reported to be restricted to only female offspring and the males are sub-fertile (Nor Azman, 1989). Numerous studies provide evidence for the role of Robertsonian translocation or the centric fusion of chromosomes in reduced fertility (Dyrendhal and Gustavsson, 1979; Rangel-Figueiredo and Iannuzzi, 1993). Despite being phenotypically normal, translocation carriers experience a decrease in reproductive potential, predominantly due to the production of chromosomally unbalanced gametes. Due to the fertility problem of the male hybrids, the project of selembu breeding was abandoned and hybrids were kept with the dairy herd. The breeding record of the selembu therefore became unavailable. The remaining group was then transferred to a deer farm (Pusat Ternakan Haiwan) in Lenggong, Perak and kept freely in a fenced paddock in the farm.

A synthetic breed of beef cattle could be produced by controlled breeding involving the female gaur x cattle hybrids, which were reported to be fertile (Nor Azman, 1989) with cattle bulls. The backcrosses should then be

selected by cytogenetic screening to identify a balanced chromosome configuration for further breeding with bulls or heifers. This study was undertaken to investigate the karyotype (number and morphology) of the remaining Selembu and their parental species using conventional, giemsa (G-banding), reverse (R-banding), and constitutive heterochromatin (C-banding).

The main objectives of this study were:

1. To investigate the karyotype status of the remaining Selembu and their parental species.
2. To determine similarities and/or differences of genetic materials possessed by both species and their hybrids via their chromosome banding patterns.

CHAPTER 2

LITERATURE REVIEW

Zoological Position of the Gaur and Cattle

Bovidae and six other family of sub-order ruminantia belong to the zoological order of the artiodactyla or even-number toe ungulates (Vaughan *et al.*, 2000). The family is most diverse with 45 genera and 137 species worldwide. Under the genus *Bos*, there are five species namely *gaurus*; gaur, *grunniens*; yak, *javanicus;* banteng, *taurus* and *indicus*; cattle.

Bos taurus (taurine) and *Bos indicus* (zebus) are the major species of cattle. *Bos taurus* is mainly of temperate origin while *Bos indicus* is of Asian or African origin. There are fundamental anatomical differences justifying their separation. The hump over the withers and the presence of dewlap below the neck are the distinguishing characteristics of the zebus. The zebu also have larger ears, skin folds, shorter hair coat, more pigmented skin, longer legs and more angular body frame as compared to the taurine (Warwick and Legates, 1979). The *Bos taurus* are developed either as dairy or meat animal while the *Bos indicus* was utilized for triple purpose before mechanization. Apart from its purpose as beef and milk resources, the zebu was used as work animal for ploughing paddy fields or pulling carts.

The gaur was reported to have an extensive range from the hilly-forested country of the Indian peninsular, Burma to Indochina and down to Malaysia (Conry, 1989; Walker, 1964). Three subspecies of the gaur were classified by their geographical location, which are India-Nepal, Indochina and Malaysia-Thailand gaur, respectively (Byers *et al.*, 1995; Conry, 1981).

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CLASS

Mammalia (warm blooded animals)

SUBCLASS

Eutheria

ORDER

Artiodactyla (mammals with even number toes)

SUBORDER

Ruminantia (true ruminants)

FAMILY

Bovidae (Hollow horn bovine)

SUBFAMILY

Bovinae

GENUS	**COMMON NAME**
Bison	Bison (Europe, North America)
Bos	Cattle, gaur, banteng, Yak
Boselaphus	Nilgai (Asia)
Bubalus	Asiatic buffalo (Asia)
Syncerus	African Buffalo (Africa)
Taurotragus	Eland (Africa)
Tetracerus	Four-horned antelope (Asia)
Tragelaphus	Bushbuck, Nyala, Kudu, Bongo (Africa)

Figure 1. Zoological Position of the Gaur and Cattle.
(Adapted from Vaughan *et al.*, 2000)

The Gaur

The gaur is a large muscular animal with sturdy limbs and a striking muscular ridge that slope down to the middle of the back. It is also known as Indian bison or locally as Seladang and can be found in Bangladesh, Cambodia, China, India, Malaysia, Myanmar, Nepal, Thailand and Vietnam. They are the native cattle of the rainforests of Southeast Asia, and one of the many wild cattle species currently listed as vulnerable and endangered (IUCN, 2008).

Previously it was deliberated that there are three subspecies of gaur: Indian gaur (*Bos gaurus gaurus*), Indochinese gaur (*Bos gaurus readei*) and Malayan gaur (*Bos gaurus hubbacki*) (Lekagul and McNeely, 1977). Recently, the gaur has been recognized with three subspecies: *Bos gaurus gaurus* (India, Nepal), *Bos gaurus laosiensis* (Myanmar to China) and *Bos gaurus hubbacki* (Malaysia, Thailand) by the Asian Wild Cattle Conservation Assessment and Management Plan (Byers *et al.*, 1995). These animals were classified as endangered species and vulnerable by the International Union for the Conservation of Nature and Natural Resources (Simon, 1966). The global population of wild gaur ranges from 13,000 to 30,000 (IUCN, 2008; Nguyen *et al.*, 2007).

Figure 2. A family of Malayan gaur (*Bos gaurus hubbacki*).

As with other bovidae, chromosome studies indicated that gaur evolved from a wild ancestor from which the domestic taurine and zebu cattle originated (Buckland and Evans, 1978; Wurster and Benirschke, 1968). Centric fusion between chromosomes 2 and 28 of the ancestral cattle karyotype of 58 acrocentric chromosomes gave rise to the Indian gaur karyotype (2n=58), consisting of 27 pairs of acrocentric and a pair of submetacentric autosomes (Mastromonaco *et al.*, 2004).

The Malayan Gaur (*Bos gaurus hubbacki*), the second largest animal in Malaysia can weigh up to 1000kg for the male and 500-700kg for the female (Bongso *et al.*, 1988; Ebil, 1982). Huge head, massive body and sturdy limbs are the characterization of vigor and strength of the gaur. It was estimated

that there were 472 individuals in 1980 (DWNP, 1981) and a minimum population of 273-333 based on data collected in 1994 to 2005 (DWNP, 2005). This impressive largest wild cattle which live in isolated and fragmented populations mostly in Pahang, Perak, Kelantan, Terengganu and Taman Negara inhabit forests near to the foothills (Ebil, 1982). Studies indicated that the number was globally declining, may be due to habitat loss, poaching and diseases from domestic cattle (Conry, 1981; Conry, 1989; DWNP, 2005; IUCN, 2008; Nguyen *et al.*, 2007) and Malaysia has the most rapidly declining population (IUCN, 2008).

Developments including agricultural activities have forced the animal from their preferred areas to marginal parts of the habitat (Conry, 1989; Ebil, 1982). This may support the interesting incident in 1983 at southern part of Peninsular Malaysia where a wild gaur was reported to mate with a group of domestic cattle in a farm. The distribution of Malayan gaur is shown in Table 1, with the biggest population being in the state of Pahang.