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Genetic variation and relationship between Kedah-Kelantan, Thai, Brahman and Charolais cattle

(Variasi dan kaitan genetik antara lembu tempatan Kedah-Kelantan, lembu Thai, Brahman dan Charolais)

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Key words: RAPD analysis, genetic relationship, beef cattle

Abstrak

Variasi dan kaitan genetik antara lembu Kedah-Kelantan (KK), Thai, Brahman dan Charolais telah dikaji dengan menggunakan analisis DNA polimorfik teramplifikasi rawak (RAPD). Sebanyak 120 ekor lembu KK, Thai, Brahman dan Charolais digunakan untuk kajian ini. Corak dan kekerapan jalur polimorfik yang dihasilkan oleh lapan primer rawak menunjukkan variasi genetik yang banyak di dalam dan antara keempat-empat baka lembu tersebut. Anggaran jarak genetik menunjukkan kaitan genetik antara baka-baka lembu pedaging dengan jelas. Dendrogram membahagikan baka-baka lembu kepada dua kumpulan utama iaitu *Bos indicus* (lembu KK, Thai, Brahman) dan *Bos taurus* (Charolais). Indeks persamaan Nei menunjukkan lembu KK dan lembu Thai adalah sama dari segi genetiknya, memungkinkan baka lembu KK dan Thai adalah daripada baka yang sama, tetapi diberi nama berlainan. Penanda RAPD sangat berguna untuk mengkaji variasi dan kaitan genetik antara baka-baka lembu.

Abstract

Genetic variation and relationship between the indigenous Kedah-Kelantan, Thai, Brahman and Charolais cattle was studied using random amplified polymorphic DNA (RAPD) analysis. A total of 120 heads of KK, Thai, Brahman and Charolais cattle were used in the study. DNA band patterns and frequencies generated by the 8 random primers indicated that there were considerable genetic variation within and between the four breeds of beef cattle. The estimated genetic distance matrix delineated the genetic relationship between the four breeds of cattle. This was indicated by the dendrogram which clearly divided the four beef cattle breeds into two main groups, namely, the *Bos indicus* which include the KK, Thai cattle and Brahman; and the *Bos Taurus,* the Charolias cattle which is of European origin. Nei's index indicated that the KK and Thai cattle are genetically similar, suggesting that the KK and Thai cattle are of the same breed but with different names. RAPD markers are useful for assessing genetic variation and establishing the genetic relationship between different breeds of beef cattle.

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Introduction

The major breeds of beef cattle in Malaysia are the Kedah-Kelantan (KK) cattle, Brahman crosses and European-Kedah-Kelantan crossbred types (Ariff 1998). The KK cattle with a population of about 559 375 heads (DVS 1999) is the most important indigenous livestock in Malaysia. They represent unique resources in the world's tropical fauna and are popular with Malaysian livestock farmers due to their high fertility, tick resistance and ability to thrive under poor conditions. For decades, this breed was reared primarily for draft purposes but now are bred primarily for beef production. In view of the importance of KK as a genetic resource for beef production in Malaysia, various studies have been carried out to assess and improve the inherent characteristics and performances of this breed (Ahmad et al. 1985; Dahlan 1985; Devendra and Lee 1978; Johari et al. 1994; Tan et al. 1996).

Phenotypically KK are similar to the Thai cattle and other native breeds found in South East Asia. However, information on the genetic uniqueness of these native breeds is lacking. This information is important for planning a breeding programme for the improvement and conservation of native breeds. Presently, KK are crossed with exotic beef breeds such as Brahman and Charolais cattle. It is therefore, important to assess the genetic differences between the KK populations and other beef breeds used in the breeding programme. Indications of their genetic differences will be of value in planning a selective breeding programme to obtain optimum heterosis.

Various molecular techniques have been used to assess genetic differences and variations between animal species at the DNA level (Avise 1994). Among these techniques, random amplified polymorphic DNA (RAPD) has been used widely for assessing the genetic variations within and between population of wild and domesticated species (Welsh and McClelland 1990; Willams et al. 1990; Caetano-Anolles et al. 1991, 1995; Michelmore et al. 1991; Huff et al. 1993; Plotsky et al. 1995). The RAPD assay has been used to generate polymorphic markers for cattle breed differentiation and identification (Gwakisa et al. 1994; Kemp and Teale 1994) for evaluating genetic variation between populations or breeds (Bardin et al. 1992; Kantanen et al. 1995), and for detecting gene introgression (Teale et al. 1995).

The objective of this study was to determine the genetic variation within and between populations of KK, Thai, Brahman and Charolais cattle and to establish a genetic relationship between the four beef cattle breeds. The RAPD fingerprinting analysis was chosen for these purposes because it is technically simple, rapid and cheap.

Materials and methods

Sample collection and extraction of DNA

A total of 120 heads of KK, Thai, Brahman and Charolais cattle were used in the study. The KK cattle were from two different areas in Peninsular Malaysia, ie., East Coast (KK1, n = 30); and West Coast (KK2, n = 30). The Thai cattle (TH, n = 30) were imported from Southern and Central Thailand while Brahman (BR, n = 30) and Charolais (CH, n = 10) cattle were imported from Texas, USA and New Caledonia, respectively.

Blood samples from these cattle were collected in heparinized-tubes and frozen at –20 °C until analysed. DNA was extracted using Genomic DNA Purification Kit (Promega, CAT # A1120). The DNA concentration was measured with DyNA Quant 200 (Hoefer, Pharmacia Biotech., USA) and diluted with distilled water to 25 ng/µL working solution for PCR amplification. Five pooled DNA samples were made by mixing equal volumes of DNA from 15 samples each for KK1, KK2, TH, and BR populations**,** and 10 samples for CH.

Random amplified polymorphic DNA (RAPD) assay

A total of 100 random primers with a G + C content of approximately 50–70% (Genosys Biotechnology Inc.) were initially screened on pooled DNA samples. Amplifications were performed according to the method optimized for vertebrate DNA (Bielawski et al. 1995). The PCR reaction mixture consisted of the followings: 2.5 µL of PCR buffer 10x (10 mM Tris-HCL, 1.0% gelatine, 50 mM KCL), 2.0 µL of 2.0 mM $MgCl_2$, 0.5 µL of 0.2 mM dNTP mixed, 0.2 µL of *Taq* DNA polymerase (Promega), 2.0 µL of 25 ng of genomic DNA, 2.5 µL of 0.5 µM primers, and finally brought to a total volume of 25.0 µL with distilled water. A negative control containing all the components mentioned above except DNA was included in all amplification series to ensure against contamination of reagents with DNA.

All amplifications were carried out in a Programmable Thermal Cycler (Biometra) at 94 °C for 30 sec, 36 °C for 30 sec and 72 °C for 2 min for 45 cycles. The amplification products were electrophoresed at 70 V in 1.5% agarose gel and stained in ethidium bromide. The RAPD-DNA fingerprinting was visualized and documented using Image Analyzer (Phamacia Biotech. USA). Individual DNA bands were scored as present (1) or absent (0) in each amplification profile.

Data analysis

The frequency of the RAPD markers was expressed as percentage of animals in each breed showing the visible loci. The genetic distances between individuals in the breeds and between breeds were computed using RAPDistance Package (Version 1.04) provided by Dr. John Armstrong, Australian National University, Canberra, Australia. A total of 50 pairwise genetic distances between individuals within and between breeds were compared using ANOVA (SAS System, 6.12). The identity index was computed and a dendrogram was constructed using UPGMA method modified from Neighbor procedure of Phylip (Nei 1978) in Popgene package program (Version 1.31, provided by the Center for International Forestry Research, CIFOR, Canada).

Results and discussion

Polymorphism in KK, Thai, Brahman and Charolais cattle

A total of 100 10-mer primers were screened of which 65 primers generated amplification products ranging from 0.35–2.0 kb in size. Thirty-five of these primers produced apparent specific bands in the pooled DNA samples of KK, TH, BR or CH cattle. Eight primers that generated prominent and reproducible apparent specific bands for KK, Thai, Brahman or Charolais cattle were then selected for further analysis. Each of the eight primers produced a different banding pattern for the four breeds of beef cattle. However, each primer produced very similar band patterns of individuals within each population or breed. The number of DNA bands amplified by each primer varies from 3 to 11 with an average of 6.36 markers per primer *(Table 1)*.

A total of 93 DNA fragments were generated by the eight primers. The size of the DNA fragments ranged from 0.35 to 1.8 kbp for primer 1-50-09; 0.40 to 2.3 kbp for primer 1-50-42; 0.5 to 1.8 kbp for primer 1-50-51; 0.45 to 1.1 kbp for primer 1-50-57; 0.55 to 1.4 kbp for primer 1-50-20; 0.4 to 2.0 kbp for primer 2-50-22; 0.4 to 1.2 kbp for primer 1-60-02 and 0.4 to 2.5 kbp for primer 1-50-16. Only RAPD fragments between 0.35 kbp and 2.0 kbp were scored since these were reproducible and prominent. Of the 93 DNA fragments generated, 19 bands were scored as monomorphic and shared between the breeds. The rest of the markers were polymorphic and were shared within and between breeds. A total of 38 polymorphic markers were scored in KK cattle, 33 polymorphic markers in Thai cattle, 27 polymorphic markers in Brahman cattle and

Table 1. Number and average of RAPD bands generated by eight random primers

No. of bands (x)	1-50-20	1-50-09	1-50-16	2-50-22	1-50-42	1-50-51	1-50-57	1-60-02
KK1(30)	4–7(5.2)	5–10(7.1)	4–9(6.3)	3–11(9.7)	4–10(7.1)	3–7(4.5)	5–10(6.5)	3–7(4.4)
KK2(30)	4–7(5.0)	5–11(7.5)	4–8(6.0)	4–11(9.4)	4–10(7.4)	4–7(5.0)	5–10(6.2)	3–7(4.5)
TH (30)	4–7(5.9)	6–11(8.2)	4–8(5.7)	5–9(7.2)	4–11(7.8)	4–8(4.0)	5–11(7.0)	3–7(5.2)
BR (30)	5–7(6.4)	5–12(9.5)	3–10(6.7)	6–11(8.4)	5–10(5.5)	4–9(5.0)	4–10(5.5)	4–7(5.2)
CH (10)	4–7(5.2)	5–9(6.5)	3–9(5.9)	5–10(7.0)	4–10(5.3)	3–8(4.5)	5–10(6.0)	3–7(5.0)

Table 2. Primer sequences and number (proportion) of polymorphic DNA markers scored

Primer	Sequence 5' - 3'	No. of polymorphic markers				
		KK1	KK2	TH	BR	CH
1-50-09	AGAAGCGATG	5(0.33)	5(0.33)	3(0.20)	2(0.13)	2(0.13)
1-50-42	GTGAAATGGC	4(0.36)	4(0.36)	4(0.36)	3(0.27)	3(0.27)
1-50-51	AGGGAAACAC	3(0.27)	3(0.27)	3(0.27)	4(0.36)	4(0.36)
1-50-57	ATATTTGGGC	6(0.46)	6(0.46)	5(0.38)	6(0.46)	5(0.38)
2-50-22	CGAAACAGTC	8(0.57)	8(0.57)	5(0.35)	3(0.21)	2(0.14)
1-50-20	AATCACACCC	5(0.50)	5(0.50)	5(0.50)	3(0.30)	2(0.02)
1-50-16	AGTGAATGCG	2(0.20)	2(0.20)	2(0.20)	3(0.30)	2(0.20)
1-60-02	GTCCTACTCG	4(0.44)	5(0.55)	6(0.66)	3(0.33)	2(0.22)
Total		37(0.37)	38(0.41)	33(0.35)	27(0.29)	22(0.24)

22 polymorphic markers in Charolais cattle *(Table 2)*. KK cattle shared 12 common bands and 28 polymorphic bands with Thai cattle. KK, Thai and Brahman cattle shared 7 common bands and 25 polymorphic bands. Brahman cattle shared 2 common bands and 17 polymorphic bands with Charolais cattle.

The eight primers generated more polymorphic markers from KK and Thai cattle than from Brahman or Charolais cattle. This indicated that genetic polymorphism was higher in KK and Thai cattle than those in Brahman or Charolais cattle. Higher genetic variation within KK or Thai cattle is expected since the KK and Thai cattle have evolved and developed through natural selection while the Brahman and Charolais are established beef breeds developed after intensive selection by cattle breeders.

The frequency of polymorphic markers varied between the four breeds of cattle ranging from 0.13 to 0.88, with a mean band frequency of approximately 0.46 *(Table 2)*. The wide variation in frequency of polymorphic markers scored between the breeds showed that there was substantial genetic variation between the four breeds of cattle.

Apparent breed-specific RAPD markers were also observed. For example, primer 1-50-20 produced a 0.60 kbp band that was present in all KK samples and 33% in samples of Thai cattle while primer 1-50-51 produced a 0.95 kbp band in all samples from KK but absent in other populations. Primer 2-50-22 generated a product of 0.90 kbp which was present in all Brahman samples but absent in other populations *(Table 3)*.

These apparent specific RAPD markers will be of value for the identification of breeds or for studying their association with production traits (Tercic et al. 1998). Kemp and Teale (1994) using RAPD fingerprinting assay generated polymorphic markers that distinguished between *Bos indicus* and *Bos taurus* subspecies of domestic cattle.

Table 3. Frequency of apparent breed specific RAPD bands

Primers	Band Size (kbp)	KK1	KK2	TH	BR	CH
1-50-09	0.60	1.00	1.00	0.16	0	0
1-50-42	0.65	0.83	0.73	0	0	0
1-50-51	0.95	1.00	1.00	0	0	0
1-50-57	0.50	1.00	1.00	0.67	0	0
1-50-20	0.55	1.00	1.00	0.33	0	0
2-50-22	0.90	0	0	0	1.00	0
1-60-02	0.45	0	0	0.43	0	0.80
1-50-16	1.80	0	0	0	0	1.00

Table 4. Nei's genetic distance between five populations of beef cattle breeds (KK, Thai cattle, Brahman and Charolais)

Population	KK1	KK2	TH	BR	CH
KK1	0.135±0.013c				
KK2	0.136±0.002c	0.136±0.003c			
TH	0.197±0.002b	0.179±0.002b	0.135±0.012c		
BR	0.323±0.002a	0.319±0.014a	0.320±0.003a	0.135±0.003c	
CH	0.527±0.013d	0.519±0.013d	0.524±0.015d	0.570±0.012d	0.136±0.003c

Different letters indicate differences at $p < 0.01$

Genetic relationship between breeds

The pairwise RAPD distances matrix within and between the four breeds of beef cattle were computed using RAPDistance and Popgene program *(Table 4).* The genetic distances matrix showed substantial genetic differences within breeds but was significantly shorter ($p < 0.01$) compared to between breeds. The genetic distances for KK and Thai cattle were 0.197 and 0.179 respectively and was significantly shorter ($p < 0.01$) compared to the genetic distance for KK and Brahman cattle. These indicated that the KK and Thai cattle are genetically closer to each other than to the Brahman cattle. The genetic distances for KK and Brahman cattle and for Thai and Brahman cattle were 0.323 and 0.320, respectively. The Charolais is very distant to the other breeds with a value ranging from 0.519 (KK-CH) to 0.570 (BR-CH).

To show the genetic relationship between the 5 populations, a dendrogram was drawn based on the genetic distance matrix. The dendrogram cleary differentiated the beef breeds into two main groups: the *B. indicus* of Asiatic origin which include the KK, Thai and Brahman cattle, and *B. taurus* of European origin, the Charolais *(Figure 1).* These results are in agreement with several reports (Gonzalez and Tunon 1987), which pointed out the two different branches of various breeds of domestic cattle.

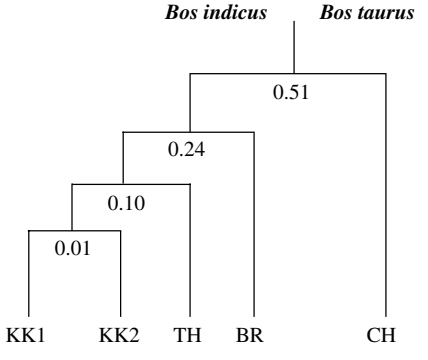

Figure 1. Dendrogram showing genetic relationship among the 4 breeds of beef cattle

To complement the analysis above, Nei's estimate of genetic identity based on the number of shared amplification products (Nei and Li 1979) was used to generate identity indices *(Table 5).* The identity indices were 0.863 within the KK1 population; 0.856 within the KK2

Table 5. Identity indices within and between populations of beef cattle

	KK1	KK2	TC	BR	CH
KK1	0.863				
KK2	0.855	0.856			
TC	0.813	0.814	0.857		
BR	0.679	0.677	0.703	0.832	
CH	0.549	0.546	0.584	0.552	0.870

population; 0.857 within the Thai cattle population, 0.832 within the Brahman cattle and 0.870 within the Charolais population. Generally the interbreed identity indices are lower than the intrabreed identity indices indicating that the genetic variation between individuals in the population is smaller than the variation between breeds. The identity indices between and within the KK and Thai cattle were not different, indicating the two breeds were genetically similar. The SI between KK and Thai cattle and between KK and Brahman are larger than the SI between KK and Charolais or between Brahman and Charolais. These indicated substantial genetic differences between KK, Thai or Brahman and Charolais cattle.

The magnitude of within breed variation in KK, Thai or Brahman cattle was similar to that reported in other Zebu breeds. The magnitudes of identity indices between KK and Brahman cattle (0.679) and between Thai and Brahman cattle (0.703) were also similar to those reported between other Zebu breeds (about 0.6) (Gwakisa et al. 1994). High genetic identity between KK and Thai cattle is consistent with morphological evidence reflecting the familial relationship between the two cattle breeds. Although comparative studies to evaluate the phenotypic variations between KK and Thai cattle are lacking, independent studies indicated phenotypic similarities between the two native breeds. Since the KK and Thai cattle are phenotypically and genetically similar, as indicated by the present study and the genetic distance between them are very close, the KK and Thai cattle are therefore perhaps the same breed but with different names found in the region of South East Asia.

Conclusion

The present study showed that RAPD fingerprinting analysis is an effective method for generating polymorphic DNA markers in local beef cattle populations. These polymorphic markers are also useful for estimating genetic distances and subsequently establishing the genetic relationships between local beef cattle breeds. RAPD fingerprint analysis will be practical in cattle breeding and conservation of indigenous breeds. It provides a mean to differentiate breed groups that are genetically dissimilar and members within the groups that are genetically similar. This will be useful in selective breeding programme to obtain optimum heterosis.

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References

Ahmad, A., Kamal, M.K, Ariff, M.O. and Shokri, A. (1985). Variation in weights of Kedah-Kelantan cattle from birth to one year of age. *MARDI Res. Bull.* ***13****(1):* 81–6

Ariff, M.O. (1998). Recent development in animal genetics and breeding in Malaysia. *Proc. Third National Cong. on Genetics.* UKM. Bangi, Malaysia p. 66–74

Avise, J.C. (1994) *Molecular markers, natural history and evolution*. New York: Chapman and Hall

Bardin, M.G., Bandi, C., Comincini, S., Domiani, G. and Rognoni, G. (1992). Use of RAPD markers to estimate genetic variations in bovine population. *Animal Genetic* ***23****,* (Suppl.): 57

Bielawski, J.P., Noack, K. and Pumo, D.O. (1995). Reproducible amplification of RAPD markers from vertebrate DNA. *Biotechniques* ***5:*** 856–60

Caetano-Anolles, G., Bassam, B.J. and Greenhoff, P.M. (1991). DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. *Biotechnology* ***9:*** 553–6

Caetano-Anolles, G., Callahan, L.M., Williams, P.E., Weaver, K.R. and Gresshoff P.M. (1995). DNA amplification fingerprinting analysis of bermudagrass (Cynodon): genetic relationships between species and interspecific crosses. *Theor. Appl. Genet*. ***91:*** 228–35

Dahlan, I. (1985). Gowth performance, body conformation, carcass characteristics and palatability of Kedah-Kelantan cattle and its crosses. M. Sc. Thesis. University of Agriculture, Malaysia

Devendra, C. and Lee, K.C. (1978). Studies on Kedah-Kelantan cattle. III. Body characteristic and carcass composition. *MARDI Res. Bull.* ***4:*** 81–8

DVS (1999). *Malaysian Livestock Statistics*. Kuala Lumpur: Department of Veterinary Services

Gonzalez, P. and Tunon, M.J. (1987). Genetic relationships between seven Spanish native breeds of cattle. *Animal Genetics* ***18:*** 249–56

Gwakisa, P.S., Kemp, S.J. and Teale, A.J. (1994). Chariacterization of zebu cattle breeds in Tanzania using randomamplified polymorphic DNA markers. *Animal Genetics* ***25:*** 89–94

Huff, D.R., Peakall, R. and Smouse, P.E. (1993). RAPD variation within and among natural populations of outcrossing buffalo grass. *Theor. Appl. Genet*. ***86:*** 927–34

Johari, J.A., Ariff, M.O. and Adnan, S. (1994). Reproductive performance of Kedah-Kelantan crossbred cattle. *MARDI Res. J.* ***22(1):*** 91–5

Kantanen, J., Vikki, J., Elo, K and Maki-Tanila. (1995) Random amplified polymorphic DNA in cattle and sheep: application for detecting genetic variations. *Animal Genetics* ***26:*** 315–20

Kemp, S.J. and Teale, A.J. (1994). Randomly primed PCR amplification of pooled DNA reveals polymorphism in a ruminant repetitive DNA sequence which differentiates *Bos indicus* and *Bos taurus. Animal Genetics* ***25:*** 83–8

Michelmore, R.W., Paran, I. and Kesseli, R.V. (1991) Identification of markers linked to disease resistance gen by bulked segregant analysis – a rapid method to detect markers in specific genome regions by using segregating populations. *Proc. National Academy Sciences USA*. ***88:*** 9825–32

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* ***89:*** 583–90

Nei, M. and Li, W.H. (1979). Mathematical modelling in studying genetic variation in term of restriction endonucleus. *Proc. National Academy Sciences USA* ***78:*** 2411–14

Plotsky, Y., Kaiser, M.G. and Lamont, S.J. (1995). Genetic characterization of highly inbred chicken lines by two DNA methods: DNA fingerprinting and polymerase chain reaction using arbitrary primers. *Animal Genetic.* ***2:*** 163–70

Tan, H.S., Kassim, H. and Mak, T.K. (1996) Reproductive performance of indigenous cattle in Malaysia. *Nuclear and Related Techniques in Animal Production and Health* p. 189–203

Teale, A.J., Wambugu, J., Gwakisa, P.S., Stranzinger, G., Bradley, D. and Kemp, S.J. (1995). A polymorphism in randomly amplified DNA that differentiates the Y chromosomes of *Bos indicus* and *Bos taurus*. *Animas Genetics* ***26:*** 243–8

Tercic, D., Holeman, A. and Dove, P. (1998) Three RAPD markers for growth performance in chicken. *Proc. Plant and Animal Genome VI Conf.* San Diego, Canada p. 73

Welsh, J. and McClelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acid Res*. ***18:*** 7213–8

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Res*. ***18:*** 6531–65

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