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Phylogenetic Relationships among Different Breeds of Domestic Chickens in Selected Areas of Peninsular Malaysia Using RAPD Markers

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ABSTRACT

The present investigation was carried out to determine the phylogenetic relationships among different breeds of domestic chickens in selected areas of Peninsular Malaysia. Four RAPD primers were used to characterise the genetic similarity among twenty-seven chicken samples from Peninsular Malaysia. Phylogenetic analysis, using neighbour-joining (NJ), generally produced six clusters which were completely distinguished based on the locality of the chickens. A comparatively high level of genetic variation was observed among the village chickens from different locations. A high level of genetic variation was also observed between commercial broiler chicken from selected localities in Peninsular Malaysia and between the commercial broiler and layer chicken in Peninsular Malaysia. Hence, the results indicated the effectiveness of RAPD to detect similarity between chicken lines and their applicability in establishing genetic relationships among chicken populations.

Keywords: Phylogenetic, Random Amplified Polymorphic DNA (RAPD), chickens

INTRODUCTION

Over the last decade, molecular markers have been used to distinguish the different species of *G. gallus*. Prior to the introduction of biochemical and molecular markers, the jungle fowl species were distinguished by morphology and metric quantitative traits (Moiseveya *et al.*, 2003). Based on the archaeological discoveries, there are two hypotheses of the origins of chicken domestication, with one proposing a monophyletic origin and the other, multiple origins from several *Gallus* subspecies (Crawford 1990). In Malaysia, the chickens are basically divided into two main categories, namely village chickens and commercial chickens, which consist of commercial broiler and layer. In particular, village chicken (*kampung* chicken) is a type of chicken that is characterized by medium or low productivity in terms of egg production, egg size, growth, survivability, and inefficient utilisation of feed as compared to commercial broiler. Village chicken is often maintained in a small population in rural areas, such as village, forest, plantation of tropical and subtropical countries and it is not confined in cage (Ramanov & Weigend, 2003). Meanwhile, in the category of commercial chickens, the commercial layer is a type of chicken that is bred for egg production under strong artificial selection (Pirany *et al.,* 2007). Both groups of broilers and layers are maintained under strong artificial selection (Pirany *et al.,* 2007).

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Currently, the breeding schemes for commercial chickens are focused on specialised production lines, derived by an intense selection from a few breeds and very large populations, with a great genetic uniformity of traits under selection (Notter, 1999). Intense selection is mainly concentrated on one or a few traits and thus the approach to homozygosity at same loci may be rapid (Dunnington & Siegel, 1994). As a population approaches homozygosity, the ability to respond to further selection may be impaired. Theoretically, the intense selection may reduce or even exhaust the genetic variability in such populations, thereby reducing progress or rendering further selection ineffective. In poultry breeding, therefore, it is important for the poultry industry to evaluate the levels of genetic diversity in elite pure stocks of the village and commercial chickens (Dunnington & Siegel, 1994; Moiseyeva *et al.*, 2003).

The RAPD markers have particularly been used for genetic and molecular studies, as it is a simple and rapid method used to determine the genetic diversity and similarity in various organisms (Salem *et al.*, 2005). Another interesting characteristic of the RAPD markers is that no prior knowledge of the genome under research is necessary (Fischer *et al.*, 2000; Klinbunga *et al.,* 2000). Meanwhile, the RAPD analysis has extensively been used for genome mapping in chickens (Levin *et al.*, 1993; 1994; Cheng *et al*., 1995), genetic characterisation of highly inbred chicken lines (Potsky *et al*., 1995), identification of specific markers (Zhang *et al*., 1995), genetic diversity, and parentage analysis in chickens and turkeys (Smith *et al.*, 1996), estimation of genetic diversity in inter and intra populations of poultry breeds (Sharma *et al.*, 2001), estimation of genetic diversity of Chinese native chicken breeds (Zhang *et al.*, 2002), genetic similarity between pure lines of chickens in Turkey (Okumus & Kaya, 2005), as well as evaluation of genetic variability and distances among five Iranian native chicken populations (Dehghanzadeh *et al.*, 2009). Currently, the chicken genetic map contains about 1,965 loci within 50 linkage groups and it is estimated that the entire chicken genome contains about 4,000 centimorgans (cM). According to Salem *et al.* (2005), about 235 of these loci have homology with known human or mammalian genes, while the remaining loci are anonymous molecular DNA markers. Sharma *et al.* (2001) have shown the effectiveness of the RAPD in detecting polymorphisms between chicken populations and in establishing the genetic relationship among the chicken populations. Ali & Ahmed (2001) and Ali *et al.* (2003) have demonstrated the potential use of the RAPD analysis for a wide range of applications in poultry breeding. Thus, the author attempted to establish the genetic relationships among different breeds of domestic chickens in selected areas of Peninsular Malaysia using the RAPDs markers.

METHODS

Sample Collection and DNA Extraction

A total of 27 samples were collected from several locations in Peninsular Malaysia (Table 1). The total genomic DNA was extracted from the quill of the feathers using the Wizard Genomic® DNA Purification Kit. The DNA concentration and its purity were examined using the spectrophotometric analysis, based on 260 and 280 nm absorbance and agarose gel electrophoresis analysis.

Random Amplified Polymorphic DNA – Polymerase Chain Reaction (RAPD-PCR)

The RAPD-PCR reaction mixture (25 µL) consisted of 13.3 µL of dH*2*O, 2.5 µL of 10x *Taq* DNA buffer with $(NH_4)_2SO_4$, 2.5 µL of 2.5 mM dNTPs, 2.5 µL of 10 µM of RAPD primer (5'-CGC TGT CGC C-3', 5'-AGT CCT CGC C- 3', 5'-TGG TGG ACC A-3', 5'- GAA TGC GAC G-3'), 0.2 µL of 5U/ µL *Taq* polymerase, 2.0 µL of 25mM $MgCl_2$ and 2.0 µL of 100 ng/ µL DNA template. The final reaction mixture was placed into a thermal cycle (Eppendorf). The RAPD-PCR profile included an initial denaturation step at 94°C for 2 min, followed by 45 cycles at 94°C of 30 sec for DNA denaturation, annealing step at optimised temperature for 30 sec, extension at 72°C for 30 sec and the final extension at

TABLE 1
Sample coding, type of chicken, gender, and locality for each individual

Sample coding	Chicken type	Gender	Locality
VJY1	Village	Male	Pulau Pinang
VJY2	Village	Male	Pulau Pinang
VJY3	Village	Male	Pulau Pinang
VJY4	Village	Male	Pulau Pinang
VJY5	Village	Male	Pulau Pinang
BJY6	Broiler (Cobb)	Male	Johor
VJY7	Village	Female	Perak
VJY8	Village	Female	Perak
BJY9	Broiler (Cobb)	Male	Perak
VJY10	Village	Male	Perak
VJY11	Village	Male	Perak
VJY12	Village	Male	Perak
LJY13	Layer (Hisex)	Female	Perak
VJY14	Village	Male	Perak
VJY15	Village	Male	Johor
BTK16	Broiler (Ross)	Female	Pahang
BTK17	Broiler (Ross)	Female	Pahang
BTK18	Broiler (Ross)	Female	Pahang
BTK19	Broiler (Ross)	Female	Pahang
VTK20	Village	Female	Selangor
VTK21	Village	Female	Selangor
VTK22	Village	Male	Selangor
VTK23	Village	Male	Selangor
VTK24	Village	Male	Selangor
VM25	Village	Male	Kuala Lumpur
VM26	Village	Male	Kuala Lumpur
VM27	Village	Male	Kuala Lumpur

72°C for 10 min were carried out. Meanwhile, the electrophoresis was carried out at 80 V for 110 min. The amplified DNA patterns were analysed on a 2.5% agarose gel electrophoreses and stained with ethidium bromide. The amplified DNA patterns were visualised on a UV transilluminator and photographed using the gel documentation system.

RAPD Data Analysis

The first step in the data analysis was to examine the band patterns of all the DNA samples that were compared with each primer. The presence or absence of the band in each RAPD pattern was recorded using the RAPDistance 1.04 (Armstrong *et al.*, 1994). The presence of the band within each RAPD pattern was scored as one, while the absence of band was scored as zero, in which they were used to estimate the genetic variability within, as well as between the populations. The genetic distances among the different breeds of domestic chickens in selected areas of Peninsular Malaysia were determined according to the Jaccard algorithm. The RAPD distance matrix was used to construct the dendogram of the neighbour-joining (NJ) tree. For each RAPD primer, the genetic similarities among the different breeds of domestic chickens in selected areas of Peninsular Malaysia were determined according to Jaccard similarity coefficient (Rao *et al*., 1996; Romesburg, 2004).

Then, the average of genetic similarity among the different breeds of domestic chickens in the selected areas of Peninsular Malaysia was determined.

RESULTS AND DISCUSSION

Four RAPD primers were screened and yielded distinct polymorphic RAPD profiles in six populations with a total of 104 of polymorphic band patterns and one monomorphic band pattern. These amplified band patterns were found to range from 180 bp to 1530 bp. As expected, the genetic distances and the genetic similarity were the lowest within the intra population and were the highest within the inter population. Furthermore, the genetic distances and genetic similarity were the lowest within similar breed types and were the highest within the dissimilarity breed types.

From the dendogram (*Fig. 1*) that was generated based on the RAPD-PCR fingerprints, the Malaysian village, layer and broiler chicken breeds were clustered into six groups which completely distinguished the locality of each chicken. The first, second and third groups were formed mainly by the village chickens taken from Pulau Pinang, Selangor and Kuala Lumpur, respectively. In this study, high levels of genetic variation were observed among the Malaysian village chickens from various localities. In fact, only the village chickens from the same locality demonstrated low level of genetic variation. The major reason that could have led to the high level of genetic variation among the Malaysian village chickens was the natural selection or breeding. Hence, a high level of genetic variation among the Malaysian village chickens indicates that there is a high level of genetic diversity among the village chickens in Malaysia.

Based on the dendogram, the village chickens from Perak were clustered into Group 4, Group 5, and Group 6. The village chickens taken from Perak and Johor were grouped together in Group 4, whereas the village chickens and the commercial broiler chickens from Perak were grouped together with the commercial broiler chicken from Johor in Group 6. As observed within Group 5, the village chickens and the commercial layer chicken from Perak were grouped with the commercial broiler chickens from Pahang. This grouping was probably due to the import of the commercial chickens between the states in Peninsular Malaysia. In counterpoint to the village chickens, the broiler chickens from Pahang and a commercial layer chickens from Perak were clustered together within Group 5 (*Fig. 1*). The classification of the Malaysian commercial broiler and layer chickens was in contrast to a previous study conducted by Shen *et al.* (2002), whereby the Japanese Chunky broilers and commercial layers were grouped into two different groups.

In this study, a high level of genetic variation observed among the broiler chickens in Peninsular Malaysia was found to be with the finding of Shen *et al.* (2002), whereby a high level of genetic variation was observed among the Japanese Chunky broilers. Nevertheless, the finding of this study is inconsistent with the previous studies conducted by Sharma *et al.* (2001) and Ali *et al.* (2003). According to Sharma *et al.* (2001), a low level of genetic variation (genetic dissimilarity) was observed among the commercial broilers. In addition, Ali *et al.* (2003) also reported that a high level of genetic similarity was observed among the commercial broiler chickens from different localities. One of the reasons that could have led to the high level of genetic variation among the broiler chickens was the different breeds of broiler chickens were used for breeding strategies. Similarly in this study, a high level of genetic variation was also observed between the commercial broiler and layer chickens in Malaysia. Zhang *et al.* (2002) reported that a great difference of genetic variation was observed between the broiler and layer chicken breeds. Hence, the finding of this study is compatible with the study by Zhang *et al.* (2002). Nevertheless, this study was found to be inconsistent with the study conducted by Ali *et al.* (2003) who observed a high level of genetic similarity between the commercial broiler and layer chickens from different localities. The

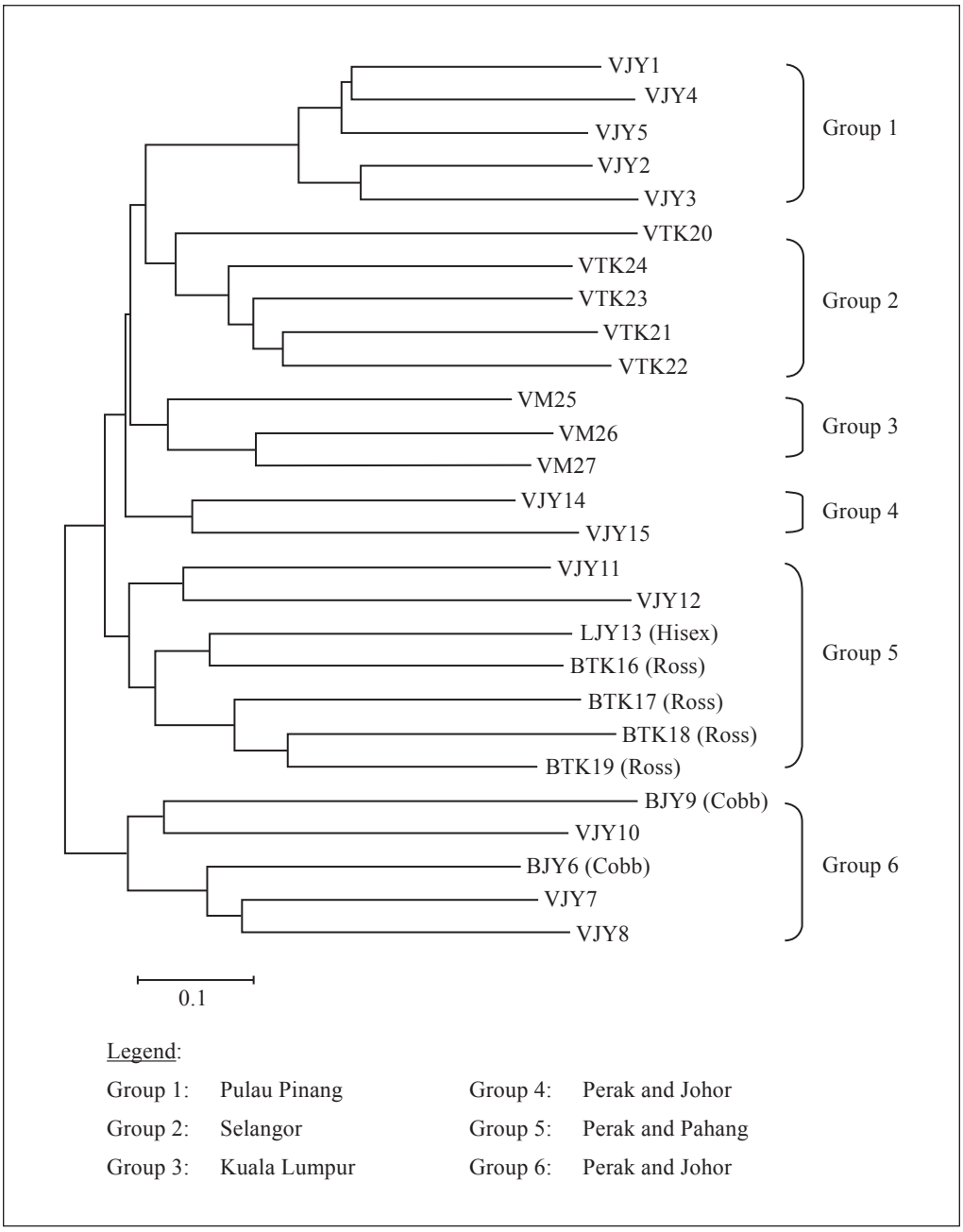


Fig. 1: The dendogram of different breeds of domestic chickens from the selected areas in Peninsular Malaysia generated based on the RAPD-PCR finger print patterns using four RAPD primers

reasons that could have led to the high level of genetic variation among the commercial broiler and layer chicken were the different breeds of chicken, whereby the broilers were bred for meat production and the commercial layers were bred for egg production (Sharma *et al.*, 2001; Shen *et al.*, 2002).

Another finding of this study is that high level of genetic variation was observed between the Malaysian village and commercial (broiler and layer) chickens. As compared to the study by Sharma *et al.* (2001), a high level of genetic variation was observed among the native chicken breed (Kadaknath) and the exotic chicken breeds in India. Furthermore, Zhang *et al.* (2002) revealed that the genetic variation between the Chinese native chicken breeds and commercial layer chickens was relatively high based on the RAPD analysis. Hence, this study is in agreement with the studies of Sharma *et al.* (2001) and Zhang *et al.* (2002).

The estimation of the genetic variability in populations is a critical prerequisite for optimising breeding strategies and regulating germplasm conservation which requires routine detection and monitoring methods (Sharma *et al*., 2001). Apart from the different breeds of village chickens and commercial chickens, one of the reasons that could have led to the high level of genetic variation among the domestic breeds was the natural and artificial selections, whereby the selection (natural or artificial) could have altered the allele frequency by reducing the likelihood that one or more genotypes would contribute to the next generation (Delany, 2003). However, if the poultry breeders continue to use population selection without taking the genetic selection schemes into consideration, the genetic variability in such population might become exhausted or even cause undesirable correlated responses to occur (Nordskog, 1965; Siegel & Dunnington, 1997). Hence, the maintenance of the genetic variation and genetic diversity among the Malaysian village chickens and commercial chickens is a crucial step to protect the Malaysian chickens against the present and unforeseen threats of avian disease. In addition, the knowledge of genetic similarity between the populations is prerequisite for establishing crossbreeding systems (Sharma *et al*., 2001). In this study, it has proven that the RAPD can provide an efficient system in determining the genetic diversity and genetic similarity of chickens due to its simplicity, speed and low cost. Moreover, Sharma *et al.* (2001) revealed the effectiveness and reliability of the RAPDs as a potential genetic marker in determining the genetic diversity and genetic similarity due to the common bands with very high degree of sequence homology, and this thus may be allelic to each other.

CONCLUSION

The present results demonstrated the efficacy of the RAPD markers in detecting polymorphism and establishing genetic relationships among the chicken breeds (Cobb, Ross, Hisex, and village chickens) in Peninsular Malaysia. Moreover, the present results demonstrated the ability of the RAPD markers to distinguish the locality of chickens from Peninsular Malaysia**.**

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