GENETIC CHARACTERIZATION BY MICROSATELLITE POLYMORPHISM IN THAI NATIVE CHICKEN COMPARE WITH BROILER NAD LAYER FOWLS

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การจำแนกลักษณะทางพันธุกรรมในไก่พื้นเมืองไทยเปรียบเทียบกับไก่เนื้อและไก่ไข่ โดยใช้ลักษณะ MICROSATELLITE MARKER

ชัชวาล สิงหะพล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Genetic characterization by microsatellite polymorphism in Thai native chicken compare with broiler and layer fowls

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree

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ไก่พื้นเมืองไทยมีความสำคัญต่อระบบเศรษฐกิจพื้นฐานของประเทศ อย่างไรก็ตามการจัดกลุ่มประชากรในปัจจุบันยังอาศัยเฉพาะหลักการคัดเลือกด้วยลักษณะภายนอกที่สังเกตเห็น เช่น สีขน และลักษณะต่างๆของร่างกายเป็นต้น ปัจจุบันยังไม่มีการจำแนกลักษณะและความหลากหลายทางพันธุกรรมในระดับโมเลกุลของไก่พื้นเมืองอย่างเพียงพอและถูกต้อง ในการทดลองนี้ได้ตรวจสอบลักษณะทางพันธุกรรมของไก่พื้นเมืองไทยในตระกูล *Gallus gallus domesticus* สองสายพันธุ์ คือ สายพันธุ์เหลืองหางขาวและประดู่หางดำ ในการทดลองได้ทำการสกัด genomic DNA ของไก่จำนวน 90 ตัวและนำดีเอ็นเอมาตรวจสอบโดยใช้ microsatellite DNA markers จากการทดลองพบว่าค่า number of allele แปรผันในช่วง 5 ถึง 25 โดยที่ microsatellite marker ทั้งหมดแสดง ลักษณะ polymorphic สูง ค่า highest H_{obs} พบใน loci MCW240 ค่า highest H_{exp} พบใน loci B154 เมื่อตรวจสอบค่า genetic diversity และ dendrogram พบว่าสามารถจำแนกกลุ่มของไก่ที่ผสมพันธ์แบบมีระบบแต่ในไก่ของเกษตรกรที่เลี้ยงทั่วไปไม่สามารถจำแนกออกจากกันได้อย่างชัดเจน กลุ่มของ microsatellite marker ที่ใช้ในการทดลองนี้สามารถแสดงความแตกต่างของไก่พื้นเมืองไทยสองสายพันธุ์ที่เลี้ยงในฟาร์มที่มีระบบการผสมพันธุ์ชัดเจนและเมื่อเปรียบเทียบกับไก่เนื้อลูกผสมพื้นเมืองและไก่ไข่ทางการค้าพบว่าไก่พื้นเมืองไทยมีสักษณะทางพันธุกรรมใกล้เคียงกับไก่เนื้อลูกผสมพื้นเมืองมากกว่าไก่ไข่ทางการค้า

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CHATCHAWAN SINGHAPOL: GENETIC CHARACTERIZATION BY MICROSATELLITE POLYMORPHISM IN THAI NATIVE CHICKEN COMPARE WITH BROILER AND LAYER FOWLS.
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The genetic variation of two main varieties of Thai native fowl *Gallus gallus domesticus*, Loenghangkhao and Praduhangdam were evaluated using sixteen microsatellite markers. All microsatellite were found to be polymorphic, the number of alleles varied from 5 to 25 per locus. The highest H_{obs} was found in MCW240. The highest H_{exp} was in B154. Native fowl that have full breeding program can show the clear differentiation of the subpopulation within total population. However, the native fowl in the open group cannot be separated. All results shown highly genetic diversity within the population. Both Thai native fowl are closely to hybrid native broiler more than commercial layer fowl. The results indicate the usefulness of microsatellite for the research of genetic variation and divergence. This group of microsatellite markers can be use to separate two main varieties of the Thai native fowl if they are bred from intense breeding program.

School of Biotechnology

Academic Year 2003

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Co-advisor's Signature .....

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Chatchawan Singhapol

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LIST OF ABBREVIATIONS

ATCG	=	nucleotide containing the base adenine, thymine, cytosine, and guanine, respectively
^{o}C	=	degree celcleus
DNA	=	deoxyribonucleic acid
EDTA	=	ethylene diamine tetraacetic acid
Kb	=	Kilobase
mg	=	milligram
ml	=	millilitre
mM	=	millimolar
M	=	molar
$MgCl_2$	=	magnesium chloride
ng	=	nanogram
μL	=	microlitre
μM	=	micromolar

CHAPTER I

INTRODUCTION

Poultry industry is an agricultural activity which has been related to Thai people for a very long time. Recently, poultry industry in Thailand has become a very large industry and is very important for the economy of the country. The actual number of poultry in Thailand is very large and they far exceed the number of pigs, buffalo and cattle. Chicken can give both meats and eggs as cheap protein source. There are fewer religious or social taboos associated with them when compare to pig and cattle, thus products produced from poultry provide an acceptable form of animal protein for most people. Commercial chicken products come from commercial layer, commercial broiler and hybrid native strains. The hybrid native strains are chicken, mated between native chicken breed and exotic chicken breed (imported commercial chicken). Thailand can produce more than 700 million broilers and 42 million layers a year. However, most of the parental stock and chicken line are imported (Apichai, 1998). Thus, the hybrid native strain seems to be more interesting because of the appropriate characters to Thailand environment and lower cost investment.

Chicken has been domesticated for a very long time. They have been manipulated for at least 2000 years. It is possible that poultry breeders in China established breeds of chickens as early as 1400 B.C. (Darwin, 1896). The native chicken *Gallus gallus domestticus* are believed to be selected and domesticated by human from

the ancestor, Red Jungle Fowl *Gallus gallus gallus* (Rose, 1997). There are two popular varieties of Thai native chicken (based on the colors of the feather) which are very well known among Thai people. These two varieties are the native chicken variety loenghangkhao and the native chicken variety praduhangdam. These two Thai native varieties have many excellence characters for example good body condition, beautiful color and appearance, high quality meat. They also can tolerate extremely high temperature and several diseases. The luenghangkhao fighting cock is one of the most popular variety and widely known because the long history of this variety. There are evidences that loenghangkhao variety has been manipulated since Ayoda era (Apichai, 1998). The number of luenghangkhao is also very high in Thailand. Therefore, this variety of chicken would be easy for the breeding selection of the excellence characters and breeding development.

At present, domestic chickens have been selected and developed to human demand. Native chickens have high potential for breeding program. They have many special characteristics such as good body condition, big and strong bone, beautiful color and appearance, high quality meat, good maternal ability and tolerate extremely high temperature and several diseases (Karnsomdee, 1999). However the weak point of native chickens is that they have slow growing rate and aggressiveness. They need about 6-8 months for maturation. With these excellent characters and to reduce the weak characters, native chicken is appropriate to use as parental stock to cross with the commercial stock for improvement of the good characteristics. The breed between native and commercial chicken will have the good characters from both commercial chicken and native chicken. Therefore, the study of genetic of Thai native chicken whether it is closer to broiler or layer is very interesting and would be beneficial to the breeding

program of the commercial chicken. (Etches and Gibbins, 1993) To improve and develop the commercial line on the right track, the basic genetic and physiology information of Thai native chicken is needed (Kaiser et al., 2000). Genetic information of Thai native chicken is not much known. Thus, the study of the relationship between morphology and genetic information for identify the variety of chicken is important.

Most of the developments of the native chicken in the past are the selection mainly by phenotypic but not genotypic characterization. Recently, the new method of molecular biology is one of the important techniques used to develop and increase the breeding efficiency. However, the genetic diversity of Thai native chickens has not been well studied. Thus, the present status of genetic diversity of Thai native chicken is still unclear. It is important to study the molecular genetic diversity and variability among individuals and population. This study should benefit the poultry breeding and development of long-term sustainable Thai poultry industry.

In general, the genetic variability can be studied by two methods. First is by indirect method such as morphological characterization. This method is relatively inexpensive, simple and less time consuming (Karnsomdee, 1999). However, the morphological characteristic is phenotype which is related to the gene expression and gene-environment interaction. The environment might effect and make the bias error. The second method is by molecular genetic technique which is a direct method. Although this method is more complex, expensive and time consuming, it can show the direct variation at the DNA level and can explain the genetic variation clearer.

One of the techniques used to study the genetic variation of the population is microsatellite polymorphism. Microsatellites, the highly repetitive sequences, are tendemly repeated tracts of DNA composed of 2-6 base pair unit. Microsatellite can be found anywhere in the genome, both in protein-coding and noncoding regions. Microsatellite patterns of coding and noncoding regions in eukaryotes show divergence that can be explained on the basis of the differential selection. The variation dependence of repeat unit length suggests fundamental differences between mechanisms of generation and fixation of simple repetitive DNA (Toth et al, 2000). Microsatellite DNA marker technique is one of the techniques that is appropriate for the study of the genetic variation of the native chicken population, due to its sensitivity of detection of the mutation. It can also detect genetic variation among species, population and individual. (Smith et al., 1990; Cheng, 1994; Cheng, 1997; Crooijmans et. al., 1996a; Toth et al., 2000)

This study specifies the genetic characterization of Thai native chicken variety loenghangkhao and Thai native chicken variety praduhangdam compared with commercial layer and hybrid native broiler by using sixteen microsatellite which is high polymorphism. This research also gave some basic information about the genetic variation of the native chicken population between different farms. The result of this study will show the present status of genetic diversity of Thai native chicken among the farm that we studied. The morphology of Thai native chicken variety loenghangkhao and praduhangdam were also studied. The result from this study can be used in the breeding program to maintain the genetic diversity for sustainable livestock and used for genetic information for future research in breeding development.

Research Objectives

1. To find the appropriate microsatellite markers which can distinguish Thai native chicken.
2. To characterize the genetic characters of Thai native chicken variety Loenghangkhao and Praduhangdam compare with commercial layer and hybrid native broiler by microsatellite marker.
3. To study and characterize the morphological characteristic of Thai native chicken variety Loenghangkhao and Praduhangdam

Anticipated Benefit

1. The appropriate microsatellite markers which can distinguish Thai native chicken will be discovered.
2. Some genetic characters of Thai native chicken variety Loenghangkhao and Praduhangdam will be known.
3. We will also identify whether the genetic characterization of Thai native chicken variety Loenghangkhao and Praduhangdam are closer to broiler or layer chickens.
4. This knowledge will be able to use in the decision to further develop the Thai native chicken.

CHAPTER II

LITERATURE REVIEW

2.1. *Gallus gallus gallus*

The history of poultry species has intrigued scholars for years. They have been interested in identifying the wild ancestors of domestic birds. Chickens have been domesticated for a very long time. They have been manipulated for at least 2000 years (Crawford, 1990). From Asia they gradually spread to the east, and to the west from where they eventually encircled the globe. However, not all scholars accept this majority view. Some believe that another wild *Gallus* species may have contributed to the domestic bird. Other believes that Southeast Asian chicken stocks were domesticated separately, and may have been derived from one or more extinct species of jungle fowl. However the first archaeological evidence appeared that breeds of chicken were established by poultry breeders in China as early as 1400 B.C. (Darwin, 1896 *in* Crawford, 1990). The native chicken *Gallus gallus domesticus* are believed to be selected and domesticated by human from the ancestor, Red Jungle Fowl *Gallus gallus gallus* (Rose, 1997). At present, the red jungle fowl has without doubt been a major contributor to domestic chicken. It is less certain whether the other species are ancestral to chicken. In addition, red jungle fowl can still be found in the forest in Southeast Asia and China (Crawford, 1990, Pramong, 1998).

Today, chicken appeared to have an important role in food economic of the world. Domestic chickens have been selected and developed to serve human demand. Chickens are bred to have varieties of strains (Dettelaff and Vassetzky, 1991). In Thailand, native chickens have been adapted and modified in characteristic and behavior for the development and conservation of the standard breeds (Sawatt, 1996).

2.2. Classification of Domestic Chicken

The taxonomic status for domestic chicken *Gallus gallus domesticus* belongs to:

Kingdom Animalia

Subkingdom Metazoa

Phylum Chordata

Subphylum Vertebrate

Class Avian

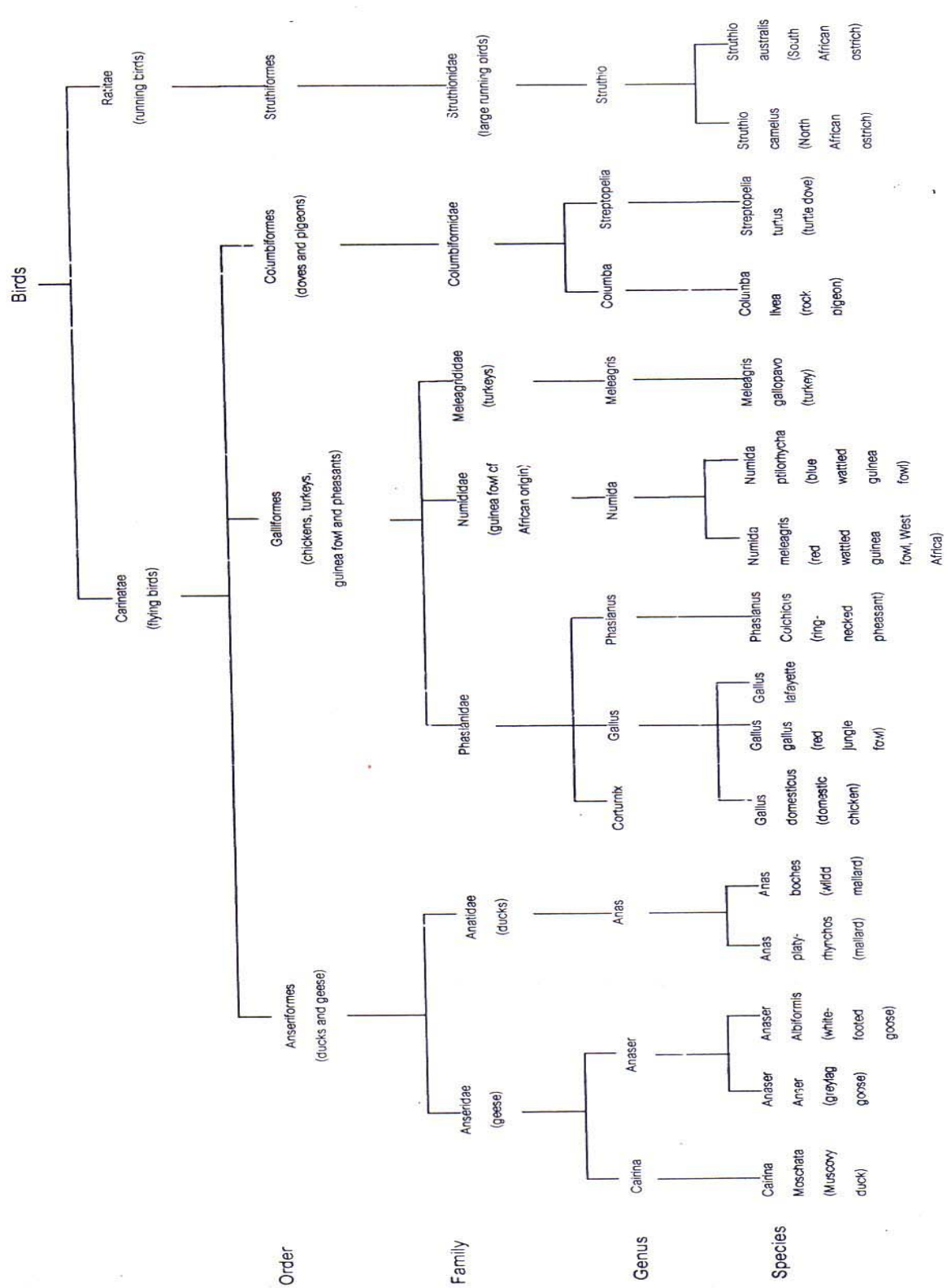
Order Galliformes

Family Phasianidae

Genus Gallus

Species *Gallus gallus domesticus* (Fumihito et al., 1995)

Common name: Fowl, Poultry, Chicken



Figger 2.1 shows the taxtonomic order of Thai native chickens (Smith, 1990)

The ancestor of the native chickens was reported that they were originated from jungle fowl from China and Southeast Asia. There are 4 main jungle fowl species that still can be found in the forest in Southeast Asia and China which is *Gallus gallus, Gallus lafayettii, Gallus sonnerati* and *Gallus varius* (Alongkod, 1998).

2.3. Characteristic of Thai Native Chicken

Although Thai native chicken have been manipulated for a long time but there are no standard of varieties identification which widely accepted. However, base on the project of the livestock division of Thailand, this study interested in 2 varieties which is the most common variety and widely known fighting cock in Thailand. These two varieties are Thai native chicken variety loenghangkhao and praduhangdam. These chickens are popular for specific breeding for chicken fighting sport. The morphology of fighting cock variety loenghangkhao and praduhangdam are described as follows:

2.3.1. Thai Native Chicken Variety Loenghangkhao

Thai native chicken variety loenghangkhao is one of the most popular fighting cock in Thailand. The adult males of this variety have mainly black color on the ventral part with white dorsal plumage including the neck, hackle, saddle, back and wing bow region. Some feather at the middle of rectric and primary wing are colored on the web. The comb of loenghangkhao is pea type which is a circular form which is controled by chromosome 1 (Etches and Gibbins, 1993). Loenghangkhao adult females have smaller body. The color of the female is mainly black. Some of the female have white dorsal plumage around the body.

2.3.2. Thai Native Chicken Variety Praduhangdam

Thai native chicken variety praduhangdam is another popular breed for fighting cock. The adult males of this variety have dark-brown fringed feathers on the saddle, neck, hackles, back and wingbow region. Restrict, primary and secondary wing feathers of this variety are black. The comb of praduhangdam is also pea type. Praduhangdam adult females have smaller body. The color of the female is black.

2.4. Native Chicken Breeding Situation in Thailand

The Thailand Research Fund and Livestock Division of Thailand are combining their effort to try to breed 4 native varieties of Thai native chicken to obtain pure line. Four varieties of this breeding development are Thai native chicken varieties loenghangkhao which are bred by the Animal Research and Development Center in Krabinburi, Thai native chicken varieties praduhangdam by the Animal Research and Development Center in ChiangMai, Thai native chicken varieties dang by the Animal Research and Development Center in Surad-Thani and Thai native chicken varieties Shee by the Animal Research and Development Center in Tha-Pra. This project started in 2003 and will be finish in 2008. At present (December 2003), each center combines the mature parent stock from the farmer around their area and breed the parent stock to get the G1 generation. The main characterization of the parent stock was mainly base of the phenotype characterization. The main breeding plan was to use 70 male and 350 female from each variety. The characterization of male and female were based on the color appearance, tarsal, beak and comb's color. One male will be bred with 5 female by

artificial insemination. Each female will produce about 12 G1 chicks. The crossing will avoid the inbreeding effect. The G1 chicks will be selected base on morphological characteristic as mention above. At present, each center has pass through the G1 generation. They obtain 3,630 loenghangkhao, 5,580 praduhangdam, 3,467 Shee and 5,138 Dang in G1 generation. However, these breeding programs have not yet including the genetic characterization. Thus, if the next breeding step is added with the molecular genetic characterization along with the phenotypic characterization, this method should help to reduce the number of chicken in the experiment and the cost of the program.

2.5. The Chicken Genome

Chicken genetic has a rich history spanning almost 100 years, since Spillman (1908) showed the barring was sex linked. The early classical maps of chicken genome were based on feather color, morphological, immunological and physiological genetic markers (Schmid et. al., 2000). It is expected that the number of classical mutants mapped with molecular markers will increase in the near future, provided that carriers of these mutants are still kept as part of chicken genetic resources.

Chicken have 39 pairs of chromosomes, a mode of 78 is obtained from the somatic cells count, and 39 bivalents and synaptonemal complexes are detected in meiotic preparations (Etches and Gibbins, 1993). Similar to most eukaryotic DNA, the chicken DNA can divided into 3 types, unique sequences, moderately unique sequences and highly repetitive sequences (Stevens, 1996).

The chicken's genome comprises of all the genetic materials present in the haploid cell include all the nuclear and mitrochondrial genome. Chicken's genome has 1.2 x 10^9 base pairs in the haploid genome (Smith and wood, 1991). The chicken karyotype comprises 39 pairs of chromosomes which divided into eight pairs of cytological distinct chromosomes 1-8 along with the Z and W sex chromosomes and 30 pairs of small, cytological indistinguishable "microchromosome" (Schmid et. al., 2000). In the domestic chicken, approximately 70% of DNA is distributed amongs the macrochromosomes and 30% among the microchromosomes (Stevens, 1996). The number of genes generally mapped to macrochromosomes and microchromosomes in the chicken has also been used to determine gene density of each set of chromosome. Microchromosomes of the chicken constitute 23% of GC-rich and have the CpG content higher than the macrochromosomes (Hartl, 2000). Since 60-70% of know chicken genes are associated with CpG-island, it has been postulated that the microchromosomes may represent a genedense fraction of the chicken genome.

2.6. Molecular Genetic Method

After molecular genetic method and polymerase chain reaction (PCR) technique have been developed, the study of systematic and population genetic have been increase explosively (Schmid et al., 2000). This method has high resolution power. It also permits exceedingly detailed description of genetic variation in DNA and molecular component (Crawford, 1990). There have been increasing the number of studied in vertebrate genome, especially in the chicken genome. This technique and method can use to apply for chicken genomic study in many fields such as genome mapping, genetic diversity, and breeding program.

2.7. Genetic Variation Determining Method

The breeding of poultry differs from the breeding of four footed farm animals. It is more flexible due to the greater numbers and more rapid reproduction (Crawford, 1990). The major requirement of any progress of chicken breeding is the genetic variation within and between varieties of individuals (Etches and Gibbins, 1993). The genetic variation should be maintained for developing sustainable production system. Thus, the genetic variation of Thai native chickens should be highly maintained. However, the status of genetic diversity of Thai native chicken is not clearly observed. Thus there are two main characterization methods that are widely use to determine the variation.

2.7.1. Phenotypic Characterization

Some characters of the chicken are generally determined. The size, shape, color, behavior or tissue enzyme content are mainly interesting. The characteristic of the chicken can be subdivided into two parameters, firstly morphology and secondly morphological traits. These characteristic traits can be compared between different species, populations and individuals. Morphology is a comparative based on plumage color and pattern polymorphism, where as morphological trait is comparative based on body dimension polymorphism (Lovette, 1998). It is recognized, however, that the expression of hereditary characters may be modified by the environment to which an individual is exposed, and that some characters are affected by environment more than other. The evolution process and rate of evolution may not be related with and different

from molecular variation (Zink and Blackwell, 1996). The genetic variation by only morphological characteristic may be resulted differently from molecular genetic method. The study of variation should used morphological characteristic together with molecular genetic method.

2.7.2. Genotypic Characterization

Molecular genetic is a new tool for genetically improvement of chicken. It emerged in the early 1980s as a result of discoveries in molecular genetic and understanding of the DNA (Crawford, 1990). Molecular genetic characterization offers potential benefits, such as providing a means of increases genetic variation and developing the genetic more rapidly in poultry production. Many genetic methods have been studied to serve the understanding of chicken's genome such as the study about allozymes for the genetic relatedness. However, this method has low genetic variability and the heterozygosity of loci make this technique unsuitable for parentage assignment or within group relatedness determination (Feraris and Palumbi, 1996). The polymorphism of genetic DNA fragment have been used to study about the genetic diversity of the population. Microsatellite DNA marker has received considerable attention for the genome analysis (Innis et al., 1999). The microsatellite marker should be presenting as Mendelian fashion, selectively neutral and resulting in high polymorphism. This microsatellite DNA marker method have been used to study the relatedness estimation of several poultry populations (Khatib et al., 1993; Cheng and Crittenden, 1994; Cheng et al., 1994; Crooijmans et al., 1996b; Vanhala et al., 1998; Zhang et al., 2002).

2.8. Microsatellite DNA

This new technique permit molecular biologists and geneticists to systematically evaluated and compare the large area of the genome. Most of this analysis based on the PCR amplification of microsatellite DNA. This technique has become the focus of the search for hypervariable single locus marker and being abundant and widely dispersed in the eukaryotic genomes (Tautz, 1989). Microsatellite DNA is a short tandemly repeating sequences found to be highly polymorphism in the genome (Mindell, 1995). The repeat units ranging in the size from 50 to about 500 bp from stretches of DNA referred to as variable number tandem repeats or microsatellites. The repeat units ranging in the size from 2 to 6 base pair from stretches of DNA referred to as short tandem repeats or microsatellite (Crawford, 1990). Avian genome contains large amounts of repetitive DNA sequences, an increasing number of which are being identified as stretches of tandem repeat unit (Innis et al., 1999). Microsatellite DNA have the variation in numbers of repeating unit that thought to be due to slippage strand missing, unequal crossing over, slippage during DNA replication and replication error (Wright, 1994). Thus, it is highly polymorphism genetic markers in the animal texa. The development of microsatellite as markers has been used for wide variety of animal such as chicken (Cheng et al., 1996; Crooijmans, 1996a,b; Cheng and Crittenden, 1994; Cheng et al., 1994; Vanhala et al., 1998; Zhang et al., 2002), cattle (Machugh et al., 1994), pig (Moran, 1993), etc.

Microsatellite have been particularly useful for comparative genetic and genomic mapping since their first description because of several fortuitous characteristics. First, microsatellite sequences show a high degree of length polymorphism. Second, microsatellite sequences are abundant and evenly distributed throughout the genome

(Hoelzel, 1998). Microsatellite DNA markers in the reference population fowl commonly having more than 12 alleles at a single locus and heterozygosity level up to 90% (Tayler et al., 1994). The DNA sequences flanking the repetitive arrays can be used for design primers for PCR amplification (Tayler et al., 1994). Microsatellite analysis relis on size determination of the entire PCR product consisting of the microsatellite stretch and flanking region. If the information on the sequence of the flanking region is available, the number of repeats may be calculated by subtraction of the flanking nucleotide and dividing the remaining base pairs by the size of the repeat unit. Sizing of PCR products is commonly achieved by polyacrylamide gel electrophoresis.

Recently, the mapping of at least 2000 loci (including Microsatellite loci) has been identified in the chicken genome database (Schmid et al., 2000). Microsatellite markers have been used in several studies for example they are used for gender determination in bird (Mindell, 1995). The microsatellite polymorphism have been used to study the genetic variation comparing between domestic sheep and big horn sheep (Forbes et al., 1995). With in the fowl genomes, microsatellite markers have been used in several studies (Khatib et al., 1993; Cheng and Crittenden, 1994; Cheng et al.,1994; Crooijmans et al., 1996b; Vanhala et al., 1998; Zhang et al., 2002). Among individuals, this method is a good choice for assisting parentage and relatedness among individuals because of the contribution of relative inclusive fitness (Blouin et al., 1996). Among population, microsatellite is the major element analyses in avian, because screening is usually confined to perfect repeats. Microsatellite DNA markers were used to study about the genetic variation of Red Jungle Fowl between Northern and Southern of Thailand (Pramong, 1998) and study among varieties of Thai Native Chicken (Karnsomdee, 2000).

Microsatellite can also be used to investigate animal breeding and preservation for the natural resources (Crooijmans et al., 1997).

2.9. Measures of the Microsatellite Variation

Microsatellite polymorphism is based on the short sequences that are repeated in tandem at one or more places in the genome. Each location in a chromosome that contains core repeats may have different number (n) of copies of the repeat. This means that at any particular location, a microsatellite repeat has multiple allele in the population, where each allele different in its value of number. With this approach, each genome yield a different fragment size due to the differing number of core repeat, and therefore yields a distinct allele in the gel electrophoresis.

Alleles in natural populations usually differ in frequency from one allele to the next. The allele frequency of a prescribed allele among a group of individuals is defined as the proportion of all allele at the locus that are of the prescribed type. The frequency of any prescribed allele in a sample is therefore equal to twice the number of genotypes homozygous for the allele (because each homozygote carries two copies of the allele), plus the number of genotypes heterozygous for the allele (because each heterozygote carries one copy), divided by two times the total number of individuals in the sample.

The allele frequency in a population for diploid organism can be estimated the by equation below. In generally, suppose that among N individuals sampled from a population the numbers of AA, Aa, and aa genotypes are NAA, NAa, and Naa,

respectively. If p and q represent the allele frequency of A and a , respectively, with p + q = 1. the estimate of allele frequency in the population can be equated as

$$\text{Allele frequency} = \frac{2NAA + NAa}{2N}$$

and the estimated sampling variance is

$$[\text{Var}(p)] = \frac{(p)(1-(p))}{2N}$$

from the equation, NAA, Naa were represented the number of homozygous at allele A and a, and NAa was represented the number of heterozygous, for such allele, N is the number of investigated individuals.

The genotype frequencies for a gene with two alleles can be deduced. Assume that genotype frequencies of *AA,Aa* and *aa* in the parental generation are *D, H* and *R*, respectively, where $D+H+R = 1.0$. The allele frequencies of *A* and *a* are given by

$p = (2D+H)/2 = D + H/2$ and $q = (2R + H)/2 = R + H/2$

The new genotype frequencies are calculated as the sum of the cross products shown.

$D' = D^2 + 2DH/2 + H^2/4 = (D + H/2)^2 = p^2$

$H' = 2DH/2 + 2\,DR + H^2/2 + 2HR/2 = 2(D + H/2)(R + H/2) = 2pq$

$R' = H^2/4 + 2HR/2 + R^2 = (R + H/2)^2 = q^2$

The new genotype frequencies P', Q' and R' simplify to :

AA : D' = p^2 Aa : H' = 2pq Aa : R' = q^2

a result known as the Hardy-Weinberg principle after Godfrey Hardy and Wilhelm Weinberg (1908).

The Hardy-Weinberg principle provides the foundation for many theoretical investigations in the population genetics. One of the most important implications emerges when we calculate the allele frequencies *p'* and *q'* of A and a. the allele frequencies of A and a are (Hartl, 2000)

$$p' = (2D' + H')/2 = (2p^2 + 2pq)/2 = p(p + q) = p$$
$$q' = (2R' + H')/2 = (2q^2 + 2pq)/2 = q(p + q) = q$$

Because the allele frequencies remain the same generation after generation, so do the genotype frequencies in the proportions p^2, $2pq$ and q^2 , which is often called the Hardy-Weinberg equilibrium (HWE).

For Chi-square test for HWE, the mere fact that observed genotype frequencies may happen to fit HWE cannot be taken as evidence that all of the assumptions in the model are valid. The principle is not very sensitive to certain kinds of departures from the assumptions, particularly those pertaining to a very large population size with no migration, mutation, or selection. On the other hand, the relative insensitivity tp departures from its assumptions gives the principle some robustness, because it implies

the HWE can be valid to a first approximation even when one or more of the assumptions is violated.

The usual test for goodness of fit of observed data to HWE is a chi-square test. The test statistic is usually symbolized X^2, and under the hypothesis of HWE the X^2 has approximately a chi-square distribution. The value of X^2 is calculated as

$$X^2 = \Sigma \frac{(obs - exp)^2}{exp}$$

For linkage disequilibrium, HWE, statistically, means that the alleles present at a locus are in random association with each other in the genotypes. It therefore may seem paradoxical that two gene, *A* and *B*, present in the same population may each obey HWE individually, yet the allele *A* and *B* can remain in *nonrandom* association in the *gametes* that from each generation. The measure of linkage disequilibrium have a predefined range is

$$p = D / \sqrt{p1p2q1q2}$$

The correlation between linkage the *A* and *B* alleles present in gametes and can range from –1 to +1. This measure has the convenient feature that the X^2 value for goodness of fit to the hypothesis that D = 0 is given by (Hartl, 2000)

$$X^2 = p^2n$$

In *F* coefficients, Wright (1965) developed an approach to partitioning the genetic variation in a subdivided population that provides an obvious description of differentiation. This approach consists of three different *F* coefficients used to allocate

the genetic variability to the total population level (T), subdivisions (S) and individuals (I). These three values, F_{ST}, F_{IT}, and F_{IS} are interrelated so that

$$F_{ST} = F_{IT} - F_{IS} / 1 - F_{IS}$$

F_{ST} is a measure of the genetic differentiation over subpopulations and is always positive. F_{IT} and F_{IS} are measures of the deviation for Hardy-Weinberg proportions within subpopulations and in the total population, respectively, where positive value indicate a deficiency of heterozygotes, and negative values indicate an excess of heterozygotes (Headrick, 2000).

2.10. Microsatellite Genotyping in Domestic Chicken.

Microsatellite DNA markers have been used to detect and determine the genetic variation of the domestic chicken in many studies. For example, 77 new microsatellite markers were map for the preliminary linkage map of the chicken (*Gallus domesticus*) (Crooijmans et al., 1996b). Two hundred and seventy five microsatellite markers were polymorphic in 93% of Wageningen resource population, 57% of East Lancing, and 44% in Campton chicken. The average allele number from all groups was 4 (Crooijmans et al., 1993). The 151 microsatellite markers were developed and used to detect the polymorphism in two reference populations and their resource population (Cheng et al., 1994).

CHAPTER III

MATERIALS AND METHODS

3.1. Specimens

Thai native chicken

Two main varieties of Thai native chicken *Gallus gallus domesticus,* 2 hybrid line broiler and commercial layer from different location were used in this experiment. All chicken can be separated into 6 groups as follow;

15 male Thai native chickens variety Praduhangdam (PM)

15 female Thai native chickens variety Praduhangdam (PF)

15 male Thai native chickens variety Loenghangkhao (LM)

15 female Thai native chickens variety Loenghangkhao (LF)

15 two hybrid line broilers (BR)

15 commercial layers (LA)

3.1.1. Group I : Male Thai native chicken variety Praduhangdam (PM)

Male Praduhangdam number 1-5 were from Research and Development center, Charoen Pokphan Northeastern Public Co., Ltd, Ban-Grang farm and number 6-15 were from Choke-Bancha farm, Nakhon Prathom.

3.1.2. Group II : Female Thai native chicken variety Praduhangdam (PF)

Female Praduhangdam number 1-12 were from Research and Development center, Charoen Pokphan Northeastern Public Co., Ltd, Ban-Grang farm and number 13-15 were from Choke-Bancha farm, Nakhon Prathom.

3.1.3. Group III : Male Thai native chicken variety Loenghangkhao (LM)

Male Loenghangkhao number 1-7 were from Research and Development center, Charoen Pokphan Northeastern Public Co., Ltd, Ban-Grang farm. Male Loenghangkhao number 8-12 were from Choke-Bancha farm, Nakhon Prathom and male Loenghangkhao number 13-15 were collected from Ban-gramg farm, Pissanuloke.

3.1.4. Group IV : Female Thai native chicken variety Loenghangkhao (LF)

All 15 female Thai Loenghangkhao (number 1-15) were from Research and Development center, Charoen Pokphan Northeastern Public Co., Ltd, Ban-Grang farm.

3.1.5.Group V : Hybrid line Brioler (BR)

All 15 two hybrid line brioler (number 1-15) were from Research farm, Suranaree University of Technology, Nakhon-Ratchasima. These chickens are line crossing of 50% Native chicken and 50% Layer chicken

3.1.6.Group VI : Commercial Layer

All 15 commercial layer (number 1-15) were from Research farm, Suranaree University of Technology, Nakhon-Ratchasima.

3.2. Materials

3.2.1. Equipments and Consumables

- Disposable syringe 1.0 ml. with needle gauge number 26 (Nipro)
- Disposable syringe 3 ml., 5 ml., 50 ml. (Nipro)
- needle gauge number 21 (Nipro)
- needle gauge number 26 (Nipro)
- Autoclave
- Water bath (Maxi-Shake, Heto-Holten, Denmark)
- Microcentrifuge tube 0.2 ml. (Somport, Canada)
- Microcentrifuge tube 0.5 and 1.5 ml. (Sorenson, USA)
- Centrifuge models 5415C (Eppendrof)
- Centrifuge model 400R (Heraeus instruments)
- Centrifuge tube 15 ml. and 50 ml. (TTP, Switzerland)
- Dry keeper dessicator (Sanplatech)
- Vortex genie-2 (Scientific Industries, USA)
- Micropipette 0.5-10 µL, 2-20 µL, 20-200 µL, 200-1000 µL (Nichiryo, Japan)
- White/Ultraviolet transilluminator (UVP, USA)
- Spectrophotometer Ultraspec 2000 (Phamacia Biotech, USA)
- Laminar flow hood UV light (HBB 2448, Holten Lamin Air, USA)
- PCR Thermal cycler Gene Amp® PCR System 9700 (PE applied Biosystems)
- PCR Thermal cycler Gene Amp® PCR System 2400 (PE applied Biosystems)
- Vertical sequencing gel electrophoresis apparatus (Bio-RAD Laboratory, USA)
- Vertical sequencing gel electrophoresis apparatus (Scie-Plas Limited, UK)
- Power supply (Power Pac 3000 Bio-RAD Laboratory, USA)

- Power supply (Power Pac 300 Bio-RAD Laboratory, USA)
- 20°C Freezer (Heto Lab Equipment, Denmark)
- 20°C Freezer (Sharp FC-27, Japan)
- pH/mV/°C meter pH 500 series (Cole Parmer, Singapore)
- Glass bottle 50 ml. 100 ml. 250 ml. 1000 ml. (Scott Duran, Germany)
- Hood, Fume Cupboard (Newlab®, Trane International CO., Ltd., Thailand)
- Shaker HS250 basic (IKA Laboratechnik, Germany)
- Variomag Electronic poly15 (H+P Labortechnik GmbH, Germany)
- Ice box (Scientific plastic CO., Ltd.,USA)
- Plastic Tank
- Electronic clock timer model CT-30 (Canon CO., Ltd., Japan)
- etc.

3.2.2. Chemicals

All chemicals used were molecular grade or otherwise specified

- Sodium Hydroxide Anhydrous pellets (Carlo Erba)
- 25% Ammonia (Rhedel-de Haën)
- Sodium Chloride (Sigma®)
- Absolute Ethyl Alcohol (Carlo Erba)
- Sodium Dodecyl Sulfate (Sigma®)
- Thermophelic DNA poly 10X Buffer (Promega corporation, USA)
- Magnesium Chloride (Promega corporation, USA)
- 100 mM. dATP, dGTP, dCTP, dTTP (Promega corporation, USA)

- Tris-(Hydroxy Methyl) Aminomethane (Promega corporation, USA)
- Boric Acid (Carlo Erba)
- Ethylenediaminetetracetic Acid Disodium Salt (Carlo Erba)
- Urea (Promega corporation, USA)
- N, N-methylene-bis-acrylamide (Sigma®)
- Amonium Persulfate (Gibco BRL)
- TEMED (ICN Biomedical Inc.)
- Fomamide (Merck)
- Formaldehyde (Sigma®)
- Agarose (Promega corporation, USA)
- Acrylamide (ICN Biomedicals)
- Bromophenol Blue (Phamacia Biotech)
- Xylene Cyanol (Amersham)
- Repel Silene (Phamacia Biotech)
- Bind Silene (Phamacia Biotech)
- Silver Nitrate (Carlo Erba)
- Sodiumcarbonate Anhydrous (Carlo Erba)
- Glacial Acetic Acid (Sigma®)
- Glycerol (Carlo Erba)

3.2.3. Enzymes

- Taq DNA polymerase (Promega corporation, USA)
- Proteinase K (Promega copporation, USA)

3.3. Method

3.3.1. Morphology Data Collection

Information about the morphology of Thai native chicken variety loenghangkhao and praduhangdam including: wing length (WL), nape length (the beak-basement to nape, NNL), beak length (BL), taros-metatarsus length (TL), Neck length (NL), Chest wile (CW) and colour appearance were collected. Sexes, maturity and body weight was also recorded. All data from each parameters were compared and analyed for variance by one-way ANOVA method (Paisarn, 1997).

3.3.2. Blood Sample Collection

Blood sample from each individual of Thai native chicken variety loenghangkhao, praduhangdam, native broiler and commercial layer fowls were collected by radial venipuncture (wing vain) using syring with 25G needle contained 100 µl 0.1M EDTA (anticoagulant). The blood were cooled under ice and transfer to the laboratory and kept at –20 ^{o}C until use.

3.3.3. DNA Extraction

The DNA extraction method by proteinase K was developed from the method of Lipkin et al., 2002. This method avoids the use of phenol choloform but use hight salt concentraton to remove protein.

This method is breifly explain as follow;

Frozen blood were thawed and 50 µl of packed red cells were resuspeded in 3 ml of lysis buffer containing :

0.1 M Tris HCl pH 7.6

0.4 M NaCl

2 mM EDTA

To each sample, 200 µl of 10% SDS solution and 16 µl of proteinase K solution (20 mg/ml) were added. Each sample was incubated at 62 ^{o}C for 3 hours or 35 ^{o}C for 18 hours. After incubation, 1 ml of 5 M NaCl were added to each sample then shake vigorously.

The blood sample were centrifuged at 14000 rpm for 15 min. The DNA were then precipitated in ethanol and centrifuged at 14000 rpm for 15 min. The genomic DNA were resuspend in 1 ml Tris-EDTA.

3.3.4. DNA Concentration and Quality Determination

The extracted genomic DNA were loaded into the 0.8% agarose gel. This 0.8% agarose gel is the medium for the electrophoresis separation and carries out for visualization of the quality of isolated DNA samples. After electrophoresis, the gel was

stain by ethedium bromide dye and visualized under ultraviolet light. The standard DNA marker was also loaded into the gel for size estimation.

The concentration of the extracted DNA were measured and estimated by ultraviolet absorption of the spectrophotometer at 260 nM (OD_{260}). Extracted DNA absorbs UV light at the 260 nm wave length (OD_{260}), whereas protein absorbes UV light at the 280 nm wave length (OD_{280}). The purity of extracted DNA can be assessed by determining ratio of the absorbed OD at 260 nm over 280 nm. If the ratio is lower than 1.5, the purity is poor which because of the protein or other contamination in the extracted solution. However, if the ratio is greater than 1.5, the absorption is probably due to the nucleic acid (Innis et al., 1999).

The DNA concentration of each sample can be calculated by the assumption:

DNA concentration (mg/mL) = (OD_{260}) X dilution factor X 50 mg/mL

3.3.5. Amplification of Microsatellite DNA

The DNA were amplified by PCR method using microsatellite primers. The appropriate pair of primers does not contain complementary sequence to each other. They were at similar length and melting temperature (Innis et al., 1999). The DNA production were about 100-400 bp in length (Feraris and Palumbi, 1996). If the PCR fails to yield product at one of the annealing temperature, it were repeated at lower temperature.

Nineteen suitable highly polymorphic primers were selected from both Thai and international native chicken related publications (Khatib et al., 1993; Cheng, 1994; Cheng, 1997; Crooijmans et al., 1996a; Schmidt et al., 2000). The primers were selected from chicken genomic libraries base on the ability to amplify and show high allelic number, high polymorphism and unambiguous amplification pattern in the East Lancing reference family (Khatib et al., 1993), Campton reference family (Cheng, 1994, Cheng, 1997) and Wagnengen resource population (Crooijmans et al., 1996a).

Only sixteen of the nineteen microsatellite primers tested were select to amplify the whole population of 90 chickens in this experiment. The microsatellite loci selected consisted of LEI94, LEI116, LEI234, ADL23, ADL192, ADL278, ADL228, B42, B71, B76, B85, B154, B259, MCW87, MCW240 and IL103. The characteristic of all selected primers are listed in the table below:

Locus name	Forward sequence	Reverse sequence	size (bp)	Tm (°C)
ADL23	5'-CTT CTA TCC TGG GCT TCT GA-3'	5'- CCT GGC TGT GTA TGT GTT GC-3'	20	62
ADL278	5'-CCA GCA GTC TAC CTT CCT AT-3'	5'-TGT CAT CCA AGA ACA GTG TG-3'	20	58
LEI73	5'-CCATATCATTTGTCAAGCACC-3'	5'-AATTCCTGACCTCCATGATAC-3'	21	47
LEI94	5'-GATCTCACCAGTATGAGCTGC-3'	5'-GATCTCACCAGTATGAGCTGC-3'	21	45
LEI116	5'-CAGCGCTTCTTGCTTGCC-3'	5'-TGCACACGCGTTTCTATGAG-3'	18/20	58
LEI192	5'-TGCCAGAGCTTCAGTCTGT-3'	5'-GTCATTACTGTTATGTTTATTGC-3'	19/23	49
LEI194	5'-TCCTTGGCATGTACATATGA-3'	5'-TCCTTGGCATGTACATATGA-3'	20/21	49
LEI228	5'-GCTGGGTTATTTCAATATGTGG-3'	5'-AGCGTACCTGATAATGATGAGC-3'	22	50
LEI234	5'-ATGCATCAGATTGGTATTCAA-3'	5'-CGTGGCTGTGAACAAATATG-3'	21/20	47
MCW87	5'-ATTTCTGCAGCCAACTTGGAG-3'	5'-CTCAGGCAGTTCTCAAGAACA-3'	21	50
MCW240	5'-CAAAACCGGTGTCACCTACTG-3'	5'-GGTTATTTCTTCAGTGACTTCC-3'	21/22	47
IL103	5'-TCTTGTTTTCCTTTTGTTGT-3'	5'-GCATACGGCTCCTTCAGTTG-3'	20	52
B42	5'-CGTGGTGTTGTGTATCATTT-3'	5'-CTCTTTTGCAGTCCTCCTAC-3'	20	56
B206	5'-GGTTAGCTCCCTCCTTCCAG-3'	5'-TCACTCCAGCTTGAGACAGG-3'	20	62
B259	5'-ACTATTAGCCTGGGGAGAGC-3'	5'-AAGGAAACAAAGAGAAATCC-3'	20	54
B154	5'-TCAGCTCTTCAGGCAAAAAG-3'	5'-AACTTGGACCACAATCTTAT-3'	20	54
B71	5'-GGTCCGACTGAAAGCATTAT-3'	5'-TTAAGACTGAAGCCAACCAG-3'	20	58
B76	5'-TGGCATGGTTGAGGAATACA-3'	5'-TAGGTGCTGCACTGGAAATC-3'	20	58
B85	5'-TGGCAGAAATAAGGCAGTGC-3'	5'-ATTCATCGCTGGCATCTTGC-3'	20	60

Table 3.1 shows Microsatellite marker information for PCR amplification

3.3.6. Polymerase Chain Reaction

The Polymerase Chain Reaction (PCR) is an *in vitro* amplification of specific DNA sequence by primer extension of complementary strand of DNA template (Karnsomdee, 1999). PCR has great potential for DNA-level studies of conservative and population genetic. The generally requirement for PCR reaction consist of DNA

template, Buffer, magnesium chloride, deoxynucleotides, microsatellite primer and DNA polymerase enzyme (Hoelzel, 1998). The efficiency of PCR is controlled by many parameters, such as polymerase type, buffer type, primer concentration and stability (T_m), dNTPs concentration, cycling parameters, and complexity and concentration of starting template (Innis et al., 1999). All selected microsatellite loci were able to amplified the genomic DNA of the sample extracted by protenase K/high salt method. In the PCR reaction, 50 ng of genomic DNA of *Gallus gallus domesticus* were used for DNA template.

Approximately 50 ng of genomic DNA were use in the amplification reaction. In the total 25µL reaction typically contained 1 unit Thermophillic DNA polymerase, 10X buffer without magnesium (Promega), 200µM dNTPs, 1.5-3 µL $MgCl_2$, 1 unit *Taq* polymerase in enzyme storage buffer B (Promega) and 100 pmoles of each primer. The composition of storage buffer B is 20mM Tris-HCl (pH 8.0 at 25 ºC), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, 0.5% Tween® 20 and 0.5% Nonidet® -P40. After diluted in PCR mixture, 1X Thermophillic DNA polymerase buffer without magnesium consist of 10 mM Tris-HCl (pH 9.0 at 25 ºC), 50 mM KCl and 0.1% Triton® X-100.

One parameter which effect the PCR reaction is melting temperature (Tm). For amplification of all loci, more complicated PCR amplification program are required. The melting temperature for each primer is examined by the Wallance's rules as follow;

Tm(ºC) = [4 (total number of G and C) + 2(total number of A and T)]

The amplification condition consist of 3 minutes denaturation at 94 ºC. The amplification went through 45 cycles of 60s 94ºC denaturation, 60s annealing (temperature depend on primer composition, table 3.1) and 90s 72ºC extension. The final extension was 10 min at 72ºC. After PCR reaction, the sample are called PCR products.

3.3.7. Six Percents Denaturing Polyacrylamide Gel Electrophoresis

PCR products were detected by 6% denaturing polyacrylamide gel. The sequencing plate (Bio-rad®) was used for running the electrophoresis. In the first step, the sequencing plates were washed by water and detergent and rinsed with distilled water and then wiped with kimwiped® until dry. After that the sequencing plates were cleaned by 70% ethanol. The binding plate was siliconised with bind silane® and air-dried. The cover plate was siliconised with repel silane® and air-dried.

The standard six percents denaturing polyacrylamide gel was prepared for detecting of the PCR products of microsatellite DNA of *Gallus gallus domesticus*. The gel were pre run for 45 minutes before loading the PCR product and DNA ladder marker. After pre running the running wells were flush with TBE buffer to remove any excess urea. The PCR products and DNA ladder marker were mix with 10X loading dye which composed of 0.05% bromophenol blue, 0.05% xylene cyanol and 99.9% formamide. The mixture were heat at 95ºC for 5 minutes and immediately dip into the 0ºC ice's water. Sixteen microlitres of the PCR products loading dye mixture and DNA ladder marker were load into each running well of the pre ran denaturing gel. The pre ran electrophoresis was run under 1X TBE buffer carried out with 400W constant, 250 V, 350 mA for 45 minutes. After loading the electrophoresis were ran at the same condition

for 220 minutes. When electrophoresis was completed, gels were stained with silver staining method.

3.3.8. Silver Staining

When electrophoresis was completed, the power supply was terminated and the gels were prepared for visualization by silver staining. The silver nitrate staining were done as follow;

1. After electrophoresis was completed, the electrophoresis plate which siliconized by repel silane was remove.
2. Rinse the gel which is stuck to the electrophoresis plate (bind silane siliconized) with water for 3 minutes.
3. Wash the gel under 0.1% CTAB and shaking for 30 minutes.
4. During CTAB washing, prepare amonium solution by diluted 3 ml of 25% ammonium solution with 1 L water.
5. After wash the gel under 0.1% CTAB for 30 minutes, move the gel to wash with amonium solution for 15 minutes.
6. Prepare silver nitrate for silver nitrate staining as in appendix II.
7. After 15 minutes, move the electrophoresis plate and the gel to staining by silver. Wash the gel under silver nitrate solution and shaking for 20 minutes.
8. Preparing the develop solution (as in appendix II) and adding formaldehyde.
9. After 20 minutes, dip the gel in water 3 times and move the gel to the developing solution. Wash the gel and shaking under developing solution for about 5-15 minutes.

10. After the clear band appeared, move the gel to stop the developing reaction by washing under water for 30 minutes.
11. Protecting the gel from cracking by rinse under 3% glyceral for 20 minutes after the stoping solution.

After staining, the alleles (band) were score by eye. This method is suitable for denaturing gel. In the experiment the stutter bands were observed (Luqmani et al., 1997). The advantage of using silver stain over radioactive labeling is that the concentration of dNTP's does not have to be lowered in order to facilitate incoporatation of P32-labeled dNTP (Innis et al., 1999). Pairwise comparison between two varieties is used for band scoring. The DNA ladder marker was used for a standard when comparing bands between different gels. The gel aligns well with the fragments of the adjacent molecular weight. Numerical (1 to n) was use to designate the alleles, 1 represent the smallest alleles and n represent to the largest alleles observed in the gel. This method was applied from Ciampolini et al (1995).

3.3.9. Analyze of the Raw Data

After PCR, the DNA were run on polyacrylamide gel electrophoresis and detect by silver staining procedure. Band (alleles) were scored by eyes for the highly polymorphic results and calculated. The difference microsatellite allele frequencies among the chicken were assessed using Fisher's exact test in GENEPOP program.

The allele frequency in a population for diploid organism can be estimated by the equation below. In generally, suppose that among N individuals sampled from a

population the numbers of AA, Aa, and aa genotypes are NAA, NAa, and Naa, respectively. If p and q represent the allele frequenct of A and a , respectively, with $p + q = 1$ the estimate of allele frequency in the population can be equated as

$$\text{Allele frequency} = \frac{2NAA + NAa}{2N}$$

and the estimated sampleing variance is

$$[\text{Var}(p)] = \frac{(p)(1-(p))}{2N}$$

from the equation, NAA, Naa were represented the number of homozygous at allele A and a, and NAa was represented the number of heterozygous, for such allele, N is the number of investigated individuals.

The allele frequency result were calculated by software GENEPOP. The unique allele was observed for each locus and all varieties. When the unique allele has high allele frequency (approximate 0.90) it can be used to search for population specific markers (Crooijmans et al., 1996b).

Heterozygosity is the statistic parameter used to evaluate the informative of a genetic variation.When the variation is in Hardy-Weinberg equilibriun, heterozygosity were calculated from heterozygous alleles frequencies at a given locus.

Expected from Hardy-Weinberg assumption were calculated by GENEPOP program. $H_{(exp)}$ of verall locus was calculated for a comparison of genetic variation among a group by Non-paracentric statistic of Wilcoxon sign rank test.

The genetic information of Thai native chicken variety loenghangkhao and Praduhangdam were compared with the commercial broiler and layer fowls.

CHAPTER IV

RESULTS

4.1. Morphological Characterization

Morphological information from all individuals of 2 main varieties of Thai native chicken variety Loenghangkhao and Praduhangdam were compared. The comparison separated between sex and breed which is: male loenghangkhao (LM), female loenghangkhao (LF), male praduhangdam (PM), and female praduhangdam (PF). Morphological traits among 15 individuals were analyzed by measured data of wing length (WL) head length (HL) beck length (BL) leg length (LL) neck length (NL) back length (BAL) chest round (CR) and tarsometatarsus length (TL). These eight morphological parameters are interesting because normally farmer do not pay much attention to some of these parameters. They often concentrate on only color appearance and the fighting related traits.

In morphological data collection, the body weight and color appearance data were also collected as shown in appendix I. The color appearance of each Thai native chicken groups was collected by checking the color on each part of the body and scoring as 10 points. In this experiment, the average color score of all native chickens were 8.53. The average color score of male loenghangkhao was 8.33. Some native chickens in this group contain more white spot appearance than normal color of this variety. On the leg and the beak of some chickens in this group show black spots which is the traits of praduhangdam. The average color score of female loenghangkhao was 7.33. The lowest

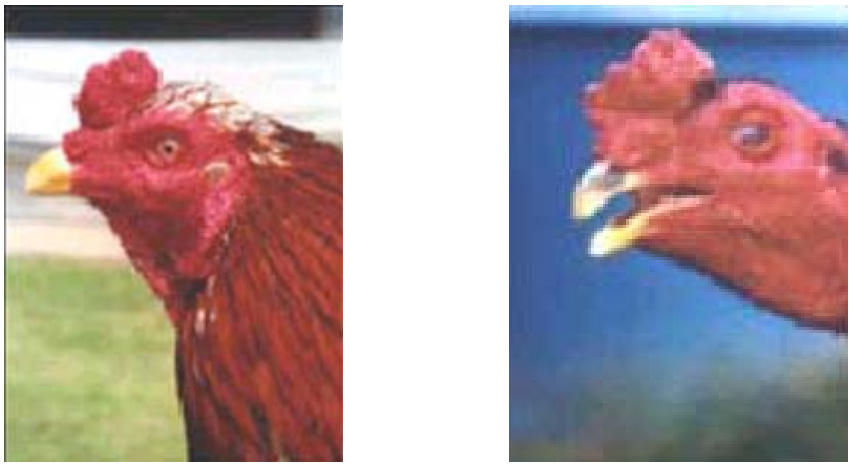
average color score was shown in this group because almost all native chickens in this group have more white spot appearing than normal color of this variety. These white spots might have an effect to the amount of white color of the newborn. The average color score of male praduhangdam was 8.86. In this group, the color of some native chickens changed from dark brown-black to green black. Especially in native chickens which have high fighting ability, the color appearance appeared to be more green than black. The color of the beak and the leg of some native chicken in this group appear as the color of loenghangkhao. The average color score of female praduhangdam was 9.60. Some native chickens in this group shown the loenghangkhao color on the beak and tarsometatarsus.

The analysis of eight parameters data (WL, HL, BL, LL, NL, BAL, CR, and TL) of four groups of Thai native chickens used one-way ANOVA of SAS program. The result from data analysis is shown in Table 4.1. The significant difference level of the analysis was on the level of 95%.

Morphological traits	df	Mean of square	C.V.	F Value	P-value
WL	3	16.75	7.358	6.35	<0.005
HL	3	10.02	7.651	22.35	<0.001
BL	3	0.30	10.537	2.87	<0.05
LL	3	35.67	7.308	19.32	<0.001
NL	3	33.57	6.510	14.75	<0.001
BAL	3	45.45	5.765	24.58	<0.001
CR	3	137.76	8.270	14.3	<0.001
TL	3	11.76	9.189	12.53	<0.001

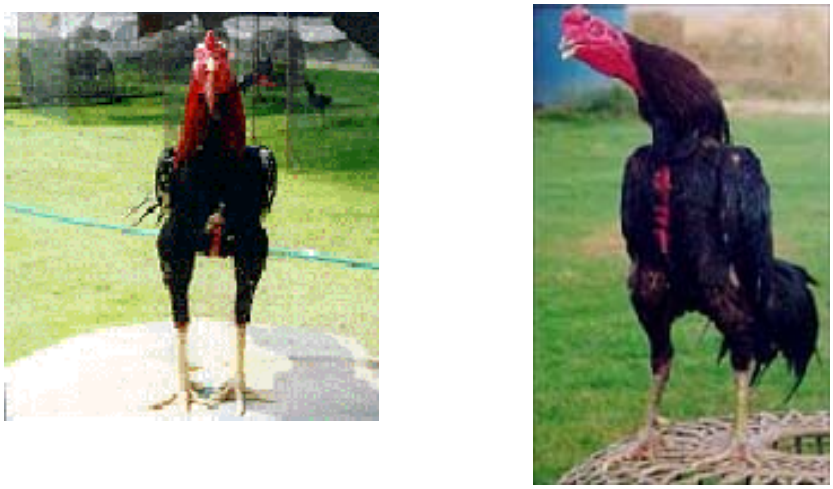
Table 4.1 The morphological data (WL, HL, BL, LL, NL, BAL, CR, and TL) of four groups of Thai native chickens (*Gallus gallus domesticus*) analyzed using one-way ANOVA. The significant level of the data was at 95%.

We compare the data base on variety traits and sex traits to find the traits that correspond to each variety of the native chicken. The experiment show different morphological traits were related to sex rather than variety in all native chicken group. Male and female native chickens show different average of the morphological data ($P < 0.01$). However, the experiments show the different of head length (HL) and leg length (LL) traits were related to the variety trait. The average head length (HL) and leg length (LL) were different between loenghangkhao and praduhangdam ($P < 0.05$) as shown in table 4.2 and figure 4.1.



A

B



C

Figure 4.1 Head length (HL, A) neck length (NL, B) and leg length (LL, C) of Thai native chicken variety loenghangkhao (left) and Thai native chicken variety praduhangdam (right)

Traits	Level of species	Level of Sex	Means	SD
WL	L*	F^{B}	21.4	0.8338
	L*	M^{A}	23.0	2.5317
	P*	F^{B}	20.9	1.7133
	P*	M^{A}	22.9	2.2055
HL	$L^{A'}$	F^{B}	8.2	0.3716
	$L^{A'}$	M^{A}	9.6	0.9536
	$P^{B'}$	F^{B}	7.9	0.5732
	$P^{B'}$	M^{A}	9.2	0.8632
BL	L*	$F^{A'}$	2.9	0.1290
	L*	$M^{A'}$	3.2	0.4911
	P*	$F^{B'}$	2.9	0.2288
	P*	$M^{B'}$	3.2	0.2439
LL	$L^{A'}$	F^{B}	17.3	0.8164
	$L^{A'}$	M^{A}	18.9	2.5345
	$P^{B'}$	F^{B}	17.5	0.5163
	$P^{B'}$	M^{A}	20.6	1.0431
NL	$L^{A'}$	F^{B}	22.1	0.6399
	$L^{A'}$	M^{A}	25.4	1.5976
	$P^{B'}$	F^{B}	22.3	1.2198
	$P^{B'}$	M^{A}	22.8	2.2886
BAL	L*	F^{B}	21.9	0.9722
	L*	M^{A}	25.4	1.5290
	P*	F^{B}	22.3	1.1751
	P*	M^{A}	24.7	1.4743

Traits	Level of species	Level of Sex	Means	SD
CR	L*	F^B	34.3	5.1915
	L*	M^A	39.7	2.1369
	P*	F^B	35.6	1.8790
	P*	M^A	40.5	2.2076
TL	L*	F^B	9.9	0.5936
	L*	M^A	11.6	1.2130
	P*	F^B	9.7	0.7973
	P*	M^A	10.9	1.5908

Table 4.2 The different morphological traits (WL, HL, BL, LL, NL, BAL, CR, and TL) of four groups of Thai native chickens (*Gallus gallus domesticus*) related to sex and variety traits. All data were analyze by Duncan's Multiple Range Test (DMRT);

* = not significant difference

A'B' = significant level at 95%

AB = significant level at 99%

4.2. DNA Extraction and Determination

The DNA of all individuals from Thai native chicken variety loenghangkhao, Thai native chicken variety praduhangdam, hybrid broiler and commercial layer were extracted by proteinase K/High salt extraction (Lipkin et al., 2000). The quality of the extracted genomic DNA was visualized by 2% agarose gel electrophoresis. The result of the extracting method which compared between incubation of the blood with proteinase K at 35 ^{o}C for 18 hours and incubated with proteinase K at 65 ^{o}C for 3 hours is shown in figure 4.2. The quantity of the extracted DNA from 3 hours incubation with proteinase K at 65 ^{o}C was less than the quantity of the extracted DNA from 18 hours incubation with proteinase K at 35 ^{o}C. However, the quantity of the DNA was enough for PCR. Thus, this experiment chose the 3 hours incubation extraction method to reduce the extraction time.

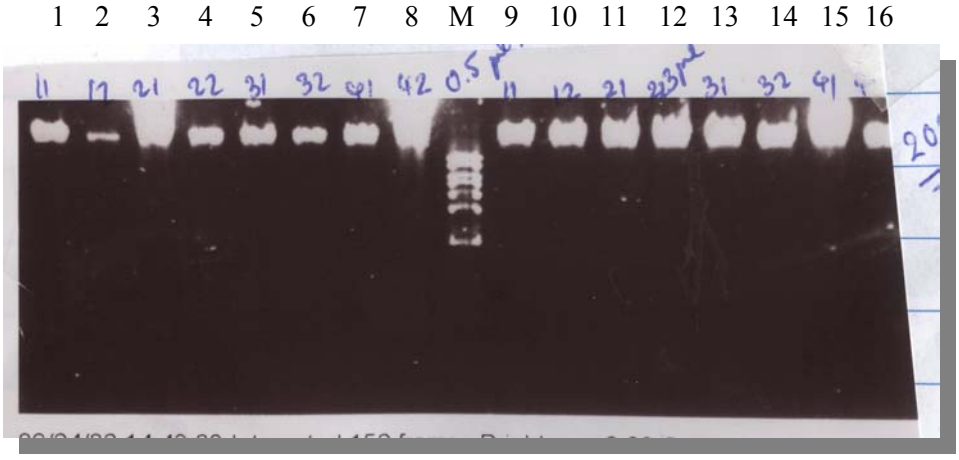


Figure 4.2 Genomic extraction results from blood of some individuals in the experiment. The genomic DNA was run on 2% agarose gel and staining by ethidium bromide.

lane M is 100 bp standard ladder marker

lane 1-8 are the gemonic DNA extracted by incubation with proteinase K at 65 ^{o}C for 3 hours

lane 9-16 are the gemonic DNA extracted by incubation with proteinase K at 35 ^{o}C for 18 hours

Extracted DNA from all individuals were measured by testing the DNA absorption (OD) by spectrophotometer at OD 260 nm and 280 nm. The concentration of the genomic DNA were calculated. The concentration of the extracted genomic DNA from 3 hours incubation with proteinase K at 65 ^{o}C ranging from 0.5 mg/ml to 3 mg/ml. The concentration of the extracted genomic DNA from 18 hours incubation with proteinase K at 35 ^{o}C ranging from 1 mg/ml to 4 mg/ml.

4.3. Optimization Condition for PCR Amplification

All 19 microsatellite loci shown in table 4.3 were used for this research.

Locus	Reaction mixture			
	$MgCl_2$(mM)	Forward Primer	Reverse primer	Anneal. (^oC)
MCW240	3	0.1	0.1	55
B259	2.5	0.1	0.1	49
IL103	1.5	0.1	0.1	44
MCW87	2.5	0.2	0.2	53
B76	1.5	0.1	0.1	50
ADL23	3	0.1	0.1	45
B85	3	0.1	0.1	55
B71	1.5	0.1	0.1	53
B154	2.5	0.1	0.1	47
B42	2.5	0.1	0.1	47
ADL228	3	0.2	0.2	48
LEI234	1.5	0.1	0.1	53
LEI116	2.5	0.1	0.1	48
ADL192	2.5	0.2	0.2	53
ADL278	2.5	0.2	0.2	48
LEI94	3	0.2	0.2	55

Table 4.3 The optimal condition for PCR amplification of male loenghangkhao (LM), female loenghangkhao (LF), male praduhangdam (PM), female praduhangdam (PF), two hybrid line broiler (BR) and commercial layer (LA) by microsatellite primers. Each PCR reaction has 25 μL total volume with 1 X buffer, 0.2 mM dNTPs, 1 U Taq polymerase.

The optimization of PCR condition of 16 microsatellite primers were investigated. The PCR condition which reported in the experiment previously (Cheng, 1994; Cheng, 1997; Crooijmans et al., 1996a; Kaiser et al., 2000; Khatib et al.,1993; Rico et al.,1997; Rohlf,2000; Tautz,1989) did not work well in Thai native chicken population. Thus, this experiment adjust the PCR condition to find the optimize PCR condition in Thai native chicken by varying the annealing temporature, $MgCl_2$ concentration and primer concentration. The new optimize condition of 16 microsatellite loci with Thai native chicken are shown in table 4.3.

4.4. Genetic Variation

From 16 microsatellite loci, high genetic variation and polymorphism were observed in all population of Thai native chicken and hybrid layer. The number of allele per population in each locus ranged from 5 to 25. The average allele number per primer was 13.38. The highest allele number was found at B259 locus and the lowest allele number was found at MCW240 locus. The mean number of allele frequency of all 6 populations (male loenghangkhao (LM), female loenghangkhao (LF), male praduhangdam (PM), female praduhangdam (PF), two hybrid line broiler (BR) and commercial layer (LA)) of ADL 192 locus are shown as an example in table 4.4 and figure 4.3. The mean number of allele frequency of other 15 locus are shown in Appendix I.

allele number	Locus ADL192					
	PM	PF	LM	LF	BR	LA
1	0.07142857	0.0625	0.117647	0.315789	0	0
2	0.07142857	0.25	0.058824	0.157895	0	0
3	0.21428571	0	0.058824	0.105263	0	0
4	0.14285714	0.3125	0.176471	0.315789	0	0
5	0.07142857	0.3125	0.235294	0.052632	0	0.2
6	0.14285714	0	0.058824	0.052632	0	0
7	0	0	0	0	0	0.2
8	0.07142857	0	0.235294	0	0	0.2
9	0.14285714	0	0.058824	0	0	0.1
10	0.07142857	0.0625	0	0	0.125	0.2
11	0	0	0	0	0.25	0.1
12	0	0	0	0	0.25	0
13	0	0	0	0	0.125	0
14	0	0	0	0	0.25	0

Table 4.4 The mean number of allele frequency of 6 populations in locus ADL 192.

LM = male Thai native chicken variety loenghangkhao

LF = female Thai native chicken variety loenghangkhao

PM = male Thai native chicken variety praduhangdam

PF = female Thai native chicken variety praduhangdam

BR = two hybrid line broiler

LA = commercial layer

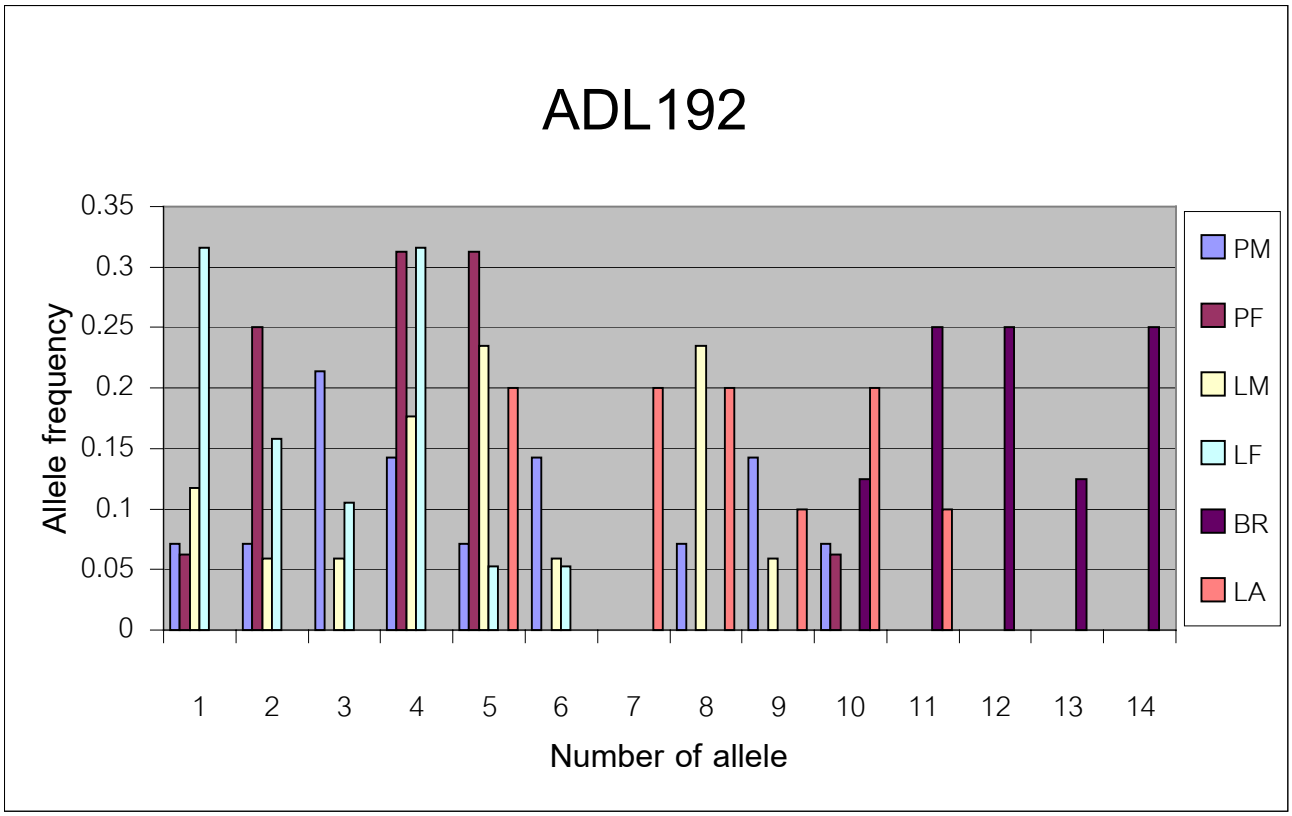


Figure 4.3 The histogram of the allele frequency of 6 populations in locus ADL 192.

LM = male Thai native chicken variety loenghangkhao

LF = female Thai native chicken variety loenghangkhao

PM = male Thai native chicken variety praduhangdam

PF = female Thai native chicken variety praduhangdam

BR = two hybrid line broiler

LA = commercial layer

Some allele distribution of Thai native chicken variety loenghangkhao and praduhangdam were similar as shown in the figures and histogram of all 16 microsatellite loci in table 4.4, figure 4.3 and appendix I. In locus ADL 23 both male and female Loenghangkhao have shown specific allele at allele 2 and both male and female Praduhangdam have shown specific allele at allele 7. However the allele frequency of both two populations were low (LM = 0.062, LF = 0.157 and PM = 0.125, PF = 0.058). In locus IL103 male Loenghangkhao shown specific allele at allele 15 and 16 (0.066 and 0.066, respectively). In locus LEI94 the specific allele of Thai native chicken variety Loenghangkhao and Praduhangdam and separate into 2 patterns. Both male and female Loenghangkhao shown specific allele at allele 2, 3, and 5 (LM = 0.058, 0.058, 0.176 and LF = 0.187, 0.187, 0.125) and both male and female Praduhangdam shown the specific allele at allele 13 and 14 (PM = 0.071, 0.285 and PF = 0.066, 0.133). In locus MCW87 Thai native chicken variety Praduhangdam shown the specific allele at allele 11 (PM = 0.066, PF = 0.062) In ADL228 locus both male and female praduhangdam show the specific allele at allele 2. The allele frequency is 0.125 in male praduhangdam and 0.066 in female praduhangdam.

4.5. Hardy-Weinberg assumption

From Hardy Weinberg assumption, the heterozygosity value varied from 0 to 0.933. Almost all values of expected heterozygosity were higher than observed heterozygosity. The highest expected heterozygosity was 0.917 found in female Thai native chicken variety praduhangdam at B154 locus. The highest expected heterozygosity of male praduhangdam was 0.896 at B154 locus. The highest expected heterozygosity of male loenghangkhao was 0.898 at B154 locus. And the highest

expected heterozygosity of female loenghangkhao was 0.912 at IL103 locus. The highest expected heterozygosity of hybrid broiler was 0.814 at B154 locus. The highest expected heterozygosity of commercial layer was 0.884 at B154 locus. The highest observed heterozygosity was 0.933 in Thai native chicken variety loenghangkhao and praduhangdam at MCW240 locus. The heterozygosity value from all 16 loci show high variation in all population.

The Hardy Weiberg assumption was carried out using an exact test. The P-value of male praduhangdam confirmed to the Hardy Weiberg assumption at all microsatellite loci except B42, ADL23 and LEI94. In female praduhangdam, the P-value confirmed to the Hardy Weiberg assumption at MCW240, IL103, B71, B42, ADL228, LEI234, ADL192, ADL278 and LEI94 loci. In male loenghangkhao the P-value confirmed to the Hardy Weiberg assumption at all microsatellite loci except B76, ADL23 and ADL192. In female loenghangkhao the P-value confirmed to the Hardy Weiberg assumption at B76, MCW87, B42 and LEI94. The estimation of Hardy Weiberg assumption is shown in table 4.5. The confirmation using an exact test of Hardy Weiberg assumption is shown in table 4.6.

Locus	PM		PF		LM		LF		LY		BR	
	H_{obs}	H_{exp}	H_{obs}	H_{exp}	H_{obs}	H_{exp}	H_{obs}	H_{exp}	H_{obs}	H_{exp}	H_{obs}	H_{exp}
MCW240	0.9333	0.6276	0.9333	0.4840	0.9333	0.7241	0.9333	0.5172	0	0.6025	0.3333	0.3538
B259	0.6000	0.7420	0.2666	0.6381	0.3333	0.6286	0.1333	0.5614	0.4666	0.6533	0.2666	0.6317
IL103	0.2000	0.7547	0.4000	0.6286	0.3333	0.7072	0.6000	0.9126	0.4000	0.5683	0.0666	0.2381
MCW87	0.1333	0.6827	0.1333	0.7802	0.2000	0.7827	0.4666	0.7120	0.4000	0.6551	0.4666	0.7160
B76	0.6000	0.8321	0.1333	0.7147	0.3333	0.8074	0.3000	0.4400	0.7333	0.8173	0.2666	0.6413
ADL23	0.2000	0.7173	0.2000	0.7850	0.2000	0.7852	0.4000	0.7173	0.0666	0.5652	0	0.5206
B85	0.2666	0.8713	0.0666	0.7760	0.1333	0.7951	0.3333	0.7839	0.2000	0.5227	0.2666	0.4877
B71	0.2000	0.6960	0.7333	0.5893	0.1333	0.7149	0.6666	0.7816	0	0.7264	0.4666	0.7013
B154	0.5333	0.8966	0.6000	0.9172	0.7333	0.8989	0.4000	0.8736	0.9333	0.8846	0.6000	0.8148
B42	0.0666	0.7309	0.6000	0.6889	0.2666	0.5407	0.1333	0.5149	0.2666	0.5014	0	0.4863
ADL228	0.4000	0.6095	0.1333	0.6107	0.2666	0.7307	0.2666	0.8000	0.2666	0.6435	0.2000	0.5474
LEI234	0.2000	0.7728	0.2000	0.2970	0.2000	0.4666	0.1333	0.4877	0.4000	0.6725	0.2666	0.4622
LEI116	0.2000	0.5524	0.2666	0.8138	0.1333	0.4784	0.2666	0.7040	0	0.5827	0.5333	0.5368
ADL192	0.3333	0.5412	0.2666	0.5913	0.4000	0.7467	0.4000	0.6933	0.0666	0.4311	0.2000	0.4154
ADL278	0.2000	0.2889	0.4000	0.6522	0.2000	0.7654	0.3333	0.6960	0.5333	0.6695	0.1333	0.4980
LEI94	0.3333	0.5059	0.3333	0.5333	0.2000	0.5930	0.4000	0.5789	0.6666	0.6286	0.2000	0.2519

Table 4.5 The Observed hererozygosity (H_{obs}) and Expected heterozygosity (H_{exp}) of all six populations in the experiment.

Locus	PM		PF		LM		LF		LY		BR	
	P-val	S.E	P-val	S.E	P-val	S.E	P-val	S.E	P-val	S.E	P-val	S.E
MCW240	1^{ns}	0.0000	1^{ns}	0.0000	1^{ns}	0.0000	1^{ns}	0.0000	<0.0001*	0.0000	0.2708	0.0066
B259	0.0198^{ns}	0.0100	0.0001^{ns}	0.0001	0.0002^{ns}	0.0002	<0.0001*	0.0000	0.0049^{ns}	0.0015	<0.0001*	0.0000
IL103	<0.0001*	0.0000	0.0013^{ns}	0.0008	<0.0001*	0.0000	<0.0001*	0.0000	0.0099^{ns}	0.0024	0.0062	0.0019
MCW87	0.0002	0.0002	0.0012^{ns}	0.0005	<0.0001*	0.0000	0.0009^{ns}	0.0005	0.0133^{ns}	0.0024	0.0304	0.0031
B76	0.0009^{ns}	0.0007	<0.0001*	0.0000	0.0004^{ns}	0.0004	0.0182^{ns}	0.0019	0.0818^{ns}	0.0092	<0.0001*	0.0000
ADL23	0.0001^{ns}	0.0001	<0.0001*	0.0000	<0.0001*	0.0000	0.0152^{ns}	0.0022	<0.0001*	0.0000	<0.0001*	0.0000
B85	<0.0001*	0.0000	<0.0001*	0.0000	<0.0001*	0.0000	0.0002^{ns}	0.0002	0.0001^{ns}	0.0000	0.0264	0.0022
B71	0.0006^{ns}	0.0006	0.2479^{ns}	0.0062	<0.0001*	0.0000	0.0232^{ns}	0.0021	<0.0001*	0.0000	0.0044	0.0011
B154	<0.0001*	0.0000	0.0009^{ns}	0.0009	0.1894^{ns}	0.0237	<0.0001*	0.0000	0.6806^{ns}	0.0182	0.0018	0.0009
B42	<0.0001*	0.0000	0.0128^{ns}	0.0020	0.0478^{ns}	0.0030	0.0050^{ns}	0.0004	0.0615^{ns}	0.0020	<0.0001*	0.0000
ADL228	0.0780^{ns}	0.0078	<0.0001*	0.0000	<0.0001*	0.0000	<0.0001*	0.0000	0.0007^{ns}	0.0005	0.0007	0.0007
LEI234	<0.0001*	0.0000	0.0999^{ns}	0.0071	0.0012^{ns}	0.0006	0.0004^{ns}	0.0004	0.0241^{ns}	0.0035	0.0019	0.0007
LEI116	<0.0001*	0.0000	<0.0001*	0.0000	<0.0001*	0.0000	0.0002^{ns}	0.0001	<0.0001*	0.0000	0.3121	0.0182
ADL192	0.0069^{ns}	0.0025	0.0025^{ns}	0.0010	0.0007^{ns}	0.0005	<0.0001*	0.0000	<0.0001*	0.0000	0.0062	0.0015
ADL278	0.1531^{ns}	0.0075	0.0015^{ns}	0.0006	<0.0001*	0.0000	0.0022^{ns}	0.0006	0.1580^{ns}	0.0084	0.0002	0.0002
LEI94	0.0295^{ns}	0.0051	0.0275^{ns}	0.0049	<0.0001*	0.0000	0.0474^{ns}	0.0047	0.8172^{ns}	0.0071	0.1231	0.0081

Table 4.6 The estimation under Hardy-Weiberg assumption of 6 groups of individual by 16 microsatellite loci.

Where as

* Significant level was further adjusted by using Bonferoni method

^{ns} no significant different (P<0.005)

The exact test for genotypic linkage disequilibrium showed significance between locus pair of B42 and B85, and B42 and B71, where other locus pairs confermed to the genetic linkage disequilibrium. The exact test for genotypic linkage disequilibrium result shown in table 4.7.

Locus pair	P-value	Chi^2
MCW240 & B259	0.772 ^{ns}	6.493
MCW240 & IL103	0.990 ^{ns}	1.657
B259 & IL103	0.975 ^{ns}	3.233
MCW240 & MCW87	0.418 ^{ns}	10.264
B259 & MCW87	1.000 ^{ns}	0
IL103 & MCW87	0.987 ^{ns}	3.786
MCW240 & B76	0.129 ^{ns}	17.574
B259 & B76	1.000 ^{ns}	0
IL103 & B76	0.270 ^{ns}	12.228
MCW87 & B76	0.383 ^{ns}	14.932
MCW240 & ADL23	0.182 ^{ns}	16.205
B259 & ADL23	0.789 ^{ns}	9.632
IL103 & ADL23	0.966 ^{ns}	4.745
MCW87 & ADL23	0.520 ^{ns}	13.084
B76 & ADL23	0.078 ^{ns}	24.577
MCW240 & B85	0.259 ^{ns}	10.085
B259 & B85	0.981 ^{ns}	5.296

Locus pair	P-value	Chi^2
IL103 & B85	1.000 ns	0.646
MCW87 & B85	0.069 ns	22.475
B76 & B85	0.063 ns	22.84
ADL23 & B85	0.302 ns	18.385
MCW240 & B71	0.021 ns	21.006
B259 & B71	0.975 ns	4.396
IL103 & B71	0.776 ns	8.116
MCW87 & B71	0.110 ns	23.115
B76 & B71	0.357 ns	17.455
ADL23 & B71	0.034 ns	27.694
B85 & B71	0.078 ns	24.577
MCW240 & B154	0.724 ns	7.017
B259 & B154	0.617 ns	9.991
IL103 & B154	1.000 ns	0
MCW87 & B154	0.945 ns	6.711
B76 & B154	0.755 ns	8.377
ADL23 & B154	0.860 ns	8.526
B85 & B154	0.898 ns	7.827
B71 & B154	0.735 ns	10.359
MCW240 & B42	0.234 ns	15.145
B259 & B42	0.769 ns	9.906
IL103 & B42	0.866 ns	6.873
MCW87 & B42	0.640 ns	11.577
B76 & B42	0.076 ns	22.119
ADL23 & B42	0.089 ns	24.013
B85 & B42	0.001*	36.859
B71 & B42	0.005*	31.505
B154 & B42	0.680 ns	11.072
MCW240 & ADL228	0.827 ns	7.449

Locus pair	P-value	Chi^2
B259 & ADL228	0.989 ns	2.628
IL103 & ADL228	1.000 ns	0
MCW87 & ADL228	0.681 ns	12.883
B76 & ADL228	0.991 ns	5.706
ADL23 & ADL228	0.435 ns	16.264
B85 & ADL228	0.517 ns	13.116
B71 & ADL228	0.798 ns	9.495
B154 & ADL228	0.996 ns	2.853
B42 & ADL228	0.975 ns	5.64
MCW240 & LEI234	0.996 ns	2.293
B259 & LEI234	0.977 ns	3.173
IL103 & LEI234	1.000 ns	0
MCW87 & LEI234	0.596 ns	12.127
B76 & LEI234	0.966 ns	2.416
ADL23 & LEI234	0.582 ns	12.299
B85 & LEI234	0.906 ns	7.666
B71 & LEI234	0.796 ns	7.856
B154 & LEI234	0.981 ns	3.036
B42 & LEI234	0.363 ns	13.089
ADL228 & LEI234	0.533 ns	12.914
MCW240 & LEI116	0.874 ns	3.805
B259 & LEI116	0.953 ns	5.137
IL103 & LEI116	0.731 ns	5.246
MCW87 & LEI116	0.526 ns	14.987
B76 & LEI116	1.000 ns	0.533
ADL23 & LEI116	0.371 ns	15.105
B85 & LEI116	0.594 ns	10.246
B71 & LEI116	0.234 ns	19.693
B154 & LEI116	0.537 ns	8.952

Locus pair	P-value	Chi^2
B42 & LEI116	0.293 ^{ns}	14.116
ADL228 & LEI116	0.321 ^{ns}	15.882
LEI234 & LEI116	0.993 ^{ns}	3.259
MCW240 & ADL192	0.511 ^{ns}	5.263
B259 & ADL192	0.863 ^{ns}	3.936
IL103 & ADL192	1.000 ^{ns}	0
MCW87 & ADL192	0.724 ^{ns}	7.01
B76 & ADL192	0.737 ^{ns}	3.556
ADL23 & ADL192	0.032 ^{ns}	19.74
B85 & ADL192	0.898 ^{ns}	4.889
B71 & ADL192	0.672 ^{ns}	7.562
B154 & ADL192	1.000 ^{ns}	1.018
B42 & ADL192	0.209 ^{ns}	13.264
ADL228 & ADL192	0.999 ^{ns}	1.361
LEI234 & ADL192	0.160 ^{ns}	11.801
LEI116 & ADL192	0.964 ^{ns}	2.455
MCW240 & ADL278	0.995 ^{ns}	0.662
B259 & ADL278	0.998 ^{ns}	2.595
IL103 & ADL278	0.859 ^{ns}	3.982
MCW87 & ADL278	0.985 ^{ns}	5.083
B76 & ADL278	0.992 ^{ns}	3.44
ADL23 & ADL278	0.773 ^{ns}	9.858
B85 & ADL278	1.000 ^{ns}	1.515
B71 & ADL278	0.512 ^{ns}	11.196
B154 & ADL278	0.991 ^{ns}	2.504
B42 & ADL278	0.379 ^{ns}	8.575
ADL228 & ADL278	0.383 ^{ns}	10.674
LEI234 & ADL278	0.867 ^{ns}	6.859
LEI116 & ADL278	0.915 ^{ns}	6.012

Locus pair	P-value	Chi^2
ADL192 & ADL278	0.922 ^{ns}	1.976
MCW240 & LEI94	0.933 ^{ns}	3.022

Table 4.7. The pairwise comparison of genetic linkage disequilibrium among 16 microsatellite loci of 6 investigated groups.

* = Significant level was futher adjusted by using a Bonferioni method

^{ns} = no significant

For the genetic divergence of the population, the fixation coefficients of subpopulations within the total population (F_{ST}) for the sixteen loci varies from –0.3196 to 0.7860, with the mean being 0.4885. The fixation indices of individuals within the total population (F_{IT}) ranged from 0.0274 to 0.2111, with the mean being 0.1682. Individual's fixation indices within the subpopulation (F_{IS}) varied from –0.0909 to 0.8068, with the mean being 0.5745. The fixation coefficient result was shown in table 4.8.

Locus	Fwc(is)	Fwc(st)	Fwc(it)
MCW240	-0.3196	0.1733	-0.0909
B259	0.4699	0.0956	0.5206
IL103	0.538	0.0325	0.553
MCW87	0.5689	0.0598	0.5947
B76	0.4189	0.0435	0.4442
ADL23	0.786	0.0975	0.8068
B85	0.6612	0.1163	0.7006
B71	0.3974	0.1717	0.5009
B154	0.2832	0.0274	0.3028
B42	0.6454	0.2111	0.7203
ADL228	0.6738	0.1134	0.7108
LEI234	0.5696	0.0711	0.6003
LEI116	0.5978	0.1536	0.6596
ADL192	0.5434	0.1309	0.6032
ADL278	0.5498	0.0456	0.5703
LEI94	0.2999	0.0967	0.3676
All:	0.4885	0.1682	0.5745

.

Table 4.8. The fixation coefficient result among 16 microsatellite loci of 6 investigated groups.

4.6. Dendrogram construction

Figure 4.4 and 4.5 shows the genetic variation within the group of male Thai native chicken variety loenghangkhao (LM) and praduhangdam (PM) by dendrogram constructed using UPGMA. In both populations the dendrogram can be separated into two main groups. The first group is Thai native chicken located at Chonburi province and the second group is Thai native chicken located at Phisanuloke and Nakhon Prathom province.

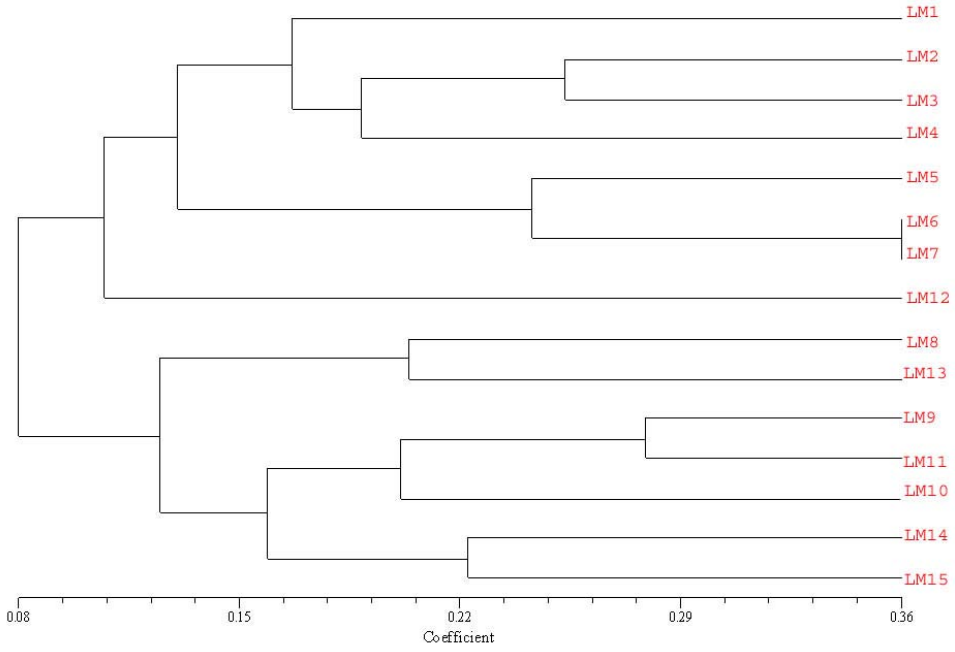


Figure 4.4. The UPGMA dendrogram within the population of male Thai native chicken variety loenghangkhao (LM 1-7; Chonburi, LM 8-12; Nakhon Prathom, LM 13-15; Phisanuloke).

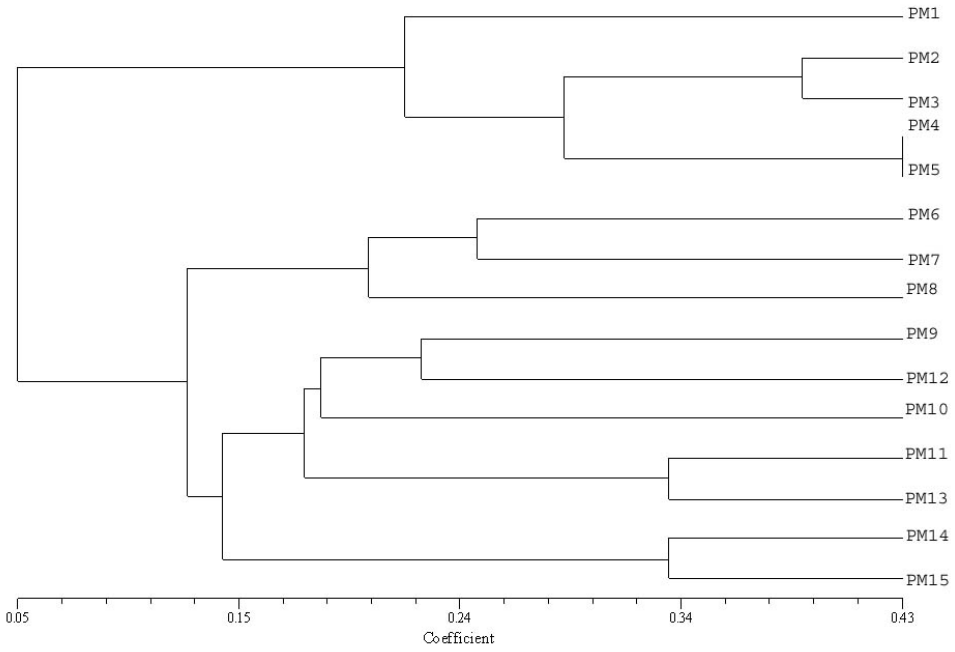


Figure 4.5. The UPGMA dendrogram within the population of male Thai native chicken variety praduhangdam (PM 1-5; Chonburi, PM 6-15; Nakhon Prathom).

When compare the different between the population of male Thai native chicken variety praduhangdam (PM) and male Thai native chicken variety loenghangkhao (LM) and between female praduhangdam (PF) and female loenghangkhao (LF), the chicken can be separated into 2 main groups. The first group is the Thai native chicken located at Chonburi province and the second group was Thai native chicken located at Phisanuloke and Nakhon Prathom province as shown in figure 4.6 and 4.7.

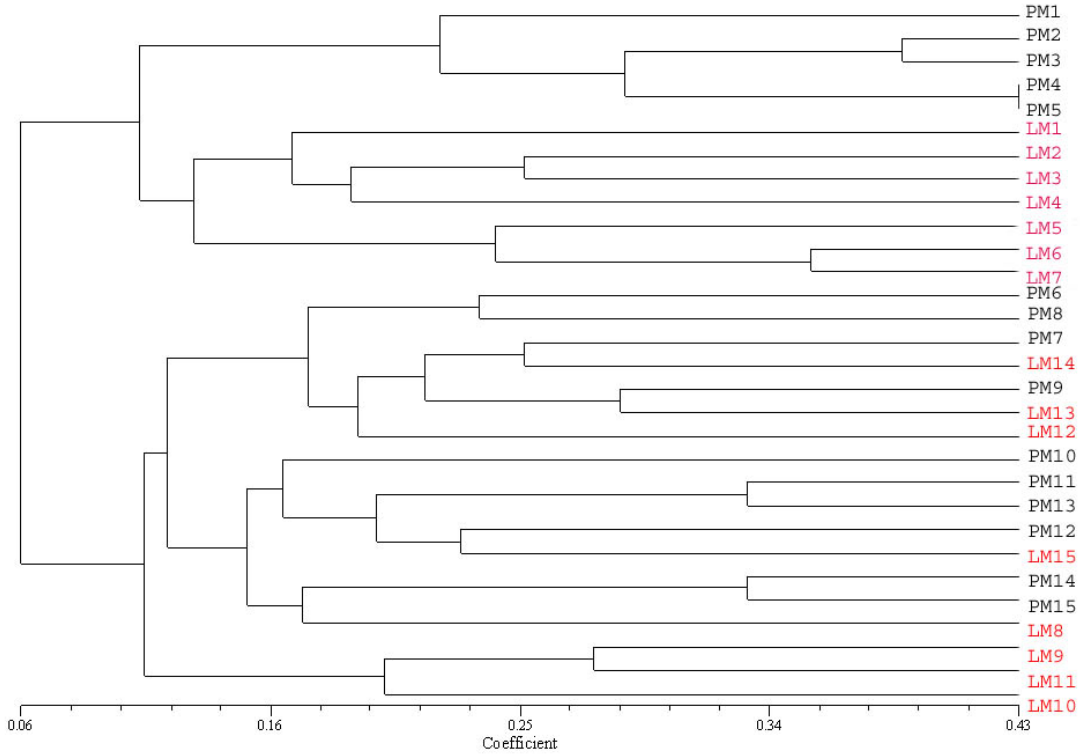


Figure 4.6. The UPGMA dendrogram between the population of male Thai native chicken variety praduhangdam (PM) and male Thai native chicken variety loenghangkhao (LM).

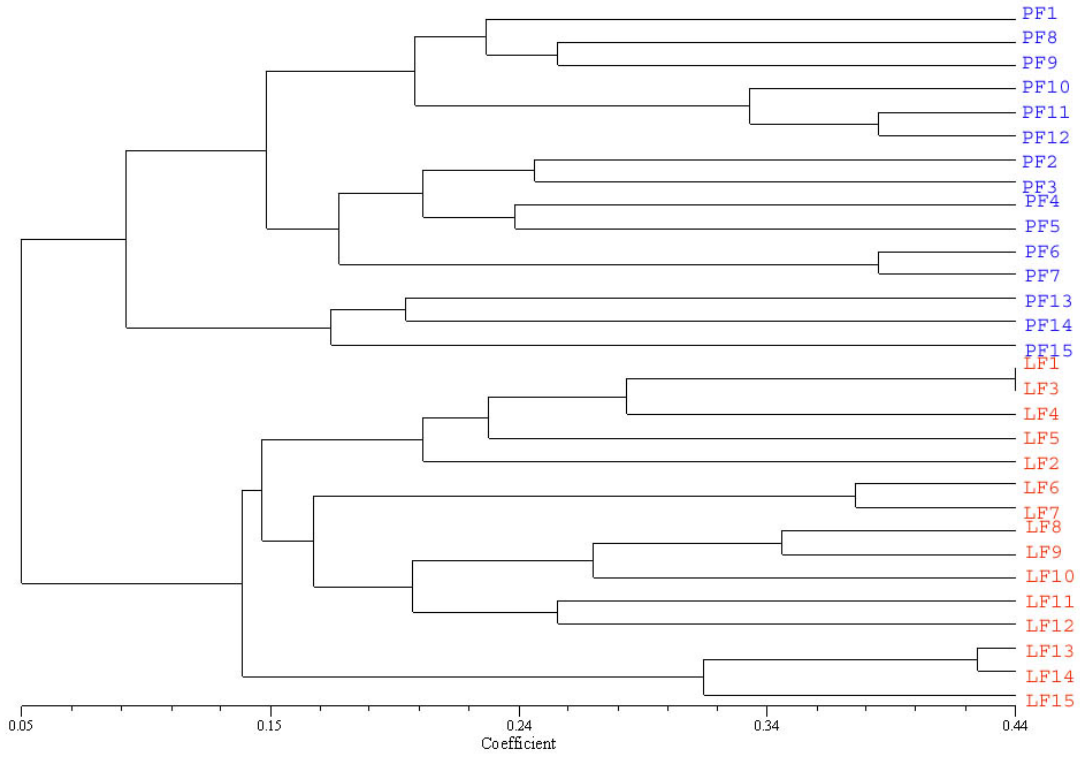


Figure 4.7. The UPGMA dendrogram between the population of female Thai native chicken variety praduhangdam (PF 1-12; Chonburi, PF 13-15; Nakhon Prathom) and female Thai native chicken variety loenghangkhao (LF 1-15; Chonburi).

With in the population of male Thai native chicken variety praduhangdam (PM) and male Thai native chicken variety loenghangkhao (LM), dendrogram was separate into two main groups but did not show clearly different between varieties. However, the dendrogram results were separated into 2 groups of native chicken from different location. The first group was Thai native chicken located at Chonburi province and the second group was Thai native chicken located at Phisanuloke and Nakhon Prathom province. With in the group of Thai native chicken located at Chonburi province, male

Thai native chicken variety loenghangkhao (LM 1-7) can be separated from male Thai native chicken variety praduhangdam (PM 1-5). In the population of Thai native chicken located at Phisanuloke and Nakhon Prathom province, the separation did not clearly different.

With in the population of female Thai native chicken variety praduhangdam (PF) and male Thai native chicken variety loenghangkhao (LF), dendrogram was separate into two main varieties. Although the origins of the native chicken are different, the different between varieties were clearly separated. However, the population of female Thai native chicken variety loenghangkhao (LF) located at Chonburi province is more distanct far from female Thai native chicken variety loenghangkhao (LF) located at Phisanuloke and Nakhon Prathom province. When compare the population of Thai native chicken variety praduhangdam and loenghangkhao with two-line hybrid broiler and commercial layer, as shown in figure 4.8, the population of Thai native chicken was closer to broiler than layer.

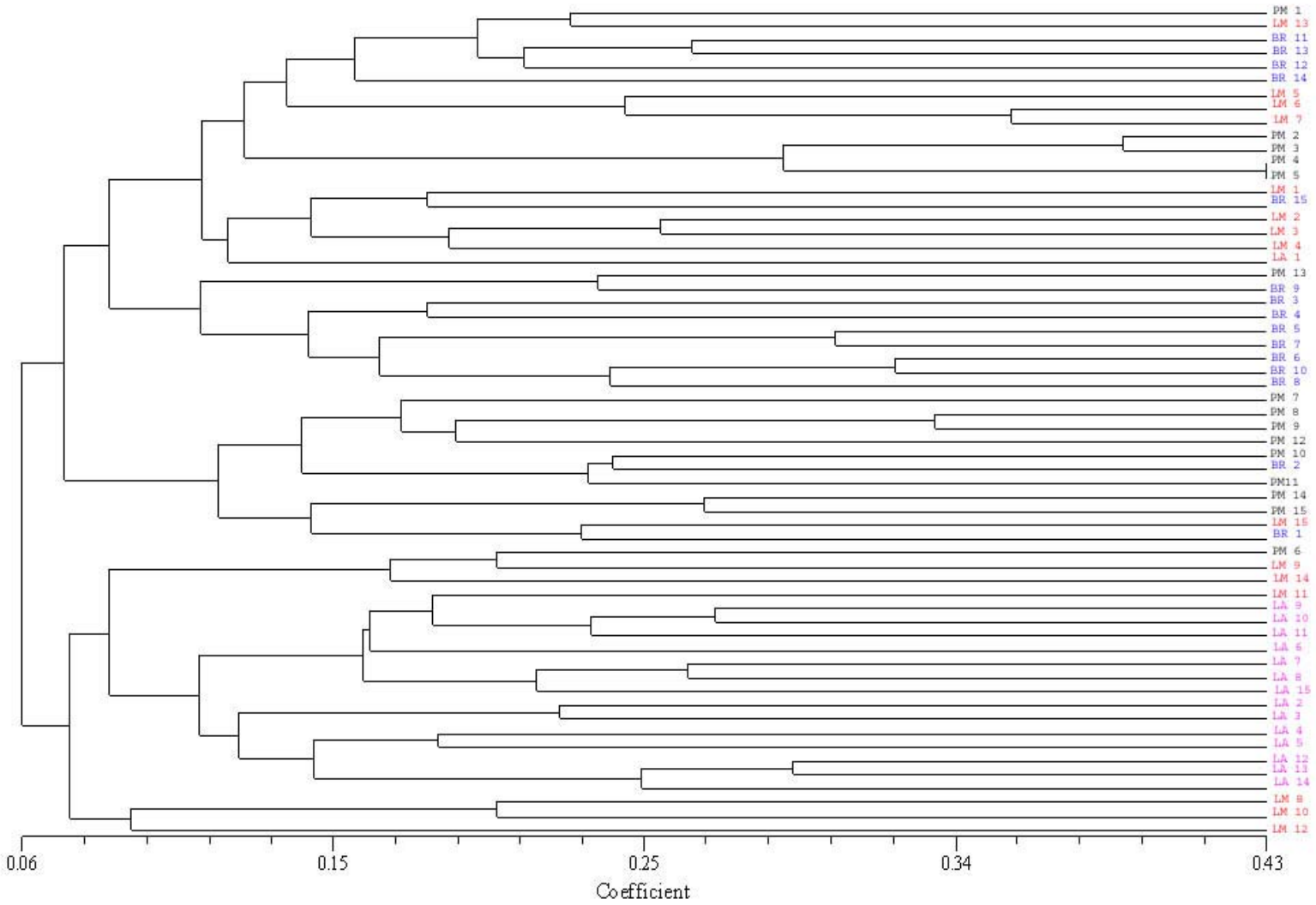


Figure 4.8. The UPGMA dendrogram aomng the population of Thai native chicken variety praduhangdam, loenghangkhao, twoline hybrid broildr and commercial layer.

CHAPTER V

DISCUSSION

The native chicken bloods in this experiment were collected from radial venipuncture (wing vain) and the anticoagulant 0.1M EDTA was added. The blood were kept under –20 ^{o}C until use. This method is convenient, powerful and not expensive. The blood sample can be kept for a long time. Lipkin et al. (2002) reported the DNA extraction result from blood which were fresh extract, short time frozen and long-time frozen. The densitomeric genotyping of DNA samples were effective for both amplifications from fresh extract and long-term frozen and thawed blood. Thus, the blood collection method in this experiment can be developed and appropriate to make a blood storage bank. This method is appropriate to keep the blood for future DNA extraction.

The morphological polymorphism of male and female Thai native chicken variety loenghangkhao and praduhangdam were analyzed. Morphological traits among individuals were analyzed by measured data of wing length (WL) head length (HL) beck length (BAL) leg length (LL) neck length (NL) back length (BL) chest round (CR) and tarsometatarsus length (TL). Some morphological traits were different between loenghangkhao and praduhangdam. In avian, normally, the different of phenotypic traits are associated with type of food and food consumption method (Zing and Balckwell, 1996). Karnsomdee (1999) reported the different of beak length, tarsal length, wing length and head length among the population of fighting cock, bantam cock and red

jungle fowl. The size of fighting cock was larger than bantam cock and red jungle fowl. In this report, when compare the morphological traits of Thai native chicken variety loenghangkhao and praduhangdam, the different were shown between head length (HL) leg length (LL) and neck length (NL) (P<0.05, table 4.2). Loenghangkhao have longer HL, LL than praduhangdam in both male and female population. In NL, male loenghangkhao show longer neck than praduhangdam but in female, praduhangdam show longer neck than loenghangkhao. The larger size of the fighting cock may be due to the selection of the structure for fighting ability. The selection for this ability generally based on tarsal length (TL), wing length (WL), beak length (BL) and the strength of the cock. The characters differences between loenghangkhao and praduhangdam may use as cooperate selection parameters along with color appearance selection to help in the breeding program.

The color appearance is the main factor associated to the decision of the breeder in the native chicken-breeding program. Color appearance of Thai native chicken variety loenghangkhao and praduhangdam were analyzed. The colors in each part of the body were checked and compare with the phenotype information reported by the livestock division of Thailand. Most of the colors were score as ten points. The score of most native chicken in this experiment were lower than 10. The average color score of all native chicken was 8.53 (appendix II). The color of some male loenghangkhao has more white spot than normal. This white color appearance might be due to the female loenghangkhao which have lots of white spot were used as mother. Some chicken in this group have dark color on the beak and tarsal as in praduhangdam. This meant that some loenghangkhao in the experiment have some genotype of praduhangdam. They were not truly pure breed. In female loenghangkhao group, some of them have more white spots.

This might be the cause of high number of white color in male loenghangkhao population. In the population of male praduhangdam chicken, the feather of some chicken were not normal dark brown red color. They appeared to be dark-green color especially in the group of fighting cock collected from farms from both Chonburi and Phisanuloke. On the beak and tarsal of some chicken in this group did not have any dark color. The color of the beak and tarsal appeared to be white-yellow as in loenghangkhao phenotype. This might be due to the genetic of loenghangkhao chicken mixed in. The genetic of loenghangkhao might contaminate in the genetic of the parent stock of these praduhangdam. In this experiment, just only few female praduhangdam have dark-green feather. The color appearance of all female praduhangdam did not show much variation from the information of livestock division of Thailand database. Therefore, the color score of this group was the highest. However, some chicken did not show dark color on the beak and/or tarsal. The genetic mixing between these two varieties might be due to breeders cross this two varieties to increase the heterosis level in hoping to increase the fighting ability. The measuring data of the morphological characters of both male and female loenghangkhao and praduhangdam show close relationship.

From the result using 16 microsatellite primers, these groups of chicken show high variation and high allele distribution. The high distribution of the number of allele per population may be due to, firstly, the primer selected for this experiment have high polymorphism. Most primers were reported as highly polymorphism in the reference poultry populations (Khatib et al., 1993; Cheng, 1994; Cheng, 1997; Crooijmans et al., 1996b). The high polymorphism of the DNA can distribute the different alleles and show the rare alleles which have less allele frequency. Secondly, the high distribution may be due to the native chicken in the experiment were collected from three different farms

from different location. The chicken from farms in Phisanuloke and Nakhon Prathom are commercial farms. But the chicken from Chonburi farm are bred under breeding inprovement. Along with the limitation of the number of native chicken in each farm, the native chicken cannot be equally collected from each place. Native chicken from many location can have an effect to the number of allele. These might effect to distinguish of the specific allele of the population. Although the phenotype of the native chicken were similar, the separation of the development makes high variation in the genetic level of the native chicken population.

Normally the unique allele frequency level should be about 0.9 to be able to use it as a marker allele. This high number of frequency indicate that this allele can be use as a genetic marker to identify the variety. Some allele distributions of loenghangkhao and praduhangdam population were close together. Most of the specific allele of all varieties in the experiment was not high enough to use as variety identification allele. Thus, the next experiment should concentrate in some of these alleles and increase the number of the pure breed individuals. In the experiment of Karnsomdee (2002), the specific alleles which can be use as variety identification was reported. However, the individual in this experiment did not show this specific allele. The different result might be because the different group of chicken used. The chickens were developed from the different area.

From the experiment, the analyzed data shows differences between two main varieties of Thai native chicken, Loenghangkhao and Praduhangdam. The Hardy-Weinberg test was tested for all loci. The expected and observed heterozygosity can be used to compare the genetic diversity within and between groups and can identify different heterozygosity within each individual. This value can used to reduce the

estimation error of rare alleles. This different of the heterozygosity between individual was due to high mutation rate in the chicken population. The lowest heterozygosity was found in the population of two line crossing broiler and commercial layer chicken. This is because both reference populations were developed and the breed were more pure than the native chicken. In native chicken, some alleles in the experiment were founded in all groups. This is because some microsatellite DNA can show the same size in different varieties (Rico et al., 1997). The expected heterozygosity in all population shows similar value. This indicate that most of the chicken in the experiment contain high genetic variation but not much different from each other. The high distribution of allele frequency and high heterozygosity in the population indicate the high genetic variation in native chicken population.

The Hardy-Weinberg Equilibrium was tested by *P-value*. The loci in this study are mainly in agreement with Hardy-Weinberg test. However, the result shows some loci deviated from the assumption. The depression of Thai native chicken from Hardy-Weinberg may be due to several parameters such as mutation process at the loci not confirming to the infinite allele model of mutation. Given the high mutation rate of microsatellite loci and individual may be homozygous for a pair of alleles of the same size, which may not be the product of the single mutation event (Rico et al., 1997). However, the most likely explanation for the deviation is wrong genotyping. Another parameter, the gel conditions may not have been accurate enough to separate two or three basepairs different between large fragments and thus some heterozygotes may have been ignored. This explanation is supported by the fact that the locus contained several different alleles which are heterozygous but appearing on the gel as homozygotes.

The different region of microsatellite loci can effect to genetic linkage on the allele distribution. The genetic linkage disequilibrium was used to prove the different region of microsatellite loci. Closely associated loci are often found to be genetically link, thus give more genetic linkage disequilibrium when compare to different region loci (Ciampolini et al., 1995). Almost all of the loci showed no significance of the parewise comparison of the genetic linkage disequilibrium among 16 microsatellite loci. The no significant different was showed between B42 and B85 and B42 and B71. These explain that both couple may locate on close distance region. Therefore, B42, B85 and B71 loci were not independent from each other and show that allele of the locus were not random association.

According to the genetic divergence of the population, the *F* statistic show high genetic variation of the individual in the subpopulations and total population. This is because the native chicken in the experiment were collected from many places. The genetic variations among them were high. These show the same results as in the heterozygosity. However, the *F* statistic did not shown clear differences in the subpopulation within the total population. The native chicken in the experiment cannot be separate clearly between each variety. Non-different in all population might be because most of the native fowl were developed as a fighting cock. The main target was to increase the fighting ability. Thus, the appearance was the second target that breeder interest.

From phenotypically characterization, male loenghangkhao and male praduhangdam have similar morphology and phenotype. The similar morphology and phenotype also was observed for female loenghangkhao and female praduhangdam. The

main different of the male chickens are the color. However, for female they are pretty much the same. In this study within the group of male loenghangkhao (figure 4.4), the genetic characterization dendrogram can separate them into two groups. The first group are the loenghangkhao from Chonburi province (LM 1-7). The second group are the loenghangkhao from Nakhon Prathom (LM 13-15) and Phisanuloke province (LM 8-12). The mainly different of the individual in this two groups is the chicken from farm in Chonburi are under full breeding program control but the chicken from farms from Nakhon Prathom and Phisanuloke does not have full control. They are from the commercial farm of the farmer which aims in increasing the fighting ability. However, one individual from Phisanuloke have similar genetic characteristic as the group of Chonburi chicken. This similarlity might be because this chicken might have come from the same origin. This is possible because, by the limitation of the farm, the experiment cannot identify the ancestor information and the origin of the native chicken in no breeding contril group. In the blood sample collection, the native chickens were chosen from the good and highest score phenotypic appearance from each farm. In another individual's group, native chicken from farm in Phisanuloke province were more genetic related to the native chicken from Nakhon Prathom province and cannot be separated clearly. This might because Thai native chicken variety loenghangkhao are widely known and popular. It is possible that some commercial exchanging, buying or selling from one province to another. This might makes the close microsatellite distribution. However, in the group of the native chicken which have full breeding program, the chicken have been cross to make the pure breed stock. Thus, it is less chance to get the genetic interrupting from other native chicken group. And it means that the breeding program have high effect to the genetic diversity.

In the popularion of male praduahngdam, the genetic diversity was similar to loenghangkhao population. The dendrogram can separate them into two groups which is praduhangdam from far, from Chonburi province (PM 1-5) and praduhangdam from Nakhon Prathom farm (PM 6-15) (figure 4.5). However, within the group of individual from Nakhon Prathom which did not have full breeding control, the native chicken can be separated into two subgroups (PM 6-8 and PM 9-15). When compare this separation with the morphological data, the native chicken number 6-8 have dark brow black feather color different from number 9-15 which have dark green feather color. And both group have different fighting capacity. The chicken number 9-15 has higher fighting character when compare to the chicken number 6-8. This dark green feather color was similar to the feather color of loenghangkhao chicken. This might be because these native chickens have the genetic contamination from loenghangkhao population and effect to the microsatellite polymorphism and fighting ability.

When compare the dendrogram between male loenghangkhao and praduhangdam (figure 4.6), the dendrogram can separate into two groups which is the native chicken which have full breeding program and the native chicken which did not have breeding control. In the group chicken which have full breeding control, loenghangkhao and praduhangdam can be separated clearly from each other. However, in the group of the chicken which did not have full breeding control, native chicken cannot be separated between varieties. This means that the breeding program have an effect to the development and breed of the native fowl. The genetic of loenghangkhao and praduhangdam in the experiment were close together. Thus, the number of native chicken population in the experiment have effect to the genetic variation of the

population. Thus, the next experiment should use enough individual of the same phenotype.

When compare the dendrogram between female loenghangkhao and praduhangdam (figure 4.7), dendrogram can separate then into two groups by variety, which is the native chicken variety loenghangkhao and the native chicken variety praduhangdam. In the group of female praduhangdam, native chicken can be separate into two subgroups as the breeding control and non breeding control group. This means that the breeding control have high effect to the microsatellite and genetic variation of the native chicken.

When compare the dendrogram between loenghangkhao and praduhangdam with the population of commercial layer and two line crossing broiler (figure 4.8), both variety of native chicken were closer to two line crossing between native and broiler chicken than commercial layer fowl. Thus, the genetic of the native chicken were closer to the two-line crossing broiler than commercial layer.

CHAPTER VI

CONCLUSION

The expected and observed heterozygosity can be used to compare the genetic diversity within and between groups of population. The population of loenghangkhao and praduhangdam has high heterozygosity. When focus on the genetic diversity, the results can indicate high variation of the native chicken within and between subpopulation. Although the experiment did not find any allele specific for each variety and cannot separate the genetic of loenghangkhao and praduhangdam clearly, the result shows difference between populations in each group. The result indicated that this set of microsatellite can be use to identify the variation of two main native chicken of Thailand which are loenghangkhao and praduhangdam. When compare the phenotype between Thai native chicken variety loenghangkhao and praduhangdam in this experiment, both varieties have different head length (HL), leg length (LL) and neck length (NL) ($P<0.05$) as they can also separated by genetic variation. Thus, the breeding program should use both genetic and phenotypic characterization for characterizes the native chicken.

These experiments were not able to identify any allele specific to the variety. This is because the chicken populations in this study are not pure line. The background of the chicken were not clearly marked. Therefore, future work on Thai native chicken diversity should make sure that the chicken population in the study are pure line if discovery of the molecular marker to identify each stain is the aim of work.

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APPENDIX

APPENDIX I

The Mean Number of Allele Frequency of 6 Populations in 16 Loci.

The mean number of allele frequency of 6 populations in 15 loci.

LM = male Thai native chicken variety loenghangkhao

LF = female Thai native chicken variety loenghangkhao

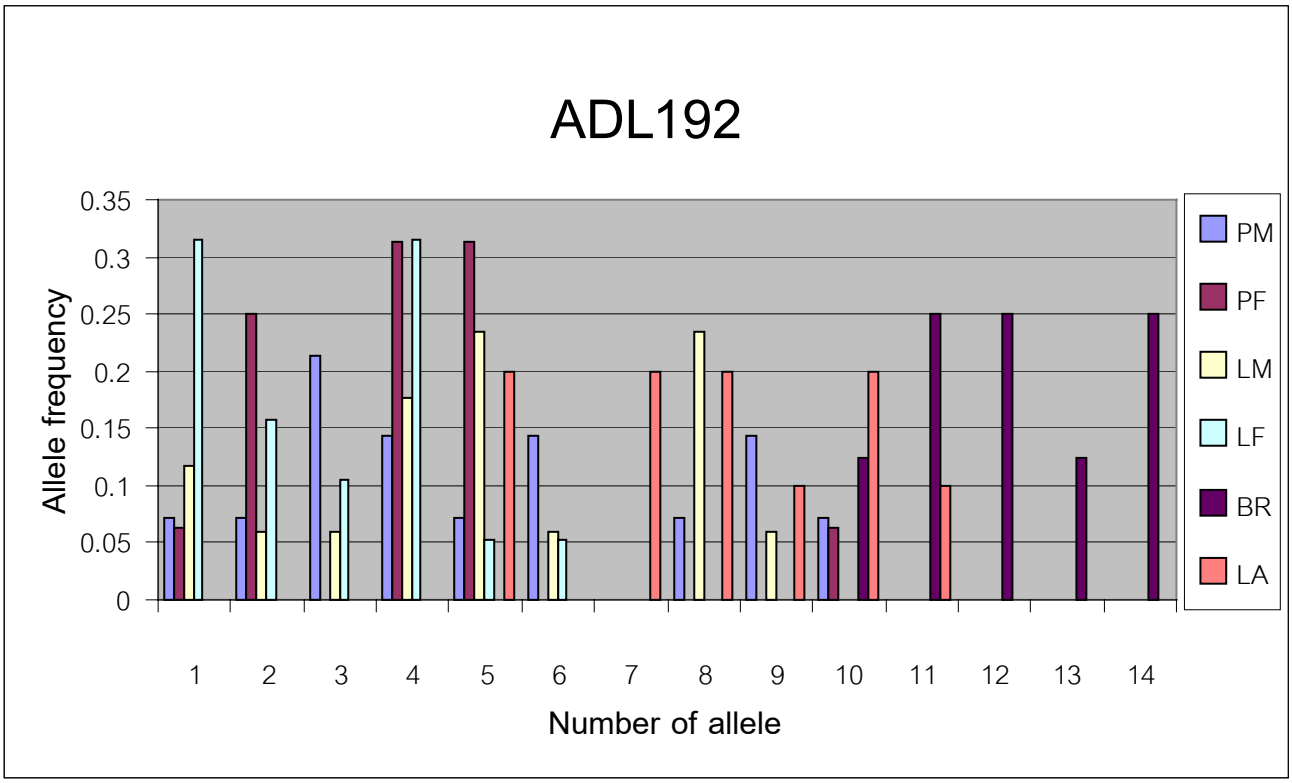
PM = male Thai native chicken variety praduhangdam

PF = female Thai native chicken variety praduhangdam

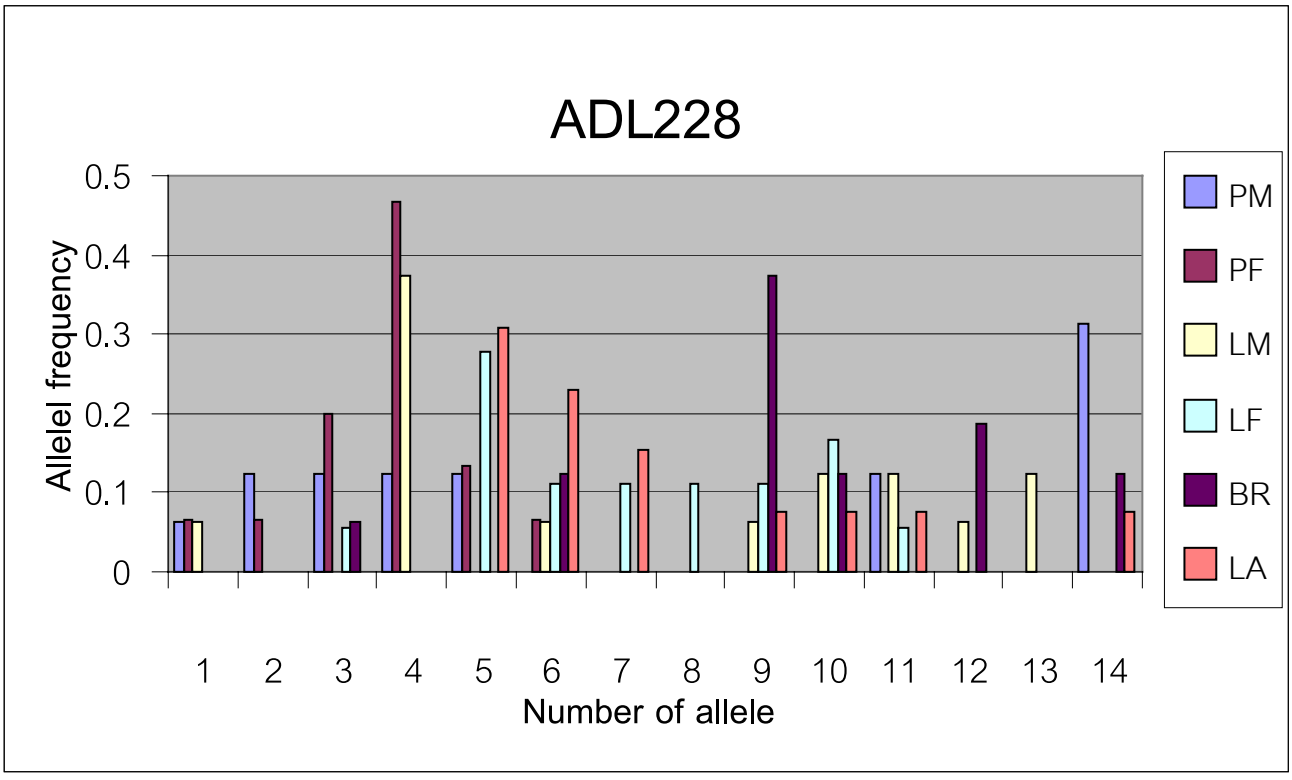
BR = two hybrid line broiler

LA = commercial layer

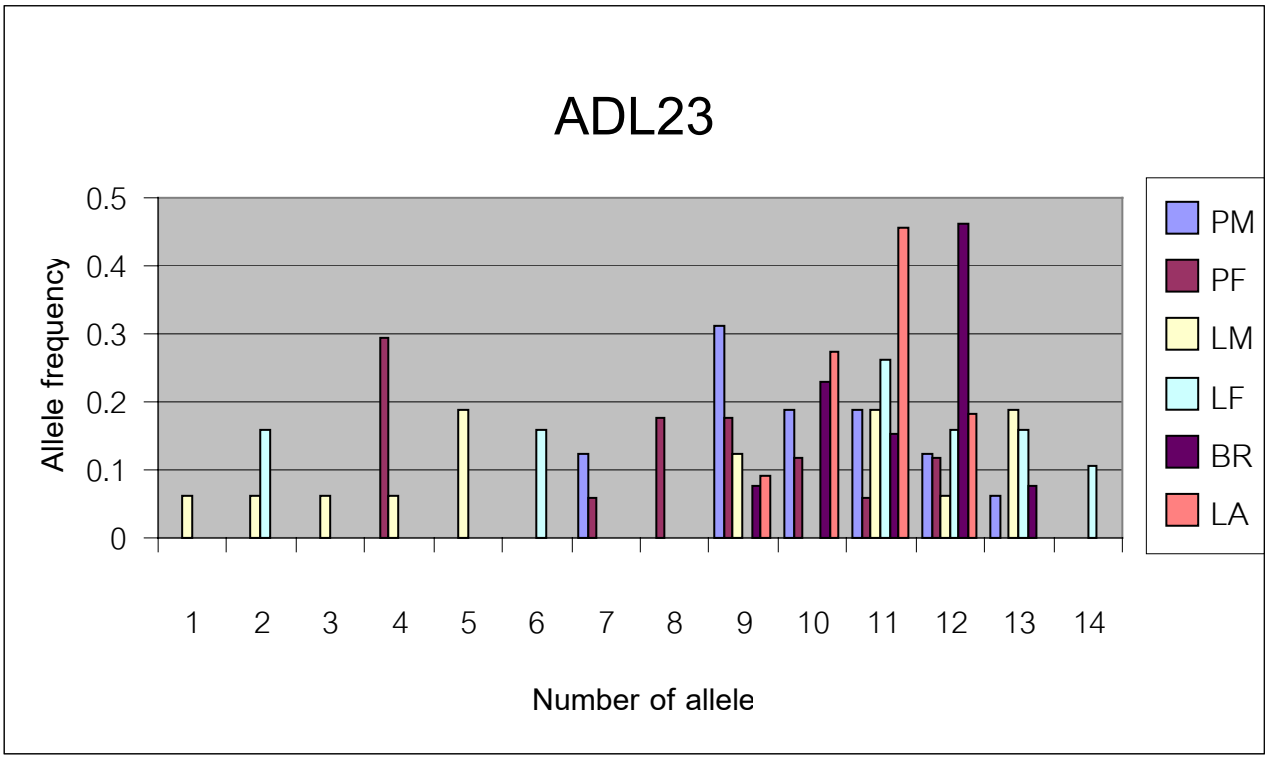
allele number	Locus ADL192					
	PM	PF	LM	LF	BR	LA
1	0.07142857	0.0625	0.117647	0.315789	0	0
2	0.07142857	0.25	0.058824	0.157895	0	0
3	0.21428571	0	0.058824	0.105263	0	0
4	0.14285714	0.3125	0.176471	0.315789	0	0
5	0.07142857	0.3125	0.235294	0.052632	0	0.2
6	0.14285714	0	0.058824	0.052632	0	0
7	0	0	0	0	0	0.2
8	0.07142857	0	0.235294	0	0	0.2
9	0.14285714	0	0.058824	0	0	0.1
10	0.07142857	0.0625	0	0	0.125	0.2
11	0	0	0	0	0.25	0.1
12	0	0	0	0	0.25	0
13	0	0	0	0	0.125	0
14	0	0	0	0	0.25	0



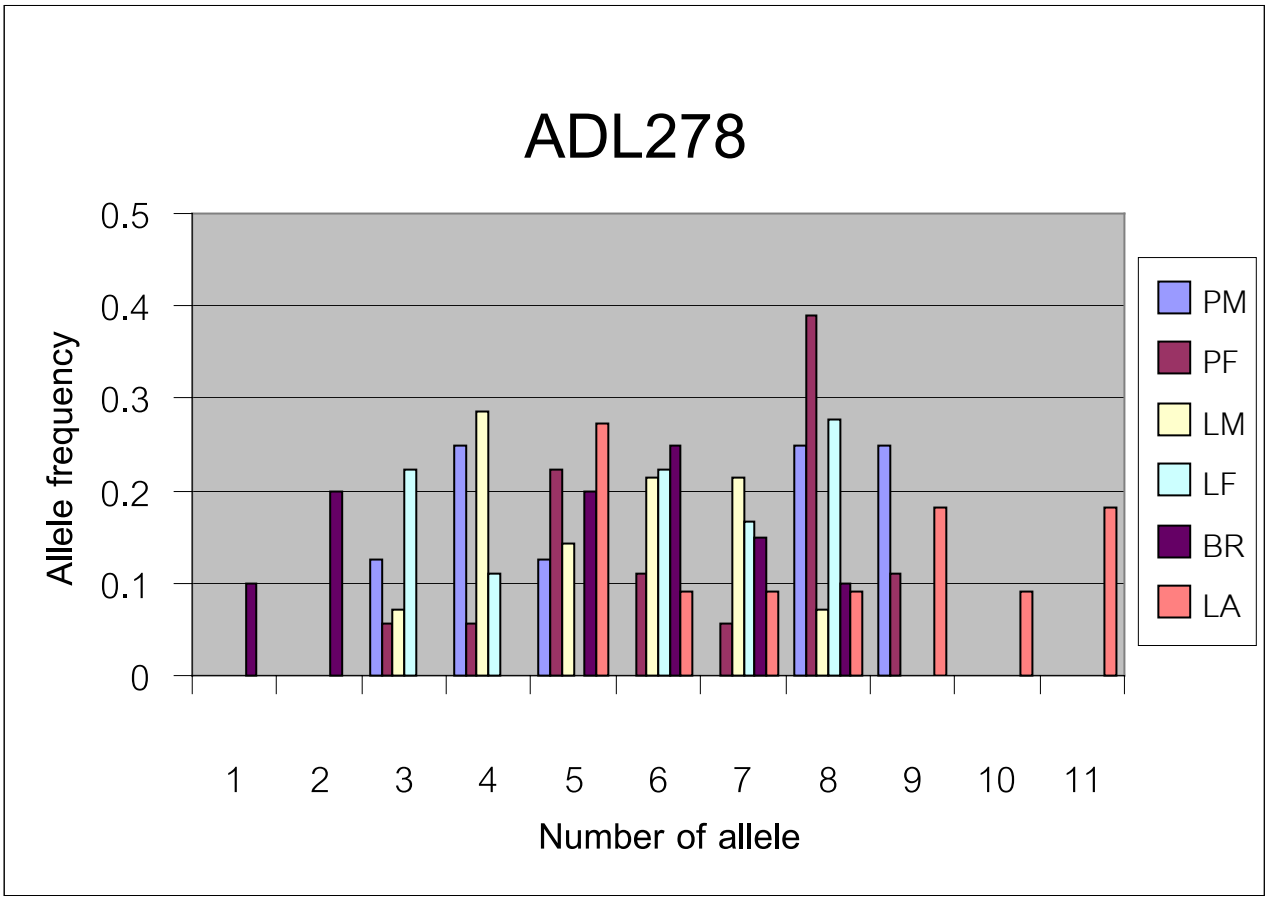
allele number	Locus ADL228					
	PM	PF	LM	LF	BR	LA
1	0.0625	0.066667	0.0625	0	0	0
2	0.125	0.066667	0	0	0	0
3	0.125	0.2	0	0.055556	0.0625	0
4	0.125	0.466667	0.375	0	0	0
5	0.125	0.133333	0	0.277778	0	0.307692
6	0	0.066667	0.0625	0.111111	0.125	0.230769
7	0	0	0	0.111111	0	0.153846
8	0	0	0	0.111111	0	0
9	0	0	0.0625	0.111111	0.375	0.076923
10	0	0	0.125	0.166667	0.125	0.076923
11	0.125	0	0.125	0.055556	0	0.076923
12	0	0	0.0625	0	0.1875	0
13	0	0	0.125	0	0	0
14	0.3125	0	0	0	0.125	0.076923



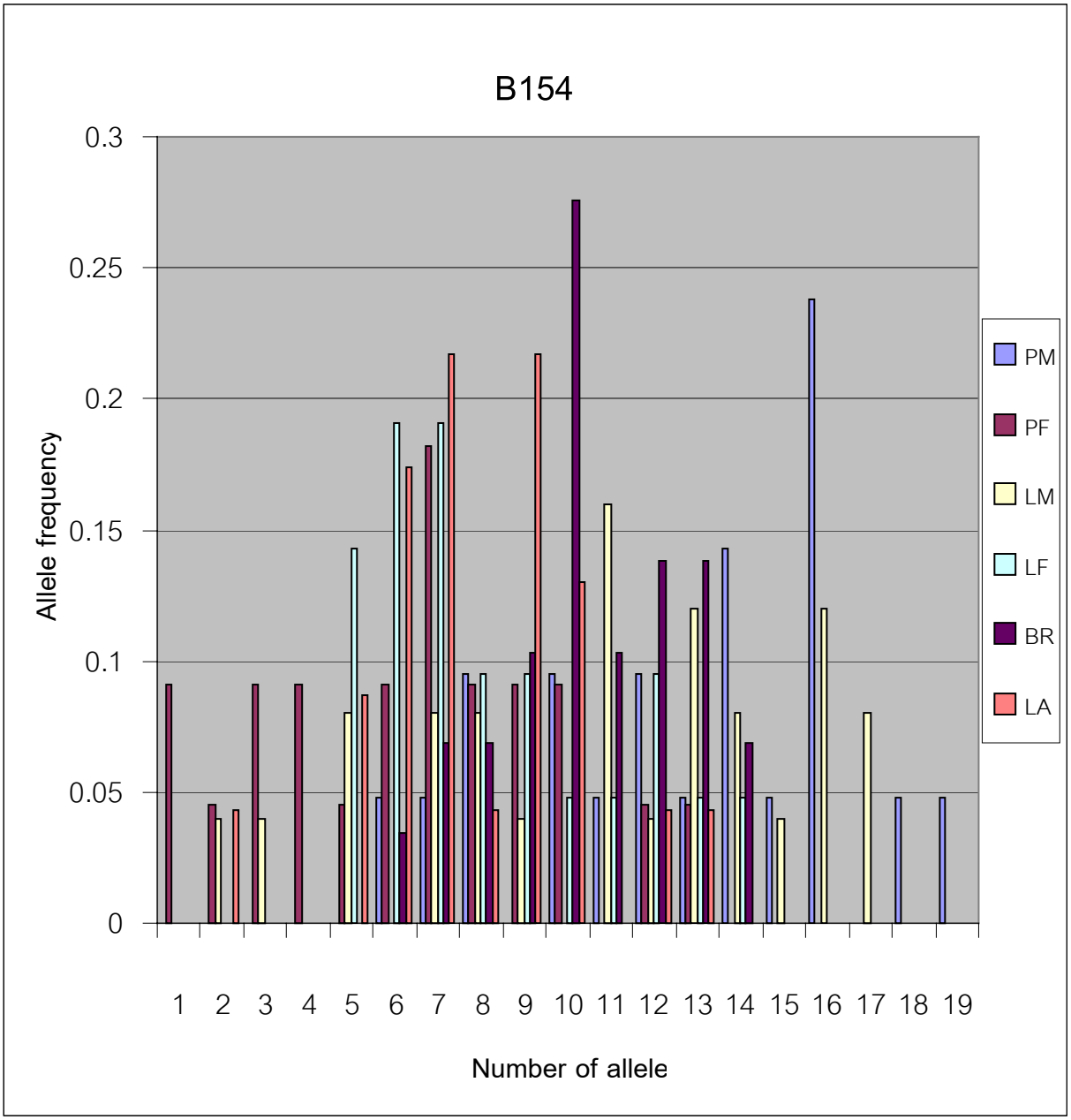
allele number	Locus ADL23					
	PM	PF	LM	LF	BR	LA
1	0	0	0.0625	0	0	0
2	0	0	0.0625	0.157895	0	0
3	0	0	0.0625	0	0	0
4	0	0.294118	0.0625	0	0	0
5	0	0	0.1875	0	0	0
6	0	0	0	0.157895	0	0
7	0.125	0.058824	0	0	0	0
8	0	0.176471	0	0	0	0
9	0.3125	0.176471	0.125	0	0.076923	0.090909
10	0.1875	0.117647	0	0	0.230769	0.272727
11	0.1875	0.058824	0.1875	0.263158	0.153846	0.454545
12	0.125	0.117647	0.0625	0.157895	0.461538	0.181818
13	0.0625	0	0.1875	0.157895	0.076923	0
14	0	0	0	0.105263	0	0



allele number	Locus ADL278					
	PM	PF	LM	LF	BR	LA
1	0	0	0	0	0.1	0
2	0	0	0	0	0.2	0
3	0.125	0.055556	0.071429	0.222222	0	0
4	0.25	0.055556	0.285714	0.111111	0	0
5	0.125	0.222222	0.142857	0	0.2	0.272727
6	0	0.111111	0.214286	0.222222	0.25	0.090909
7	0	0.055556	0.214286	0.166667	0.15	0.090909
8	0.25	0.388889	0.071429	0.277778	0.1	0.090909
9	0.25	0.111111	0	0	0	0.181818
10	0	0	0	0	0	0.090909
11	0	0	0	0	0	0.181818



allele number	Locus B154					
	PM	PF	LM	LF	BR	LA
1	0	0.090909	0	0	0	0
2	0	0.045455	0.04	0	0	0.043478
3	0	0.090909	0.04	0	0	0
4	0	0.090909	0	0	0	0
5	0	0.045455	0.08	0.142857	0	0.086957
6	0.04761905	0.090909	0	0.190476	0.034483	0.173913
7	0.04761905	0.181818	0.08	0.190476	0.068966	0.217391
8	0.0952381	0.090909	0.08	0.095238	0.068966	0.043478
9	0	0.090909	0.04	0.095238	0.103448	0.217391
10	0.0952381	0.090909	0	0.047619	0.275862	0.130435
11	0.04761905	0	0.16	0.047619	0.103448	0
12	0.0952381	0.045455	0.04	0.095238	0.137931	0.043478
13	0.04761905	0.045455	0.12	0.047619	0.137931	0.043478
14	0.14285714	0	0.08	0.047619	0.068966	0
15	0.04761905	0	0.04	0	0	0
16	0.23809524	0	0.12	0	0	0
17	0	0	0.08	0	0	0
18	0.04761905	0	0	0	0	0
19	0.04761905	0	0	0	0	0


B154
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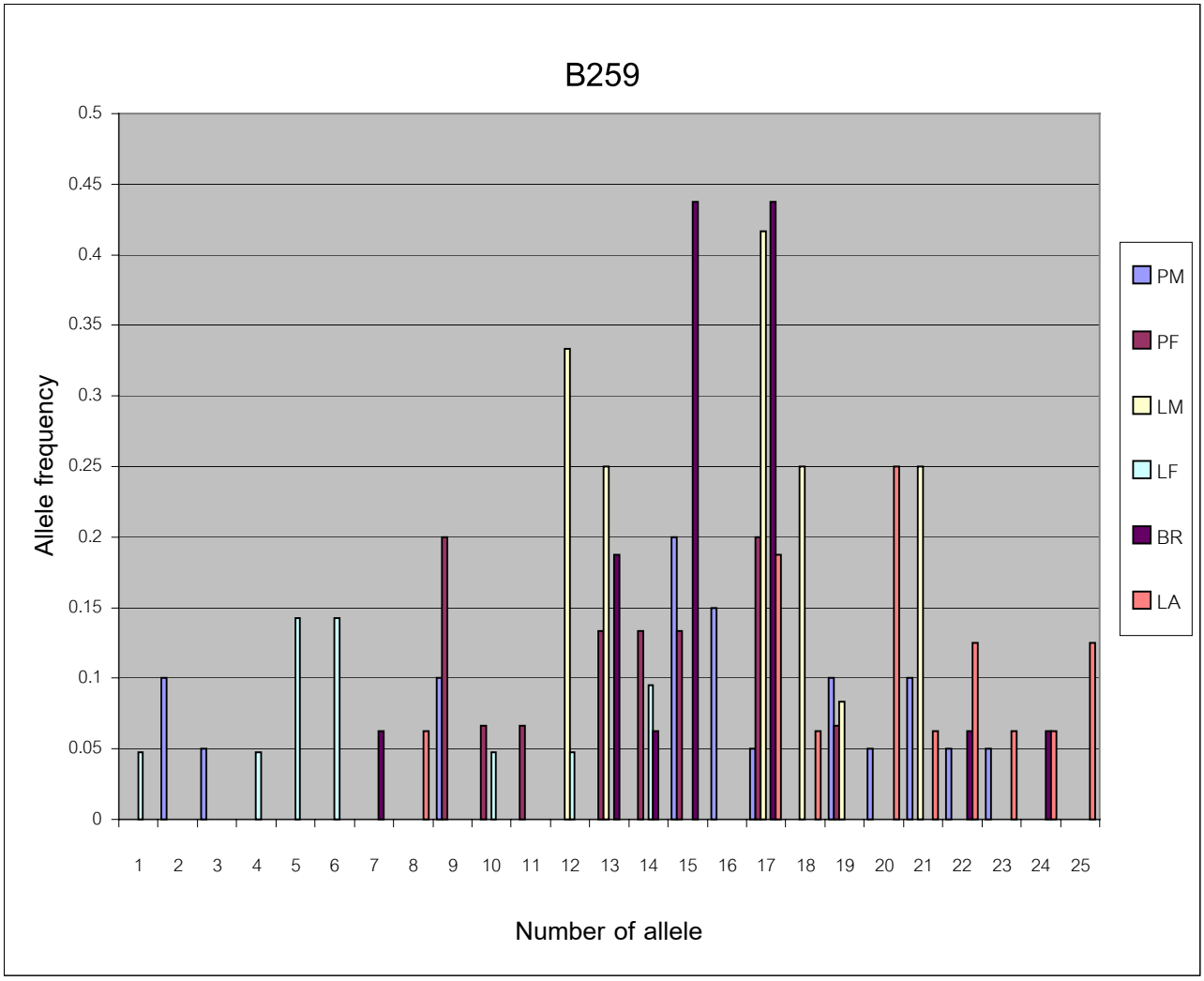
0.25
0.2
0.15
0.1
0.05
0
Allele frequency
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Number of allele
PM
PF
LM
LF
BR
LA

allele number	Locus B259					
	PM	PF	LM	LF	BR	LA
1	0	0	0	0.047619	0	0
2	0.1	0	0	0	0	0
3	0.05	0	0	0	0	0
4	0	0	0	0.047619	0	0
5	0	0	0	0.142857	0	0
6	0	0	0	0.142857	0	0
7	0	0	0	0	0.0625	0
8	0	0	0	0	0	0.0625
9	0.1	0.2	0	0	0	0
10	0	0.066667	0	0.047619	0	0
11	0	0.066667	0	0	0	0
12	0	0	0.333333	0.047619	0	0
13	0	0.133333	0.25	0	0.1875	0
14	0	0.133333	0	0.095238	0.0625	0
15	0.2	0.133333	0	0	0.4375	0
16	0.15	0	0	0	0	0
17	0.05	0.2	0.416667	0	0.4375	0.1875
18	0	0	0.25	0	0	0.0625
19	0.1	0.066667	0.083333	0	0	0
20	0.05	0	0	0	0	0.25
21	0.1	0	0.25	0	0	0.0625
22	0.05	0	0	0	0.0625	0.125
23	0.05	0	0	0	0	0.0625
24	0	0	0	0	0.0625	0.0625
25	0	0	0	0	0	0.125


B259
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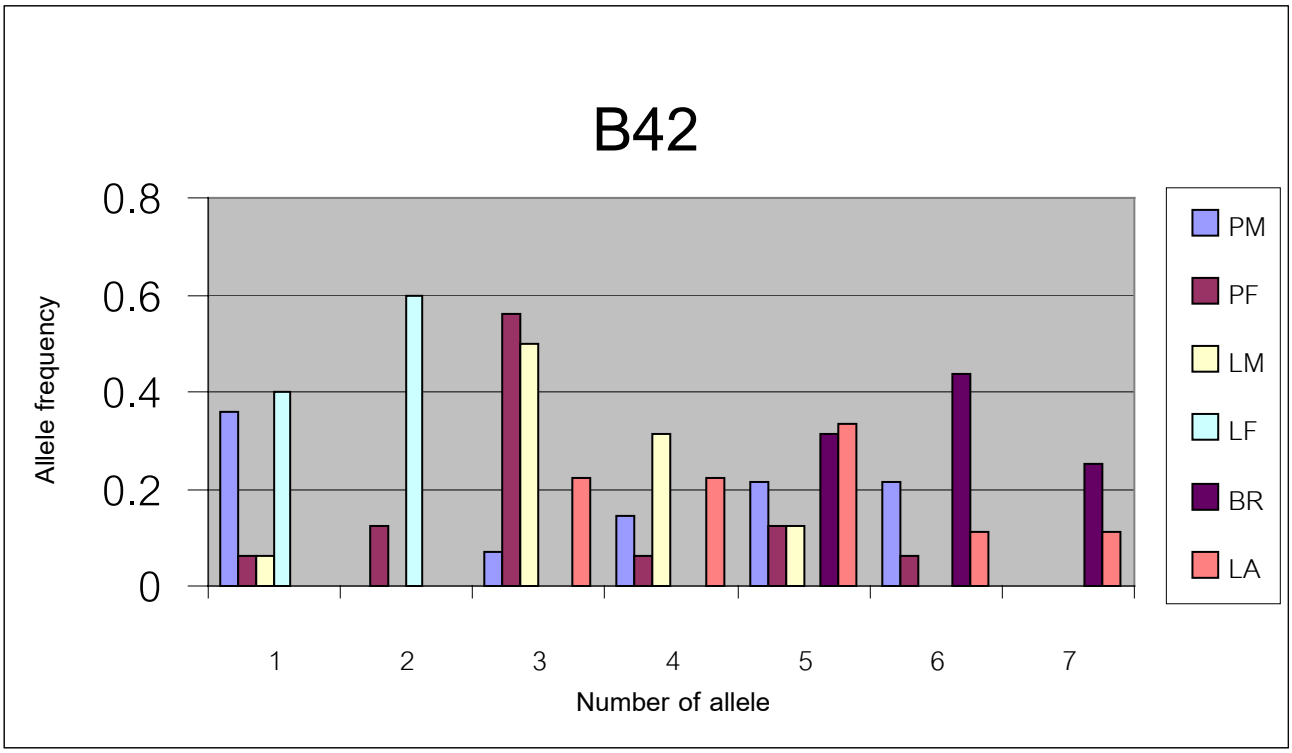
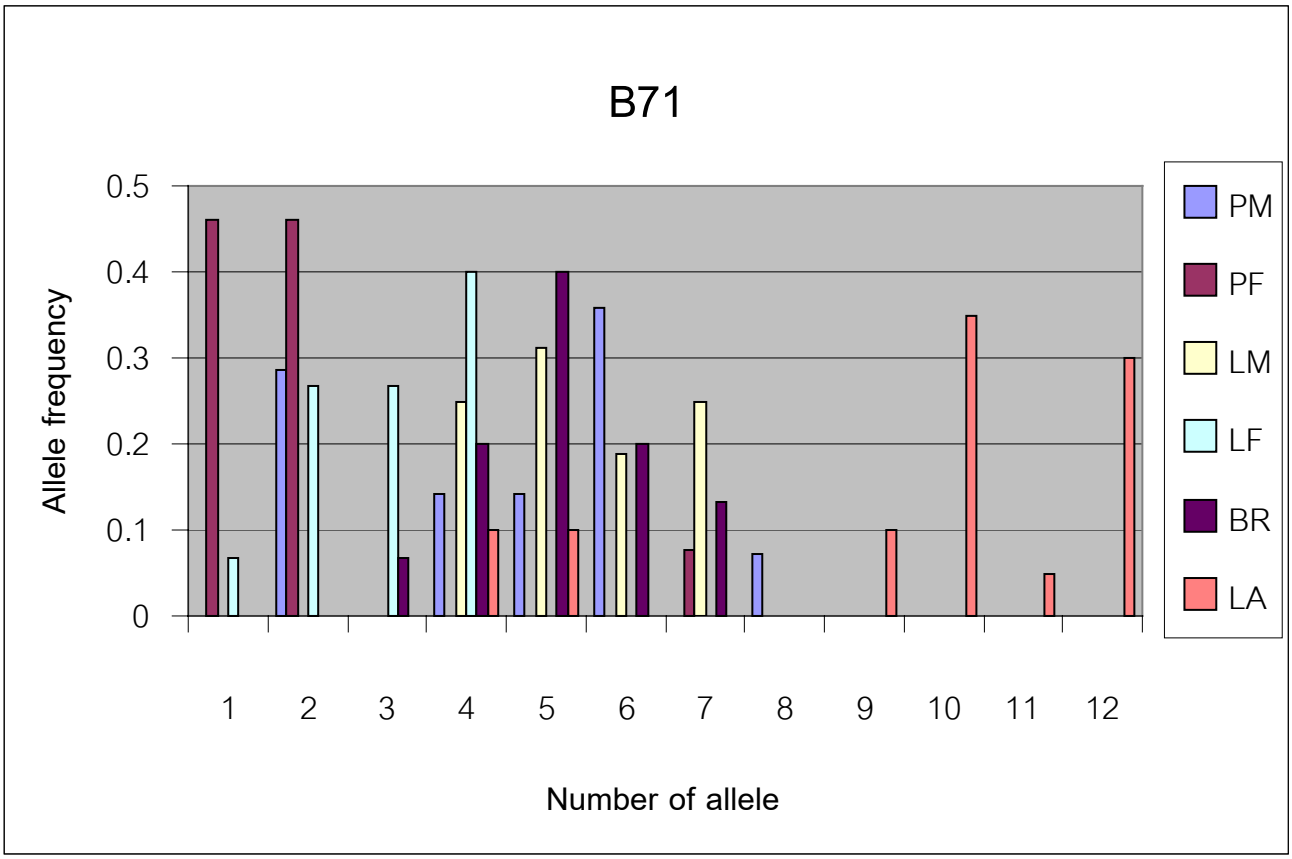
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Number of allele
PM
PF
LM
LF
BR
LA

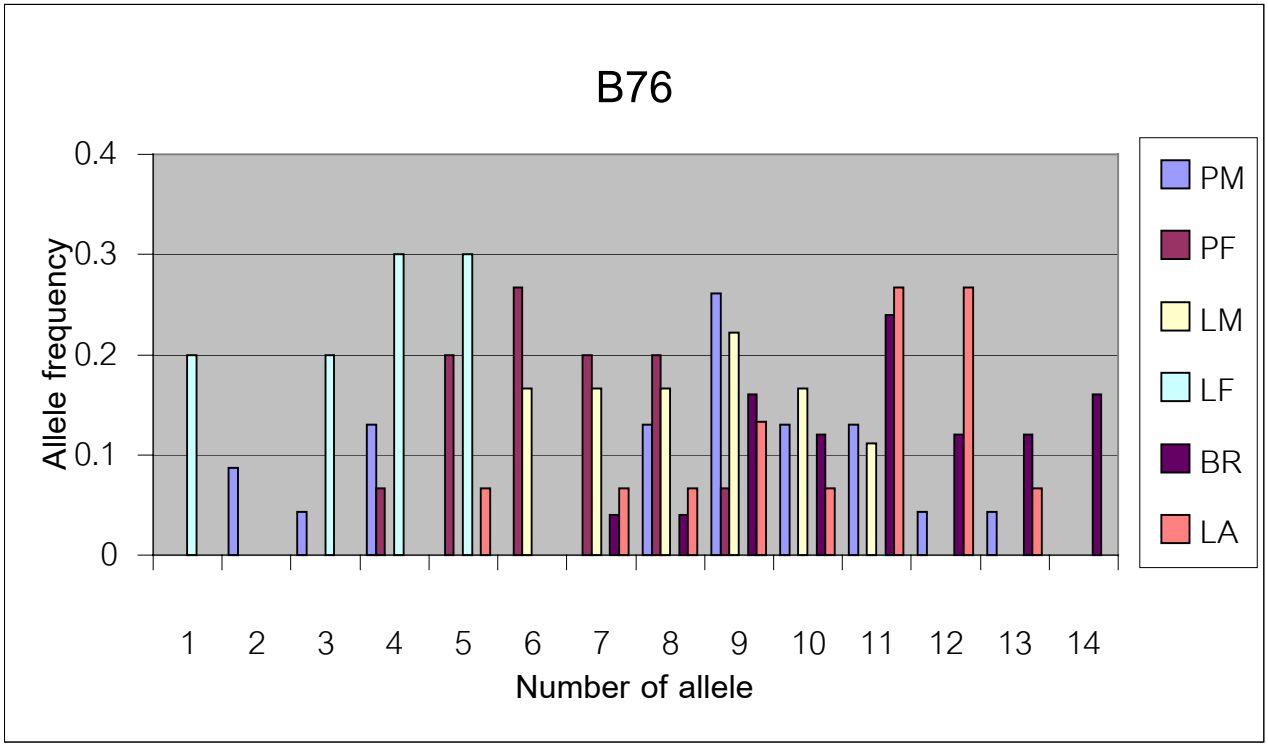
allele number	Locus B42					
	PM	PF	LM	LF	BR	LA
1	0.35714286	0.0625	0.0625	0.4	0	0
2	0	0.125	0	0.6	0	0
3	0.07142857	0.5625	0.5	0	0	0.222222
4	0.14285714	0.0625	0.3125	0	0	0.222222
5	0.21428571	0.125	0.125	0	0.3125	0.333333
6	0.21428571	0.0625	0	0	0.4375	0.111111
7	0	0	0	0	0.25	0.111111



allele number	Locus B71					
	PM	PF	LM	LF	BR	LA
1	0	0.461538	0	0.066667	0	0
2	0.28571429	0.461538	0	0.266667	0	0
3	0	0	0	0.266667	0.066667	0
4	0.14285714	0	0.25	0.4	0.2	0.1
5	0.14285714	0	0.3125	0	0.4	0.1
6	0.35714286	0	0.1875	0	0.2	0
7	0	0.076923	0.25	0	0.133333	0
8	0.07142857	0	0	0	0	0
9	0	0	0	0	0	0.1
10	0	0	0	0	0	0.35
11	0	0	0	0	0	0.05
12	0	0	0	0	0	0.3



allele number	Locus B76					
	PM	PF	LM	LF	BR	LA
1	0	0	0	0.2	0	0
2	0.08695652	0	0	0	0	0
3	0.04347826	0	0	0.2	0	0
4	0.13043478	0.066667	0	0.3	0	0
5	0	0.2	0	0.3	0	0.066667
6	0	0.266667	0.166667	0	0	0
7	0	0.2	0.166667	0	0.04	0.066667
8	0.13043478	0.2	0.166667	0	0.04	0.066667
9	0.26086957	0.066667	0.222222	0	0.16	0.133333
10	0.13043478	0	0.166667	0	0.12	0.066667
11	0.13043478	0	0.111111	0	0.24	0.266667
12	0.04347826	0	0	0	0.12	0.266667
13	0.04347826	0	0	0	0.12	0.066667
14	0	0	0	0	0.16	0



allele number	Locus B85					
	PM	PF	LM	LF	BR	LA
1	0	0.142857	0.058824	0	0	0
2	0	0	0.235294	0.315789	0	0
3	0	0.142857	0.117647	0.315789	0	0
4	0	0.071429	0.058824	0.105263	0.0625	0
5	0.15789474	0.214286	0	0.157895	0.0625	0
6	0.15789474	0.142857	0.058824	0.105263	0.1875	0.285714
7	0	0.142857	0	0	0.5625	0.285714
8	0	0.071429	0	0	0	0.357143
9	0.05263158	0.071429	0.058824	0	0.125	0
10	0.21052632	0	0.235294	0	0	0.071429
11	0.15789474	0	0.117647	0	0	0
12	0.05263158	0	0	0	0	0
13	0.10526316	0	0	0	0	0
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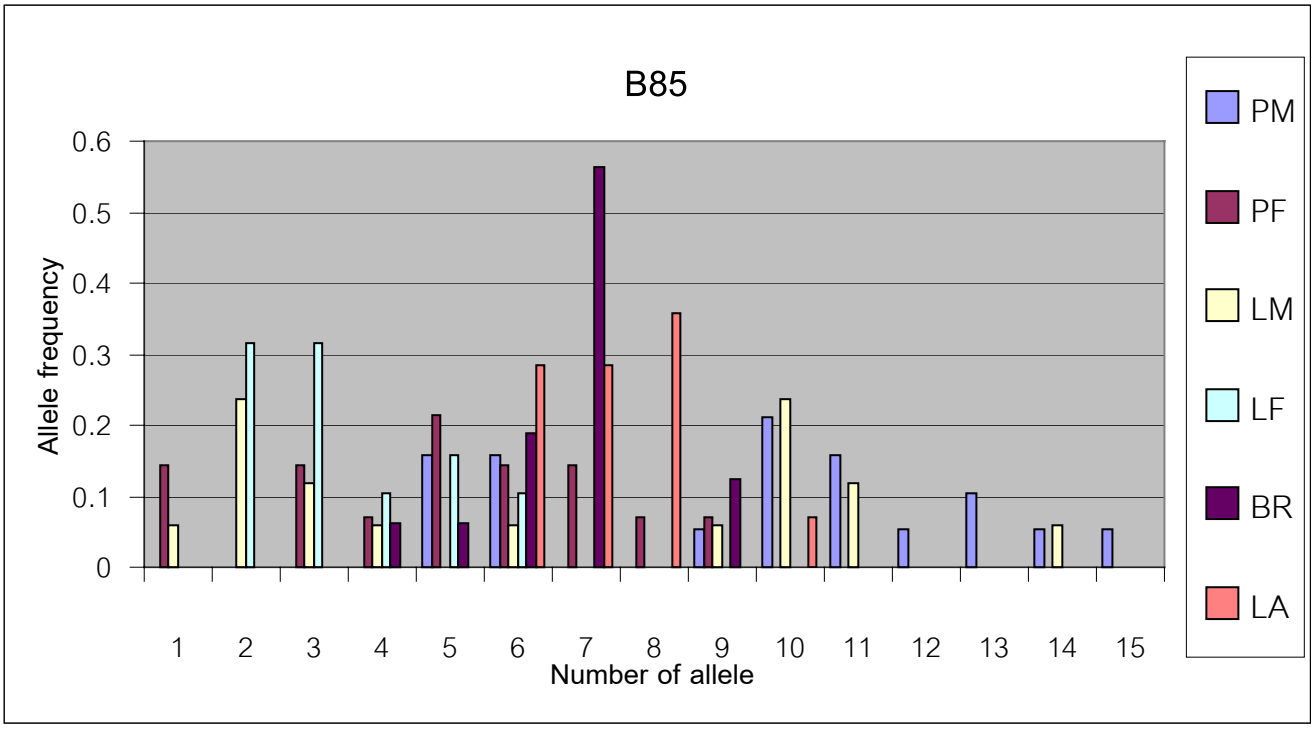
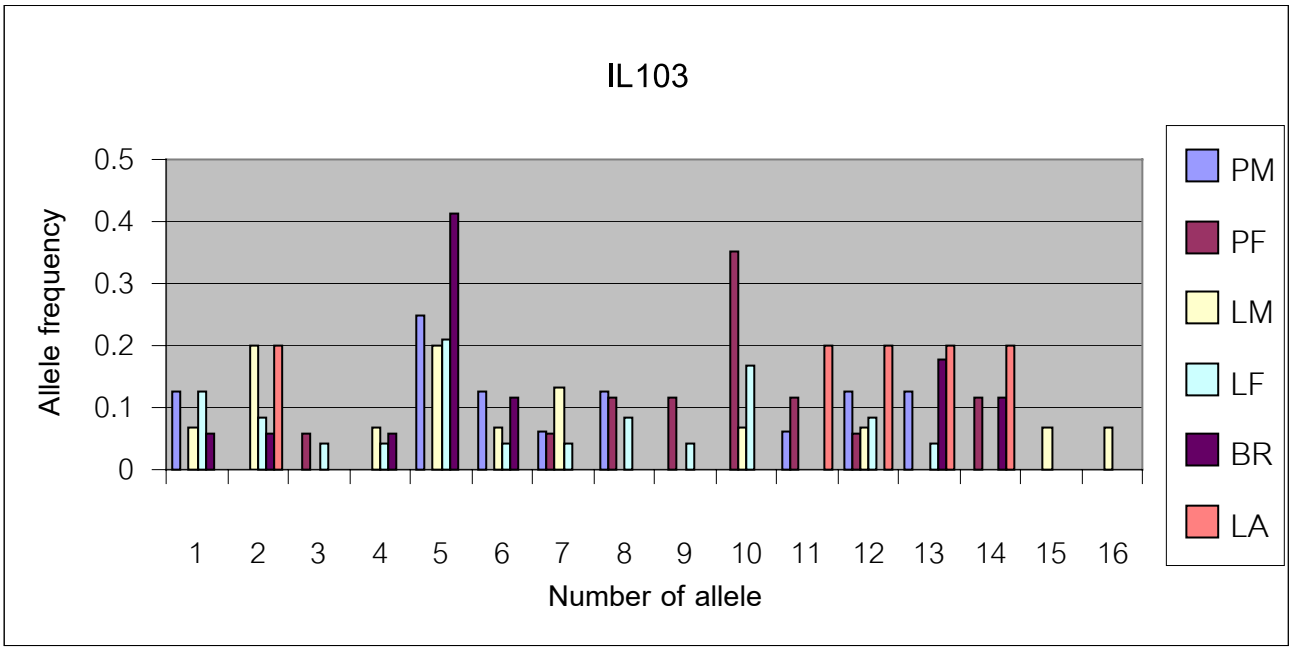
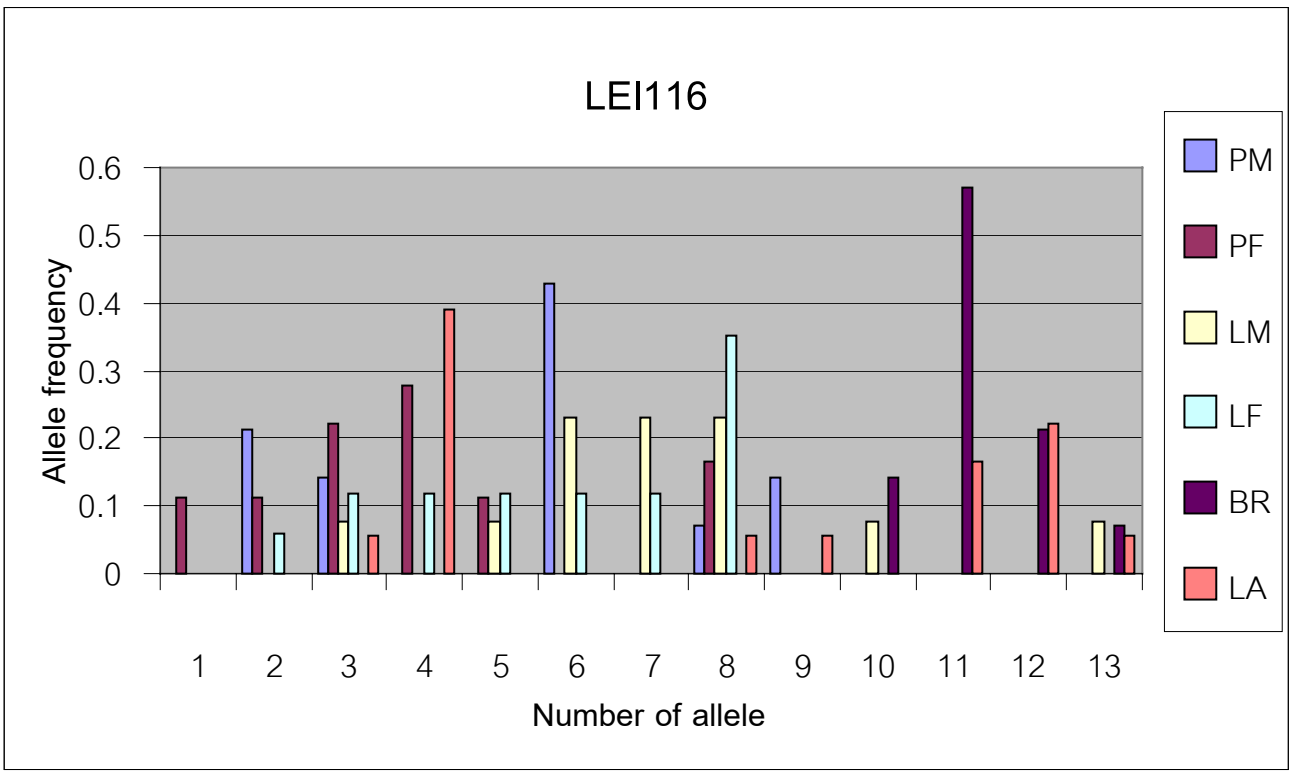
15	0.05263158	0	0	0	0	0



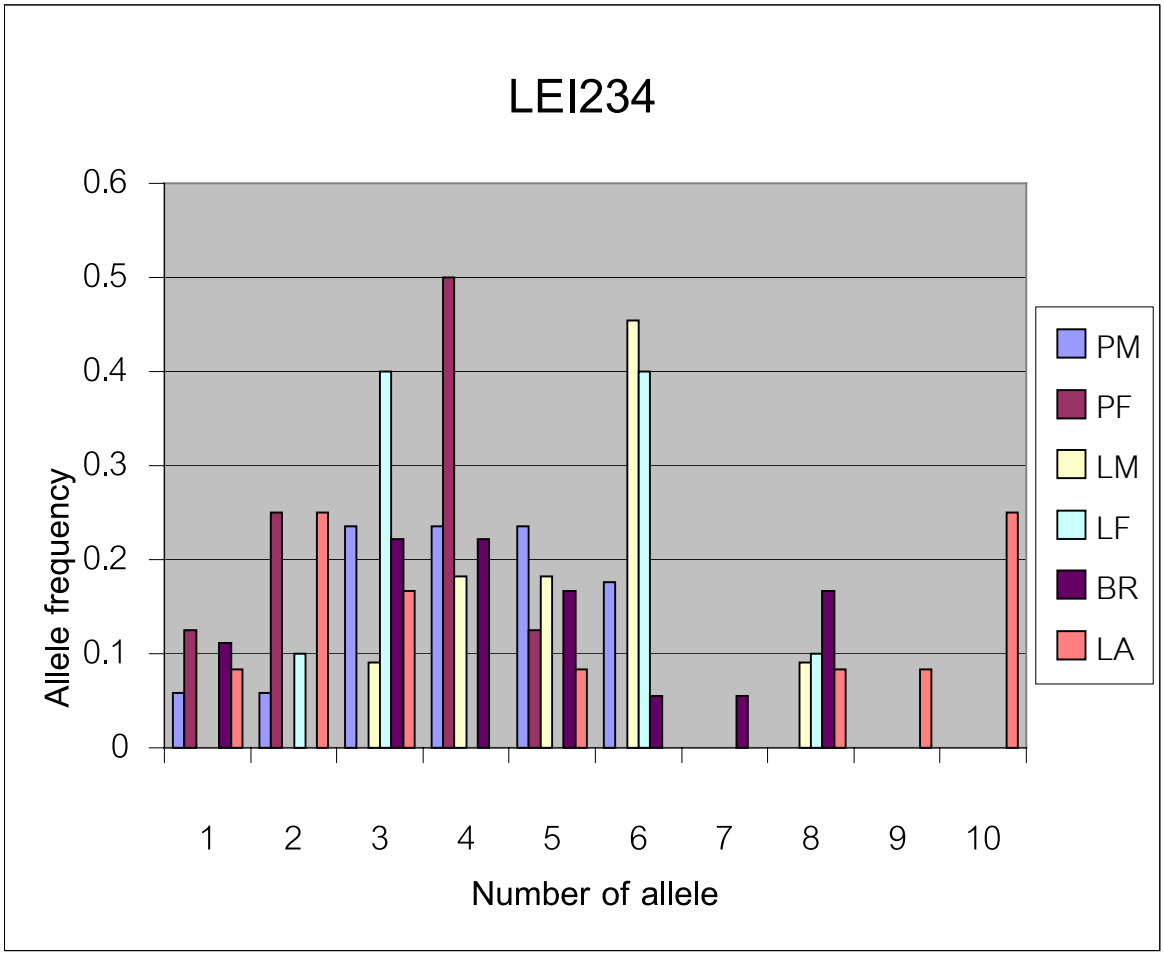
allele number	Locus IL103					
	PM	PF	LM	LF	BR	LA
1	0.125	0	0.066667	0.125	0.058824	0
2	0	0	0.2	0.083333	0.058824	0.2
3	0	0.058824	0	0.041667	0	0
4	0	0	0.066667	0.041667	0.058824	0
5	0.25	0	0.2	0.208333	0.411765	0
6	0.125	0	0.066667	0.041667	0.117647	0
7	0.0625	0.058824	0.133333	0.041667	0	0
8	0.125	0.117647	0	0.083333	0	0
9	0	0.117647	0	0.041667	0	0
10	0	0.352941	0.066667	0.166667	0	0
11	0.0625	0.117647	0	0	0	0.2
12	0.125	0.058824	0.066667	0.083333	0	0.2
13	0.125	0	0	0.041667	0.176471	0.2
14	0	0.117647	0	0	0.117647	0.2
15	0	0	0.066667	0	0	0
16	0	0	0.066667	0	0	0



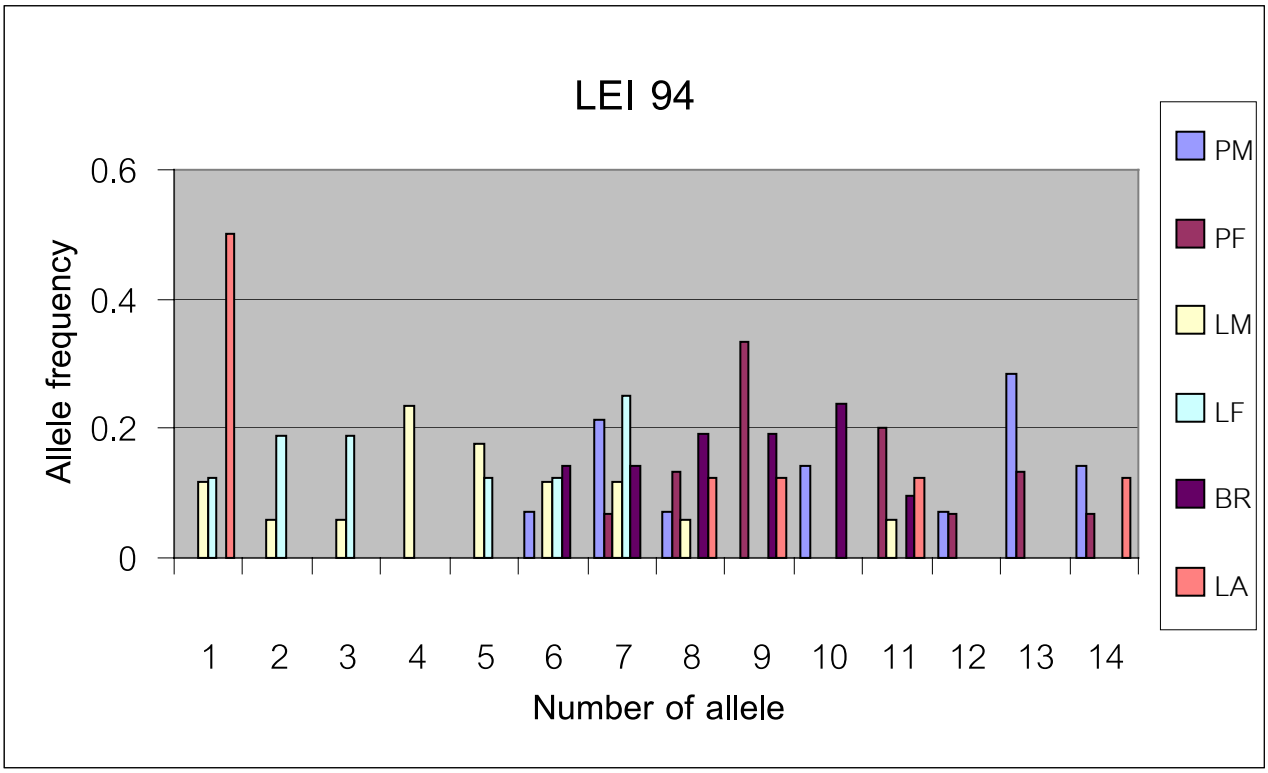
allele number	Locus LEI116					
	PM	PF	LM	LF	BR	LA
1	0	0.111111	0	0	0	0
2	0.21428571	0.111111	0	0.058824	0	0
3	0.14285714	0.222222	0.076923	0.117647	0	0.055556
4	0	0.277778	0	0.117647	0	0.388889
5	0	0.111111	0.076923	0.117647	0	0
6	0.42857143	0	0.230769	0.117647	0	0
7	0	0	0.230769	0.117647	0	0
8	0.07142857	0.166667	0.230769	0.352941	0	0.055556
9	0.14285714	0	0	0	0	0.055556
10	0	0	0.076923	0	0.142857	0
11	0	0	0	0	0.571429	0.166667
12	0	0	0	0	0.214286	0.222222
13	0	0	0.076923	0	0.071429	0.055556



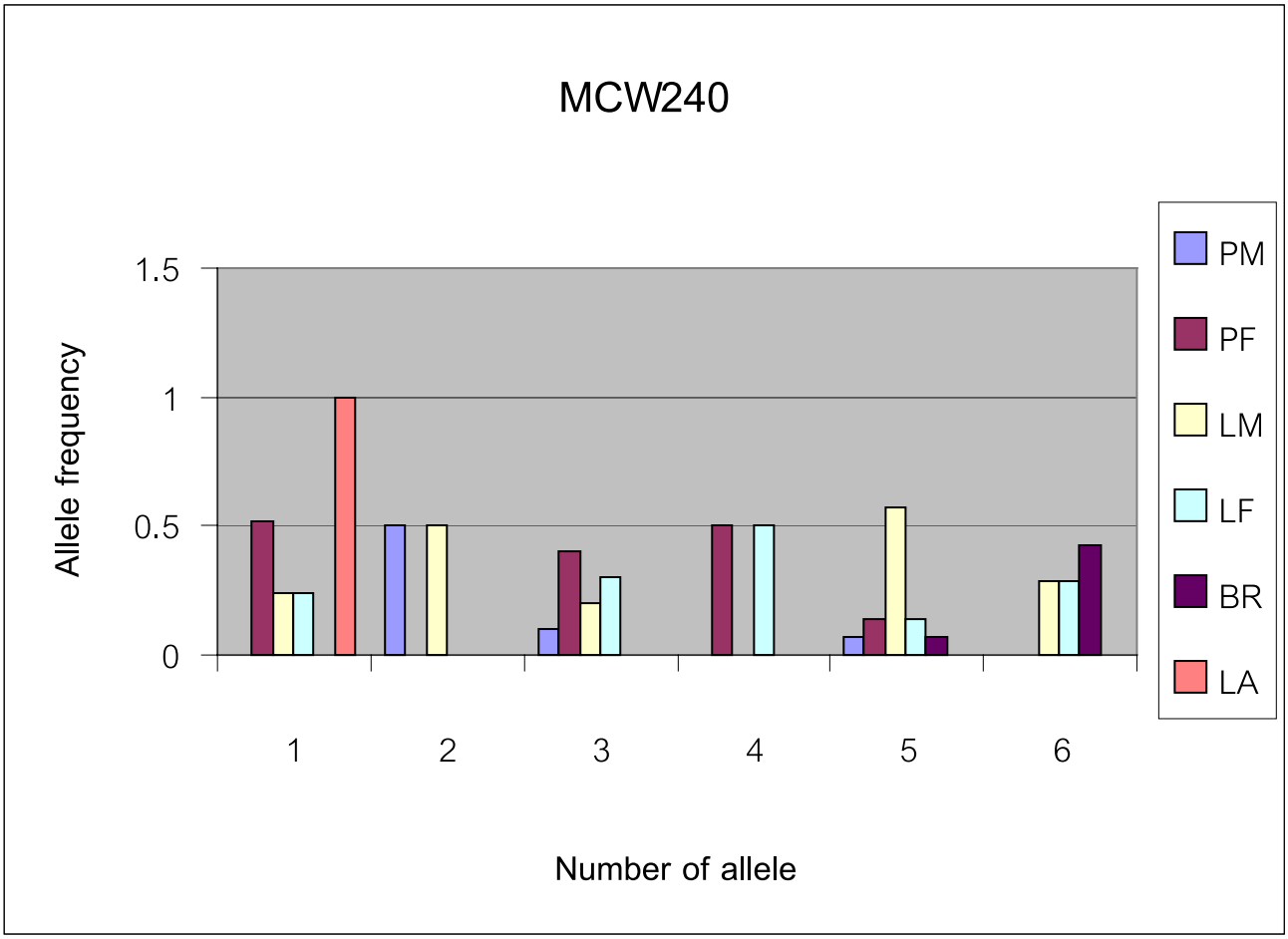
allele number	Locus LEI234					
	PM	PF	LM	LF	BR	LA
1	0.05882353	0.125	0	0	0.111111	0.083333
2	0.05882353	0.25	0	0.1	0	0.25
3	0.23529412	0	0.090909	0.4	0.222222	0.166667
4	0.23529412	0.5	0.181818	0	0.222222	0
5	0.23529412	0.125	0.181818	0	0.166667	0.083333
6	0.17647059	0	0.454545	0.4	0.055556	0
7	0	0	0	0	0.055556	0
8	0	0	0.090909	0.1	0.166667	0.083333
9	0	0	0	0	0	0.083333
10	0	0	0	0	0	0.25



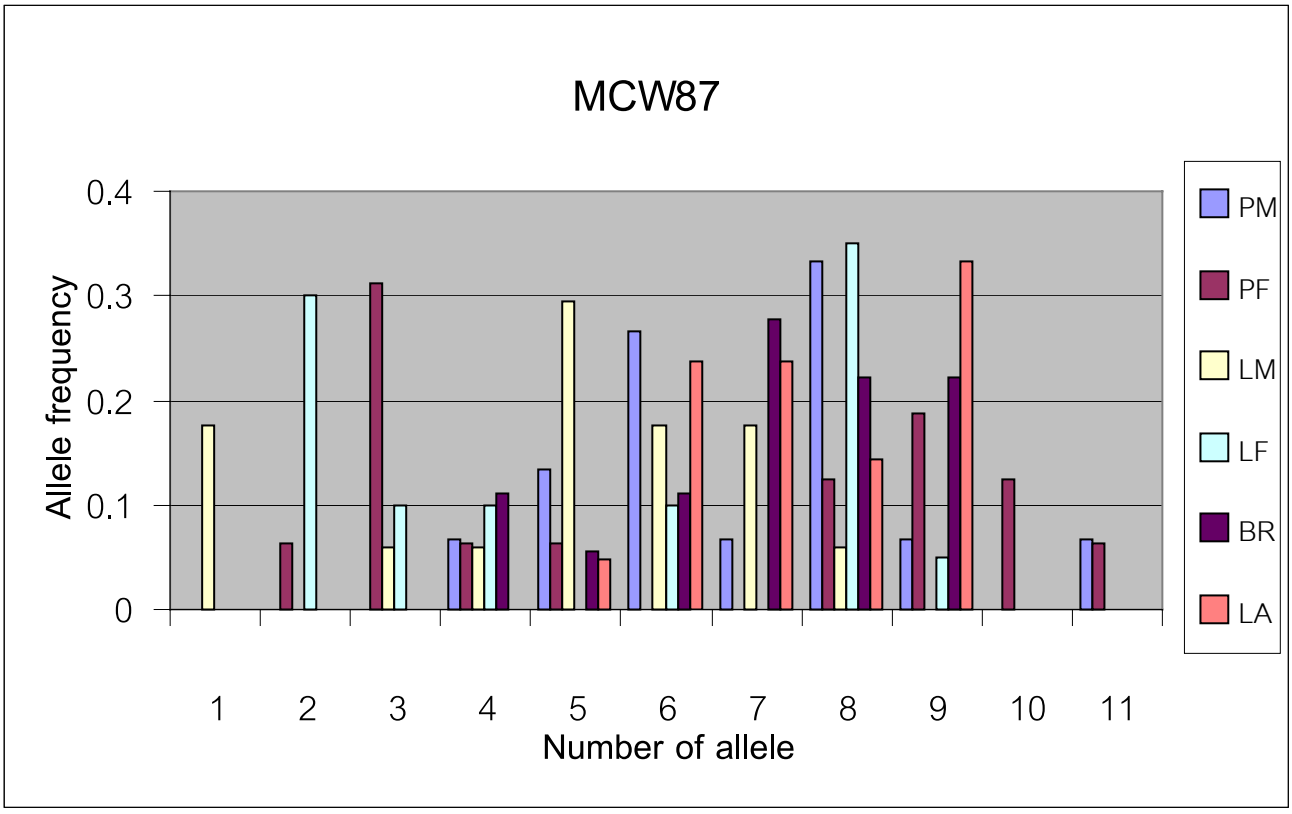
allele number	Locus LEI94					
	PM	PF	LM	LF	BR	LA
1	0	0	0.117647	0.125	0	0.5
2	0	0	0.058824	0.1875	0	0
3	0	0	0.058824	0.1875	0	0
4	0	0	0.235294	0	0	0
5	0	0	0.176471	0.125	0	0
6	0.071429	0	0.117647	0.125	0.142857	0
7	0.214286	0.066667	0.117647	0.25	0.142857	0
8	0.071429	0.133333	0.058824	0	0.190476	0.125
9	0	0.333333	0	0	0.190476	0.125
10	0.142857	0	0	0	0.238095	0
11	0	0.2	0.058824	0	0.095238	0.125
12	0.071429	0.066667	0	0	0	0
13	0.285714	0.133333	0	0	0	0
14	0.142857	0.066667	0	0	0	0.125



allele number	Locus MCW240					
	PM	PF	LM	LF	BR	LA
1	0	0.5	0.1	0	0.071429	0
2	0.517241	0	0.4	0.5	0.142857	0
3	0.241379	0.5	0.2	0	0.571429	0.285714
4	0.241379	0	0.3	0.5	0.142857	0.285714
5	0	0	0	0	0.071429	0.428571



allele number	Locus MCW87					
	PM	PF	LM	LF	BR	LA
1	0	0	0.176471	0	0	0
2	0	0.0625	0	0.3	0	0
3	0	0.3125	0.058824	0.1	0	0
4	0.066667	0.0625	0.058824	0.1	0.111111	0
5	0.133333	0.0625	0.294118	0	0.055556	0.047619
6	0.266667	0	0.176471	0.1	0.111111	0.238095
7	0.066667	0	0.176471	0	0.277778	0.238095
8	0.333333	0.125	0.058824	0.35	0.222222	0.142857
9	0.066667	0.1875	0	0.05	0.222222	0.333333
10	0	0.125	0	0	0	0
11	0.066667	0.0625	0	0	0	0



APPENDIX II

Morphological Information

The table show morphological information of all Thai native chicken in the experiment

The measured data contain wing length (WL), head length (HL), beck length (BL), leg length (LL), neck length (NL), back length (BL), chest round (CR) and tarsometatarsus length (TL).

CB; Chonburi , NP; Nakhon Prathom, PL; Phisanuloke.

Name	Exp Code	source	Sex	WL	HL	BL	LL	NL	BAL	CR	TL	COLOR	W
L26983M	LM1	CB	M	27	10	3	22	27	25	41	13	9	3.96
L41528M	LM2	CB	M	25	8	2.5	21	26	24.5	42	12	8	4.14
L2M	LM3	CB	M	25	9.5	3	21	24	26	40	13	7	3.92
L55606M	LM4	CB	M	23	9	3	19	24	22	39	11	9	3.1
L55454M	LM5	CB	M	25	9	3	22	25	25	39	12	8	3.58
L1M	LM6	CB	M	23	9	3	22	25	26	46	13	9	4.7
L58737M	LM7	CB	M	25	9	3	21	25	26	40	12	8	3.75
LP1M	LM8	PL	M	21	10.5	3	16.5	25	26.5	38	14	9	2.5
LP2M	LM9	PL	M	24	8.5	3.2	16.5	26.5	28	38.5	11	9	3
LP3M	LM10	PL	M	23.5	11	3.5	15.5	22	28	38.5	10.5	10	2.8
LP4M	LM11	PL	M	22.5	11	3.5	16	27	25	37.5	10	9	3
LP5M	LM12	PL	M	23	11	3.5	15.5	28.5	25	37.5	11	9	2.8
LN1M	LM13	NP	M	22	10	4	20	26.5	25.5	40	10.5	7	4.2
LN2M	LM14	NP	M	16.5	10	3	17	24	24	40	10.5	7	3.6
LN3M	LM15	NP	M	20	9	4.5	19	25	24	39	10.5	7	3.9
L46850F	LF1	CB	F	22	8	3	19	22	22	37	10	8	2.8
L26506F	LF2	CB	F	21	8	3	17	22	23	35	10	7	2.35
L55612F	LF3	CB	F	21	9	3	17	22	21.5	37	10	7	2.79
L19949F	LF4	CB	F	22	8.5	3	18	22	22	35	9	7	2.65
L48354F	LF5	CB	F	22	8.5	3	17	23	23	36	11	7	2.41

Name	Exp Code	source	Sex	WL	HL	BL	LL	NL	BAL	CR	TL	COLOR	W
L27266F	LF6	CB	F	21	8	3	18	22	22	34	10	7	2.28
L26621F	LF7	CB	F	22	8	3	17	22	21.5	36	10	8	2.46
L25927F	LF8	CB	F	21	8.5	3	17	22	23	35	10	7	2.35
L42247F	LF9	CB	F	21	8	3	17	23	22	36	10	7	2.31
L9922F	LF10	CB	F	20	8	3	16	21	20	38	9	9	2.3
L37218F	LF11	CB	F	22	8	3	17	22	20	34	10	7	2.12
L11164F	LF12	CB	F	22	9	3	18	23	21	16	10	8	2.71
L40053F	LF13	CB	F	22	8	3	18	23	22	36	10	7	2.23
L49922F	LF14	CB	F	23	8	3	18	22	23	35	11	7	2.51
L48374F	LF15	CB	F	20	8	2.5	16	21	22	35	9	7	2.38
P58751M	PM1	CB	M	26	9	3.5	22	26	25.5	41	13	9	3.97
P54766M	PM2	CB	M	23	9	3	20	24	23.5	38	12	10	3.18
P49776M	PM3	CB	M	25	9	3	21	25	24	40	13	9	3.6
P50008M	PM4	CB	M	25	9	3	22	25	26.5	43	13	9	4.4
P40470M	PM5	CB	M	26	9	3	22	25	25	39	13	10	3.68
PN1M	PM6	NP	M	20.5	7.5	3.5	21	25.5	26	40.5	12.5	10	4.2
PN2M	PM7	NP	M	20.5	8	3	20	22	24	40	9.5	9	3.4
PN3M	PM8	NP	M	26	10	3	22	23	28	43.5	10	10	4.2
PN4M	PM9	NP	M	22.5	8.5	3.5	20.5	24	26	41.5	10	10	3.8
PN5M	PM10	NP	M	20	10.5	3	19.5	20.5	24	40	9.5	8	3.7
PN6M	PM11	NP	M	22	9	3	19	20	23.5	37.5	9.5	8	3.3
PN7M	PM12	NP	M	22.5	10	3	19.5	22	25	39	9	8	3.8
PN8M	PM13	NP	M	22.5	10	3	19.5	19	25	38	9.5	7	3.5
PN9M	PM14	NP	M	20	9.5	3.5	20.5	21.5	22.5	40.5	10.5	8	4.1
PN10M	PM15	NP	M	22	10.5	3.5	21	20	23	45.5	10	8	4.1
P37468F	PF1	CB	F	23	8	3	18	24	24	39	11	8	2.98
P49372F	PF2	CB	F	22	8	3	17	22	21	34	10	10	2.09
P27351F	PF3	CB	F	22	8	3	17	21	22	38	10	10	2.61

P82888F	PF4	CB	F	21	8	3	17	22	21	34	10	10	2.05
Name	Exp Code	source	Sex	WL	HL	BL	LL	NL	BAL	CR	TL	COLOR	W
P44418F	PF5	CB	F	20	8	3	17	21	22	33	9	9	1.93
P53905F	PF6	CB	F	21	8	3	17	22	22	34	10	9	2.11
P47632F	PF7	CB	F	22	8.5	3.5	18	22	22	35	10	10	2.08
P58809F	PF8	CB	F	21	8	3	17	22	21	35	10	10	2.26
P52703F	PF9	CB	F	21	8	3	18	22	22	34	10	10	2.19
P53903F	PF10	CB	F	21	8	3	17	22	22	35	10	10	2.21
P25639F	PF11	CB	F	22	8	3	18	22	22	38	10	10	2.83
P40609F	PF12	CB	F	22	8.5	3	18	23	22	36	10	9	2.55
PN1F	PF13	NP	F	21	7.5	2.5	18	23.5	23	37.5	9.5	9	2.35
PN2F	PF14	NP	F	18.5	6	2.5	18	25.5	24	37	8	10	2.8
PN3F	PF15	NP	F	16	8	3	17	21	25	34	8	10	2.3

APPENDIX III

Color Appearance Information

The table show color appearance information of all Thai native chicken in the experiment

The highest score of the color in the experiment is 10.

CB; Chonburi , NP; Nakhon Prathom, PL; Phisanuloke.

Name	Exp Code	source	Sex	COLOR	Information
L26983M	LM1	CB	M	9	Dark on the beak
L41528M	LM2	CB	M	8	Dark on the beak, no white feather at the tail
L2M	LM3	CB	M	7	No white on the wing and head, Dark on the beak and tarsal
L55606M	LM4	CB	M	9	Dark on the beak
L55454M	LM5	CB	M	8	White on the head, Dark on the beak and tarsal
L1M	LM6	CB	M	9	No white on the head, Dark on the beak
L58737M	LM7	CB	M	8	Dark on the beak, White feather on neck
LP1M	LM8	PL	M	9	Less white feather contaminate
LP2M	LM9	PL	M	9	Less white feather contaminate
LP3M	LM10	PL	M	10	Similar to the reference
LP4M	LM11	PL	M	9	No white feather on the wing
LP5M	LM12	PL	M	9	Less white feather contaminate
LN1M	LM13	NP	M	7	Too much white feather contaminate
LN2M	LM14	NP	M	7	Too much white feather contaminate, Dark color on tarsal
LN3M	LM15	NP	M	7	Too much white feather contaminate
L46850F	LF1	CB	F	8	Dark color on the beak, too much white feather contaminate
L26506F	LF2	CB	F	7	Dark color on the beak, too much white feather contaminate
L55612F	LF3	CB	F	7	Dark color on the beak, too much white feather contaminate
L19949F	LF4	CB	F	7	White color on the head, white tarsal, too much white feather
L48354F	LF5	CB	F	7	Dark color on the beak, less white feather contaminate
L27266F	LF6	CB	F	7	Dark color on the beak, less white feather contaminate
L26621F	LF7	CB	F	8	Dark color on the beak and tarsal, too much white feather
L25927F	LF8	CB	F	7	Dark color on the beak, too much white feather

Name	Exp Code	source	Sex	COLOR	Information
L42247F	LF9	CB	F	7	Dark color on the beak and tarsal, too much white feather
L9922F	LF10	CB	F	9	Dark color on the beak
L37218F	LF11	CB	F	7	Dark color on the beak and tarsal, too much white feather
L11164F	LF12	CB	F	8	Too much white feather
L40053F	LF13	CB	F	7	Dark color on the beak and tarsal, less red feather on neck
L49922F	LF14	CB	F	7	Dark color on the beak and tarsal, too much white feather
L48374F	LF15	CB	F	7	Dark color on the beak, too much white feather
P58751M	PM1	CB	M	9	Less yellow color on the beak
P54766M	PM2	CB	M	10	Similar to the reference
P49776M	PM3	CB	M	9	Less yellow color on the beak
P50008M	PM4	CB	M	9	Less yellow color on the beak
P40470M	PM5	CB	M	10	Similar to the reference
PN1M	PM6	NP	M	10	Similar to the reference
PN2M	PM7	NP	M	9	Less dark-green feather contaminate
PN3M	PM8	NP	M	10	Similar to the reference
PN4M	PM9	NP	M	10	Similar to the reference
PN5M	PM10	NP	M	8	Dark green color contaminated as loenghangkhao
PN6M	PM11	NP	M	8	Dark green color contaminated as loenghangkhao
PN7M	PM12	NP	M	8	Dark green color contaminated as loenghangkhao
PN8M	PM13	NP	M	7	Yellow on the tarsal
PN9M	PM14	NP	M	8	Dark green color contaminated as loenghangkhao
PN10M	PM15	NP	M	8	Dark green color contaminated as loenghangkhao
P37468F	PF1	CB	F	8	White color on the comb and white feather on the head
P49372F	PF2	CB	F	10	Similar to the reference
P27351F	PF3	CB	F	10	Similar to the reference
P82888F	PF4	CB	F	10	Similar to the reference
P44418F	PF5	CB	F	9	White color on the beak
P53905F	PF6	CB	F	9	Less white feather contaminate

Name	Exp Code	source	Sex	COLOR	Information
P47632F	PF7	CB	F	10	Similar to the reference
P58809F	PF8	CB	F	10	Similar to the reference
P52703F	PF9	CB	F	10	Similar to the reference
P53903F	PF10	CB	F	10	Similar to the reference
P25639F	PF11	CB	F	10	Similar to the reference
P40609F	PF12	CB	F	9	No comb
PN1F	PF13	NP	F	9	White color on the beak
PN2F	PF14	NP	F	10	Similar to the reference
PN3F	PF15	NP	F	10	Similar to the reference

APPENDIX IV

DNA Extraction

ProteinaseK/High salt concentration extraction

1. Blood sample of each individual was kept under –20 ^{o}C and 3% EDTA anticoagulant was added.
2. 50 µL blood was thawed and extract the DNA by High salt extraction method of Lipkin et al.,2002 as follow;
3. Add 3 ml of lysis buffer which contain

   1 M Tris pH 7.5,

   0.4 M NaCl,

   2mM EDTA pH 8.0
4. After that 200 µL of 10%SDS was added into the blood sample solution.
5. Add 16µL of 20 mg/ml proteinase K into the blood sample solution.
6. Incubate under 62 ^{o}C water in the water bath 3 hours or 35 ^{o}C overnight.
7. After incubation, centrifuge the mixture under 14000 rpm for 15 minutes.
8. Add 1 ml 5M NaCl in the solution.
9. Take the supernatant into the eppendrof.
10. Centrifuge the supernatant at 14000 rpm for 15 minutes.
11. Precipitate the DNA by adding ethanol into the supernatant.
12. Check the quality and quantity of the DNA by spectrophotometer and electrophoresis.

APPENDIX V

Chemical Preparation

1. 6% polyacrylamide gel

The preparation of 6% polyacrylamide gel for 1 side of vertical gel electrophoresis composed of;

- 5X TBE 9 ml
- 29:1 acrylamide:bisacrylamide 9 ml
- Urea 17.36 g
- 10% APS 412 µl
- TEMED 22.5 µl
- H_2O 10.94 ml
- repel silane
- bind silane
  - bind silane 10 µl,
  - glacial acitic acid 7.5 µl
  - 95% ethanol 1.5 ml
- 95% ethanol
- glacial acetic acid

Apply the description previously as follow:

1. Mix 5X TBE, 29:1 acrylamide:bisacrylamide, water and urea together. Melt the solution by microwave for 20 second or until most of the urea were dissolved.
2. Wash the sequencing plates (Bio-rad®) by water and detergent and rinsed with distilled water and then wiped with kimwiped® until dry.

3. Clean the sequencing plates by 95% ethanol.
4. The binding plate was siliconised with 0.5 ml bind silane® and air-dried.
5. The cover plate was siliconised with 0.5 ml repel silane® and air-dried.
6. After dried, set the vertical gel electrophoresis apparatus.
7. Add 10% APS 412 µl into the solution and mix together.
8. Add 22 µl TEMED and load the solution into the electrophoresis apparatus. Beware the bubble happened.
9. Insert the comb into the apparatus.
10. Polymerization process was take about 1-2 hours.
11. Remove the comb when the gel was completely solidified and polymerize.

2. 5X Tris Boric EDTA (5X TBE)

1 liter of 5X TBE composed of ;

- Tris base	54 g
- Boric acid	27.5 g
- 0.5M EDTA pH 8.0	20 ml
- H_2O	up to 1 L

Apply the description previously as follow:

1. Mix Tris, EDTA and boric acid into 1000 ml beaker.
2. Add the water until equal to 1000 ml.
3. Mix all chemical together and keep under room temperature.

3. 10% APS

The preparation of 1 ml 10% APS can be done by mix 100 mg APS with 1 ml water in the eppendrof.

4. 0.1% Cetyltrimethyl ammonium bromide (CTAB)

The preparation of 2 L 0.1% CTAB can be done by mix 2 g CTAB with 2 L water in the 2000 ml Beaker.

5. Silver nitrate for gel staining

1 liter of Silver nitrate solution composed of ;

- Silver nitrate 1.6 g
- 3M NaOH 1.3 ml
- H_2O
- 25% ammonia

Apply the description previously as follow:

1. Add 1.6 g silver nitrate into the 1 L beaker.
2. Add 1 L water and mix together.
3. Add 3M NaOH 1.3 ml into the solution until the solution was brown.
4. Add ammonium solution until the color was disappearing. Use the mixture for silver staining.

6. Developer solution

1 liter of Develop solution can be compared as ;

1. Put 20 g of Sodium carbonate anhydrous into the 1 L beaker.
2. All 1 L of water and mix together.
3. Cool the solution by keeping less than 4 ^{o}C. The cold solution will help to reduce too much black color of the staining.
4. Before use the develop solution, add 600 µl of 35% formaldehyde.
5. Use the solution to develop the gel.