Chapter V

Discussion

Non invasive method was used in this experiment, feathers from museum specimens which were collected when they shed out feathers after breeding season. It is convenient and does not cause the pain to animals that live in nature.

The visualization of microsatellite alleles in this experiment was used the silver staining method. It is not handled with radioactive material (Luqmani et al., 1997). Therefore, this method is more safety and less expensive method than radioisotope. The silver staining method offer advantage of sensitivity over ethidium bromide (detecting in pictogram of quantities of DNA), the result of this method can also be detected very quickly and can be used for qualitative assessment of microsatellite allelic bands. Nevertheless, this method still causes a problem with variable background (Koreth, O' leary and McGee, 1996).

Eight percents of polyacrylamide gel electrophoresis was used to separate the sizes of PCR products. It is the effective ranges of separation from 60 to 400 base pairs (Smith and Wood, 1991). PCR products of two populations were loaded into the same plate of denaturing sequencing gel and run electrophoresis together (for pairwise comparison between varieties), according to compare the similar size of PCR products (similar alleles) of every variety with the same primer. It helped estimate the error of band scoring, and the set of 100 bp + 1.5 Kb DNA ladder standard marker was used to reference sizes.

The band scoring of microsatellite was detected by naked eyes, but the sequencing marker was not used. Therefore, numerical symbol was designated to score the alleles. This method can not be estimated the real sizes of each observed alleles. It can use program Image - Pro Express to identify homozygous or heterozygous allele. Now, sequencing marker for silver staining method has been develop from Promega

Corporation (Zink and Blackwell, 1996), which helped to indicate the real sizes of observed alleles.

In this experiment, numbers of microsatellite allele were high (2-4 alleles). This result may be caused by selected the high polymorphic primer. Therefore, that so good because each locus of this study showed many different alleles and some rare alleles which have low allelic frequency. The comparison of mean number of alleles between the varieties showed no significant mean difference (P=0.317). It can be explained that between populations of Green Peafowl in northern and western have nearly of allele number. Moreover, the mean effective number of alleles was compared between populations of Green Peafowl showed no significantly mean difference, too (P=0.498).

Green Peafowl from western has shown unique or private allele and has allele frequency at MCW034 locus (allele number 3rd) is 0.014. Unique allele is specific region of each population. Generally, if the unique allele frequency is approximate 0.9, this allele is possible to be for identifying its own out from others (Lovette, 1998). However, most of unique allele in this study has low allele frequency.

Any population, which has high the number of alleles and high heterozygosity, this population, shows highly genetic variation. However, heterozygosity can be calculated only heterozygous allele frequency but not assessed the number of alleles. In addition, number of alleles per locus is not assessed the allele frequency of rare allele, which can permit the bias. Although the number of alleles per locus and heterozygosity value can be determined the genetic variation, the effective number of alleles is likely to be a good parameter for the study of genetic variation because it can calculated heterozygous allele frequency of all observed alleles (Wolfus, Garcia and Warren, 1997)

Heterozygosity is commonly extended to refer to the population as a whole, i.e., the fraction of individuals in a population that are heterozygous for a particular locus. It can also refer to the fraction of loci within an individual that are heterozygous. Typically, the mean observed (H_O) and expected from Hardy-Wienberg (H_E) heterozygosities are

compared, defined as follows for diploid individuals in a population: from this study, the mean observed (H_O) and mean expected (H_E) heterozygosity showed no significant difference (P=0.180), that mean heterozygosity was no different in population level. Therefore, H_E can be used to compare genetic diversity between populations, because it reduces estimation error of rare alleles. The result showed no significant difference of H_E between populations (P=0.889). This result was caused by microsatellite DNA has highly mutation rate, it permits high polymorphism of alleles and it has different size of alleles, which these alleles increase the chance to find heterozygous allele. Moreover, microsatellite DNA is still permitted the band sharing between varieties. Thus, each examined variety can be found the same heterozygous alleles and it is highly heterozygosity (Mindell, 1997; Rico *et al.*, 1997).

The Hardy-Wienbreg assumption was tested for all loci and all populations. The result showed all microsatellite loci conformed to Hardy-Wienbreg assumption (P>0.05). The Hardy-Weinberg assumption states that both allele and genotype frequencies in a population remain constant (equilibrium from generation to generation). Static allele frequencies in a population across generations assume: random mating, no mutation (the alleles don't change), no migration or emigration (no exchange of alleles between populations), infinitely large population size, and no selective pressure for or against any traits (Hartl and Clark, 2007). The departure from Hardy-Wienbreg may be due to several parameters including (1) mutation process at this locus not conforming to the infinite allele model of mutation (Rico *et al.*, 1997). Given the high mutation rate of microsatellite locus and individual may be homozygous for a pair of alleles of the same size, which may not be the product of a single mutation event. (2) Non-amplification of one of the two alleles presented in heterozygote, because the locus is not suitable for amplification this populations. Therefore, further study should be developed primers for species-specific (Lessios, 1992; Wolfus, Garcia and Warren, 1997)

The different region of microsatellite loci can effect to genetic linkage on the allelic distribution (Ciampolini *et al.*, 1995). Genetic linkage disequilibrium was used for provide the different region of microsatellite loci. The microsatellite DNA at HUJ002,

LEI166, MCW034, MCW069, MCW080, MCW098, MCW295 and MCW330 locus are located at chromosome 17th, 5th, 2th, E46C08W48, 15th, 4th, 4th and 17th, respectively. For MCW098 - MCW295 locus, which locate on the same chromosome, showed no significance of the linkage disequilibrium (P=0.423). It explained that both loci may locate on far distance region. Therefore, MCW098 locus is independent from MCW295 locus. But for HUJ002 - MCW330 locus, which locate on the same chromosome showed significance of the linkage disequilibrium (P=0.070). It explained that HUJ002 locus is not independent from MCW330 locus, and showed that the alleles of these loci are not random association. Generally, closely region of locus is often found genetic linkage disequilibrium more than far region (Ciampolini *et al.*, 1995).

Twelve of twenty-four pairs of pairwise comparison between populations of Green Peafowl and Red Jungle Fowl showed no significantly genetic different, whereas four of eight pairs between populations of Green Peafowl from northern and western showed no significantly genetic differentiation. It may explain that each population of Green Peafowl has alike genetic information that has period of time not enough for clearly revealed genetic differentiation between populations. Therefore, the geographic isolation can not separate Green Peafowl population to two sub-populations but in long time Green Peafowl population can separate to sub-population. In addition, rate of evolution of each population of Green Peafowl may be different depend on the trait of each population such as in northern part (Huai Hong Khrai) that has small population but showed no significantly genetic differentiation with western part, may be Green Peafowls at Huai Hong Khrai bred with captive Peafowls that increased genetic variation in northern population. Furthermore, selected microsatellite loci exhibited many different alleles, so it increased chance to show significant genetic differentiation within and between populations. Thus, genetic differentiation within population is more than between populations (Mindell, 1997). It supported that Green Peafowl from northern and western have same common ancestor and they are same subspecies (*Pavo muticus imperator*).

According to this study, the results of microsatellite analysis found that Green Peafowl from northern and western are in the same group (genetic distance between varieties 0.1956). This result of molecular approach should be occurred, because both Green Peafowl from northern and western are not separated to be other subspecies. It may be resulted from the selected microsatellite loci have many different size of alleles, high mutation rate and small number of sample sizes. Thus, it is possible to find the difference of genetic distance between the same Green Peafowl.

In this study, only eight microsatellite loci were determined for genetic variation analysis. In addition, each microsatellite loci showed high number of alleles, and that some alleles showed low allele frequency (lower than 0.1). Beside microsatellite DNA markers are neutral marker that not under the selection process and show a high mutation rate, leading to many different alleles, which low allele frequency (Wolfus *et al.*, 1997). Moreover, different mutation process between microsatellite markers may be provided opportunities and probabilities of complicated for analysis of population. The stepwise mutation process whereby repeats was usually added one at a time suggested that similarity of length reflected allelic relatedness and showed band sharing between population (Mindell, 1997). As a result, the study at populations level over which microsatellite provide sufficient resolution may be narrower than that mutate more slowly, such as mitochondrial DNA. It may be showed clearly genetic differentiation between population levels.

In further study, more other technique and other gene such as the D-loop mitochondrial DNA sequencing of Green Peafowl from northern and western should be studied to confirm the genetic information from microsatellite DNA and should be increased number of sample size for cover all individual in each area. Therefore, following the commentary in this thesis should be examined.