

## CHAPTER IV

### DISCUSSION

#### 4.1 Sample collection and DNA extraction

In this study, bloodstain and feather papillae were collected for DNA extraction because it is a non invasive method and more ever easy to collect and carried for a less time in the field. Bloodstains can be easily managed in the laboratory, convenience for working in the field and need not use dry ice or liquid nitrogen for preservation. A bloodstain is also appropriate for avian sample collection, because red blood cells of avian contain genomic DNA in their nuclei and bloodstains can be kept for a long time (Karnsomdee, 1999). Although genomic DNA extracted from bloodstains had higher quality and yield than that from feathers (Wutthivikaikarn, 2003), but some case blood samples can not be taken from the wild. In the other way, there were also several problems found from genomic DNA extracted from a feather pulp but in this study the extracted DNA from feather were not got any problem after amplification. However, in natural habitat, feathers are easier to be collected along their trails. The advantage of collection both bloodstain and feather are non invasive method and need not to kill the animals.

## 4.2 RAPD analysis

In previously, lots of molecular research in gallopheasants and partridge were work on cytochrome b (Randi, 1996; Meckvichai et al., 1997), mitochondrial D-loop control region (Randi and Lucchini, 1998; Plubcharoensook, 2000), micosattelite, RFLP and RAPD. In this MSc Thesis was used randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique for studying genetic diversity in five different species of gallopheasants and partridge because it is a simple and rapid method for determining genetic diversity and similarity in various organisms. It also has the advantage that no prior knowledge of the genome under research is necessary (Fischer et al., 2000).

In this study, 5% screening primer can be use for amplifying genomic DNA of gallopheasants and partridge. Amplification among in interspecies and intraspecies are the same avian family, they are in different genera. Optimization of each PCR component is therefore very essential. From the PCR results, optimize concentration of magnesium, *Taq* DNA polymerase, template and annealing temperature were found to be important. In the case of magnesium chloride concentration may be adjust and vary concentration for choosing appropriate concentration for this study. Although increasing magnesium concentration can be increase yields of PCR amplification excess magnesium tends to cause non specific reactions. The annealing temperature was suggested to be the first-choice variable to optimize for PCR amplification condition and conclusively recommended to be  $T_m-5$  °C.

### 4.3 Genetic distance and phylogenetic analysis

#### 4.3.1 Interspecies

From this studies the Great Argus, crested wood partridge, Siamese Fireback and Red Junglefowl have close relationship while the Green Peafowl shown high genetic diversity and separated out from other species when compared by using average genetic distance. The results in this study gave same result with Fumihito et al. (1995) that used base sequence data of mitochondrial DNA control region (mtDNA D-loop). They were reported Green Peafowl have different genetic relationship with Red Junglefowl. And same as the results of Meckvichai et al. (2001), studied genetic relationship of Hume's Pheasant, Malay Brown Peacock Pheasant, Grey Peacock Pheasant, Great Argus and Green Peafowl by using cytochrome *b* (cytb) were found Hume's Pheasant and Green Peafowl separated out from Great Argus, Malay Brown Peacock Pheasant and Grey Peacock Pheasant.

The result same as the results of Kimball et al. (1999; 2001) studied Cytochrome *b* and D-loop nucleotide sequences for studying patterns of molecular evolution and phylogenetic relationships between the pheasants and the partridges, which are thought to form two closely related monophyletic galliform lineages. They were able to establish six major lineages containing pheasant and partridge taxa, including one lineage containing both pheasants and partridges (*Gallus*, *Bambusicola* and *Francolinus*). The Green Peafowl and Great Argus have closely genetic relationship while the Red Junglefowl have closely related to partridge, Genus *Lophura*, Great Argus and Green Peafowl respectively. In my studies shown different results, Red Junglefowl has closely genetic distance with Great Argus, Siamese Fireback and Crested Wood Partridge respectively. The Green Peafowl have different genetic distance with Great Argus.

### 4.3.2 intraspecies

#### In the Great Argus

From neighbor-joining tree constructed from average genetic distances between pairs of each population indicated three separate groups. The population of Great Argus from Hala Bala Wildlife Sanctuary and Soi Dao wildlife research and breeding station has genetic distance lower than other population (0.0706) because both of them are in the same office and may be got from same origin according to genetic distance was lower than others. The population from Pratabchang wildlife research and breeding station and Sattaheep show highly different in genetic distance it could be considered that both populations are possibly came from different origin. However, the genetic diversity of Pratabchang wildlife research and breeding station has more diversity than the individual from Sattaheep farm.

#### In Red Junglefowl

The data shown population from Huay Yang Parn wildlife research and breeding station separated out from the others. Genetic diversity of this population was higher than population from Huay Kha Khaeng wildlife sanctuary, Patthalung wildlife research and breeding station, and Satun. This population is capture from the wild that were distributed in Indochina area.

From the analysis of genetic distance, the population from Patthalung wildlife research and breeding station and Satun has closed genetic relationship. Both are capture from the wild and distributed in the south of Thailand which have separated by geographic barrier by Bantad Mountain and village. The population from Satun dwells in the southwest and population from Patthalung wildlife research and breeding station are existing in the southeast. However, in the old day both populations are possibly come from the same origin because they have closely genetic distance.

Habitat of population from Huay Kha Kheang Wildlife Sanctuary was ecotone and overlapped between Indochina and Malay peninsular. They shown genetic distance closely related to Red Junglefowl from Patthalung wildlife research and breeding station and Satun more than population from Huay Yang Parn wildlife research and breeding station.

#### **In Siamese Fireback**

This species could be separated into three groups. The first group was the population from Phu Kiew wildlife research and breeding station and Sattaheep which were very closely related because they have closed genetic distance, 0.0801.

While the second population from Pratabchang wildlife research and breeding station and the third group was the population from Soi Dao wildlife research and breeding station. However, Siamese Fireback from Soi Dao wildlife research and breeding station shown higher average genetic distance more than other populations. High genetic diversity of Soi Dao population may be more different genetic breed than other population. Almost all of Siamese Fireback in this study was collected from many wildlife researches and breeding station is from the captive breeding.

#### **In Green Peafowl**

Green Peafowl could be separated into two groups. The first group was divided into 4 subgroups. The first subgroup was the population from Huay Yang Parn wildlife research and breeding station and Pong which was capture from the wild show closely related in genetic distance (0.0710). The second subgroup was the population from Pratabchang wildlife research and breeding station, Chiangkhong wild and Surin which was capture from the wild. They showed closely genetic distance. The third subgroup was the population from Lumpang which was capture from the wild and Phu kiew wildlife research and breeding station, they have closely genetic distance. The fourth subgroup was the population from Soi Dao wildlife research and breeding station. From

this studies population from Soi dao wildlife research and breeding station has closely genetic distance with Green Peafowl from Pong wild

The second group is the population from Ping watershed because average genetic distance of Green Peafowl between all populations has genetic distance more different than the others. This population can be comparable with genetic distance of interspecies and they may be other subspecies of Green Peafowl from Burma because the location of collecting site near Burma country or may be these population was endemism species in this area only that shown high genetic diversity more than others.

#### **In Crested Wood Partridge**

Crested Wood Partridge could be separated into four groups. The first group was Crested Wood Partridge from Khok Mai Rua wildlife research and breeding station, Narathivas province. This population is a purebred in the wild from the south of Thailand. The second group was Crested Wood Partridge from Pratabchang wildlife research and breeding station. The third group was Crested Wood Partridge from individual farm in Sattaheep and the last group was Crested Wood Partridge from individual farm in Bangkok.

In this studies could be found candidate population specific bands in intraspecies for using to characterize between different populations of some gallopheasant and partridge. When was used OPA-18 primer its could be separated Great Argus from Hala Bala wildlife sanctuary out from other population at 800 bp, OPC-02 primer could be separated Great Argus from Hala Bala wildlife sanctuary out from others at 650 bp and separated population from Pratabchang out from others at 500 and 700 bp , and OPP-03 primer could be separated population from Pratabchang at 1400 bp. For Green Peafowl were found only UBC-133 primer which could be separated population from Ping watershed out from others at 700 bp. In Crested Wood Partridge when was used OPP-03 primer its could be separated population from Pratabchang out from others at 1200 bp and UBC-135 primer could be separated population from Khok

Mai Rua out from other population at 500 bp. Candidate population specific bands that were found in this studied can be used for characterize some gallopheasants and partridge from the different population site.

However, phylograms constructed by neighbor-joining. Genetic differences analysis using pairwise exact test shown population among inter- and intraspecies non significant genetic differences ( $P \leq 0.05$ ). In the future need number of samples more than this study (each population need more than 30 individuals for studying in population genetic). However, genetic diversity of four gallopheasants and a partridge both in natural and domesticated stocks should be regularly monitored by various molecular approaches such as microsatellite, mtDNA and RAPD.

Nowadays, the royal Thai government has given permission to set pheasant farm for breeding as commercial domestic animals. RAPD markers suggested from this research can help tagging any gallopheasants and partridge pedigree for planning of a better breeding program. In another case that could happen soon, the government would plan to reintroduce gallopheasants and partridge from wildlife research and breeding stations to the natural sources. The RAPD markers can check genetic variation of gallopheasants and partridge before and after released them.