Chapter II

Literature Review

2.1 The characteristics of Green Peafowl

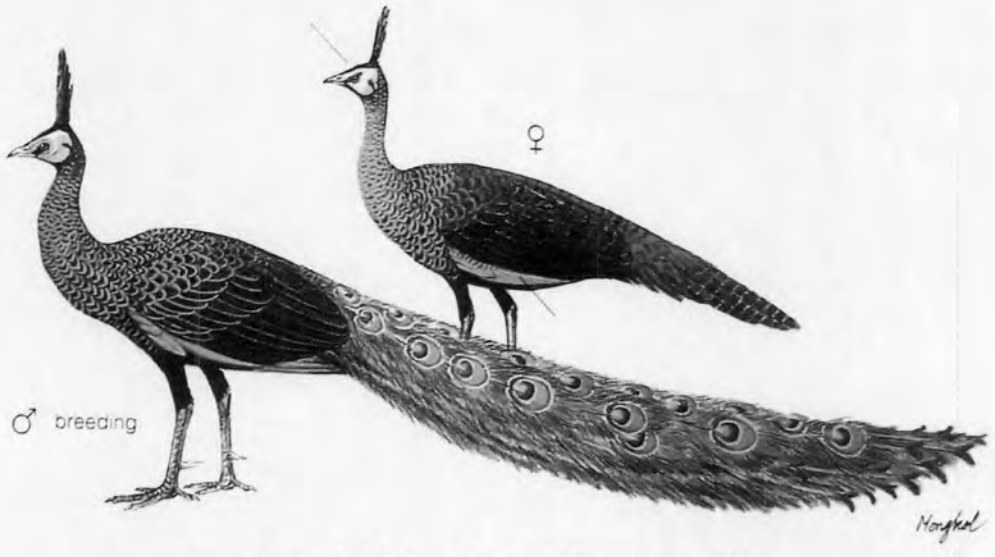

Figure 2.1 Green Peafowl (*Pavo muticus*)

Green Peafowl is very beautiful bird. The male Green Peafowl is larger and more colorful than female. Its brilliant green crest is composed of a long narrow tuft of feathers more upright in stance and its plumage is even more colorful. The neck, breast and mantle are scale-appearance. Wing feather is bright blue and is with a broad metallic green border. The train is brighter emerald green. The wing coverts are bright metallic blue and green, and the bare facial skin is pale blue and yellow beneath the eye. The mantle, back and tail of Green Peafowl are more brilliant and coppery. The primaries are bright chestnut, the dark green abdomen and the grey vent and under tail coverts. The secondaries are blackish-brown on the inner web and dark blue and green on the outer. The female Peafowl is similar to the male, but is only slightly duller. There is no train in female but it is replaced by short greenish-brown feathers with buff. The female can be distinguished from the young males by the brown patch instead of bluish-black local one between an eye and the bill. Also, distinguished by the primaries, the

chestnut speck of female is bright and brown, whereas the chestnut of male have not speck.

Green Peafowl are even more wary and less prone to live near human habitation. They are often found in a jungle, usually in the vicinity of a river or open clearing. The flock is small, except during the breeding season. Adult males fight to defend their territory and band of female (Wayre, 1969).

2.2 Distribution of Green Peafowl

Green Peafowl have been reported in many areas; about 1500 Peafowls are only confirmed in Huai Kha Khaeng Wildlife Sanctuary in the western part of country that is the highest population. In northern, about 500 Peafowls are found along Ping, Ing, Yom and Nan basin such as Mae Yom National Park, Doi Phu Nang National Park and Wiang Loh Wildlife Sanctuary. Furthermore, Green Peafowl had been found at Huai Hong Krai Royal Development Study Center. There are about 56 birds (Meckvichai *et al.*, 2007) and population increase from near-vanishing point due to abandonment of farmland, regeneration of secondary forest, reduced hunting and improved protection.

2.3 Biology of Green Peafowl

Green Peafowl often flock into a small group of 3-5 birds. It uses variety of habitats, including an open forest which is preferable, a coastal scrub, a riverbank, a teak, a forest edge and clearing, an area with dense secondary growth near shifting agriculture, and other. It can fly weakly so it spends most of its time on the ground looking for food or perching. It can omnivorous. It likes to eat berries and other fruits, including rice-grain and seedling such as grass seed. Also, it can eat crickets, dragonflies, small moth, ticks and termites, lizards, frogs and other aquatic small animals, etc. (Ponsena, 1988).

Peafowl are polygamous in which four or five females could be mated to a male. Moreover, it has special behavioral characteristics. During the breeding season,

the dominant male will move to sand bars along the main stream and creates a breeding territory. It tires to defend its territory from other males. A female usually moves in its flock ranging from 2 to 6 individuals. Their feeding range at this season may cover 2 to 4 male's territory. Mating usually occurs in the morning and in the late afternoon. A mated begins to lay eggs at 22 months of age (Humphrey and Bain, 1990). A female usually lays 4-6 eggs in a shallow hole dug on the ground. The female incubates the eggs for approximately 26-28 days by herself. After hatching, the young chicks follow the mother, even though they are capable of foraging on their own. The young Green Peafowl can fly within two weeks of hatching, but will remain with the adults until the next breeding season (Collar, 2001).

2.4 Study areas

Huai Hong Khrai Royal Development Study Center

Huai Hong Khrai Royal Development Study Center is located in Doi Saket District, Chiang Mai Province. The area is about 1,360 hectares. It is between 18° $53'$ to 18° $56'$ N in latitude and 99° $14'$ to 99° $16'$ E in longitude. This center is the project under initiation of His Majesty the King. Its original purpose was to offer educational courses that were appropriate to the Northern river and watering area. This center composes of seven sections such as forestry, agricultural techniques, livestock rising, fisheries, consolidation through planting grasses and conservation work around Huai Hong Khrai in Mae Kuang Forest.

Huai Kha Khaeng Wildlife Sanctuary

Huai Kha Khaeng Wildlife Sanctuary is situated mainly in Uthai Thani Province overlapping Nakhon Sawan Province, covering 257,464 hectares. It is between 15°00' to 15°50'N in latitude and 99°00' to 99°28'E in longitude. It is contiguous with Thung Yai Naresuan wildlife sanctuary (320,000 hectares) in the west. Although these sanctuaries are administered separately, they are essentially a single conservation area representing the largest legislated protected area in mainland in South-east Asia.

2.5 Genetic variation

Low genetic variation in endangered populations is of conservation concern because genetic variation is the raw material required for future adaptive evolution. Low levels of genetic variation can be the consequence of recent population declines, or it can represent an ancestral state (Wandelera, Hoecka and Keller, 2007). Genetic variation is highly desirable characteristic (Woodruff, 1990). Genetic variation can be monitored directly and indirectly in a number of ways. Studies of allozyme variation have been the most commonly employed approach during the last thirty years. In this technique, the allelic variants of soluble enzymes and other proteins that can be visualized biochemically on a gel after electrophoresis are counted directly. At present, there are several molecular genetic approaches to monitoring genetic variation and determine relationships between individual, population, species including mitochondrial DNA (mtDNA), restriction fragment length polymorphism (RFLP) analysis, randomly amplified polymorphic DNAs (RAPD), amplified fragment length polymorphism (AFLP), whole genomic DNA-fingerprinting and microsatellite DNA. Such techniques facilitate very fine detail analysis of microevolutionary process (Avise, 2004).

2.6 Genetic in the conservation of biodiversity

In the past, all studying DNA level variation required the large amount of tissue or blood and the large-scale extraction of high molecular weight DNA. Furthermore, collecting tissue samples from free-ranging animal was difficult. When the polymerase chain reaction (PCR) technique has been develop, it enables investigators to amplify very small amounts of tissue, thus eliminating the need for large blood or tissue samples which are difficult to handle in the field (Woodruff, 1990). DNA can be extracted and amplified from nanogram samples of pulp from feathers and microgram amounts of tissue from museum skin or preserved specimen (Wandelera, Hoecka and Keller, 2007). Appropriate molecular genetic markers can be chosen by matching the level of innate variability of a gene to the level of ecological of evolutionary resolution required (Proulx, Promislow and Phillips, 2005).

2.7 Molecular genetic methods to study Green Peafowl genome

Since, many molecular genetic methods have been developed; there have been increasingly studied in vertebrate genome, especially in avian genome. These methods have highly resolution power. They also permit exceedingly detailed description of genetic variation in DNA and molecular component (Crawford, 1990). Molecular genetic method in explosively increase study about avian in systematic and population genetic are mainly at the level of DNA (Mindell, 1997). Furthermore, this method and technique is used to apply for many fields even in the Green Peafowl genome such as conservation management (Hale, Petrie and Wolff, 2004) and genetic diversity (Bao *et al.*, 2006).

2.8 Method for determining genetic variations

Phenotypic method

Several animal species are grouped on the basis of their phenotypic attributes. The common parameter taken from different characters is morphological characteristics. It is subdivided into two parameters as morphology and morphometric traits that are compared between different species, populations and individuals (Stevens, 2004). Morphology is a comparative based on plumage color and pattern polymorphism, where as morphometric trait is comparative based on body dimension polymorphism e.g.: bill depth at base and nares, bill length, wing length, tarsus length, mid-toe length (Haywood and Bull, 2007). In addition, the phenetic analyses of morphological characteristic might track phenotypic evolution. The evolution process and rate of evolution may not be related with, and differed from molecular variation (Zink and Blackwell, 1996). For example, the study of molecular phylogeny and evolution of morphology of the *Piranga* genus found that morphology and morphometric trait are not related with genetic variation (Burns, 1998). In addition, the study of allozyme, mtDNA and morphometric variation of sparrow genera found that molecular variation is not related with morphometric variation (Zink and Blackwell, 1996). Thus, the genetic variation by only morphological characteristic may be resulted differently from molecular

genetic method. As a result, the study of variation should be used morphological characteristics together with molecular genetic method.

Genotypic method

Molecular genetic is now appropriate for solving important question. Many molecular genetic methods have been studied for example; allozymes have been used intensively in studied of genetic relatedness. Nevertheless, low genetic variability and a paucity of loci make this technique unsuitable for parentage assignment or within group relatedness determination (Ferraris and Palumbi, 1996). DNA-DNA hybridization has clearly contributed to avian molecular and systematic (Sibley and Anlquist, 1990). Also polymorphism pattern of mitochondrial DNA (mtDNA) has aided in differentiation studies of species and population (Mindell, 1997), it is maternal inheritance. However, it is not suitable for discrimination of closely related population. Polymorphism of random genetic DNA fragment has been used to resolve questions about polymorphism (Ferraris and Palumbi, 1996). In addition, DNA fingerprinting using minisatellite or synthetic simple repeats probes to prove the most sensitive method for determining the genetic relationship of individuals in population (Pornsuksiri, 1995). In addition, it is a reliable technique for parentage exclusion within family groups, but it is not suitable for examining larger or across group, because of the high variability of this technique and difficulties associated with marking comparisons between gels (Ferraris and Palumbi, 1996). Microsatellite DNA marker has received considerable attention in genome analysis (Ellegren, 1992). Furthermore, it is used for parentage assignment and relatedness estimation, ideal marker would be Mendelian fashion, selectively neutral, and high polymorphic. Scores would be unambiguous enough to be evaluated against a standard across gels. Microsatellite meets these criteria (Ferraris and Palumbi, 1996).

2.9 Microsatellite DNA marker

Microsatellite DNA marker is a genetic tool applied to study population genetics. It has become a focus of the search for hypervariable single locus marker, being abundant and wildly dispersed in the eukaryotic genomes (Tautz, 1989)

Microsatellites DNA refers to a class of codominant DNA markers which are inherited in a Mendelian fashion. Microsatellites are highly polymorphic and abundant sequences dispersed throughout most eukaryotic nuclear genomes (Lee and Cho, 2006). Microsatellite DNA belongs to the family of repetitive non-coding DNA sequence (Koreth, O' leary and McGee, 1996) and also short tandem repeats (STRs). 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). 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). Therefore, it is highly polymorphism genetic markers in the animal taxa (Crooijimans *et al.*, 1993). Microsatellite DNA marker is commonly having more than 12 alleles at a single locus and heterozygosity level up to 90% (Taylor, Sherwin and Wayne, 1994). In addition, the DNA sequences flanking the repetitive arrays can be used to design primer for PCR amplification, because it has small of the total size of the arrays, generally less than 300 base pairs (Taylor, Sherwin and Wayne, 1994). The mutation rate of microsatellite DNA is estimated to range between 10^{-3} to 10^{-5} per gamete per generation (Mindell, 1997). The products of PCR amplification are visualized by polyacrylamind gel electrophoresis. The development of microsatellite marker has proceeded for a wide variety of animal, including bee (Kukuk *et al.*, 2002), buffalo (Soysal *et al.*, 2005), elephant (Siripunkaw, 2003), chicken (Singhapol, 2003; Ambady, Cheng and Ponce de Leo´n, 2002) and Green Peafowl (Wutthivikaikan and Meckvichai, 2003; Bao *et al.*, 2006). There are having a range of 1-15 alleles and 40-90% heterozygosity for the avian microsatellite DNA, but it seems to have fewer microsatellites DNA in their genome than other vertebrates (Ferraris and Palumbi, 1996).

Microsatellite markers are used in several scales. For example, among gene, they are used for gender determination in bird by using microsatellite loci on W and Z chromosome (Mindell, 1997). Among individuals, they are good choice for assessing parentage and relatedness among individuals because of the contribution of relative to

inclusive fitness (Blouin *et al.*, 1996). Among population, microsatellite is the major element analyses in avian, because screening is usually confined to perfect repeats. In population level study, validity of a number of repeats concerning the mutation process and the balance between mutation and drift (Allen *et al.*, 1995). The sampling necessary to be examines variation between populations. Population genetic of microsatellite DNA marker was used to study about genetic variation of Red Jungle Fowl between northern and southern Thailand (Becthaisong, 1998). Between species, they are used to study of comparing level of genetic variation in bat *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* (Kanuch *et al.*, 2007). Between genera and higher taxa, they are examined genetic distance that will probable be less studied at lower taxonomic level. Because it has problem about homoplasy that constrains on repeating number, non linear divergence and high mutation rate are likely to make such analysis unrewarding in comparison to those using other molecular more suited to these coarse grained analysis (Mindell, 1997). In addition, microsatellite can be used to investigate for animal breeding and preservation of natural resource, it can be subdivided a breed into subpopultion that are genomic homogeneous, and these can be tested for morphological or functional differences (Ciampolini *et al.*, 1995).

Furthermore, microsatellite DNA markers are the most likely to conform with the assumption of neutrality and proved to be powerful in differentiation geographically isolated population, sibling species and subspecies (Rico, Ibrahim, Rico and Hewitt, 1997). It is also shown an appropriate tool to detect linkage between markers and genes controlling productive, disease and morphological traits (Ciampolini *et al.*, 1995). Not only are these markers useful for genome mapping, but they can also be used in animal breeding.

The advantages of microsatellite DNA markers over traditional methods showed that population survey of microsatellite variations yield 5 or more time higher heterozygosity level than it can be detected by using allozyme electrophoresis (Srikwan, Field and Woodruff, 1996). Also, multilocus minisatellite VNTRs resolved by whole genomic DNA fingerprinting, becomes in contrast to microsatellite DNA marker, which is

a small size of amplified products. Thus, temporal changes in allele frequencies can potentially be studied directly through non-invasive DNA sampling methods based on degraded DNA in museum specimens, plucked feathers and faces (Taylor *et al.*, 1994; Mundy *et al.*, 1997).

2.10 Utilization of microsatellite genotyping in Green Peafowl

In Green Peafowl, microsatellite DNA markers are used for detection and determination genetic variation in many cases. For example, 13 microsatellite tracts had polymorphism, when it was examined in Indian Peafowl (*Pavo cristatus*) for maternity assignment and to separate maternal from paternal effects. The remaining 13 loci were polymorphic enough to determine (Hale, Petrie and Wolff, 2004). Microsatellite 23 loci of chicken were used for study genetic diversity in Green Peafowl population. The results showed the microsatellite locus HUJ2 was the best to use in this study (Wutthivikaikan, 2003). The 29 microsatellite primers from chicken were determined genetic diversity in Green Peafowl and blue peafowl. The results showed 14 of 29 pairs of microsatellite primers from chicken could amplify peafowl DNA and produce specific allele patterns (Bao *et al.*, 2006).