CHAPTER I

INTRODUCTION

Peafowls are the most beautiful and well-known birds for centuries. These birds symbolize elegance and nobility. They have been famous in arts, letters, poems and literatures and have been admired by comparing to a beautiful woman. In addition, peafowls appear in the niras (Thai literary work about voyage and lover) of Thummatibes, H.M. and Pra-ya Trang. Peafowls were mentioned in the old Testament of the Bible. In the historical record, the ancient painting of peafowl was found on a cave wall of Spain (Thera, 1997). The peafowls were brought from Egypt since the fourteenth century; these birds could be found all over Europe, India, China and around the Mediteranean Sea, but they are still quite rare and confined mostly to royalty and persons of means. Furthermore, it is interesting to note that the early church and temple valued the peacock as a religious symbol. In the past, a green peafowl was a resident bird and could be found in many areas in the north of Thailand. Most of temples in that region have a peafowl as symbol of pure commandment. In Burma, green peafowl is a forbidden animal for Karen huntsman (McGowan *et al.*, 1999). Nowadays, peafowls have become one of the most popular and graceful additions to many parks and gardens. (Figure 1.1)

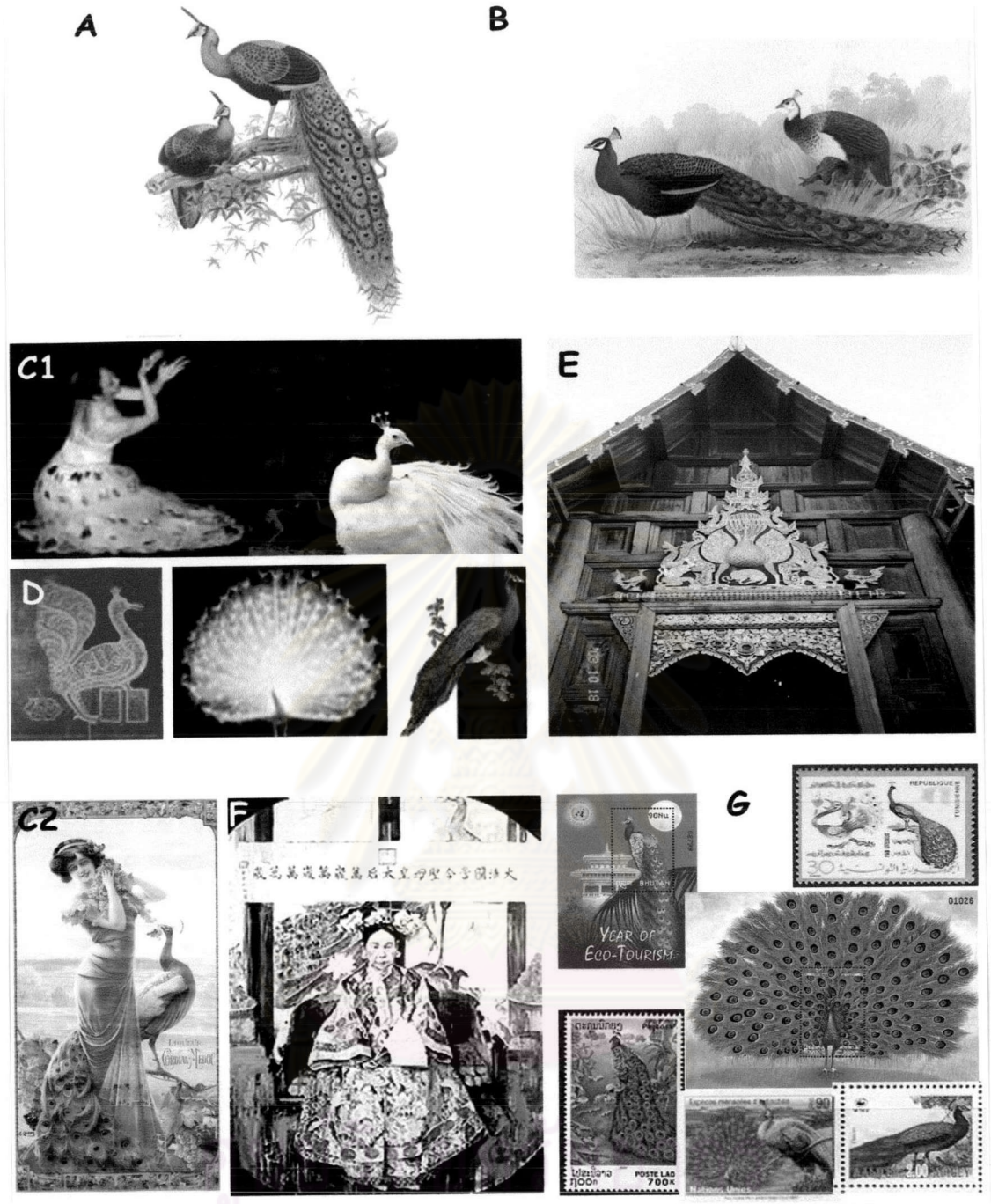

Figure 1.1 Peafowl illustrations and peafowls were mentioned in many ways. (A) green peafowls. (B) Indian peafowls. (C1) an albino Indian peafowl and a beautiful woman. (C2) a woman and a blue peafowl. (D) an ancient picture of peafowl. (E) a peafowl at the front door of Pan Tao temple in Chiang Mai province. (F) an elegant peafowl painting of the Royal Family of Chinese. (G) peafowl stamps from India, Bhutan, Laos and World Wild Found of Nature (WWF).

Remark (A) available from www.scientificillustrator.com
(B) available from www.wildlifeofpakistan.com/Indian_Peafowl.htm
(C) available from www.icm2002.org.cn/L/musicdance.htm,
www.inapet.com and www.wtv-zone.com/7742/animals/peacock.gif
(D) available from www.cmes.uchicago.edu/geninfo.htm
(E) photographed by Waree Wutthivikaikan
(F) available from www.bernicesteinbaumgallery.com/images/peacock.jpg
(G) available from www.bird-stamps.org

Peafowls belong to the family Phasianidae, the same family of birds as pheasants and chickens. There are three species of peafowl: the Congo peafowls (*Afropavo conginsis*) in Congo, central Africa, the Indian peafowls (*Pavo cristatus*) from India and often called blue peafowls, and the green peafowls (*Pavo muticus*) from southeast Assam through Burma and Thailand to the China Sea and southwards to the Malaysian Peninsula and Java. Additionally, there are distinguishing characteristics separating the green peafowls in a wild to be three different subspecies. *Pavo muticus spicifer*, with more bluish color, is distributed in the western Burma. *Pavo muticus imperator* from Indochina has some wing barrings. And *Pavo muticus muticus* is distributed in some parts of Thailand from Kra and Java.

In the past, both two subspecies of *Pavo muticus*, *Pavo muticus imperator* (Indochina peafowl) and *Pavo muticus muticus* (Java peafowl), were found in Thailand. But in the present, *Pavo muticus imperator* is the only one subspecies found in the country.

Green peafowls (*Pavo muticus*) are classified as an endangered species in Thailand because of their small population sizes. Once they used to be found through out the kingdom in any place below nine-hundred meter high, except in the central valley of Chao Phraya River. However, over-hunting has eliminated most of them from Thailand. Hunters intensively trap them for their feathers and as pet trade. Villagers also take them as food as well as collect their eggs from the forests. Not only their habitats were destroyed, but also separated into small areas. In addition, they have been in danger from contaminated chemicals released from agricultural farms beside the forests (Arrathrakorn, 2001) (Figure 1.2).

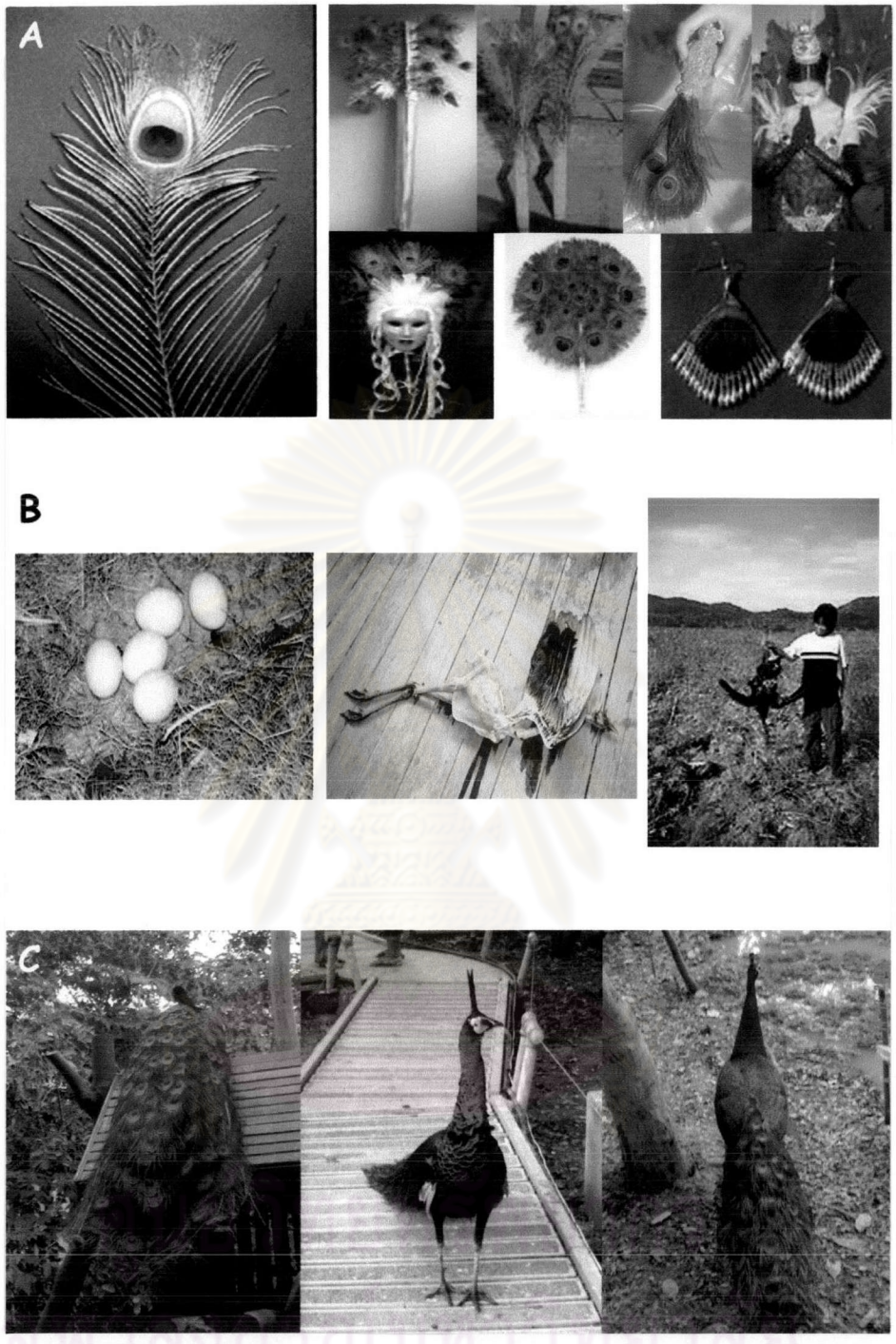

Figure 1.2 Some causes of declination of green peafowl population and peafowls in natural zoo (A) Feathers are the major cause. (B) Collected eggs from the forest (left) and dead green peafowls by poison from agricultural farms beside Doi Phu Nang national park (middle and right). (C) Green peafowls in the natural zoo at Bueng Cha-wak, Supanburi province.

Remark (A) available from www.byu.edu/projects/microscopy/image/peacock58.gif and www.artafx.com/images/artwork/Peacock.jpg
(B) photographed by Thiti Sukapan and bangkokpost
(C) photographed by Waree Wutthivikaikan

Currently, IUCN (International Union for The Conservation of Nature and Natural, 1998) has classified green peafowl as vulnerable species. In Thailand, they are also classified as a protected and endangered species. Furthermore, they have been added into the Appendix II of CITES (The Conservation on International Trade in Endanger Species of World Fauna and Flora, 1996). Green peafowls were found as small groups individual in national parks (NP), wildlife sanctuaries (WS) and other protected areas.

To make a proper conservation of any wildlife, it requires information about ecology, biology and behavior for species survival. Genetic variation within and among populations is also the important information for conservation. Genetic variation is the basic requirement for wildlife breeding and for a long-term sustention in endangered species. It is important to investigate genetic variation both among individuals and among populations of studied species. Till now, genetic variation of green peafowl has never been reported or studied before, either by microsatellites or Random amplified polymorphic DNA (RAPD) techniques. Thus, the present status of genetic diversity of green peafowl is little known.

In general, genetic variability of birds can be studied by two methods. The first method is an indirect method using morphological characteristics (e.g. body dimension, color and pattern of plumages). This method is relatively inexpensive, simple and less time consuming. However, morphological characteristics which represent phenotype of the species, is a product of gene expression and interaction between genes and environment. Therefore, environment could affect the expression of the gene and then morphological traits. Furthermore, the genetic variation resulting from morphological characteristics may be different from molecular

genetic characteristics. As a result, genetic variation studies by morphological method would not be sufficient, especially in the case of green peafowls.

The second method is a molecular genetic technique which can be considered as a direct method. Although the molecular genetic technique is more complex, expensive and time-consuming, it has been more performed for genetic variation study. That is because the molecular technique can detect genetic variation directly at DNA level and then explain such variation better than using morphological characteristics. Therefore, this method is now widely used for solving problems in many biological fields such as evolution, taxonomy, phylogeny, speciation, genetic diversity and animal breeding. In addition, it should be suitable to use for short- and long-term conservation planning for green peafowls.

One of the genetic markers commonly used is microsatellite DNA. Microsatellite DNA is very useful for studying the genetic variation because it is a hypervariable genetic marker. It can be used to detect genetic variation among populations and individuals. Moreover, polymerase chain reaction technique (PCR) can be added to improve the microsatellite marker by eliminating problems of small amount or degraded DNA sample. Small amount of DNA collected in the field can be amplified at a specific DNA region using a thermal cycling PCR machine. In addition, PCR can handle samples from fresh and preserved tissue easily, for example oral-swabs, bloodstain and even museum species. One can choose a non-invasive DNA sampling method such as collecting hair follicles or feces for PCR amplification. To study population genetic of green peafowl by cross-species amplification, the first step is to screen appropriate PCR primers and to establish optimum condition for PCR amplification.

Many previous studies have demonstrated that sequences flanking the repeats of microsatellite loci are often conserved between closely related taxa, thus allowing cross-species amplifications. Peafowls and chickens belong to the same family of Phasianidae. Therefore, peafowl DNA may allow cross-species amplification by using chicken primers.

In this study, twenty-three microsatellite DNA marker loci from *Gallus gallus* (chicken) were screened on green peafowls DNA from many areas of Thailand. The result reveals an appropriate microsatellite marker for further population genetic study.

Another genetic marker used in this study is Random amplified polymorphic DNA (RAPD). Technically, it is a simple method to determine of genetic variation using arbitrarily primed PCR-based technique. The advantages of using RAPD markers are as follow. First, RAPD is a simple, rapid and inexpensive method for detecting DNA polymorphism. Secondly, RAPD does not require knowledge of the genome under investigation. RAPD is also a PCR-based method and does not require the use of radiolabelled probes for hybridization. Finally, unlimited numbers of RAPD primers can be screened for suitable molecular markers of various applications within a short period of time. In this study, sixty primers were screened on green peafowl DNA.

The genetic variation of green peafowl gained from both markers can be used to plan genetic resource conservation in order to maintain for sustention genetic diversity. It can also be used as genetic information for future research about breeding development in a wildlife research and breeding station.

Objectives

The aims of this thesis are to introduce chicken microsatellite PCR method, to optimize condition for green peafowl amplification and to screen appropriate primers for green peafowl RAPD amplification. In addition, both microsatellite and RAPD analyses were compared for effectiveness.

Anticipated benefits

The knowledge gained from this study would be basic information for future study on genetic variation of green peafowl populations in Thailand. Moreover, the optimized microsatellite technique should be able to reveal any inappropriate breeding or evaluate management program in wildlife research and breeding. Finally, the ecology, biology, behavior and genetic variation information could be used to plan a wildlife management program for sustainable conservation of green peafowl in natural sources.