CHAPTER III

LITERATURE REVIEWS

2.1 Taxonomy of *Pavo muticus imperator*

The taxonomic definition of *P. muticus imperator* is as follows

Kingdom Animalia

Subkingdom Metazoa

Phylum Chordata

Subphylum Vertebrata

Superclass Tetrapoda

Class Aves

Subclass Neonithes

Order Galliformes

Suborder Galli

Family Phasianidae

Subfamily Pavoninae

Genus *Pavo*

Species *Pavo muticus*

Subspecies *Pavo muticus imperator*

(Ponsena, 1988)

Scientific name : *Pavo muticus imperator* (Linnaeus, 1766)

Common name : Peacock, Peahen, Green- necked Peafowl

Genbank common name : Green Peafowl

2.2 Green Peafowl and Indian Peafowl

There are three species of peafowl, the Congo peafowl (*Afropavo Conginsis*), the Indian peafowl *(Pavo cristatus)*, often called blue peafowl, and the green peafowl *(Pavo muticus)*. In addition, there are three subspecies of green peafowl (Asian peafowl); Burmese green peafowl *(Pavo muticus specifer)*, Indochinese green peafowl *(Pavo muticus imperator)* and Javanese green peafowl *(Pavo muticus muticus)* (Wayre, 1969).

The characteristics of two Asian peafowl are similar but green peafowl are bigger than blue peafowl. Furthermore, the feather of blue peafowl's body coverts are blue and of it's wings are light black and white. The other's feather is brown. The facial skin of blue peafowl is white while green peafowl is blue and yellow. Blue peafowls's crest is fan shaped while green peafowl's crest is erect standed (Figure 2.1A).

Green peafowl has a reputation of being flighty with savage or unfriendly dispositions more than Indian peafowl (Marion, 1999).

The two species of peafowls, the Indian (*P. cristatus*) and the green (*P. muticus*), cross freely and the offspring is entirely fertile, like that of the Golden and Lady Amherst's pheasants, so that hybrids with various proportions of both bloods can be produced. Birds issued from the first cross intermediate in shape and colors, very handsome, hardy and as good tempered as pure Indian peafowl. Moreover, they also cross occasionally with Guinea-fowls, Monals and domestic chicken of the larger breeds, such hybrids being sterile (Delacour, 1977).

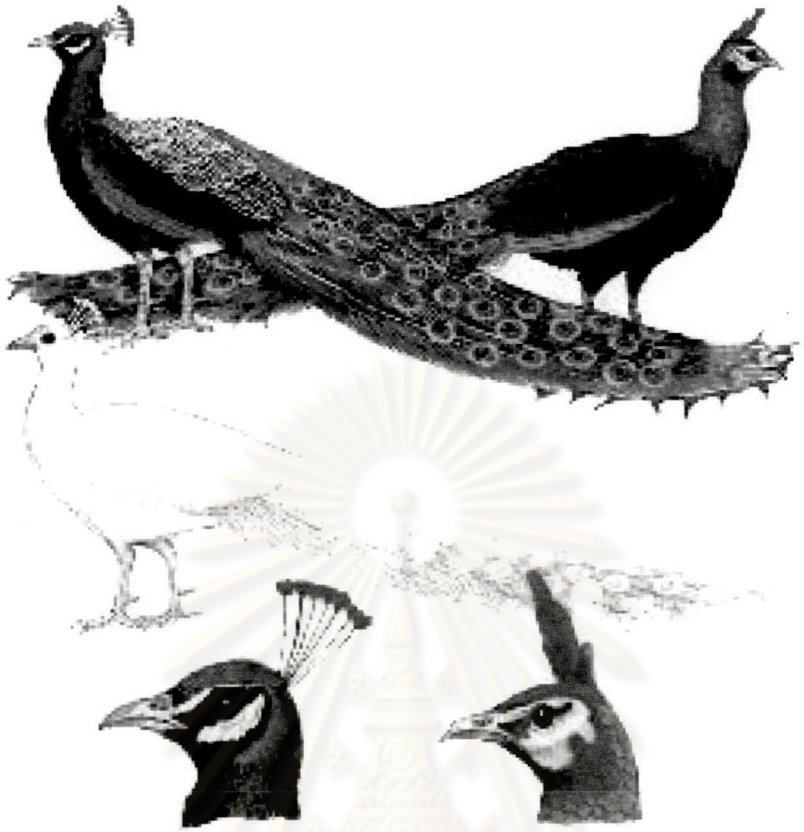


Figure 2.1A Indian peafowl and green peafowl

Available from www.bangalorenet.com/system1/rahul/Variety.html

Figure 2.1B Feathers of Indo-Chinese green peafowl are more coppery than other green peafowl.

Photographed by Waree Wutthivikaikan

2.3 The Characteristic of green peafowl

Green Peafowl is gorgeous bird more than blue peafowl because green peafowl has longer leg and straight body when it stands. The peafowl is a bird that has large size, brilliant colors, erect crest and long train make it striking objects. Other characteristics are a small head, a long neck, rounded wings, the first primary shorter than the tenth, and strong tarsi armed with a thick spur. The flat tail is composed of twenty graduated rectrices which moult from the fifth pair outwards and inwards, are dull colored, of moderate sized and hidden by tremendously long and highly ornate coverts (Delacour, 1977).

The male green peafowl is larger and more colorful than the Indian peafowl. Its brilliant green crest is composed of a long narrow tuft of feathers and its plumage is even more colorful. The neck, breast and mantle are scaleapparent. Each feather is bright blue and is with a broad metallic green border. The train is of a brighter emerald green. The wing coverts are bright metallic blue and green, and the bare facial skin is pale blue and yellow. The mantle, back and tail of the green peafowls are the same as in the Indian peafowls, but only 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 and outer web are black or dark blue.

The female peafowl is similar to the male, but are 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 loral one between an eye and the bill. Also, distinguished by the outer web of primaries, the female's chestnut with black bar, whereas the male's chestnut is pure (Sukapan, 2004).

Immatures and first-year young males are rather the female. However, the males are showing more color on the neck and female have a black bar on alular(Sukapan, 2004). They assume that the adult dress is the third year while in their second year they are like the adult in color but without the train.

Green peafowls live in the same habitat like Indian peafowls. Green Peafowls 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).

2.4 *Pavo muticus imperator* (*P. m. imperator*)

Pavo muticus imperator or Indo-Chinese green peafowl is one of two subspecies in Thailand. It is rare resident; much reduced by human persecution (Boonsong, 1991). Size of male is 180-250 cm and 3,850-5,000 grams; female is 110-110 cm, weight less than 4,000 gm (Zoological Parks and Gardens Board of Victoria, 2003).

Males are similar to *Pavo muticus muticus* but are not quite brilliant generally. The fringes of the neck, upper back, and breast feathers are more coppery, but are not so golden green. Lower breast and flanks are duller and darker. Mantle and back are slightly more bluish and less golden. Wing coverts and the outer web of secondaries are bluer and little duller, and less green on the borders. (Figure 2.2)

Females differ from *Pavo muticus muticus* in having the borders of the breast feathers. They are more heavily marked with buff, less green, and the wing-coverts are less brilliant (Delacour, 1977). The female has 18 tail feathers while the male has 20 tail feathers.

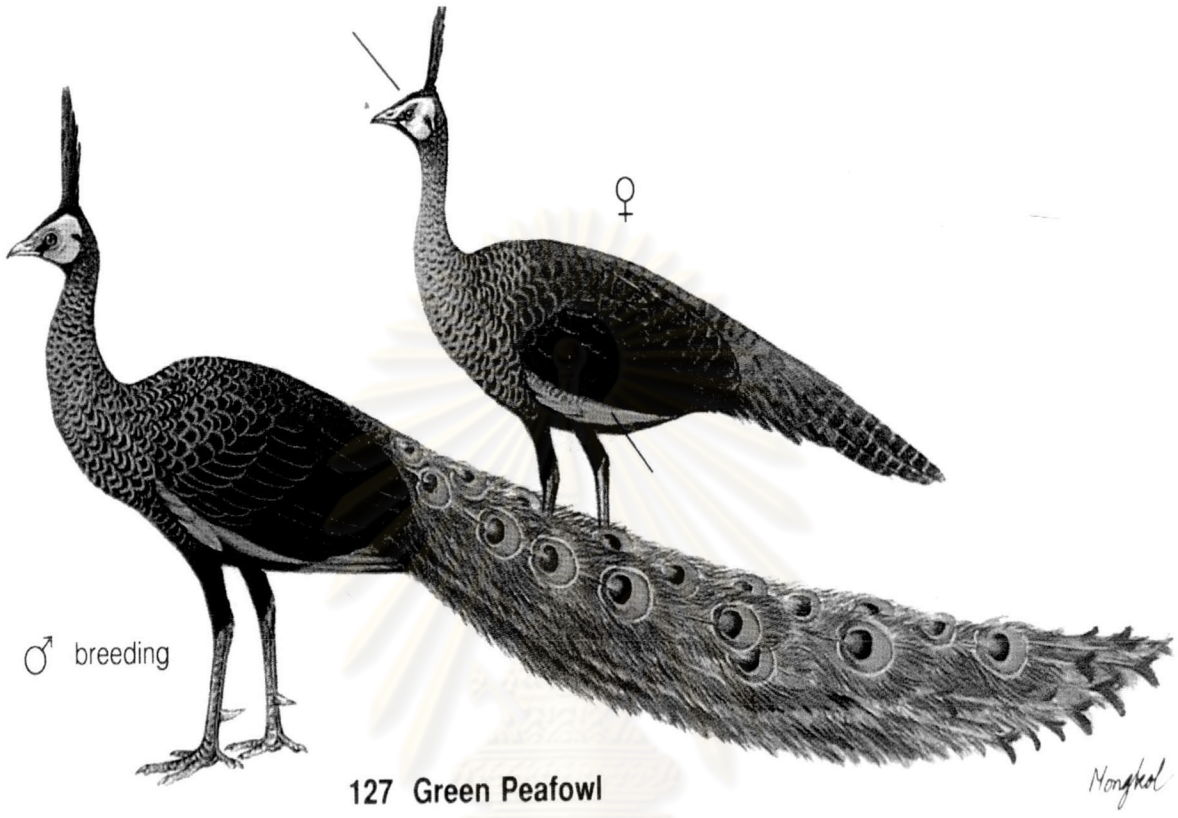


Figure 2.2 Green Peafowl *(Pavo muticus)*

Illustrated by Mongkol Wongkalasin in A guide to the birds of Thailand

2.5 Distribution of Green peafowl

The last decade, Asian peafowl had inhabited in Ceylon, India, the Indo-Chinese countries, Malaya and Java, being absent from Sumatra and Bornea (Delacour, 1977). The map is shown in Figure 2.3.

The present, green peafowl is interested by many researches so Red data book threatened birds of Asia (2003) reported the distribution of green peafowl in Figure 2.4 and 2.5.

Once, green peafowl had been found through out Thailand below 900 meter except in the central valley of the Chaophraya river and the northeastern provinces. At present, this bird is classified as one of the threatened species. The decreasing of its population is due to habitat destruction, environmental pollution and hunting. (Rojanadelok *et al.,* 1986).

They have been reported in many areas, but about 300 peafowls are only confirmed in Hwai Kha Kaeng wildlife sanctuary (Somchai, 1993). According to a report on ecological effects of Kaeng Sua Ten Dam project, there are one of two possibly surviving wild population of green peafowls in the north of Thailand at Doi Phu Nang national park. Furthermore, green peafowls had been found at Wieng Lor wildlife sanctuary and Sri Na Kha Rin national park. (Meckvichai *et al.,* 2001).

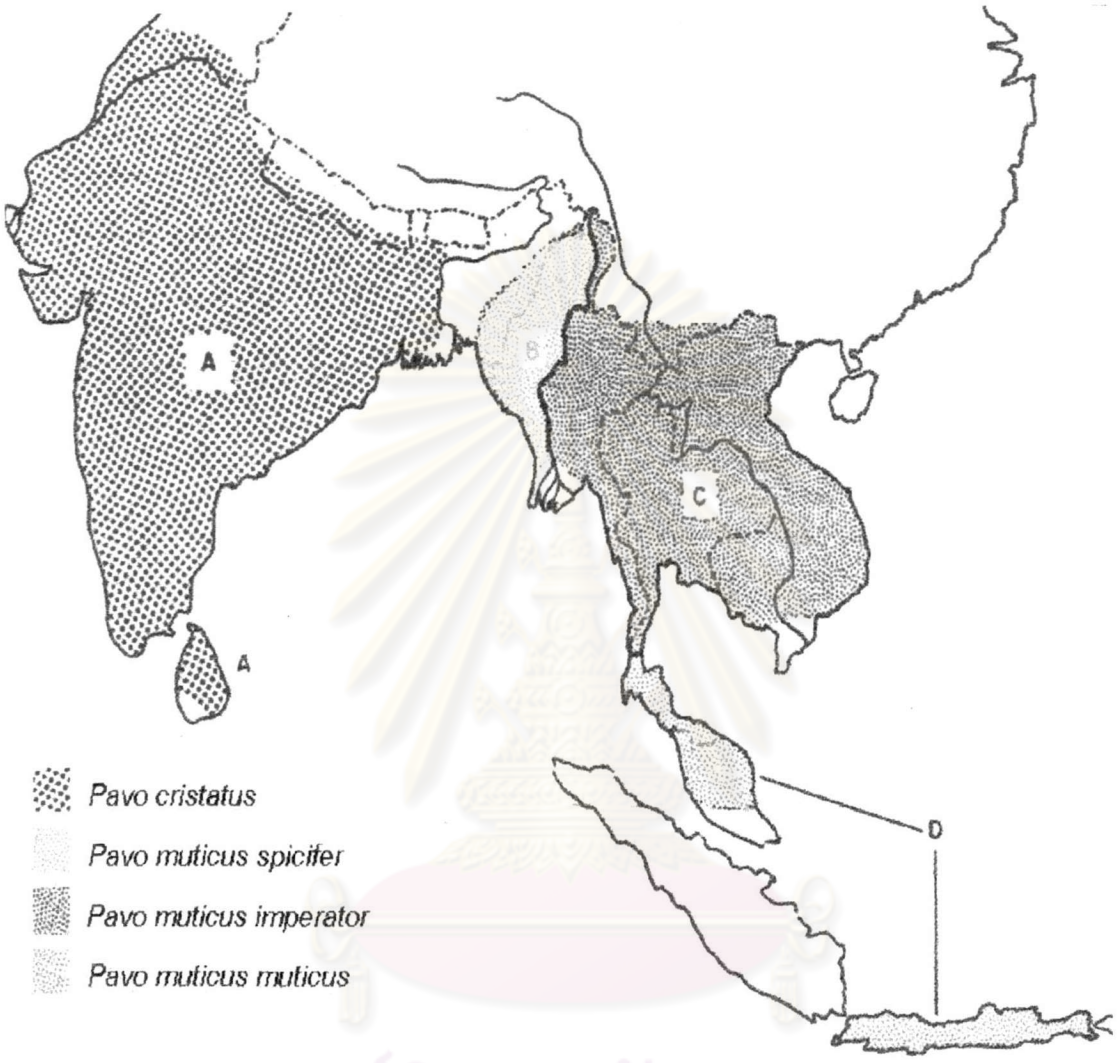


Figure 2.3 Distribution of the *Pavo* sp. (Delacour, 1977)

The distribution of Green Peafowl *Pavo muticus* (map opposite): (1) Dingba Qu; (2) Kamen He; (3) Deqen county; (4) Zhonghe; (5) Zhiju; (6) Liuku; (7) Yao'an county; (8) Weishan county; (9) Nanhua county; (10) Lufeng county; (11) Tengchong county; (12) Changning county; (13) Zhongyishe; (14) Yingjiang county; (15) Tuodian; (16) Maolan; (17) Longling county; (18) Ainishan; (19) Wuliang Shan Nature Reserve; (20) Jinping; (21) Husa; (22) Ejia; (23) Qingping; (24) Mile county; (25) Tongbiguan Nature Reserve; (26) Yingpan; (27) Zhelong; (28) Nongba; (29) Zhefang; (30) Wenjing; (31) Xiaomengtong; (32) Laochang; (33) Ailaoshan National Nature Reserve; (34) Mayidui; (35) Xingfu; (36) Zhehou; (37) Shuitang; (38) Yongkang; (39) Daxue Shan Nature Reserve; (40) Mengbang; (41) Ruili county; (42) Longwu; (43) Longpeng; (44) Zhenyuan county; (45) Matai; (46) Tuantian; (47) Baoxiu; (48) Mengsa; (49) Minle; (50) Xinfu; (51) Pingcun; (52) Jianshui county; (53) Quannei; (54) Gengma county; (55) Shuangjiang county; (56) Mengzi county; (57) Tongguan; (58) Nangunhe Nature Reserve; (59) Longtan; (60) Mengban; (61) Pu'er county; (62) Weiyuan river; (63) Simao county; (64) Zhengnuo; (65) Meng'a; (66) Mengyang; (67) Mengla county; (68) Manipur valley; (69) Churachandpur district; (70) Garjania; (71) Hukawng valley; (72) Kachin hills; (73) Chipwi; (74) Kaunghein; (75) Pidaung Sanctuary; (76) Talawgyi; (77) Homalin; (78) Bhamo; (79) Upper Chindwin; (80) Thabyebin; (81) Yuwa; (82) Mohlaing; (83) Tagaung; (84) Mabein; (85) Ingade; (86) Bandi; (87) Maymyo; (88) Maubin; (89) Thabyegyin; (90) Southern Shan States; (91) Kule; (92) Monnyin; (93) Magwe; (94) Payadaung; (95) Nattaung; (96) Kayinlegyin; (97) Gwethi Reserve; (98) Kyundawzu; (99) North Nawin Reserved Forest; (100) Paukkaung; (101) Chaunggwa; (102) Myohla; (103) Padaung; (104) Sandoway district; (105) Paungde; (106) Sabyin; (107) Papun; (108) Shwegyin; (109) Henzada; (110) Taikkyi; (111) Thaton district; (112) Insein; (113) Wimpong; (114) Myawadi; (115) Kawkarieka; (116) Ataran valley; (117) Salween river; (118) Hmawbi; (119) Tavoy; (120) Yabu; (121) Mergui; (122) Pakchan; (123) Chiang Saen; (124) Doi Chiang Dao Wildlife Sanctuary; (125) Doi Saket district; (126) Mae Yom National Park; (127) Doi Inthanon National Park; (128) Khun Tan; (129) Salawin Wildlife Sanctuary; (130) Chom Thong district; (131) Den Chai; (132) Ban Mut Ka; (133) Nam Jarim; (134) Li district; (135) Pak Pan; (136) Kamphaeng Phet; (137) Ban Salak Phra; (138) Umphang; (139) Huai Kha Khaeng Wildlife Sanctuary; (140) Thung Yai Naresuan Wildlife Sanctuary; (141) Srisaket province; (142) Non Luam; (143) Huey Sak; (144) Ban Kok Klap; (145) Lawa cave; (146) Ban Nakloea; (147) Ban Yaha; (148) Ban Houayxai; (149) Xiang Khouang; (150) Nam Xan; (151) Nam Ngiap; (152) Ban Nakhay; (153) Xe Banghiang; (154) Phou Xiang Thong NBCA; (155) Tha Teng; (156) Bolaven plateau; (157) Dong Hua Sao NBCA; (158) Dong Khanthung proposed NBCA; (159) Dong Kalo; (160) Taveng district; (161) Koh Ker; (162) Kaoh Nhek; (163) Ratanakiri; (164) Phumi Yuon Dong; (165) Snoul Wildlife Sanctuary; (166) Kirirom National Park; (167) Bokor National Park; (168) Sin Ho district; (169) Muong Lay district; (170) Dien Bien district; (171) Song Ma district; (172) Moc Chau district; (173) Luong Son district; (174) Ky Son district; (175) Da Bac district; (176) Kim Boi district; (177) Mai Chau district; (178) Tan Lac district; (179) Lac Thuy district; (180) Lac Son; (181) Quy Chau district; (182) Tan Ky district; (183) Huong Khe district; (184) Ky Anh district; (185) Tuyen Hoa district; (186) Thua Luu; (187) Bach Ma National Park; (188) Pleiku; (189) An Khe district; (190) Mang Giang district; (191) Dac Doa forest; (192) Xomgom; (193) A Yun Pa district; (194) Chu M'lang; (195) Ya Lop; (196) Ea H'Leo district; (197) Ea Sup district; (198) Buon Don district; (199) Yok Don National Park; (200) Cu Jut district; (201) Ea Kar district; (202) Ban Me Thuot; (203) Eaktur; (204) Dak Mil district; (205) Killplanol district; (206) Bu Gia Map Nature Reserve; (207) Da Lat; (208) B'srê; (209) Dac Nong district; (210) Dak R'Lap district; (211) Hon Quan; (212) Di Linh; (213) Bao Loc district; (214) Cat Tien National Park; (215) Bac Binh; (216) Kedah; (217) Bukit Jong; (218) Lenggong; (219) Ulu Sungai Soh; (220) Kuala Kangsar; (221) Ulu Pahang; (222) Kemaman river; (223) Kuala Tembeling; (224) Kempadang; (225) Pahang river; (226) Air Hitam Forest Reserve; (227) Bangi; (228) Mersing; (229) Cape Romania; (230) Pulau Panaitan; (231) Ujung Kulon National Park; (232) Merak; (233) Cikepuh; (234) Cilowa; (235) Pelabuhanratu; (236) Sampora; (237) Ciseureuh; (238) Tapos; (239) Gunung Gede-Pangrango National Park; (240) Ciogong; (241) Karawang; (242) Tanjung Sedari; (243) Purwakarta; (244) Cikelet; (245) Leuweung Sancang Wildlife Reserve; (246) Buahdua; (247) Cikawung; (248) Indramayu; (249) Cirebon; (250) Nusa Kambangan; (251) Pemalang; (252) Dieng plateau; (253) Kendal; (254) Alas Roban; (255) Gedangan; (256) Penawangan; (257) Banjaran; (258) Colo; (259) Gundih; (260) Purwodadi; (261) Clering; (262) Pati; (263) Wirosari; (264) Kradenan; (265) Ngaringan; (266) Randublatung; (267) Mantingan; (268) Cepu; (269) Alas Sengok; (270) Walikukun; (271) Paringan; (272) Padangan; (273) Pulung; (274) Jatirogo; (275) Besuki; (276) Nganjuk; (277) Tuban; (278) Jombang; (279) Wonosalem; (280) Kebonagung; (281) Lebakharjo; (282) Ranu Darungan; (283) Hyang plateau; (284) Gunung Ringgit; (285) Meru Betiri National Park; (286) Gunung Raung; (287) Krepekan; (288) Lijen; (289) Baluran National Park; (290) Alas Purwo National Park.

○ Historical (pre-1950) ◎ Fairly recent (1950–1979) ● Recent (1980–present) □ Undated

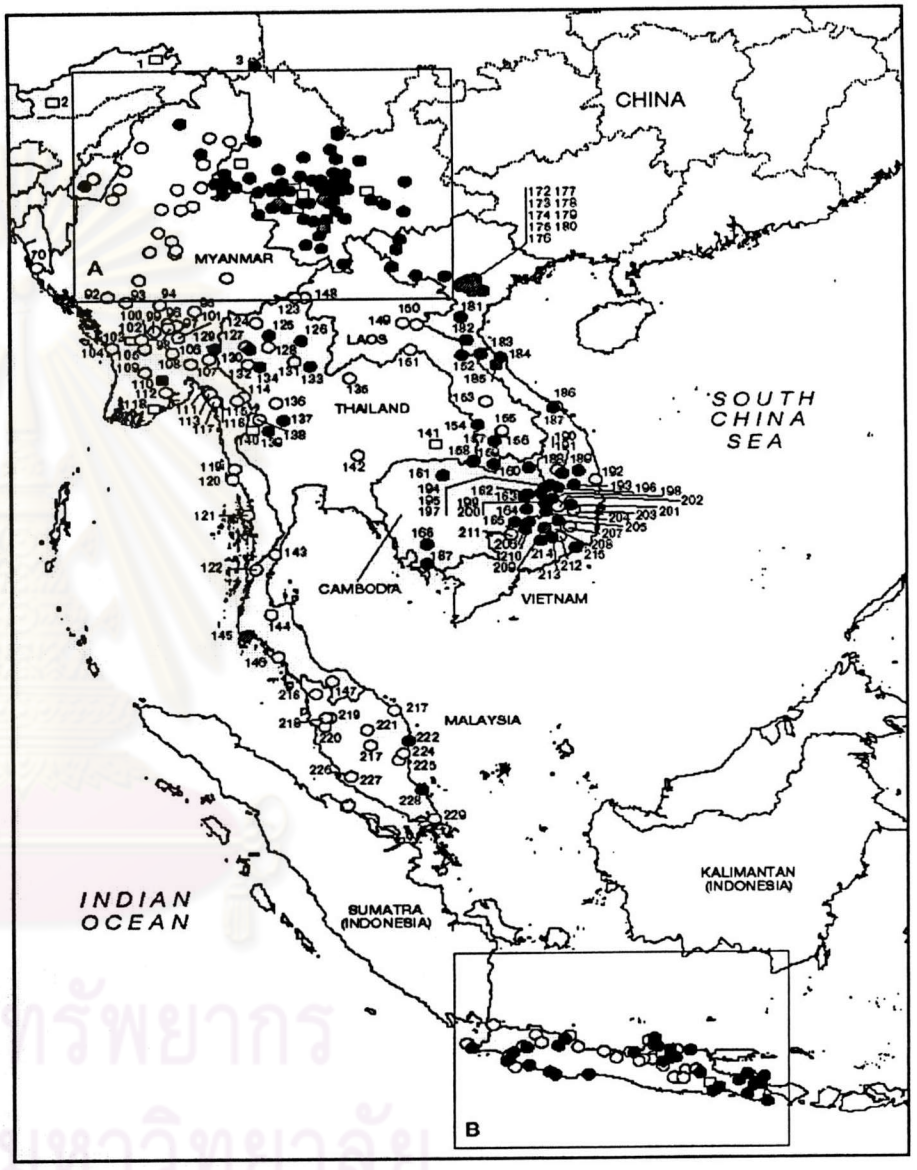
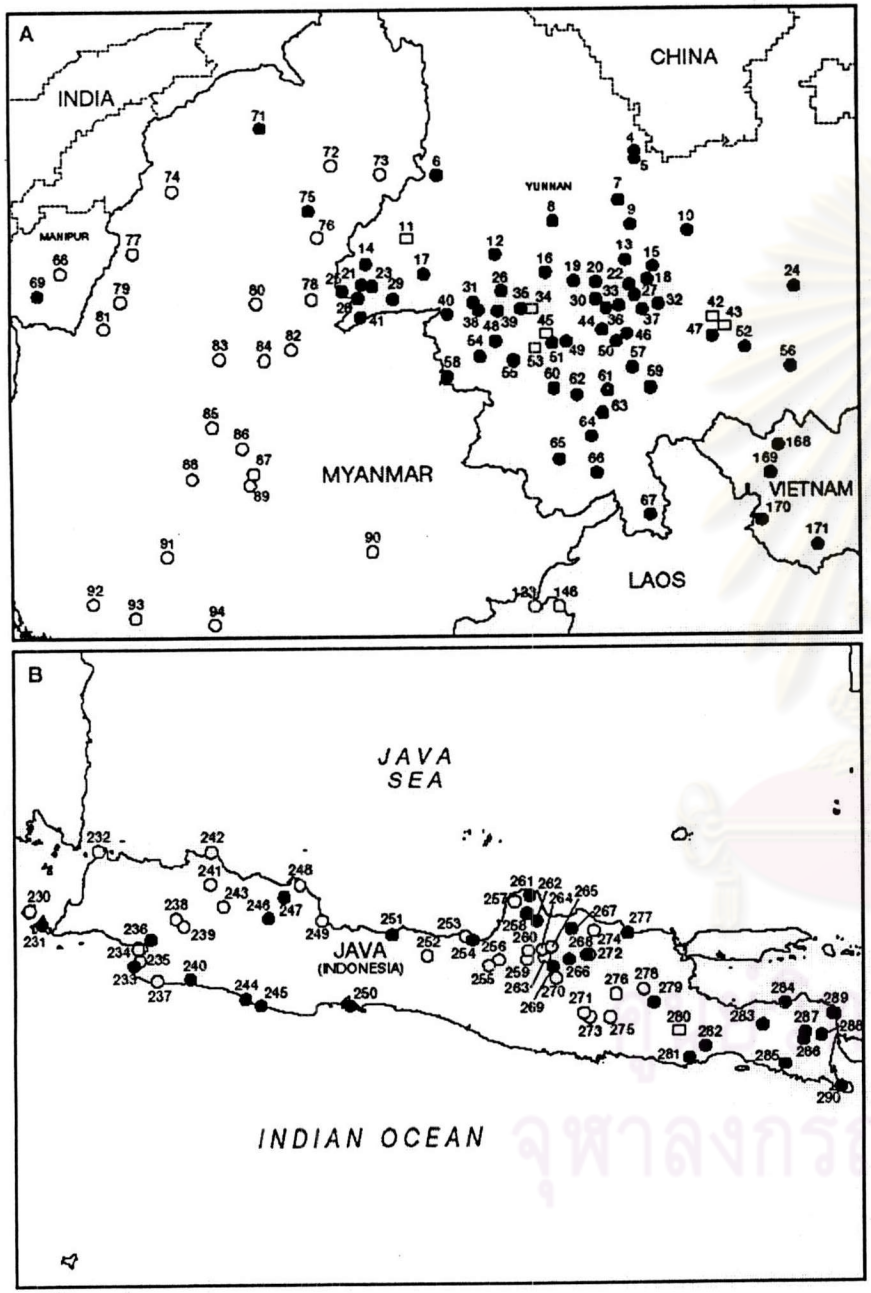


Figure 2.4A Distribution of Green Peafowl *Pavo muticus* from Red Data Book (BirdLife Internatiional, 2003)



The distribution of Green Peafowl *Pavo muticus* (map A opposite): (4) Zhonghe; (5) Zhiju; (6) Liuku; (7) Yao'an county; (8) Weishan county; (9) Nanhua county; (10) Lufeng county; (11) Tengchong county; (12) Changning county; (13) Zhongyishe; (14) Yingjiang county; (15) Tuodian; (16) Maolan; (17) Longling county; (18) Ainishan; (19) Wuliang Shan Nature Reserve; (20) Jinping; (21) Husa; (22) Ejia; (23) Qingping; (24) Mile county; (25) Tongbiguan Nature Reserve; (26) Yingpan; (27) Zhelong; (28) Nongba; (29) Zhefang; (30) Wenjing; (31) Xiaomengtong; (32) Laochang; (33) Ailaoshan National Nature Reserve; (34) Mayidui; (35) Xingfu; (36) Zhehou; (37) Shuitang; (38) Yongkang; (39) Daxue Shan Nature Reserve; (40) Mengbang; (41) Ruili county; (42) Longwu; (43) Longpeng; (44) Zhenyuan county; (45) Matai; (46) Tuantian; (47) Baoxiu; (48) Mengsa; (49) Minle; (50) Xinfu; (51) Pingcun; (52) Jianshui county; (53) Quannei; (54) Gengma county; (55) Shuangjiang county; (56) Mengzi county; (57) Tongguan; (58) Nangunhe Nature Reserve; (59) Longtan; (60) Mengban; (61) Pu'er county; (62) Weiyuan river; (63) Simao county; (64) Zhengnuo; (65) Meng'a; (66) Mengyang; (67) Mengla county; (68) Manipur valley; (69) Churachandpur district; (71) Hukawng valley; (72) Kachin hills; (73) Chipwi; (74) Kaunghein; (75) Pidaung Sanctuary; (76) Talawgyi; (77) Homalin; (78) Bhamo; (79) Upper Chindwin; (80) Thabyebin; (81) Yuwa; (82) Mohlaing; (83) Tagaung; (84) Mabein; (85) Ingade; (86) Bandi; (87) Maymyo; (88) Maubin; (89) Thabyegyin; (90) Southern Shan States; (91) Kule; (92) Monnyin; (93) Magwe; (94) Payadaung; (123) Chiang Saen; (148) Ban Houayxai; (168) Sin Ho district; (169) Muong Lay district; (170) Dien Bien district; (171) Song Ma district;

(map B opposite): (230) Pulau Panaitan; (231) Ujung Kulon National Park; (232) Merak; (233) Cikepuh; (234) Cilowa; (235) Pelabuhanratu; (236) Sampora; (237) Ciseureuh; (238) Tapos; (239) Gunung Gede-Pangrango National Park; (240) Ciogong; (241) Karawang; (242) Tanjung Sedari; (243) Purwakarta; (244) Cikelet; (245) Leuweung Sancang Wildlife Reserve; (246) Buahdua; (247) Cikawung; (248) Indramayu; (249) Cirebon; (250) Nusa Kambangan; (251) Pemalang; (252) Dieng plateau; (253) Kendal; (254) Alas Roban; (255) Gedangan; (256) Penawangan; (257) Banjaran; (258) Colo; (259) Gundih; (260) Purwodadi; (261) Clering; (262) Pati; (263) Wirosari; (264) Kradenan; (265) Ngaringan; (266) Randublatung; (267) Mantingan; (268) Cepu; (269) Alas Sengok; (270) Walikukun; (271) Paringan; (272) Padangan; (273) Pulung; (274) Jatirogo; (275) Besuki; (276) Nganjuk; (277) Tuban; (278) Jombang; (279) Wonosalem; (280) Kebonagung; (281) Lebakharjo; (282) Ranu Darungan; (283) Hyang plateau; (284) Gunung Ringgit; (285) Meru Betiri National Park; (286) Gunung Raung; (287) Krepekan; (288) Lijen; (289) Baluran National Park; (290) Alas Purwo National Park.

○ Historical (pre-1950) ◎ Fairly recent (1950–1979) ● Recent (1980–present) □ Undated

Figure 2.4B Distribution of Green Peafowl *Pavo muticus* from Red Data Book (BirdLife Internatiional, 2003)

2.6 Biology of green peafowl

The green peafowl's voice is loud, harsh and disagreeable. A loud braying; toong-hoong or "aow- aaw", emphasis on second syllable, given by the male. The female has a more nasal "aa-ow" call with emphasis on the first syllable. Most frequently uttered in early morning and at dusk (Boonsong, 1991). Furthermore, Ponsena reported that "kruk kruk kruk" is meaning an alert and site locating, "tok tok tok" is alarming call, "tak tak tak" is warning and high degree alarming.

The Green Peafowl often flock into a small group of 2-6 birds (Ponsena, 1988; Rojahadelok *et.al,* 1986; Arrathrakorn, 2001). It uses a wide variety of habitats, including an open forest which is preferable, a riverbank, a coastal scrub, a teak, a tea and coffee gardens, a forest edge and clearing, an area with dense secondary growth near shifting agriculture, and others (Delacour, 1977; Rojahadelok *et.al,* 1986; Ponsena, 1998). It can fly weakly so it spends most of its time on the ground looking for food or perching. It is omnivorous. It likes to eating berries, pears, and other fruits, including rice-grain and seedling such as grass seed. Also, it can eat crickets, dragonflies, small moth, etc., and frogs and lizards, etc. (Humphrey and Bain, 1990). At Doi Phu Nang national park, it can be also fed on *Heteropgon contortus, Antidesma ghesimbia* , *Onchna integerrina* , *Vegna mungo and Zea mays* (Arrathrakorn and Meckvichai, 2000). Green peafowl frequently takes small pebbles or sand for helping in digestive system (Ponsena, 1988).

The green peafowl sleeps on the roost when light concentration is less than 8 lux (Subramanian and John, 2001). It is also found that sometimes there are more than one peafowl roosting in a single tree (Rojahadelok *et al.,* 1986). Generally in breeding season, the bird will return to sleep at the same tree for a long period of

time except when it has some disturbances, in this case it will move to a nearly taller tree (Ponsena, 1988).

In breeding season, the male peafowl will display the fan. The fan spread for up to 15 minutes. The events sequence of display is into 3 steps, wing-fanning, shiver and hoot-dash.

Peafowl is polygamous, so four or five females may be mated to one male. Moreover, it has especially behavioral characteristic. During the breeding season, the dominant male from Huay Kha Kheng will move to sand bars along the main stream and create a breeding territory but the male from the north will move to hill ridge for creating a breeding territory. It tries 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 territories. The male uses calling signals and displays to induce females to come into his territory. Mating usually occurs in the morning and in the late afternoon. A mated begins to lay eggs at 2 months of age (Humphrey and Bain, 1990). A Female usually lays 3-5 eggs in a shallow hole dug on the ground. The female incubates the egg for approximately 28 days by herself. After hatching, the young chicks follow the mother, even though they are capable of foraging on their own. Arrathrakorn and Meckvichai (2000) reported that a breeding season of green peafowl at Doi Phu Nang is from November to April. They create breeding territories on the top of the hill and also found at the bottom of the hill. The clutch sizes are for 4-6 eggs. The chicks grow quickly, able to fly good distances at two weeks of age (Zoological Parks and Gardens Board of Victoria, 2003). The nestling and hatching are abundantly found in May. In captive breeding of Chiang Mai Zoo, green peafowl has a breeding season from December to April (Rattanawiboon, 1986). Green peafowls have a

good sense of seeing and bearing. They usually can run away from human from far distance. It prefers to run away from an enemy but, for sudden alarm, they will run for a short distance and then fly up into the air (Rojahadelok *et al.,* 1986).

2.7 Sample collection in an endangered species

The late 1980's, 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 animals was difficult. The development of the polymerase chain reaction (PCR) technique 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, feces of an extinct or endangered species and microgram amounts of tissue from museum skin or preserved specimen.

Collection of DNA from feces are becoming widespread due to the fact that feces is a readily available animal material that can be collected completely non-intrusively (without requiring permission for sampling and transport), and from species that are difficult to trap or encounter in the wild. But systematic with Bonobo (*Pan paniscus*) have shown some potential risks associated with PCR amplification from feces. By re-extracting the same feces sample and performing multiple PCRs, it has been shown that some heterozygous individuals falsely appear to be homozygous following PCR (Gerloff *et al.,* 1995).

After breeding season (in March), Green peafowl starts to shed its train off in the wild so the feather sample is taken in this month.

But blood sample is collected from Wildlife and Breeding Station (captive).

2.8 Genetic variation

The loss of genetic variation is caused by reduced population size and habitat fragmentation which are important for biodiversity conservation. Genetic variation is a highly desirable characteristic (Woodruff, 1990). Genetic variation can be monitored directly and indirectly in a number of ways. Several genetic markers have previously been proven to be useful. Studies of allozyme variation have been the most commonly employed approach during the last twenty years. More recently, there are several molecular genetic approaches to monitoring genetic variation and determine relationships between individual birds, population, species including mitochondrial DNA (mtDNA), restriction fragment length polymorphism (RFLP) analyses, whole genomic DNA-fingerprinting and direct sequencing of mtDNA and nuclear DNA loci. Random amplified polymorphic DNA (RAPD) and Variable Number of Tandem Repeats (VNTR). Such techniques facilitate very fine detail analysis of microevolutionary process (Avise, 1994). However, these techniques have many different advantages and disadvantages. They are mentioned in the next topic. Some appropriate molecular genetic markers will choose to use for study genetic variation and genetic population in *P. muticus*.

2.9 Genetic markers in the conservation of biodiversity

2.9.1 Allozymes

Allozymes are the variant protein products of allelic genes in the same chromosomal DNA locus (Park and Moran, 1994). Polymorphism of allozymes can be detected by the different migration of investigated proteins in an electrical field. Protein bands can be visualized by histochemical stains specific for

the enzymes. Allozyme electrophoresis has been used as a predominant molecular technique to characterize population level genetic variation in many avian researches because this technique is a relatively easy, inexpensive and fairly rapid to perform on a large scale. Furthermore, a number of loci can be screened simultaneously.

Some avian species have adequate demographic data for estimating effective genetic dispersal and N_e. In the house sparrow (*Passer domesticus*), Fleischer (1983) used demographic data to predict F_{st}, and then tested the prediction with allozyme data and concluded that these birds approximated a stepping stone model of genetic structure.

However, this technique has certain limitations. Because of redundancy in the DNA code that dictates protein sequences, changes in some base at the DNA level may not change amino acid residues in polypeptide chain. Besides, this technique is based on the different net charge of investigated proteins. Theoretically, sixteen of the common amino acids are electrostatically neutral consequently, nonsynonymous mutation from one neutral amino acid to other neutral base does not change the net charge of the polypeptide chain (Nei, 1987). Accordingly, many genetic variants are not detected by protein electrophoresis. Interpretation of allozyme data also pose other problem. Since allozyme electrophoresis only detects actively expressed proteins that react to histochemical stain (Hunter and Markert, 1957) and these genes constitute only a small percentage of the whole genome of an animal therefore only a small proportion of DNA sequence variability can be detected. Besides, this molecular marker used tissue sampling for investigation so it does not appropriate method to study genetic information in rare or in endangered species such as *Pavo muticus*.

2.9.2 Mitochodrial DNA

Mitochondrial DNA is DNA that found in mitochondria of eukaryotes. Each mitochondria contains 5-10 copies of double-standed circular DNA. This extrachromosomal DNA is about 15,700-19,500 bp in length (Brown *et al.*, 1979) composed of 13 protein coding genes, 2 genes coding for ribosomal RNAs (12S and 16S ribosomal RNA), 22 transfer RNA (tRNA) coding genes and the control region which containing initiation site for replication and transcription.

Mitochondrial DNA evolves 5-10 times faster than single copy nuclear DNA (Brown *et al.*, 1979). This property provides the magnifying ability to distinguish and identify the differences between populations and between closely related taxa. Since evolution rates of each region are various, mtDNA can be used for study in multilevel.

For instance, the extremely conserved in protein coding genes, three subunit of cytochrome c oxidase (COI, II and III) and cytochrome b, are appropriate for the studies of population genetic questions in distantly related taxa. While the evolutionary rate of 12S and 16S rRNA genes is useful for evolutionary studies between distantly related species (Meyer and Wilson 1990; Meyer and Dolven, 1992, cite in Meyer, 1994).

Mitochondrial has been used as a molecular technique to characterize population level genetic variation in many avian researches.

2.9.1.1 Avian mitochondrial DNA

The first complete sequence of an avian mitochondrial genome was published from chickens by Desjardins and Moriais (1990) (Figure 2.5). It showed highly conserved features when compared to other vertebrate mtDNA.

Since many features are the same in all the vertebrate mtDNAs, the avian genome has some remarkable differences. First, the avian gene order is novel when compared to the mammalian's. The ND5 gene is followed by cytochrome b gene, $tRNA^{Thr}$ and $tRNA^{Pro}$ genes, ND6 and $tRNA^{Glu}$ genes in the 5'→3' direction of the avian L-strand (Desjadins & Morais 1990, Quinn & Wilson, 1993). Second, the L-strand replication origin that is found between $tRNA^{Cys}$ and $tRNA^{Asn}$ genes in other vertebrates is absent in the avian genome (Desjadins & Morais, 1990).

2.9.1.1.1 Cytochrome b

Cytochrome b is one of the cytochromes involved in the electron transport system. It is the only cytochrome coded by mitochondrial DNA. The cytochrome b gene is the most widely used gene for phylogenetic. Although it evolves slowly in terms of nonsynonymous substitutions (Irwin *et al.,* 1991). Cytochrome b is variable enough to study at a population level, and conserved enough to clarify phylogenetic relationships in deeper details.

2.9.1.1.2 Control region of mtDNA

The mtDNA control region is the only large non-coding region in avian mitochondria. It varies from 1,044-1,227 bp in *Gallus domesticus* (Desjadin &

Morais, 1990). It contains the heavy strand replication origin. This region is divided into three domains identified by Desjadeans and Morais (1990) and Quinn and Wilson (1993) from *Gallus domesticus*. The first domain at the 5' end of the control region contains C-stretch and high variation. C-stretch is specific to the 5' terminus of the avian control region. It is present in various forms at least in Anatidae, Phasianidae and Poridae (Quinn & Wilson 1993, Desjadins & Morais 1990). The central domain is the most conserved. The most variable part is usually the third domain at the 3' end of the control region. Also, it is highly variable in other birds (Wenick *et al.,* 1993*)*. This variability has lead to the expanding usage of control region sequence to examine questions ranging from population structures to phylogenetic relationships. This region has already been proven to be quite a powerful tool in elucidating the global population structures in shorebirds (Wenick *et al.,* 1993) and fringilline finches (Marshall and Baker, 1997), DNA polymorphism in two local populations of blue tit *Parus caeruleus* (Taberlet *et al.,* 1992), phylogeography studies of Nearctic songbirds (Mila *et al.,* 2000) in revealing recent mixing of maternal lineages in snow geese (Quinn, 1992) and inevaluating gene flow between social groups and populations in babblers (Edwards, 1993), Moreover, this region can be used in geographic analysis of many species such as song sparrow (Adam *et al.,* 1998), blue chaffinch (pestano *et al.,* 2000).

However, since mtDNA is haploid and uniparentally inherited therefore effective population size is reduced to one fourth that of nuclear DNA (Nie and Li, 1979; Birky *et al.*, 1989 cited in Ward and Grewe, 1995). Thus, due to a single-parent inheritance mode of mtDNA, testing against Hardy-Weinberg proportion and other most standard population analyses impossible.

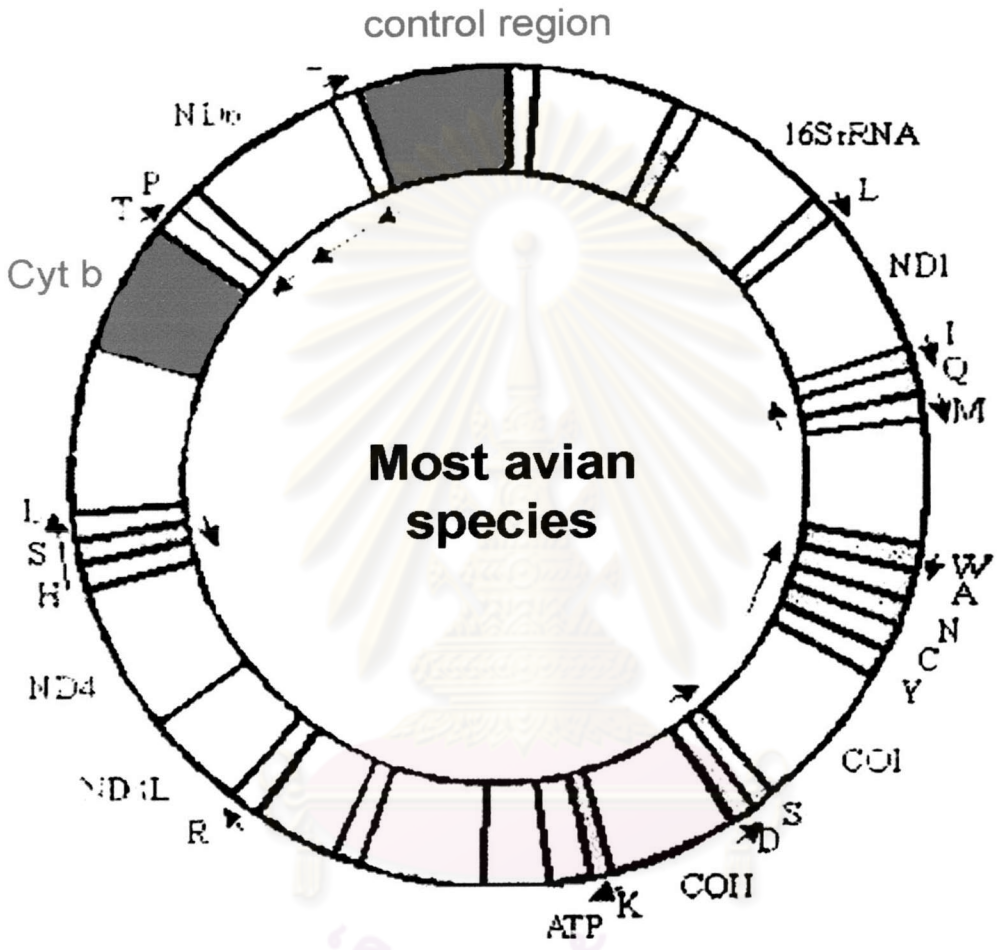


Figure 2.5 Mitochondrial genomes of birds. The outer circle represents the heavy (H) strand and the inner circle represents the light (L) strand. Polarity of transcription and the transcribed strand is shown with arrowheads (Desjadeans and Morais, 1990).

2.9.3 Variable Number of Tandem Repeat (VNTR)

VNTR markers are characterized by a core sequence that consists of a number of identical repeated sequences. They can be divided into three categories based on their repeat length including satellite, minisatellite and microsatellite.

2.9.3.1 Satellite

Satellite DNA is the first of tandem repeated DNA sequence to be discovered. It was named by its appearance as minor of "satellite" band that separated from the "bulk" DNA upon buoyant density gradient centrifugation (Britten *et al.*, 1968). The basic repeat unit of satellite can vary from hundreds to thousands of base pairs. Satellite DNA may occur as million of copies per genome in some mammals (Alberts *et al.*, 1983). Nevertheless, they are not as variable in size within population as the other members of highly repetitive DNA family are.

2.9.3.2 Minisatellites

Minisatellite is a tandemly repeated nucleotide sequence which ranging between 9-65 bp per repeat unit and typically 0.1 to 20 kb in size (Wright, 1993). Minisatellite is called variable numbers of tandem repeats (VNTR) because increases and decreases in the lengths of the repeat array was found that resulted from changes in the number of repeat copies residing in the region (Avise, 1994). Minisatellites are found within noncoding regions of genomic DNA tending to cluster at telomeric region of chromosome where known as "hot spot" for homologous recombination (Wahls *et al.*, 1990). The mechanism generating variability in minisatellites is still inconclusive. Several models have been suggested including unequal crossing over between homologous chromosomes at

meiois or mitosis, replication, slippage and gene conversion within repeat units (Wolff *et al.*, 1989).

The variation of minisatellite loci can be detected due to difference in length between conserved restriction sites. The approach to detect this was developed first by Jeffrey and colleagues at Leicester University (1985) known as DNA fingerprinting. This method involved hybridizing restriction enzymes digested DNA with repetitive probes. The pattern of DNA is specific for each individual and is inherited in a Mendelian fashion. These characteristics of minisatellites have allowed their application in pedigree construction (Wright, 1993). The principal problem of DNA fingerprinting was that the pattern of DNA generated by multilocus probes (about 20 or more bands per individual). There were no practical ways of knowing which pairs of bands in the fingerprint represented alleles at a particular minisatellite locus. Therefore, it was impossible to calculate allele frequencies. This problem seriously constrain attempts to estimate gene flow or other population parameter employing the usual statistical algorithms that require straightforward Mendelian markers (Avise, 1994).

Two approaches have been developed for single locus analysis, use of refined DNA probes (Georges, 1991) and PCR-based methods (Jeffrey *et al.*, 1988; Jeffrey *et al.*, 1994 and Galvin *et al.*, 1995b). The problems of the first one are finding of a suitable probe and using Southern blotting. The latter is more convenient, using specific primers flanking the array. PCR products are separated by standard gel electrophoresis, and can be visualized by a variety of methods. However, in conventional PCR, fragments much larger than 4 of 5 kb are not readily amplified and detected thus the large minisatellite loci can not be done. Moreover, highly difference in size of the large allele and small allele make it

difficult to separate and determine size of alleles by electrophoresis manner (Budowle *et al.*, 1991 and Taylor *et al.*, 1994).

2.9.3.3 Microsatellites

Microsatellites are short DNA consisting of short repeats (1-6 nucleotides) which arrayed in tandem repeated manner for approximately 10-50 copies (Hearne *et al.*, 1992). Most observed mutations at microsatellite loci involve increase or decrease in copy number of repeat unit (Hastbacka *et al.*, 1992; Mahtani and Willlard, 1993; Weber and Wong, 1993 cited by O'Reilly and Wright 1995). Due mainly to high mutation rate of the microsatellite loci (10^{-6} to 10^{-2} per generation), arrays of the repeat have been found to vary dramatically in length (from several to hundreds of basepairs) providing a plentiful source of allelic polymorphism (Anger and Bernatchez, 1997).

Microsatellite arrays are embedded in unique DNA sequence, sometimes within the coding region of genes, but more commonly in the untranslated regions of the genome (Valdes *et al.*, 1993). Like single locus analysis of minisatellites, variation of these arrays can be determined by using PCR-based method. The method for microsatellite marker developing consists of genomic library construction, screening for microsatellite clones by hybridization with repetitive probe, sequencing and primer design (Tautz, 1989 and Weber and May, 1989). Different from minisatellite, microsatellite alleles vary in size by decrease or increase of small-sized repeat unit therefore the length of a microsatellites allele at a specific locus can be easily determined by size fractionation with acrylamide gels. Microsatellites are represented as the markers of choice because there are some advantages of utilizing microsatellites over other markers, which make them

desirable. First, they are abundant widely spread throughout the chromosome and are highly polymorphic in eukaryotic genomes. It was estimated that one microsatellite locus may be found every 10 kb in eukaryotic genomes (Tautz, 1989). This characteristic provides utility in genome mapping. Second, by PCR approach, a very small quantity of DNA (e.g., many social insects) and low quality or degraded DNA can be used for analysis. Besides this, the utility of a PCR approach also allow the possible processing of a large number of samples generally required for population surveys and large breeding programs (Brooker *et al.*, 1994). Third, since microsatellites have a high rate of mutation which causing extensive allelic variation, the power of discrimination between closely related populations is increased (Wright and Bentzen, 1994). Microsatellites have been found to be variable even in populations that have low levels of allozyme and mitochondrial variation (Paetkau and Strobeck, 1994). Nevertheless, there are several report show that some microsatellite loci are conserved enough to allow various applications in closely related species. Finally, co-dominant Mendelian inheritance makes microsatellites more informative in pedigree studies, as well as in population studies (Wright and Bentzen, 1995).

At present, a lot of publications show the application of microsatellite in birds or other animals. Publications of Microsatellite analysis in bird such as the study of microsatellite variation in the critically endangered San Clemente Island loggerhead shrike between mainland and island (Nicholas *et al.*, 1997). Zhou reported genetic characterization of biodiversity in highly inbred chicken lines by microsatellite markers (1999). The difference of domestic pigeon and wild rock pigeon were investigated by using microsatellites (Traxler *et al.*, 2000). In addition, five microsatellite markers are tools for conservation in Japanese marsh warbler

and examine their allelic variation in natural population and cross-species amplification in other related species of warblers (Yusuyuki *et al.*, 2000).

In addition, the development of microsatellite analysis in bears from three Canadian interpopulation differences in genetic variation in black bears from three Canadian National Park, they found low variation in three Canadian National Park (Paetkau and Strobeck, 1994). The pattern of microsatellite polymorphism were compared in chimpanzees and endangered great ape for understanding genetic structure of great ape for management conservation program in Africa (Reinartz, 2000). Furthermore, microsatellite was used in many organisms such as cattle (Moore *et al.*, 1991), primate (Reinartz *et al.*, 2000), sheep (Ivanna *et al.*, 2002), lizard (Natasha *et al.*, 2000), fungi (Johnnesson *et al.*, 2000), rice (Brondani *et al.*, 2000) and eelgrass (Thorsten and Reusch, 2000).

Publication from birds (Primmer *et al.*, 1996) and cattle (Moore *et al.*, 1991) suggest a 50% success rate in cross-amplification and polymorphism detection in species which diverged from 10 to 20 Million year ago. This is in agreement with the empirical finding that cross-species amplification works for closely related taxa such as species belonging to the same genus or to recently separated genera (Zane *et al.*, 2002). In 2001, Baratti *et al.* found the common pheasant can cross-species amplification from turkey and chicken primers and in the same year, Randi shown the Ruffed pheasant can cross-species amplification from domestic fowl primers. In this reason, it demonstrated that sequences flanking the repeats of microsatellite loci are often conserved between closely related taxa, thus allowing cross-species amplifications. Peafowl and chicken are belong to the same family of Phasianidae. Thus cross-species amplification in *P. muticus* may occur by using chicken primers.

2.9.4 Random amplified polymorphic DNA (RAPD) marker

RAPD markers are produced by PCR using short oligonucleotide primers (typically 10 bp long) which have random sequence. (Welsh and McClelland, 1990 and William *et al.*, 1990). Unlike conventional PCR technique, this technique uses only a single primer for amplification. Because the amplified fragments are those regions of the genome that are flanked by "inward-oriented" sequences complementary to the primer. Under low stringency condition, a number of PCR products are generated from random locations within the genome (Dear, 1997 cited by Hillis, 1996). Allelic variation depends on the presence or absence of these particular amplification products, which can be separated on agarose gels stained with ethidium bromide. Polymorphism of alleles may result from mutation of a primer recognition site which prevent its amplification or from insertion that change the size of DNA segment (William *et al.*, 1990 and Avise, 1994)

RAPD method is quick, simple, relative inexpensive and numerous markers can be developed easily by changing sequences or number of nucleotide in the primer. However, since RAPD markers are mostly inherited as dominant alleles, information on the parental origin of alleles may be inaccessible (Lewis and Snow, 1992). Owing to short length of primer and low stringency of PCR condition, RAPD markers may produce some artifact of amplification products therefore careful control of DNA quality and amplification conditions is necessary to ensure reproducible banding patterns (Carson *et al.* 1991; Riedy *et al.*, 1992 and Scott *et al.*, 1993). RAPD was used in many organism, it was investigated in estimating the intra and inter genetic variation in three varieties of guinea fowl (Deepak, 1998) and using in shrimp(Anchalee, 1998) and oyster (Klinbunga, 2001).

2.10 Genetic marker in *Pavo muitcus imperator*

Only one report studied the genetic variation in *P. m. imperator* (Plubcharoensook, 2000). Two populations of green peafowl between Doi Phu Nang national park and Wieng Lor wildlife santuary were inveatigated genetic variation which these two areas are unbounded with small roads and villages. The aim of Plubcharoensook's study was examined to segregate of 2 green peafowl population. Pattra reported that genetic variation of *Pavo muticus* between Doi Phu Nang national park and Wieng Lor wildlife santuary populations by 330 bp D-loop nucleotide sequences. Doi Phu Nang national park has 26 variable sites, 14 informative sites, 16 transition sites and 8 transversion sites. Genetic distance was 0.0000-0.0513. Furthermore, the D-loop sequence analysis shown that *P. m. imperator* within Doi Phu Nang national park are higher divergent and more variable than *P. m. imperator* within Wieng Lor wildlife sanctuary population. From phylogenetic tree is divided into two groups, all of the samples did not separated clearly. In addition, there are some green peafowl begin to be diverged. It is possible that both areas are located closely and two populations can still be involved. Furthermore, these populations were separated not for long time by geographical barriers. There are mountains and roads separating two populations. These populations still share more character of ancestors, because in the former, these two populations may be continuous areas. At present, both populations were separated by two roads. There are many villages along two side of the road. However, the study found that both populations begin to separate into subpopulation. In the future, two populations may be completely separated. Green peafowls from these populations can not move and flow back and forward.

Then, the conservation may be considered because metapopulation or habitat fragmentation may occur.

In the sustainable conservation of *P. muticus*, we need more some informations such as pedigree analysis for breeding and release them to the forest. But mitochondrial DNA marker does not give this information.

Moreover, there are many reports that shown advantage of microsatellite more than mitochondrial DNA such as the problem of unmask hybrids from *C. pictus* (golden pheasant) with only paternal introgression of *C. amherstiae* (Lady Amherst pheasant). As the mt-DNA is maternally inherited, it can detect only maternal hybridisation. Randi (2001) is currently checking. 50 different microsatellite loci, which were originally isolated in the domestic fowl. These microsatellites will be used to obtain information on eventual paternal hybridisation.

Thus, the chosen genetic markers are microsatellite marker and RAPD analysis for study the genetic variation in *Pavo muticus imperator* in Thailand. Microsatellite is chosen because it exhibits high levels of allelic polymorphism. It can determine genetic variation at intraspecific level when populations of such a species are inbred and can carry out pedigree analysis in selective breeding programs (Gamow, 1954). In the case of RAPD, it is chosen because RAPD is a genetic marker that does not require the prior knowledge of *P. m. imperator* genome.