CHAPTER I

INTRODUCTION

Pheasants are the most beautiful birds and in the present we can domesticate pheasants to commercial domestic animals in house or farm under Thai protected animal and endangered species law (1992). The breeder can make a profit by selling to interested people who love and need to domesticate them as a hobby or entertainment. They are classified in Family Phasianidae. They are terrestrial bird with plump body, strong and unfeathered leg; and strong bills. They can fly strongly on broad, rounded wings, although they can fly but most species prefer to run away from danger or enemies. They are highly secretive and difficult to observe, though some species have distinctive calls. The foods of them are seeds in primary or destruction or fragmentation of their forest habitats, combined with illegal hunting or capture. The world gallopheasants are 189 species while 8 species are distributed in Thailand. (Lekagul and Round, 1991). In this study are included five different species in four gallopheasants: Great Argus (*Argusianus argus argus*), Red Junglefowl (*Gallus gallus spadiceus*), Siamese Fireback (*Lophura diardi*), Green Peafowl (*Pavo miticus imperator*) and a partridge: Crested Wood Partridge (*Rollulus roulroul*). For these studies' species IUCN classified as near threatened species; Great Argus, Siamese Fireback, vulnerable species: Green Peafowl and least concern species; Red Junglefowl and Crested Wood Partridge. In 2003 ministry of Natural Resource and Environment allow to domesticate 42 pheasant species as commercial domestic animals and five species in this thesis into this list.

1.1 Classification of five different species in four gallopheasants: Great Argus, Red Junglefowl, Siamese Fireback, Green Peafowl and a partridge: Crested Wood Partridge.

Kingdom Animalia
Phylum Chordata
Subphylum Vertebrata
Class Aves
Subclass Neonithes
Order Galliformes
Suborder Galli
Family Phasianidae

Great Argus:
Genus Argusianus
Species *Arusianus argus*
Subspecies *Arusianus argus argus*
Common Name: Great Argus, Argus pheasants.

Red Junglefowl:
Genus Gallus
Species *Gallus gallus*
Subspecies *Gallus gallus spadiceus*
Common Name: Red Junglefowl, Burmese Red Junglefowl

Siamese Fireback:
Genus *Lophura*
Species *Lophura diardi*
Common Name: Siamese Fireback pheasant

Green Peafowl:
Genus Pavo
Species *Pavo muticus*
Subspecies *Pavo muticus imperator*
Common Name: Green Peafowl

Crested Wood Partridge:
Genus Rollulus
Species *Rollus roulroul*
Common Name: Crested Wood Partridge, Roul-roul partridge, Green wood partridge or Red-crowned wood partridge

1.2 Biology of five different species in gallopheasants and partridge

1.2.1 The Great Argus

Genus Argusianus

The Great Argus is a very large, tropical pheasant in which sexual dimorphism is highly developed (Johnsgard, 1999). There are three subspecies of Great Argus. Firstly was Malay Great Argus (*Argusianus argus argus* Linnaeus, 1766) distributed in the Malay Peninsula, North currently only to about the southern part of Thailand and Sumatra Island. Secondly was Bornean Great Argus (*Argusianus argus grayi* Elliot, 1865). They are only found in the island of Borneo, Indonesia and thirdly were Double-banded Great Argus (*Argusianus argus bipunctatus* Wood, 1871) this subspecies was considered by Delacour (1977) and Beebe (1918-1922) to be come extinct. In the present, they have only a single feather's sample of unknown origin but assumed they may be possibly from Tioman Island, Malaysia (Johnsgard, 1999).

The Great Argus is one of the most highly specialized pheasants, and, though the male may look dull and somewhat ungainly when seen walking in the open area but his display is one of the most remarkable in the bird world. The main feathers of the genus are the tremendous length and width of the secondaries with their intricate pattern and beautiful ocelli, the complicated design of the primaries and the length of the two central rectrices. Great Argus is found in the Malay Peninsula, Sumatra and Borneo, the two last-named forming subspecies. This pheasant is a bird found from sea-level up to 4,000 feets, far from the haunts of man. It prefers old forest growing on steep dry hillsides or ridges and, since it is an extremely shy and wary bird, it is very difficult to observe. The male has the well-known habit of making a display or dancing arena. Which he guards jealously during the breeding season. The size of such a display ground varies, but it is usually roughly circular and 4 to 5 yards in diameter. From it the male assiduously removes all sign of leaves, twigs and forest debris, keeping the surface as a clean as if it had been raked. At this time the arena and its immediate

vicinity becomes his territory. The females come to the male's dancing ground when they feel the urge to mate and are attracted by his loud call. After his specialized plumage a display, the male's voice is the Argus's most singular characteristic. Various descriptions are given in the literature, some of which are contradictory. Wayre (1969) have studied carefully and made tape recordings of the calls of an adult male Argus which was caught in Borneo and has lived in the Pheasant Trust's collection for six years. During the spring and summer this bird roosts high up on the wire-netting overhang surrounding his enclosure and when the weather is fine he calls frequently during the months May to October. As dusk approaches his loud cry can be heard more than half a mile away. He continues to call intermittently as darkness falls, them more frequently up to midnight or even later if there is bright moon. His normal call is a single penetrating hoot, which reminds one of Tawny Owl or a Gibbon. It is remarkably loud and consists of two syllables 'wow-wow'. The second being a semitone higher and louder than the first. This call is repeated at intervals of fifteen to thirty seconds or longer, and the bird can sometimes be persuaded to reply to sudden loud noise.

Malay Great Argus (*Argusianus argus argus*)

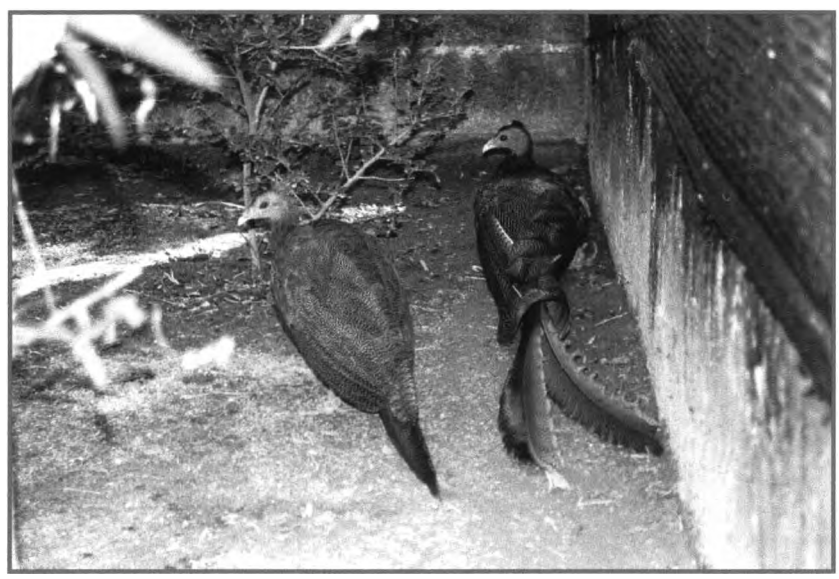

Figure 1.1 Malay Great Argus (*Argusianus argus argus*) at Soi Dao wildlife research and breeding station, Chantaburi province photographed by Miss Sudthida Sudhtharmvilai.

The male Malay Great Argus has a velvety-black crown with a small crest and the nape and hind neck are covered with narrow grey and black feathers. The rest of the head and neck is naked and blue, with a few thin feathers, the ear coverts are brown and the mantle and wing coverts very dark brown spotted with buff; the back is bright ochrous yellow with black spots. The tertiaries are large, spotted with dark reddish-brown, white and buff, the inner web being grey and the outer having large ocelli similar to those of the secondaries, but less perfect. The secondaries far exceed the primaries in length and are broad and almost square tipped; these feathers are highly specialized and of a most intricate pattern. The inner web is grayish, shading to white along the edge, and is covered with dark brown spots circled with pale buff. The border of the outer web is buff with blackish-brown spots and the centre buff with wavy longitudinal lines of blackish-brown. Along the shaft lies a chain of large and beautiful ocelli, each being a mixture of purplish-brown shading through a greenish tint to yellow and grey; each is circled with black and buff, and they are separated by small areas of buff irregularly marked with black. The broad tip is grayish-brown spotted with white, each spot being circled with black. The primaries are of normal length and are stiff. The shaft is cobalt-blue covered with elongated spots of dark brown and rufous. The inner web is wide, the outer half being pale grayish-buff covered with elongated single or double dark brown spots circled with light chestnut. Along the shaft is a narrow pale grey band with a chain of yellow spots separated by thin black lines, and between these areas is a wide band of reddish-chestnut with numerous tiny white spots, each narrowly encircled with blue. The shorter tail coverts are yellowish-buff spotted with black, the longest grey and brown reticulated with white. The two central rectrices are often over 1,100 millimeters long and between 80 millimeters and 90 millimeters wide. The tip is twisted and pale grey, the shaft black and the outer web dark grey, shading to deep chestnut on the border and profusely covered with white spots circled with black. The inner web is grey, darker along the shaft, with irregularly shaped white spots, those nearer the shaft being circled with black. The five pairs of short rectrices are very broad and have blunt tips of the same color as the centre ones, only duller. The upper breast is dark chestnut and the rest of the under parts are brown, streaked with black or buff. The legs are reddish-brown.

Young males attain adult plumage in their third year; the secondaries and central rectrices increase in length at each moult up to the six or seventh year. The female has a well-developed black occipital crest and brown crown spotted with buff. The neck, upper back and upper breast are dark chestnut, the mantle dark brown reticulated with buff and the primaries chestnut with elongated black spots. The back and tail are coarsely barred with black and vermiculated with buff and brown; the under parts are chestnut-brown finely vermiculated with black. Soft parts are as in the male, but duller. *Argusianus argus argus* and *Argusianus argus grayi* in Borneo are very similar but slightly smaller and greyer. The male's back is buff, not yellowish. (Wayre, 1969).

The Argus pheasant has a very interesting mating ritual. The male will build a small arena in a clearing of sticks and twigs. When finished constructing his arena he will call the female pheasant into the ring. The male will then begin to circle around the female getting closer as he does so and flapping his wings. The female is an egg layer usually laying a clutch of about 3-8 eggs and incubation period is 24-25 days. One nest incubated by a female had an incubation period of 24 days 18 hours, whereas artificially incubated eggs required from 24 to 26 days to hatch (Johnsgard, 1999). The Argus pheasant has also been found to be monogamous in the wild.

1.2.2 The Red Junglefowl

Genus Gallus

The Junglefowl are the ancestors of modern poultry, having been domesticated for more than 4,000 years. Domestic fowls were known around 2500 B.C. in the Indus Valley and had been carried westward in to Europe via Mesopotamia by 1500 B.C. They were well established in north-western Europe and Britain at the time of the Roman invasion. As one would expect, Junglefowls closely resemble domestic chickens, especially Leghorns, in general appearance. The cock has a comb on top of the head and two lappets below the bill. Its tail is much compressed and composed of

fourteen or sixteen rectrices, the central pair elongated and curved downward. The legs are long and armed with a large curved spur. The hen is smaller and duller and lacks the curved tail feathers and the spurs.

Junglefowls are found all over the warmer parts of Asia, but are lacking in Borneo. They inhabit a wide diversity of country from low-altitude forest, dry scrub and bamboo groves to small woods and rough ground near villages. There are always wild and extremely wary, so that, despite persecution by man, they continue to survive. They are often found in family parties consisting of one cock and several hens during the breeding season, and they congregate in larger flocks during the winter. The male's courtship is similar to that of the domestic fowls, his oblique attitude resulting in a conspicuous display of the bright neck and saddle feathers to the hen. His sudden circling, accompanied by the rasping sound produced by the movement of the lowered primaries, stimulates the hen to crouch, after which copulation may follow. The hen makes her nest on the ground under the shelter of vegetation. The number of eggs in the clutch depends upon the species. All Junglefowl feed chiefly upon seeds, grain, shoots and buds as well as insects and particularly on the eggs and larvae of termites. The crow of the male Junglefowl is similar to a domestic cock's, but only the Red Junglefowl resembles it closely. The genus consists of four distinct species were Cochin-Chinese Red Junglefowl (*Gallus gallus gallus* (L), Tokinese Red Junglefowl (*Gallus gallus jabouillei Delacour and Kinear*), Indian Red Junglefowl (*Gallus gallus murghi* Robinson and Kloss) and Javan Red Junglefowl (*Gallus gallus bankiva* Temminck).

Red Junglefowl (*Galus gallus spadiceus*)

Figure 1.2 Red Junglefowl (*Galus gallus spadiceus*) at Satun province photographed by Mr. Sathit Malawong.

Red Junglefowl is found in South-western Yunnan, Burma, Thailand (except extreme east), northern Laos, Malaya and northern Sumatra. Integrades with *jabouillei*, *gallus*, *murghi* and *bankiva* near their respective boundaries. It is probable that the world's domestic poultry originates from this species alone and therefore of all birds the Red Junglefowl has been of unrivalled importance to mankind.

Red Junglefowl (*Galus gallus spadiceus*) exist in the genuine wild state from north-west India, through Assam, Burma, Thailand and Malaya, to Indo-China in the east and also to South China, Hainan, Sumatra and Java. All fives races are superficially similar in appearance. This Junglefowl has been introduced to several island groups of south-east Asia, where wild populations now occur. The male has a long, pointed crown and neck feathers of golden-brown to fiery red in color; the mantle and wing coverts are of metallic green and the scapulars, back and median wing coverts dark reddish-brown to orange-red on the rump, where the feathers are elongated and pointed. The primaries are blackish-brown and the secondaries rufous; the tail and its coverts are dark metallic green. The under parts are dull black. The comb is largish and serrated; there are two wattles which, with the naked throat and face, are scarlet. The female is much duller. The crown and nape are reddish, the neck feathers long and dull brown in color with

yellow borders, the upper parts dull brown with black penciling. The breast is reddish-brown and the rest of the under parts are paler. The comb is very small and the face thinly feathered. They have the same general habits as other members of the genus, but are inclined to gather in large flocks. They lay well in captivity, but genuine wild birds remain shy and are not easily tamed. There are very few birds of truly wild stock in captivity in Britain today. Some wild specimens of Tonkinese race, *Gallus g. jabouillei*, have been imported by the Pheasant Trust, and many young have been raised. In birds of pure stock the males undergo a molt into eclipse plumage in the summer. They have a short shrill crown which ends abruptly. The hens show neither comb nor lappet, and both sexes hold the tail almost horizontal. The eggs are white or cream, clutch sizes are apparently normally five or six eggs. Incubation is done by the female alone. Under natural tropical conditions it takes about 18-21 days. Red Junglefowl is usually found in well-watered areas and the hill tracts of Bhutan and Assam. Junglefowl are not always monogamous because a cock frequently accompanied by two or three hens. (Wayre, 1969)

1.2.3 Siamese Fireback (*Lophura diardi*)

Figure 1.3 Siamese Fireback (*Lophura diardi*) at Soi Dao wildlife research and breeding station, Chantaburi province photographed by Mr. Thiti Sukapan.

Genus Lophura

Lophura diardi (Bonaparte), Siamese Fireback are found in Indo-China north to central Vietnam and to Luang-Prabang (Laos) on the Mekong; eastern Thailand as far west as the River Me-Yom and Klum-Tan mountains. Slimmer and proportionately longer-legged than the other gallopheasant. Facial skin red in both sexes. In Male have dark grey upperparts, neck and breast; coppery-reddish rump and upper tail coverts; golden-yellow patch on lower back conspicuous in wing-whirring display. Tail blackish, glossed green. They are very beautiful bird that will attain its adult plumage the first year. The crest is long and made up of purple-black feathers. The facial wattles are bright red, the throat, head and face behind the wattles are black. The breast, neck and upper back is gray with very fine vermiculations. The middle of the back is bright yellow (hence the name "fireback"), the lower back is metallic blue with chestnut fringes. The tail is long and curved with metallic black, blue and green sheens. The wings are gray with black and white streaks; the belly and lower areas are black. The bill is yellow, legs and feet red. Like other species of Lophura, they can grow fairly long spurs that the keeper will need to keep trimmed to prevent injuries to the hen when breeding. In the Female easily distinguishec from other female pheasants by broad, black and buff bars on wings and tail. Rufous-chestnut outer tail feathers and underparts. Fireback hens, despite not being colorful, their unique markings make them more attractive than other pheasant hens. The Siamese Fireback hen has no crest, her facial wattles are smaller than the male's, but just as bright. The head, throat, chin and neck are grayish-brown; the upper back and upper breast are bright chestnut. The lower back, wings and tail are chestnut, vermiculated with white and black. The bill is dark gray and the legs and feet are red. In captivity, the incubation period is 24-25 days, which is typical for the genus Lophura. The clutch size in captivity ranges from five to eight eggs. This is probably the normal clutch for wild birds as well, given the limited information from the wild. (Johnsgard, 1999). Habitat of them were evergreen forest, chiefly of the plains and foothills, though occationally found up to 800 m. Uncommon to rare resident; now much reduced (Lekagul and Round, 1991).

1.2.4 Green Peafowl (*Pavo muticus imperator*)

Figure 1.4 Green Peafowl (*Pavo muticus imperator*) at Soi Dao wildlife research and breeding station at Chantaburi province photographed by Assoc. Prof. Wina Meckvichai.

Genus Pavo

Peafowl have been famous in arts and letters for several tens of centuries around in India, China and the Mediteranean Sea (Delacour, 1977). There are three species of Peafowls, the Congo Peafowl (*Afropavo conginsis*), live in Congo, central Africa, the Indian Peafowls (*Pavo cristatus*) from India, often called Blue Peafowls and the Green Peafowls (*Pavo muticus*) which live in southeast Assam through Burma and Thailand to the China sea and Southwards to the Malaysian Peninsula and Java. It does not occur in Sumatra or Borneo. The characteristic of two Asian Peafowls are similar but Green Peafowls are larger, more dignified and more colourful than the Indian Peafowls. Besides, the feathers of Blue Peafowl's body coverts are blue and of its wings are light black and white. The other's feather is brown. Blue Peafowl have white facial skin while Green Peafowl have blue and yellow facial skin. Green Peafowl's crest is erect standard while Blue Peafowl's crest is fan shaped.

In Green Peafowl have brilliant green crest is composed of a long narrow tuft of feathers and his plumage is even more colourful; the neck, breast and mantle have a scaly appearance, each feather is being bright blue 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 are as in the Indian Peacock, only more brilliant and coppery. The secondaries are blackish-brown on the inner web and dark blue and green on the outer. The primaries are bright chestnut, the abdomen and flanks dark green and the vent and under tail coverts grey. The green Peahen is similar to the cock, only slightly duller and she lacks a train, which is replaced by short greenish-brown feathers barred with buff. She can be distinguished from young males by the brown instead of bluish-black loral patch between the eye and the bill and by the primaries, which are bright chestnut black bar on outer web, whereas they are pure chestnut in the males (Sukapun and Meckvichai, 2004).

Second-year males are like the adult, but have a short eyeless train. First-year males are similar, but less bright and with tail coverts barred with buff. Green Peafowl have similar habits to the Indian, and prefer the same type of country. They are even more wary and less prone to live near human habitation, often being found in dense jungle, usually in vicinity of river or open clearing. They live in flocks or small groups, except during the breeding season, when adult males fight to defend their territory and band of hens. The male's voice is similar to the Indian's, but rather less discordant and with a greater variety of notes. The Green Peacock must be counted among the most beautiful birds in the world and for this reason has long been popular in captivity. Its management and feeding should be the same as described for the genus, the only difference being that it is less hardy and needs to be confined to a warm shelter in severe weather. The male must be kept separate owing to their extremely aggressive temperament. There are three subspecies of Green Peafowl; *Pavo muticus specifer* distributed in the western Burma. *Pavo muticus imperator* lives in eastern Burma, Thailand and Indo-China. In the southern apart of Thailand from Kra and Java were founded *Pavo muticus muticus*. There is only one species of *Pavo muticus* in Thailand.

There are two subspecies, the Indo-Chinese Green Peafowl (*Pavo muticus imperator*) and the Javanese Green Peafowl (*Pavo muticus muticus*). *Pavo muticus imperator* Delacour, Indo Chinese Green Peafowl. Whole of Indo-China, extreme south of Yunnan, Thailand south to Kra and eastern Burma, west to the Salween-Irrawaddy divide. (Wayre, 1969). Green Peafowl in Thailand were classified as an endanger species reflected from small population. Humprey and Bain (1990) were found throughout Thailand below nine hundred meter except in the central valley of Chao Phraya and Southeastern of the northwest. Hunters intensively trap it for the feather, pet trade and collect egg from the forest. Over hunting threatens to eliminate this species from Thailand. IUCN (Internatinal Union for the Conservation of Nature and Natural Resources 2004) classified as vulnerable species. In Thailand have been classified as a protected animal and endangered species and classified into Appendix II by CITES (The Conservation on International Trade in Endanger Species of World Fauna and Flora 1998). Green Peafowls were founded in small individual at the following National Park (NP) and Wildlife Sanctuaries (WS) (Humprey and Bain, 1990). Clutch size of hens was four to six eggs. Incubation is done by a female only and lasts for 28-30 days; the chicks grow quickly, able to fly good distances at two weeks of age.

1.2.5 Crested Wood Partridge (*Rollulus roulroul*)

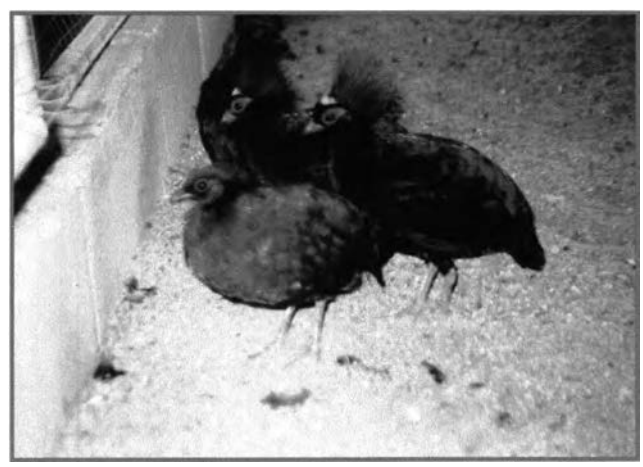

Figure 1.5 Crested Wood Partridge (*Rollulus roulroul*) at Sattaheep farm, Chonburi province photographed by Mr. Sathit Malawong.

Genus Rollulus

The Roul-roul Partridge is also known as the Crested Wood Partridge, Green Wood Partridge or Red-crowned Wood Partridge. They are native to the tropical rain forests of Burma and Thailand, south to Malaysia, Borneo and Sumatra and despite not often kept in captivity, they are charming aviary birds. The Roul-roul Partridge is the sole member of the Genus Rollulus and has no known subspecies. These birds are small and quail-like, with no spurs and no claw on the hind toe. In male: Feathers on the upper body are a brilliant dark green, while the head, neck and underparts are dark blue, almost black. The crest consists of permanent filoplumes that are scarlet with a few black filoplumes in front; the crest is not erectile. The wings are dark brown with orange primary feathers, and the tail feathers are black. The legs are red. Facial skin is red with a black or red bill and hazel eyes. In female: Coloration is similar to the male, but muted. The underparts are green, and the wings are chestnut. The female's bill is all black, and she has no crest, but does have the black filoplumes (feathers that look like stiff hair, like ostrich eye lashes). Unlike the Rock Partridges, Genus Alectoris, the sexes are different. The male, seen in the front in the photograph, has a large red crest, red orbital skin, overall glossy bluish-purple body and red feet and bill. The hen is much drabber in comparison. She lacks the bright crest, has an overall greenish color with chestnut-brown wings, black bill and red feet.

Breeding of this species, if kept in an indoor, tropical environment, Roul-roul may lay at anytime during the year. The males seem to do most of the nest building, using pine needles, branches or straw to create a tunnel like nest. Clutches range from about 4 to 6 eggs. Incubation is done by the female and lasts 18 to 22 days. In captivity, the chicks of this species may have problems learning to eat. Provide mealworms and place a few small size chicks such as button quail in the brooder to help "teach" the little partridge to eat. Roul rouls are monogamous, the male simply keeping watch. The hen builds a nest of leaves and twigs that is completely roofed over. There is a small entry hole that is usually closed when the nest is occupied. Four to six cream-colored eggs are laid and sat by the hen. Incubation is about 18 days. Though precocial, the chicks

are fed (some sources say by only the hen, others say by both parents) for the first 5 or 6 days, after which they scratch and pick for themselves. After 10 days or so the chicks can flutter, and they are perching as necessary at a month, at which time the males are beginning to show their red crest. They stay with the parents approximately 3 months, and then disperse gradually.

Roul rouls are not often kept and bred in aviaries and remain somewhat expensive. They are not recommended for the beginning breeder and are still a challenge for even experienced breeders. They are prone to develop many poultry diseases, and need to be kept away from ground where other game birds have been kept. They also do not seem to do well on wire, often developing foot and leg problems. It is recommended that they are kept in pairs in aviaries with sand bottoms and well planted. Roul-roul is not very winter hardy and need heated quarters during extremely cold weather.

1.3 Molecular genetic markers as tools for genetic diversity studies in birds or in gallopheasants.

Molecular genetic markers, the direct translated products of gene or intact genetic materials, particularly Mendelian inherited, are important to address population genetic questions. The common technique which concerning the direct translated products of gene is protein electrophoresis such as allozyme analysis. To obtain information of more exactly genetic backup of population, nuclear DNA and organelle DNA (Mitochondrial DNA) are analyzed by various method such as in many genetic studies on animal populations, mitochondrial DNA (mtDNA) was investigated to getting evidence of ancestry at the population level (Avise, 2000; for a review about mtDNA recombination, see Rokas et al., 2003). Specifically, Cytochrome b gene (Cyt-b) and D-loop Control Region (D-loop) were employed as markers (Randi, 1996; Randi and Lucchini, 1998), restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD) and DNA fingerprinting.

1.3.1 Allozyme

Since the 1960s (May, 1992) allozyme electrophoresis technique has been successfully applied to various organisms from bacteria to animals and plants. Extracted tissue were prepared and electrophoresed on supporting media. Proteins are separated by their net charges and sizes. Protein bands can be visualized by a specific histochemical stain of investigated enzymes. When the electrophoresed gel is stained, the status of homozygosity or herterozygosity at such a locus can be examined. The number of band is reflected from configuration of enzyme molecules. The position of polymorphic band are genetically informative (Weising et al., 1995).

Allozyme electrophoresis technique is one of the most important tools used for genetic variation studies at intraspecific level even though it is being increasingly replaced by direct DNA analysis. The advantages of this method that nondenatured proteins with different net charge (rather than size and shape) migrate at different rate through starch or acrylamide gel to which an electric current is applied (Avise, 1994) and due to its speed and cost-effective. Data from hundreds of individuals at several loci can be assessed within a few days or weeks. Although interpreting gel patterns usually requires considerable experience, operator can be trained quickly. Disadvantages of this method are the strict requirement of large amount of fresh tissue specimen for protein extraction or good quality frozen tissue, the need of more material than most DNA methods, and a small proportion of protein coding sequences that can be investigated. Nevertheless, their has remained a debate about whether allozyme variation is selectively adaptive or neutral. This latter point results to an assumption that genetic drift is responsible for population differentiation. Thus, this method is not suitable for studies of endanger species (Carvalho and Pither, 1995).

Randi et al. (1991) were studied Phylogenetic relationships among 12 galliform species belonging to the superfamily Phasianoidea were studied with multilocus protein electrophoresis. Nei's standard genetic distances ranged from D = 0.081 between *Alectoris rufa* and *A. graeca*, to D = 1.292 between *Pavo cristatus* and

Lagopus mutus. Dendrograms were generated using distance matrix and discrete character states methods. The phylogenies obtained were generally congruent with previous estimates based on protein electrophoresis, DNA-DNA hybridization, nuclear DNA restriction maps and karyology, but they were not in accordance with phylogenies suggested by current classifications. In their study *Numida and Pavo* were distantly related to the other taxa studied and the Phasianidae, appears to be a genetically highly heterogeneous group. Taxonomically, the Phasianidae could be split into two lineages: the first one comprising *Meleagris*, *Phasianus*, *Perdix* and the grouse; the second one linking the partridges of the genus *Alectoris* with the Old World and the New World quails. The relative rate test suggested consistent homogeneity of the rate of protein evolution in all lineages obtained using analyses of distance data, except the one including *Numida*. The latter showed a slowdown, possibly due to underestimation of its genetic distances with the other taxa, or to erroneous classification of its fossil ancestor. A calibration of the molecular clock based on a study of nuclear DNA produced the relation 1*D* = 22.9 million years (Myr), which was used to estimate the divergence times between lineages. Divergence times were compared with the utilizable fossil record. These results suggest that multilocus protein electrophoresis is a reliable method of studying bird phylogenetic relationships spanning from the subspecific to the intraordinal level.

Schreiber et al. (2000) and Baker et al. (2001) were studied on allozyme analysis of 24 loci in 154 red kites (*Milvus milvus*) and 36 black kites (*Milvus migrans*) from the Hakel forest revealed a Nei's interspecies genetic distance of D=0.009. Of the observed genetic variance of four polymorphic enzymes, 15.4% referred to the differentiation between the kite species, but 84.6% were contributed by the ingroup polymorphism within these species. Allozymes permit the identification of some 78% of samples as orignating from *M. milvus*, but only of 5.4% of samples obtained from *M. migrans*. Although the genetic distance is slight, the *Milvus* kites are valid biospecies which, despite occasional instances of hybridization, coexist sympatrically and may breed in mixed breeding aggregations. Mate choice in the largely separate winter quarters of these migratory birds or chromosomal incompatibility are hypothetical

isolation mechanisms stabilizing the species boundary. Moreover, the range sympatry could have developed fairly recently with the spread of human agriculture.

1.3.2 Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) is one of several techniques used to determine DNA variation based on the assumption that digested DNA fragments having identical migration distance on the gel are the same restriction fragment resulted from the homologous fragment. For conventional RFLP approach, the target DNA digested with restriction endonucleases are size-fractionated by agarose gel electrophoresis and transferred to a supporting membrane (usually nylon or nitrocellulose membranes). The investigated fragment (s) is identified by hybridization with the specific radiolabeled prob (Davis et al., 1996).

Restriction analysis was extensively used for molecular population genetics of animal taxa has emphasized surveys of genotype frequencies, diversit and population based on polymorphism of mitochondrial genome. Genotype frequencies can be quantified by the presence or absence of restriction sites among individuals. This method can be directly carried out if pure mtDNA was digested with appropriate restriction enzymes followed by gel electrophoresis. On the other hand, the large and complex molecule like nuclear DNA can not be directly analyzed after restriction enzyme digestion. The labeled DNA prob is required for hybridization and can be labeled using either isotopic or non-isotopic methods. The RFLP markers are regarded as a Type I marker in linkage maps. Accordingly, RFLP markers have been extensively used to develop genetic maps and phylogenetic trees. (Yu et al., 1993). Studies on mtDNA variation are primarily carried out by restriction analysis of the entire mitochondrial genome. Nevertheless, analysis of the entire mtDNA by restriction enzymes is increasingly replaced by PCR-RFLP where the specific regions of mtDNA are amplified in vitro through the polymerase chain reaction (PCR), and the PCR products are digested with restriction endonucleases. Alternatively, the PCR amplified product can be further analyzed by direct sequencing (Chapman and Brown, 1990).

1.3.3 Microsattelite

Microsatellite DNA marker is one of genetic tools for applied to study in the population genetics, and it has become the focus of the search for hypervariable single locus marker, being abundant and widely dispersed in the eukaryotic genomes. 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). Microsatellites are highly informative repetitive sequences of 2-6 bp, dispersed throughout the eukaryotic genome (Morgante and Olivieri, 1993; Taramino and Tingey, 1996). Polymorphism at microsatellite loci can be efficiently assessed by PCR. The alleles are usually separated and identified by high resolution polyacrylamide gel electrophoresis. Characteristics such as convenient analysis through PCR, a high number of alleles per locus, precise allele identification through the use of allelic ladders and the accurate comparison of data among researchers and laboratories make SSR markers one of the most informative techniques for genome mapping, DNA fingerprinting and population studies (Weber and May, 1989).

Microsatellites can serve as genetic markers at the level of genes in at least two ways: sex determination (Hanotte et al., 1997) and gene mapping (Bailey et al., 1997; Crooijmans, 1996; Zheng et al., 1993; Dietrich et al., 1992 and Wissenbach et al., 1992). At individual's level, microsatellites have been successfully applied to parentage and relatedness testing in diverse organism (Ellegren, 1992; Keane, Dittus and Melnick, 1997 and Bowling et al., 1997). Because they are nonfunctional, they are not subjected to strong selection or selectively neutral and accompanying with their characteristic described above, have made microsatellite as an ideal class of genetic markers for population genetic studies. The population survey of microsatellite variability have been done in many organism (Paetkau and Strobeck, 1994; Lade et al., 1996; Mundy et al., 1997; Valsecchi, 1997 and Estoup et al., 1995). An additional, microsatellite data can

provide useful evidence in assessing relationship among species or at above specials level (McDonald and Potts, 1997). Generally, developing microsatellite loci for new species requires that one constructs a genomic library of clones bearing one or more tandem repeats. At present, numerous microsatellite survey of diverse organisms especially domestic species have been carried out (e.g. Swinburne et al., 1997; Dolf et al., 1997; Goodman, 1997 and Dawson et al., 1997).

1.3.4 Randomly amplified polymorphic DNA (RAPD)

Random amplified polymorphic DNA (RAPD) has already found their application in the typing of prokaryotic as well as eukaryotic genomes. RAPD-PCR employs usually short (10–15 base long) arbitrarily chosen primers to amplify DNA fragments at low-stringency conditions that are species-specific (Welsh and McClelland, 1990 and William et al., 1990). Beside an unlimited number of RAPD primers can be screened for desired molecular markers within a short time. These make this method ideal for searching for useful genetic markers in various species (Weising et al., 1995). Under low stringency condition, a number of PCR products are generated from random locations within the genome 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)

RAPD analysis is a simple, rapid technique, relative inexpensive and numerous markers can be developed easily by changing sequences or number of nucleotide in the primer (Hadrys et al., 1992). However, since RAPD markers are mostly inherited as dominant alleles, information on the parental origin of alleles may be inaccessible. 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. 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), using in Mud crabs and Cupped Oysters (Klinbunga, 2000; Klinbunga, 2001). Wutthivikaikarn (2003) studied on screening of 60 RAPD primers in some Green Peafowl in Thailand, 39 primers could not amplify RAPD-PCR product, 19 primers could amplify RAPD-PCR product without polymorphism and only 2 primers (the primer 1 and 13) could amplify RAPD-PCR product with polymorphism. However, the primer 1 gave low allelic polymorphism in Green Peafowl from 7 sites.

There are some disadvantages to use RAPD-PCR approach for population genetic, genetic mapping, and taxonomic studies. First, many fragments (especially those arising from mispairing of a primer with the genomic DNA) may not be reproducible among different laboratories because amplification is sensitive to slight changes in temperature cycles. Second, in contrast to RFLP, which is usually inherited codominantly, most RAPD-PCR amplified fragments are inherited in the dominant fasion. Therefore, homozygotes and heterozygotes can not be differentiated. Third, RAPD bands of the same size may not actually identical, therefore, comigrating RAPD bands may not be allelic (Narang et al., 1994)

1.4 Objective

The objectives of this study were screen primers and studying genetic diversity in five different breeds in gallopheasants and partridge in Thailand by using random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR).