Chapter 2

Literature Reviews.

2.1 The Red Junglefowl

Red Juglefowl was first described by Linnaes as *Phasianus gallus* in 1758. Its scientific names has been changed several time before the latest revised name, *Gullus gallus* O.Grant (nec.Linn) was accepted (Rothschild, Hartert , and Jordan, 1926). The present classification of Red Junglefowl is:

Kingdom Animalia
Phylum Chordata
Subphylum Vertebrata
Class Aves
Order Galliformes
Family Phasianidae
Genus *Gallus*
Species *Gallus gallus*

The genus *Gallus* (Junglefowl) consists of four distinct species (Wayer, 1969); *Gallus gallus* (Red Junglefowl), *Gallus lafayettei* (Ceylon Jungle), *Gallus sonneratı* (Gray Junglefowl) and *Gallus varius* (Green Junglefowl). Red Junglefowls are also subdivided into five subspecies comprising *G. g. gallus*, *G. g, spadiceus*, *G. g. jabouillei*, *G. g. murghı*, and *G. g. bankiva*.

In general appearance, these junglefowls are similar to each other. The cock has comb on the top of the head and two lappets below the bill. Its tail is much compressed and composed of fouteen or sixteen rectrics. The central pair is elongated and curved downward. The legs are long and armed with a large curve spur. However, It is distinguishable by their plumages colour pattern (Figure2-1). Wayre (1962) detailed the characters of these birds and described the Red Junglefowl as:

The male has a long, pointed crown and neck feathers of golden-brown to fiery red in colour; 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

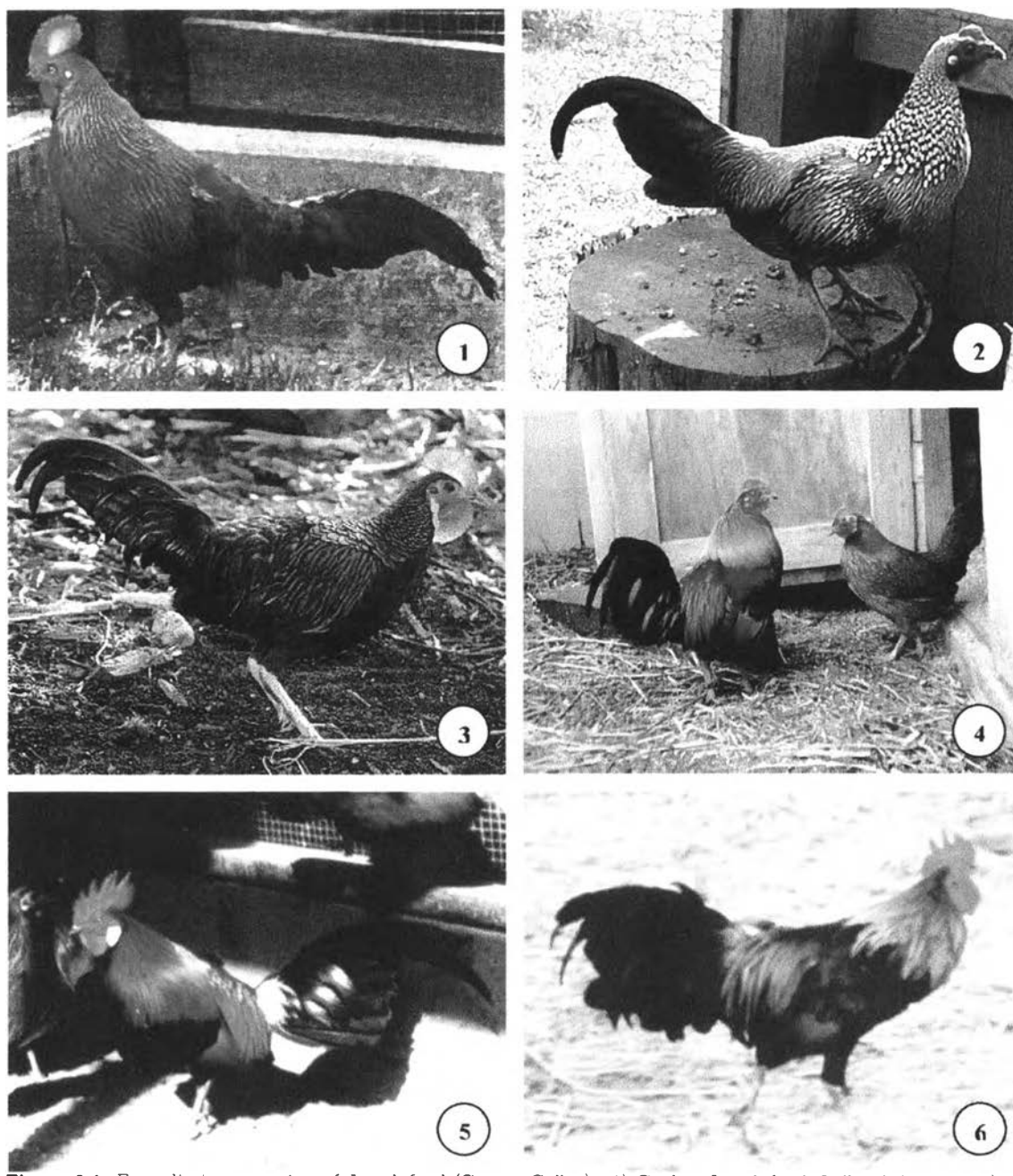

Figure 2-1 Four distinct species of Junglefowl (Genus *Gallus*). 1) Ceylon Junglefowl *Gallus lafayettei* 2) Grey Junglefowl *G. sonneratı* 3) Green Junglefowl *G. varıus* and 4) Red Junglefowl *G. gallus* which two subspecies 5) *G. g. gallus* and 6) *G. g. spadiceus* are found in Thailand.

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 include the naked throat and face are scarlet. He undergoes a moult into eclipse plumage in the summer (breeding season). Since junglefowl's wings are rounded, curved and fit close to body. They are able to fly swiftly, but cannot sustain flight for very long. When flying away the most noticeable feature of this cock is the white patch on a blue black except for the red wattle on the head. Nevertheless, female of this genus is closely resemble and is much duller, brown in basic colour. The colour pattern of precocial downy chick of Red Junglefowl was described by Lord Medway and Well (1976) as: buff, with a broad median band from crown to rump, a band from the eye to the side of the beck and a band on each side of the back, dark reddish brown bordered with black; throat, ear coverts and underparts pale buff, with a darker band across the upper breast; wings rufous brown. As a result, the colour of her and her chick make it difficult for predator to see them. The call of both sexes resemble those of domestic fowl, but the cock's crow is shorter, the last note being cut off abruptly.

In comparison with other species, Red Junglefowl are likely to be polygamous rather than monogamous. While the other species are usually found singly or in pair (sometime with their young), Red Junglefowl generally live in small groups that comprise a dominant cock, one or more hens, and immature birds. During the mating and rearing phase, the hens separate off within the group territory so that the cock is left alone or with non-broody-hen. When the hen is ready to lay an egg, she gives a nesting call, inviting her mate to join her in finding a nest site together. They find and create a nest by pulling and flinging around themselves twings feathers, hay, leaves and loose dirt, after they have scraped a depression with their beaks and feet. The hen usually makes the nest on the ground under the shelter of vegetation. Zuk, Johnsen and Maclarty (1995) pointed out that female Red Junglefowl prefered to mate with male longer, redder comb, but paid little attention to plumage characters. Male Red Junglefowls generally perform food calling during courtship, with both edible and inedible object, when they want to attract or want to contract with a female. The male's courtship behavior is similar to that of domestic fowl, his oblique attitude resulting in a conspicuos 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 lay 6-12 eggs, resemble small domestic hen's eggs. Most eggs are probably laid from March to May, old nest are found up to October, so that the breeding season may be said to extend through out the year. While hen is incubating her egg, She will occasionally leave the nest very briefly to feed, drink, preen or defecate. After 21 days, the eggs hatch, hen and her chick form a unit independent from her original flock and for the large past away from any males although males may occasionally consort with these hens. Chick mortality is extremely high since there are many predators including lizards, birds of prey, and carnivores such as wild cats and civets (Beebe, 1921) Red Junglefowl are almost exculsively ground living, flying only to safety, to roost, or when chasing or being chased by another Junglefowl (Sullivan,1991).

Junglefowls are natural foragers. They have long strong legs with four-clawed feet. The leg and bill are adapted for scratching in the ground for food. They spend long hours in early morning and late afternoon for scratching away at the covering of leaves that hides their favorite food. All junglefowl feed chiefly upon seeds, grain, shoots and bud as well as insects especiallly on the eggs and larvae of termites.

Red Junglefowl has been found from sea level to approximately 2000 metres. Their prefered habitat usually is open forest. Forest edge, lightly logged and particularly bamboo forest are all typical habitats in which they are found (Johnsgard, 1986).

All Junglefowl are found in only warmer parts of oriental region as shown in figure 2-1. Ceylon Junglefowl are endemic to several different habitats on the island of Sri Lanka (formerly Ceylon). Green Junglefowl inhabit in Java and neighboring islands. Gray-Junglefowl are found in western and southern India up to in the north-west and east to the River Godavari, central India and Rajputana (Wayre,1988). The most widespread species, Red Junglefowl, is range from northern India in the Southern edge of Himalayas westward

to southern Sikim, Napal and Kashmir. Eastward it is commonly found in the hilly portion of Bengal and, throughout Burma including Pegu and Tennessarim, Yunnan, Siam (now Thailand), Cochin-China and southward to the Malay Peninsula and Indonesia including Bali, but absent from Island of Singapore and Borneou (Beebe, 1921). Five subspecies of this species are differently found in this area as Wayre (1969) describe as:

1. *Gallus gallus gallus* (L), Cochin-Chinese Red Junglefowl are found in Cochin-China, Cambodia and nearby island, Vietnam (except extreme north) central and lower Laos, eastern Thailand with in northern Vietnam.
2. *Gallus gallus spadiceus* (Bonnaterre), Bermese Red Junglefowl inhabit south-western Yunnan, Burma, Thailand (except extreme east), northern Laos, Malaya and northern Sumatra. Integrates with, *G. g. murghi* and *G. g. bankiva* near their respective boundaries.
3. *Gallus gallus jabouillei* Delacour and Kinnear, Tokinese Red Junglefowl live in Tokin, north of Veitnam, extreme southeast of Yunnan, Kwangsi and. Integrade with *G. g. spadiceus* in north-western Tokin.
4. *Gallus gallus murghi* Robinson and Kloss, Indian Red Junglefowl dwell in northern and north-eastern India, the lower ranges of the Himalayas from southern Kashmir to Assum, northern and eastern central India. Meets *Gallus sonnerati* in the Central Province and hybrid have been found
5. *Gallus gallus bankiva* Temminck, Javan Red Junglefowl exist on southern half of Sumatra, Java and Bali.

In Thailand, subspecies with whitish ear spot, *Gallus gallus gallus* exist in north-eastern and eastern parts of country. Burmese Red Junglefowl that have clear red earlobe distributed form Laos border down to southwest.

2.2 Habitats of the Red Junglefowl are on Progressively Declined

The biodiversity crisis in Thailand is similar to those prevailing in many other tropical countries. Deforestation is the one of the most important problem, causing loss of biodiversity. Thailand's forest cover been reduced from 53% in 1960's to about 22.8% or

111,010 km^2 at present (Bhumibamon, 1986; FAO, 1997; cited in Forest Restoration Research Unit,1998), proceeding the rate of more than 2% per year. A ban on commercial logging since 1989, has help to slow down the destruction, but the deforestation rate then increase and still exceeds 1,000 km^2 per year or about 0.8% annually (RFD statistic), due to illegal logging, agricultural expansion and development projects. Consequently, progressive loss of wildlife habitats and food source which are results of deforestation and forest degradation have strong negative effects on wildlife's population viability. Much of remains of Thailand's remarkable flora and fauna are being threaten with extinction in the next decade (Woodruff, 1990). Populations surviving on small and fragmented habitats potentially loss of genetic variation, future adaptation and evolutionary ability. The non-migratory species such as Red Junglefowl are subject to this alteration more severely. Thus conservation activities of these speciesis of immediately concern.

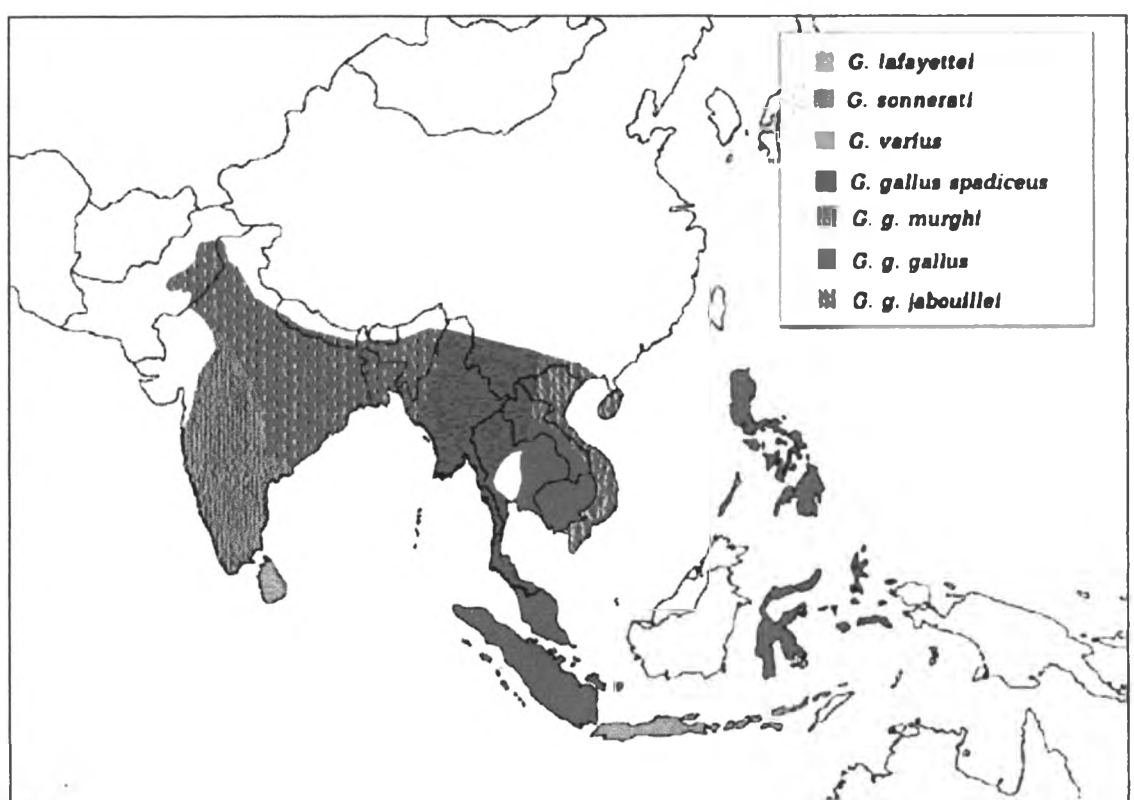

Figure 2-2 Distribution of Junglefowl. All junglefowls are found in onlythe warmer parts of Asia. (After Beebe, 1921)

2.3 All Varieties of Domesticated Chicken are Descendant of the Red Junglefowls.

In terms of economic and cultural importance to mankind, chicken, the single mat obvious species of bird, have played an important role in the evolution of human civilization since prehistoric time. Chicken have been domesticated for their meat, egg as well as for recreational purpose such as cock fighting and ornamentation. Now chicken is the most important economic animal of the world as the main source of food. There are many kinds of domestic chickens exist in all over the world. The progenitor of domestic fowls is seemingly more clear, but there is still no clear answer to question of when and where chicken were first domesticated.

As one would expect, domestic fowls closely resemble Junglefowls in general appearance. Accordingly, a belief that domestic fowls originated from wild resemblance is broadly except. The Junglefowls consist at least of four living species such as Ceylon Junglefowl, Gray Junglefowl, Green Junglefowl, Red Junglefowl and two fossilized species. Since human civilization had begun in thousand of year ago, thus domestic chicken should been domesticated from these living species. Controversy about the origin of domestic fowl is whether it is monophyletic or polyphyletic. Polyphyletic origin hypothesis propose that diverse breed of chicken descended from different wild species. Unlike, monophyletic one state that domestic fowl originates from the Red Jungle Fowl alone. Okada (1994) reviewed this issue based on crossbreeding data, plumage pattern, spectrogram analysis of crowing voice, analysis of blood group and protein polymorphism, including mendelian genetic studies. Most of the data indicated that Red Junglefowl was a species most closely related to domestic fowl and the most reasonable conclusion was that Red Junglefowl is the main progenitor of domestic fowls, although some gene might have been introduced into domestic chicken by hybridization with other species of Junglefowl.

To elucidate greater detail of this view, Fumihito et al. (1994, 1996) analysed the restriction fragment length polymorphism (RFLP) and sequence of the first 400 base in noncoding control region or displacement loop (D-loop) of mitochrondrial DNA extracted from various domestic breeds and all of four species of Junglefowl. They found that both sequences and RFLP haplotype of Red Junglefowl were closer to all breeds of domestic

chicken than another Junglefowl. In conclusion, they suggested that Red Junglefowl alone had been sufficient to yield all the diverse breeds of domesticated chicken.

2.4 Red Junglefowl as Genetic Resources for Future Use in Chicken Breeding.

Genetic diversity is the major requirement of progress in animal breeding. Genetic diversity in commercial line is limited by the selection for production trait whereas those of local animal and wild animal are relatively high.. Under evolutionary consideration, all wild gene pools may hold potential benefits. Wild ancestor of domesticated animal may be an important sources of genetic diversity for future breeding program (Gaston, 1992).

According to chicken lineage describe above, as well as high fertility of artificial hybrids between domestic fowl and Red Junglefowl (Crittenden et al. 1993; Okada, 1994) and the occurrence of hybrid between local native chicken and Red Junglefowl in forest villages indicate that introduction of the Red Junglefowl's genes to domesticated chicken's gene pool is possible. For effective breeding programme and sustainable use of genetic diversity in these birds, evalution of genetic variability and genetic status of both ancestor and domesticated descendant are considerately necessary. Genetic variation within and among different commercial chicken lines including the native breeds were examined (Okada, 1994; Dunnington et al., 1994; Crooijmans et al., 1996; Wei, Dontine and Bitgoud, 1997; Wimmers and Horst, 1998; Takahashi et al., 1998, Vahala et al., 1998; Delany and Krupkin, 1999). For comparative study, their accepted ancestor, Red Junglefowl is usually added to these investigation such as the works of Crittendon et al. (1993) and Dunnington et al., (1994). However, genetic variation within wild ancestor's natural population has scarcely examined. Thus, fulfillment this requirement must be carried out.

2.5 Measurement Genetic Variation in Natural Population

Early studies of general variation in natural population concentrated on easily detected and/or quantifiable variations. Although these were important-variants, they did not allow an estimate of the total amount of genetic variation in the genome of the studied population (Hendrick, 1983). Molecular revolution in population genetics and evolutionary biology, which gained great momentum with the introduction of allozyme (isozyme) method

in the mid-1960s (Avise, 1996), when Lewohtin and Huby estimated the average genomic heterozygosity in population of *Dorsophila pseudoobscur* (O'Brien, 1994). In the late 1970s, attention shifted to method of DNA analysis, primarily through restriction enzymes, and in 1980s, mitichondrial DNA analysis and DNA fingerprint approaches gained immense popularity (Avise, 1994). PCR-mediated DNA sequencing had been widely employed in early of this decade. Recently, analyses of simple tandem repeat loci, microsatellite was just applied into this field.

Barrowclough (1989) pointed out advantages of molecular techniques over more traditional types of characters. First, molecular methods allow us to examine the direct translated products of a gene or the gene themselves. Problem of pleiotropy or complex genetic-environmental interaction during ontogeny are greatly reduced. Second, molecular methods yield information concerning the state of single gene which can be analyzed in term of a well developed population genetic theory. Unlike, thoery of polygenic and quantitative traits for traditional types of characters are more complicated and not yet very well developed.

2.6 Molecular Genetic Markers

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 genes is protein electrophoresis such as allozyme analysis. To obtain information of more exractly genetic backup of population, nuclear DNA and organelle DNA (mitochrondrial DNA and chloroplast DNA) are analysed by various method such as restriction fragment length polymorphism (RFLP), randomly amplified pholymorphic DNA (RAPD), DNA fingerprinting, DNA sequencing and microsatellite analysis.

2.6.1 Allozyme

This method takes the advantage of the fact that nondenatured proteins with different net charge (rather than size and shape) migrate at different rate through starch or arcrylamide (or other supporting media) to which an electric current is applied (Arise, 1994).

Because of the low cost and safety this method are employed widely. For example, Darrells, Roseberry and Klimstra (1989) used 19 allozymic loci to examine genetic structure and gene flow in the northen Bobwhite (*Colinus virginianus*), Petersen (1992) analyse phylogeny and splitting date of the Aphelocoma Jays (Corvidae): *Aphelocoma coerulescens, A. unicolour* and *A. ultramarina* ,by using 28 loci data; Browne et al. (1993) estimate heterozygosity and genetic distance among population of the Hawaiian Duck, Lysan Duck and Mallard. Genetic variation and distance of various breed of chicken were studied by many researchers. Fortunately, relationship among Junglefowl species was documented by Hashiguchi et al. (1981) (cited in Ponsuksili, 1995). However, only histochemical stains available for investigated enzyme can be carried out. Since requirement of large amount of fresh tissue specimen for protein extraction, investigated animals frequently invasive.Thus,this method is not sutible for studies of endanger species.

2.6.2 Mitochondrial DNA

Since there are several predominant feature of mitochondrial DNA, an extrachromosomal DNA found in mitochondria that make it attractive for use in studying population genetics. First, mtDNA is haploid and transmitted through maternal lines in most species. Thus independent assortment and recombination are absent. Difference in their nucleotide sequence are caused by mutation alone. As a result, interpretion of mtDNA data are easier than those of nuclear DNA. Second, mtDNA evolves rapidly accumulating mutations 5-10 times faster than does single-copy nuclear DNA reflecting its potential to be used for the determination of intraspecific genetic variation among geographically different populations. Finally most mutational difference between mtDNA clones are selectively neutral (Shields and Helmbychowski, 1988)

To detect variation in mtDNA, RFLP or PCR-mediate RFLP and nucleotide sequencing are usually applied. Comparison of the restriction fragment patterns assumes that the great majority of mutations in mtDNA (that due to base substitution) prevent or allows an investigated enzyme to cleave at such position and thus produce the different number and size of DNA fragments from investigated individuals. Different fragments are separated by agarose gel electrophoresis and then detected either by chemical staining or radioactive

labelling (Tegelstrom, 1992). Based on this technique, various avian mtDNA were analysed, such as Chickadee (Mack et al. 1986), Phalaropes (Dittmann and Zink, 1991), Goose (Quin, Shield, and Wilson, 1991), Snow Goose (Avise et al., 1992), Noeth American Crested Titmice (Gill and Slikas, 1992), Francolins (Crowe et al., 1992), Alaskan Song Sparrow (Hare and Shields, 1992), Streaked Saltator (Seutin et al., 1993), Chhpping Sparrow (Zink and Dittmann, 1993), Oilbird (Gutierrez, 1994), and various sibling species (Avise and Zink, 1998). Ball and Avise (1992) to address question of the validity of subspecies designations of six avian species with continental wide distributions in North America. At present, analysis of the entire mtDNA by restriction endonuclease is replaced by PCR-RFLP.

Alternatively, the specific regions of mtDNA are amplified through the polymerase chain reaction (PCR) and further analysed by direct sequencing (Quinn and White, 1987). The hypervariable region such as cytochrome *b* gene and noncoding control region or displacement loop (D-loop) are frequently sequenced. Today, a number of documentation of avian mtDNA sequencing have been obtained by many authors, for example, Krajewski and Fetzner (1994), Lanyon and Hall (1994), Morin, Messier and Woodruff (1994), Leisler et al. (1997), Quin and Wilson (1993), Meckvichai, Malaivijitnond and Tirawatnapong (1997)

In domestic chicken, Desjardins and Morais (1990) cloned and sequenced the entired 16,775 base pair mitochrondrial genome of white Leghorn chicken. Fumihito et al. (1994), Fumihito et al. (1995) and Fumihito et al. (1996) used the first 400 base pairs and RFLP data to elucidate genetic link between domestic chicken and Junglefowl. They found that Thai Red Junglefowls were very close to all breeds of domestic chicken and sufficed as the monophylatic ancestor of all domestic breeds. Boripat Siriaroonrat (1997) amplified and sequenced mtDNA of Thai red Junglefowl. Significant difference in genetic diversity between two subspecies, *G. g. gallus* and *G. g. spadiceus*, was found. However, genetic divergence within these subspecies was small (0-2.25% in *G. g. gallus* and 0-6.54% in *G. g. spadiceus*). Thus, sensitivity of this marker may not be enough for the discrimination of closely related populations which seems to be the case of present study.

2.6.3 High variable nuclear DNA

2.6.3.1 Single copy nuclear DNA

Single copy nuclear DNA is a sequence which occurs with a frequency of one per haploid genome. Restriction analysis of single-copy nuclear DNA or scnDNA traditionally relied on Southern blotting procedure. The raw data are in many respects analogous to those provided by protein electrophoresis. The major advantage over protein electrophoresis is that, in principle, a nearly unlimited pool of genetic variants may be tapped. Despite, disadvantage in comparison to protein electrophoresis is that, this method are expensive, both in effort and meterials. The difficulties of genotype determination, evolutionary relationship among scnRFLP allele of a particular locus are seldom accomplished (Avise, 1994). Thus, this technique may not be suitable for intraspecific relationship analysis.

2.6.3.2 Ribosomal RNA genes and other Middle-Repetitive Gene families.

The ribosomal DNAs in the nuclear genome of the eukaryotes usully exist as tandem repeated unit. It is classified as one of the moderately repetitive gene families. The coding sequences of these genes are approximately 6 kb in total length and highly conserved, whereas the non-coding sequence are more variable. The most important factor which causes difficulties in the use of rDNA as a genetic marker is the mechanism of concerted evolution. The concerted evolution refer to the tendency of the copies of rDNA sequence to become homogeneous initially among gene copies within a genome and among individuals within population through unequal cross over or gene conversion. Hence, the extent to which different family members can be view as providing independent phylogenetic information (Avise, 1994).

2.6.3.3 Highly repetitive DNA sequence

The highly repetitive DNA sequences are devided into two groups on the basis of their arrangements in the genome. The first group is the dispersed repetitive DNA which contains the repeat unit dispersed throughout the genome. The second class is clustered repetitive DNA made up of repeat sequence arranged into long tendam array. This type of repetitive DNA is generally devided into three categories depending on the length of the

clusters and the short nucleotide sequence motif or repeat unit (Brown, 1992; Koreth, O'Leary and McGee, 1996):

(a) Classical satellite DNA. This is the first type of satellite DNA to be identified. The sequence arrays with repeat size ranging from 5 to 100 bp, characteristically organized in clusters between 100 and 5000 kb in length. They are located in the heterochromatin near chromosomal centromeres and telomere and are not as variable in size within population as the other of this family.

(b) Minisatellite DNA. Minisatellite DNAfrom shorter clusters, between 100bp and 30kb which array with repeat size of 15-70bp.Minisatellites are found in euchromatic region of the genome and are hightly variable in repeat size within population.

(c) Microsatellite DNA. There is overlap between the lengths of minisatellite and microsatellite cluster, but the latter are distinguished by the short length of repeat unit. Microsatellite contains a repeat motif of 1-6 bp which array in tandemly repeated manner for approximately 10-50 copies (Hearne, Ghosh and Todd, 1992), and subsequently, also called as simple sequences length polymorphism (SSLPs), simple sequence repeats (SSRs) or short tandem repeats (STRs), (McDonald and Potts, 1997). These repititive sequences are highly variable in size, ranging around the mean value of 100 bp (Koreth, O'Leary and McGee, 1996) Microsatellite are highly abandant and randomly dispersed in euchromatin and allele size of populations characteristically exhibit multiple size classes distributed about the population mean. It was estimated that one microsatellite locus may be found in every 10 kb in eukaryotic genome (Taulz, 1989; Koreth, O'Leary and McGee, 1996).

Due to variation in the number of tandemly repeated element, hence, a general designation for both micro and minisatellite is the variable number of tandem repeat loci (VNTRs).

The mutational mechanism causing VNTRs loci to be hypervariable is completely understood. Several models have been hypothezised including strand slippage, gene conversion and unequal crossing over between homologous chromosome during miosis (Wolff et al,1989). The predominant means which new length alleles are generated, is

thought to be intra-allelic polymerase slippage during replication (Schlotterer and Taugz,1993).A replicating DNA strand can slip one or more repeat units within a repeat and resume perfect base pairing. The resulting bulge can then be repaired, resulting in the the addition or deletion of the nonpaired based. 'There is evidence that the addition or deletion usually invloves a single repeat unit, which some evidence for rarer event of larger effect. This type of stepwise mutational process means that allelic variants of similar size are more closely related. VNTR alleles differ in length (number of repeat), are easier to identify than markers differing only by sequense since they can be readily discriminated on the basis of their differential electrophoretic mobility (Mcdonald and Potte,1997). Conventional Southern blotting techniques have been used to reveal minisatellite variation at multiple loci simultaneously to produce the bar code-like multilocus DNA fingerprints or using specific probes to reveal variation at single loci (single locus-DNA fingerprint). An obvious advancement in the efficiency of VNTR analysis utilized the PCR and system were developed for application to specific mimisatelite loci (also called minisatelite variant repeat-PCR, MVR-PCR or digital DNA fingerprinting Jeffreys et al,1991) However, only a limited subset of variation can be analysed by PCR due to the large size of many minisatellite alleles.

A system of highly polymorphic sequences having allele size smaller than 1 kb and varying over narrow size range was desirable because variability in this loci could be assayed by PCR combined with gel electrophoresis, avoiding the need for Southern blotting. Thus, variation could be assessed from minute amounts of material that might contain highly degrade DNA such as forensic or ancient samples. Microsatellite sequence fitted these criteria well, and due to their ubiquity,Mendelian co-dominant inheritance and extreme polymorphism have made them become the most important tool for addressing question at variety of scale, ranging from the extremely fine grained to the fairly coarse grained.(Bruford and Wayne,1993,McDonald and Potts,1997)

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, 1995; Zheng et al., 1993; Dietrich et al., 1992 and Wissenbach et al.,1992). At individuals

level, microsatellites have been sucessfully applied to parentage and relateness testing in diverse organism (Ellegren, 1992; Keame, Dittus and Melnick, 1997 and Bowling et al., 1997). Because they are non-functional, 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. In recent years, population servey 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 species level (McDonal 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, numeruos 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)

2.7 Avialable chicken microsatellite marker

In chicken, the number of microsatellite markers have been characterized and mapped on international reference population. Khatip and Soller (1992a and 1992b) and Cruuigmans et al. (1993) have started to develop microsatellite markers in pooling. The former studies have reported one mono- and dinucleotide repeat polymorphism in functional gene. While Moran (1993) attemping to search for mono-, di-, tri- and tetranucleotide repeat sequence from genebank database, polymorphic (TG/AC)n markers obtained from those database and genomic library screening in the East Lansing and the Compton reference population carried out by Khatib et al. (1993). The East lansing poultion is a cross between a single male of junglefowl origin and four females of highly inbred White Leghorn line (Critteden at al., 1993). The Comton population a cross between an out breded (N) and in breded (I_5) White Leghorn line (Burnstead and Palyga, 1992). Coincidence study of Crooijmans et al. (1993) also screened for polymorphic (TG/AC)n in White Leghorn line. Later, a large number of microsatellite markers of domestic chicken was additionally reported (Cheng and Crittenden, 1994; crooijman et al., 1994, 1995, 1996; Gib et al., 1997, 1997; Ruyter-spira et al., 1996; Dufour et al.,1997). In addition, Crooijmans et al. (1997) optimized a number of

microsatellite markers for automated fluoresent genotyping. Investigation of the use of utility chicken microsatellite markers in other galliformes was carried out by Hanotte et al. (1997)

2.8 Detection of microsatellite variability

Once microsatellite-flanking PCR primers are obtained, PCR can be used to identify genotype of interested individuals. After amplification, the products are fractionated by their different lengths using agarose (usually for tetranucleotide repeat) or polyacrylamide gel (for mono-,di-, and trinucleotides repeats) with radioactive and non-radioactive methods (Kareth,O; Leary and Mcgec, 1996)

2.8.1 Radioactive method

Tridítional autoradiography detection (labelling amplification, electrophoresis of products, fixed, exposed gel with x-ray film, and film developing) of PCR-amplified microsatellite, either by the direct incorporation of single labelled deoxynucleoside triphosphate like [α-^{32}P] dCTP during thermal cycle (internal labelling) or a single 5' [γ-^{32}P] ATP end labelled primer in the PCR mix (end labelling) are more sensitive and common. The internal labelling method is easier to perform and more sensitive whereas the end-labelled approach minimize addition (stutter) band and produces cleaner result.

2.8.2 Non-radioactive methods

Non-radioactive methods are composed of ethidium bromide staining, silver staining, and fluorescence detection. Resolution of the products on acrylamide gel either non-denaturing or denaturing sequencing type gels followed by ethidium bromide staining is the simplest means of visualization. The disadvantages include low sensitivity (only > 10 ng of double stand DNA can be detected) and subjective quantitation unless additional image processing is undertaken. The use of silver stains to visualize DNA offers advantages of sensitivity over ethidium bromide (detecting pg quantities of DNA) but there are problem with variable background and non linear deposition of silver. Fluorescent labelling of the PCR products in combination with automated fluorescent DNA fragement analysers yeild significantly more rapid and reliable result and allows data to be recorded automatically for multiple markers (Ziegle et al., 1992)