Comparative Genomics Among Red Junglefowl, Thai Native Chicken and Commercial Line

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ABSTRACT

The objectives of this study were to investigate the genetic variation of Red junglefowl (RJF), indigenous chicken, commercial broiler and layers using microsatellites and to compare microsatellites and functional genes for genetic assessment before utilizing the chicken genetic resources with efficiency. For the first objective, four Thai indigenous strains and three commercial lines were genotyped genetic variability and divergence using twenty microsatellite loci of which sixteen are recommended by Food and Agriculture Organization. The highest (0.81) and lowest (0.77) average of expected heterozygosities were observed in Indigenous chicken (Dang; DG) and commercial layer (Isa Brown; IB), respectively. Four genetic clusters were detected: first group consisted of layers (IB and White Leghorn, WL); second group was broiler; third group consisted of non-black feather indigenous chicken (Chee; CH, DG and Leung Hang Khoa; LK); and the fourth group was black feather indigenous chicken (Pradu Hang Dam; PD). This study also revealed that PD is suitable to be developed as a meat type chicken due to lower genetic distance between PD and broiler. Moreover, eighteen microsatellites revealed Bhutanese native chickens; Yuebjha Narp (Black plumage chicken) represented the lowest genetic variability. A Neighbor-Joining tree was constructed to show genetic relationship while principal component analysis plot revealed Bhutanese native chickens should be prioritized for conservation because of their genetic distinctiveness. When, we compared the efficiency of genetic characterization of chicken populations that had been under different intensities of selection using selective functional gene versus microsatellite marker analyses. A neighbor-joining tree from Nei's genetic distance was constructed to show genetic relationships. A similar pattern was found in both functional genes and microsatellites: three groups were formed, consisting BR and WH separated into two groups and the third group was RJF and TIC. We tried to confirm tree by a principal component plot based on individual similarity using Dice's coefficient based on functional gene analysis also gave three clusters. However, a different result was found between the cluster from neighbor-joining and principal component analysis when using microsatellite. According to, neighbor-joining showed BR separated from GG but principal component formed BR and GG in the same group. Thus, we showed that genetic characterization with functional genes is superior compared to microsatellites, especially when a different genetic makeup among populations under selection.

Key words: functional genes, genome comparisons; genetic variability**,** microsatellites

INTRODUCTION

Genetic diversity refers to the existence of genetic variants among genomes of individuals, families, strains and populations. Rich genetic resources must be maintained because it will provide for unforeseen breeding requirements to satisfy both farmer and consumer demands in the future. Indigenous chicken may be regarded as much diversified populations due to long-term adaptation from their ancestor (**RJF**) with response to varied agro-ecological zones. Moreover, Thai indigenous chicken (**TIC**) are generally preferred for the quality of the meat (Teltathum and Mekchay 2009), especially as healthy food because of lower triglyceride and cholesterol compared to exotic breeds (Jaturasitha et al. 2008) consensus**,** Bhutanese native chickens have socio-cultural and economic importance to the livelihood of many rural populations. For instance, they are slaughtered to please local deities, entertain guests, and sustain the health of women during pregnancy and after birth through egg and meat production (Nidup *et al.,* 2005) while the commercial lines are superior in terms of growth or egg production. However, under evolution or genetic selection, this may cause native chicken had change in genetic makeup, and even the repair or loss of genes associated with specific characteristics. Consistency, many genetic studies reported the decrease in genetic diversity of native chicken populations. It is because the unique and valuable genotypes and traits of native populations are at greater risk of being lost, with consequent threat to food security (Nassiri *et al.,* 2007). Thus, an assessment of genetic variations and genetic distances among original indigenous and commercial strains is essential**.** The objectives of this study were to investigate the genetic variation of RJF, indigenous chicken, commercial broiler and layers using microsatellites and to compare microsatellites and functional genes for genetic assessment before utilizing the chicken genetic resources with efficiency.

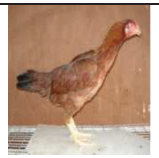
MATERIALS AND METHODS

Animals and DNA isolation

Chicken with no genetic relationships (no common ancestors) were randomly selected. The minimum sample size suggested by Tadano *et al*. (2007) has been considered in this study. one ml of blood samples were drawn from ulnar vein in a microtube containing 0.5 M EDTA from birds two subspecies of RJF, *Gallus gallus gallus* (**GG**) and *Gallus gallus spadiceus* (**GS**) from the Thailand National Park, Wildlife and Plant Conservation Department in collaboration with the Wildlife Conservation Office; TIC names are based on male plumage (Table 1); Pradu Hang Dam (**PD**) from Research and Development Network Center for Animal Breeding (Native chickens) of Khon Kaen University, Leung Hang Khao (**LK**), Chee (**CH**) and Dang (**DG**) from Department of Livestock, Bhutanese native chickens (Seim, Yuebjha Narp, Khuilay and Phulom) and three commercial lines (Isa Brown, **IB**; Broiler, **BR** and White Leg Horn, **WL**) from private Thai company. The DNA was extracted from whole blood by Guanidium Hydrocloride protocol as described in Goodwin et al. (2007). Spectrophotometer was used to adjust the genomic DNA concentration to 50ng/µl.

Table 1. Characteristics of Red Junglefowl, Thai and Bhutanese indigenous chicken, subspecies of used in this study

Population	Male	Female	Distribution	Morphology features		
				Comb type	Plumage	Shank and beak
G. g. gallus[1,2]			Northeast of Thailand	Single	Red jungle fowl like, greenish feathers tailed and sickle shaped, male are golden brown, sometimes reddish brown saddle; female are brownish red with dark-greenish strip following at each feather.	Black, yellowish
G. g. spadiceus [3,4]			North of Thailand	Single	Male has uniform golden yellow covered neck to lower back; tail feathers are greenish black with white patches; female are dark brown, yellowish plumage designed for camouflage, red ear lobe.	grey, yellowish
Pradu Hang Dam			Northeast	Pea	• Both adult are completely black • Male has dark-brown fringed feather on the saddle	Black, Black
Leung Hang Khoa			East and Central	Pea	• Males are mainly black on ventral part while dorsal plumage is yellowish. • Rarely primary wing is coloured on the web. • Female are usually black with whitish dorsal plumage.	
Chee			Central	Pea	• Both adult entire plumage is white	Yellowish, Yellowish

Population	Male	Female	Distribution	Morphology features		
				Comb type	Plumage	Shank and beak
Dang			South	Pea	• Male are reddish brown. • Female has blackish plumage around the neck.	Yellowish, Yellowish
Seim			Throughout Bhutan	Rose, pea, single	• Red jungle fowl like, greenish feathers tailed and sickle shaped, male are golden brown, sometimes reddish brown saddle; female are brownish red with dark-greenish strip following at each feather.	Black, yellowish
Yuebjha Narp			Southwest, West of Bhutan	Rose, pea	• Both sexes are entirely black; name derived from morphology.	Blackish, slate
Khuilay (Naked neck)			South, Southwest of Bhutan	Rose, pea, single	• Generally soft-feather red, diverse plumage color occurs (such white, partridge), featherless at neck.	Yellowish, whitish
Phulom (Frizzle)			Southwest, South of Bhutan	Rose, pea	• Feathers faced outwards (various colour such as Seim, black).	Yellowish, black

[1]*Gallus gallus gallus* male (Bao, 2009); [2]*Gallus gallus gallus* female (Tsai, 2010); [3]*Gallus gallus spadiceus* male; [4]*Gallus gallus spadiceus* female (Somchai, 2009)

Microsatellites and functional genes genotyping

The microsatellite markers were selected based on having more than four alleles (Nassiri et al. 2007; Nassiry et al. 2009). Twenty microsatellites loci were genotyped to compare genetic variation among four TICs and three commercial lines. Microsatellites eighteen loci were used to classify two RJF, two TIC (PD and CH), Bhutanese native chickens and broiler. Eighteen microsatellites were versus with five function genes (six loci) in two RJF, PD, broiler and WL.

Statistical analysis approach

The alleles were computed and analyzed to examine mean number of alleles (MNA), observed heterozygosity (H_O) and expected heterozygosity (H_E).

A Neighbor-Joining method (Saitou and Nei, 1987) of Numerical Taxonomy System (NTSYSpc) Version 2.10 package was used to construct a phylogenetic tree based on Nei (1978) unbiased genetic distance. Principal component analysis (SAS, 1998) based on individual Dice genetic distance was employed to visualize genetic relationships and detect geographical clines that may not be apparent from the phylogenetic tree.

RESULTS AND DISCUSSION

Genetic diversity between Thai indigenous and commercial chickens

The regular parameters used to assess population variations are Mean number allele (MNA), Observed (direct count) heterozygosity (H_O) and expected heterozygosity (H_E) (Tadano et al. 2007). MNA per locus was 11.35 for seven populations and 14.17 for ten populations. Genetic variability for every microsatellite loci were analyzed and summarized in Table 2 and 3.

The results of genetic diversity for seven populations are summarized in Table 2. The MNA examined minimum and maximum for IB (7.60) and CH (8.80), respectively. The MNA and H_E for all Thai chicken populations were greater than the commercial lines except for PD. Among the Thai chicken populations, CH and DG exhibited for superior H_E (CH: H_E = 0.80; DG: H_E = 0.81). On contrary, IB was inferior in H_E (0.77) though H_O (0.71) was the highest. This study showed considerable genetic diversity in the populations. MNA is another form of reporting genetic diversity (Toro et al. 2009; Nassiry et al. 2009) intended for conservation. The MNA value is determined by sample sizes (Toro et al. 2009), hence, H_E and H_O are fundamental parameters extended to infer the population diversity (Nassiry et al. 2009; Toro et al. 2009). Compared to the commercial lines, the TIC populations had greater H_E. This implies that random mating is frequent within population and also with the wild RJF. The higher H_O:H_E ratio in commercial lines, particularly in IB, depicted that the population size was relatively small at the beginning (Tadano et al. 2007). Among the TIC populations, the highest heterozygosity was found in CH and DG, represented the greater genetic diversity. Conversely, PD exhibited lower MNA and heterozygosity which showed that slight selection pressure might occur. The H_E (~0.8) in TIC populations was higher than H_E (0.58) found in *Mazandaran* chicken populations using same 20 microsatellite loci (Nassiri et al. 2007), reflecting that the Thai indigenous chickens retained the rich of genetic diversity.

Table 2. Estimation of average number of alleles and genetic variation using twenty microsatellite primers for seven populations (four TICs and three commercial lines)

Primer	Allele	PD		DG		CH		LK		BR		IB		WH	
		H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E
MCW 14	12	0.73	0.84	0.90	0.85	0.88	0.88	0.60	0.81	0.37	0.87	0.73	0.84	0.50	0.81
MCW 34	13	0.87	0.87	1.00	0.83	0.88	0.89	0.90	0.87	0.67	0.85	0.90	0.90	0.93	0.89
MCW 37	11	0.47	0.69	0.62	0.73	0.72	0.80	0.83	0.81	0.57	0.58	1.00	0.84	0.43	0.80
MCW 69	9	0.33	0.75	0.90	0.80	0.50	0.80	0.53	0.80	0.20	0.58	0.87	0.79	0.80	0.81
MCW 81	10	0.87	0.82	0.75	0.88	0.72	0.81	0.83	0.82	0.50	0.80	0.90	0.75	0.60	0.78
MCW 104	15	0.23	0.83	0.79	0.88	0.69	0.87	0.77	0.88	0.67	0.86	0.93	0.80	0.20	0.70
MCW 111	6	0.30	0.68	0.55	0.66	0.44	0.64	0.53	0.65	0.47	0.69	0.17	0.61	0.27	0.68
MCW 123	8	0.47	0.76	0.72	0.82	0.63	0.80	0.70	0.80	0.43	0.73	1.00	0.67	0.57	0.76
MCW 183	16	0.37	0.85	0.48	0.92	0.34	0.88	0.47	0.87	0.57	0.87	0.50	0.83	0.60	0.91
MCW 222	14	0.83	0.86	0.59	0.85	0.75	0.89	0.87	0.89	0.77	0.89	0.43	0.64	0.43	0.82
MCW 248	12	0.43	0.77	0.14	0.79	0.25	0.80	0.37	0.72	0.10	0.83	0.40	0.78	0.03	0.84
MCW 295	8	0.13	0.72	0.48	0.77	0.31	0.76	0.40	0.77	0.47	0.81	0.43	0.68	0.37	0.56
ALD 112	13	0.73	0.78	0.24	0.79	0.22	0.69	0.23	0.77	0.40	0.77	0.80	0.78	0.60	0.73
ADL 123	10	0.17	0.72	0.38	0.62	0.38	0.56	0.57	0.69	0.47	0.84	0.70	0.75	0.53	0.88
ADL 127	9	0.90	0.77	0.45	0.77	0.16	0.84	0.47	0.77	0.53	0.80	0.77	0.78	0.50	0.81
ADL 147	12	0.87	0.86	0.69	0.89	0.63	0.86	0.77	0.87	0.60	0.86	0.97	0.85	0.43	0.80
ADL 268	8	0.50	0.77	0.69	0.74	0.38	0.66	0.13	0.67	0.33	0.73	0.73	0.83	0.50	0.77
ADL 372	10	0.77	0.73	0.62	0.87	0.34	0.83	0.67	0.85	0.47	0.77	0.03	0.63	0.73	0.72
LEI 94	15	0.97	0.91	0.69	0.81	0.94	0.87	0.80	0.85	0.90	0.87	0.93	0.78	0.90	0.80
LEI 166	16	0.70	0.84	0.86	0.90	0.78	0.88	0.60	0.88	0.53	0.86	0.93	0.79	0.60	0.85
Mean	11.35	0.58	0.79	0.63	0.81	0.55	0.80	0.60	0.80	0.50	0.79	0.71	0.77	0.53	0.79

Note: PD = Pradu Hang Dam; DG = Dang; CH = Chee; LK = Leunghangkhoa; BR = Broiler; IB = Isa Brown; WH = White Leg Horn. H_O and H_E = observed and expected heterozygosity respectively.

Table 3. Genetic variability estimates (mean ± SD) from eighteen microsatellite loci for 10 chicken populations

Population	MNA ± SD[a]	H_O ± SD[b]	H_E ± SD[c]
Gallus gallus spadiceus	9.28 ± 0.66	0.47 ± 0.06	0.81 ± 0.02
Gallus gallus gallus	9.50 ± 0.59	0.52 ± 0.06	0.82 ± 0.01
Seim	9.33 ± 0.72	0.51 ± 0.06	0.82 ± 0.01
Yuebjha Narp (Black chicken)	7.94 ± 0.40	0.44 ± 0.05	0.79 ± 0.02
Khuilay (Naked neck)	9.50 ± 0.68	0.49 ± 0.05	0.83 ± 0.02
Phulom (Frizzle)	8.50 ± 0.57	0.55 ± 0.04	0.81 ± 0.01
Pradhu Hang Dam (Black chicken)	9.78 ± 0.69	0.59 ± 0.06	0.83 ± 0.02
Chee (White chicken)	10.83 ± 0.85	0.58 ± 0.04	0.84 ± 0.02
Broiler	9.28 ± 0.77	0.49 ± 0.06	0.82 ± 0.02
White Leghorn	8.67 ± 0.82	0.45 ± 0.06	0.78 ± 0.02

[a] Mean number of alleles per locus, [b] observed heterozygosity, [c] expected heterozygosity

Genetic diversity among Red Junglefowl, Thai indigenous, Bhutanese indigenous and commercial chickens

The levels of genetic variations across ten populations were assessed (Table 3). They were greater for CH (MNA, 10.83 ± 0.85; H_O, 0.58 ± 0.04; H_E, 0.84 ± 0.02) and Khuilay (MNA, 9.50 ± 0.68; H_O, 0.49 ± 0.04; H_E, 0.83 ± 0.02). By contrast, Phulom (MNA, 8.50 ± 0.57; H_O, 0.55 ± 0.04; H_E, 0.81 ± 0.01) and Yuebjha Narp (MNA, 7.94 ± 0.40; H_O, 0.44 ± 0.05; H_E, 0.79 ± 0.02) tends to contain lower genetic variations compared to the control populations. For all loci, the mean H_E was higher than mean H_O describing the sampling biasness or possibly inbreeding mating system. Low observed heterozygosity may lead to positive assortment or a situation of high homozygosity.

Evidently, data regarding the breeds and their specific adaptations, distinct phenotypes, performance level, demography (includes effective population size, local or transboundary, geographical distribution, level of enlargement), and description databases are also required to assess decision on the breeds for conservation and breeding programs (Groeneveld *et al.,* 2010). Nevertheless, the genetic data is a fundamental method to indicate the existence of biodiversity (Nassiri *et al.,* 2007; Semik and Krawczyk, 2011).

The environmental influences on individual and geographical barrier possibly explain the presences of very high number of alleles at various loci but also fairly high F_{IS} values. Though mean F_{IS} value was high, the test for HWE indicated non-significant deviation from HWE in native chickens and Junglefowl chickens. On the other hand, eight loci (Broiler) and two loci (WL) deviating HWE informs commercial populations were intensively selected decades for morphology and production, genetic subdivision then occur. It was possible that some loci might be associated with genes that might be lost due to genetic drift this could explain for a few loci with a strong genetic differentiation and others slightly. However, mean F_{ST} value indicates that subpopulation division is moderate and 8.4% of the total genetic variation is caused by population differences while 91.6% corresponds to differences within populations.

Comparable population variations were observed for Seim and Khuilay with original and ancestor fowl populations. Strain Seim is commonly reared by Bhutanese farmers while Khuilay has highly diversified plumage colour (soft-red, white, black, partridge, and speckled) and possible gene flow from Indian Naked neck populations. The major issue of

concern is for Yuebjha Narp population which has low variations. The possible reasons could be the least diversified morphology and finite population sizes (approximated average 20 to 25 individuals per village). As expected, the H_E for the two subspecies of RJF across the loci, was higher than the WH, even more than those obtained by Hillel *et al.* (2003) and Granevitze *et al.* (2007). The present study shows that the wild progenitor of the domestic chickens contains considerable genetic variation as reported in RJF of Northern India (Mukesh *et al.,* 2011). The wild ancestors of major livestock species considered to be genetic diversity reservoirs are either extinct or low in numbers (Hanotte and Jianlin, 2005). Therefore, putative wild ancestors of our present-day chickens must be conserved because they are threatened to extinction by the habitat loss, fragmentation, and poaching. On contrary, commercial lines were developed from few breeds. Thus, the commercial lines has low genetic base and in other words lower genetic variations than the native and Junglefowl populations. Interestingly the result revealed substantial genetic variation content was observed similarly as reported that enable further genetic progress (Pirany *et al.,* 2007).

Phylogenetic relationships

A phylogenetic tree was reconstructed exclusively based on Nei's unbiased genetic distance (Figure 1), four Thai indigenous chickens and three commercial lines split into four clusters (two clusters each represented by Thai chicken populations and commercial lines. IB and WL branched together to form an egg layer and commercial broiler represented another group. Among the Thai chicken populations, PD clustered separate from CH, DG and LK. It confirmed that the high pressure on selection for meat (broiler) or egg (IB and WL) could differentiate the genetic structure from the unselected TIC (*G. domesticus*). This result was in agreement with Tadano et al. (2007a) for 12 commercial lines. Among Thai chicken populations, PD formed different cluster from the others and it might be related with special characteristics of black plumage, shank and beak while the other TIC were white, yellow or red plumage with yellow shank and beak. The tree from this study also revealed that based on genetic clustering, the group of CH, LK, and DG were closer to the group of layers. On the contrary, the relative genetic distance between PD was closer to commercial broiler (0.044) than commercial layers (0.055). These result suggested that PD could justify to be improved for meat type while the others TIC should consider for egg type.

Moreover, the genetic classification of RJF, TIC, Bhutanese chicken, broiler and layer chicken, illustrated that one Khuilay (Bhutanese naked neck) was most closely related to to PD (Thai native black). The other three Bhutanese strains, Seim (RJF like), Yuebjha (black feather), and Phulom (frizzle) were in a separate group with a node connect to PD. According to the results, Bhutanese native chickens should be classified genetically close to Southeast Asian domestic chicken. This study also showed that Bhutanese native chicken and TIC (*Gallus gallus domesticus*) were related to *Gallus gallus spadiceus*, the red earlobe RJF (Figure 2). The relatedness of Khuilay and PD, and separate genetic group of the other Bhutanese native chicken were confirmed in the PCA plot, however, the result from phylogenetic tree and PCA showed a silent difference (the data not shown).

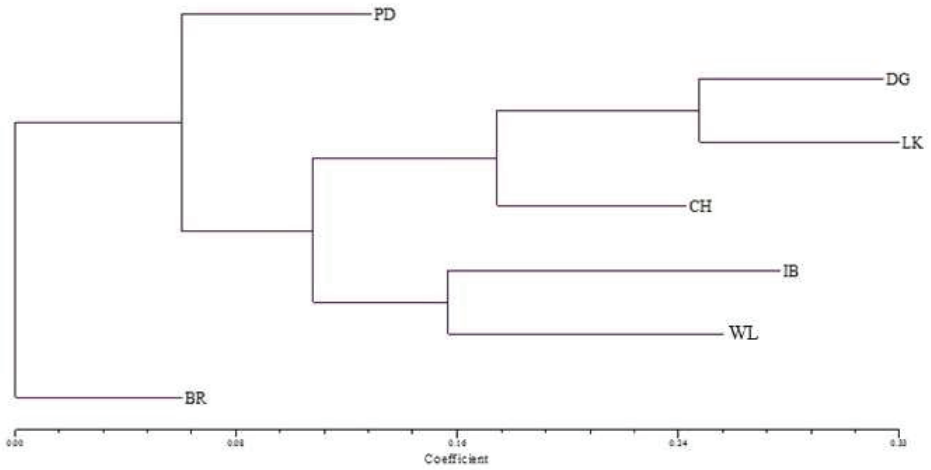


Figure 1. A phylogenetic tree among seven subpopulations (four TICs and three commercial lines) based on Nei's unbiased genetic distance method. (PD = Pradu Hang Dam; DG = Dang; CH = Chee; LK = Leunghangkhoa; BR = Broiler; IB = Isa Brown; WL = White Leg Horn)

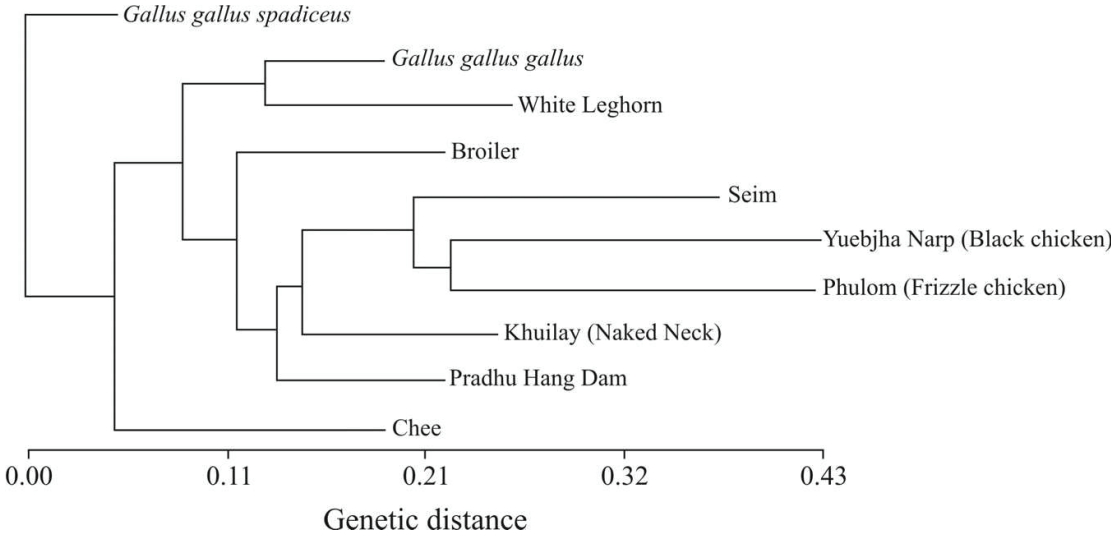
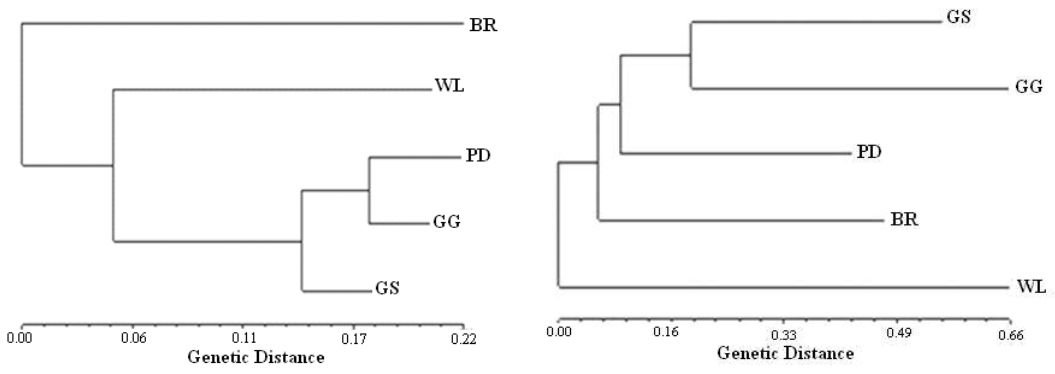


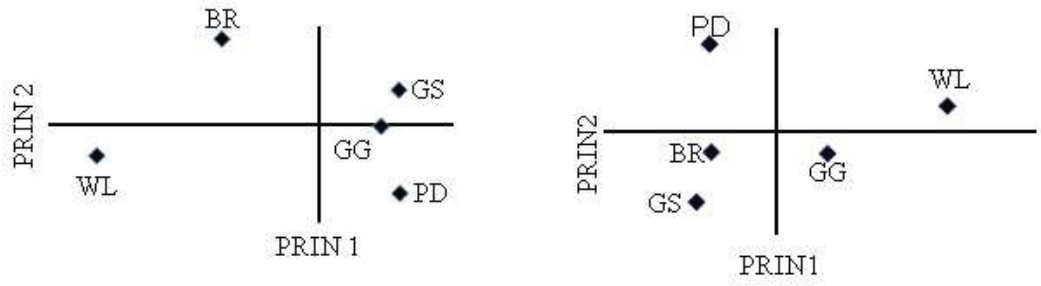
Figure 2. A phylogenetic tree based on Nei's genetic distance (Nei, 1978) for ten chicken populations

In addition, genetic comparison between microsatellites and functional genes in RJF, TIC and two commercial chickens revealed phylogenetic tree and PCA plot derived from microsatellites and functional genes were similar (Figure 3 and 4). Overall, the genetic comparison for RJF, PD and commercials line with functional genes was highly efficient in detecting genetic differences between populations. Thus, the appropriate set of functional genes may be regarded as useful tools, taking into consideration populations that are under different degrees of selection.



(a) **(b)**

Figure 3. A phylogenetic tree based on Nei's Unbiased distance from six loci of functional genes (a), and eighteen microsatellite markers (b) for *G. gallus gallus* (GG), *G. gallus spadiceus* (GS), Pradu Hang Dam (PD), Broiler (BR) and White Leghorn (WL)



(a) **(b)**

Figure 4. Two-dimention principal components plot among 5 populations based on Dice's genetic similarity of six loci of functional genes (a), and eighteen microsatellite markers (b) for *G. gallus gallus* (GG), *G. gallus spadiceus* (GS), Pradu Hang Dam (PD), Broiler (BR) and White Leghorn (WL)

CONCLUSIONS

Despite the bias in comparing with previous report we may conclude that Thai indigenous chicken seems to have good genetic diversity with DG showing the highest variations followed by CH and LK. If we consider the relatively small genetic distance between PD with broiler, it is suitable for PD to be developed as a meat type. The other Thai indigenous chickens might be developed as an egg type due to closely genetic clustering. Principal component analysis plot revealed Bhutanese native chickens should be prioritized for conservation because of their genetic distinctiveness. The comparison between microsatellites and functional genes showed appropriate set of functional genes may be regarded as distinguished alternative tools for consideration populations that are under different degrees of selection.

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