Genetic Variation and Phylogeographic Analysis of Native Chicken Populations in Myanmar and Thailand

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Native chickens in Myanmar and Thailand have been being domesticated for generations and yielded a wide variety of chickens. The objective of the research is to analyze the genetic variation and relationships of native chicken populations in Myanmar and Thailand. A total number of 249 genomic DNA samples from a total nine populations including the commercial lines, were genotyped using 98 autosomal SNP markers. The average heterozygosity of each population was in the range of 0.181–0.262. The neighbor-joining trees constructed by pair-wise F_{ST} estimates corresponded that the native chickens in Myanmar and Thailand were at a distance from the commercial chickens. The STRUCTURE analysis revealed that the nine chicken populations used in this study might be derived from six genetic populations ($K=6$). The AMOVA showed significant value of F_{ST} with 79–90% of the total genetic variation found within populations. The F_{CT} value was significant but accounted for only 4.45% of the total variability among countries. Finally, the mantel test for Isolation by Distance result ($r=0.5964$; $P<0.01$) suggested the contribution of geographic distance to the genetic structure of native chicken populations in Myanmar and Thailand.

Key words: genetic variation, genetic relationship, heterozygosity, native chicken phylogeography

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Introduction

Native chickens in Myanmar and Thailand have been being domesticated for generations and yielded a wide variety of chickens. Their genetic characteristic may also vary along with the history of their migration and new ecosystems. However, either the breeds or ecotypes are mainly recognized based on the local knowledge and the geographic locations. It is assumed that the assessment of the genetic variation which is partitioned in a certain geographic scales would be useful to disclose their genetic variations with respect to the ecological background.

Several studies using molecular markers spread across autosome had been conducted to examine the population structure covering various levels of geographic regions in human (Ramachandran *et al.*, 2005; Wang *et al.*, 2007),

sheep (Lawson *et al.*, 2007), and native chicken (Mwacharo *et al.*, 2007). Particular to the native chicken, the study was conducted in East Africa, where each area is ecologically diverged. However, no report is found with respect to the geographical aspects for the native chickens in Myanmar and Thailand, which is known to be domesticated in the same region with their origin, red junglefowl. It is reported that chickens became domesticated from red junglefowl in South East Asia, including Myanmar and Thailand (Nishibori *et al.*, 2005; Sawai *et al.*, 2010). The domestication of chicken has been part of a long custom and tradition of people in these countries, such as cockfight breed in Thailand.

In the previous study, we have genotyped and analyzed the genetic diversity of the Indonesian native chickens using 98 autosomal SNP markers (Riztyan *et al.*, 2011). The SNP markers would also be useful to provide the genetic variation data for the native chickens in the other regions in South East Asia, particularly in Myanmar and Thailand. This study was aimed to analyze the genetic variation and relationships of native chicken populations in Myanmar and Thailand. It is expected that the analysis, which included the variation data and geographical aspects, would provide information to

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maintain the genetic resources of the native chickens in these regions.

Materials and Methods

Sample Collection and DNA Extraction

A total number of 249 genomic DNA samples were obtained from nine populations from Myanmar, Thailand and commercial lines (Fig. 1). The blood samples of Myanmar native chickens were collected in Yangon, Mandalay, and Bago. In Thailand, the blood samples of native chickens were collected in Nan, Chiang Rai and Lampang (northern area), Kanchanaburi (western area), and in Trat and Chantaburi (southeastern area). In commercial lines i.e. Cobb, White Leghorn and Rhode Island Red, as reference breeds, blood samples were collected from commercial farms in Kagoshima, Japan. The sampling code and its amount are presented in Table 1. Genomic DNA was purified from these samples using the proteinase K and phenol-chloroform method (Sambrook *et al.*, 1989).

SNP Markers and Genotyping

Genomic DNA samples were genotyped using 98 SNP markers across chicken autosome. Genotyping was conducted using the standard polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Information on the validated SNPs and the detail PCR-RFLP conditions could be found in our previous study (Riztyan *et al.*, 2011).

Data Analysis

Average expected heterozygosity ($\overline{He}$) was estimated by referring to Nei (1978). Population structure was investigated by using the Bayesian clustering algorithm implemented in the STRUCTURE 2.3.3 application (Pritchard *et al.*, 2000) to cluster 438 individuals (13 populations) on the

basis of their genotypes at 98 SNP loci. We applied a burn-in period of 20,000 followed by 20,000 iterations of Markov chain Monte Carlo (MCMC) inference (Pritchard *et al.*, 2000). The probable number of genetic clusters (K) was set to the range of $1 \leq K \leq 20$, with 20 independent runs for each value of K . For the inference of the K value, we used the estimated likelihood of the probability of data ($\ln \Pr(X|K)$) and the second order rate of change of the likelihood function with respect to K (ΔK). $\ln \Pr(X|K)$ was expressed by the mean $\pm$ SD of $\ln \Pr(X|K)$ over 20 runs for each K . The ΔK value was calculated using Structure Harvester 0.56.3 application (Riztyan *et al.*, 2011). The optimal K value was determined by the greatest value of ΔK as described in Evanno *et al.* (2005). The DISTRUCT 1.1 application (Rosenberg, 2004) was used to display the assignment probabilities for population subdivisions, because individuals were assumed to have ancestry from only one population. Subdivisions were represented as colors, and individuals were depicted as bars partitioned into colored segments that corresponded with the membership coefficients for the population.

The genetic variation was compared among countries, among populations within a country and within populations after 20,000 permutations using a hierarchical analysis of molecular variance (AMOVA) by referring to Excoffier *et al.* (2005) in Arlequin 3.5. Significance levels were determined by default settings for genotypic data. The mantel test for Isolation by Distance (IBD) was performed between genetic differentiation [$F_{ST}/(1-F_{ST})$] (Slatkin, 1995) and geographic distances (km) among populations using IBDWS 1.52 (Bohonak, 2002). Geographic locations were determined by referring to the United States Geological Survey (USGS) latitude and longitude decimal data. The pairwise distance matrix (F_{ST}) (Weir and Cockerham, 1984) was estimated using the GENEPOP 4.0 program (Rousset, 2008). Neighbor-joining trees for individuals and among populations were constructed using MEGA4 (Tamura *et al.*, 2007). R^2 was calculated to determine whether a tree's topology and branch lengths accurately reflect the genetic distances in the genetic distance matrix (Kalinowski, 2009).

Results

Genetic Diversity Within Population

The genetic diversities of each population across geographic regions in Myanmar and Thailand are compared in Table 1. The results suggested that the greatest (0.262 ± 0.031) and the smallest (0.181 ± 0.038) expected heterozygosity ($\overline{He}$) were found respectively in the native chicken populations in the north part of Thailand (TN) and the commercial line, RIR. The greatest deficit and excess of heterozygosity was found by 0.217 and -0.419 in the TSE and WL populations, respectively.

Differentiation Among Populations and Admixture Analysis

Global F_{ST} across 98 loci was 0.261, while all pairwise values ranged significantly ($P < 0.05$) from 0.018 (YG-BG) to 0.397 (TW-RIR) (Table 2). In average, native chickens in Myanmar and Thailand mostly showed moderate genetic

Fig. 1. Sampling locations in Myanmar and Thailand built on ArcMap 9.3 based on Peel *et al.* (2007). The gradation of color from white to gray represents each Koppen-Geiger climate group and criteria. Abbreviations for populations are the same as in Table 1.

Table 1. Genetic diversity across 98 SNP loci in the Myanmar and Thailand native chickens and commercial lines

Group	Population	Code	n ($\Sigma=249$)	$P_{poly} \pm SE$	$\overline{H_e} \pm SE^{1)}$	$\overline{H_o} \pm SE^{2)}$	$F_{IS}^{3)}$
Myanmar	Yangon	YG	25	0.816 ± 0.078	0.259 ± 0.041	0.258 ± 0.028	0.004
	Mandalay	MN	40	0.806 ± 0.063	0.250 ± 0.032	0.264 ± 0.050	-0.056
	Bago	BG	15	0.765 ± 0.112	0.255 ± 0.059	0.249 ± 0.029	0.024
Thailand	Nothern	TN	34	0.888 ± 0.055	0.262 ± 0.031	0.251 ± 0.114	0.042
	Western	TW	4	0.551 ± 0.252	0.228 ± 0.131	0.226 ± 0.030	0.009
	South Eastern	TSE	41	0.816 ± 0.062	0.244 ± 0.025	0.191 ± 0.035	0.217
Average				0.774 ± 0.104	0.250 ± 0.053	0.240 ± 0.048	
Commercial	Cobb	CB	30	0.755 ± 0.079	0.254 ± 0.035	0.283 ± 0.042	-0.114
	White leghorn	WL	30	0.541 ± 0.092	0.198 ± 0.039	0.281 ± 0.062	-0.419
	Rhode island red	RIR	30	0.510 ± 0.092	0.181 ± 0.038	0.203 ± 0.048	-0.122
Average				0.602 ± 0.088	0.211 ± 0.037	0.256 ± 0.051	

¹⁾ Mean expected heterozygosity $\pm$ SE; ²⁾ Mean observed heterozygosity $\pm$ SE; ³⁾ Inbreeding coefficient.

Table 2. Pairwise distance matrix (FST) between populations

Population	YG	MN	BG	TN	TW	TSE	CB	WL	RIR
YG									
MN	0.068								
BG	0.018	0.066							
TN	0.089	0.061	0.081						
TW	0.100	0.076	0.093	0.054					
TSE	0.123	0.104	0.135	0.047	0.061				
CB	0.112	0.099	0.127	0.104	0.125	0.112			
WL	0.234	0.214	0.248	0.237	0.261	0.265	0.209		
RIR	0.331	0.293	0.357	0.299	0.397	0.323	0.278	0.376	
Mean	0.134 ± 0.033	0.123 ± 0.028	0.141 ± 0.037	0.122 ± 0.031	0.146 ± 0.04	0.146 ± 0.032	0.146 ± 0.021	0.256 ± 0.018	0.332 ± 0.014

All *P*-values applied to pair-wise FST were significant at $P < 0.05$; Abbreviations for populations are the same as in Table 1.

differentiation (0.122–0.146) whereas commercial lines except CB line showed much greater genetic differentiation ($F_{ST} \geq 0.146$). The neighbor-joining (NJ) tree derived from pairwise F_{ST} distance between the nine populations revealed that native chickens in both countries were clustered close to each other whereas the commercial lines, particularly RIR, diverged at greater distance (Fig. 2). The R^2 value (0.984) was near 1.0 and represented a good summary of the genetic relationships shown in the distance matrix (Kalinowski, 2009).

The STRUCTURE analysis revealed that the $\ln \Pr(X|K)$ increased sharply between $K=2$ and $K=6$, and slightly increased to reach a maximum likelihood value of -16663.4 at $K=15$. These results indicated that the appropriate value of K would be between $K=6$ and $K=15$. However, the mean of $\ln \Pr(X|K)$ were deviated from $K=7$ to $K=15$, and given the slight difference in the $\ln \Pr(X|K)$ within this range, the smaller K ($K=6$) was estimated to be the value that captured the major structure in the data (Pritchard *et al.*, 2000). In addition, according to the method of Evanno *et al.* (2005) for the inference of optimal K values, the K value was calculated to be six with a maximum value for the *ad hoc* statistic ΔK

($\Delta K=5.92$). This value was much greater than the ΔK at $K=5$ and $K=7$ by 0.64 and 0.50, respectively (Fig. 3). Therefore, the probable number of clusters (K) was thought to be six. The contribution of the six assumed ancestral populations ($K=6$) to the nine populations is presented in Figure 3. Similar admixture and proportions of membership, which suggested a common origin between populations, were detected between populations in each country. An admixed structure was found in MN, TN, and TW, while a sub-structure was found in TSE population. In contrast, the low level of admixture was detected in all commercial lines, YG and BG populations.

Genetic Variation Among Countries and Populations

The hypothetical model of population structure by country and population using AMOVA (Table 3) showed significant differences among populations with around 79–90% of the total genetic variation found within populations. Geographic distance of native chickens in Myanmar and Thailand had a significant contribution to the genetic variation but accounted for only 4.45% of the total variability. Population grouping based on the native and commercial type had a significant contribution with greater variation (6.09%) of the total vari-

Fig. 2. **Neighbor-joining tree among populations.** Abbreviations for populations are the same as in Table 1.

Fig. 3. **Individual assignment according to the estimated membership coefficient at $K=5$, $K=6$, and $K=7$.** Abbreviations for populations are the same as in Table 1.

Table 3. **Analysis of molecular variance (AMOVA) by country and population groups**

Group	n	Variance of components (%)			F statistics		
		Among groups	Among populations within group	Within populations	$F_{CT} (P)$	$F_{SC} (P)$	$F_{ST} (P)$
Country							
Myanmar	3						
Thailand	3						
Overall	6	4.45	5.27	90.28	0.045***	0.055****	0.097****
Population							
Commercial chickens	3		28.61	71.39			
Population							
Myanmar and Thailand native chickens	6						
Commercial chickens	3						
Overall	9	6.09	14.34	79.57	0.061****	0.153****	0.204****

*** $P < 0.001$; **** $P < 0.0001$

Fig. 4. Correlation between genetic differentiation [$F_{ST}/(1-F_{ST})$] and geographic distance (Ln) among six native chicken populations in Myanmar and Thailand. Abbreviations for populations are the same as in Table 1. (1: YG-MN, 2: YG-BG, 3: YG-TN, 4: YG-TW, 5: YG-TSE, 6: MN-BG, 7: MN-TN, 8: MN-TW, 9: MN-TSE, 10: BG-TN, 11: BG-TW, 12: BG-TSE, 13: TN-TW, 14: TN-TSE, 15: TW-TSE).

ability. The mantel test for Isolation by Distance resulted in a significant level of correlation between genetic differentiation and geographic distances (Fig. 4).

Discussion

In average, expected heterozygosity were mostly greater in Myanmar and Thailand native chickens than in commercial lines. However, we found evidence for heterozygote deficit in most native chickens. Particular to the TSE population, we observed that these results could be explained by subdivision among flocks (the Wahlund effect). It might result from our sampling method that included several flocks with small number of individuals. This occurrence was consistent with our previous report for the Indonesian native chickens (Riztyan *et al.*, 2011). In commercial lines, the observed heterozygosity were slightly greater, which was in agreement with the result obtained by Whittaker *et al.* (2004) and Lawson *et al.* (2007) for the commercial sheep and domestic sheep, and Zanetti *et al.* (2010) for the commercial chicken (brown layer) population and Italian breeds of chickens, where heterozygosity were greater than their wild relatives. They suggested that selective breeding are generally successful in maintaining diversity.

Pairwise distance matrix (F_{ST}) provided evidence of significant differentiation between populations despite the close genetic relationships and similar admixtures. This finding was clearly shown between YG and BG in Figs. 2 and 3. We observed that the geographic distance has a significant con-

tribution to the genetic differentiation between YG and BG (Fig.4). Interestingly, a small component of admixture from Myanmar populations was found in Thailand populations. The populations were presumably diversified due to the long history of domestication within the two countries that caused a restricted gene flow. It was reported that chickens became domesticated from red junglefowl in South East Asia nearly 10,000 years ago (Sawai *et al.*, 2010).

The AMOVA and Isolation by Distance results provided evidence for the contribution of geographic distance to the genetic structure of the native chicken populations in Myanmar and Thailand. Our result (Fig. 4) showed a lower correlation value (r) between genetic differentiation [$F_{ST}/(1-F_{ST})$] and $\ln$ (geographic distance) in comparison with the findings in East African indigenous chickens ($r=0.736$) by Mwacharo *et al.* (2007). However, according to the Koppen-Geiger climate classification (Peel *et al.*, 2007) these regions are characterized by tropical and arid. It was presumed that natural selection for adaptation to arid conditions such as high temperature and scarcity of water or feed could have contributed to the observed genetic divergence of Marsabit chickens from the other Kenyan and Ugandan chicken populations (Mwacharo *et al.*, 2007). Our sampling areas in Myanmar and Thailand are all under the same tropical (A) group (Fig. 1), which ecologically supported the distribution of all native chickens within the regions.

In summary, the result of this study suggested that the genetic differentiation between native chicken populations in Myanmar and Thailand might be due to the long history of domestication and distantly isolated location that caused a restricted gene flow.

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