Genetic Characterization of Taiwan Commercial Native Chickens Ascertained by Microsatellite Markers

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Taiwan commercial native chickens have played a vital role in the domestic market due to Taiwanese traditional cooking style and culture. This study investigated the genetic characterization and population structure of 10 Taiwan commercial native chicken populations, together with two exotic breeds and one population of red jungle fowl, using 22 microsatellites. The results showed that Taiwan commercial native chickens generally harbored high genetic diversity but lower than that of red jungle fowl population in terms of number of alleles and gene diversity. The neighbor-joining tree revealed a poor resolution with only two branches showing bootstrap values above 70%. Based on Bayesian clustering approach, thirteen populations were inferred into eight distinct clusters namely Game bird, B strain, L2 strain, White Broiler and White Leghorn with an average proportion of membership higher than 0.90 and the values higher than 0.85 for red jungle fowl, Hakka chicken and Hakka strain while four remaining breeds were closely related together. The population structure showed Taiwan commercial native chickens are more admixed, in contrast to occidental highly productive breeds. The high genetic variation within breed as shown in the results of the analysis of molecular variance, facilitated by gene exchanges, did not allow discriminating in an efficient way. This suggests that the genetic pool of Taiwan commercial native chickens is well distributed among breeds and therefore there is a good potential for adaptation to new environmental conditions or markets. Some populations, namely L2 strain and B strain showed very high inbreeding coefficient and thus could be considered at risks. Therefore, management needs to be taken into account for the populations, to prevent inbreeding depression and maintain genetic diversity.

Key words: Bayesian clustering approach, genetic diversity, microsatellite, population structure, Taiwan

J. Poult. Sci., 50: 290–299, 2013

Introduction

Chicken meat and egg play an important role in Chinese traditional cuisine in Taiwan. There was 40 to 50% chicken meat from imported exotic White broiler (USDA, 2006) while the remaining meat production comes from Taiwan

commercial native chickens (TCOM). For instance, Red feathered and Black feathered chickens produce about 80% of TCOM and the remaining 20% comes from commercial slow-growing local breeds such as Silkies, Naked Neck, Game bird, Hakka chicken, Golden chicken and Classical chicken (Lee, 2006). Taiwan native chickens were frequently crossed with imported exotic breeds (broiler-type rooster) to produce TCOM and selected by local farmers for high body weight, feed efficiency and their ability to adapt to local conditions since 1960s (Lee, 2006).

Genetic diversity of chicken genetic resources provides the

Received: March 25, 2012, Accepted: March 7, 2013

Released Online Advance Publication: April 25, 2013

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basis for genetic improvement in order to increase productivity but also to adapt domestic populations to changes in production environments as well as in markets, management practices, and disease challenges (Tixier-Boichard *et al.*, 2009; Boettcher *et al.*, 2010). Moreover, the understanding of the constitution of breeds, effective management and traceability of breed origin is needed for potential utilization their genetic resources (Dalvit *et al.*, 2007; Nakamura *et al.*, 2010).

Population structuring might reflect the management status and breeding histories of chickens. Using 18 microsatellite markers, Berthouly *et al.* (2009) recently reported that the scavenging Vietnamese Ha Giang chicken breed was highly variable and in some localities even exchanged genes with the wild relatives, red jungle fowl. In contrast, the study of six Taiwan native chicken breeds under a conservation program at Chung-Hsing University (NCHU) showed breed specific features with a multi-criteria approach combining 24 microsatellite markers, the melanocortin 1 receptor (*MC1R*) locus, a major histocompatibility complex (*MHC*) marker and phenotypic data (Chen *et al.*, 2004; Chen *et al.*, 2005; Chang *et al.*, 2012). Similarly, Takahashi and Nakamura (2007), using 24 microsatellites, suggested that four strains of the Nagoya breed were highly differentiated. These four Nagoya strains were established for egg and meat production since many years in Japan.

Taiwan commercial native chickens have been developed by local farmers for improvement of production efficiency as well as meat quality, but little information on their genetic relationships and population structuring is known. This research aims to investigate the genetic characterization and population structure for Taiwan commercial native chicken breeds.

Material and Methods

Populations

The TCOM have prevalently appeared to have a big erected comb, blue shanks, big legs, and a smaller breast with less fat under skin than exotic broiler, and produce small eggs but with larger proportion of yolk. They grow faster than native chickens (i.e., Hua-Tung, Hsin-Yi, Ju-Chi and Quemoy) and are preferred by Taiwanese consumers because its meat quality suits to the traditional cooking style and similar plumage color compared to Taiwan native chickens (Lee, 2006). Red feathered chicken was developed in early 1980's through continuously introgression of imported exotic breed into the small Red feathered chicken and selection for early maturity and production efficiency. The smaller body-size Black feathered chickens with white hackles were developed by farmers in Changhua County in the late 1980's. In recent years, Golden chickens were produced by crossing Red feathered male with Black feathered female. Simultaneously, Classical chickens with red-black plumage and strong shanks were also created. Game bird was a fighting chicken and local farmers only used hens and capons for food consumption.

Conservation of native chickens in Taiwan started from

1982, when native chickens were collected around the islands and conserved at NCHU. In the same year, this university began to establish chicken lines for specialized purposes. Both L2 and B strains were selected by NCHU from the same Taiwan native chicken population (Lee, 2006). L2 and B strains have now been selected for 24 and 26 generations, respectively, and have been extensively used in research as well as in production (Chao and Lee, 2001; Chen *et al.*, 2007). Both strains were closed populations since their establishment in 1983 while B strain was a male line and L2 was female line for crossing to produce commercial meat-type chicken. L2 strain has higher egg production, but B strain is better for meat production after a long-term selection (Chen *et al.*, 2007). Likewise, Hakka people preferred large chickens so their chickens had low egg production (Lee, 2006). Thus, NCHU used Hakka chicken sires crossed with L2 strain dams to establish the synthetic Hakka strain in order to improve its egg performance since 2005.

Sampling and Genotyping Microsatellite

A total of 493 chickens from 10 Taiwan commercial native chicken breeds, two exotic breeds and one population of red jungle fowl with pure white earlobes (*G. g. gallus* type C) inhabiting in the continental Southeast Asia (Nishida *et al.*, 2000; Nishibori *et al.*, 2005) kept in Taiwan were analyzed together. Red jungle fowl (JF, $n=22$) came from Hualien preserved station (Fig. 1). Two exotic highly productive breeds were: a parent stock of White broiler (BR, $n=24$) and of White Leghorn (LG, $n=54$). Ten TCOM were investigated: B strain (BS, $n=45$), L2 strain (LS, $n=51$) and Hakka strain (HS, $n=27$) conserved at NCHU experimental farm; Hakka chicken (HC, $n=45$, from one farm), Black feathered (BF, $n=46$, from three farms), Red feathered (RF, $n=49$, from three farms), Golden chicken (GC, $n=13$, from one farm), Classical chicken (CC, $n=26$, from one farm), Game bird (GB, $n=42$, from two farms) and Naked Neck (NN, $n=49$, from two farms). The required laws and regulations regarding the use of chickens have been followed in scientific research. Blood samples were taken from the wing vein into the sterile blood collection tubes (BD Vacutainer, Franklin Lakes, NJ USA) containing 7.2 mg K2 EDTA and stored at 4°C and subsequently genomic DNA was extracted using the salt extraction method (Miller *et al.*, 1988) and stored at -20°C. Twenty two microsatellite markers distributed on 12 autosomal chromosomes (Table 1) previously used in the AVIANDIV project were chosen for genotyping 493 individuals. These all 22 markers were polymorphic in 52 chicken populations from different management practices and breeding histories (Hillel *et al.*, 2003). PCR products were analyzed by 3730 DNA Analyzer of Applied Biosystems and genotypes were read using GENEMAPPER version 4.0.

Data Analysis

The presence of null alleles was tested using FreeNA software (Chapuis and Estoup, 2007) in which loci with the estimated frequencies of null alleles ($r \geq 0.2$) were considered to be potentially problematic for calculations. The number of alleles, effective number of alleles (Weir, 1990) and

Fig. 1. The distribution of 10 Taiwan commercial native chicken populations, two exotic breeds and one red jungle fowl population all over the country. B strain (BS), L2 strain (LS), Hakka strain (HS), Hakka chicken (HC), Black feathered (BF), Red feathered (RF), Golden chicken (GC), Classical chicken (CC), Game bird (GB) and Naked Neck (NN); White broiler (BR) and White Leghorn (LG); red jungle fowl (JF). This map was obtained from <http://www.infoplease.com/atlas/country/taiwan.html> (Accessed on November 9, 2011).

private alleles were calculated by GENALEX 6.41 package (Peakall and Smouse, 2006). The observed heterozygosity, expected heterozygosity and Polymorphic Information Content (PIC) values (Botstein, 1980) were performed by the CERVUS version 3.0.3 (Kalinowski *et al.*, 2007). Moreover, the F_{IS} values according to Weir and Cockerham (1984) and exact test for deviation from Hardy-Weinberg equilibrium (HWE; Guo and Thompson, 1992) were computed by the GENEPOP software version 4.1 (Rousset, 2008). Allelic richness (AR), using a rarefaction method, was estimated with FSTAT 2.9.3 (Goudet, 2002).

The modified Cavalli-Sforza chord distance D_A (Nei *et al.*, 1983) and Reynold's genetic distance D_R (Reynolds *et al.*, 1983) were computed by the POPULATIONS package 1.2.32 (Langella, 1999). The phylogenetic tree was constructed using neighbor-joining (NJ) clustering method (Saitou and Nei, 1987) based on pairwise genetic distances D_R (Reynolds *et al.*, 1983) for the 13 populations by the PHYLIP version 3.69 (Felsenstein, 2009). Bootstrap resampling values of 1,000 was performed to test the robustness of

the tree topology. Analysis of molecular variance (AMOVA) was computed using ARLEQUIN version 3.5.1.2 (Excoffier and Lischer, 2010) and variance components were estimated among and within the population groups.

Additionally, ten TCOM populations, two exotic breeds and one red jungle fowl population were used to investigate the genetic structuring between populations. The Bayesian clustering method implemented in STRUCTURE 2.3.3 (Pritchard *et al.*, 2000) was performed with admixture model and with correlated allele frequency to infer the population structure (Falush *et al.*, 2003) on the BIOPORTAL (Kumar *et al.*, 2009). In the first step, we ran Structure from $K=1$ to $K=16$ as the number of assumed genetic clusters, 50 independent runs for each K value with 1×10^6 MCMC iterations after a burn-in period of 5×10^5 repetitions. The evaluation of the best K genetic cluster was based on ΔK from $K=2$ to $K=15$, 50 runs each K value following Evanno method (Evanno *et al.*, 2005) using STRUCTURE HARVESTER v0.6.91 application (Dent and Bridgett, 2012). Then CLUMPP 1.1.2 (Jakobsson and Rosenberg, 2007) was used

Table 1. The profile of 22 microsatellite markers, allele range (bp), annealing temperature (T_A , °C), observed number of alleles (N_A), expected heterozygosity (H_E), Polymorphic Information Content (PIC), null allele frequencies (r) and private alleles across 10 Taiwan commercial native chicken populations, two exotic breeds and one red jungle fowl population

Locus	GGA	Allele range	T_A	N_A	H_E	PIC^1	r (Null)	Private alleles ²
ADL0112	10	122 – 130	59	5	0.619	0.564	0.022	
ADL0268	1	102 – 114	60	6	0.774	0.736	0.060	
ADL0278	8	99 – 123	59	9	0.680	0.622	0.056	99 (0.02); 109 (0.04)
LEI0192	6	234 – 332	60	25	0.688	0.665	0.109	236 (0.01); 262 (0.01); 268 (0.07); 286 (0.02); 304 (0.02); 314 (0.02); 332 (0.02)
MCW0014	6	165 – 189	60	10	0.583	0.513	0.109	166 (0.32); 171 (0.45); 183 (0.06); 187 (0.02)
MCW0034	2	215 – 245	59	14	0.597	0.574	0.128	215 (0.11); 219 (0.23); 221 (0.09); 241 (0.02); 243 (0.04)
MCW0037	3	152 – 158	62	4	0.576	0.524	0.024	
MCW0067	10	174 – 186	60	7	0.642	0.578	0.037	174 (0.09); 176 (0.02); 186 (0.18)
MCW0069	26	155 – 177	60	12	0.752	0.716	0.059	162 (0.04)
MCW0078	8	136 – 144	60	5	0.550	0.508	0.064	
MCW0081	5	107 – 133	60	7	0.636	0.565	0.101	107 (0.02); 132 (0.01)
MCW0098	4	255 – 265	60	4	0.498	0.379	0.014	265 (0.03)
MCW0103	3	264 – 276	62	6	0.397	0.351	0.086	272 (0.07); 276 (0.02)
MCW0111	1	96 – 106	60	6	0.620	0.556	0.036	96 (0.05)
MCW0183	7	292 – 319	60	11	0.764	0.730	0.080	307 (0.02); 309 (0.09); 317 (0.10); 319 (0.10)
MCW0206	2	221 – 241	60	8	0.750	0.712	0.029	231 (0.70); 241 (0.39)
MCW0216	13	136 – 150	60	7	0.598	0.521	0.067	140 (0.11)
MCW0222	3	219 – 225	60	4	0.556	0.511	0.030	
MCW0248	1	216 – 224	59	4	0.411	0.378	0.023	218 (0.32)
MCW0284	4	234 – 242	58	5	0.508	0.419	0.028	236 (0.07)
MCW0295	4	81 – 99	60	9	0.764	0.725	0.085	81 (0.08)
MCW0330	17	246 – 286	60	8	0.797	0.768	0.121	246 (0.43)
Mean				8.0	0.625	0.573	0.069	

¹ $PIC > 0.50$ indicating a high level of polymorphism, $0.25 < PIC < 0.50$ indicating a medium level of polymorphism and $PIC < 0.25$ indicating a low level of polymorphism;

² Values in brackets indicated the frequency (%) of private alleles.

to estimate the highest similarity coefficient (Rosenberg *et al.*, 2001) over all runs for different value of K using LARGKREEDY algorithm to compute the similarity function G' . The Q matrix with the highest similarity was used for graphical representation of individual assignments by DISTRICT 1.1 program (Rosenberg, 2004). The second step applied the method suggested by Rosenberg *et al.* (2001) for the evaluation of individual breed assignment. At $K=2$, Game bird was differentiated from other 12 populations so we ran structure on the 12 remaining populations using $K=1$ to 15 as number of genetic clusters following the procedures in the first step. The third step, we analyzed separately the subcluster involving four breeds (i.e., Classical chicken, Black feathered, Red feathered and Golden chicken) from $K=1$ to 6 and the last subcluster involving two breeds (i.e., Hakka strain and Naked Neck) from $K=1$ to 5 as the procedures in the first step to quantify the admixture pattern.

Results and Discussion

Polymorphism of Microsatellite Markers

The null allele frequency of all 22 markers was lower than 0.20 (Table 1) so we assumed that null alleles were absent (Chapuis and Estoup, 2007). As a result, these 22 markers were used for further analyzes. Among 22 microsatellite

markers, a total of 176 alleles were detected across 13 populations. The mean number of alleles per locus was 8 and ranged from 4 to 25. The expected heterozygosity per locus averaged 0.625 and varied from 0.397 for locus MCW0103 to 0.797 for locus MCW0330. On average, the PIC value was 0.573 suggested that those 22 markers showed polymorphism in 10 TCOM populations, two exotic breeds and the red jungle fowl population (Botstein, 1980).

Genetic Diversity within Chicken Breeds

Within 10 TCOM populations, the average number of alleles per breed was 4.0 and ranged from 3.2 for Game bird to 4.8 for Black feathered chickens (Table 2). The number of alleles was similar to that of red jungle fowl population (3.8, Table 2) but higher than that of two exotic breeds (2.9 in White Leghorn and 3.2 in White broiler, respectively) in this study. The mean effective number of alleles was 2.4, the lowest value (2.0) was found in B and L2 strains while the highest value was 2.7 in Classical chicken. This averaged value was equal to the one found in red jungle fowl population (2.4) but lower compared to the value of 3.7 found in Indian chickens using 9 microsatellite markers (Pirany *et al.*, 2007). Among 13 populations, two populations, Hakka chicken and White broiler had no private alleles. The observed number of private alleles averaged 2.6 for overall

Table 2. Number of samples (n), observed number of alleles (N_A), effective number of alleles (N_E), number of private alleles (N_P), allelic richness (AR), observed (H_O) and expected (H_E) heterozygosity, Polymorphic Information Content (PIC), inbreeding coefficient (F_{IS}) and number of loci deviating from Hardy-Weinberg equilibrium test ($dHWE$) within 10 Taiwan commercial native populations, two exotic breeds and one red jungle fowl population based on 22 microsatellites

Population	n	N_A	N_E	N_P	AR	H_O	H_E	PIC^1	F_{IS}	$dHWE$
Red jungle fowl (JF)	22	3.8	2.4	10	3.4	0.419	0.536	0.468	0.223	3
White broiler (BR)	24	3.2	2.2	0	2.9	0.541	0.502	0.433	-0.057	0
White Leghorn (LG)	54	2.9	2.1	3	2.6	0.476	0.429	0.377	-0.129	1
Taiwan commercial native chickens (TCOM):										
B strain (BS)	45	3.3	2.0	1	2.7	0.363	0.444	0.381	0.220	4
L2 strain (LS)	51	3.6	2.0	3	2.8	0.322	0.419	0.372	0.218	4
Hakka strain (HS)	27	3.6	2.3	1	3.0	0.473	0.521	0.442	0.090	0
Black feathered (BF)	46	4.8	2.6	3	3.6	0.502	0.582	0.522	0.145	3
Classical chicken (CC)	26	4.5	2.7	3	3.8	0.481	0.592	0.522	0.169	2
Game bird (GB)	42	3.2	2.2	4	2.8	0.435	0.485	0.415	0.108	0
Golden chicken (GC)	13	3.8	2.6	1	3.6	0.430	0.607	0.522	0.283	3
Hakka chicken (HC)	45	4.0	2.5	0	3.3	0.506	0.578	0.509	0.125	2
Naked Neck (NN)	49	4.7	2.5	6	3.5	0.457	0.547	0.488	0.166	3
Red feathered (RF)	49	4.5	2.4	4	3.4	0.419	0.532	0.475	0.217	7
Mean of TCOM		4.0	2.4	2.6	3.3	0.439	0.531	0.465	0.174	2.8

¹ The $PIC > 0.50$ indicating a high level of polymorphism, $0.25 < PIC < 0.50$ indicating a medium level of polymorphism and $PIC < 0.25$ indicating a low level of polymorphism.

10 TCOM populations. This value is lower than 10 alleles in red jungle fowl (Table 1) and 9.8 alleles in Indian chickens (Pirany *et al.*, 2007). Allelic richness, based on minimum sample size of nine diploid individuals, averaged 3.3 which varied from 2.7 in B strain to 3.8 in Classical chicken, respectively. This mean value is lower than in red jungle fowl (3.4) but higher than that of two exotic breeds (2.6 in White Leghorn and 2.9 in White broiler, respectively), but also higher compare to Vietnamese local chickens (2.9, Berthouly *et al.*, 2009). The average values of the observed and expected heterozygosity harbored 0.439 and 0.531, respectively, which are very similar to the values of 0.419 and 0.536 in red jungle fowl as well as the value of 0.488 found in six Taiwan conserved chicken breeds using 22 loci (Berthouly *et al.*, 2008), but higher than the values ranging from 0.296 to 0.344 and from 0.341 to 0.395 found in four Nagoya strains, respectively (Takahashi and Nakamura, 2007). This can be partly explained as Nagoya strains were established in 1919 and have been selected for meat and egg since then. In contrast, TCOM populations have been managed in a way that enhances gene flow between flocks, and they have combined gene pools from different breeds (Lee, 2006; Tadano *et al.*, 2007) and thus increase their genetic diversity. For example, Hakka people prefer to keep their native chickens but they do not reproduce their own chicks, they always purchase large and sturdy chicks from hatchery or salesman irrespective to a specific breed (Lee, 2006). Such results are also consistent with values observed in village chickens in both Zimbabwe (Muchadeyi *et al.*, 2007) and Ethiopia (Goraga *et al.*, 2012) where local chickens have not been selected for performance traits and are managed in a

free-range system. The F_{IS} values ranged from 0.090 in Hakka strain to 0.283 in Golden chicken, which is a much larger range than the range observed in Taiwanese conserved chicken breeds (-0.053 to 0.068) by Berthouly *et al.* (2008). The present values of F_{IS} indicate a high inbreeding in Golden chicken, red jungle fowl and the remaining nine TCOM populations but an excess of heterozygotes in White Leghorn and White broiler populations. On average, 2.8 (0-7) of 22 loci deviated significantly from HWE suggesting a slight loss of heterozygosity. The high inbreeding rates reflect the fact that TCOM populations were developed from a small number of chicken and/or a strong selection for production efficiency and meat quality.

Breed Relationship and Population Clustering

The NJ tree based on D_R pairwise genetic distance presented a poor resolution with only two branches showing bootstrap values above 70%. Hakka and L2 strains were closed to Naked Neck with bootstrap values of 76.4%, and then clustering with White broiler with 100% bootstraps (Fig. 2). Hakka strain is a crossbreed between Hakka chicken and L2 strain. This strain seemed to be closer to L2 strain than Hakka chicken, which would indicate a higher contribution of L2 strain than Hakka chicken into Hakka strain. However, the D_A and D_R genetic distances amongst these three breeds were quite similar. The D_A genetic distances between Hakka strain and Hakka chicken, and Hakka and L2 strains were 0.165 and 0.150, respectively, while D_R genetic distances between Hakka strain and Hakka chicken, and Hakka and L2 strains were 0.161 and 0.189, respectively. L2 and B strains originate from the same original population while the D_A genetic distance between L2 and B strains was

Fig. 2. Neighbor-joining tree of 10 Taiwan commercial native chicken populations, two exotic breeds and one red jungle fowl population based on D_R genetic distance. Bootstrap values ≥ 70 were presented. The abbreviation of chickens is: red jungle fowl (JF); White Leghorn (LG); White broiler (BR); 10 Taiwan commercial native chickens: Game bird (GB), Classical chicken (CC), B strain (BS), Hakka chicken (HC), Naked Neck (NN), Hakka strain (HS) and L2 strain (LS), Black feathered (BF), Red feathered (RF) and Golden chicken (GC).

0.189 (Table 3). This genetic distance is higher than the one found between two Nagoya strains (0.140; Takahashi and Nakamura, 2007). Despite this common origin, they did not cluster in a significant way in the tree. A possible explanation to this is a strong effect of different selection objectives: meat vs. egg production lead to a clear genetic divergence between subpopulations (Takahashi and Nakamura, 2007). Moreover, small population size and therefore high inbreeding rates contribute to genetically differentiate subpopulations having the same origin (Frankham, 1996; Allendorf *et al.*, 2010).

Table 4 represented the analysis of global AMOVA for 12 Taiwan domestic chicken populations. When 10 TCOM populations were considered separately and grouped as TCOM vs. CEB (commercial exotic breeds), a non-significant genetic variation was obtained among groups (only 5.8

%, $P > 0.05$) indicated a very low genetic variation among two groups. Within groups, either TCOM or CEB, the highest genetic variation was found within populations ($> 75\%$). Overall, this indicates a small genetic variation between populations within and among groups and it is therefore in agreement with the poor resolution that we found with the NJ tree.

The well-known Bayesian clustering approach (Pritchard *et al.*, 2000) detects the population structure based on the proposition of admixture between genetically divergent populations without *a priori* information of breeds (Allendorf *et al.*, 2010; Tadano *et al.*, 2011) and differs from traditional methods such as phylogenetic trees which assume genetic separation between breeds (Granevitze *et al.*, 2009; Tixier-Boichard *et al.*, 2009). The highest ΔK value was found at $K=2$ (data not shown) according to Evanno *et al.* (2005). At $K=2$, Game bird was the most distinguished from other populations (Fig. 3A). Using Rosenberg *et al.* (2001) approach, the highest ΔK values for the remaining 12 populations were at $K=3$, $K=7$ and $K=12$. Leroy *et al.* (2009) suggested that the highest values for small number of K are biased with Evanno method as the number of breeds was crucial in the dataset. At $K=3$, only White Leghorn could be distinguished from the remaining populations. With regard to $K=7$, Hakka strain, Naked Neck and White broiler were clustered together. For $K=12$, the male-line B strain, the female-line L2 strain, White broiler and White Leghorn were clearly assigned to their own cluster with average proportion of membership higher than 0.90 (Table 5) where a few individuals exhibited a larger degree of admixture (Fig. 3B). Red jungle fowl, Hakka chicken and Hakka strain could be assigned to their own cluster (Figs 3B and 3D) with proportion of membership higher than 0.85 suggesting more recent admixed ancestry as found in Swiss goats (Glowatzki-Mullis *et al.*, 2008). Naked Neck population surprisingly shared more than a third of its genetic pool with Hakka strain. These results are similar to those found in the NJ tree. The four admixed populations (i.e., Red feathered, Black feathered, Golden chicken and Classical chicken), when analyzed apart, still could not be distinguished with the low proportion of membership (Fig. 3C). As their known breeding history, they were genetically related together. Similarly, a substructure was found in Naked Neck chicken (Fig. 3D), this chicken breed had a complex genetic background and individuals were assigned to diverse clusters. Muchadeyi *et al.* (2007) also reported that Zimbabwe chicken population was absent of substructuring among five ecotypes due to extensive gene flow among populations. Furthermore, breeds with high-within-breed diversity (H_E) revealed a low assignment by using Bayesian cluster approach as suggested by Leroy *et al.* (2009).

The population structure results showed that Taiwan commercial native chickens are more admixed, in contrast to occidental highly productive breeds. The high genetic variation within breed as shown in AMOVA results, facilitated by gene exchanges, did not allow discriminating in an efficient way for all the populations. The results suggest that the

Table 3. The D_A genetic distance below the diagonal and D_R above the diagonal between pairs of 10 Taiwan commercial native chicken populations, two exotic breeds and one red jungle fowl population

Populations ¹	JF	GB	CC	RF	GC	HS	HC	BF	BS	LS	LG	BR	NN
JF	—	0.350	0.259	0.270	0.207	0.331	0.283	0.207	0.382	0.471	0.447	0.347	0.307
GB	0.374	—	0.166	0.267	0.208	0.348	0.257	0.193	0.351	0.525	0.361	0.389	0.272
CC	0.324	0.194	—	0.112	0.065	0.150	0.124	0.042	0.157	0.292	0.250	0.188	0.122
RF	0.306	0.227	0.112	—	0.043	0.164	0.143	0.059	0.206	0.281	0.279	0.170	0.145
GC	0.297	0.216	0.098	0.072	—	0.137	0.063	0.026	0.158	0.284	0.209	0.124	0.117
HS	0.357	0.306	0.163	0.158	0.168	—	0.161	0.140	0.270	0.189	0.283	0.153	0.087
HC	0.351	0.252	0.140	0.154	0.120	0.165	—	0.089	0.175	0.252	0.213	0.137	0.112
BF	0.278	0.202	0.071	0.072	0.073	0.154	0.121	—	0.147	0.247	0.212	0.144	0.110
BS	0.351	0.294	0.164	0.175	0.142	0.231	0.165	0.150	—	0.269	0.287	0.270	0.186
LS	0.398	0.423	0.237	0.214	0.225	0.150	0.211	0.211	0.189	—	0.387	0.284	0.155
LG	0.422	0.316	0.255	0.270	0.217	0.269	0.234	0.234	0.220	0.310	—	0.251	0.202
BR	0.370	0.346	0.173	0.149	0.140	0.149	0.146	0.150	0.190	0.237	0.236	—	0.153
NN	0.318	0.247	0.121	0.128	0.131	0.093	0.110	0.100	0.167	0.160	0.201	0.123	—

¹ Red jungle fowl (JF), Game bird (GB), Classical chicken (CC), Red feathered (RF), Golden chicken (GC), Hakka strain (HS), Hakka chicken (HC), Black feathered (BF), B strain (BS), L2 strain (LS), White Leghorn (LG), White broiler (BR) and Naked Neck (NN).

Table 4. AMOVA partitions of 10 Taiwan commercial native chicken populations and two exotic breeds based on 22 microsatellite markers

Groups	Source of variation (%)		
	Among groups	Among populations within groups	Within populations
All 12 breeds — in one group		20.24 (0.000)	79.76 (0.000)
Ten TCOM ¹ — in one group		19.37 (0.000)	80.63 (0.000)
CEB ² — in one group		24.68 (0.000)	75.32 (0.000)
TCOM vs. CEB	5.83 (0.058)	17.64 (0.000)	76.53 (0.000)

¹ Ten Taiwan commercial native chickens were consisted of B strain (BS), L2 strain (LS), Hakka strain (HS), Hakka chicken (HC), Black feathered (BF), Red feathered (RF), Golden chicken (GC), Classical chicken (CC), Game bird (GB) and Naked Neck (NN).

² Commercial exotic breeds were consisted of White broiler (BR) and White Leghorn (LG). The significance tests were computed based on 1,023 permutations; *P* values (Probability level) were given in parentheses.

genetic pool of Taiwan commercial native chickens is well distributed among breeds and therefore there is a good potential for adaptation to new environmental conditions or markets. Therefore, management needs to be taken into account for the populations, especially B strain, Golden chicken and L2 strain with F_{IS} values above 0.20, to prevent inbreeding depression and maximize genetic diversity as suggested by Eding *et al.* (2002) and Toro *et al.* (2009) methods.

Acknowledgments

The authors would like to thank National Science Council, Taiwan for its financial supports (Project number NSC-3111-Y-466-005). The first author would gratefully thank for support from Taiwan Scholarship Program. We would like to thank two anonymous reviewers for useful comments.

Fig. 3. **Genetic structure of 10 Taiwan commercial native chicken populations, two exotic breeds and one red jungle fowl population.** A: Clustering diagrams of 10 Taiwan commercial native chicken populations, two exotic breeds and red jungle fowl population obtained from $K=2$, using Q matrices over 50 runs with the highest similarities. B: Clustering diagrams of nine Taiwan commercial native chicken populations (i.e., after removing Game bird population), two exotic breeds and red jungle fowl population obtained from $K=3$, $K=7$ and $K=12$. C: Clustering diagrams of four populations (i.e., Classical chicken, Black feathered, Red feathered and Golden chicken) were at $K=4$. D: Clustering diagrams of two populations (i.e., Naked Neck and Hakka strain) at $K=3$. The black lines separate individuals of different populations: red jungle fowl (JF), Game bird (GB), Classical chicken (CC), Black feathered (BF), Red feathered (RF), Golden chicken (GC), Hakka chicken (HC), Naked Neck (NN), B strain (BS), L2 strain (LS), Hakka strain (HS), White Leghorn (LG) and White broiler (BR).

Table 5. **The proportion of membership of each of the nine Taiwan commercial native chicken populations, two exotic breeds and one red jungle fowl population (after removing Game bird population) in the 12 inferred clusters using STRUCTURE analysis**

Populations ¹	Inferred clusters ²												<i>n</i>
	1	2	3	4	5	6	7	8	9	10	11	12	
JF	0.019	0.008	0.006	0.007	0.006	0.006	0.895	0.015	0.005	0.003	0.015	0.015	22
CC	0.556	0.018	0.013	0.080	0.016	0.037	0.005	0.103	0.031	0.014	0.052	0.075	26
BF	0.185	0.036	0.023	0.024	0.015	0.019	0.008	0.482	0.023	0.013	0.042	0.130	46
RF	0.015	0.013	0.035	0.015	0.011	0.013	0.005	0.110	0.013	0.008	0.474	0.288	49
GC	0.193	0.059	0.033	0.009	0.010	0.088	0.010	0.097	0.010	0.044	0.296	0.150	13
NN	0.028	0.028	0.046	0.402	0.023	0.023	0.011	0.036	0.321	0.018	0.011	0.053	49
HC	0.012	0.868	0.013	0.024	0.016	0.011	0.003	0.011	0.009	0.010	0.008	0.013	45
BS	0.006	0.008	0.011	0.012	0.013	0.901	0.006	0.007	0.010	0.011	0.010	0.006	45
LS	0.005	0.005	0.005	0.008	0.924	0.010	0.004	0.010	0.014	0.004	0.005	0.006	51
HS	0.016	0.008	0.039	0.017	0.021	0.006	0.003	0.007	0.858	0.005	0.009	0.011	27
LG	0.003	0.003	0.003	0.005	0.004	0.005	0.002	0.003	0.004	0.962	0.003	0.003	54
BR	0.006	0.008	0.905	0.009	0.009	0.007	0.003	0.008	0.013	0.012	0.013	0.008	24

¹ Red jungle fowl (JF), Classical chicken (CC), Black feathered (BF), Red feathered (RF), Golden chicken (GC), Hakka chicken (HC), Naked Neck (NN), B strain (BS), L2 strain (LS), Hakka strain (HS), White Leghorn (LG) and White broiler (BR).

² The contribution higher than 0.80 are in **bold**; *n*: Number of samples.

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