GENETIC VARIATION AND RELATIONSHIPS AMONG VISAYAN NATIVE CHICKEN GENETIC GROUPS BOHOLANO AND DARAG *(Gallus gallus L.)*

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ABSTRACT

The present study was conducted to evaluate the genetic diversity and relationships of two Visayan native chicken genetic groups (Boholano and Darag), the native chicken (Labuyo) and commercial breed layer chicken (Lohman). Thirteen microsatellite or simple sequence repeats (SSR) markers were used covering 8 linkage groups or chromosomes. Four to eight alleles per locus were detected across all the breeds. The highest PIC value (0.773) was detected in primer ADL0268 and the average across primers was 0.6114. The mean number of alleles per locus (MNA), the observed heterozygosity (Ho), expected heterozygosity (He) and the inbreeding coefficient (FIS) were obtained for the Boholano (5.08, 0.6859, 0.6433, and -0.0998 respectively), the Darag (5.61, 0.6667, 0.6646, and -0.0225 respectively), the Labuyo (4.46,0.5944, 0.6486, and 0.0711 respectively) and the Lohman (2.46, 0.5, 0.3943, and 0.0258 respectively). The degree of genetic distance of the Boholano was moderate from Darag but great from Labuyo; but close between Darag and Labuyo. These indicate that the Darag, but not the Boholano, may have originated from the Labuyo. The high MNA, Ho, He, negative inbreeding coefficient and high interpopulation genetic differentiation (FST) [D1] values between genetic groups shows the richness of our native chicken genetic resource in Bohol and Panay Islands."

Key Words: Boholano, Darag, Labuyo, microsatellite, native chicken

INTRODUCTION

The jungle fowl is a native of tropical Asia. Previously, it was theorized that the modern-day chickens descended from the mating of at least four subspecies of jungle fowls or the polyphyletic theory: *Gallus bankiva* or red jungle fowl, *Gallus sonneratti* or gray jungle fowl, *Gallus lafayetti* or Ceylon jungle fowl, and *Gallus javanica* or green jungle fowl. However, the monophyletic theory has gained popularity as scientists are convinced that modern-day chickens are descendants of the red jungle fowl of Southeast Asia before the sixth millennium BC (West and Zhou, 1989).

The Philippine native chicken was also believed to have descended from the wild red jungle fowl (Lambio and Gay, 1993) domesticated by Filipino ancestors even before the Spanish colonizers arrived in the Philippines. Historical accounts by Jesuit priest Pedro Chirino (1604) in his book *Relacion de las Islas Filipinas* state that at that time, Filipinos were raising native chickens and he also observed that they were in the thousands, roaming the fields and mountains.

Another report of the importance of the Philippine native chickens is by Thomson *et al*. (2014) who studied the migration of the Polynesian people 3,250 – 3,100 years ago. Their study used ancient chicken DNA to follow the route of the ancient migrant seafarers as they colonize the Polynesian islands. Their report concluded that the Philippines was

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a potential source or "homeland" of the Polynesian chickens. This has implication on the importance of the native chicken as a genetic resource. This claim is not farfetched because there are reports that the pre-colonial Visayan people may have had contact with the Polynesians based on some similarities of some cultural practices (Jagor, 1875).

In the present time, the native chicken, including gamefowl, dominates the chicken population in the Philippines which comprises 45.19% of the total chicken population. However, the commercial broiler and layer chickens contribute 35.57% and 19.24% of the total chicken inventory (BAS, 2013).

Due to the archipelagic nature of the Philippine geography, several strains or genetic groups of Philippine native chicken have survived, reproduced and evolved in different areas of the country. These genetic groups are phenotypically unique from each other but not all of these strains are recognized nor identified. There are only few documented strains such as the "Banaba" from Batangas and Quezon provinces, "Bolinao" from Pangasinan province, "Camarines" from Bicol region, "Darag" from Panay Island, and "Paraoakan" from Palawan Island (Lambio, 2000). Newly described genetic strains are "Boholano" from Bohol Island, (Salces *et al.*, 2013), "Joloanon" from Basilan Island (Lambio, 2010) and "Labuyo" which is found all over the country (Bejar *et al.*, 2012).

The current study aimed to supply further information of the Philippine native chicken and to evaluate their genetic diversity. The genetic variability between Boholano and Darag genetic groups of Philippine native chicken breeds was studied using known microsatellite or simple sequence repeats (SSR) markers. The objective of the study was to estimate the genetic diversity within and between populations of Boholano and Darag native chicken genetic groups using the SSR marker analysis.

MATERIALS AND METHODS

Three genetic groups of Philippine native chicken and a layer-type chicken of foreign origin were used in this study (Table 1). The Darag native chicken samples (n=30) were collected from various points in Panay Island, specifically: the municipalities of San Joaquin, Alimodian and Calinog (Iloilo province); municipalities of Patnongon and

Table 1. Genetic groups of chicken used in the study, their country of origin, sources of samples and general phenotypic characteristics of each group.

Genetic Group (No. of samples)	Country of Origin	Sources of Samples	Specific Phenotypic Characteristics	
			Rooster	Hen
Darag (30)	Philippines	Panay Island	red-laced plumage	brown penciled plumage
Boholano (24)	Philippines	Bohol Island	red-plain plumage	red laced plumage
Labuyo (12)	Philippines	Provinces of Pangasinan, Batangas and Surigao; Islands of Palawan and Siquijor	thin bright gold and bronze hackle feathers	brown penciled plumage
Lohman (10)	USA	Batangas province	white	white

Barbaza (Antique province); municipalities of Buruanga and Malay (Aklan province); and municipality of Sigma (Capiz province). While the Boholano native chicken samples (n=24) were from the municipalities of Bilar, Calape, Sikatuna, Duero and Ubay (Bohol province).

Demographic data, production data, morphometric data (e.g. live weight, wingspan, body length, body height, heart girth and shank length) and morphological data (e.g. plumage pattern, plumage color, shank color, skin color, comb type, earlobe color and iris color) including digital images were taken for each animal specimen and recorded in the local animal genetic resources library.

Table 2. ISAG-FAO recommended microsatellite markers (FAO, 2011).

Name	Chromo-some	Primer Sequence (5' → 3') Forward Reverse	Annealing Temp (^{O}C)	Allele Range	Multi-plex Group
ADL 0268	1	CTCCACCCCTCTCAGAACTA CAACTTCCCATCTACCTACT	60	102-116	1
MCW 0216	13	GGGTTTTACAGGATGGGACG AGTTTCACTCCCAGGGCTCG	60	139-149	1
LEI 0166	3	CTCCTGCCCTTAGCTACGCA TATCCCCTGGCTGGGAGTTT	60	354-370	2
MCW 0111	1	GCTCCATGTGAAGTGGTTTA ATGTCCACTTGTCAATGATG	60	96-120	2
MCW 0014	6	TATTGGCTCTAGGAACTGTC GAAATGAAGGTAAGACTAGC	58	164-182	3
MCW 0183	7	ATCCCAGTGTCGAGTATCCGA TGAGATTTACTGGAGCCTGCC	58	296-326	3
MCW 0104	13	TATTGGCTCTAGGAACTGTC GAAATGAAGGTAAGACTAGC	60	190-234	4
MCW 0123	14	CCACTAGAAAAGAACATCCTC GGCTGATGTAAGAAGGGATGA	60	76-100	4
MCW 0098	4	GGCTGCTTTGTGCTCTTCTCG CGATGGTCGTAATTCTCACGT	60	261-265	5
MCW 0078	5	CCACACGGAGAGGAGAAGGTCT TAGCATATGAGTGTACTGAGCTTC	60	135-147	5
ADL 0278	8	CCAGCAGTCTACCTTCCTAT TGTCATCCAAGAACAGTGTG	60	114-126	6
MCW 0248	1	GTTGTTCAAAAGAAGATGCATG TTGCATTAACTGGGCACTTTC	60	205-225	6
MCW 0222	3	GCAGTTACATTGAAATGATTCC TTCTCAAAACACCTAGAAGAC	60	220-226	7
MCW 0016	3	ATGGCGCAGAAGGCAAAGCGATAT TGGCTTCTGAAGCAGTTGCTATGG	60	162-206	7
MCW 0295	4	ATCACTACAGAACACCCTCTC TATGTATGCACGCAGATATCC	60	88-106	8
MCW 0081	5	GTTGCTGAGAGCCTGGTGCAG CCTGTATGTGGAATTACTTCTC	60	112-135	8

The FAO (2011) guidelines were used in the laboratory analysis. Fresh blood samples were extracted from the wing vein of live animal that were physically restrained using 1cc hypodermic needle. Blood samples were placed in blood storage cards (NucleoSave®, Machery-Nagel, Bethlehem, PA, USA) and dried in the laminar flow-hood overnight. Laboratory analysis for DNA extraction, purification, elution and amplification were executed at the Animal Biotechnology Laboratory, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines Los Baños, Laguna.

Using a micropuncher, at least six discs per dried card were collected and placed in labelled microcentrifuge tubes. Sample discs were washed with 200 µL of FTA Purification Reagent (Whatman Inc., Pittsburgh, PA, USA) three times. Sample discs were then dried under the laminar flow-hood overnight. Two dried sample discs were transferred into a PCR tube and added with 60 µL molecular biology grade water. DNA was eluted by incubating at 90^{O}C for 10 min.

Sixteen (16) SSR primer sets recommended by FAO (2011) were used for this study. Multiplex PCR amplification was carried out in a volume of 20 µL containing 6.0 µL of eluted DNA, 1x PCR buffer, 5.0mM $MgCl_2$, 0.35mM dNTP, 0.25 µM of each primer and 0.6 U *Taq* polymerase. PCR reactions were performed in thermal cycler: an initial step of 2 min at 94^{O}C, 35 cycles of 30 s at 94^{O}C, annealing temperature for 30 s, and 30 s at 72^{O}C, and a final step of 2 min at 72^{O}C. The PCR products were separated and visualized in native polyacrylamide gel by ethidium bromide staining (list of primer pairs used are presented in Table 2).

The genetic diversity of each breed was assessed by calculating the number of alleles per locus and its mean (MNA), observed heterozygosity (Ho), and unbiased expected heterozygosity (HE), using the POPGENE version 3.2 software package (Yeh *et al*., 1997). F-statistics FIS (fixation coefficient of an individual within a subpopulation), FIT (fixation coefficient of an individual within the total population), and FST (fixation coefficient of a subpopulation within the total population) per locus across the three Philippine native chicken and one commercial breed were also calculated using the same software.

DISPAN computer program (Ota, 1993) was used to measure genetic distances among four genetic groups of chicken. Modified Cavalli-Sforza chord distance (Nei, 1983) was used to evaluate the genetic distance. Neighbor-joining (NJ) method (Saitou and Nei, 1987) was used to construct a phylogenetic tree based on the DA genetic distances. The robustness of tree topologies was evaluated with a bootsrap test of 1,000 resampling across loci.

RESULTS AND DISCUSSION

Genetic diversity

A total of 13 microsatellite markers distributed on eight autosomes in 76 birds representing three Philippine native chickens and one commercial breed were examined. All the primers were in Hardy-Weinberg equilibrium for all the genetic groups tested. Across all breeds, the average MNA was 6.15 with the range from four (MCW0295) to eight (ADL0268, MCW0123 and MCW0222). Table 3 shows marker information and F-statistics for the three genetic groups of Philippine native chickens. The FIS, FIT and FST values ranged from -0.5594 (MCW0016) to 0.2392 (ADL0278), from -0.4702 (MCW0016) to 0.3806 (ADL0278), and from 0.0294 (MCW0016) to 0.2861 (ADL0278) with the mean values of -0.0703, 0.0210 and 0.1016, respectively. The highest Polymorphic Information Content (PIC) value was 0.773 (ADL0268) and the lowest was 0.394 (MCM0104). The PIC average across primers was 0.6114.

Genetic diversity in four genetic groups of chicken was measured using different parameters such as MNA, Ho, HE and FIS. As shown in Table 4, the MNA ranged from

Table 3. SSR primers used in the study and F-statistics per locus for three genetic groups of Philippine native chickens and one commercial breed (Lohman).

Locus Code	Locus Name	Alleles (no.)	Fis	Fit	Fst	PIC
PR01	ADL0268	8	0.006	0.1495	0.0221	0.773
PR04	MCW0295	4	0.0744	0.0766	0.0398	0.522
PR05	MCW0081	5	-0.0323	0.0193	0.0434	0.598
PR08	ADL0278	5	0.2392	0.3806	0.1159	0.656
PR10	MCW0104	7	-0.0854	-0.0416	0.0404	0.394
PR11	MCW0123	8	0.0992	0.1105	0.0664	0.696
PR14	MCW0069	7	-0.2016	-0.0385	0.0202	0.589
PR16	MCW0111	6	-0.0468	-0.0302	0.0158	0.618
PR18	MCW0034	5	-0.1706	-0.1102	0.0529	0.612
PR21	MCW0222	8	-0.098	0.0538	0.0329	0.723
PR22	MCW0016	5	-0.5594	-0.4702	0.0321	0.58
PR27	MCW0078	7	-0.1003	0.0514	0.033	0.72
PR30	MCW0216	5	0.0491	0.0553	0.0065	0.467
Mean		6.15	-0.0703	0.0210	0.0406	0.611385

Table 4. Genetic diversity parameters estimated for 13 microsatellite markers in three genetic groups of Philippine native chicken and one commercial breed (Lohman).

Genetic Group	MNA*	Ho	HE	Ho:He	FIS
Boholano	5.0769	0.6859	0.6433	1.066	-0.0998
Darag	5.6154	0.6667	0.6646	1.003	-0.0225
Labuyo	4.4615	0.5944	0.6486	0.916	0.0711
Lohman	2.4615	0.5	0.3943	1.268	0.0258

*MNA - Mean Number of Alleles HE – Expected heterozygosity
Ho – Observed heterozygosity FIS – Inbreeding Coefficient

2.46 (Lohman) to 5.6 (Darag). The Ho and HE ranged from 0.5 (Lohman) to 0.69 and from 0.39 (Lohman) to 0.66 (Darag), respectively. The FIS or inbreeding coefficient values that were positive came from Labuyo and Lohman (0.0711 and 0.0258 respectively) and the negative values came from Boholano and Darag (-0.0258 and -0.0225 respectively).

FIS is an F-statistics that is used to measure the inbreeding coefficient of a subpopulation or a genetic group. It also represents a degree of nonrandom mating or deviation from Hardy-Weinberg equilibrium. A positive value means that there could be a deviation from Hardy-Weinberg equilibrium that may be caused by inbreeding. As expected, the Lohman samples showed a positive value because it is well-known that commercial chickens came from inbred lines. A surprising finding is that the value for Labuyo is also positive and even higher than the value for Lohman. This is in spite of the fact that Labuyo samples came from different islands in the Philippines such as Luzon (Pangasinan, Batangas and Palawan), Visayas (Siquijor) and Mindanao (Surigao).

The mean FST value of 0.0406 indicates that approximately 4.06% of the total genetic variation is caused by breed differences, and the remaining 95.94% is due to differences among individuals within breeds. This indicates that the genetic groups tested are not that genetically subdivided as can be expected because these genetic groups were not yet subjected to intensive selection. In comparison with other studies, Tadano *et al*. (2007) reported a 0.303 FST value among Japanese long tailed chicken breeds. This value is high and is expected because the Japanese long tailed chickens have a long history of inbreeding between related individuals or more intensive selection of fix desirable traits. Chen *et al.* (2008) studied Chinese native chickens and reported a 0.164 FST value. This is lower than the Japanese long tailed chicken but higher than the Boholano and Darag chickens.

Among the Visayan native chickens, Darag was observed to have a higher MNA (5.615) compared to Boholano (5.0769) although the difference is not that high. These values are higher in comparison with the Japanese long tailed chickens that has a highest MNA of 3.90 (Satsumadori) (Tadano *et al.,* 2007) and the Korean black strain of native chicken with an MNA of 5.07 (Kong *et al*., 2006).

The MNA of Labuyo (4.4615) was lower than the Boholano and Darag even though it came from different islands. This result may reflect the current status of the Labuyo populations. This endangered Red Jungle Fowl has very low numbers in the wild that may explain the lower diversity (lower MNA). The lowest MNA was from Lohman (2.4615) and can be expected because these birds are inbred lines.

The Ho:HE of the Lohman (1.268) was higher compared to that of the native breeds. Higher Ho:HE values suggest that the population size is relatively small at the starting point of this commercial breed. This is consistent with inbred lines wherein a small number of individuals were selected to be the parental stocks. It can also be observed that the Boholano (1.066) and Darag (1.003) had a Ho:HE ratio that reached 1.0. Although no intensive selection programs were done for these genetic groups, the sampling strategy of purposively selecting based on plumage pattern maybe the reason the Ho is slightly higher than the HE in these genetic groups. On the other hand, Labuyo with a value of 0.916 had the lowest Ho:HE ratio that reflects the diversity of the samples coming from different islands that are very far from each other. Three (3) of the twelve (12) samples were from Palawan Island that is known to have a different geologic origin from the rest of the Philippine Islands.

Genetic distance and relationships among genetic groups

Table 5 shows FST between each pair of native and commercial chickens based on 13 microsatellite primers. The FST ranged from 0.0537 (between Darag and Labuyo) to 0.2556 (between Darag and Lohman). Figure 1 shows a phylogenetic tree of the native and commercial chickens that was constructed from FST by using the NJ method. The Lohman commercial breed formed as an outgroup far from the native chicken genetic groups while Darag clustered with Labuyo.

Table 5. Genetic distance (FST) between each pair of three Philippine native chickens and one commercial breed (Lohman).

Genetic Group	Boholano	Darag	Labuyo	Lohman
Boholano	****			
Darag	0.0718	****		
Labuyo	0.1522	0.0537	****	
Lohman	0.2549	0.2556	0.251	****

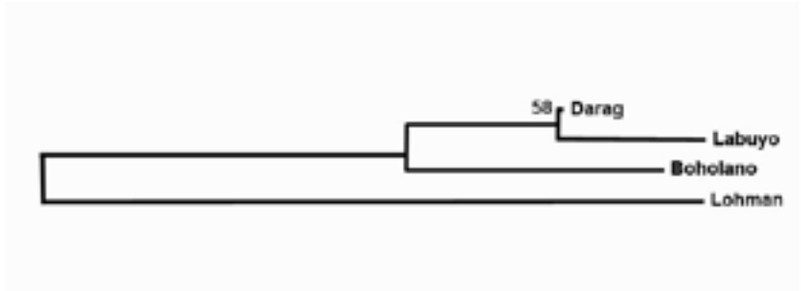

Figure 1. Neighbor joining tree of three Philippine native chickens and one commercial breed (Lohman).

Boholano and Darag native chickens showed a 0.0718 FST value or a moderate degree of genetic divergence according to the Wright (1978) F-statistics. This indicates that they can be considered as distinct genetic groups. On the other hand, Boholano and Labuyo showed a 0.1522 FST value or a great degree of genetic divergence. This may indicate that the Boholano native chicken diverged from Labuyo a long time ago or it could have a different ancestor not related to Labuyo.

The Darag genetic group showed a very low FST value with Labuyo (0.0537) bordering between little genetic divergence and moderate genetic divergence. This is a strong evidence that the Darag genetic group came from the Labuyo wild chicken. This is not surprising since the plumage pattern and color of Darag and Labuyo females are highly similar. This is in contrast with the findings of Bondoc and Santiago (2012) wherein Darag is more related to Igon native chicken than Labuyo.

CONCLUSION

The high MNA, Ho, He and negative inbreeding coefficient shows the high diversity of these native chickens in their genetic resources for Bohol and Panay Islands. FST values between genetic groups also show that Boholano and Darag native chickens are distinct genetic groups that are moderately genetically distant from each other. The Darag native chicken is closely related to the wild Labuyo chicken indicating that Darag originated from this group of chicken. On the other hand, the origin of Boholano native chicken is not yet clear because it showed a great degree of genetic distance from the Labuyo chicken.

ACKNOWLEDGMENT

The authors would like to thank the Bureau of Animal Industry for funding this project; the Bohol Provincial Government through its Bohol Native Chicken Program and the Department of Agriculture Regional Field Office No. 7-Ubay Stock Farm for the Boholano chicken samples; the West Visayas State University-Calinog campus for the Darag genetic group samples and the Department of Agriculture Regional Field Office No. 6 for coordinating and facilitating sampling for the whole Panay Island.

REFERENCES

Bejar FR, Baylon MJ, Fabillar JB, Mante LEB, Ultra AA, Aquino RR and Bejar ET. 2012. Characteristics of native chickens. *SEARCA Agriculture & Development Discussion Paper Series*: *Management Practices and Morphological Characterization of Indigenous (Native) Chickens in Samar Province* 3: 1-5.

Bondoc OL and Santiago RC. 2012. The use of DNA barcodes in the evolutionary analysis of domestic breeds and strains of chicken (*Gallus gallus domesticus*) in the Philippines. *Philipp Agric Sc* 95(4):358-369.

Bureau of Agricultural Statistics (BAS). 2013. *Chicken Industry Performance Report*. Quezon City: Bureau of Agricultural Statistics, Department of Agriculture.

Chen G, Bao W, Shu J, Ji C, Wang M, Eding H, Muchadeyi F and Weigend S. 2008. Assessment of population structure and genetic diversity of 15 Chinese indigenous chicken breeds using microsatellite markers. *Asian-Aust J Anim Sci* 21(3): 331-339.

Chirino P. 1604. (translated to English by Echevarria R, 1969). *Relacion de las Islas Filipinas (The Philippines in 1600)*. Manila: Historical Conservation Society 15.

FAO. 2011. Molecular Characterization of Animal Genetic Resources. *FAO Animal Production and Health Guidelines 9*. Rome.

Jagor F. 1875. *Lakbay-lakbay sa Pilipinas*. London, England.

Kong HS, Oh JD, Lee JH, Jo KJ, Sang BD, Choi CH, Kim SD, Lee SJ, Yeon SH, Jeon GJ and Lee HK. 2006. Genetic Variation and relationships of Korean native chickens and foreign breeds using 15 microsatellite markers. *Asian-Aust J Anim Sci* 19(11):1546-1550.

Nei M. 1987. *Molecular Evolutionary Genetics*. New York: Columbia Univ. Press.

Lambio AL and Gay EC. 1993. The indigenous chickens of the Philippines. *Anim Prod Technol J* 8: 8-9.

Lambio AL. 2000. Germplasm and new cultivars or breeds: The Philippine native chickens. *Philipp Agri Sci* 83: 112-17.

Lambio AL. 2010. *Poultry Production in the Tropics*. Diliman, Quezon City: The University of the Philippines Press.

Ota T. 1993. *DISPAN: Genetic Distance and Phylogenetic Analysis*. Pennsylvania State Univ., University Park.

Salces AJ, Quirog LM, and Chatto ED. 2013. Participatory Approach in Definition of Breeding Objective Traits for Boholano strain of Native chicken. *Philipp J Vet Anim Sci* 39(2): 165.

Saitou N and Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4: 406–425.

Tadano R, Sekino M, Nishibori M and Tsudzuki M. 2007. Microsatellite marker analysis for the genetic relationships among Japanese Long-Tailed Chicken Breeds. *Poultry Sci* 86 (3): 460-469.

Thomson VA, Lebrasseur O, Austin JJ, Hunt TL, Burney DA, Denham T, Rawlence NJ, Wood JR, Gongora J, Flink LG, Linderholm A, Dobney K, Larson G and Cooper A. 2014. Using ancient DNA to study the origins and dispersal of ancestral Polynesian chickens across the Pacific. *P Natl A Sci*. 111(13): 4826-4831.

West B and Zhou B. 1989. Did chickens go north? New evidence for domestication. *World Poult Sci J.* 45: 205-16.

Wright S. 1978. *Evolution and the genetics of populations variability within and among natural populations.* 4th ed. Chicago: University of Chicago press.

Yeh FC, Yang RC, Boyle TBJ, Ye ZH and Mao JX. 1997. *POPGENE, the user-friendly shareware for population genetic analysis*. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alberta, Canada.