ORIGINAL ARTICLE

PHENOTYPIC AND GENETIC CHARACTERISTICS OF BOHOLANO GENETIC GROUP OF PHILIPPINE NATIVE CHICKEN (*Gallus gallus domesticus,* L.)

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ABSTRACT

A total of 130 heads of native chicken from the entire province of Bohol were randomly sampled to determine the phenotypic characteristics, including qualitative and quantitative traits, of the Boholano genetic group of the Philippine native chicken. Results showed variations in the qualitative traits of the Boholano genetic group. However, it is noted that Boholano genetic group was predominantly of single comb, orange-colored iris, white skin, yellow shank and red-plain plumage. Although the quantitative traits of Boholano genetic group were not significantly different across the province of Bohol, the roosters were significantly heavier and had higher body measurements than the hens (P<0.01). Twenty four native chickens and a commercial layer strain were used to evaluate the genetic characteristics of the Boholano genetic group. Sixteen microsatellites were used but only 13 microsatellite markers were found to be polymorphic. The 13 microsatellites, distributed to eight linkage groups, had 4-8 alleles detected per locus. The high mean number of alleles per locus, observed heterozygosity and expected heterozygosity, negative inbreeding coefficient and high fixation coefficient of a subpopulation within the total population values show the high diversity of Boholano genetic group of Philippine native chicken.

Keywords: Bohol, genetic characterization, phenotypic characterization, Philippine native chicken

INTRODUCTION

In rural areas, rearing native chickens is one of the common activities of farmers. It is a cheap source of meat and eggs for home consumption and provides cash for the family members by selling eggs or few heads of chicken. The present native chickens reared in the traditional way, which is based on scavenging, are the products of a long and continuous process of natural crossing without selection between the improved breed and indigenous chicken. Due to continuous interbreeding of native chicken with the improved breeds, the indigenous chicken exhibits wide variability in terms of qualitative and quantitative traits (Avante, 1989).

In the Philippines, there are several genetic groups of native chickens. The emergence of these genetic groups which are found in different provinces in the country can be attributed to genetic isolation (Arboleda, 1985). In 2000, Lambio stated that there are several native chicken genetic groups in the country namely: “Banaba”, “Bolinao”,

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"Camarines", "Egon", "Paraoakan", and "Darag". Newly discovered genetic groups are "Joloanon" from Basilan (Lambio, 2010) and "Boholano" from Bohol, (Salces *et al.*, 2013) and there are many out there waiting to be discovered.

Bohol's reputation as a premiere eco-cultural destination aims to position native chicken in the niche market as a unique Boholano product. The Provincial Government of Bohol in 2011 allocated funds to support a breeding program entitled "Community-Based Native Chicken Conservation, Development and Utilization for Meat and Egg Production for the Province of Bohol". Native chickens in the Province of Bohol are not studied and there is a need to verify and determine their phenotypic and genetic characteristics as a component of the breeding program for further development, hence this study.

MATERIALS AND METHODS

Sampling procedure and design

Four coastal or exterior towns and two interior towns of Bohol were sampled in the study. Exterior towns included Calape, Garcia Hernandez and Duero where farmer breeders were located and Ubay, which is the location of the institutional flock. The towns of Bilar and Sikatuna were classified as interior towns.

Phenotypic characterization

On-farm investigations and surveys were conducted using a pre-drafted and pre-tested questionnaire. All the chickens in each breeder farmers were measured and characterized. Measurements were done early in the morning to avoid the effect of feeding and watering on the animal's size and conformation. Quantitative traits that were measured in the mature birds included body length, shank length, wingspan and chest circumference. The qualitative traits that were observed were the type of comb, plumage morphology (plumage pattern and color), and colors of the shank, skin, eye and ear lobe.

The data on qualitative traits of the native chickens were tabulated and frequency distribution was computed. The quantitative traits were analyzed using PROC MIXED (SAS) version 9.3 following the statistical model:

$$Y_{ijk} = \mu + C_i + S_j + L_k + (SxL)_{jk} + \varepsilon ijk$$

Where: Y_{ijk} = least square mean on the observation of a trait on the ith chicken of the jth sex of the kth location.

μ = mean
C_i = random effect of the ith chicken
S_j = fixed effect of the jth sex (j = 1, 2)
L_k = fixed effect of the kth location (j = 1, 2)
SxL_{jk} = the interaction between sex and location
εijk = the error term associated with the environment.

Genetic characterization

Twenty four native chickens and ten chickens of commercial layer strain were randomly chosen to be used in this study. Fresh blood samples were extracted from the wing vein of the chickens with proper physical restraint. Blood samples were placed in NucleoSave blood storage cards (Machery-Nagel, USA) and dried in the laminar flowhood overnight. The FAO (2011) guidelines were used in the laboratory analysis. Laboratory analysis for DNA extraction, purification, elution and amplification were conducted at the Animal Biotechnology Laboratory, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines Los Baños, Laguna.

For DNA extraction and purification, 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., USA) three times. Sample discs were then dried under the laminar flowhood 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.

For DNA amplification, 16 SSR primer sets recommended by FAO (2011) were used for this study. A multiplex PCR amplification was carried out in a volume of 20 µl containing 6.0 µ; 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 a thermal cycler: an initial step of 2 min at 94^{O}C, 35 cycles of 30 sec at 94^{O}C, annealing temperature for 30 sec, and 30 sec 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 1.

The genetic diversity of each chicken group 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 v.3.2 (Yeh *et al.*, 1997). The F-statistics such as fixation coefficient of an individual within a subpopulation (FIS), fixation coefficient of an individual within the total population (FIT) and fixation coefficient of a subpopulation within the total population (FST) per locus were also calculated using the same software.

RESULTS AND DISCUSSION

Phenotypic characterization

The 130 chickens that were analyzed comprised of 78% female and 22% male. There were 60 chickens sampled from the interior towns while 70 heads were sampled from the coastal towns (Table 2). All of the phenotypic characteristics, which include qualitative and quantitative traits, were analyzed separately for each sex and towns.

Qualitative traits

<u>Shank color</u>

Yellow was the most prominent color observed (Table 3), with 53% of all the hens and 62% of roosters belonging to this group.The second most predominant color was white, 36% and 31% for hens and roosters, respectively. Only the hens in both interior and exterior towns possessed the green colored shank. The trend in the occurrences of shank color is different from the findings of similar studies conducted in other countries (such as Cambodia, Ethiopa, Dekina, Nigeria, and Daikwo) by Daikwo *et al.* (2011). Likewise, in the study conducted by Cabarles *et al.* (2012) in Western Visayas, the most prominent shank color was yellow followed by white and then slate.

According to Smyth (1990) the diversity in shank color may be due to the interactions of major modifier genes. The homozygosity of the *black extension factor* (*E*) expresses

the black shank. With the interaction of *dermal melanin* (*id*$^{+}$) and *E* with *dominant white* (*I*), chickens will express slate or green shank. Moreover, the presence of *autosomal white* (*W*$^{+}$) interacting with melanin will appear as blue or slate shank and the *w* for green.

Skin color

Only two different skin colors were recorded, 96% of hens and 85% of roosters had white, while the yellow skin was possessed by the remaining 4% and 14% of hens and roosters, respectively. Interior and exterior towns had similar pattern of distribution in

Table 1. ISAG-FAO recommended microsatellite markers that were used in the study.

Name	Chro-mo-some	Primer Sequence (5' -> 3') Forward Reverse	Annealing Tempera-ture (^{O}C)	Allele range	Mul-tiplex Group
ADL 0268	1	CTCCACCCCTCTCAGAACTA CAACTTCCCATCTACCTACT	60	102-116	1
MCW0216	13	GGGTTTTACAGGATGGGACG AGTTTCACTCCCAGGGCTCG	60	139-149	1
LEI0166	3	CTCCTGCCCTTAGCTACGCA TATCCCCTGGCTGGGAGTTT	60	354-370	2
MCW0111	1	GCTCCATGTGAAGTGGTTTA ATGTCCACTTGTCAATGATG	60	96-120	2
MCW0014	6	TATTGGCTCTAGGAACTGTC GAAATGAAGGTAAGACTAGC	58	164-182	3
MCW0183	7	ATCCCAGTGTCGAGTATCCGA TGAGATTTACTGGAGCCTGCC	58	296-326	3
MCW0104	13	TATTGGCTCTAGGAACTGTC GAAATGAAGGTAAGACTAGC	60	190-234	4
MCW0123	14	CCACTAGAAAAGAACATCCTC GGCTGATGTAAGAAGGGATGA	60	76-100	4
MCW0098	4	GGCTGCTTTGTGCTCTTCTCG CGATGGTCGTAATTCTCACGT	60	261-265	5
MCW0078	5	CCACACGGAGAGGAGAAGGTCT TAGCATATGAGTGTACTGAGCTTC	60	135-147	5
ADL0278	8	CCAGCAGTCTACCTTCCTAT TGTCATCCAAGAACAGTGTG	60	114-126	6
MCW0248	1	GTTGTTCAAAAGAAGATGCATG TTGCATTAACTGGGCACTTTC	60	205-225	6
MCW0222	3	GCAGTTACATTGAAATGATTCC TTCTCAAAACACCTAGAAGAC	60	220-226	7
MCW0016	3	ATGGCGCAGAAGGCAAAGCGATAT TGGCTTCTGAAGCAGTTGCTATGG	60	162-206	7
MCW0295	4	ATCACTACAGAACACCCTCTC TATGTATGCACGCAGATATCC	60	88-106	8
MCW0081	5	GTTGCTGAGAGCCTGGTGCAG CCTGTATGTGGAATTACTTCTC	60	112-135	8

Table 2. Number of hens and roosters sampled in Bohol.

Location	Roosters		Hens		Total
	Freq	%	Freq	%	
Interior	12	20	48	80	60
Coastal	17	24	53	76	70
Total	29	22	101	78	130

which most of the chickens had white skin. The white skin originated from the red jungle fowl (*G.gallus*) while the yellow skin was from the grey jungle fowl (*G. sonneratii*). The diversity in skin color can be due to mode of inheritance and hybridization. The findings of this study was similar to the results of the study conducted in Cambodia (FAO, 2009) and Cabarles *et al.* (2012), wherein the most prominent skin colors were white and yellow.

Earlobe color

Red was the most prominent earlobe color, accounting for 64% of hens and 88% of roosters (Table 3).The variation in the earlobe color may be due to ancestral lineages and mutations (Cabarles *et al.,* 2012). Smyth (1990) explained the the presence of white pigment in the earlobe was because of purine bases and not of melanin or carotenoid. It was inherited as a polygenic trait. The possibility of mutations on genes responsible for the expression of melanin and carotenoids was also considered given the occurrences of other earlobe colors.

The result of this study was similar with the one conducted in Western Visayas, wherein, 57.41% of the characterized chickens had red with white earlobes, 37.53% had red, 2.22% had white and 1.85% had turquoise earlobes and the remaining percentages comprised chickens with black, black with red and yellow earlobes (Cabarles *et al.*, 2012). In the study conducted in Cambodia, 82% of traditional chickens had red with white earlobes (FAO, 2009). This was followed by 12.16% having red with yellow, 2.82% having blue or turquoise, 2.26% having white and 0.2% having red colored earlobes. The observation in Ethiopia showed that 67.0% had white, 17.9% had red with white, 18.6% had red and 0.7% had black earlobes (Duguma, 2006). The differences in the distribution of earlobe colors were due to the adaptability of chickens with specific earlobe color to local conditions.

Iris color

From the data gathered, orange was the most common iris color accounting for 63% of the birds (Table 3). A very similar trend was found in both interior and exterior towns. According to Cabarles *et al.* (2012), the results on diversities of iris color may be attributed to the interactions of melanin and carotenoids, ingestion and utilization of xanthophylls, and its correlation with other genes expressing colors to other parts of the chicken body. The diversity in iris color of hens can be due to the presence of carotenoids in ingested feeds and its utilization for egg yolk production as explained by Smyth (1990). The brighter colored iris of roosters can be due to excess carotenoids reacting with the melanin. In addition, eye color was closely correlated with shank color and can be modified by genes associated with plumage color. Similar studies conducted in the provinces of Cambodia

showed that 70.74% of the chickens had orange; brown, 10.20%; pearl, 15.42% and red, 3.48% iris color (FAO, 2009). The findings of Duguma (2006) in Ethiopia showed that all their chickens had black-colored eye. The observed differences were probably due to the absence of standardized characterization guide for traditional chickens. The phenotypic characterization guide drafted by FAO was released only in November 2010 (FAO, 2012).

Comb type

Ninety one percent of the birds had a single comb. Only hens in interior towns had pea comb (Table 3). Duguma (2006) stated that single comb was dominant among traditional chickens in tropical regions for it helps reduce 40% of body heat. This explains the dominant occurrence of single comb in the province. The results on the trends of distribution of single comb were in accordance to the findings of Egahi *et al.* (2010) in Markudi, Nigeria but in contrast to those reported by Dana *et al.* (2010) in Ethiopia. The observed differences may be attributed to the prevailing conditions in the place and the frequency of genes carrying such comb expression.

Table 3. Proportion of hens and roosters according to shank, skin, earlobe and iris colors and comb type, by location in Boholano genetic group of Philippine native chicken.

Parameter	Interior		Coastal		Total	
	n= 48 Hen %	n= 12 Rooster %	n= 53 Hen %	n= 17 Rooster %	n= 101 Hen %	n= 29 Rooster %
Shank color						
Yellow	50	60	55	63	53	62
Gray-blue	0	0	0	12	0	7
White	39	40	32	25	36	31
Green	4	0	4	0	4	0
Slate	7	0	9	0	8	0
Skin color						
White	92	100	100	76	96	85
Yellow	8	0	0	24	4	14
Earlobe color						
White	8	0	0	12	4	7
Red	51	87	77	88	64	88
Red and white	41	13	14	0	27	5
Black	0	0	9	0	5	0
Iris color						
Red	21	10	0	24	10	18
Orange	79	50	46	76	61	65
Brown	0	27	28	0	15	11
Pearl	0	13	26	0	14	5
Comb type						
Single	66	100	100	100	82	100
Pea	34	0	0	0	18	0

Plumage pattern

As shown in Table 3, majority of the birds had plain plumage pattern, accounting to 56%, followed by pencilled which was 24% of the total samples. Some of the birds observed had laced or mottled plumage pattern. Diversities in plumage patterns can be attributed to feather developmental mechanisms, genes of chickens and raisers selection practices (Cabarles *et al.*, 2012). According to Smyth (1990), the color patterns were due to the distribution of eumelanin and the presence or absence of pheomelanin at feather developmental stage. The kind and concentration may vary among cells because of molecular gradients at the feather follicles. The position of feather in the body may also affect the expression of color pattern because of differences in intensity of melanin pigmentation in the skin. These are also governed by different gene actions (Smyth, 1990). Raisers may have retained chickens with attractive color patterns as replacement stocks.

Plumage color

The most predominant plumage color for the chickens was red (Table 4). However, in both interior and exterior towns, the color ranged from light to dark brown, brown and wheaten. Cabarles *et al.* (2012) indicated that the higher occurrences of red plumage among roosters and brown plumage in hens may be inherited from their progenitor – the red junglefowl and through natural selection. These colors enable them to mimic dry leaves and debris which is important especially when threatened by dangers. This is the same with the chickens in Guimaras having black and slate plumages which make it easy for them to hide, when threatened, in the grey to black bark of mango trees. The preferences of raisers for other colors further increase diversity in plumage colors.

Studies conducted in the Maison District of Sonla Province in Northwest Vietnam on the H'mong chickens showed that 70.66% had brown, 14.78% had black and 14.56% had white plumages (Cuc *et al.*, 2006). The trends in brown and black plumage occurrences among these chickens differ from the present findings. The observed differences in the magnitude of plumage color occurrences may be due to limited color variations among chickens and selection preferences of raisers.

Quantitative traits

Body weight

Roosters were significantly heavier compared to hens ($P<0.01$; Table 5). Chickens in the interior towns weighed 1.36 kg and in the coastal towns 1.25 kg although the 100 grams difference was not statistically significant.

Chest circumference

Birds sampled had chest circumference of 24.90 cm for hens and 28.99 cm for roosters (Table 5). In both locations, male birds had higher chest circumference than females ($P<0.01$)

Table 4. Proportion of hens and roosters according to plumage pattern and color and distributed to either interior or coastal towns of the province of Bohol, 2014.

Parameter	Interior		Coastal		Total	
	n= 48 Hen %	n= 12 Rooster %	n= 53 Hen %	n= 17 Rooster %	n= 101 Hen %	n= 29 Rooster %
Plumage pattern						
Plain	40	80	67	18	54	44
Laced	30	0	0	23	14	13
Pencilled	20	0	18	47	19	28
Mottled	10	20	15	12	13	15
Plumage color						
Red	55	60	60	48	58	53
Brown	10	20	15	20	13	20
Light brown	15	0	15	20	15	12
Wheaten	20	20	10	12	14	15

Wingspan

Roosters (38.57 cm) had wider wingspan compared to hens (37.82 cm) (P<0.01). Birds from interior towns also had wider wingspan compared to those of exterior towns.

Shank length

Evidently, roosters had longer shanks compared to hens, 10.05 cm and 8.60 cm, respectively (P<0.01). There was no significant effect of location on the quantitative traits of the Boholano genetic group of native chicken. These findings were similar with the result of the study conducted by Avante and del Fierro (1991) wherein the male Paraoakan weighed 2.0–2.5 kg, the Banaba and Camarines had 1.5–2.0 kg body weight, the weight of female ranged from 1.4 to 1.6 kg, which was slightly higher than those measured in the study. Cabarles *et al.* (2012) also reported a similar result wherein the roosters (ranged from 1.54 to 2.04 kg) had higher live weight compared to hens (ranged from 1.23 to 1.31 kg). Observed differences were probably due to the expression of genes associated with body weight. The dimorphism in chicken started at egg cells before fertilization given the heterozygosity (ZW) of hens as governed by different genes and hormones. Accordingly, the overall phenotypic expression of the native chickens wherein male is larger than female in larger species and female is larger than male in smaller species follows the Rensch's rule (Lopez *et al.*, 2013).

Genetic characteristics

Sixteen microsatellites were used but only 13 microsatellite markers were found to be polymorphic. The 13 microsatellites, distributed to 8 linkage groups, had four to eight alleles detected per locus. All the primers were in Hardy-Weinberg equilibrium for all the genetic groups tested. The average MNA was 6.15 with the range from 4 (MCW0295) to

Table 5.Least square means ± standard error of quantitative measurements of Boholano strain of Philippine native chicken.

	PARAMETERS					
	Body weight (kg)	Body height (cm)	Body length (cm)	Chest Circumfer-ence (cm)	Shank length (cm)	Wing span (cm)
Sex**						
Hen	1.15 ± 0.05	24.15 ±0.58	20.45± 0.42	24.90 ±0.60	8.60 ±0.16	37.82± 0.88
Rooster	1.51 ± 0.11	28.06 ±1.18	23.25 ±0.85	28.99 ±1.23	10.05± 0.33	38.57 ±1.77
Location^{ns}						
Interior	1.36± 0.08	26.90±0.85	21.89± 0.61	28.18±0.88	9.55±0.24	39.03±1.29
Coastal	1.25± 0.08	25.31±0.83	21.80±0.59	25.71±0.87	9.09± 0.23	37.35±1.24

8 (ADL0268, MCW0123 and MCW0222).

Genetic diversity was measured using parameters such as MNA, Ho, HE and FIS. As shown in Table 6, the MNA of Boholano genetic group and commercial layer strain were 5.0769 and 2.4615, respectively. Inbreeding coefficient values (Fis) for Boholano was -0.0998 and for the commercial layer strain was 0.0258. The FIS is an F-statistic that is used to measure the inbreeding coefficient of a subpopulation or a genetic group. A positive value means that there could be a deviation from Hardy-Weinberg equilibrium that is a consequence of inbreeding. As expected, the layer samples showed a positive value because commercial chickens came from inbred lines.

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 Boholano genetic group is not that genetically subdivided since no selection has yet been done. The result of this study is higher compared to the reports of Chen *et al.* (2008) in their study with Chinese native chickens which was 0.164 FST value.

The Ho: HE of the Layer (1.268) was higher compared to the Boholano genetic group which was 1.066. This is consistent with inbred lines wherein a small number of individuals are selected to be the parental stocks. Although no intensive selection programs were done, 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.

CONCLUSION

Boholano genetic group was predominantly of single comb, orange-colored iris, white skin, yellow shank, and red-plain plumage. Although the quantitative traits of Boholano genetic group were not significantly different across the province of Bohol, the roosters were significantly heavier and had higher body measurements than the hens. The high mean number of alleles per locus, observed heterozygosity and expected heterozygosity, negative inbreeding coefficient and high fixation coefficient of a subpopulation within the total population values show the high diversity of Boholano genetic group of Philippine native chicken.

Table 6. Genetic diversity parameters estimated for 13 microsatellite markers in Boholano genetic group of Philippine native chicken and 1 commercial breed.

Genetic Group	MNA	Ho	HE	Ho:He	FIS
Boholano	5.0769	0.6859	0.6433	1.066	-0.0998
Layer (Lohman)	2.4615	0.5	0.3943	1.268	0.0258

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

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