Proceedings, 10th World Congress of Genetics Applied to Livestock Production

Population Genetic Structure of Sri Lankan Backyard Chicken Flocks: Implication for Conservation and Genetic Improvement Programs

A. M. Samaraweera*, P. Silva†, N. D. F. Abeykone†, M. N. M. Ibrahim‡, A. M. Okeyo‡ and *J. L. Han*‡
*Faculty of Animal Science and Export Agriculture, Uva Wellassa University, Badulla, Sri Lanka,
†Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka,
‡Internationa Livestock Research Institute (ILRI), Nairobi, Kenya

ABSTRACT: Traditional 'random' sampling strategy for molecular characterization has revealed low genetic differentiation and weak genetic structure among Asian and African indigenous, non-descript chicken populations although they carried high within-population phenotypic and molecular genetic diversity. In the present study, 192 backyard chickens collected following a nearly 'complete' sampling strategy for all adult birds within flocks of 75 households in five villages at two sites in Sri Lanka were genotyped using 20 microsatellite markers. The phenotypes and history of these birds were also recorded. A link of phenotypic composition and flock size with specific genetic structure of the backyard chicken populations was explored. The results suggest that the households who used to keep large flocks of indigenous backyard chickens of mixed genotypes should be included in in-situ conservation program to effectively maintain and sustainably utilize these important chicken genetic resources.
Keywords: Backyard chicken; Genetic structure; Complete sampling

Introduction

A pattern of the genetic makeup of all individuals within a population is referred to as genetic structure. The genetic structure can therefore be used to infer the genetic constitution of an individual by studying other members of the population. It is expected that all populations have genetic structures which are characterized by their genotype or allele frequencies. In a natural population, there may not be any recognizable subdivision of individuals or substructure at genetic level because the distribution of individual demes (herds/flocks) is assumed to be continuous. However, different herds/flocks across the distribution range of a population can have different allele frequencies because the whole population is not panmictic even if it is effectively continuous. Inbreeding, selection and migration are the major evolutionary forces that cause the deviations from Hardy-Weinberg equilibrium or panmixia. Therefore understanding of the genetic structure of a population is fundamental to obtaining a deeper insight into its evolutionary process.

The rural farmers in Asia and Africa generally keep five to 50 indigenous chickens with a sex ratio of one cock to three up to five hens per household. An indigenous hen usually lays 10 to 20 eggs per clutch and can have two to six clutches in a year (Aini 1990; Gunaratne et al. 1993; Abdelqader et al. 2007; Sarkar and Golam 2009; Hossen 2010; Moges et al. 2010; Moreki 2010; Dessie et al. 2013). Therefore population genetic structure is expected to exist among indigenous backyard chicken flocks because most birds owned by a particular household are related to each other following the 'clonal' reproduction mode of one or a few hens sharing the same breeding cock(s). However, previous studies on molecular genetic characterization of Asian and African village chickens identified no or very weak population genetic structure within several eco-types of a particular country or several local non-descript populations in a large geographic region or one indigenous breed across relatively large geographic space (Muchadeyi et al. 2007; Berthouly et al. 2009; Leroy et al. 2012; Berima et al. 2013; Mwacharo et al. 2013). On the other hand, most of well-established local chicken breeds in Asia and Europe, highly selected commercial egg layer and broiler breeds/lines and inbred experimental lines have distinct genetic structures (Chen et al. 2008; Granevitze et al. 2009; Tadano et al. 2007, 2010; Wilkinson et al. 2011; Ceccobelli et al. 2013; Pham et al. 2013; Seo et al. 2013).

'Random' sampling strategy for un-related individuals of known history/pedigree or for only a few individuals, e.g. one adult male and female each from a village chicken flock per household to avoid the relatedness of birds has been adopted in all previous molecular characterization studies. This leads to a disconnection of the dynamic flock structure and highly mixed phenotype composition from potential genetic structure of backyard chickens. The consequence is that the resulting research findings can hardly inform sustainable conservation and genetic improvement programs to benefit individual flocks. In the present study, a nearly complete sampling strategy was used to collect morphological data and blood samples from all adult birds (≥ six months of age) within a backyard chicken flock of selected households. This approach offers an opportunity to establish the link between flock composition (e.g. phenotypes) and genetic structure of backyard chickens.

Materials and Methods

Sampling. A total of 192 birds were sampled from 75 households in five villages at two geographically distinct sites of Thirappane (80.5039-80.6331 E, 8.1185-8.2202 N) in North Central Province and Karuwalagaswewa (79.5395-80.5042 E, 8.0047-8.0692 N) in North West Province, Sri

Lanka. The geographic distance between the two sites is around 90 km. All birds in backyard chicken flocks of the selected households were first documented for their age, sex and breeding history etc. during an initial survey. Every bird older than six months was selected for recording their morphological and morphometric data following the guidelines of FAO (2012). The same adult birds were also included for blood sampling. Number of adult birds ranged between one and eight per household (Table 1). Blood samples were collected on the Whatman FTA® filter paper (Whatman Bio-Science, Maidstone, UK) and stored at room temperature.

This is part of the GEF/UNEP supported project titled "Development and Application of Decision-support Tools to Conserve and Sustainably Use Genetic Diversity in Indigenous Livestock and Wild Relatives", implemented in four Asian countries (Bangladesh, Pakistan, Sri Lanka and Vietnam) by ILRI.

Table 1. Sampling information of backyard chickens from five villages at two sites in Thirappane and Karuwalagaswewa, Sri Lanka.

No. of households in each village	No. of adult birds sampled								Total
	1	2	3	4	5	6	7	8	
Thirappane									
Dematagama	8	2	2	2	1	1			37
Labunoruwa	3	4	2				2	1	39
Ooththupitiya	5	4	5	1				1	40
Karuwalagaswewa									
Tabbowa	5	7	4					1	39
Thewanuwara	5	3	2	2		2			37

Genotyping. DNA was extracted from blood collected on the FTA paper. Around 15 discs punched from each FTA paper were added to 100 µl of distilled water and then heated at 90 °C for 10 min. The resulting solution was directly used in PCR amplification. The DNA quantification was carried out using the NanoDrop spectrophotometer ND1000 (Thermo Fisher Scientific, Wilmington, DE, USA). PCR amplification was carried out for chicken DNA using 20 microsatellite markers selected from a panel of 30 ISAG–FAO recommended microsatellite markers for chicken molecular characterization. They included *LEI0094, MCW0069, ADL0268, MCW0034, LEI0166, MCW0248, MCW0216, LEI0234, ADL0278, MCW0222, MCW0016, MCW0295, MCW0037, MCW0206, MCW0111, MCW0067, MCW0183, MCW0014, MCW0330* and *MCW0081* (FAO 2011). The PCR amplicons were separated by size using the ABI 3130XL Genetic Analyzer (Applied Biosystems, Carlsbad, California, USA). The GeneScan™-500 LIZ® Size Standard was used as an internal lane size standard (Applied Biosystems). Allele sizing calling was carried out using the third-order least squares method implemented in the GeneMapper® software version 3.7. following the procedure of Mburu et al. (2003).

Statistical analyses. The morphological data was phenotyped following the guidelines of FAO (2012). Population genetic structure was determined using a model-based clustering for assigning individuals based on multilocus genotypes to a cluster with the STRUCTURE software version 2.3.4 (Pritchard et al. 2000). The analyses involved an admixture model with correlated allele frequencies following a burn-in period of 50,000 steps and 100,000 iterations of Markov Chain Monte Carlo algorithms. Individuals were grouped into the predefined number of clusters with 10 independent runs repeated for each K value ranging from 2 to 6 and the results were interpreted with STRUCTURE HARVESTER (Earl and von Holdt 2012). A plot of the mean likelihood value per K value was prodcuced and the highest value of the second-order rate of change (ΔK) was calculated according to the method of Evanno et al. (2005) to detect the number of K clusters that best fitted the data. The results of 10 replicates at $K = 2$ to 6 were post-processed using CLUMPP program version 1.1.2 (Jakobsson and Rosenberg 2007) following the FullSearch algorithm to align the multiple outcomes and to determine the optimal clustering which was finally visualized using DISTRUCT software version 1.1 (Rosenberg 2004).

Results and Discussion

Six different phenotypes were recognized as follows: (1) indigenous village chickens having a red, black, brown or white plumage (Gam kukullu); (2) birds with a naked neck (Peda kapapu); (3) birds with long legs (Pora kukullu); (4) crown headed birds (Konda kikili); (5) crosses resulting from mating indigenous chickens with commercial birds; and (6) others including commercial layers or birds with frizzled feathers. There were 75, 53, 30, 6, 7 and 21 birds of each of these six categories, respectively. The 26 adult birds, each owned by an individual household, exhibited various phenotypes across the five villages. One particular household in Labunoruwa village had seven adult birds of only two phenotypes (five naked neck and two indigenous village chickens). The distribution of phenotypes among all remaining backyard chickens suggested a general pattern that the larger the flock size is, the more phenotypes the flock has, indicating a rather mixed phenotypic composition within most of flocks in backyard chicken system.

The best fit K value as the most likely number of genetic clusters among 192 Sri Lankan backyard chickens was identified at 3 based on their genotyping data of 20 microsatellite markers. No obvious signal of genetic differentiation among the five village chicken populations and between the two sites was observed (top three rows in Figure 1). This was in agreement with the observation of Muchadeyi et al. (2007), Berthouly et al. (2009), Leroy et al. (2012), Berima et al. (2013) and Mwacharo et al. (2013). However, some population sub-structuring patterns emerged and were associated with specific chicken phenotypes and flocks within nearly all villages. For example, the genetic background in high proportion of blue color in birds from 29 to 35 across two flocks/households in Dematagama village and from 61 to 66 (except for 62) of one household

in Labunoruwa village was all linked with the specific genotypes of commercial layer chickens and associated crosses (see bottom row in Figure 1).

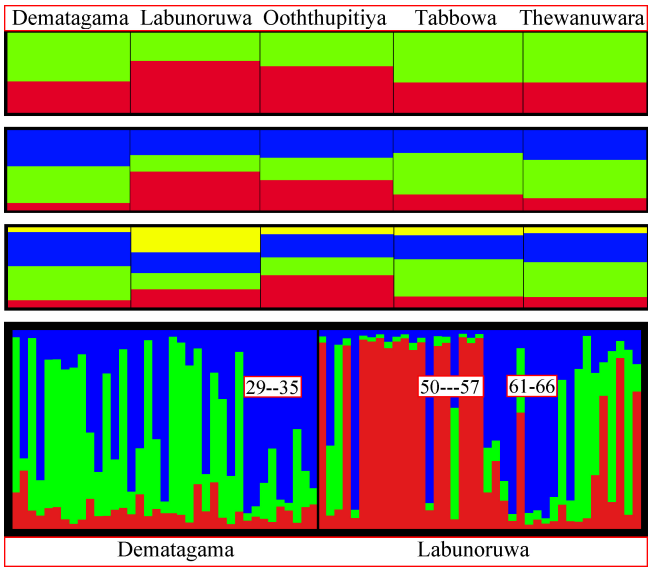

Figure 1: Genetic structure shown in average Q-values of five village chicken populations (top three rows for *K* = 2, 3 and 4) and also in multiple lines with each vertical line representing an individual of 76 genetically admixed chickens in two villages at *K* = 3 (bottom row)

One household which contributed eight adult birds had phenotypically different birds. The genetic backgrounds of these birds were also quite admixed (as shown in different colors from 50 to 57 in bottom row in Figure 1). Such birds are therefore ideal conservation candidates because they represent major and specific genetic variations among the backyard non-descript chicken population. These households should be encouraged and considered as a major part of the in-situ conservation program because they often have rich experience and strong passion to keep a large flock of indigenous non-descript chickens. They are the guardians to effectively protect and sustainably utilize these unique chicken genetic resources.

Conclusion

The nearly complete sampling strategy for all adult birds (≥ six months of age) within a flock in each household that was implemented in this study unveils the hidden genetic structure among backyard chicken flocks/populations. The results of combined analysis of phenotypic and flock size data together with genetic structuring information suggest that households who keep large flocks of indigenous non-descript chickens harbor enough important and unique variations/alleles that can be harnessed through targeted genetic improvement and conservation programs.

Literature Cited

Abdelqader, A., Wollny, C B. A. and Gauly, M. (2007). *Trop. Anim. Health Prod.* 39:155–164.

Aini, I. (1990). *World's Poult. Sci. J.* 46:51–57.

Berima, M. E. A., Yousif, I. A., Eding, H., et al. (2013). *Afr. J. Biotechnol.* 12:6424–6431.

Berthouly, C., Leroy, G., Nhu Van, T., et al. (2009). *BMC Genetics* 10:1.

Ceccobelli, S., Di Lorenzo, P., Lancioni, H., et al. (2013). *Italian J. Anim. Sci.* 12:e66.

Chen, G. H., Bao, W. B., Shu, J. T., et al. (2008). *Asian-Aust. J. Anim. Sci.* 21:331–339.

Dessie, T., Esatu, W., Waaji, L. V., et al. (2013). *Village chicken production in the central and western highlands of Ethiopia: characteristics and strategies for improvement*. Nairobi, Kenya: International Livestock Research Institute.

Earl, D. and Vonholdt, B. (2012). *Conserv. Genet. Res.* 4:359–361.

Evanno, G., Regnaut, S. and Goudet, J. (2005). *Mol. Ecol.* 14:2611–2620.

FAO. (2011). *Molecular genetic characterization of animal genetic resources*. FAO Animal Production and Health Guidelines. No. 9. Rome.

FAO. (2012). *Phenotypic characterization of animal genetic resources*. FAO Animal Production and Health Guidelines. No. 11. Rome.

Granevitze, Z., Hillel, J., Feldman, M., et al. (2009). *Anim. Genet.* 40:686–693.

Gunaratne, S. P., Chandrasiri, A. D. N., Hemalatha, W. A. P., et al. (1993). *Trop. Anim. Health Prod.* 25:249–257.

Hossen, M. J. (2010) *Livestock Research for Rural Development* 22:Article #192. http://www.lrrd.org/lrrd22/10/hoss22192.htm

Jakobsson, M. and Rosenberg, N. A. (2007). *Bioinformatics* 23:1801–1806.

Leroy, G., Kayang, B. B., Youssao, I. A. K., et al. (2012). *BMC Genetics* 13:34.

Mburu, D. N., Ochieng, J. W., Kuria, S. G., et al. (2003). *Anim. Genet.* 34:26–32.

Moges, F., Mellesse, A. and Dessie, T. (2010). *Afr. J. Agri. Res.* 5:1739–1748.

Moreki, J. C. (2010). *Livestock Research for Rural Development* 22:Article #46. http://www.lrrd.org/lrrd22/3/more22046.htm

Muchadeyi, F. C., Eding, H., Wollny, C. B. A., et al. (2007). *Anim. Genet.* 38:332–339.

Mwacharo, J. M., Nomura, K., Hanada, H., et al. (2013). *Mol. Ecol.* 22:2683–2697.

Pham, M. H., Chang, W. H., Salazar, C. B., et al. (2013). *J. Poult. Sci.* 50:290–299.

Pritchard, J. K., Stephens, M. and Donnerly, P. (2000). *Genetics* 155:945–959.

Rosenberg, N. A. (2004). *Mol. Ecol. Notes* (4):137–138.

Sarkar, K. and Golam, M. (2009). *World's Poult. Sci. J.* 65:251–259.

Seo, D. W., Hoque, M. R., Choi, N. R., et al. (2013). *Asian-Aust. J. Anim. Sci.* 26:316–322.

Tadano, R., Nishibori, M., Nagasaka, N., et al. (2007). *Poult. Sci.* 86:2301–2308.

Tadano, R., Kinoshita, K., Mizutani, M., et al. (2010). *Exp. Anim.* 59:511–514.

Wilkinson, S., Wiener, P., Teverson, D., et al. (2011). *Anim. Genet.* 43:552–563.