IDENTIFYING POPULATIONS OF PAPUA NEW GUINEA'S INDIGENOUS CHICKENS FOR PRIORITY CONSERVATION

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ABSTRACT

The possible decline in genetic diversity of Papua New Guinea's (PNG) indigenous chickens and its consequences for food production and other concerns has necessitated a discussion of measures to conserve this genetic resource. As an initial step to investigate the genetic diversity and provide a theoretical basis for a conservation program of PNG's indigenous chickens, calculation and analyses of genetic diversities were carried out in this study using mitochondrial DNA (mtDNA) D-loop sequence variations in three populations of indigenous chickens. The results indicate that all of the Alotau, Madang and Port Moresby chicken populations in PNG have unique haplotypes and high genetic diversity. Priority for conservation of these populations has been suggested on the basis of their contributions to genetic diversity.

Key words: *Papua New Guinea, indigenous chicken, DNA, genetic, conservation*

INTRODUCTION

Genetic diversity in indigenous chickens will be required in the future to meet production needs in various environments, to allow sustained genetic improvement and to facilitate rapid adaptation to changing breeding objectives. However the need for conserving animal genetic resources (AnGR) is generally not well appreciated. According to the FAO (2007a) even though 321 world-wide chicken breeds have been found to be not at risk, 467 breeds are either extinct, critical, critical-maintained, endangered or endangered-maintained. FAO (2005, 2007a, 2007b) has therefore included indigenous chicken breeds in its AnGR conservation programs. However, the production potential of indigenous breeds in some developing countries is often inadequately documented and utilized (Philipsson and Okeyo 2006). The indigenous chickens of PNG have never been considered in AnGR conservation programs. Indigenous chickens were probably introduced into PNG between 2000 to 3000 years ago (Bellwood, 1978). The breed is currently found distributed throughout all parts of PNG and is the most important poultry species in the rural areas for food security and other socio-economic purposes. Currently these populations of indigenous chickens are threatened by many factors including genetic erosion by crossbreeding with imported exotic breeds, intensification of production systems and loss of habitat due to increasing human population and activity (Turner, 1972; Bilong 1990; Moat and Bilong 1999). Genetic characterization of these indigenous breeds for conservation and rational use is therefore necessary and urgent. In this study therefore, the genetic diversity of some populations of indigenous chickens of PNG were analysed and assessed for the first time using mitochondrial DNA (mtDNA) D-loop sequences and priorities for conservation of these indigenous chicken populations are discussed.

MATERIALS AND METHODS

Sampling

Blood samples were collected on FTA cards (Whatman, Inc.) from a total of 92 indigenous chickens in three different geographical regions of Papua New Guinea: Alotau (35 birds), Madang (35 birds) and Port Moresby (22 birds). Within each region, samples were collected from several birds from multiple households in different villages. To minimize the chances that the birds used from each village were closely related, a single bird was sampled from each household. The households within each village from which birds were used were approximately 0.7–1.5km apart.

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PCR amplification, purification and DNA sequencing

This work was done at the CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Beijing and the PNG University of Technology, Biotechnology Centre.

The D-loop region was amplified directly from the genomic DNA by polymerase chain reaction (PCR). The primer pair, L16750 (5'-AGGACTACGGCTTGAAAAGC–3') and H547 (5'- ATGTGCCTGACCGAGGAACCAG-3'), described by Niu et al. (2002), was used to amplify the first 510bp segment of the D-loop hypervariable region. In the primer names, L and H refer to the light and heavy chains, respectively, and the number designates the position of the 3'-end of the primer on the complete chicken mtDNA sequence (Desjardins and Morais, 1990). PCR reactions were carried out in 50µl volumes using 1× buffer, 1.5 mM MgCl2, 2.5 mM dNTP, 10 pM of each primer and 1 unit Huitian Taq polymerase. The PCR cycle included the initial denaturation at 94°C for 10 min followed by 30 cycles of denaturation at 94° C for 30 sec, annealing at 61°C for 30 sec and extension at 72°C for 30 sec with a final extension at 72°C for 10 min using GenAmp 9700 (Applied Biosystems, CA, USA). The PCR products were purified with Tiangen® PCR purification kit according to the manufacturer's instructions. Sequencing of the DNA was performed by using Big Dye Terminator Cycle Sequencing Ready Reaction Kit (v3.1, Applied Biosystems, CA, USA) and electrophoresis was done by a ABI3130XL DNA Genetic Analyzer (Applied Biosystems, CA, USA).

Data analysis

The mtDNA nucleotide sequences obtained in this study were aligned by using the ClustalX program (http:// www.igbmc.ustrasbg.fr/pub/ClustalX; Jeanmougin *et al.*, 1998) and identical sequences were considered as the same haplotypes. Calculation of haplotype frequency and Genetic diversity analyses were performed using Dnasp software version 4.10.3 (http:// www.ub.es/dnasp).

RESULTS AND DISCUSSIONS

Variant sites analysis

Analysis of the mtDNA D-loop sequences from the 92 samples showed a total of 28 nucleotide changes which could be grouped into 22 haplotypes (Table 1). The two largest haplotype groups consisted of 34 and 13 individuals while the remaining 20 haplotypes contained less than 10 individuals each. The nucleotide changes were characterized by transitions at 26 sites and transversions at 2 sites and no deletions or insertions.

Twenty eight polymorphic sites were found in the 397bp sequenced giving an average of 7.05% polymorphic sites in the 92 samples. This value is higher than those reported by other authors. *Liu et al.* (2004), Niu *et al.* (2001) and Fu *et al.* (2001) who reported the average percentage of polymorphism in D-loop region to be 6.4 and 4.45 (33 and 21 polymorphic sites in the same fragment of 397bp), respectively, for a Chinese native chicken breed. *Lee et al.* (2007) reported that the average percentage of polymorphic sites was 3.53 for 510bp (17 polymorphic sites in the same fragment of 397bp) for Korean Ogol chickens and attributed this lower level of polymorphism to sampling from one location where selection pressure continued for a long time. Moreover, low percentage of polymorphic sites in mtDNA D-loop sequences may be due to an evolutionary bottleneck during the course of domestication (Moritz, 1994). Therefore the higher level of polymorphism found in this study suggests that the samples have wide representation among the indigenous chickens of Papua New Guinea, and that these indigenous chickens could have experienced a milder bottleneck compared with those of other localities.

Distribution of haplotypes

The frequency of 22 haplotypes (H1-H22, Table 1) found in this study ranged from 1.09% to 36.96%, and the diversity of haplotypes was 23.91%. Only one haplotype, H4, was found in all the three regions of PNG while four haplotypes were observed in two of three regions and 17 haplotypes were detected in only one region. The wide distribution of haplotype H4 in all three regions suggests that this haplotype had higher fitness during the long history of domestication and so it might be an ancestral haplotype among PNG's indigenous chickens. Haplotypes 4, 12 and 1 were unique to Alotau, Madang and Port Moresby regions respectively, giving a total of 17 unique haplotypes. This distribution pattern of unique haplotypes indicates the genetic distinctiveness of the three indigenous chicken

Table 1: Polymorphic sites of mtDNA D-loop of indigenous chicken in Papua New Guinea

Haplotypes	Variable Sites*	Frequency			
	11112222222222223333333333 67791122445668990011234566 9 97179275836615136690524243 71 GTTATGTCCCCCTCGTCCCTCTG	Alotau	Madang	Port Moresby	Total
H1	GATCTC		1	5	6
H2	.....T.....		1		1
H3	.....T.....		9		9
H4	.....T.T.....	20	7	7	34
H5	.....CT.T.....		2		2
H6	A.....T.T.....	1**			1
H7	.....T.TC...G....	9		4	13
H8	.....T.TC..AG....	1			1
H9	A.....T.TC...G....	1			1
H10	.....T.TC.....		2		2
H11	.....T.T....G....	1	1		2
H12	.....T....G....		1		1
H13	...T.....T....T....G.T..		1		1
H14	.....TC.....T.....		1		1
H15	....C.C.....A..TT.....		4		4
H16	.....C.....A..TT.....C.		2		2
H17	.....C.....A..TT.....	1		5	6
H18	....AC.....A..TT.....		1		1
H19	..C..A.....A.TTT..A.CT..		1		1
H20	..C..A.....A.TTT..A.CT.T		1		1
H21	.C.....T.T.TC.A..TTC.....	1			1
H22	.....A...TTTC.A..TTCT.....			1	1
	Total	**35**	**35**	**22**	**92**

*Numbers indicate nucleotide base position in mitochondrial D-loop region and hyphen represents the identical nucleotide with the H1 sequence; ** The italic number indicate the unique haplotypes.

populations. Thus the Madang chicken population with 12 unique haplotypes is the most distinctive followed by the Alotau and Port Moresby chicken populations.

Genetic distinctiveness of populations is an important criterion used when populations are selected for conservation. The highest priority for conservation is often given to population with highest genetic distinctiveness Moritz (1994). Parker *et al.* (1999, 2001) selected distinct highest priority for conservation followed by the Alotau and Port Moresby chicken populations.

Genetic diversity

Genetic diversity indices calculated for the three chicken populations are shown in Table 2. Nucleotide diversity indices ranged from 0.00446 to 0.00862 while haplotype diversity indices were between 0.620 and 0.889. Values of both indices were lower than those calculated

by Silva et al (2008) for indigenous chicken populations of Sri Lanka The difference in diversity indices between PNG and Sri Lankan indigenous chickens could be explained by the fact that Sri Lanka, located in Southeast of the Indian subcontinent, is an important centre of origin of indigenous chicken (Fumihito *et al.* 1996; West and Zhou 2002; Liu *et al.* 2006) and forms a confluence or exchange centre of other centres from where indigenous chickens were distributed to other parts of Southeast Asia (including PNG) and Africa (Muchadeyi *et al.* 2008). Therefore Sri Lankan indigenous chickens can be expected to have generated and accumulated higher levels of genetic diversity after several centuries of domestication. However, nucleotide diversity indices observed in this study were higher than those estimated by Liu *et al.* (2006) for certain clades of chickens from Europe, Middle East, Southeast and East Asia, and by Oka *et al.* (2007) for Japanese native chickens. Higher genetic diversity in chickens is indicative of center of species origin and confluence (Chen *et al.* 2002) where genetic variation has been generated and accumulated over long periods of time. Japanese native chickens are believed to have been established from native chicken populations of other East and Southeast Asian countries and this account for the lower genetic diversity of the foundation populations of Japanese native chicken compared to original populations of other Asian countries. Furthermore, most Japanese native chickens are ornamental breeds that have low productivity. Therefore the number of individuals in some of these Japanese breeds could be decreasing, and this could be one of the causes of reduction in genetic diversity (Oka *et al.* 2007). Several authors have suggested that Southeast Asia is another centre of origin of indigenous chicken (Fumihito *et al.* 1996; West and Zhou 2002; Liu *et al.* 2006) and that one of three major maternal lineages of the modern Chilean chicken breeds is from the South Chinese/ Indonesian/Japanese area (Gongora *et al.* 2008). Because PNG has a common border with Indonesia to the west it might be part of the Southeast Asian centre of origin of indigenous chickens. This could explain the higher genetic diversity of the indigenous chickens of PNG observed in this study. The genetic diversity generated because of originality of centre is the most important for conservation (Chen *et al.* 2002).

Another approach used by some authors is to give higher priority for conservation to populations that show higher genetic diversity (Chen *et al.* 2002). In this study, the diversity indices Pi, k and Hd for the Madang chicken population were 0.00862, 3.422 and 0.889 respectively (Table 2) and these were the highest values among the three populations. The Port Moresby chicken population had the next highest indices while the Alotau population had the lowest. These results also indicate the order of genetic richness in the three indigenous chicken populations of PNG and consequently the order to be followed in prioritizing these populations for con-

Table 2 Diversity parameters of Indigenous chicken in Papua New Guinea

Population	S	Hn	Hu	Pi	k	Hd+SD
Alotau	35	8	4	0.00446	1.771	0.620±0.074
Madang	35	15	12	0.00862	3.422	0.889±0.034
Port Moresby	22	5	1	0.00835	3.316	0.797±0.039

S; The size of populations' Hn: The number of haplotypes; Hu: The number of unique haplotypes, Pi; Nucleotide diversity, k; Average number of nucleotide differences; Hd: Haplotypes (gene) diversity, SD; Standard deviation

Genetic contribution analysis

Due to differences in the size of populations, scarcity of funds for species conservation and conflict between conservation and economic development, deciding what and where to conserve is an essential step in managing important species. Generally, the main aim is usually to protect the genetic resources as much as possible, on the basis of both genetic distinctiveness and diversity. However, the genetic distinctiveness-based approach chooses populations with more genetic uniqueness for priority conservation. It does not consider genetic variation within populations (Chen *et al.* 2002; Pennock and Dimmick 2002), while the genetic diversity-based approach chooses populations with high genetic variation for priority conservation without considering genetic distinctiveness. Thus some haplotypes unique to some populations with low genetic variation may not receive sufficient attention for conservation in some cases. Therefore, Petit *et al.* (1998) put forward the approach of genetic contribution, a synthesis that considers genetic diversity. This approach appears to be the most appropriate for selecting populations for conservation (Chen *et al.* 2002; Ping *et al.* 2004). The contributions of genetic diversity ($R_{S\,(k)}$) and genetic distinctiveness ($R_{D\,(k)}$) are combined to get the total genetic contribution ($R_{T\,(k)}$) of the k^{th} population

$$R_{S(k)}=\frac{R_k}{n};\ R_{D(k)}=\sum_i^{R_k}\frac{n-n_i}{nn_i}\ \text{and}\ R_{T(k)}=R_{S(k)}+R_{D(k)}=\sum_i^{R_k}\frac{1}{n_i}$$

Where n represents the total of populations studied and n_i represents the number of populations with the i^{th} haplotype. Similarly the rates of contribution attributed to genetic variation ($C_{S(k)}$) and genetic distinctiveness ($C_{D(k)}$) to the total genetic contribution rate ($C_{T(k)}$) of the k^{th} population with R_k haplotypes are obtained by using the formulae:

$$C_{RS(k)}=\frac{R_{S(k)}-\bar{R}_S}{R_T};\ C_{RD(k)}=\frac{R_{D(k)}-\bar{R}_D}{R_T}\ \text{and}\ C_{RT(k)}=\frac{R_{T(k)}-\bar{R}_T}{R_T}$$

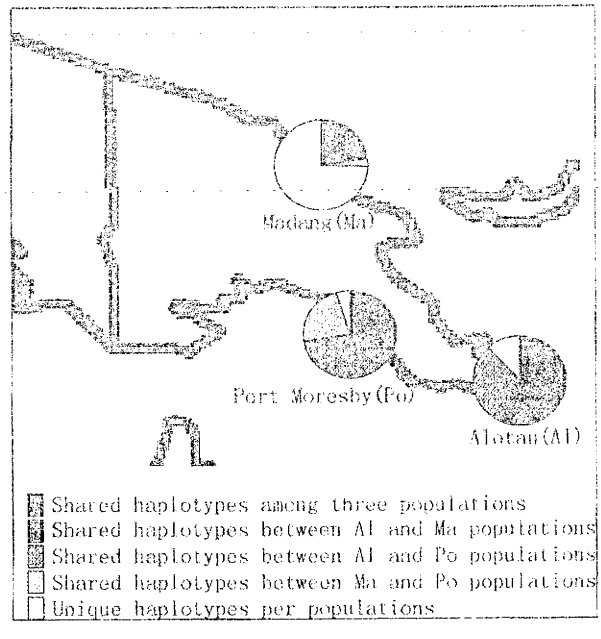
Where R_T represents the total of haplotypes, $\bar{R}_S=\sum_k R_{S(k)}/n$, $\bar{R}_D=\sum_k R_{D(k)}/n$, $\bar{R}_T=\sum_k R_{T(k)}/n$. The total contribution rate $C_{RT(k)}$ can be partitioned into two components, $C_{RS(k)}$, which is the rate of contribution of the k^{th} population due to its own diversity and $C_{RD(k)}$, the contribution due to its divergence, i.e. $C_{RT(k)}=C_{RS(k)}+C_{RD(k)}$, $\sum_k C_{RS(k)}=0$, $\sum_k C_{RD(k)}=0$, $\sum_k C_{RT(k)}=0$ (Table 3).

Table 3 Genetic contribution of three indigenous chicken populations in Papua New Guinea

Populations	$R_{S(k)}$	$R_{D(k)}$	$R_{T(k)}$	$C_{RS(k)}$ (%)	$C_{RD(k)}$ (%)	$C_{RT(k)}$ (%)
Alotau	2.667	3.167	5.834	-0.020	-0.048	-0.068
Madang	5.000	8.334	13.334	0.086	0.187	0.273
Port Moresby	1.667	1.167	2.834	-0.066	-0.139	-0.205

The values obtained for R_S and R_D (and therefore R_T) were highest for the Madang chicken population and lowest for the Port Moresby chicken population. The value of $C_{S(k)}$, $C_{D(k)}$ and $C_{T(k)}$ provide relative overall criteria for setting priorities for conservation of PNG's indigenous chicken populations. The positive values of $C_{S(k)}$, $C_{D(k)}$ and $C_{T(k)}$ in Madang chicken population show that the genetic variation contribution rate, genetic distinctiveness contribution rate and the total of genetic contribution rate were higher than the average of the three populations, thereby indicating that the Madang chicken population could contribute most to improving the genetic variation and haplotypes richness of PNG's indigenous chicken followed by the Alotau and Port Moresby indigenous chicken populations respectively.

Figure 1: Geographical distribution of three chicken populations in Papua New Guinea



This order could therefore be followed in the conservation of PNG's indigenous chicken population given that human, material and financial resources are limited. This order is the same as the order based on genetic distinctiveness and implies that the priorities for conservation of PNG's chicken genetic resources depends to a great extent on the genetic distinctiveness of the chicken populations.

CONCLUSION

Papua New Guinea's indigenous chicken populations have unique haplotypes and high genetic diversity and probably belong to the Southeast Asia centre of domestication of indigenous chickens and are therefore an important genetic resource which needs to be considered for conservation. This need is all the more urgent in the light of global climate change and its consequences for food production especially in developing countries, genetic erosion and intensification of production systems in the country. This study has concentrated on three indigenous chicken populations and suggests that the highest priority for conservation should be given to the Madang chicken population followed the populations from Alotau and Port Moresby in that order.

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