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Genetic Analysis of Haimen Chicken Populations Using Decamer Random Markers

O. Olowofeso[1, 2, *], J. Y. Wang[1], P. Zhang[1], G. J. Dai[1], H. W. Sheng[1], R. Wu[1] and X. Wu[1]

[1]Department of Animal Genetics, Breeding and Reproduction, Yangzhou University, Yangzhou, 225009, P. R. China

ABSTRACT : Through a screening and selection approach method, decamer random markers were used in a technique called random amplified polymorphic DNA (RAPD) assay with 252 genomic DNAs isolated from four major Haimen chicken populations: Rugao (62), Jiangchun (62), Wan-Nan (63) and Cshiqishi (65). A total of 3-score decamer random primers (S241-S260, S1081-S1100 and S1341-S1360) were employed in the preliminary RAPD-polymerase chain reaction (RAPD-PCR) assay with 50 random template DNA samples from all the populations. Four (6.67%) of the primers that produced obvious polymorphic patterns, interpretable and reproducible bands were selected and used with both the individual DNAs from each population and with pooled DNA samples of the four populations in subsequent analyses. The selected primers produced a total of 131 fragments with molecular size ranging from 835 to 4,972 base pairs (bp) when used with the individual DNAs; 105 (80.15%) of these fragments were polymorphic. With the pooled DNAs, 47 stable and characteristic bands with molecular size ranging from 840 to 4,983 bp, of which 23 (48.94%) polymorphic, were also generated. The band-sharing coefficient (BSC) calculated for the individuals in the population and among populations of bulked samples was between 0.8247 (Rugao) and 0.9500 (Cshiqishi); for pairwise populations, it was between 0.7273 (Rugao vs. Wan-Nan) and 0.9367 (Jiangchun vs. Cshiqishi) chicken populations. Using the BSC for individual and pairwise populations, the Nei's standard genetic distances between the chicken populations were determined and ranged from 0.0043 (Jiangchun vs. Cshiqishi) to 0.1375 (Rugao vs. Cshiqishi). The reconstructed dendrogram linked the Jiangchun and Cshiqishi chickens as closely related populations, followed by Wan-Nan, while the Rugao was the most genetically distant among the populations. (**Key Words :** Genetic Analysis, Haimen Chickens, Random Markers)

INTRODUCTION

Over the years, there have been several methods developed for examining genetic information of several species populations. Available molecular methods provide an opportunity for a powerful and reproducible approach of estimating genetic characteristics within and among strains based on DNA variation. Among the numerous methods available, the random amplified polymorphic DNA (RAPD) assay is the most suitable assay for genetic analysis of species especially the chickens. The advantages of RAPD are manifolds. It is a simple and easy method to detect polymorphisms based on the amplification of random DNA segments with single primer of arbitrary nucleotide sequence according to (Welsh et al., 1990; Williams et al., 1990). The technique is faster and less expensive than other kinds of DNA fragment analyses and can be used with any quantity of DNA. It is even suitable for work on anonymous genomes. The method is also capable of sampling genome randomly like the allozyme and the RFLPs (Wang and Dai, 2001). It had also been used to develop genome maps (Levin et al., 1993). Several authors including Smith et al. (1996), Zhang et al. (2002) have tested the usefulness of the assay method with chickens. In literature, genetic information on the large population of Haimen chickens in China is completely not available. These chicken populations are rich in China and provides easy source of protein and income to rural farmers in this region of the world. In order to integrate these chicken populations to the existing common populations and for conservation purposes, a detail genetic analysis of these chickens is therefore imperative. In the present work, we adopted the RAPD technique, generate and analyzed the genetic population data in the Haimen chicken populations. Here, we report the band-sharing coefficient, heterozygosity, the genetic

* Corresponding Author: O. Olowofeso. E-mail: jideolowofeso@yahoo.ca

[2] Department of Animal Production and Health, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria.

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Table 1. Haimen chicken populations, origin and sample size by sexes

Population(s)	Origin	Individuals/sample size		
		Hens	Cockerels	Total
Rugao chicken	China	40	22	62
Jiangchun chicken	China	42	20	62
Wan-Nan chicken	China	40	23	63
Cshiqishi chicken	China	40	25	65
Total		162	90	252

distances between the chicken populations and reconstruct the dendrogram based on the RAPD assay technique.

MATERIALS AND METHODS

Experimental chickens and sample size

The chickens used were breeds that have been maintained at the Haimen Integrated Poultry Company, Jiangsu Province, China. A total of 252 blood samples comprising individuals from the populations were collected and used for this study. The information on population, origin and samples collected by sexes in the examined chicken populations are presented in Table 1.

Blood collection, DNA isolation and purification

Birds confined in cages were sampled and veinous bloods were collected aseptically into haemotocrit tubes using heparinzed 13 mm, 27 gauge needle and were stored at -80°C using sequestering agent (ethylenediamine-tetraacetic acid-EDTA) and heparin as anticoagulants. Approximately 1 ml of blood was collected from each of the bird into 1.5 ml microfuge tube and was allowed to settle for 48 h before DNA extraction.

Samples were carefully centrifuged at 200 g for 2 minutes and DNAs were isolated by saturated salt procedure described by Miller et al. (1988). Total genomic DNA was isolated from the blood beginning with cell lysis and 200 µl whole blood was pipette into a 1.5 ml microfuge tube and re-suspended in 300 µl lysing buffer (44 mM NH_4Cl, 10 mM NH_4HCO_3) with centrifugation at 1,000 g for 10 minutes to pellet cells, 20 µl RNase A stock solution (Sangon Company, Shanghai, China) was added to the sample, followed by thorough shaking. The resultant supernatant was removed and replaced with 1 ml SET buffer (10 mM Tris-HCl, PH = 8.0, 200 mM common salt (NaCl), 0.1 M EDTA and 0.5% SDS) and the samples were digested with 0.5 µg/µl Proteinase K stored dried at 4°C. For efficient lysis and yield of homogeneous solution, samples and buffer were thoroughly mixed and vortexed for 15 seconds. The resultant mixture was placed in hot water bath, incubated overnight at 55°C with slight shaking. After overnight incubation, high molecular weight DNAs was extracted twice with phenol-chloroform-isoamyl (25:24:1 v/v)-(phenol chloromethane and iso-amyl alcohol). In each case, phenol-chloroform-isoamyl were added to the resultant filtrate collected into new tubes after repeated centrifugations and finally the DNA samples were precipitated by 70% ethyl alcohol absolute. The tubes containing the resultant DNA samples were air-dried for 10 minutes so that it can easily re-dissolve. The air-dried DNA pellets were dissolved in 300 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH = 8.0) to prevent protein and other contaminants that may inhibit PCR reactions and the mixtures were carefully vortexed. The content of each microfuge tube containing the DNA was diluted with sterilized water before the purity and the concentration of each DNA sample was carried out. Following the determination of the $OD_{260}/_{280}$ value of the samples using spectrophotometer, the concentration of the total DNAs were adjusted to 100 ng/µl and exactly 1 µl of the DNA samples were used as template for polymerase chain reaction.

Table 2. The code, sequence of markers (5'-3' flanking region) and their molecular weight

S/No.	Code	Sequence of markers	Molecular weight (g)
1	S249	CCACATCGGT	2,977
2	S1083	CCCACCCTTG	2,913
3	S1088	GTCGCCCTCA	2,953
4	S1092	CCCAGGCTAC	2,962

RAPD-PCR composition and programme

The PCR constituents used with the final volume of 25 µl included 2.5 µl of 10×PCR Buffer, 2.2 µl of 25 mmol/L $MgCl_2$, 1 µl of dNTPs (25 mM dNTPs), 1.5 µl (10 p/mol) of each random primer, 0.2 µl *Taq* DNA polymerase (5 U/µl) (Sangon Company, Shanghai, China), 16.6 µl sterilized distilled water and 1 µl template DNA from each individual in each population was used. PCR was performed in a Touchdown Hybaid Express system 9600 (Perkin Elmer) with the following programme; initial denaturation at 94°C, 60 s; 45 cycles of denaturation at 94°C, 30 s; annealing at 36°C for 60 s and 72°C for 120 s; the final extension was at 72°C for 600 s.

The four selected random decamer markers with their code; sequences and molecular weight are presented in Table 2.

Preparation of pooled DNAs and PCR

Exactly 3 µl of each of the individual DNA samples whose concentration has been adjusted were bulked together. Individuals of the Rugao population were bulked into a bigger microfuge tube and labeled, and same were

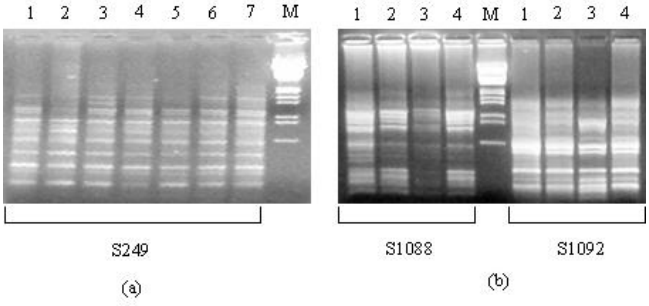

Figure 1. (a) Electrophoresis result of primer S249 with individual DNAs of Cshiqishi chicken population, 1, 2, 3, 4, 5, 6 and 7 are amplified PCR products and M is λ DNA/Hind III+EcoR I marker. (b) Electrophoresis result of primers S1088 and S1092 with pooled DNAs of Rugao (1), Jiangchun (2), Wan-Nan (3) and Cshiqishi (4) chicken populations and M is λ DNA/Hind III+ EcoR I marker.

carried out for Jiangchun, Wan-Nan and Cshiqishi populations, respectively, to have fewer representative samples. The four bigger microfuge tubes containing the pooled DNAs were vortexed for 10 s and centrifuged for another 60 s to ensure homogenous mixture of samples. The PCR conditions and programme used was replica to those used for the individual DNAs and 1 µl bulked sample in each population was used as PCR component.

Submerged agarose gel electrophoresis (SAGE)

The RAPD fragments amplified by the polymerase chain reaction were separated by high-resolution gel electrophoresis using 1.4% agarose gel with a drop (0.5 µg/ml) of ethidium bromide used as staining agent. Gels were run in Submarine Agarose Unit containing a 15×10-cm white tray. First, 1 µl mobility marker (6×bromophenol red) was placed on cellophane paper and 20 µl of the amplified product added, mixed and loaded into each capillary well of the prepared gel. The Agarose Unit was connected to a D.C source and lasted for 1 h at 100 V, 20 mA. The buffer used to run the gel was 0.5×TBE, while 15 µl λ DNA/Hind III+EcoRI was used in the same gel as molecular marker to estimate the size of the amplified products. Following electrophoresis, gels were visualized under UV-transilluminator and the RAPD profiles photographed. A portion of the gel result of the primer S249 with individual DNAs of Cshiqishi chicken population and S1088 and S1092 with the pooled DNAs of the four chicken populations are represented in Figure 1 (a and b), respectively.

Statistical analyses

For the estimate of the band-sharing coefficients (BSC) in each population and in pairwise populations using the amplified fragments, three steps were followed; band clearly present and scorable were assumed 1, band completely absent were scored zero and band appearing weak and cannot easily be scored were assumed negligible.

The band-sharing coefficients was estimated as:

$$BSC_{XY} = Pb = \frac{2N_{XY}}{N_X + N_Y}$$

where N_X and N_Y are the number of bands scored in X and Y individuals and N_{XY}, is the number of bands which both individuals in population have in common. For the pairwise populations, the above formula was also used, but both N_X and N_Y, redefined as number of bands which populations X and Y have in common and N_{XY} in such case represents the number of bands common to two populations (Jeffreys et al., 1985; Lynch and Milligan, 1994; Mohd-Azmi et al., 2000). Heterozygosity (H) was calculated as:

$$H = \frac{2(1-q)}{2-q}$$

with average frequency for a band

$$(q) = 1 - \sqrt{1 - BSC} = 1 - \sqrt{1 - Pb}$$

The genetic distances (D_{ij}) between the chicken populations were estimated using Nei (1975) equation defined as:

$$D_{ij} = -\ln \frac{\overline{S_{ij}}}{\sqrt{\overline{S_i} \times \overline{S_j}}}$$

where D_{ij} = genetic distance between populations, $\overline{S_{ij}}$ = average band-sharing coefficients of individuals of two populations in pair, $\overline{S_j}$ and $\overline{S_j}$ = average band-sharing coefficients of individuals of the populations i and j, respectively. Dendrogram from the D_{ij} estimates of RAPD patterns of the selected primers for which $\overline{S_{ij}}$ between various combinations of populations were different and less than 1 was constructed using Sandra (2003) method.

RESULTS

The four criterion primers (S249, S1083, S1088 and S1092) produced polymorphic band patterns, which were clear, scorable and interpretable in all the chicken populations. The primers produced a total of 131 bands across all the chicken populations when used with individual DNAs, with size ranged from 835 to 4972 base pairs (bp), of which 105 (80.15%) were polymorphic and the same primers produced 47 fragments out of which 23

Table 3. The band-sharing coefficients in each and in pairwise populations (BSC), average frequency for a band (q) and heterozygosity (H) in the Haimen chicken populations

Population(s)	BSC	q	H
Rugao	0.8247	0.5813	0.5903
Jiangchun	0.9314	0.7381	0.4151
Wan-Nan	0.8334	0.5918	0.5797
Cshiqishi	0.9500	0.7764	0.3655
Rugao vs. Jiangchun	0.8219	0.5780	0.5935
Rugao vs. Wan-Nan	0.7273	0.4778	0.6861
Rugao vs. Cshiqishi	0.7714	0.5219	0.6469
Jiangchun vs. Wan-Nan	0.8267	0.5837	0.5879
Jiangchun vs. Cshiqishi	0.9367	0.7484	0.4020
Wan-Nan vs. Cshiqishi	0.8611	0.6273	0.5430

Table 4. Genetic distances (below) and identity similarity matrices (above) the diagonal between four Haimen chicken populations using RAPD markers

Population	Rugao	Jiangchun	Wan-Nan	Cshiqishi
Rugao	0.0000	0.9378	0.8773	0.8715
Jiangchun	0.0642	0.0000	0.9384	0.9957
Wan-Nan	0.1309	0.0636	0.0000	0.9677
Cshiqishi	0.1375	0.0043	0.0328	0.0000

(48.94%) were polymorphic with the pooled DNAs across populations and size ranged from 840 to 4,983 bp (data not presented). Using the band patterns produced by the primers, the calculated band-sharing coefficients (BSC) for each population ranged from 0.8247 (Rugao) to 0.9500 (Cshiqishi) and for pairwise populations across all primers ranged from 0.7273 (Rugao vs. Wan-Nan) to 0.9367 (Jiangchun vs. Cshiqishi). Heterozygosity (H) for each population was between 0.3655 (Cshiqishi) and 0.5903 (Rugao) and for pairwise populations; it ranged between 0.4020 (Jiangchun vs. Cshiqishi) and 0.6861 (Rugao vs. Wan-Nan) populations (Table 3).

Using Nei's standard genetic distance approach, the genetic distances (D_{ij}) calculated ranged between 0.0043 (Jiangchun vs. Cshiqishi) to 0.1375 (Rugao vs. Cshiqishi) populations (Table 4).

The dendrogram (Figure 2) revealed that chicken populations (Jiangchun vs. Cshiqishi) are closely related and formed the first group and this linked with Wan-Nan forming the second cluster and later linked to Rugao forming the third group.

DISCUSSION

The four decamer primers used for the RAPD-PCR with Haimen chicken populations produced relatively large number of fragments totaling 131 with individual DNAs across populations and 47 fragments with pooled population DNAs. With RAPD primers, large numbers of fragments are often been generated as earlier reported by Murayama et al. (1999) and Bartfai et al. (2003). RAPD assay is capable

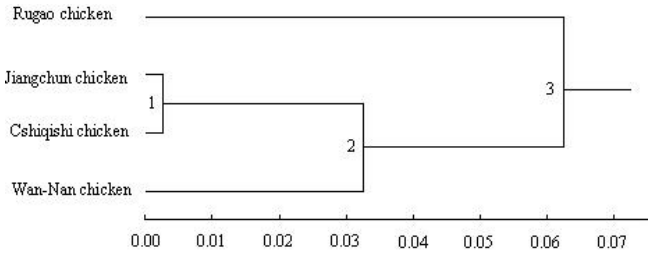

Figure 2. Dendrogram obtained using the D_{ij}-values generated with random primers with Haimen chickens.

of showing the specific differences existing between populations even in closely related species (Wang and Dai, 2001). With four primers used in this study, there were real differences in the patterns of fragments produced. The differences in both the number and size of the amplified fragments indicate genomic DNA diversity among the Haimen chicken populations. In this study, the band-sharing coefficients were all very high (0.8247 to 0.9500) among populations and it was (0.7273 to 0.9367) when the DNAs were pooled. This resulted in the lower heterozygosity among and between populations. Using RAPD technique, Chen et al. (2002) reported that higher band-sharing rate, a term analogous to the band-sharing coefficient used in the present study means lower heterozygosity and vice-versa. Zhang et al. (2002) have equally obtained a very low heterozygosity values among and between Chinese chicken populations with RAPD technique. Here, higher values of band-sharing coefficients bring about lower heterozygosity among and between various combinations of chicken populations, meaning that results of this work were consistent with previous studies of RAPD with chickens.

Results of this experiment therefore, supports that RAPD assay is effective in the genetic analysis of any species particularly chicken populations band-sharing coefficients and heterozygosity. It also serves as a quick assay method in determining the genetic distances in Haimen chicken populations. The reconstructed dendrogram linked the Jiangchun and Cshiqishi chickens as closely related populations, followed by Wan-Nan, while the Rugao is the most genetically distant among the populations.

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