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Research Paper

Investigation of Genetic Diversity in the Kashmir Faverolla and Red Jungle Fowl of chicken by using RAPD

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Abstract— In the present study, The Kashmir Faverolla and red jungle fowl chicken were compared for DNA band variations using the randomly amplified polymorphic DNA (RAPD) approach. Fifteen oligonucleotide primers, DNA polymorphisms between Kashmir Faverolla and red jungle fowl were found. These primers resulted in DNA band loss ranging from 36% to 60%. According to data, RAPD markers could detect DNA variants.

Keywords— Kashmir Faverolla, Red Jungle Fowl, RAPD, DNA

1. Introduction

One of the most prevalent domesticated bird species in the world is the chicken Gallus, "*gallus domesticus*". They are primarily responsible for this flexibility of transportation through portability, movement (migration) of people, trading in markets, and development in agronomic methods [1,2], mainly because of their usage, since there are no religious or cultural restrictions on nutrition reservations. Asian civilizations domesticated chickens was recorded by 5400 BC and possibly as early as 8000 BC [3,4]. Recently, research has been conducted on the chicken diaspora, which is of great importance to production. Depending on their existence and habitat dominance, they can attract considerable attention. The Indigenous breeds are more valuable due to their regional diversity. A broad spectrum of Indigenous species is near to extinct due to human negligence. In high altitude areas, especially in Jammu and Kashmir, an important native breed of backyard-raised Kashmir poultry called the Kashmir Faverolla is well adapted to the region's temperature swings and is predominantly raised for its meat and eggs [5]. Due to the introgression of domestic DNA through opportunistic mattings with domestic chickens, Red Jungle fowls (RJFs), the wild ancestor of contemporary chickens (DCs), are thought to be genetically endangered [6]. However, a genomic analysis of some domestic chickens' genomes revealed the existence of an autosomal DNA sections from the Grey Jungle fowl [7]. The domestic chicken exhibits a wide range of phenotypic variations, but the domesticated Red Jungle Fowl lacks traits that are as a result of domestication, such as colour of plumage and extra morphological traits, behavioural traits, and production traits, as well as traits that allow for adaptation to various ecosystems and fixed human selection for both production and aesthetic qualities [8, 9].

The variation of alleles and genotypes within the genome is referred to as genetic diversity [1]. Common molecular markers used to assess genetic diversity in domestic livestock include RAPD, ISSR, SCAR, Genomic mitochondrial Deoxyribonuclease sequences (DNA), single-nucleotide polymorphisms (SNPs) and Simple Sequence repeats (SSRs) found at commercial levels, and full meta-genomic sequences. Williams *et al.*, 1990 established the technique for DNA analysis which is known as random amplified polymorphic DNA (RAPD) [10] which is used for assessing genetic deviation and relatedness within and between species. Random amplified DNA fragments (RAPD) are among one the most widely used tool for estimating biodiversity, according to Weigend and Romanov (2001) [11]. RAPD is a considered technique of genome analysis that, in addition to genotype identification, also makes estimations of genetic diversity and affinity between them. It is evidence that it is polymorphic. Some authors suggested, the most novel technique RAPD for establishment of phylogenetic relationships and diversity in chicken [12, 13, 14].

DNA markers can be amplified randomly using short random oligonucleotide primers and separated by gel electrophoresis following PCR (Welsh and McClelland (1990) [15]. Because it is an easy and quick way to identify genetic diversity and similarities among species, it has been employed notably for genetic and molecular studies. **Sharma *et al.* (2001) [16]** reported on the efficacy of random amplified polymorphic

DNA in identifying polymorphism among chicken populations, their relevance in population genetic studies, and their potential to establish genetic connections among chicken populations.

In this recent study, we collected invasive blood samples by legal approach to increase their productivity, cultural influence, and to prevent genetic erosion. It is worth mentioning that this technique is used to distinguish between the genetic resources of economically significant farms and polities. In addition to genomic mapping, RAPD is employed to determine specific markers to determine genetic relatedness between different breeds. We strongly emphasise RAPD-PCR examination to identify band differences between RJFs and Kashmir Faverolla based on RAPD markers.

2. Materials and Methods

(2a) Bird sample collection

For present investigation, Total number of eight individuals from two chicken breeds was examined. Individuals included four local Kashmir Faverolla and four Red Jungle Fowl blood samples were collected from different regions of Kashmir valley to understand their genetic traits. (Included geostationary map location).

Figure 1a: Location where RJF's samples are collected. [Google Maps]

Figure 1b: Photograph of RJF's in the morning and evening time for the searching of food. The area which is marked by the red has the maximum probability of having Red Jungle fowl. This possibility is due to the maximum availability of food. [From website Birds of the world]

Figure 1c: Location where Kashmir Faverolla samples are collected. [Google Maps]

Figure 1d: Photograph of Kashmir Faverolla in the Remote area of Kulgam District. The region which is enclosed in red, from where we collected the Kashmir Faverolla. The people of remote areas domesticate them for the purpose of income.

3. Blood sampling and PCR amplification procedures

The Sharma et al. (2000) [17] approach was used to extract DNA, and it was done as follows. Samples of venous blood were combined with the anticoagulant EDTA and kept at -20°C. 700 ml of lysis buffer and 60 g of proteinase K were added to an aliquot of 50 ml blood after it had been thawed. After vortexing, the mixture was left to sit at 37°C for the night. Equal amounts of phenol-chloroform (1:1) and chloroform-isoamyl alcohol were used to extract the DNA (24:1). Adding 0•1 volume of 3 M sodium acetate and 2 volumes of cold ethanol, DNA was precipitated. The pellet was air-dried, wash away with 70% ethanol, and then dissolved in TBE buffer. The DNA concentration and relative purity were calculated via a spectrophotometer at 260 and 280 nm absorbance, respectively.

4. Analysis of random amplified polymorphic DNA (RAPD)

The following random primers were applied to random amplified polymorphic DNA (RAPD) are well illustrated in Table 1.

The 25 L polymerase chain reaction mixture contained 40 µg of genomic DNA, 25 pmolar dNTPs, 0.8 units of Taq DNA polymerase, and 0.8 Units of random primer. A DNA thermal cycler was used to put the final reaction mixture (Perkin Elmer 9700). The PCR procedure consisted of a first stage of DNA denaturation at 94°C for 2 min, followed by 45 cycles of DNA denaturation at 94°C for 30 seconds, an annealing at 36°C for 30 seconds, using all 15 primers, extension at 72°C for 30 seconds, and a final extension at 72°C for 10 min. On a 2.5% agarose gel, the amplified DNA fragments were separated, and ethidium bromide was used to dye them. On a UV trans-illuminator, the enhanced pattern was seen and photographed.

Table 1: Selected random primers with nucleotide sequences of chicken with standard GC content.

Primer	Sequence 5'- 3'	GC Content
1	AAA GCT GCG C	60%
2	ACC GCC GAA G	70%
3	AGG CCT CGC C	80%
4	AGT CAG CCA C	60%
5	GGA TGC GAC G	70%
6	CTG GGA AGT G	60%
7	ATG ACG TTG G	50%
8	GGT GCC GCA G	80%
9	ACC GGG AAG C	70%
10	GAA CGG ACT C	60%
11	AGG CCC CTG T	70%
12	ATG CCC CTG T	60%
13	CGC TGT CGC C	80%
14	GGC ACT GAG G	70%
15	AGC CAG CGA A	60%

5. Results and Discussion

Table 2: The aggregate number of bands, polymorphic bands, polymorphism and PIC between RJFs and Kashmir Faverolla using dissimilar random RAPD primers.

Primer	Total Bands	Polymorphic Bands	Polymorphic %	PIC
1	7	0	0	0
2	6	4	66.6	0.16
3	5	5	100	0.1
4	4	1	25	0.6
5	10	4	40	0.48
6	8	4	50	0.32
7	10	2	20	0.36
8	9	7	77.7	0.8
9	13	5	38.46	0.16
10	6	2	33.3	0.18
11	7	1	14.28	0.16
12	8	4	50	0.32
13	3	2	66.6	0.04
14	11	1	9.09	0.2
15	7	0	0	0

The Calculation of polymorphism % was calculated by the formula given below:

The polymorphism percentage is equal to the number of polymorphic bands divided by total number of bands and multiply by Hundred.

Polymorphism percentage (%) = (Number of

Polymorphic band/Total number of band) ×100.

The formula was used to estimate the polymorphic information content (PIC) of each marker:

$$PIC = 2fi (1 - fi)$$

Where fi is the frequency of the amplified allele (i.e., the present band), and (1 - fi) denotes frequency of the null allele (i.e., the absent band) [18].

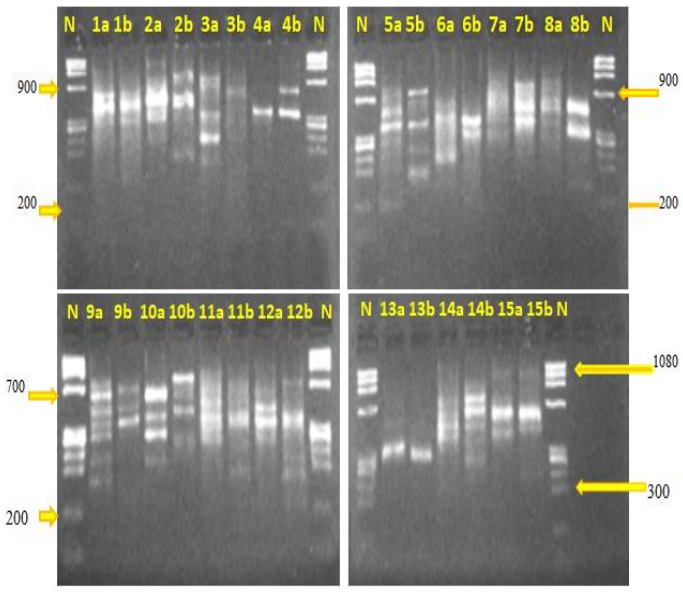

Figure 2: Different random primers (1a to 15b) generate amplification of RAPD products. Lane N: DNA marker (base pair N=φX174). The two lanes of each primer are: RJFs (1a-15a) and Kashmir Faverolla (1b-15b). The RAPD primers band ranges from 200bp to 1080bp.

From above data in Table 2 and Figure 2 by which we find, the aggregate number of bands, the number of polymorphic bands, and the proportion of polymorphism among Kashmir Faverolla and RJFs via several arbitrary primers. With the exception of two primers, all fifteen produced polymorphic bands (1a and 15b). A 9.09 to 100% polymorphism range was observed. For all the RAPD primers, total amplified bands are 114 out of which 42 bands show high polymorphic in nature. For RAPD primers, the observed Amplified PCR output size distribution was >200-1080bp. The polymorphism ratio in the RAPD study was 36.8%. Additionally, the PIC (polymorphic information content) value was obtained to evaluate each RAPD primer marker's capacity to reveal polymorphic loci

for such a sample, and this is displayed in Table 2. Among all the primers, primer no.8 shows the maximum PIC value of 0.80, while primer no.13 shows the lowermost PIC value of 0.04. The maximum amplified bands are obtained by primer no.8, where 7 bands are polymorphic nature.

The examination and explanation of banding patterns of genetic markers in poultry were done using DNA profiling. By comparing the banding patterns of several species, it was possible to identify the inherits of band, which were genetic traits that were constant over time. The degree of gene pool was assessed using a DNA fingerprinting approach (Kuhnlein et al., 1990) [19] , a result similar to that of the Manchurian pheasant (P. colchicus) population in the area when RAPD was used (P = 79.4%) [20]; also, wild Iran and captive Azerbaijan Pheasant populations (P = 86.70%) [21]. Muhammad et al. (2010) did find a percentage of polymorphic loci for Prinia (Prinia burnesii) ranging from 48.11 to 93.07[22].

6. Conclusion

Utilized to create phylogenetic trees between various chicken breeds and estimate degrees of variety and diversity, RAPD markers are sufficiently effective. The conclusions of the RAPD analysis conducted in this study were useful in determining the level of genetic polymorphism present in the RJF and Kashmir Faverolla populations. High priority approach and a strategy should be established to ensure the protection of RJFs and Kashmir Faverolla in general by avoiding the selective eradication of males from populations for the improvement of commercial breeds, which may further erode the breeds' genetic diversity.

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References

[1] D.Wragg, JM Mwacharo, JA Alcalde, PM Hocking, O Hanotte. Analysis of genome-wide structure, diversity and fine mapping of Mendelian traits in traditional and village chickens. *Heredity (Edinb).*; Vol.**109** pp**.6–18, 2012**.

[2] J. M Mwacharo, G.Bjørnstad , V. Mobegi , K.Nomura, H. Hanada, T.Amano, & Hanotte, Mitochondrial DNA reveals multiple introductions of domestic chicken in East Africa. *Molecular phylogenetics and evolution*, vol**.58**, Issue **2**, pp.**374-382, 2011.**

[3] D. M Fitzpatrick, & K. Ahmed, Red roving fowl. “Down Earth” 9, 28 (2000).

[4] R. D.Crawford, (ed.) Poultry Breeding and Genetics (Elsevier, Amsterdam, 1995).

[5] Henna Wani, Mohammad Maqbool Darzi, Shayaib Ahmad Kamil, Shakil Ahmad Wani, Ziaul Hassan Munshi, Abdul Shakoor, Tariq Ahmad Raja, Shabu Shoukat, Bisma Kashani and Aazima ShahHistological and histochemical studies on the reproductive tract of Kashmir faverolla *chicken Journal of Entomology and Zoology Studies*.Vol**. 5**, Issue **.6** pp.**2256-2262, 2017**.

[6] M.Thakur, M. Fernandes, S. Sathya Kumar, S. K.Singh, R.K.Vijh, J.Han, & Y. P. Zhang, Understanding the cryptic introgression and mixed ancestry of Red Junglefowl in India. *PLoS One*, Vol**.13**, Issue.**10**, **2018**.

[7] J.Eriksson, G.Larson, U.Gunnarsson, B.Bed'Hom, Tixier-Boichard, Strömstedt, & L.Andersson, Identification of the yellow skin gene reveals a hybrid origin of the domestic chicken. *PLoS genetics*, Vol**.4**, Issue.**2, 2008**.

[8] K. E. Schütz, B.Forkman, & P.Jensen, Domestication effects on foraging strategy, social behaviour and different fear responses: a comparison between the red junglefowl (Gallus gallus) and a modern layer strain. *Applied animal behaviour science*, Vol.**74**, Issue.**1,** pp**.1-14, 2001.**

[9] M.Tixier-Boichard, Bed’hom, B., & Rognon, X. Chicken domestication: from archeology to genomics. *Comptes rendus biologies*, Vol.**334**, Issue**.3**, pp.**197-20,2011**.

[10] J. G.Williams, A. R. Kubelik, K. J. Livak, J. A. Rafalski, & S.V. Tingey, DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic acids research*, Vol.**18**, Issue. **22,** pp.**6531-6535, 1990**.

[11] S.Weigend, & M.N.Romanov, Current strategies for the assessment and evaluation of genetic diversity in chicken resources. *World's Poultry Science Journal*, Vol**.5**, Issue.**3**, pp.**275-288, 2001**.

[12] M B.R.Mollah, F. B. Islam, M. S.Islam, M. A.,Ali, & M. S. Alam, Analysis of genetic diversity in Bangladeshi chicken using RAPD markers. *Biotechnology*, Vol**.8**, Issue.**4,** pp.**462-467,2009**.

[13] T. S.Rabie, & A. M. Abdou, Genetic diversity and relationship among Egyptian indigenous chicken strains using random amplification of polymorphic DNA. *Egypt. Poult. Sci*, Vol.**30**, pp.**473-482**, **2010**.

[14] R.Al-Atiyat, Diversity of chicken populations in Jordan determined using discriminate analysis of performance traits. *Int. J. Agric.* Biol, Vol.11, pp.374-380,2009.

[15] J.Welsh, & M.McClelland, Fingerprinting genomes using PCR with arbitrary primers. *Nucleic acids research*, Vol.**18**, Issue.**24**, pp**.7213-7218,1990**.

[16] D.Sharma, K.B.C.Appa Rao, and S.M.Totey, Measurement of within and between population genetic variability in quails. *Br. Poult. Sci*. Vol.**41**, pp**.29– 32, 2000.**

[17] D.Sharma, K.B.C.Appa Rao, R.V.Singh, and S.M. Totey, Genetic diversity among chicken breeds estimated through randomly amplified polymorphic DNA. Anim. Biotechnol. Vol.**12,** pp**.111-120,2001.**

[18] M.Lynch,"The similarity index and DNA fingerprinting". *Mol. Biol. Evol*., Vol.**7**, pp.**478-484**,**1990.**

[19] U. Kuhnlein, D. Zadworny, Y. Dawe, R. W. Fairfull, & J. S. Gavora Assessment of inbreeding by DNA fingerprinting: development of a calibration curve using defined strains of chickens. *Genetics*, Vol.**125**, Issue.**1**, pp.**161-165,1990**.

[20] I.V. Kulikova, G. N. Chelomina, & Y. N. Zhuravlev, RAPD–PCR Analysis of Genetic Diversity in the Manchurian Pheasant. *Russian Journal of Genetics*, Vol.**38**, pp**.699-703, 2002**.

[21] G. Elyasi Zarringhabaie , A. Javanmard, & O. Pirahary, Random amplified polymorphic markers as indicator for genetic conservation program in Iranian pheasant (Phasianus colchicus). *The Scientific World Journal***, 2012**.

[22] S.Muhammad, A. A.Khan, M. Babar, M.Riaz, N.Akhtar, & I. Khaliq, Population genetic structure of Rufous-Vented Prinia (Prinia burnesii) in Pakistan. African. *Journal of Biotechnology*, Vol. **9**, no.**53**, pp.**9077-9081, 2010.**

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