Comparison of the Effectiveness of Decamer and Microsatellite Markers with Chicken Populations in Ogun and Ondo States, Nigeria

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Abstract
For the purpose of comparing the effectiveness of random amplified polymorphic deoxyribonucleic acid (DNA) (RAPD) and microsatellite markers (MS) with DNAs obtained from five chicken populations, a. total of eighty genomic DNAs with sixteen from each of Frizzle feathered (FF), Normal feathered (NF), Naked neck (NN), Bovan nera (BN) and Black harco (BH) chickens sampled in Ogun and Ondo States, Nigeria were subjected to 45 and 35 cycles of amplification in a polymerase chain reaction (PCR). PCR products obtained with four RAPD and four MS markers were subjected to agarose and polyacrylamide gel electrophoreses. Considering the RAPD markers, fragment sizes and their proportions were obtained, used to compute band-sharing coefficient and genetic distances (GD). Using the MS with DNAs, allele frequencies were generated with Microsatellite Analyzer version 4.05 and used to compute GD among populations. With RAPD markers, GD obtained ranged from 0.3249 (FF vs. NF) to 0.4180 (FF vs. BN); while that of MS markers ranged from 0.3224 (BN vs. BH) to 0.7294 (FF vs. NN). The MS revealed abundant genetic information. Phylogenetic analysis based on MS showed a more accurate cluster than RAPD, demonstrating the superiority of MS to RAPD markers in examining genetic background of chicken populations.

Keywords: genetic distances; genetic information; phylogenetic study; relationship

1.0 Introduction

There is an increase in the demand for poultry products all over the world, poultry breeders are more interested in maintaining genetic variation within and between commercial lines and exotic populations. The development of DNA-based marker has had a revolutionary impact in gene mapping and more generally on all animal and plant genetics. In present day genetics, DNA markers are widely used for genome mapping, identification of genes controlling commercial traits and analysis of long-term selection and inbreeding (Darvasi, 1998; Heyen *et al.,* 1999). Advances in techniques for PCR-based analysis have greatly increased the ability to understand the genetic relationships among species at molecular level. These DNA molecular techniques have been more efficient in demonstrating genetic diversity, since they detect the variability of DNA in both the coding (suspected) and non-coding regions (unsuspected open reading frames). Several PCR-based techniques have been developed. Some of these are the DNA fingerprinting, arbitrary primed PCR (Welsh and McClelland, 1990), and random amplified polymorphic DNA (RAPD) (Williams *et al.,* 1990). In birds, RAPD and microsatellite markers have been employed for several purposes such as evaluating genetic variation within and among populations (Smith *et al.,* 1996; Padilla *et al.,* 2000), identification of geographic populations of migratory species (Haig *et al.,* 1997), distinguishing segregation of alleles in cross between inbred lines (Wei *et al.,* 1997), sex determination (Lasselles and Mateman, 1998), identification of quinolone-resistant *Escherichia coli* isolated from chickens (Warren *et al.,* 2008) and evaluating genetic diversity (Pawel *et al.,* 2009). These markers can, in addition, be used to generate data suitable for estimation of cumulative power of discrimination of any population including the avian species (Olowofeso, 2005).

With DNA-based marker, it is possible to exploit the entire diversity in DNA sequence that exists in the genome. This study therefore aimed at comparing the effectiveness of RAPD or decamer and microsatellite markers with DNAs isolated from five chicken populations in Ogun and Ondo States, Nigeria.

2.0 Material and Methods

2.1 Animals and markers

A total of eighty blood samples were collected in Ogun and Ondo States, both in South-West Nigeria, with sixteen from each of the chicken population (that is Frizzle feathered, FF; Normal feathered, NF; Naked neck, NN; Bovan nera, BN and Black harco, BH), from which DNAs were isolated and used for this study. The first three chicken populations have previously been described by Adeleke *et al.* (2011) and phenotypic features as well as the performance characteristics of the other two chicken populations have been reported by Olowofeso *et al.* (2011). Four RAPD markers reported in literature for chickens and same number of MS markers was used with the DNAs. The MS markers were selected from the International Society for Animal Genetics-Food and Agriculture Organization list recommended for chickens and also taken into cognisance the recommendation of Baumung *et al.* (2004) that more markers be used with animal genetic resources including chickens. The code, sequence and molecular weight of these markers are presented in Tables 1 and 2, respectively.

2.2 Blood collection, polymerase chain reaction and electrophoresis

Approximately 2 ml of blood were collected from the wing veins of individual bird using disposable syringe into 10 ml haemotocrit tube containing ethylene diamine tetra acetic acid used as an anticoagulant. The collected blood was transferred to the laboratory for the isolation of DNA. DNA was isolated from the chicken blood using Spin-Column protocol. DNA was quantified using ultraviolet spectrophotometer (Spectronic^{R} Genesis^{TM}) based on the absorbance at 260 and 280 nm respectively, and quality was checked by electrophoresis in a mini gel. Analysis was carried out at the Biotechnology Centre, Federal University of

Agriculture, Abeokuta, Nigeria. The RAPD-Polymerase reaction (PCR) mixture were made up of 2.50 µl of 10 x PCR buffer, 2.20 µl of 25 mm/mol Mg^{2+}, 1.00 µl of dNTPs (25 mM dNTPs), 1.50 µl of each RAPD marker, 0.20 µl *Taq* DNA polymerase (5 U/µl), 16.60 double distilled water (*dd*H_2O) and 1.00 µl each of template DNA. Amplification was carried out in a thermal cycler for 45 cycles with the following reactions: initial denaturation at 94℃ for 3 minutes, denaturation at 94℃ for 1 minute, annealing at 34.7℃ to 41.7℃ for 1 minute, extension at 72℃ for 1 minute and final extension at 72℃ for 7 minutes. The MS-PCR conditions and programme used with total volume of 25.00 µl contained 1.00 µl template DNA, 2.50 µl of 10 x PCR buffer, 1.00 µl of 25 mM dNTPs, 2.00 µl (1.00 µl forward and 1.00 µl reverse form) of each pair of the marker, 0.20 µl of (5 U/µl) *Taq* DNA polymerase, 2.20 µl of 25 mm/mol Mg^{2+} and 16.10 µl *dd*H_2O. Amplification was carried out in a thermal cycler for 35 cycles and the reaction conditions were as follows: initial denaturation at 94℃ for 5 minutes, denaturation at 94℃ for 1 minute, annealing temperature ranged from 46℃ to 55℃ (Table 2) for 1 minute, extension at 72℃ for 1 minute and final extension at 72℃ for 10 minutes.

The RAPD and MS fragments amplified by the PCR were separated by gel electrophoresis using 1% agarose gel with a drop of 0.5 µl ethidium bromide used as intercalating agent. Running of the gels were done in Submarine Agarose Chamber containing 20 x 20 cm tray. The gadget was connected to direct current source and lasted for 90 minutes at 100 V and 20 mA with 0.50 x TBE used as running medum, while 1 µl 100 basepair (bp) ladder DNA marker was used in the same gel to estimate the size of the amplicons. For MS, buffer used to run the gel was 1.00 x Tris-boric- ethylene diamine tetra acetic acid (TBE), while 1 µl of *pBR*322DNA/Mspl was used in the same gel as ladder to determine the fragment size. Following electrophoreses, gels were visualized under ultraviolet trans-illuminator and RAPD and MS profiles were examined. Portions of results generated are presented in Plates 1 and 2, respectively.

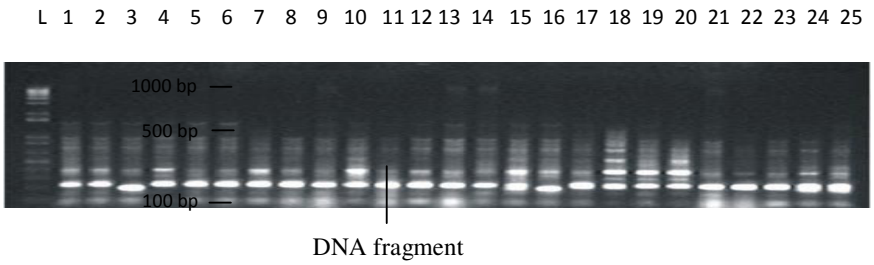

Plate 1: Portion of the agarose gel electrophoresis result of twenty five sample DNAs with RAPD marker OPG 16 and L is 100 bp ladder DNA marker.

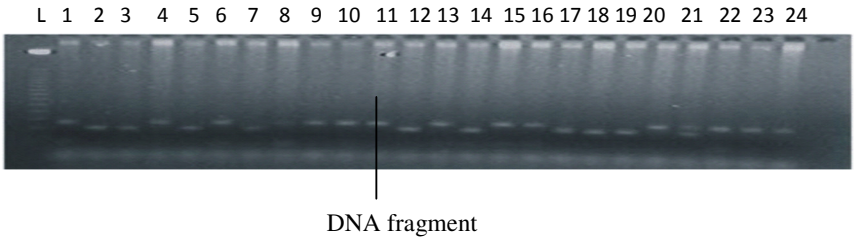

Plate 2: Portion of the agarose gel electrophoresis result of twenty four sample DNAs with MS marker ADL 171 and L is *pBR*322DNA/Mspl.

2.3 Scoring of fragments and analyses

The fragment sizes were scored manually based on band absence (0) and presence (1), band-sharing coefficient (BSC), average frequency of band (q), and heterozygosity of each marker and between pairwise populations were estimated using the formula suggested by Smith *et al.* (1996), considering the four RAPD markers. With MS markers, data were analysed with Microsatellite Analyser (MSA) version 4.05 developed by Dieringer and Schlotterer (2003). Genetic information (i.e. allele frequencies, allele number, allele size range (bp), homozygosity and heterozygosity levels for each marker and each chicken population were computed). The observed (H_O) and expected (H_E) heterozygosities were obtained using MSA. Using Neighbor-Joining method, the phylogenetic trees were constructed for the five chicken populations.

3.0 Results and Discussion

Band-sharing coefficient of each marker, heterozygosity (H), and average frequency of band (q) generated considering all comparisons among individuals in the same chicken population and mean of these parameters for each population using the amplified bands are represented in Figure 1. The BSC ranged from 0.5239±0.11 (Normal feathered) to 0.7067±0.06 (Frizzle feathered). Using the estimated mean BSC, the average frequency of band, the heterozygosity for each chicken population estimated. The highest heterozygosity (0.8166) was observed in Normal feathered chicken population and the average frequency of band denoted as 'q' was determined in each population. The lowest (0.7072) was observed in Frizzle feathered chicken population. These indicate that the higher the band-sharing coefficient, the lower the heterozygosity and vice versa.

Using the same bands, the BSC, average frequency of band and heterozygosity among pairwise populations were estimated and it ranged from 0.5820±0.04 (Frizzle feathered vs. Bovan nera) to 0.6751±0.03 (Normal feathered vs. Black harco), 0.3535 (Frizzle feathered vs. Bovan nera) to 0.4300 (Normal feathered vs. Black harco), 0.7853 (Normal feathered vs. Black harco) to 0.7853 (Frizzle feathered vs. Bovan nera). Results generated are presented in Table 3. Total number of alleles observed across the five chicken populations was 84, the highest number of alleles recorded per population was 10 in Normal feathered chicken population produced by locus ADL 171 and lowest of 2 were observed in Frizzle feathered, Normal feathered and Naked neck by markers ADL 206 and MCW 150, respectively. Mean number of allele across markers ranged from 3.500±1.07 in Frizzle feathered chicken population to 5.00±1.73 in Normal feathered chicken population. However, ADL 171 produced the highest mean number with a value of 6.60 and lowest value of 3.20 was observed in MCW 150 and ADL 206, respectively (Table 4).

Variation in homozygosity, gene diversity, effective number of allele, observed and expected heterozygosities generated by markers across the chicken populations are presented in Table 4. Across markers, homozygosity ranged from 0.3160 (Black harco) to 0.4723 (Frizzle feathered) chicken; across populations, it ranged from 0.2640 (ADL 171) to 0.4667 (ADL 206). Highest value of homozygosity observed per population was 0.6894 (Frizzle feathered) with ADL 206, while the lowest was 0.2284 (Frizzle feathered) with ADL 171. Across markers, gene diversity ranged from 0.5190 (Naked neck) to 0.6840 (Black harco) while across populations, it ranged from 0.5914 (MCW 150) to 0.7360 (ADL 171). Across markers, effective number of alleles ranged from 2.2358 in Naked neck to 3.2821 in Normal feathered chicken population. Across populations it ranged from 2.3479 in MCW 88 to 4.1779 in ADL 171.

The highest value recorded per population was 6.8876 in Normal feathered and the lowest 0.2753 was observed in Naked neck. Observed heterozygosity across populations had mean, ranged from 0.1496 in MCW 88 to 0.6222 in ADL 171. For expected heterozygosity, it ranged from 0.5646 in ADL 206 to 0.7768 in ADL 171. Across markers observed heterozygosity ranged from 0.2848 in Frizzle feathered to 0.5449 in

Black harco chicken population and expected heterozygosity ranged from 0.5383 in Naked neck to 0.7323 in Bovan nera chicken population. However, Black harco had the highest value of observed heterozygosity of 0.9286 produced by ADL 171, while Normal feathered had the lowest value of 0.0833 generated by ADL 206. The expected heterozygosity had the highest value of 0.8862 in Normal feathered population with ADL 171 and the lowest value of 0.3730 was observed in Naked neck population with MCW 88. Expected heterozygosity across markers ranged from 0.5388±0.08 seen in Naked neck to 0.7323±0.00 obtained in Bovan nera. Polymorphism information content (PIC) across chicken populations ranged from 0.4720 in ADL 206 to 0.7004 in ADL 171 (Figure 2).

Table 5 shows the genetic distances among chicken populatons on the basis of RAPD and MS markers. For RAPD, smallest genetic distance was observed between Frizzle feathered and Naked neck, while the largest genetic distances was between Frizzle feathered and Bovan nera. For MS, Frizzle feathered and Bovan nera had the smallest genetic distances, while Frizzle feathered and Naked neck had the largest genetic distance. Using Neighbor-joining approach, phylogenetic trees based on the genetic distances among the chicken populations are represented in Figures 3 and 4, respectively. For RAPD, the cluster analysis shows that Frizzle feathered and Naked neck populations clustered together and independent from Normal feathered, Black harco and Bovan nera, respectively. For MS, the cluster analysis shows that Bovan nera and Black harco first clustered together, Frizzle feathered and Normal feathered also clustered together and independent from Naked neck.

The RAPD analysis revealed the existence of genetic diversity among chicken populations. In this work, the band-sharing coefficients were low in both wthin and among chicken populations, this may be the major reason responsible for the higher heterozygosities for both within and among populations which corroborated the earlier submissions of Chen *et al.* (2002) and Zhang *et al.* (2002). The high heterozygosity level obtained may be the result of low rates of selection pressure owing to lack of improvement programs and existence of several lineages.

The average allele number in populations across markers ranged from 3.50 or approximately 4.00 in both Frizzle feathered and Naked neck to 5.00 in Normal feathered chicken population. This is consistent with what was recommended (at least 4 alleles in population) for microsatellite markers to be used in the estimation of genetic diversity and distances by Wimmer *et al.* (2000); Hillel *et al.* (2003); Ohwojakpor *et al.* (2012). Thus, the four MS markers used in this study can be taken as been effective for genetic diversity studies in chicken populations reared in Ogun and Ondo States, Nigeria. Heterozygosity was a good measure of genetic diversity. Heterozygosity reflected the hereditary and mutation of markers in each group. The level of genetic diversities observed were similar to values reported by Chen *et al.* (2004) and Wu (2004), respectively.

Polymorphism information content provides an estimate of the discriminatory power of a locus by taking into account not only the number of alleles that are expressed, but also the relative frequency of those alleles (Smith *et al.,* 1997). Based on PIC values obtained, it means that the four microsatellite markers used were informative and hence can be employed in molecular characterization of chicken populations particularly those reared in Ogun and Ondo States, Nigeria. The effective number of alleles is the non-linear function of the expected heterozygosity in the population. It gives the idea about how wide the allele frequency is in a population. The effective number of alleles across markers ranged from 2.2358 in Naked neck to 3.2821 in Normal feathered chicken population. Similar results of these nature reported in literature were 3.09 in Assel, 3.39 in Miri, 3.15 in Nicobari (Pandey *et al.,* 2002); 2.91 in Ankaleswar (Pandey *et al.,* 2005) and 4.05 in Haimen chickens (Olowofeso *et al.,* 2005).

Phylogenetic analysis based on microsatellite markers showed a more accurate clustering of the five chicken populations sampled in Ogun and Ondo States, Nigeria than those based on RAPD markers. This

therefore confirmed that the MS marker provides abundant genetic information than RAPD marker when used with animal genetic resources.

Table 1. Random amplified polymorphic DNA markers used in this study with their sequences, annealing temperature and molecular weight

Primer	Sequence	Annealing temperature (^{0}C)	Molecular weight (g)
OPA 11	AAACGGGCGG	41.70	3,102.10
OPG 02	GGCACTGAGG	36.60	3,093.10
OPG 16	AGGGTCCTCC	38.80	2,964.00
OPM 06	CTGGGCAACT	34.70	3,028.00

Table 2. Microsatellite markers used in this study with their sequences, annealing temperature and molecular weight

Marker	Primer sequence (forward (F) and reverse (R) forms)	Annealing temperature (^{0}C)	Molecular weight (g)
ADL 171	F: ACAGGATTCTGAGATTTTT	46	6,420.20
	R: GGTCTTAGCAGTGTTTGTTT		6,330.20
ADL 206	F: TTTCTATCCTTCATCTCCAG	46	5,968.90
	R: AGACATCCTGCTTTCTCGTG		6,059.00
MCW 88	F: TTGCAAATGAACGTATCATGC	46	6,429.20
	R: TCCAATCCTAGAAGTCTTATG		6,380.20
MCW 150	F: TCCTGACTGAAATGGTACAGC	55	6,430.20
	R: CATGAAAACCTTTGCCCTCAG		6,350.20

Table 3. Band-sharing coefficient (BSC) on marker basis, their means, average frequency of band (q) and heterozygosity (H) among five chicken populations examined in Ogun and Ondo States, Nigeria using RAPD markers

Population	BSC of RAPD marker				$\overline{BSC}$±S.E.	q	H
	OPA 11	OPG 16	OPG 02	OPM 06			
Frizzle feathered vs. Naked neck	0.8485	0.5532	0.5952	0.6846	0.6704±0.06	0.4259	0.7294
Frizzle feathered vs. Bovan nera	0.5238	0.4946	0.6383	0.6712	0.5820±0.04	0.3535	0.7853
Frizzle feathered vs. Black harco	0.7083	0.5684	0.4750	0.6944	0.6115±0.05	0.3760	0.7679
Normal feathered vs. Naked neck	0.7097	0.3902	0.5926	0.7195	0.6030±0.07	0.3699	0.7731
Normal feathered vs. Bovan nera	0.6410	0.5185	0.5275	0.7453	0.6081±0.05	0.3740	0.7600
Normal feathered vs. Black harco	0.7333	0.6265	0.6234	0.7170	0.6751±0.03	0.4300	0.7261

Naked neck vs. Bovan nera	0.8353	0.6237	0.5333	0.6879	0.6201±0.03	0.3836	0.7627
Naked neck vs. Black harco	0.7423	0.5484	0.4419	0.7226	0.6138±0.06	0.3786	0.7665
Bovan nera vs. Black harco	0.6829	0.5958	0.5814	0.6447	0.6262±0.02	0.3886	0.7588

Table 4. Allele number, allele size range and frequencies, homozygosity, gene diversity, effective number of allele, observed and expected heterozygosities in five chicken populations in Ogun and Ondo States, Nigeria with four microsatellite markers*

Marker	Parameter	Population					X±SEM (across population per marker)
		Frizzle feathered	Normal feathered	Naked neck	Bovan nera	Black harco	
MCW 150	Allele number	2	2	3	6	3	3.2000±0.74
	Allele size range (bp)	146-148	146-148	145-150	145-155	146-150	
	Range of frequency	0.5000-0,5000	0.3333-0.6667	0.2500-0.4063	0.0333-0.2667	0.2188-0.5000	
	h_o	0.5000	0.5556	0.3458	0.2645	0.3770	0.4086±0.05
	h_i	0.5000	0.4444	0.6542	0.7355	0.6230	0.5914±0.05
	EN_A	2.0000	1.7999	2.8919	3.7807	2.6525	2.6250±0.35
	H_O	0.2000	0.1533	0.5000	0.6667	0.4375	0.3875±0.10
	H_E	0.5172	0.4598	0.6754	0.7881	0.6649	0.6211±0.06
MCW 88	Allele number	3	4	4	4	4	3.8000±0.02
	Allele size range (bp)	196-200	198-204	198-204	196-202	196-202	
	Range of frequency	0.0313-0.5000	0.0938-0.6250	0.0357-0.7857	0.0313-0.3438	0.1250-0.4063	
	h_o	0.4708	0.4395	0.6403	0.3448	0.3145	0.4500±0.05
	h_i	0.5292	0.5605	0.3597	0.6152	0.6855	0.5500±0.06
	EN_A	2.1241	0.2753	1.5618	2.5988	3.7197	2.3479±0.27
	H_O	0.3750	0.6250	0.2857	0.3125	0.5000	0.1496±0.06
	H_E	0.5464	0.5786	0.3730	0.6563	0.7313	0.5771±0.06
ADL 206	Allele number	2	4	2	4	4	3.200±0.49
	Allele size range (bp)	184-186	184-202	202-204	184-204	184-204	
	Range of frequency	0.1923-0.8077	0.0417-0.6250	0.3077-0.6923	0.0769-0.4615	0.1250-0.3125	
	h_o	0.6894	0.4618	0.5740	0.3373	0.2715	0.4667±0.08

	h_i	0.3106	0.5382	0.4260	0.6627	0.7285	0.5332±0.08
	EN_A	1.4503	2.1654	1.7422	2.9647	3.6832	2.4012±0.41
	H_O	0.2308	0.0833	0.3077	0.1539	0.3125	0.2176±0.03
	H_E	0.3231	0.5616	0.4431	0.7180	0.7771	0.5646±0.08
ADL 171	Allele number	7	10	5	6	5	6.6000±0.93
	Allele size range (bp)	194-204	180-204	186-192	192-204	196-204	
	Range of frequency	0.1111-0.3333	0.0357-0.2500	0.0385-0.5385	0.0313-0.4375	0.0357-0.4643	
	h_o	0.2284	0.1454	0.3640	0.2813	0.3011	0.2640±0.04
	h_i	0.7716	0.8546	0.6360	0.7187	0.6989	0.7360±0.04
	EN_A	4.3783	6.8876	2.7473	3.5549	3.3212	4.1779±0.73
	H_O	0.3333	0.7143	0.3846	0.7500	0.9286	0.6222±0.11
	H_E	0.8170	0.8862	0.6615	0.7667	0.7528	0.7768±0.04
$\bar{X}$±SEM Across marker/ population	MNA	3.5000±1.07	5.0000±1.73	3.5000±0.65	4.0000±0.58	4.0000±0.41	
	h_o	0.4723±0.10	0.4006±0.09	0.4810±0.07	0.3170±0.03	4.3160±0.02	
	h_i	0.5279±0.10	0.5994±0.09	0.5190±0.07	0.6830±0.02	0.6840±0.02	
	EN_A	2.4882±0.65	3.2821±1.21	2.2358±0.34	3.2248±0.27	3.2092±0.21	
	H_O	0.2848±0.04	0.3890±0.16	0.3695±0.05	0.4708±0.14	0.5447±0.13	
	H_E	0.5509±0.10	0.6216±0.09	0.5388±0.08	0.7323±0.03	0.7315±0.02	

* Homozygosity (h_o), gene diversity (h_i), effective number of allele (EN_A), mean number of allele (MNA), observed (H_O) and expected (H_E) heterozygosities.

Table 5. Genetic distances among five chicken populations in Ogun and Ondo States, Nigeria based on four RAPD markers (above diagonal) and based on four microsatellite markers (below diagonal)

Population	Frizzle feathered	Normal feathered	Naked neck	Bovan nera	Black harco
Frizzle feathered	0.0000	0.3595	0.3296	0.4180	0.3885
Normal feathered	0.4624	0.0000	0.3970	0.3919	0.3249
Naked neck	0.7294	0.4913	0.0000	0.3799	0.3862
Bovan nera	0.3695	0.4826	0.5689	0.0000	0.3738
Black harco	0.4231	0.4239	0.5132	0.3324	0.0000

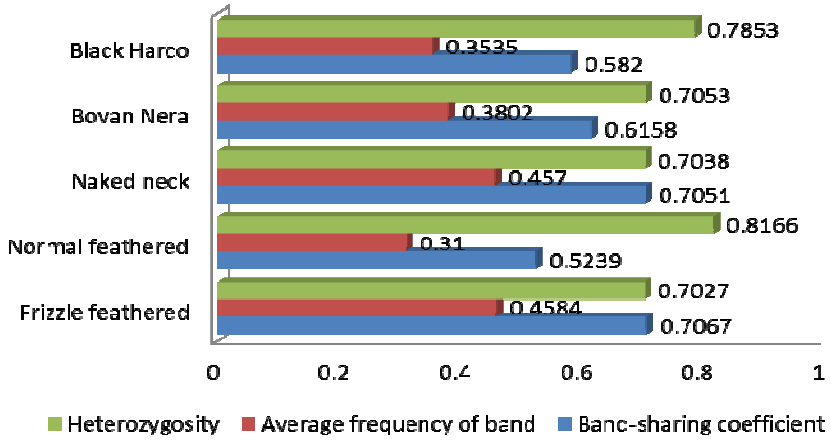

Figure 1: Band-sharing coefficient of each marker, average frequency of band and heterozygosity in the five chicken populations examined in Ogun and Ondo States, Nigeria with RAPD markers.

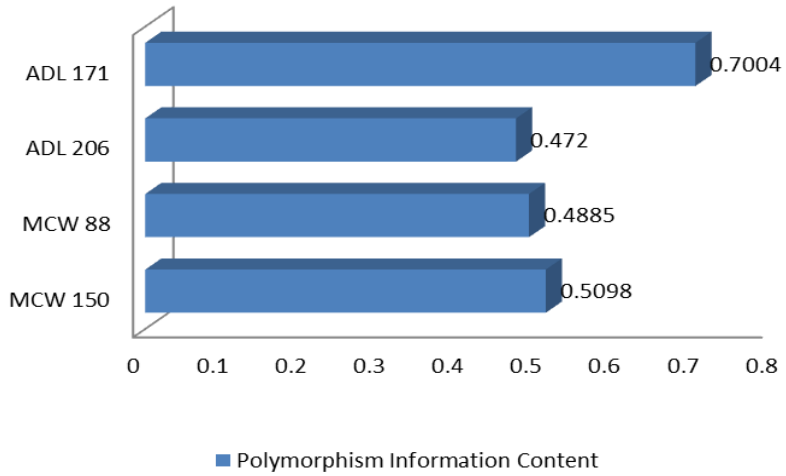

Figure 2: Polymorphism information content of four microsatellite markers used with five chicken populations in Ogun and Ondo States, Nigeria.

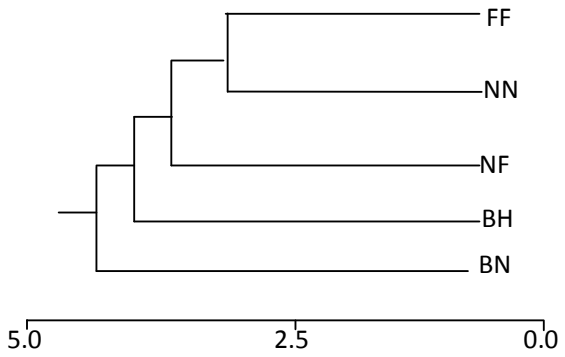

Figure 3: Phylogenetic tree based on the genetic distances among five chicken populations in Ogun and Ondo States, Nigeria with four RAPD markers (abbreviations are as defined within text).

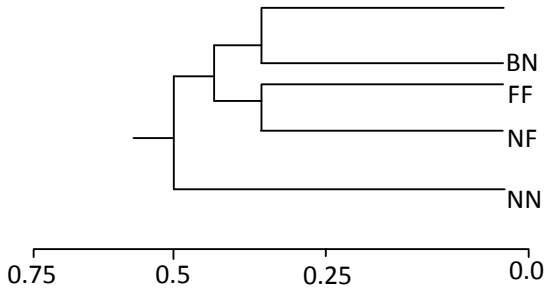

Figure 4: Phylogenetic tree based on the genetic distances among five chicken populations in Ogun and Ondo States, Nigeria with four MS markers (abbreviations are as defined within text).

4.0 Conclusion

The study revealed that there was moderate genetic diversity in the five chicken populations. Findings of this work with regards to measures of genetic diversity and population relationships of the chickens will provide excellent baseline information for further genetic improvement. The study pinpointed Normal feathered chicken population with the highest mean number of alleles. This work combined with previous molecular studies with chicken populations will ultimately lead to meta-analysis of indigenous chicken genetic diversity in Nigeria using RAPD/decamer and microsatellite markers. Based on the PIC values and other genetic information provided by the four MS markers, we concluded that the markers were informative, superior to RAPD markers and can be used for genetic characterization of chicken populations at all times.

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5.0 References

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