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Relationship between Measures of Genetic Diversity and Effective Population Size in Three Nigerian Chicken Populations Based on Microsatellite Markers

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Abstract
Microsatellites were used with three Nigerian chicken populations with objectives to determine measures of genetic diversity (mean number of allele/marker ($\overline{N}_A$) and mean expected heterozygosity ($\overline{H}_e$)), effective population size (N_e) of each chicken population based on infinite allele and stepwise mutation models (IAM/SMM) of microsatellite and to know how correlated measures of genetic diversity and their relationship with logarithm N_e. For these, eleven microsatellite markers were used with 80 genomic DNA isolated from Naked neck (*NN = 18*), Normal feathered (*NF = 35*) and Frizzle feathered (*FF = 27*) chickens. The DNA, microsatellite markers, Master Mix and double distilled water ($dd H_20$) which formed the polymerase chain reaction mixture were amplified and products electrophoresed on 12% polyacrylamide gel. Fragments in basepairs were obtained by comparing with ladder used. Data were analysed with Microsatellite Analyser version 4.05. Results were 7.00±0.82 to 9.55±0.66 and 0.76 to 0.84 for $\overline{N}_A$ and $\overline{H}_e$ in NN and NF. Logarithm N_e ranged from 3.95 to 4.18, and 4.43 to 4.75 based on IAM and SMM in NN and NF, respectively. Analysis revealed that measures of genetic diversity and logarithm N_e were positively correlated, values ranged from 0.43 to 0.96. The study pointed out a noteworthy genetic diversity, its relationship with logarithm N_e and suggested some linear models for predicting N_e.
Keywords: Chickens, Genetic information, Heterozygosity, Markers, Measures, Models

1. Introduction

Effective population size and measures of genetic diversity are important parameters that are essential for the designing of breeding programmes which will maximise the variations in populations in successive generations. Effective population size is the number of individuals in an idealised population, while genetic diversity is the total number of genetic characteristics in the genetic makeup of a species. Genetic diversity study in domestic animals is mainly for evaluating genetic variation within and among breeds and or strains for conservation purposes. Genetic diversity serves as a way for populations to adapt to changing environments. It is the fuel in breeding works and as such an important asset, giving breeders the opportunity to improve current traits or to develop new characteristics according to Talle *et al.* (2005) and Ásbjarnardóttir *et al.* (2010).

Modern molecular techniques provide the opportunity to study and obtain measures of genetic diversity within populations (i.e. mean number of alleles/marker, observed and expected heterozygosities) and among populations (i.e. genetic differentiation or fixation indices) and to identify population-specific alleles for breed characterization (Hillel *et al.,* 2003). Genetic markers particularly microsatellites are relatively robust and unaffected by environmental variation. They serves as molecular tools for exploring genetic diversity and information on genetic diversity is very germane in optimizing conservation and utilization strategies in animal genetic resources. Microsatellite markers have been very useful in product validation, pedigree control and reconstruction; predicting hybrid vigour, provide information on the whole genome, proper assignment of individual to a specific breed (Tolene *et al.,* 2012), measuring diversity, parentage analysis, evaluating degree of relatedness of individuals or groups, evaluate inbreeding levels and to look for evidence of population bottlenecks (severe or temporary reduction in population number as a result of human actions) and can be used to discover origins, connectivity and demography of populations (Girard *et al.,* 2010).

Microsatellite markers have also been used to better understand the genetic background and to estimate with ease the effective population size of animal populations even in the absence of population history (Rosenberg *et al.,* 2001; White and Searle, 2007; Berthouly *et al.,* 2008; Olowofeso, 2008; Granevitze *et al.,* 2009; Ásbjarnardóttir *et al.*, 2010; Groeneveld *et al.,* 2010; Tolene *et al.,* 2012). Genetic diversity of a population can be assessed by both the mean number of alleles per locus and mean expected heterozygosity. When the within-species genetic diversity or the coalescence time is divided by the microsatellite mutation rate, the parameter called effective population size can be generated which represent the number of individual in an ideal population with the same decrease in heterozygosity due to genetic drift (Hartl and Clark, 1989).

Using microsatellite markers with animal genetic resources (Vanhala *et al.,* 1998; Lemus-Flores *et al.,*

2001; Tolene *et al.,* 2012) have assessed the mean number of alleles per locus and the mean expected heterozygosity and use of these markers with animal genetic resources with the aim of estimating effective population size have been reported by Frankham (1996); Lehmann *et al.* (1998); Piry *et al.* (1999); Askt *et al.* (2002) and Ásbjarnardóttir *et al.* (2010). However, information on the relationship between measures of genetic diversity and effective population size in the large chicken populations in Nigeria has not been reported, which therefore ginger this study. In the study reported herein, DNA were isolated from three Nigerian chicken populations: Naked neck, Normal and Frizzle feathered chickens. The chicken populations were abundant and represent important genetic resources in Nigeria. The phenotypic features of these chicken populations have earlier been reported by Adeleke *et al.* (2011). DNA isolated from the chicken populations were amplified with eleven microsatellite markers which were selected based on their high polymorphism information content and high heterozygosity values reported by Hillel *et al.* (2003, 2007); Granevitze *et al.* (2007) and Mwacharo *et al.* (2013). The objectives of this study were to use microsatellite markers with three Nigerian chicken populations to determine measures of genetic diversity, effective population size based on two models of microsatellite evolution and to know how correlated is the measures of genetic diversity with effective population size.

2. Materials and Methods

2.1 Sampling Locations and DNA Isolation

Chicken blood samples used in this study were obtained from the South-South geo-political zone of Nigeria. The locations, marked A, B, C, D and E where chickens were sampled were as indicated in Figure 1. DNA used in this study were obtained from the 80 blood samples collected from three Nigerian chicken populations: Naked neck (*NN = 18*), Normal feathered (*NF = 35*) and Frizzle feathered (*FF = 27*) using the method described by Ohwojakpor *et al.* (2012). DNA were quality tested and their optical densities (OD) determined using ultraviolet (UV) spectrophotometer. Samples with OD values which ranged from 1.70 to 1.90 were selected for polymerase chain reaction (PCR).

2.2 Microsatellite Markers Used, PCR Mixture and Conditions

Eleven microsatellite markers used were selected from the International Society for Animal Genetics-Food and Agriculture Organization list of microsatellite markers recommended for biodiversity studies in chickens and further consideration was based on the high polymorphisms of the markers reported in literature (Hillel *et al.,* 2003, 2007; Nahashon *et al.,* 2008; Mwacharo *et al.,* 2013). The code, sequence and annealing temperatures of markers used are shown in Table 1. PCR reactions were performed using the following components (i) Master Mix made up of 10 x PCR buffer, dinucleotide triphosphate, Magnesium chloride and *Taq* DNA polymerase, (ii) Primer (forward and reverse forms), (iii) Double distilled water (*dd*H_2O) and (iv) Template DNA. Each PCR reaction comprised 2 µl Master Mix, 0.6 µl primer (0.3 µl forward and 0.3 µl reverse), 1.50 µl template DNA and 5.90 µl *dd*H_2O made up to a total volume of 10 µl in each PCR tube. Reaction profile was set as follows: initial denaturation at 94°C for 300 seconds, 35 cycles of denaturation at 94°C for 60 seconds, annealing temperatures of the eleven microsatellite markers after optimization ranged from 56 to 62^0C (Table 1), extension at 72°C for 60 seconds and final extension was at 72°C for 600 seconds. A pair of each primer (i.e. the microsatellite marker), DNA and other PCR mixture were amplified separately using the afore-stated reaction. PCR products were heat-denatured for 300 seconds at 94^0C in the thermal cycler and cooled on ice for 120 seconds before electrophoresis.

2.3 Polyacrylamide Gel Electrophoresis

Polyacrylamide gel solution (PAGE) made up of 210 g of 6 M urea, acrylamide and N'N'-methylene bisacrylamide (19:1) were prepared, added together with TBE buffer containing Tris and boric acid (2:1) with 2 ml EDTA and standardized with water. TEMED (i.e. $C_6H_{16}N_2$) and ammonium persulphate (1: 16) microlitre added to serves as cross-links. Total of 4 µl each of the PCR products were added to 1 µl loading dye containing formamide, mixture were sucked up and down before loading into gel capillary wells. Exactly 4 µl of ladder (*PBR322 DNA/MspI*) was mixed with 1 µl of loading dye and loaded into one of the wells for sizing. Detailed procedure of this electrophoresis has been reported elsewhere by Olowofeso *et al.* (2005). Amplicons generated when each marker was used with the DNA and Master Mix were electrophoresed on an ABI DNA Sequencer (Applied Biosystems), gels were then stained with a drop of ethidium bromide, photographed on UV trans-illuminator , cropped with computer and fragment sizes were determined based on ladder (Sizer) incorporated using the Fragment Manager version 1.1.

2.4 Statistical Analysis

Frequencies of the allele fragments and measures of genetic diversity produced by each marker and in each chicken population were obtained with the aid of Microsatellite Analyser (MSA) version 4.05 developed by Dieringer and Schlotterer (2003). Using the expected heterozygosity produced by each marker and in each

chicken population, the effective population size based on two models of microsatellite evolution was calculated using equations 1 and 2 below. Total values over the number of markers in which expected heterozygosity was above zero was taken as the effective population size (Askt *et al.,* 2002; Olowofeso, 2008). For each marker and each chicken population, the effective population size (N_e) based on infinite allele model (Piry *et al.,* 1999; Waldick *et al.,* 2002) was obtained with using the relation:

$$H_e = 4N_e\mu/(4N_e\mu+1) \Rightarrow N_e = \frac{H_e}{4\mu(1-H_e)} \text{---------}eq.1$$

and N_e based on stepwise mutation model (Lehmann *et al.,* 1998) was obtained as:

$$N_e = \frac{(\frac{1}{1-H_e})^2 - 1}{8\mu} \text{---------}eq.2$$

where H_e, N_e and μ are the expected heterozygosity, effective population size and microsatellite mutation rate in equations above. Microsatellite mutation rate of 10^{-4} suggested by Weber and Wong (1993) was used. Inter-population for marker designated as (G_{ST}), which measures the proportion of gene diversity that is distributed among populations was generated with MSA software. Relationship between measures of genetic diversity and logarithm effective population size were obtained using Statistical Analysis System version 9.1, and five linear models that can be used to develop line of best fit involving measures of genetic diversity and effective population size in chicken populations were formulated through least squares regression.

3. Results and Discussion

DNA fragments obtained were designated as alleles and prepared into Microsoft Excel Worksheet considering the chicken populations and markers selected. Measures of genetic diversity computed as (mean number of alleles/marker and mean expected heterozygosity); effective population sizes based on two models of microsatellite evolution and logarithm effective population sizes with other summary statistic in the chicken populations and across markers are presented in Table 2. Across markers, mean number of alleles designated as $\overline{N}_A$ ranged from 7.00±0.82 (NN) to 9.55±0.66 (NF). Similarly, mean expected heterozygosity $(\overline{H}_e)$ across markers ranged from 0.76 (NN) to 0.84 (both in FF and NF chicken populations).

High level of polymorphism was exhibited by the markers based on the number of alleles produced and expected heterozygosity. Number of alleles produced by these markers as well as their heterozygosities were consistent with what have been reported by Olowofeso (2005); Nahashon *et al.* (2008) and Mwacharo *et al.* (2013), but higher than value reported by Hillel *et al.* (2003). Mean number of alleles produced by these markers in each chicken population was above four, being the minimum suggested by Wimmers *et al.* (2000), meaning that these markers were effective with Nigerian chicken populations having elucidated the measures of genetic diversity in the populations. A lower mean number of allele, 7.00±0.82 was observed in NN and higher value of 9.55±0.66 was obtained in NF chicken population. Mean expected heterozygosities were high (0.76 to 0.84) in the chicken populations, which is an indication of high level of genetic variability among populations; a typical characteristic of native chicken populations in Africa and Asia according to Groeneveld *et al.* (2010). High heterozygosity values might therefore mean that inbreeding must have been discouraged in the chicken populations or may be due to the fact that poultry breeders in Nigeria are conversant with problems associated with indiscriminate or uncontrolled breeding in these populations which might trigger gene flow among populations, hence why Agaviezor *et al.* (2012) submitted that the trend be halted completely so as to prevent loss of Nigerian pure breeds or strains of the important animal genetic resources.

Mean number of alleles and mean expected heterozygosity obtained in this study were within the values reported for these measures of genetic diversity by Romanov and Weigend (2001); Olowofeso *et al. (*2005); Tadano *et al.* (2007); Chatterjee *et al.* (2008); Kayang *et al.* (2010) and Mwacharo *et al*. (2013) in their use of microsatellite markers with chicken and guinea fowl populations. However, the lower limits of these variables in this study were slightly higher than the lower limits reported by Tolene *et al.* (2012). Though, these differences might be as results of different species/breeds, microsatellite markers and or PCR reaction programmes employed.

Effective population size expressed as N_e based on two models of microsatellite evolution in each chicken population with their corresponding logarithm N_e produced by each marker is shown in Table 2. The logarithm effective population size ranged from 3.95 (NN) to 4.18 (NF) and 4.43 (NN) to 4.75 (NF) chickens based on IAM and SMM models, respectively. Table 3 present the relationship (correlations) between each measure of genetic diversity and logarithm effective population size as well as equations for line of best fit between variables. Correlation coefficients between variables were positive and ranged from 0.43 (both in mean

number of alleles/marker versus mean expected heterozygosity, and mean expected heterozygosity versus logarithm effective population size based on SMM) to 0.96 (mean number of alleles/marker versus logarithm effective population size based on IAM).

Significant regressions ranged from 0.03 in mean number of alleles/marker versus mean expected heterozygosity to 0.80 in mean expected heterozygosity versus logarithm effective population size. Simple linear equations for predicting measures of genetic diversity and effective population sizes were formulated using least squares regression. The two measures of genetic diversity correlates well with logarithm effective population size of each chicken population. The linear equations that were derived for the line of best fit involving these variables are presented in Table 3. Considering the level of relationship between measures of genetic diversity and effective population size based on data generated, positive correlation coefficients (0.43 to 0.96) were obtained between variables. Significant regressions, which ranged from 0.03 to 0.80, were also obtained between variables. These results further substantiates the positive correlations and significant regressions between some measures of genetic diversity and effective population size observed by White and Searle (2007) in their study with shrew *Sorex araneus* using microsatellite markers.

Using the intercepts and regression coefficients generated, five equations were obtained between measures of genetic diversity and effective population size. Linear relationship occur when the equations were used (though graphical representation of results not present here), meaning that measures of genetic diversity increases with corresponding increase in the effective population size or vice versa. This may be due to the fact that chicken populations under consideration do not have ample time to attain equilibrium. Results of this nature have been observed by Frankham (1996, 1997) in studies involving mammals and some other species.

Genetic differentiation (G_{ST}) which measures the proportion of gene diversity that is distributed among populations for the markers used is shown in Table 1. In this study, G_{ST} for each marker and across chicken populations ranged from 0.04 (MCW0206) to 0.13 (MCW0037) and overall mean G_{ST} across markers was 0.08 (Table 1). These values were very low and even lower than G_{ST} of 0.15 reported by Shahbazi *et al.* (2007) in Iranian chicken populations and G_{ST} of 0.17 obtained by Bao *et al.* (2008) in Chinese domestic fowls, respectively. Differences in these results might be adduced to the fact that the chickens were from different locations or may be due to different analytical procedures adopted in different locations.

4. Conclusion

The study revealed that microsatellite markers selected were effective to elucidate the genetic information in the three ubiquitous chicken populations in Nigeria. Overall, measures of genetic diversity were high, meaning that there is high genetic variability in the chicken populations. Our results further indicated that measures of genetic diversity were positively correlated with logarithm effective population size in the chicken populations. Significant regressions between measures of genetic diversity and effective population size also occur. The study pointed out a noteworthy genetic diversity in the chicken populations, established the level of relationship between measures of genetic diversity and effective population size and suggested some linear models that can be used for predicting measures of genetic diversity and effective population size in chicken populations.

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Table 1. Eleven microsatellite markers used, their sequences, annealing temperature and genetic differentiation value of each marker across chicken populations

Marker	Primer sequence (5'-3')		Annealing temperature (^{0}C)	Total heterozygosity in populations (H_T)	Heterozygosity in sub-population (H_S)	Genetic differentiation (G_{ST})
	Forward	Reverse				
ADL0112	GGCTTAAGCTGACCCATTAT	ATCTCAAATGTAATGCGTGC	58	0.86	0.79	0.08
ADL0268	CTCCACCCCTCTCAGAACTA	CAACTTCCCATCTACCTACT	56	0.88	0.83	0.06
ADL0278	CCAGCAGTCTACCTTCCTAT	TGTCATCCAAGAACAGTGTG	60	0.87	0.83	0.05
LEI0094	GATCTCACCAGTATGAGCTGC	TCTCACACTGTAACACAGTGC	60	0.91	0.82	0.10
LEI0234	ATGCATCAGATTGGTATTCAA	CGTGGCTGTGAACAAATATG	60	0.90	0.83	0.08
MCW0020	TCTTCTTTGACATGAATTGGCA	GCAAGGAAGATTTTGTACAAAATC	60	0.81	0.76	0.06
MCW0034	TGCACGCACTTACATACTTAGAGA	TGTCCTTCCAATTACATTCATGGG	60	0.91	0.82	0.10
MCW0037	ACCGGTGCCATCAATTACCTATTA	GAAAGCTCACATGACACTGCGAAA	62	0.89	0.77	0.13
MCW0104	TAGCACAACTCAAGCTGTGAG	AGACTTGCACAGCTGTGTACC	60	0.92	0.84	0.09
MCW0284	GCCTTAGGAAAAACTCCTAAGG	CAGAGCTGGATTGGTGTCAAG	60	0.85	0.79	0.07
MCW0206	CTTGACAGTGATGCATTAAATG	ACATCTAGAATTGACTGTTCAC	60	0.91	0.87	0.04
Mean across markers				0.88	0.81	0.08

Table 2. Number of alleles, expected heterozygosity, logarithm effective population size and their means across markers in the three Nigerian chicken populations*

Marker	Chicken Populations											
	Naked neck				Frizzle feathered				Normal feathered			
	N_A	H_e	N_e (IAM/SMM)	Log N_e	N_A	H_e	N_e (IAM/SMM)	Log. N_e	N_A	H_e	N_e (IAM/SMM)	Log. N_e
ADL0112	6	0.83	12206/42003	4.1/4.6	8	0.83	12206/42003	4.1/4.6	6	0.77	8370/22379	3.9/4.3
ADL0268	10	0.82	11389/37330	4.1/4.6	5	0.81	10658/33355	4.0/4.5	8	0.75	7500/18750	3.9/4.3
ADL0278	6	0.83	12206/42003	4.1/4.6	8	0.85	14167/54306	4.2/4.7	10	0.88	18333/85556	4.3/4.9
LEI0094	6	0.78	8864/24577	3.9/4.4	12	0.89	20227/102056	4.3/5.0	11	0.86	15357/62526	4.2/4.8
LEI0234	4	0.77	8370/22379	3.9/4.3	8	0.87	16731/72715	4.2/4.9	12	0.90	22500/123750	4.4/5.1
MCW0020	4	0.21	665/753	2.8/2.9	6	0.79	9405/27095	3.9/4.4	6	0.78	8864/24577	3.9/4.4
MCW0034	4	0.73	6759/15897	3.8/4.2	14	0.93	33214/253852	4.5/5.4	11	0.92	28750/194063	4.5/5.3
MCW0037	12	0.82	11389/37330	4.1/4.6	6	0.81	10658/33355	4.0/4.5	9	0.74	7115/17260	3.9/4.2
MCW0104	7	0.87	16731/72715	4.2/4.9	7	0.82	11389/37330	4.1/4.6	11	0.90	22500/123750	4.4/5.1
MCW0284	10	0.82	11389/37330	4.1/4.6	6	0.80	10000/30000	4.0/4.5	9	0.83	12206/42003	4.1/4.6
MCW0206	8	0.89	20227/102056	4.3/5.0	9	0.86	15357/62526	4.2/4.8	12	0.92	28750/194063	4.5/5.3
Mean across markers	7.00±0.82	0.76		3.95/4.43	8.09±0.82	0.84		4.14/4.72	9.55±0.66	0.84		4.18/4.75

** N_A = Number of alleles, H_e = Expected heterozygosity, N_e = Effective population size, Log N_e = Logarithm effective population size.*

Table 3. Correlation and regression coefficients, intercept and linear equation developed for line of best fit between (independent, X^1 and dependent, $\hat{Y}$) variables

Paired variables	Correlation coefficient (r)	Regression coefficient (b)	Intercept (a)	Equation for line of best fit
Mean number of alleles/marker versus mean expected heterozygosity	0.43	0.03	0.56	$\hat{Y} = 0.56 + 0.03\,X^1$
Mean number of alleles/marker versus logarithm effective population size based on infinite allele model	0.96	0.09	3.38	$\hat{Y} = 3.38 + 0.09\,X^1$
Mean number of alleles/marker versus logarithm effective population size based on stepwise mutation model	0.89	0.12	3.64	$\hat{Y} = 3.64 + 0.12\,X^1$
Mean expected heterozygosity versus logarithm effective population size based on infinite allele model	0.48	0.60	3.60	$\hat{Y} = 3.60 + 0.60\,X^1$
Mean expected heterozygosity versus logarithm effective population size based on stepwise mutation model	0.43	0.80	3.98	$\hat{Y} = 3.98 + 0.80\,X^1$

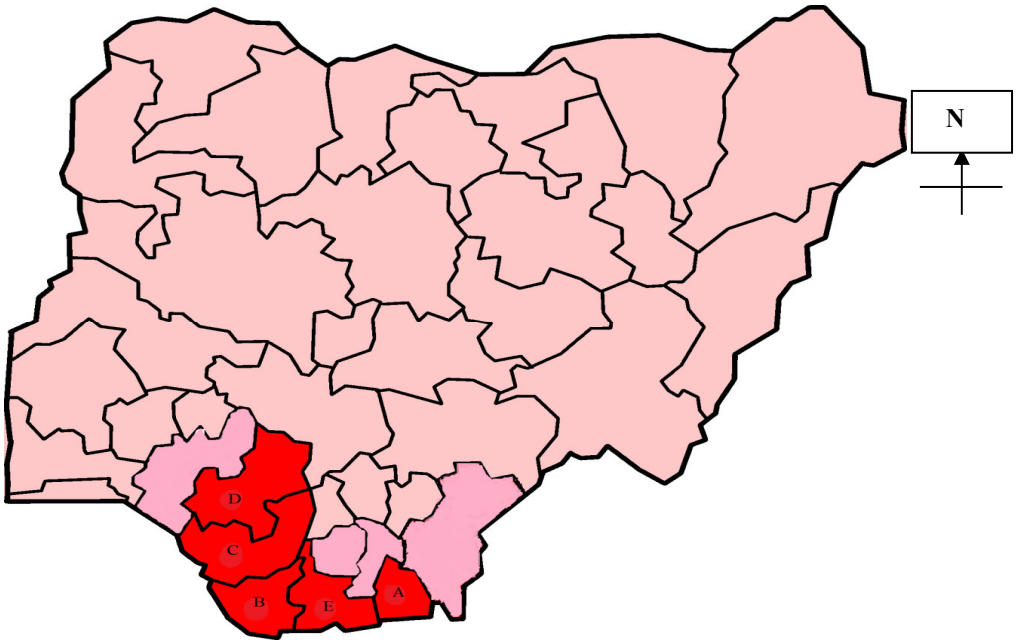

Figure 1. Map of Nigeria showing sampled population locations (A = Akwa Ibom, B = Bayelsa, C = Delta, D = Edo and E = Rivers States) for the three chicken populations.