Genetic diversity, phylogeographic structure and effect of selection at the mitochondrial hypervariable region of Nigerian chicken populations

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Abstract. In this study, the maternal genetic diversity, phylogeographic relationship and effect of natural selection on indigenous chickens from Nigeria were assessed. A total of 397-bp fragment of the mitochondrial DNA (mtDNA) D-loop region of 171 indigenous chickens from four populations of Nigeria and four commercial egg line strains (two Anak titan, one Giriraja and one Yaffa) as out-groups were analysed. Thirty-one haplotypes (28 from Nigerian chickens and three from commercial strains) and 34 polymorphic sites were identified. The mean haplotypic and nucleotide diversity were found to be 0.39 ± 0.05 and 0.02 ± 0.02, respectively. Majority of Nigerian chicken haplotypes observed were grouped into haplogroup D which originated from Indian subcontinent, suggesting a single maternal lineage. Genetic variation within and between populations accounted for 97.30 and 2.70% of the total genetic variation, respectively, which is in agreement with a recent and maternal founding effect. High number (4) of negatively selected sites observed based on single likelihood ancestral counting (SLAC) model indicated that the sampled Nigerian chicken populations were undergoing purifying selection. This study concluded that there was relatively high genetic diversity and differentiation, thus, this information will probably pave way for further evaluation studies, preservation and improvement of Nigerian chickens as genetic resources towards ensuring food security.

Keywords. genetic diversity; mitochondrial DNA; Nigerian chickens; phylogeny; selection.

Introduction

Domestic chickens are considered to be one of the most important and widely distributed avian species among poultry birds, believed to have descend from a single ancestor, the Red Jungle fowls (Gallus gallus) with Southeast Asia as the centre of origin (Fumihito et al. 1994, 1996; Yamashita et al. 1994). Chicken genetic resources comprise a wide range of breeds and populations including Red Jungle fowl, native and fancy breeds, middle-level food producers, industrial stocks and specialized lines (Wani et al. 2014).

Nigeria has many indigenous chicken breeds that together form a large proportion of the poultry consumption in the country, which are yet to be incorporated into breeding programmes as well as to exploit their genetic potentials. They are able to better survive in low input systems, being more tolerant and/or resistance to disease challenges, and variable production and climate conditions than their commercial counterparts due to their long breeding history.

Despite the importance of Nigerian indigenous chicken, little is known about their genetic diversity. There is a need to characterize their genetic structure towards revealing their uniqueness, and identify their valuable genetic resources for conservation, genetic improvement and utilization towards meeting the current production needs and future food security (Hanotte et al. 2010; Ajibike 2016). The use of molecular tools is of great assistance/importance in the conservation of endangered
breeds, particularly mitochondria DNA (mtDNA) D-loop region, because it has a strictly maternal inheritance, absence of recombination and more polymorphic compared to other regions of the mtDNA, making it a useful tool for studying the evolution of closely related species and maternal origins.

Therefore, this study was undertaken to assess the genetic diversity, phylogenetic relationship and effect of selection within and among indigenous chickens based on geographical zones since there is no information available on the genetic characteristics and distribution of types of indigenous chicken in Nigeria.

Materials and methods

Sample collection and DNA extraction

Genomic DNA was extracted from air-dried blood preserved on FTA classic cards (Whatman Biosciences) using the recommended manufacturer protocol, from 171 unrelated individual indigenous chicken from four geographical regions of Nigeria (west Africa). The samples include 22 birds from the north-western region representing population I (NW), 37 birds from the north-central region representing population II (NC), 38 birds from the north-eastern region representing population III (NE), 74 birds from south-western region representing population IV (SW) and four commercial strains as reference.

The DNA concentration and purity, A260/A280 ratio between 1.8 and 2.0 were assessed using a NanoDrop 1000 Spectrophotometer. Potential DNA degradation was visualized on 1% agarose gel. To address the possible origin of Nigerian indigenous chicken, seven reference clades reported by ILRI (2006) and the chicken mtDNA reference downloaded from the National Centre for Biotechnology Information (NCBI) (GenBank accession numbers: AB007720 and AB007718) were included in the analyses.

PCR amplification

Using the primers: L16750 (5'-AGGACTACGGTTGA AAAGC-3'; accession number NC_001323) as the forward primer (Desjardins and Morais 1990) and H547 (5'-ATGTGCGACGAACGAGAACCAG-3'; accession number AB098668) as reverse primer (Komiyama et al. 2003), PCR amplification was carried out using 30 µL reaction volume containing 2.5 mM of each dNTPs, 14 pmol of each primer, 1.5 mM MgCl2, 1 x PCR buffer comprising 10 mM Tris-HCl (pH 8.3) and 50 mM KCl and 1.25 U Taq DNA polymerase (Roche Applied Sciences, Germany). Amplifications were carried using the GeneAmp PCR system 9700 thermal cycler (Applied Biosystems, Foster city, USA) programmed as follows: initial denaturation of 94°C for 2 min, followed by 10 cycles at 94°C for 15 s, 58°C for 30 s and 72°C for 40 s.

Sequencing of the mtDNA

Two internal primers: CR-for (5'-TCTATATTCCACATT TCTC-3') and CR-rev (5'-GCGAGCATACCCAATGGG-3') were used in a 20 µL comprising ~20 ng of purified PCR product as template DNA, 3.2 pmol of primer and 8 µL of Big Dye Terminator Ready Reaction Mix (mixture of dNTPs, ddNTPs, buffer, enzyme and MgCl2), 8 µL of deionized water, 2 µL of primer and 2 µL template DNA, using an ABI 3730 XL Capillary DNA Analyzer (Applied Biosystems) programmed as: 25 cycles at 96°C for 10 s, 50°C for 5 s and 60°C for 4 min. After the last cycle, there was a rapid thermal ramp to 4°C and holding until the purification of the sequencing product.

Data analysis

A 396-bp long fragment, including the hypervariable region I (HV1), was subsequently used for analysis. Viewing and editing of the sequences was done using BioEdit software (Hall 1999). The consensus sequence was aligned against the reference (GenBank accession number AB098668) excluding all gaps using ClustalW in MEGA 6.06.

The population haplotypes and clades used in this study are defined by north-east (NE), north-west (NW), north-central (NC), south-west (SW), Giriraja (GIR), Yaffa (YAF), Anak titan (ANAK), the number signifies identification number of individual chickens, while clade I, clade II, clade IIIa, clade IIIb, clade IIIc, clade IID and clade IV, all represent different haplogroups from the Asian continent (ILRI 2006, Adebambo et al. 2010).

Sequence variations (number of haplotypes, haplotype diversity, nucleotide diversity, average number of nucleotide difference and average number of nucleotide substitutions per site between populations) were calculated using DnaSP ver. 5 (Librado and Rozas 2009).

A neighbour-joining (NJ) tree was constructed for identified Nigerian chicken haplotypes, commercial strain haplotypes and reference lineage haplotypes with a 1000 bootstrap replicates using MEGA ver. 6.06 (Tamura et al. 2013). A median-joining (MJ) network analysis was constructed using Network 4.6.1.2 (Bandelt et al. 1999).

Maternal genetic differentiation was further quantified using hierarchical analysis of molecular variance (AMOVA), mismatch distributions, Tajima’s D and Fu’s Fs were calculated using Arlequin 3.5.1.3 software (Excoffier et al. 2005). The generated mtDNA sequences were used in testing among and within selection effects based on single likelihood ancestral counting (SLAC) method using Hyphy 2.2 software (Kosakovsky Pond et al. 2005, available at http://www.hyphy.org).

The Institutional Animal Care and Use Committee of the Federal University of Agriculture, Abeokuta, Nigeria approved all experimental procedures. The field
surveys involve no endangered or protected animal species: a veterinarian helped in the blood sample collection, and manually restrained the animals; no tranquilizers or short-acting anesthetics used. Blood samples were collected using appropriate equipment. The Federal University of Agriculture, Abeokuta Animal Care and Use Committee approved the sampling procedures and number of animal sampled, as part of obtaining the field permit.

| Reference | NE33 | NE35 | NE36 | NE46 | NE57 | NE69 | NE73 | NE74 | NW9 | NW18 | NW21 | NW25 | NC4 | NC7 | NC15 | NC20 | NC27 | NC77 | NC79 | NC81 | SW12 | SW30a | SW32 | SW49 | SW55 | GIR57 | SW60 | SW62 | SW82 | ANAK109 | YAF113 |
|-----------|------|------|------|------|------|------|------|------|-----|------|------|------|-----|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
|           | GC   | GC   | GC   | GC   | GC   | GC   | GC   | GC   | GC  | GC   | GC   | GC   | GC  | GC  | GC   | GC   | GC   | GC   | GC   | GC   | TGC  | GC   | GC   | GC   | .   | .   | .   | .   |
|           | CCT  | CCT  | CTCT | CTCT | CTCT | CTCT | CTCT | CTCT | CTCT| CTCT | CTCT | CTCT | CTCT| CTCT | CTCT | CTCT | CTCT | CTCT | CTCT | TC   | TC   | TC   | TC   | TC   | TC   | TC   | TC   | TC   |
|           | ACT  | ACT  | ACT  | ACT  | ACT  | ACT  | ACT  | ACT  | ACT | ACT  | ACT  | ACT  | ACT | ACT | ACT  | ACT  | ACT  | ACT  | ACT  | ACT  | ACT  | ACT  | ACT  | ACT  | ACT  | ACT  | ACT  | ACT  |
|           |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|           |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |

Vertically oriented numbers indicate the variable sites position. ‘.‘ Identity with the reference (Ref) sequence (GenBank accession number: AB098668), while different base letters denote substitution. NE, north-east; NC, north-central; NW, north-west; SW, south-west; GIR, Giriraja; ANAK, Anak titan; YAF, Yaffa; n, number of individuals in each haplotype.

Results

Nucleotide variability and diversity indices

The pattern of 397-bp mtDNA variability revealed high variations between nucleotide 212 and 397. A total of 31 haplotypes (eight from NE population, four from NW population, eight from NC population, eight from SW population).
population and three reference populations) defined by 34 polymorphic sites with no insertion or deletions from 171 Nigerian chicken and four commercial strain sequences were found (table 1).

The result of 171 Nigerian chicken molecular diversity indices (table 2) revealed that NE chicken has the highest haplotype diversity (0.59 ± 0.09), nucleotide diversity (0.03 ± 0.02) and mean number of pairwise difference (0.90 ± 0.64) value, while SW chicken has the least haplotype diversity, nucleotide diversity and mean number of pairwise difference value of 0.25 ± 0.07, 0.01 ± 0.01 and 0.35 ± 0.35, respectively. The entire populations have mean haplotype diversity, nucleotide diversity and mean number of pairwise difference value of 0.39 ± 0.05, 0.02 ± 0.02 and 0.56 ± 0.24, respectively. The sum of square frequency was high in SW chicken (0.75) and least in NE chicken (0.43), while mean for all population was 0.62.

Mismatch distribution

The demographic change indices, and test of goodness-of-fit of the observed data to the simulated model of expansion with sum of square deviations (SSD) and Harpending’s raggedness value (r) using bootstrap approach was carried out for 171 Nigerian chicken (table 3). The highest mismatch mean (0.9) and variance (1.17) were observed in NE chicken, while the least value of 0.35 and 0.44 was observed in SW chicken, with an overall chicken population having an average mismatch mean and variance of 0.53 and 0.66, respectively. The highest time of expansion (T = 3.00) was observed in NW and SW chicken, while the least value of 0.55 was observed in NC chicken, with an overall chicken population with a mean value of 1.85.

The NE chicken have the highest sum of square deviation value of 0.01, while a least value of 0.00 was observed in NW, NC and SW chicken. The highest r of 0.35 was observed in SW chicken and the least value of 0.11 was observed in NE chicken, with an overall chicken population having a mean value of 0.20. Tajima’s and Fu’s tests of neutrality result were found to be negative for all the chicken population with SW chicken having the highest value of −2.31 and −13.83 for Tajima’s and Fu’s tests, respectively. NE chicken has the least Tajima’s value of −1.72, while NW chicken was observed with the least Fu’s value of −3.14. The overall chicken population has a mean value of −2.03 and −7.34 for Tajima’s and Fu’s tests of neutrality.

The mismatch distribution pattern observed in this study for each sampled population suggested that there was a population expansion (figure 1, a–d).

Phylogenetic analysis of haplotypes

The neighbour joining dendrogram revealed that 31 haplotypes identified in the Nigerian chicken were placed into two clusters with the domestic chicken Gallus gallus gallus (figure 2). This indicates a very close relationship between the Nigerian indigenous chickens and Gallus gallus gallus, while they are relatively genetically distanced from

### Table 2. Sampling population, sample size, population genetic diversity measure, standard deviation for each population.

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>π</th>
<th>H_d</th>
<th>SSF</th>
<th>PD</th>
<th>θ_s</th>
<th>θ_π</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE chicken</td>
<td>38</td>
<td>0.03 ± 0.02</td>
<td>0.59 ± 0.09</td>
<td>0.43</td>
<td>0.90 ± 0.64</td>
<td>2.14 ± 0.71</td>
<td>0.90 ± 0.71</td>
</tr>
<tr>
<td>NW chicken</td>
<td>22</td>
<td>0.02 ± 0.02</td>
<td>0.34 ± 0.13</td>
<td>0.68</td>
<td>0.46 ± 0.42</td>
<td>1.37 ± 0.73</td>
<td>0.46 ± 0.47</td>
</tr>
<tr>
<td>NC chicken</td>
<td>37</td>
<td>0.02 ± 0.02</td>
<td>0.43 ± 0.10</td>
<td>0.58</td>
<td>0.53 ± 0.45</td>
<td>1.92 ± 0.86</td>
<td>0.53 ± 0.50</td>
</tr>
<tr>
<td>SW chicken</td>
<td>74</td>
<td>0.01 ± 0.01</td>
<td>0.25 ± 0.07</td>
<td>0.75</td>
<td>0.35 ± 0.35</td>
<td>2.26 ± 0.87</td>
<td>0.35 ± 0.38</td>
</tr>
<tr>
<td>Mean</td>
<td>42.75 ± 22.08</td>
<td>0.02 ± 0.02</td>
<td>0.39 ± 0.05</td>
<td>0.62</td>
<td>0.56 ± 0.24</td>
<td>1.92 ± 0.85</td>
<td>0.56 ± 0.52</td>
</tr>
</tbody>
</table>

N, sample size; π, nucleotide diversity; H_d, haplotype diversity; SSF, sum of square frequency; PD, mean number of pairwise difference; θ_s, theta value based on number of segregating sites; θ_π, theta value based on the average number of pairwise differences.

### Table 3. Demographic expansion indices of Nigerian chicken.

<table>
<thead>
<tr>
<th>Population</th>
<th>MOM</th>
<th>MOV</th>
<th>T</th>
<th>θ_0</th>
<th>θ_1</th>
<th>D</th>
<th>F</th>
<th>r</th>
<th>SSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE chicken</td>
<td>0.90</td>
<td>1.17</td>
<td>0.84</td>
<td>0.00</td>
<td>999999.0</td>
<td>−1.72</td>
<td>−3.96</td>
<td>0.11</td>
<td>0.01</td>
</tr>
<tr>
<td>NW chicken</td>
<td>0.46</td>
<td>0.51</td>
<td>3.00</td>
<td>0.00</td>
<td>0.52</td>
<td>−1.99</td>
<td>−3.14</td>
<td>0.21</td>
<td>0.00</td>
</tr>
<tr>
<td>NC chicken</td>
<td>0.53</td>
<td>0.47</td>
<td>0.55</td>
<td>0.02</td>
<td>999999.0</td>
<td>−2.10</td>
<td>−8.43</td>
<td>0.12</td>
<td>0.00</td>
</tr>
<tr>
<td>SW chicken</td>
<td>0.35</td>
<td>0.44</td>
<td>3.00</td>
<td>0.00</td>
<td>0.34</td>
<td>−2.31</td>
<td>−13.83</td>
<td>0.35</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean</td>
<td>0.53</td>
<td>0.66</td>
<td>1.85</td>
<td>0.01</td>
<td>499999.71</td>
<td>−2.03</td>
<td>−7.34</td>
<td>0.20</td>
<td>0.00</td>
</tr>
</tbody>
</table>

MOM, mean observed mismatch; MOV, mean observed variance; T, time of expansion; θ_0 and θ_1, mutation parameters; D, Tajima’s neutrality test; F, Fu’s neutrality test; r, Harpending’s raggedness index; SSD, sum of square deviation.
Genetic diversity of Nigerian indigenous chicken

**Figure 1.** Mismatch distribution of: (a) north-east Nigerian chicken; (b) north-west Nigerian chicken; (c) north-central Nigerian chicken; and (d) south-west Nigerian chicken.

**Gallus gallus bankiva.** Alignment with the reference lineage haplotypes from Asia showed that all Nigerian chicken were grouped into clade IV, with one major NE33 haplotype present in 134 observations of the 171 Nigerian chicken (table 1), which is identical to our haplotype of reference clade IV (refer to haplogroup D by Hassaballah et al. 2015), while the commercial egg line strain (GIR57) studied concurrently with Nigerian chicken fell into clade IIIc.

The haplotype network analysis (figure 3) further confirms the close relationship between Nigerian chicken haplotypes. This clearly illustrates that all Nigerian chicken haplotypes and Anak titan haplotype belongs to a single expansion event centred on Clade IV (NE33).

**Analysis of molecular variance (AMOVA)**

Maternal genetic differentiation within population and among population within only Nigerian chicken was quantified using hierarchal analysis of molecular variance AMOVA on Tajima–Nei distance considering Nigerian populations as one single group. The genetic variation within population was 97.30% than the genetic differentiation among the populations was 2.70% with a fixation index ($F_{ST}$) value of 0.03 (table 4).

**Selection detection between and within Nigerian chicken populations**

The quick selection detection analysis was carried out using only the Nigerian chicken sequences based on single likelihood ancestor counting (SLAC) model proposed by Suzuki and Gojobori (1999) revealed that NE, NC and SW chicken populations have one negative selected site each, while NW chicken showed a balanced selection with no site either positively or negatively selected (table 5), while the between Nigerian chicken sequences selection analysis
Neighbour-joining tree reconstructed from the 28 haplotypes identified in the 171 Nigerian chicken sequences, three haplotypes identified in four commercial strain, two haplotypes of *Gallus* genus (GenBank accession number: AB007720 and AB007718) and seven haplotypes of references using MEGA 6.06 software. The percentage bootstrap value is represented by the numbers at the node after 1000 replications.

revealed four negatively selected sites with no positively selected site (table 6).

**Discussion**

On the basis of nucleotide sequence variation, 31 haplotypes were detected from a total of 34 polymorphic sites between Nigerian chicken sequences and Red Jungle fowl (GenBank accession number: AB098668), where sequence variation within and between populations represents the phenotypic variation among individuals in the population. The observed haplotype number (31) was lower than 35 haplotypes observed between Nigerian village chickens and Red Jungle fowl reported by Adebambo *et al.* (2010), these may be due to lower sample size and differences in sampling location used in this study.

The observed haplotypic diversity was lower than what was reported by Muchadeyi *et al.* (2008), Cuc *et al.* (2011), Hoque *et al.* (2013), Kawabe *et al.* (2014) and Hassaballah *et al.* (2015), which may be due to the low sample size, while the observed nucleotide diversity was higher than that reported by Liu *et al.* (2006), Oka *et al.* (2007), Kawabe *et al.* (2014) and Hassaballah *et al.* (2015), thus, suggesting that Nigerian chicken populations have relatively higher genetic diversity. The combination of high haplotypic diversity and low nucleotide diversity in this...
Figure 3. Median-joining network ($\varepsilon = 0$) of Nigerian chicken haplotypes based on the polymorphic sites of the mitochondrial D-loop HV1 region. Area of each circle is proportional to the frequency of the corresponding haplotype. Different classes of haplotypes are distinguished by use of colour codes (NE, green; NW, black; NC, orange; SW, yellow; Anak titan, blue; Giriraja, purple; Yaffa, lemon). The red colour between the haplotype nodes refer to the positions of median vector.

Table 4. AMOVA of Nigerian chicken.

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>SS</th>
<th>Variance components</th>
<th>Per cent variation</th>
<th>$F_{ST}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among populations</td>
<td>3</td>
<td>1.65</td>
<td>0.01 Va</td>
<td>2.70</td>
<td>0.03</td>
</tr>
<tr>
<td>Within populations</td>
<td>167</td>
<td>43.66</td>
<td>0.26 Vb</td>
<td>97.30</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>170</td>
<td>45.31</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SOV, source of variation; DF, degree of freedom; SS, sum of squares; $F_{ST}$, fixation index.

The observed negative Tajima’s $D$ indicated that the Nigerian chicken populations departed from equilibrium, and may be as a result of past or recent population expansion, bottleneck effect or heterogeneity of mutation rates (Tajima 1996). However, the observed negative Fu’s $F$ value provides a strong evidence of past population expansions, which may be due to genetic hitchhiking, background selection and evolutionary force producing the observed population expansion pattern (Okello et al. 2005; Joshi et al. 2013).

A significant $r$ or SSD value was taken as evidence of departure from the estimated sudden demographic model, which can be either a model of population expansion (if $t > 0$ and $\theta_1 > \theta_0$) or a model of population stationary (if $t = 0$ or $\theta_1 = \theta_0$). The obtained result confirms to the model of population expansion ($t > 0$ and $\theta_1 > \theta_0$) for Nigerian chicken populations. The hypothesis that the observed Nigerian chicken data fit the sudden expansion model, which was tested using SSD (Schneider and
Excoffier 1999) and r (Harpending 1994). The observed nonsignificant SSD = 0.00 and r = 0.20 mean values suggested that population expansion had occurred in Nigerian chicken.

The phylogeny and haplotype network analysis revealed that Nigerian chicken showed relatively less divergence and shared the most common haplotype (NE33) belonging to clade IV (haplogroup D), thus, suggesting a single maternal origin believed to have its root in the Indian subcontinent. These results confirm to the report of Mobegi and Chicken Diversity Consortium (2006) in African chickens, Muchadeyi et al. (2008) in Zimbabwean chickens; Adebambo et al. (2010) in Nigerian chickens; Mtileni et al. (2011) in South African chickens, Islam and Nishibori (2012) in Bangladesh and neighbouring Asian countries chickens; Lyimo et al. (2013) in Tanzanian chicken ecotypes, Wani et al. (2014) in Sudan and southern Sudan chicken breeds and Hassaballah et al. (2015) in Chad and central African indigenous chickens, who are of the opinion that the initial centre of origin for the haplogroup D found in Africa is Indian subcontinent. The low bootstrap values of NJ tree indicated no clear genetic substructuring in Nigerian chicken, which could be due to the result of high and random genetic interbreeding between the chicken populations which tends to slow down or prevent geographic differentiation process (Joshi et al. 2013).

The observed high number of negatively selected site with no positively selected site indicated that Nigerian chickens are undergoing purifying selection process, which is responsible for the preservation of adaptive characteristics under constant environmental conditions. Charlesworth (2006) opined that purifying selection reduces the frequency of deleterious allele and the genetic diversity at linked loci as well as effective population size, genetic variability and gene frequency. Thus, endangering the conservation of such chicken populations. The negatively selected site observed in NE, NC and SW chicken populations may be due to genetic drift, migration, random mating pattern, which could have resulted in heterozygous deficiency, or insufficient time to establish a balance between the occurrence of new mutations and their genetic diversity loss. The NW chicken population tends to be more adapted to their local environment, and have less gene flow as shown by having neither positively nor negatively selective sites.

**Conclusion and recommendation**

Despite the observed low fixation index, Nigerian chicken populations possessed relatively high genetic diversity and differentiation with no clear substructuring between and among them. All Nigerian chicken populations (except Giriraja) clustered under haplogroup D, which has been earlier reported to be the likely maternal origin of Nigerian chickens. Effect of natural selection gave an insight

### Table 5. Selection effect results within sampled Nigerian chicken populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Selection type</th>
<th>Site index</th>
<th>$d_N - d_S$ value</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE chicken</td>
<td>Positive</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>126.00</td>
<td>−5.61</td>
<td>0.01</td>
</tr>
<tr>
<td>NW chicken</td>
<td>Positive</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>79.00</td>
<td>−3.00</td>
<td>0.04</td>
</tr>
<tr>
<td>NC chicken</td>
<td>Positive</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>97.00</td>
<td>−3.08</td>
<td>0.03</td>
</tr>
<tr>
<td>SW chicken</td>
<td>Positive</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>126.00</td>
<td>−3.08</td>
<td>0.03</td>
</tr>
</tbody>
</table>

$d_N$, nonsynonymous rate; $d_S$, synonymous rate; $P < 0.05$.

### Table 6. Selection results between sampled Nigerian chicken populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Selection type</th>
<th>Site index</th>
<th>$d_N - d_S$ value</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nigerian chickens</td>
<td>Positive</td>
<td>79.00</td>
<td>−3.00</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>84.00</td>
<td>−3.35</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>97.00</td>
<td>−4.47</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>126.00</td>
<td>−4.47</td>
<td>0.01</td>
</tr>
</tbody>
</table>

$d_N$, nonsynonymous rate; $d_S$, synonymous rate; $P < 0.05$. 
into how well Nigerian chickens have been adapting to the ever changing production environment, uncontrolled interbreeding, migration and exchange through trade with insufficient time to establish a balance between the occurrence of new mutations and their genetic diversity loss. Therefore, it is imperative to put in place a National conservation policy and priorities as well as well-defined breeding and improvement programmes.

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