

## ***Evolutionary Divergence and Rates of Base Substitutions Across Four Populations of Nigerian Zebu Cattle at Heat Shock Protein 90 Locus***

Gbolabo O. Onasanya<sup>1,2</sup>, George M. Msalya<sup>3</sup>, Aranganoor K. Thiruvenkadan<sup>2</sup>,  
Nagarajan Murali<sup>2</sup> and Christian O. Ikeobi<sup>4</sup>

<sup>1</sup>Department of Animal Science, Federal University Dutse, Dutse, Nigeria

<sup>2</sup>Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. <sup>3</sup>Department of Animal, Aquaculture and Range Sciences, Sokoine University of Agriculture, Morogoro, Tanzania

<sup>4</sup>Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Abeokuta, Nigeria

Corresponding Author: Onasanya Gbolabo Olaitan; onasanya.gbolabo@gmail.com;

### **Introduction**

The changing effect of climate has brought threat to survival of man and animals and as such the consequences of global warming will be grave if not mitigated. In this study, we attempted to examine the ability of Nigerian zebu cattle to cope with changing effect of climate especially thermal assaults/heat stress. Heat shock protein (*HSP*) 90 is a member of *HSPs* family that are upregulated when animals is expose to thermal conditions and they help animals to tolerate/survive thermal assault (Onasanya *et al.*, 2020). Evolutionary divergence at *HSPs* loci play major role in the adaptation of animals to wide range of thermal

conditions. We unravelled thermo-tolerance potentials of four Nigerian zebu cattle raised under thermal conditions.

## Materials and Methods

To do this, we studied evolutionary divergence, codon-based Darwinian natural selection pressure and phylogenetics in four Nigerian zebu cattle populations (White Fulani: WF (4), Red Bororo: RB (4); Sokoto Gudali: SG (4) and Ambala: AM (3) at *HSP 90* locus. DNA isolation and amplifications of *HSP 90* gene were carried out according to earlier procedures of Onasanya *et al.* (2020). Sequencing of amplified DNAs and analyses of sequence data were performed according to earlier procedures of Onasanya *et al.* (2021). Primers used in this study F-GCGTCATCACGTGTCATCTT and R-CCTCCTTT GGGTTCCAGT were published earlier by Kumar *et al.* (2015) covering 450 bp within coding region in exon 3 of *HSP 90*.

## Results and Discussion

Regarding the estimation of evolutionary divergence among four Nigerian Zebu Cattle populations, the number of base substitutions/mutations between sequences are shown in Table 1. Condon-based analyses were performed using Maximum Composite Likelihood mode (Tamura *et al.*, 2004). The analysis involved 15 nucleotide sequences and codon positions included were 1st+2nd+3rd+Noncoding. There were total of 155 base positions in the final dataset. Evolutionary analyses were performed using MEGA5 (Tamura *et al.*, 2011). From our study, we estimated 1.8 as an average rates of mutation/substitutions per site among four zebu cattle populations (Table 2), this might have occurred either by negative binomial distribution or Poisson distribution with transversion or transition bias nature of mutation (Golding, 1983). Our study showed that rate of mutation /base changes were more evident among four Nigerian zebu cattle than within the breed. This is similar to earlier works of Kyazhimskiy and Plotkin (2008) and Golding (1983) who reported that rate of mutation is higher among sequences than within sequences noting that evolutionary divergence per site/base position is always higher between breeds than within breed. The variability and distribution of base changes/base substitution between mutation sites among four zebu cattle could be by gamma or lognormally distributed. The Variability in base

changes among sites/base positions could have great or greater effect than a transition/transversion bias. Irrespective of the two scenarios, variability of base substitutions is higher in among distant breeds than closely related breeds. Suffice to say that the higher the rate of mutation/base changes between adjacent nucleotide sequences the higher the evolutionary divergence and adaptation (Golding, 1983) leading to variabilities/speciation among the breeds of animals

<b>S/</b>	<b>Br</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>	<b>13</b>	<b>14</b>	<b>1</b>
<b>N</b>	<b>eed</b>															<b>5</b>
<b>1</b>	<b>W</b>															
	<b>F</b>															
<b>2</b>	<b>W</b>	1.3														
	<b>F</b>	79														
<b>3</b>	<b>W</b>	2.3	3.1													
	<b>F</b>	97	88													
<b>4</b>	<b>W</b>	0.0	1.1	2.1												
	<b>F</b>	80	28	94												
<b>5</b>	<b>AB</b>	1.3	0.0	3.1	1.1											
		79	75	88	28											
<b>6</b>	<b>AB</b>	0.0	1.1	2.1	0.0	1.1										
		80	28	94	71	28										
<b>7</b>	<b>AB</b>	1.3	0.0	3.1	1.1	0.0	1.1									
		79	09	88	28	04	28									
<b>8</b>	<b>SG</b>	0.0	1.1	2.3	0.0	1.1	0.0	1.1								
		72	51	07	87	51	91	51								
<b>9</b>	<b>SG</b>	1.3	0.0	3.1	1.1	0.0	1.1	0.0	1.1							
		79	05	88	28	88	28	83	51							
<b>1</b>	<b>SG</b>	0.0	1.1	2.2	0.0	1.1	0.0	1.1	0.0	1.1						
<b>0</b>		88	02	22	86	02	06	02	13	02						
<b>1</b>	<b>SG</b>	1.3	0.0	3.1	1.1	0.0	1.1	0.0	1.1	0.0	1.0					
<b>1</b>		56	96	92	09	06	09	46	30	06	82					
<b>1</b>	<b>RB</b>	0.0	1.1	2.1	0.0	1.1	0.0	1.1	0.0	1.1	0.0	1.1				
<b>2</b>		80	28	94	06	28	58	28	97	28	06	09				

<b>1</b>	<b>RB</b>	1.3	0.0	3.1	1.1	0.0	1.1	0.0	1.1	0.0	1.1	0.0	1.1		
<b>3</b>		79	77	88	28	09	28	01	51	81	02	86	28		
<b>1</b>	<b>RB</b>	0.0	1.1	2.2	0.0	1.1	0.0	1.1	0.0	1.1	0.0	1.0	0.0	1.1	
<b>4</b>		88	02	22	86	02	66	02	13	02	08	82	66	02	
<b>1</b>	<b>RB</b>	1.3	0.0	3.1	1.1	0.0	1.1	0.0	1.1	0.0	1.0	0.0	1.1	0.0	1.0
<b>5</b>		56	96	92	09	06	09	76	30	76	82	91	09	56	82

Table 1. Estimation of Evolutionary Divergence Among Nucleotide Sequences of four **Analysis of Darwinian Natural Selection Pressure Across Four Nigerian Cattle Populations at HSP 90 locus**

Table 2 shows the estimation of Darwinian natural selection using Maximum Likelihood analysis via codon-by-codon based Z test (Suzuki and Gojobori, 1999). Due to large volume of the estimated results, we selected few Z-test estimates to depicts the overabundance of dN over dS in four populations of Nigerian zebu cattle. For each codon, estimates of the numbers of inferred synonymous (dS) and non-synonymous (dN) substitutions are presented along with the corresponding mutation sites that are estimated to be synonymous (dS) and non-synonymous (dN). These estimates were produced using joint maximum likelihood reconstructions of ancestral states under a Muse-Gaut model (Muse, and Gaut, 1994) of codon substitution and Felsenstein 1981 model (Felsenstein, 1981). The test statistic  $dN - dS$  is used for detecting codons that have undergone positive selection and dS is the number of synonymous substitutions per site (dS) and dN is the number of non-synonymous substitutions per site (dN), a positive value for the test statistic indicates overabundance of dN substitutions (Kyazhimskiy and Plotkin, 2008). In this case, the probability of rejecting the null hypothesis of neutral evolution (P-value) is calculated (Kosakovsky and Frost, 2005; Suzuki and Gojobori, 1999). dS and dN values considered significant at  $P < 0.05$  (5%) and were selected (Table 2). Maximum Likelihood computations of dN and dS were performed using HyPhy software package (Sergei *et al.*, 2005). The analysis involved 16 nucleotide sequences. All positions containing gaps and missing data were eliminated. There was a total of 171 positions in the final dataset. Evolutionary analyses were performed using MEGA5 (Tamura *et al.*, 2011).

From our study, Darwinian selection pressure ( $w$ ) was estimated by codon-based Z test using the Suzuki and Gojobori method, and it revealed that at  $P < 0.05$ , dN mutations/base substitutions were abundantly greater than dS substitutions/mutations across the codons of *HSP 90* in the four cattle populations. This implies that *HSP 90* gene might have evolved through positive selection ( $dN > dS$ ) across the populations of four Nigerian zebu cattle, the

sweeping effect of dN in these populations is suggestive of changes in protein product occasioned by nucleotide substitutions possibly aid positive effect of the fitness on the animals' response to assault of thermal pressure (Onasanya *et al.*, 2019; Gade *et al.*, 2010). Our study showed a dN/dS ratio that was greater than across the four population cattle studied. A dN/dS ratio of less <1 is an indication of negative selection or purifying selection, thus indicating selective purgatory removal of deleterious alleles/gene from the population and a dN/dS ratio >1 indicates positive selection suggesting changes in protein product which is occasioned by single nucleotide changes/substitutions resulting in biologically positive effect on fitness of the animals (Onasanya *et al.*, 2019; Kyazhimskiy and Plotkin, 2008), and dN=dS indicate neutral evolution which implies that fitness/evolution/adaptation is not caused by natural selection but by random genetic drift.

**Table 2. Codon-based Estimates Showing Abundance of dN over dS Across Four Nigerian Zebu Cattle**

S/N	Mutation sites	Condon	dS*	dN*
1	37	GGC	6.070	7.971
2	40	AAG	5.163	7.583
3	49	ACC	4.665	5.872
4	52	ATG	3.540	4.052
5	55	CGT	3.111	5.802
6	61	GAC	3.319	5.646
7	64	CAG	2.947	4.036
8	67	GGC	4.284	5.739
9	70	AAC	3.524	4.886
10	73	CGC	2.823	2.625
11	76	ACC	3.401	5.435
12	79	ACC	2.818	4.645
13	82	CCC	2.890	3.675
14	85	AGC	3.873	3.550
15	88	TAC	2.061	4.761
16	91	GTG	2.968	2.514
17	94	GCC	4.886	5.205
18	97	TTC	2.699	3.043

*Academia Letters*, May 2022

©2022 by the authors – Open Access – Distributed under CC BY

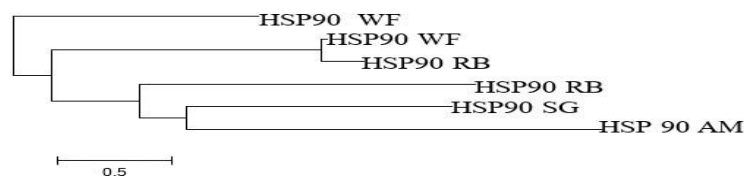
**Corresponding Author:** Dr Gbolabo Onasanya, onasanya.gbolabo@gmail.com

**Citation:** Onasanya et al., (2022). Evolutionary Divergence and Rates of Base Substitutions Across Four Populations of Nigerian Zebu Cattle at Heat Shock Protein 90 Locus. *Academia Letters*, Article 5423. <https://doi.org/10.20935/AL5423>

19	100	ACC	2.724	3.161
20	106	ACC	1.787	2.718
21	109	GAG	2.760	3.171
22	112	CGG	1.199	2.945
23	115	CTC	1.237	2.672
24	136	ATC	2.498	4.026
25	139	GGG	2.755	3.359
26	142	GAT	1.612	1.766
27	148	GCC	2.058	3.193
28	151	AAG	2.079	4.124
29	154	AAC	4.247	4.287
30	157	CAG	2.759	2.909
31	172	AAC	1.796	1.736
32	175	CCG	2.823	3.648
33	178	CAG	2.026	3.649
34	181	AAC	1.185	2.969
35	184	ACG	1.568	2.837

\* $P < 0.05$

The evolutionary history was inferred using neighbour-joining dendrogram of *HSP* 90 sequences of WF, AM, SG and RB Zebu cattle and it showed shared cluster/architecture, thus they have history of common ancestral lineage (Onasanya *et al.*, 2019).



**Fig. 1** Neighbor joining dendrogram of *HSP* 90 sequences showing ancestral relationship among White Fulani, Ambala, Sokoto Gudali and Red Bororo Nigerian zebu cattle

## Conclusions

We concluded that evolutionary divergence is evident among the breeds of Nigerian zebu cattle than within breed. These evolutionary potentials might be responsible for adaptation

*Academia Letters*, May 2022

©2022 by the authors – Open Access – Distributed under CC BY

**Corresponding Author:** Dr Gbolabo Onasanya, onasanya.gbolabo@gmail.com

**Citation:** Onasanya et al., (2022). Evolutionary Divergence and Rates of Base Substitutions Across Four Populations of Nigerian Zebu Cattle at Heat Shock Protein 90 Locus. *Academia Letters*, Article 5423. <https://doi.org/10.20935/AL5423>

/thermo-tolerance potentials of Nigerian zebu cattle. Therefore, at the mutation sites, overabundance of dN over dS across populations of Nigerian zebu cattle is evidence of positive selection ( $dN > dS$ ) at *HSP 90* locus, this suggests that changes in protein product occasioned by base substitutions/changes could have aided positive effect of fitness on the animals' response to thermal stress. The sequence diversity at the *HSP90* Locus of the Nigerian cattle breeds may be useful in the search for heat tolerance genotypes bearing these non-synonymous mutations (dS) might be responsible for thermo-tolerance potentials and adaptation of Nigerian zebu cattle to assaults of thermal conditions. Finally, we recommend further study to compare our findings with exotic cattle of temperate environment.

## Acknowledgement

The study was supported by TWAS-DBT Post-Doctoral Research Fellowship awarded to Dr. Gbolabo Olaitan Onasanya.

## References

- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Molecular Evolution*, 17:368-376.
- Golding, G. B. (1983), Estimates of DNA and Protein Sequence Divergence: An Examination of Some Assumption. *Mol. Biol. Evol.* 1(1):125-142.
- Kosakovsky Pond, S. L., and Frost, S. D. W. (2005) Not So Different After All: A Comparison of Methods for Detecting Amino Acid Sites Under Selection. *Mol. Biol. Evol.*, 22:1208-1222.
- Kyazhimskiy, S. and Plotkin, J. B. (2008) The Population Genetics of dN/dS. *PLoS Genet.*, 4(12): e1000304. doi: 10.1371/journal.pgen.1000304.
- Muse, S. V., and Gaut, B. S. (1994) A likelihood approach for comparing synonymous and nonsynonymous nucleotide substitution rates, with application to the chloroplast genome. *Mol. Biol. Evol.*, 11:715-724.

Onasanya, G. O., Msalya, G. M., Thiruvankadan, A.K., Sreekumar, C., Tirumurugaan, G. K., Fafiolu, A. O., Adeleke, M. A., Yakubu, A., Ikeobi, C. O. N. and Okpeku, M. (2021). Heterozygous Single-Nucleotide Polymorphism Genotypes at Heat Shock Protein 70 Gene Potentially Influence Thermo-Tolerance Among Four Zebu Breeds of Nigeria. *Front. Genet.*, 12:642213. doi: 10.3389/fgene.2021.642213.

Sergei, L., Kosakovsky Pond, S. D., Frost, W. and Spencer V. M. (2005). HyPhy: hypothesis testing using phylogenies. *Bioinformatics* 21:676-679.

Suzuki Y, Gojobori T. (1999) A method for detecting positive selection at single amino acid sites. *Mol. Biol. Evol.*, 16:1315-28.

Tamura, K., Nei, M. and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences (USA)* 101:11030-11035.