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Research Article
Genetic Diversity of Prolactin Gene in Two Strains of Japanese Quail (*Coturnix coturnix japonica*) in Nigeria

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Abstract

Background: Among poultry species, quail is the easiest to produce, yet no proper breeding strategy exist in Nigeria. However, data on production characteristics and genetic diversity among quail populations in Nigeria is scant. **Objective:** This study was carried out to investigate the genetic diversity, relationship and population structure in two Japanese quail strains (Albino and wild) using a restricted fragment length polymorphism marker in the prolactin (PRL) gene. **Methodology:** Fifteen quail from each strain were sampled in 5 geographical regions in Nigeria (Kano, Jos, Umudike, Port Harcourt and Ibadan). Polymerase chain reaction (PCR) and electrophoresis was used to characterize a 24 base pair (bp) insertion/deletion in a 358 bp PCR product. **Results:** The genetic variability using allele frequency, molecular variance, deviation from Hardy-Weinberg (H-W) equilibrium using the phylogenies package (PHYLIP) and analysis of molecular variance (AMOVA) were obtained. The frequency of insertion (A allele) was similar for both strains in the Ibadan, Jos and Umudike populations, however, the allele frequency was 0.73 and 0.50 for the Albino and Wild strains, respectively in Kano 0.57 and 0.70, respectively for the Albino and Wild strains in Port Harcourt. Whereas, there were no deviations from HWE for both strains, in Ibadan, Jos and Umudike, the populations in Kano and Port Harcourt deviated from H-W equilibrium. The AMOVA analysis showed 4.04% population difference, 1.17% variation among individuals and 94.25% within individuals. **Conclusion:** Prolactin is an important gene for reproduction and it's segregation could be assessed for reproductive capacity. The delineation of genetic diversity in these populations allows for innovative selective breeding and conservation strategies to be developed.

Key words: Prolactin, diversity, Japanese quail, conservation, selection

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The domestic quail (*Coturnix coturnix japonica*) has been used for biological and genetic studies. This is because of their small body size, short generation interval, high egg production and resistance to diseases[1]. Prolactin (PRL) is a peptide hormone secreted by specialized cells in the anterior pituitary gland in vertebrates. Reports have shown that PRL plays vital role in the onset of incubation and brooding behaviour[2-5]. A sudden increase in the level of circulating PRL extends the interval between the inter sequence pause between egg laying[6], leading to a decreased egg production[7]. It plays important role in the regulation of the activities of the gonad and immune responses in some species. The PRL also play roles in crop-sac development in columbi forms, induction and maintenance of brooding behaviour[8], suggesting that these loci might serve as potential genetic markers for breeding and also provide basic information into the regulation of PRL gene expression in various species of poultry.

Molecular markers have been proved to be important and effective tool in the characterization and evaluation of genetic resources, they provide useful information about the genetic variation both within and among species, strains and populations in key areas of conservation. The genetic diversity among and within the two strains (the Wild and the Albino strains) makes them potential donors of genes for breeding, selection and conservation purposes. While, studies have reported the use of morphological approaches to the study of polymorphism of cPRL gene[9,10], the correlation between prolactin and egg performance[9], genetic species or strain diversity[11], information on strain effect and environmental effect on genetic diversity is scant. The use of molecular analysis is important as it provides complementary information and increases the effectiveness of genetic diversity analyses[12]. In this study it is hypothesized that (1) There is no genetic differentiation in quails between geographical locations (2) There is no genetic differentiation among the strains and within the geographical locations in quails and (3) Sampling location/site has no effect on PRL gene.

MATERIALS AND METHODS

Sample size and blood collection: Blood samples were randomly collected from 150 Japanese quail birds comprising of two strains: Wild mottled and Albinos. Fifteen quails of each of the two strains (Albino and Wild) were sampled from 5 different regions in Nigeria, Kano, Jos, Umudike, Port Harcourt and Ibadan, for PRL loci analysis (Fig. 1). Blood were collected through jugular vein puncture into 2 mL vacutainer treated with K3-ethylenediaminetetra acetic acid (EDTA) and inverted several times to ensure proper mixing in order to prevent coagulation. Ethical permission was obtained prior to the sampling.

DNA isolation: Genomic DNA was extracted manually from 200 µL of individual blood samples using a commercial kit (Gene JET Whole Blood Genomic DNA Purification Mini kit). The DNA yield was assessed and quantified using Nanodrop ND-1000 UV/Vis Spectrophotometer (Nanodrop Technologies, Inc., DE) and gel electrophoresis on 1% agarose. The DNA concentration was adjusted to (50 ng μL^{-1}).

PCR and electrophoresis procedure: Polymerase Chain Reaction (PCR) was carried out, using the Applied BiosystemsVeriti™ GeneAmp®thermocycler and the PCR Master Kit (Thermo Scientific). The kit of master mix consisted of 2.5 µL of 10x PCR buffer, 2 mM of $MgCl_2$ and 2 mM dNTPs (each). Each reaction mixture consisted of 12.5 µL of the master mix, 0.5 U μL^{-1} of Taq DNA polymerase, 1 µL of the DNA solution (50 ng μL^{-1}), 1 µL of each primer (5 pmol μL^{-1}) and some deionized water to make up to 25 µL in PCR tubes and positioned in the interchangeable blocks of 96 wells of 0.2 mL. Amplification of PRL gene fragment (130 or 154 bp, containing the 24 bp indel at np 358) using the thermo-cycler ABI9700 was carried out using primers described by Cui *et al.*[9], PRL-F (5'-TTT AAT ATT GGT GGG TGA AGA GAC A-3') and PRL-R (5'-ATG CCA CTG ATC CTC GAA AAC TC-3') with an initial incubation and enzyme activation of 94°C for 5 min; followed by 35 cycles of 30 sec at 94°C, 30 sec at 61°C and 45 sec at 72°C and a final extension of 5 min at 72°C. The PCR-products of the 24 bp was run on 6% polyacrylamide gel, staining was done using silver nitrate (Fig. 2).

Statistical analysis: The statistical analysis involved the use of different software packages designed for the analysis for molecular data. Basic measures of genetic diversity, such as total number of alleles, allele frequencies, mean number of alleles and polymorphism information content (PIC), PRL loci informativeness in relation to expected heterozygosity were computed using[13] Powermaker version 3.25. POPGENE version 1.31 software[14] was used to determine Hardy-Weinberg equilibrium and heterozygosity. Genetic distance and gene flow values were calculated using GENPOP software[15] version 4.13. Genetic distance and identity was obtained according to the method[16]:

$$Ds = (1-J_{xy})-1/2[(1-J_x)+(1-J_y)]$$

$$Ds = In\ (J_{xy}/\sqrt{J_xJ_y})$$

Fig. 1: Map of Nigeria showing the 5 regions of Nigeria from which Japanese quails were sampled for use in this study

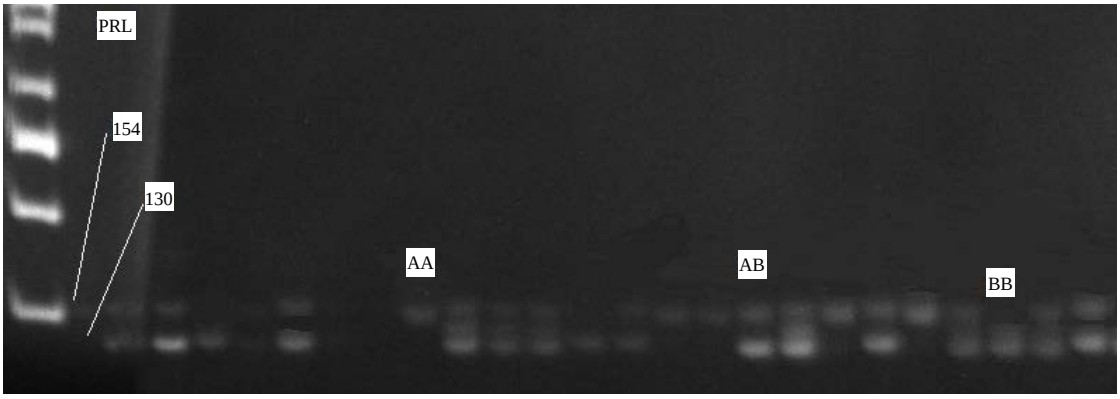

Fig. 2: Gel pictures of primers on 6% polyacrylamide gel electrophoresis (PAGE)

Where:

$J_x = (2n_x\Sigma x_i^2-1)/(2n_x-1)$

$J_y = (2n_y\Sigma y_i^2-1)/(2n_y-1)$

$J_{xy} = \sum_{xy}$

n = Population size

Analysis of molecular variance (AMOVA) was done using Arlequin software[17] version 3.0. The PHYLIP version 3.69 software program[18], was used to constructed a phylogenetic tree based on Nei genetic distance, using the neighbour joining tree method[19].

RESULTS AND DISCUSSION

A total number of 278 alleles were observed at the PRL gene loci. The PIC observed between the strains ranged from (0.32) Kano Albino to (0.37) Ibadan Albino populations. The (observed heterozygosity) Ho among populations ranged from 0.27-0.69 at Kano Albino and Ibadan Albino populations. The farthest genetic distance was recorded between 0.003 (Jos Albino) to 0.14 (Kano Wild) while the closest was between 0.009 (Ibadan Albino) and 0.003 (Jos Albino), respectively. Among populations F_{IS} ranged between -0.29 (Jos Albino) to 0.32 (Kano Albino), indicating moderate genetic differentiation among populations. Chi square result indicated that the populations were not in Hardy-Weinberg equilibrium. Phylogenetic relationships revealed two major clusters with Jos branching off, Port Harcourt and Kano clustering at point one while Umudike and Ibadan clustered at point two but all two clusters originating from a source. Clusters from the combine population suggest that PRL gene is based on individual genotype and not location. Analysis of molecular variance indicated that 4.04% of the total genetic variation was explained by population difference 1.71% by variation among individuals and 94.25% within individuals. The result suggest that study of prolactin gene polymorphism may be used as basis for decision making for selection, breeding and conservation programmes. Based on present results two alleles, [insertion (A) or deletion (B)] and three genotypes, namely, AA, AB and BB were observed in the population. The observed frequencies of alleles and genotypes for the PRL gene are shown in Table 1. The allele frequencies and genotypes of Japanese quail in Nigeria as well as gene diversity are as shown in Table 1. Heterozygosity values were calculated to determine the level of genetic variation within the populations. Results is as shown in Table 2.

The PIC values were calculated by the algorithm:

$$PIC = 1-\Sigma^{n}pi^{2}$$

Where:

I = 1

pi^2 = Frequency of the ith alleles

Paczos-Grzeda *et al.*[20] reported that polymorphism largely depends on the species, degree of differentiation of the tested materials and the primer.

In this study, the genetic variability was detected through PRL marker. The aim of the study was to explore genetic diversity with respect to PRL gene within and between the two strains of Japanese quails in Nigeria using PRL marker and to determine the phylogenetic relationship between them.

Allele polymorphism and polymorphic information content: The efficiency of PRL was determined both as the amount of polymorphism and Polymorphic Information Content (PIC) coefficient. The Polymorphic Information Content (PIC) between the two strains ranges between 0.35 and 0.38. In the majority of the study populations PRL marker was reasonably informative (Table 3). According to classification by Botstein *et al.*[21], the highly informative markers have PIC values >0.50, the reasonably informative markers have PIC

Table 1: Summary of alleles and genotypes frequency for Hardy-Weinberg equilibrium between strains (Albino A) and (Wild W) populations for PRL gene of Japanese quails in Nigeria

		Allele frequency (%)		Genotype frequency (%)		
Gene	Types	A	B	AA	AB	BB
Ibadan	A	0.42	0.58	0.08	0.69	0.23
Ibadan	W	0.39	0.61	0.14	0.50	0.36
Jos	A	0.46	0.54	0.14	0.64	0.21
Jos	W	0.50	0.50	0.29	0.43	0.29
Kano	A	0.73	0.27	0.60	0.27	0.13
Kano	W	0.50	0.50	0.25	0.50	0.25
Port Harcourt	A	0.57	0.43	0.33	0.47	0.20
Port Harcourt	W	0.70	0.30	0.47	0.47	0.07
Umudike	A	0.31	0.69	0.08	0.46	0.40
Umudike	W	0.39	0.61	0.21	0.36	0.43

A: Insertion allele, B: Deletion allele, AA: Insertion-insertion, AB: Insertion-deletion, BB: Deletion-deletion

Table 2: Summary of No. of alleles (Ne), heterozygosity chi-square (χ^2) for Hardy Weinberg Equilibrium (HWE) tests and Wright[37] fixation index (F_{IS}) between two strains of Japanese quails in Nigeria

Gene	Location	Types	Ne	Observed heterozygosity	Expected heterozygosity	GD	F_{IS}	χ^2	HWE
PRL	Ibadan	A	1.99	0.62	0.52	0.009	-0.24	0.51	NS
	Ibadan	W	1.91	0.50	0.49	0.000	-0.05	0.00	*
	Jos	A	1.99	0.64	0.52	0.003	-0.29	0.91	NS
	Jos	W	2.00	0.43	0.52	0.000	0.14	0.45	NS
	Kano	A	1.64	0.27	0.40	0.003	0.32	1.95	NS
	Kano	W	2.00	0.50	0.52	0.138	0.00	0.02	*
	Port Harcourt	A	1.97	0.47	0.51	0.108	0.05	0.11	NS
	Port Harcourt	W	1.72	0.47	0.43	0.022	-0.11	0.09	NS
	Umudike	A	1.74	0.46	0.44	0.009	-0.08	0.03	*
	Umudike	W	1.91	0.36	0.49	0.043	0.25	1.17	NS

*p<0.05, NS: Non-significant, Ne: Effective No. of alleles, χ^2: Chi-square value, GD: Genetic distance

Table 3: Major Allele frequencies, gene diversity, observed heterozygosity and Polymorphic Information Content (PIC) between two strains (Albino A) and (Wild W) populations of Japanese quails in Nigeria

Gene	Regions	Types	Major allele frequency	Gene diversity	Heterozygosity (Ho)	PIC
PRL	Ibadan	A	0.5769	0.5769	0.6923	0.3690
	Ibadan	W	0.6071	0.4770	0.5000	0.3633
	Jos	A	0.5357	0.4974	0.6429	0.3737
	Jos	W	0.5000	0.5000	0.4286	0.3750
	Kano	A	0.7333	0.3911	0.2667	0.3146
	Kano	W	0.5000	0.5000	0.5000	0.3705
	Port Harcourt	A	0.5667	0.4911	0.4667	0.3705
	Port Harcourt	W	0.7000	0.4200	0.4667	0.3318
	Umudike	A	0.6923	0.4260	0.4615	0.3353
	Umudike	W	0.6071	0.4770	0.3571	0.3633

A and W represents the strains, A: Albino, W: Wild, PIC: Polymorphic information content

value between 0.25-0.50 and the slightly informative markers have PIC value <0.25. However, lower PIC values have been reported by Kayang *et al.*[22] than those obtained in this experiment while, Amirinia *et al.*[11] recorded PIC values between 0.427 in Panda strain and 0.815 in Golden strain in a study using four strains of Japanese quail. Various PIC values have also been recorded in many chicken studies. Zhou[23] recorded 0.523-0.702 in a study of 19 Chinese native chicken breeds. Yeh *et al.*[14] also reported values between 0.560-0.641 in a study using 12 indigenous chicken populations in Southern China. In Turkish native chicken breeds, Kaya and Yildiz[24] reported 0.426-0.599 and Davila *et al.*[25] reported values ranging from 0.172-0.847 in a study with 13 Spanish chicken breeds population.

Allele frequencies and Hardy-Weinberg equilibrium: In the present study, two alleles and three genotypes were detected. Present results are in agreement with previous report by Yousefi *et al.*[26] and Lotfi *et al.*[5]. The allele frequencies among populations ranged from A: 0.31-0.73 and B: 0.27-0.69 respectively (Table 1). Present result is in agreement with previous report by Lotfi *et al.*[5], Yousefi *et al.*[26] and Emamgholi-Begli *et al.*[27] that reported two alleles (I = 0.76 and D = 0.24) in native hens and Japanese quail (I = 0.52, D = 0.48), while Alipanah *et al.*[28] also observed two alleles T and C with frequencies 0.67 and 0.33 in chicken, respectively. Ya-Bo *et al.*[29] observed two alleles (I and D) and three genotypes (II, ID and DD) for PRL gene in chicken. The frequencies of alleles I and D were reported in native and commercial chickens in a study by Cui *et al.*[9]. In their study, the frequencies of alleles I and D were reported to be 1 and 0 in White Leghorn, 0.05 and 0.95 in Yangshan, 0.20 and 0.80 in Taihe Silkies, 0.22 and 0.78 in White Rock and 0.17 and 0.83 in Nongdahe. In addition, Li *et al.*[30] studied polymorphism of PRL gene in duck and also found two alleles (A = 0.226 and B = 0.774) and three genotypes AA, AB and BB. Jiang *et al.*[4] in a study of PRL gene also observed two alleles A and B with frequencies 0.59 and 0.41 in Rhine geese while, Wan-xi White recorded 0.73 and 0.27 frequencies.

Based on individual populations from five regions in Nigeria as shown in Table 1, the allele frequency for A; Kano Albino and Port Harcourt Wild populations had the highest frequencies of 0.70 and 0.73 while, Umudike populations and Ibadan wild had the least frequency of (0.31 and 0.35) respectively for allele B; Umudike Albino population (0.69) had the highest while Kano Albino population (0.27) had the least frequencies respectively. The genotype frequencies obtained at the 24 bp indel site of prolactin gene were AA 0.27, (insertion deletion) AB 0.48 and (deletion deletion) BB 0.26. The frequency of heterozygous genotype was higher (AB 0.48) compared to homozygous (AA 0.27) and (BB 0.26) genotypes among population as shown in Table 1. This result agrees with that reported by Lotfi *et al.*[5] and Yousefi *et al.*[26] in Japanese quail with heterozygote genotypes frequencies ID 0.85. Rashidi *et al.*[31] also reported heterozygote frequency of ID 0.40 respectively in Iranian indigenous breeder hens. Among the populations, genotype AB had the highest (0.69) frequency in Ibadan population, while Kano had the least (0.27).

The result indicated that the populations were not in Hardy-Weinberg equilibrium for this region of the PRL gene (Table 2). This result is in line with that reported by Granevitze *et al.*[32] on microsatellite study in 64 chicken populations from different continents. Across the strains, the Albino populations at Ibadan Jos and Umudike showed significant deviation from HWE under the heterozygote excess assumption while Kano showed deviations under heterozygote deficit assumption, Port Harcourt was in Herdy-Weinberg Equilibrium. In the Wild populations; Ibadan and Port Harcourt showed significant deviations under heterozygote excess assumption while Jos, Kano and Umudike showed deviations under heterozygote deficit assumption. These resultssuggest that the Albino strain and wild strains were generally not in HWE except for Port Harcourt Albino

population (Table 2). This was in agreement with Amirinia *et al.*[11] who reported that for the four strains of Japanese quail studied in Iran, all locus-strain interaction deviated from Hardy-Weinberg equilibrium except in Pharach strain. Davila *et al.*[25] also reported that some Spanish chicken breeds showed significant deviations from Hardy-Weinberg equilibrium, suggesting that selection has been carried out for many years on morphological traits such as plumage. Non-random mating, mutation and migration, the presence of null alleles or genotyping error may also be factors affecting the population structure. Under the heterozygote deficit assumption, the deviations from HWE ($p<0.05$) from the overall population might be due to excess of individual migration, less mutation rate in PRL loci and less artificial selection and random breeding of birds in the population based on the study PRL gene. The heterozygote excess may be indicative of high number of heterozygote than homozygote individual migration, high mutation rate in PRL loci and reduced artificial selection and non-random breeding in the population[33] based on PRL gene. Populations from Kano and Umudike that showed significant deviations from HWE under the heterozygote deficit may be indicative of high level inbreeding and non-random mating in those populations. According to the history of the studied lines, the lines were under selection for different economic traits and thus the marker was informative to determine the genetic variation in different populations of Japanese quail effectively with respect to PRL gene.

Gene diversity and heterozygosity estimate: Takezaki and Nei[34] recommended that marker should be in the range of 0.3-0.8 in a population in order for it to be useful for measuring genetic variation. Gene diversity among populations ranged from 0.46-0.59 and 0.27-0.69 for the two strains respectively showing that this marker is useful for measuring genetic variation of PRL gene in Japanese quail. These values were higher compared to those reported by Zhou *et al.*[23].

The average direct gene count among the population was less than the expected heterozygosity. The observed heterozygosity (Ho) values ranged from 0.27-0.62 while the expected (He) value ranged from 0.42-0.52, respectively. When compared to other studies, the observed heterozygosity of Japanese quail in Nigeria was 0.47 which is higher than that reported by Jiang *et al.*[4]. The estimated heterozygosity suggests the presence of variation in the studied Japanese quail population. High values of observed heterozygosity (0.62) within the populations may be attributed to the number of allele detected in the tested loci. The gene diversity in the Albino population ranged from Kano (0.39) to Ibadan (0.57) while that of the Wild population ranged from Port Harcourt (0.43) to Kano and Jos (0.50) respectively. Ho recorded from the populations were 0.69 (Albino) and 0.50 (Wild) which were generally above average. The gene diversity values falls within the recommended range for measuring genetic variation and are therefore suitable for further use in the study of PRL gene.

The less observed heterozygosity in Kano, Port Harcourt, Umudike and wild Jos populations may be attributed to the level of inbreeding in the case of inbreeding the deficit affects all or most of the loci in a similar way. Wahlund effects may contribute to less heterozygosity, due to the presence of substructure within the populations, genotyping errors may be a contributing factor and the presence of null allele may also contribute to deficit heterozygote in the population, genetic drift, less mutation in PRL gene, selective mating within the population, small population size (population bottleneck or population dynamics that severely reduces the level of genetic variation related to that expected. High heterozygosity values in this study as recorded in the Albino population in Jos and Ibadan populations, may be an indicator of higher reproductive characteristic, it may also implies the presence of Isolate-break effect (mixing of two previously isolated populations) and the presence of store of genetic diversity irrespective of the low level of differentiation within close relatives. This agrees with the prediction of Handley *et al.*[35].

Genetic differentiation: Wright's F-statistics and other similar indices that describe the partitioning of genetic variations at different levels can be estimated for natural populations using molecular marker data[16]. The F statistics value F_{IT} and F_{ST} are measure of deviation from HWE proportion and total population. Positive values indicate a deficiency in heterozygotes while negative value indicates an excess of heterozygotes. The F_{IS} can be interpreted as a measure of inbreeding. Positive F_{IS} shows the deficiency of heterozygote in the population and the level of relationship between mates in compares with the average relationship of the population. Across the two strains, the Albino populations; Ibadan Jos and Umudike showed significant deviation from HWE under the heterozygote excess assumption while Kano showed deviations under heterozygote deficit assumption, Port Harcourt was in Herdy-Weinberg Equilibrium. In the wild populations; Ibadan and Port Harcourt showed significant deviations under heterozygote excess assumption while Jos, Kano and Umudike showed deviations under heterozygote deficit assumption. The estimated F_{ST} value, which

correspond with the amount of genetic variability accounted for by the difference among the strains and to an extent quantified by the marker may be due to proximity, breeding practise, the similarity in environment and to a large extent due to past gene flow among them. The genetic differentiation may also be due to mutation, genetic drift, selection, sampling error, genetic bottleneck of severe reduction in population size, small re-occurring population size, inbreeding, non random breeding, selective mating among individuals within the population, freely interbreeding, migration pattern and wahlund effect in the PRL gene.

Genetic distance estimate and phylogenetic relationship: Genetic distance was calculated using Nei[16] genetic distance method to evaluate inbreeding relationship among the two strains of Japanese quail in Nigeria. The bias due to unequal sample size was corrected using the bootstrap procedure by Simianer[36]. The results from Nei[16] genetic distance suggest that there were considerable distances among the populations (Table 2).

The neighbour joining tree among populations also indicates two main branching from the ancestral root, Phylogenetic relationships revealed two major clusters, however some sub-clusters were also observed, with Kano Albino population and Port Harcourt wild branching off to cluster at point one while the second branch had two sub-clusters respectively. The closest cluster was observed between Jos and Kano wild populations, Ibadan and Jos Albino populations and Ibadan and Umudike wild populations, respectively. The farthest distance was recorded between wild Jos populations and wild Port Harcourt populations. The phylogenetic relationship among all the Albino populations also revealed two main branching, from the root (Fig. 3) with Port Harcourt population branching off while Umudike on the other hand clustered with Jos, Kano and Ibadan respectively. The closest distance was recorded between Ibadan and Kano while the farthest distance was between Ibadan and Port Harcourt respectively. Among the wild populations two main branching were also observed (Fig. 4), with Jos branching off from the root while the other branch had two clusters. Kano and Port Harcourt sub-clustered at one point while Ibadan and Umudike sub-clustered on the second branch. The closest distances were recorded between Kano, Port Harcourt, Ibadan and Umudike which indicates that they were all not genetically different from one another. Across individual populations, the phylogenetic relationship revealed high similarity between the strains in Ibadan, Jos, Kano, Port Harcourt and Umudike. The closest distance was observed in Jos population while, the farthest distance was observed in Kano and Umudike population, respectively. The degree of closeness shown in clusters among populations Populations did not follow uniform clustering as shown in Fig. 5. Based on neighbour-joining tree clustering assessment high level of similarities between populations may be explained considering that they are characterized by a common breeding system. This may be indicative that the variability of PRL gene in Japanese quail in Nigeria is not affected by geographical location rather on individual genotypes.

Analysis of molecular variance (AMOVA): The AMOVA provided an estimate of the measure of population genetic differentiation within and between populations. The AMOVA carried out for the PRL data (Fig. 4), suggests that only 4.04% of the total genetic variation was explained by population difference 1.71% by variation among individuals and 94.25% within individuals. The hierarchical analysis of variance revealed an F_{ST} value of significant ($p<0.001$) Indicating the presence of genetic differentiation in the population. Variance components within individuals were highly significant ($p<0.001$). The result from AMOVA and pairwise computation

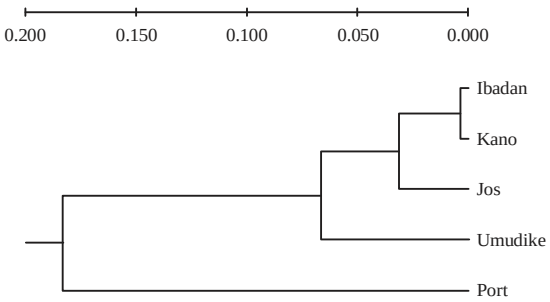

Fig. 3: Neighbour-joining tree representing all Albino populations constructed using Nei's genetic distance (1978)

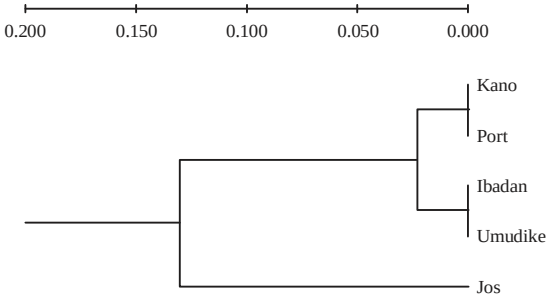

Fig. 4: Neighbour-joining tree representing all Wild populations constructed using Nei's genetic distance (1978)

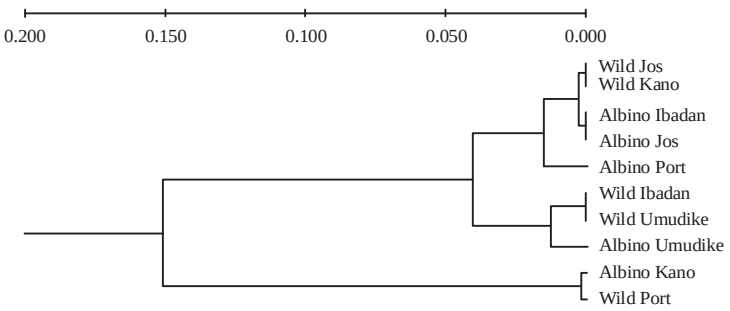

Fig. 5: Neighbour-joining tree between strains (Albino and Wild) across populations constructed using Nei's genetic distance (1978)

Table 4: Summary of analysis of molecular variance between two strains of Japanese quails in Nigeria

Source of variation	Degree of freedom	Sum of squares	Estimation of variance	Percentage of variation	F statistics (F_{ST})
Among populations	9	4.757	0.010	4.04	0.04
Among individuals	129	31.739	0.004	1.71	
Within individuals	139	33.000	0.237	94.25	
Total	277	69.496	0.252		

indicates differentiation relative to a random collection of genotypes and reflects differences in the spatial distribution in genetic variation. Wright[37] reported similar results of higher variations within populations. This suggests that PRL gene is based on individual genotype and not determined by location (Table 4).

CONCLUSION

Results from this study suggest that reasonable genetic differentiation exists among the strains within the populations. This study also reveals that there were no isolate rather all the populations were from the same decent. In addition, the results from this study can provide basic information for decision making for selection, breeding and conservation programs as the results from AMOVA suggests that breeding and selection would be more profitable within the populations. Furthermore, prolactin is an important gene for reproduction and segregation of the RFLP marker could be assessed for reproductive capacity. The delineation of genetic diversity in these populations allows for innovative selective breeding and conservation strategies to be developed.

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