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Genetic Characterization of Different Plumage Varieties of Noiler Chicken

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Abstract

This study investigated the genetic characteristics of Noiler chickens of five different plumage varieties: brown, barred, black, black-white, and brown-white. A 195-day-old noiler chicks were procured from a reputable hatchery and distributed into triplicate groups, with 13 birds per plumage variety. The chickens were raised at the Teaching and Research Farm of Adekunle Ajasin University, following standard management practices and fed a commercial diet ad libitum. At the 10th week of the study, 2 ml of blood samples were randomly collected from 10 birds per plumage variety for laboratory analysis. Protein separation was performed using the Bio-Rad Mini Protean II Cell electrophoresis system. The analysis revealed distinct protein bands, including myosin, bovine serum albumin, carbonic anhydrase, β-galactosidase, ovalbumin, and α-lactoglobulin. The detected bands were analyzed using the Paleontological Statistics software, and the resulting dendrograms illustrated the genetic similarities among the different plumage varieties. Notably, the brown and brown-white noiler chickens exhibited the closest genetic relationship.

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Introduction

Amo Hatchery, also known as Amo Farm Sieberer Hatchery Limited, developed the Noiler chicken breed in Nigeria [1]. The primary breeding objective of Noiler chickens is to create an improved breed that can replace indigenous chickens, which are known for their low-quality meat and egg production [2]. Noiler chickens perform well under various production systems and demonstrate adaptability to poor feed conditions and suboptimal nutrition [3]. Feathers are sclerotic structures with diverse developmental pathways, representing highly specialized integumentary appendages that have been the subject of extensive research for over 150 years [4]. Recent advancements in genome-wide association studies, evolutionary developmental research, and phylogenetic comparative analyses have provided new insights into the evolutionary history and genetic mechanisms governing plumage variation [5].

In poultry, particularly chickens, plumage colour serves as a genetic marker for identification due to its distinct characteristics [6]. This is because, in contrast to human, birds recognize their relatives visually, and interacting with them is crucial [7]. The feathers and non-feathered body parts, such as the beak and legs, are both covered in pigments that contribute to bird diversity. Three classes of pigments are identified in birds that contribute to changes in plumage colour: carotenoids, melanin, and unique pigments, porphyrin [6]. According to Abeyrathne et al. [8], plumage variance may be used in the genetic classification of chickens. Allele characteristics from the particular locus of bodily tissue, such as blood, egg white, and yolk, can reveal the genetic diversity [9]. Blood contains distinct protein loci [9]. Meanwhile, Sodium dodecyl sulphate polacrylamide gel electrophoresis (SDS-PAGE) is currently one of the most widely used electrophoretic techniques for protein analysis [9]. This is because a strong anionic detergent, SDS, may solubilize, denature, and dissociate the majority of proteins into single polypeptide chains when combined with chemicals that cleave disulfide bonds, like β-mercaptoethanol or Dithiothreitol (DTT) [10]. Limited research has been conducted on the genetic basis of plumage variation in noiler chickens. However, this called for comprehensive research, aimed at assessing the genetic relationship of noiler chicken of different plumage varieties.

Materials and methods

A 195-day-old noiler chicks were obtained from Amo Farm Sieberer Hatchery, Ibadan, Oyo State, Nigeria. The chicks were randomly distributed into triplicate groups, with 13 birds per plumage variety. The birds were fed *ad libitum* without restrictions on light intensity for the periods of the experiment. The study was carried out at the Teaching and Research farm of Adekunle Ajasin University, Akungba Akoko, having a mean annual rainfall of 1500-2000 mm and the average temperature ranging between 18°C and 35°C. Uniform plumage patterns were replicated; each of the plumage were housed in a wooden cage of 0.4 m by 0.3 m in a well-ventilated area.

At the 10th week, 10 birds were randomly selected from each plumage variety, and 2 ml of blood samples were collected from the wing vein of each bird using syringes. The blood samples were taken to the laboratory for analysis. Serum was separated from the whole blood after centrifugation at 4000 rpm. The supernatant from the serum sample was carefully transferred into a clean 2 ml Eppendorf microtube and stored at −4°C. The sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis was carried out using the Bio-Rad Mini Protean II (10 ml capacity). The stacking gel contained distilled water (6.1 ml), 0.5 M Tris-HCl at pH of 6.8 (2.5 ml), 10 percent SDS (100 µl), acrylamide/bisacrylamide solution (1.3 µl), 10% ammonium tetraoxosulphate (vi) (50 µl) and Initiator or N, N, N', N'-Tetra methylethylenediamine (TEMED) ($C_6H_{16}N_2$) (10 µl). B-mercaptoethanol (7.5%) in the sample buffer was used for the preparation of the various plumage varieties. The serum and sample buffer were added at a ratio of 1:2 and heated at 95°C for 5 min in a water bath. The mixture was placed inside the deep freezer for 5 min and then loaded into the wells on the gel. The separation of protein was carried out with the aid of a Bio-Rad Electrophoresis system using the Bio-Rad Mini Protean II Cell at 150 V for 2 h. The separating gel is composed of 0.375 M Tris at pH 8.8, distilled water (3.5 ml), 1.5 M Tris-HCl (2.5 ml), 10% SDS (100 µl), acrylamide /bisacrylamide (4.0 µl), 10% ammonium tetraoxosulfate (vi) (50 µl), and TEMED (5 µl).

After the electrophoresis, the gels were carefully removed under water and placed in a staining solution of 0.1% Coomassie Blue in 1:4 glacial ethanoic acid (CH_3COOH) and methanol (CH_3OH). The staining solution was later removed,

and the gels were de-stained with a solution of 40%t distilled water in 1:4 glacial ethanoic acid and methanol. The de-stained gels were then scanned, and the bands were visually scored [14]. The portion of the gel with bands was denoted as 1, while the area with the absence of bands was denoted as 0. A computer statistical package designed for protein electrophoresis data analysis, Paleontological Statistics (PAST), was adopted to develop the dendrogram produced by the unweighted pair method with arithmetic mean UPGMA [18]. The genetic distance was calculated using this formula: D=1-S. The D is the genetic distance, and S represents the similarity.

Results and Discussion

Using the molecular weight via SDS-PAGE of the serum protein analysis (Fig. 1), the comparative dendrogram for the noiler plumage varieties showed the polymorphic of the protein bands, including myosin, bovine serum albumin, Carbonic anhydrase, Beta-galactosidase, alpha-lactoglobulin, and ovalbumen. Comparing the protein bands with the blue pre-stained standard, all plumage varieties: brown, back, black-white, barred, and white-brown indicated a uniform abundance of myosin, galactose, albumin, ovalbumin, alpha-albumin, except for black, and black-white, which possess aprotinin and glutamic dehydrogenase in their protein bands (Fig. 1). Dendrogram of the Noiler chicken breed was differentiated based on the protein serum bands, utilizing the statistical software and Euclidean correlated distances among the various plumage varieties. The dendrogram showed the relationship and genetic distance of different plumage varieties via their serum protein.

Genetic similarities within the barred plumage variety as shown in Fig. 2. Samples 7, 3, 4, 5, and 8 were genetically similar with 0.0 genetic disparities; a pool of them was 1.0 genetically similar with sample 9. Samples 6, 2, 1, and 10 clades had a genetic distance of 3.1 from the initial clades. The genetic distance of 3.1 indicated some genetic divergence in barred plumage. Genetic distance among black plumage varieties showed that samples 7 and 9 were the most genetically similar. A pool of samples (7 and 9) with 8, (2 and 1), (3 and 5), and (4 and 6) had a genetic coefficient of 1.0. Pools from all the samples within black plumage had a genetic distance of 2.2 (Fig. 3). The SDS-PAGE for brown-white plumage revealed that samples (5 and 7), (4, 2, and 1), and (3, 6, and 8) had no genetic disparities, respectively. Meanwhile, the 10th sample from brown-white plumage was moderately close at 1.8 genetic distance to other samples (Fig. 4).

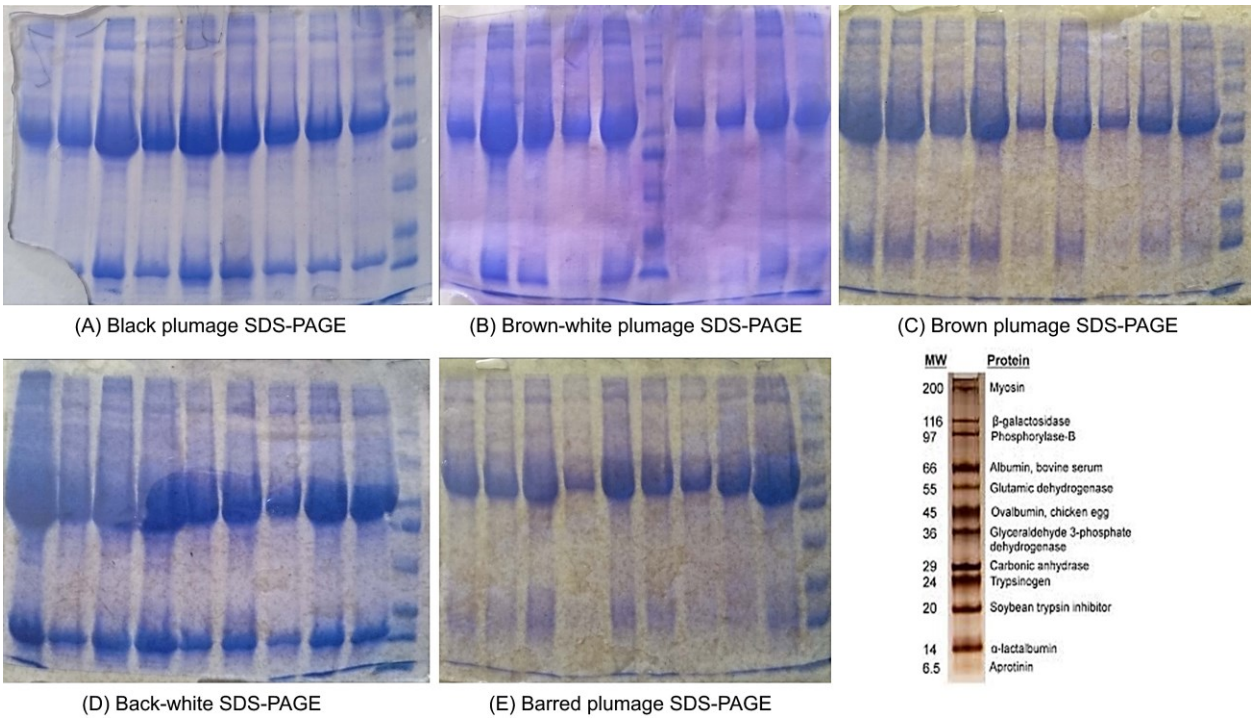

Fig. 1 SDS-PAGE analysis of five different plumage varieties: black (A), black-white (B), brown (C), black-white (D), and barrel (E).

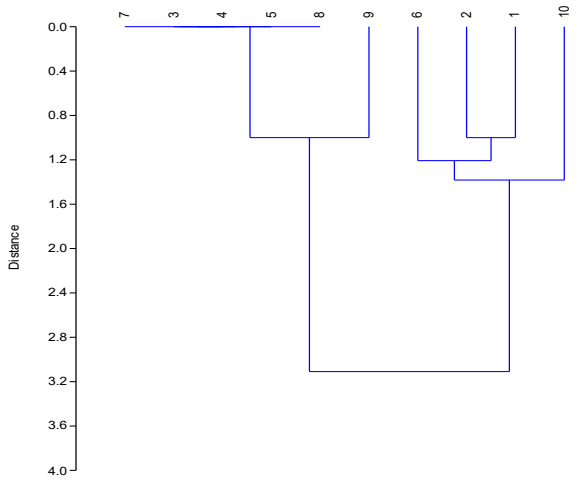

Fig. 2 Dendrogram of barred plumage variety chickens.

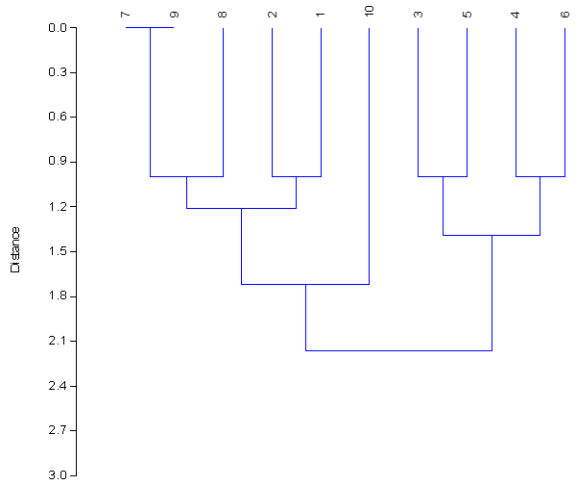

Fig. 3 Dendrogram of black plumage variety chickens.

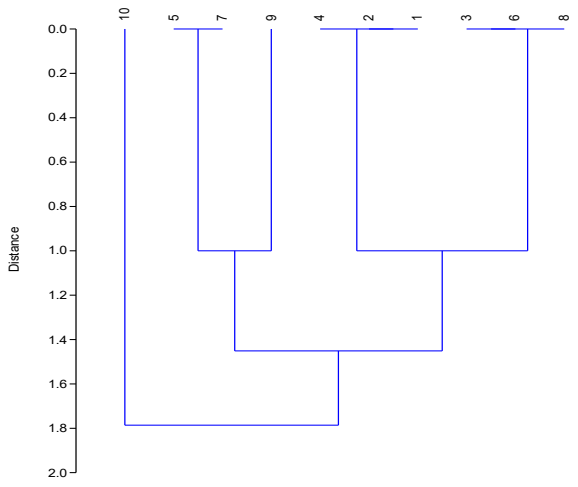

Fig. 4 Dendrogram of brown-white plumage variety chickens.

The genetic distance among brown plumage variety chickens showed that samples (2 and 3), and (5, 6, 7, and 8) had a 100% coefficient of similarity, respectively. Samples 4, 9, 1, and 10 were moderately close, having a final pool of 1.9 genetic similarities among the brown plumage variety chickens (Fig. 5). Phylogenetic tree for black-white plumage varieties chickens showed that samples (3, 4, and 5) were genetically similar, and a pool with sample 2 had a genetic distance of 1.0. Sample 1 was moderately similar at a genetic distance of 1.12 (Fig. 6). From the isolative comparison within each plumage variety of chickens, brown-white, black-white plumage had a more intimate genetic relationship and with the lowest genetic distance value as shown on SDS-PAGE Figs. 4 and 6, respectively.

A combined genetic similarity was further compared among the plumage varieties. Brown-white and brown plumage were observed to be more genetically close, with a genetic distance value of 0.08; black plumage shared the nearest genetic distance with the closest plumage varieties relationship of about 0.40. Barred plumage varieties showed the most genetic distance from other plumage varieties of 1.36 (Fig. 7). This emphasized that their ancestors are moderately related. The results indicated a very high level of genetic similarity and a general loss of genetic variation. The synthesis of different proteins of the Noiler chicken corresponds with different bands on the electrophoretic analysis, which are influenced by single or multiple polygenes. The observable trait of plumage variations in Noiler chicken can be used as a genetic marker, via the presence of different loci [11]. The presence of pigments may be attributed to genetic differences among species of chickens. Employing the Statistica software and Euclidean correlated distances among the various plumage varieties. The dendrogram showed the relationship and genetic distance of different plumage varieties via their serum protein. Each plumage variety showed a closed genetic distance similarity. This is perhaps a result of reduced heterozygosity, heterogeneity, low polymorphism, and introgression among breeds of chicken [10]. The degree of sympatry associated with plumage divergence could largely be attributed; climatic effects and some selective pressure within the shared population [12]. Ensminge [13] documented that plumage varieties had genetic and breeding functions to play other than camouflage and defense mechanisms, influencing the molecular composition. The results indicated the abundance distribution of myosin, bovine serum albumin, and carbonic

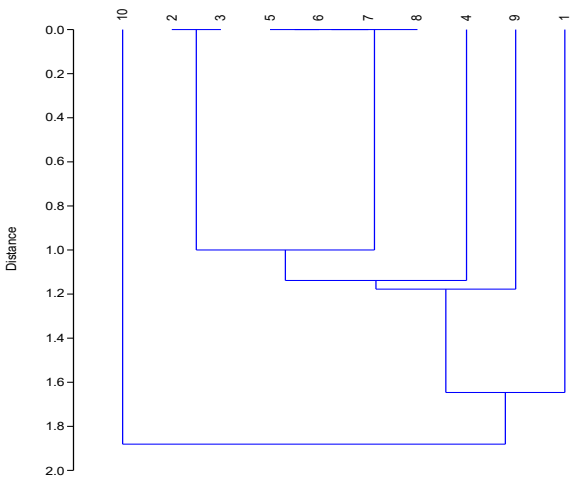

Fig. 5 Dendrogram of brown plumage variety chickens.

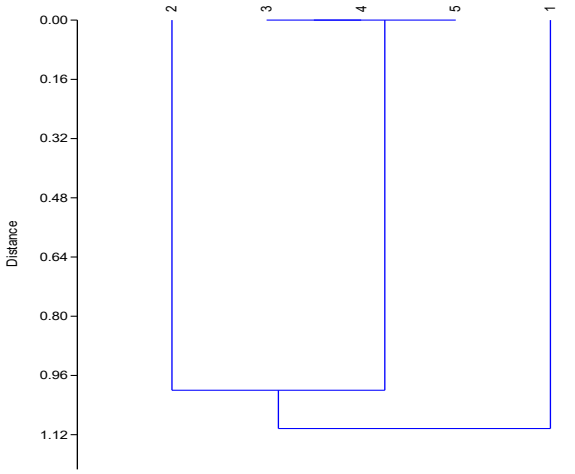

Fig. 6 Dendrogram of black-white plumage variety chickens.

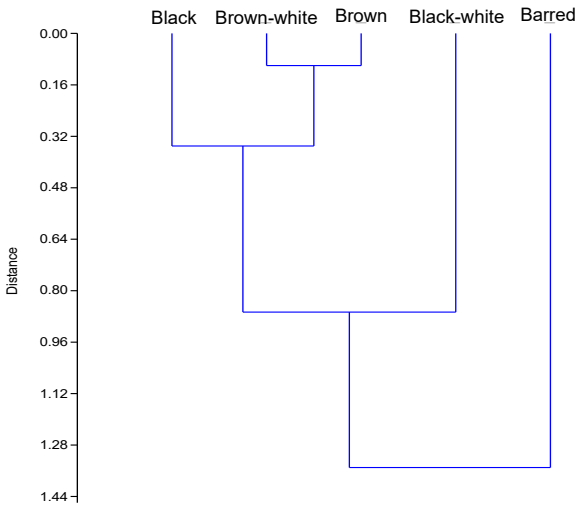

Fig. 7 Dendrogram of five Noiler plumage varieties.

anhydrase, Beta-galactosidase, ovalbumen, and alpha-lactoglobulin among Noiler chickens of different plumage varieties were considered in this research. From the obtained results, black-black-white plumage had a potential for a higher tendency for aprotinin and glutamic dehydrogenase in their protein bands, above other plumage varieties. Variations in the bands may have to do with the feather distribution gene that gives its characteristic phenotype [14]. Barred plumage type exhibited internal sub-clustering, likely due to multiple breeding lines being grouped under a phenotypic category. The overall genetic distance of 2.2 indicated moderate diversity within the black plumage variety. This observed diversity suggested a limited gene flow or selection. Brown-white plumage coloration is generally more diverse than black plumage coloration, with moderate internal differentiation, which can be linked to outcrossing in the breeding system. Final pool of 1.9 indicated a relatively homogenous but slightly more diverse than black plumage variety. The closeness suggested a stable genetic base with little recent divergence. The low genetic distance across the plumage varieties showed that there was a high level of inbreeding or narrow genetic bases within specific plumage varieties of the Noiler chicken.

Adeleke et al. [14], all Gallus and chicken populations formed a common cluster on a dendrogram, with various geographic populations of *G. gallus* occupying different domains, this was in-line with the results of this research in that, though plumage varieties of noiler chicken had low disparities on the dendrogram clusters, Brown, white-brown plumage were observed to be more genetically closed in their genetic distance. Okamoto et al. [15] had earlier affirmed a slight genetic difference in the polymorphic structure of local breeds of chickens. Andrew [16] had foremost documented that a stable polymorphism will continue to occur in a population if the fitness associated with a genotype decreases as that genotype increases in frequency. The low genetic disparity observed within various plumage can be linked to changes that might have occurred in the nucleotide chain during protein synthesis. Mutation is largely responsible for genetic drift [17]. In the absence of natural selection and genetic drift, homogeneity within various colorations of birds is maintained [16].

Conclusions

This study affirmed that there is a low genetic distance between Noiler of different plumage

varieties. The SDS-PAGE protein profiles and dendrogram clustering support the use of plumage variations as an alternative genetic marker for breed characterization and diversity assessment. Noiler chicken shows limited but distinct genetic diversity across plumage varieties, with brown and brown-white being the most closely related, while barred plumage stood out as the most genetically distinct.

Conflict of interest

The authors declare no conflict of interest.

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