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Short communication

Preliminary investigations on comparative genetic evaluation between Japanese quail (Coturnix coturnix japonica) and African Bush Fowl (Pternistis bicalcaratus) using SDS-PAGE

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Abstract

Concerns about genetics and biodiversity of game birds have been increasing throughout the World. Game birds are continuously being threatened due to increased hunting pressures, habitat destruction resulting from agricultural intensification and with fewer research activities and publications among others. Japanese quail (*Coturnix coturnix japonica*) is a small bird with obvious sexual dimorphism. The domesticated Japanese quail was derived from the wild Japanese quail (*C. japonica*). The African Bush Fowl (*Pternistis bicalcaratus*) is a species of bird that is well adapted to the rainforest and savannah ecological zones of West Africa. They are cursorial birds, sharing a group with pheasant, quails, snowcocks and turkeys. In this study, Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was utilized to investigate the genetic relationship between Japanese quail and African Bush Fowl. Sera proteins were prepared from blood samples extracted from each of the Quail and African Bush Fowl for electrophoretic analysis. After electrophoresis, data obtained were analyzed using clustering algorithm of the Unweighted Pair Group of PAlaeontological STatistics (PAST) software to evaluate their genetic relationship. Results generated from different clusters for both species showed genetic similarity coefficient of 98%. This result indicates that these two species of same Phasianidae family but different genera are very close and have non-distant genetic relationship; therefore this could be explored for enhanced breeding programs. Conclusively, both the Japanese quail and African Bush Fowl are known for their high quality of meat and for their good quality eggs, rich in proteins and vitamins, while quail eggs have many therapeutic virtues. The results obtained through this research lead to several solutions to increase these two populations since both species are exposed to hunting that has several negative effects including reducing the populations; hybridization can be done due to the genetic similarity between them, or an enhanced breeding program.

Key words: the Japanese quail, the African Bush Fowl, domestication, biology

الملخص

حظيت الدراسات الوراثية والتنوع الحيوي لطيور الصيد باهتمام واسع على مستوى العالم، إذ تواجه هذه الطيور تهديدات متزايدة نتيجة الصيد الجائر، وتدمير الموائل الطبيعية الناجم عن تكثيف الأنشطة الزراعية، بالإضافة إلى قلة الأنشطة البحثية وضعف النشر العلمي في هذا المجال. يُعد السمان الياباني (*Coturnix coturnix japonica*) طائراً صغير الحجم يتميز بوجود تمايز جنسي واضح، وقد تم استئناسه من السمان الياباني البري (*C. japonica*). أما دجاج الأدغال الأفريقي (*Pternistis bicalcaratus*) فهو من الطيور التي تتكيف جيداً مع المناطق الإيكولوجية للغابات المطيرة والسافانا في غرب أفريقيا. وهي طيور أرضية سريعة العدو تنتمي إلى المجموعة نفسها التي تضم الدراج، والسمان، والديكة الثلجية، والديك الرومي. في هذه الدراسة، استُخدمت تقنية الفصل الكهربي ببولي أكريلاميد-سلفات دوديسيل الصوديوم (SDS-PAGE) للكشف عن العلاقة الوراثية بين السمان الياباني ودجاج الأدغال الأفريقي. جرى تحضير بروتينات مصل الدم من عينات مأخوذة من كلا النوعين لإجراء التحليل الكهربي، ثم جرى تحليل البيانات باستخدام خوارزمية التجميع بطريقة المجموعة الزوجية غير المرجحة عبر برنامج PAST لتقدير مستوى القرابة الوراثية. أظهرت النتائج معامل تشابه وراثي بلغ 98%، ما يشير إلى أن هذين النوعين، رغم انتمائهما إلى جنسين مختلفين من فصيلة الدراج (Phasianidae)، يتمتعان بقرب وراثي وثيق، الأمر الذي يمكن استثماره في برامج تحسين التربية. وفي الختام، يُعرف كلٌّ من السمان الياباني ودجاج الأدغال الأفريقي بجودة لحمهما العالية وقيمة بيضهما الغذائية الغنية بالبروتينات والفيتامينات، كما يتميز بيض السمان بخصائص علاجية متعددة. وتُبرز نتائج هذه الدراسة حلولاً ممكنة لزيادة أعداد هذين النوعين المهددين بالصيد الجائر وما يترتب عليه من آثار سلبية كتناقص أعدادها؛ وذلك إما عبر التهجين استناداً إلى تقاربهما الوراثي، أو من خلال تعزيز برامج التربية.

الكلمات المفتاحية: السمان الياباني، دجاج الأدغال الأفريقي، الاستئناس، علم الأحياء

Introduction

A preliminary comparative genetic evaluation of the Japanese quail (Coturnix coturnix japonica) and the African Bush Fowl (*Pternistis bicalcaratus*) is deemed important. It will provide foundational knowledge about genetic variations within the two populations. This baseline data is essential for making accurate comparisons between different individuals or groups.

The Japanese quail and the African Bush Fowl are both important game birds; however, with decreasing population due to factors such as overhunting and habitat destruction resulting from agricultural intensification and the use of pesticides (Huisman, 2006; Aro & Akinmoladun, 2015). Moreover, fewer research plans and publications exist in comparison to other birds.

The Japanese quail belongs to the order of Galliformes and the family Phasianidae (Woodard *et al.,* 1973). During the 1900s, the Japanese bred quail for egg and meat production. Although many of these commercial lines were lost during the Second World War, new strains of quail were eventually re-established (Mills *et al.,*1997). Japanese quail was formerly considered a subspecies that originates from East Asia (Huisman, 2006); but was later categorized to be Phasianidae family (Orhan *et al.,* 2011). It performs very well in captivity with high breeding potential, and contributes greatly to household income through its chicken meat and egg production; and also serves as experimental model poultry for research (Huisman, 2006; Orhan *et al.,* 2011).

The African Bush Fowl (*Pternistis bicalcaratus*) is also a game bird that is considered to be Phasianidae family and referred to as spurfowls and francolins or otherwise double-spurred francolin that are common to the savannah ecological zone of Africa (Oluyemi and Roberts, 2000; Aro and Akinmoladun, 2015; Hunter *et al.,* 2021). However, both species appear similar in nature. Research investigation into their genetic relationship is crucial to provide insights into their evolutionary history and ecological adaptations, understanding the processes underlying speciation and identifying candidate regions for trait variation (Tella *et al.,* 2017).

Among the Galliformes, the most diverse genus of the family Phasianidae is the genus *Francolinus* (Oluyemi and Roberts, 2000). The genus comprises 41 species, 36 of which are afrotropical. Seven species including the double-spurred Francolins *(Francolinus bicalcaratus)* have been recorded in Nigeria (Keith *et al.,*1993). The double-spurred francolin otherwise known as African Bush Fowl is a species of bird that is highly adaptable to the savannah ecological zone of Africa (Oluyemi and Roberts, 2000). African Bush Fowl has a high dressing percentage and the nutritional classification of its flesh as the white meat type makes it a culinary delicacy for both rich and poor alike (Aro and Akinmoladun, 2015). However, the availability of the meat is solely from hunting proceeds and is even limited to certain times of the year when hunting is very easy (Aliou, 1986). Preliminary investigation on the domestication and adaptive breeding from clutches of the egg collected from the wild revealed an average egg weight of 26g (Aro and Akinmoladun, 2015). This is approximately 2.5 times heavier than the average quail's egg and half the size of the domestic chicken's egg.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is one of the effective tools being employed for analyzing genetic relationships at the protein level (Vivodik *et al.,* 2016). SDS-PAGE separates proteins according to their size and charge. Proteins are treated with sodium dodecyl sulfate (SDS), which adds a negative charge proportional to their size. When an electric field is applied, proteins migrate through a polyacrylamide gel matrix, with smaller proteins moving quickly and farther than bigger ones. The electromotive force is employed to move the molecules through the gel matrix (Liu *et al.,* 2006). SDS-PAGE exploits the differences in molecular size to resolve proteins differing by as little as 1% in their electrophoretic mobility through the gel matrix.

Recently, in the 20th century, the quail-like, African Bush Fowl had a torturous taxonomic history. They are now grouped into a single genus called *Pternistis*, with about 34 subspecies and 25 species with a complex eco-biogeographical history (Mandiwana-Neudani *et al.,* 2018). Moreover, African Bush Fowls and quails are medium-sized game birds from the family Phasianidae. However, they both belong to different subfamilies such as Perdicinae for true quails and Gallopheasants for spurfowls that have distinct features and ecological adaptations. Japanese quails are small ground-nesting birds grown for meat and eggs in agricultural settings while the double-spurred francolins are larger birds unique to Sub-Saharan Africa, distinguished by their two dimorphic long leg spurs and predilection for terrestrial habitats (Aro *et al.,* 2020). Therefore, this study aimed to investigate the genetic relationship at the protein level between Japanese quail and African Bush Fowl employing SDS-PAGE. The result of the preliminary investigation on comparative genetic

evaluation between Japanese quail and African Bush Fowl could be exploited positively in several ways including improving breeding program for commercial, guiding conservation efforts for wild populations.

Materials and methods

Experimental Location and Sample: This research was conducted between October and February, 2023 at Adekunle Ajasin University, Akungba-Akoko (AAUA), Ondo State and the Biotechnology laboratory of the Department of Animal Science, Obafemi Awolowo University, (OAU) Ile-Ife, Nigeria. Ten samples of each species of Quail and African Bush Fowl were used in this study. *C. japonica* was obtained from the Teaching and Research Farm, AAUA, Ondo State, and *P. bicalcaratus* were caught from the wild at Akure North Local Government, Ondo State. Electophoretic set-up was carried out at OAU, Ile-Ife, Nigeria.

Serum preparation: Blood samples from the Quail and the African Bush Fowl were obtained for the electrophoresis following (Olaniyi *et al.,* 2018). The samples of blood were drawn from veins on the wings of each bird from both species using a 2ml hypodermic syringe and needles. The blood sample was gently released into a serological glass tubes containing normal saline (0.9% NaCl), and kept for an hour at ambient temperature. Then, it was centrifuged for 10 minutes at 2,500rpm. The sera were then used for electrophoretic analysis.

Electrophoretic procedure: A discontinuous analysis was employed; 10% resolving gel was prepared (10g of SDS/90ml in distill water) at pH of 8.8; and 4% stacking gel at pH of 6.8, were used to cast the gel (Adeola and Omitogun, 2012; Olaniyi *et al.,* 2018). Blood samples of each species were denatured in a sample buffer of 7.5% β-mercaptoethanol (SIGMA) by heating at 95ºC for 5 min. 10 ml of the mixture was then loaded into the wells of the casted gel and proteins were separated at constant 150V for an hour with the aid of Bio-Rad Mini Protean II Cell and its electrophoresis power supply kit (Model 200/2.0). After the separation, the gel was removed from the kit and placed into a staining solution made up of 0.1% Coomassie Blue in 1:4 glacial acetic acid: methanol for about 2 hours. The gel, thereafter, was then de-stained in a 60% solution containing 1:4 of glacial acetic acid: methanol. Gels were then scored based on the intensity of the protein bands (Ferrier, 1981; Pasteur *et al.,* 1988). The signs "+" and "–" represent the presence and absence of band respectively.

Data analysis: The data generated from the scoring were analyzed using the clustering algorithm of the Unweighted Pairgroup average algorithm (UPGMA) of Paleontological Statistics (PAST) with the Euclidean option to evaluate their genetic relationship.

Results

Figures 1 and 2 respectively shows the representative gel of SDS-PAGE from serum protein of Quail and African Bush Fowl, while Fig. 3 shows the dendrogram of genetic distance between population of Quail and African Bush Fowl.

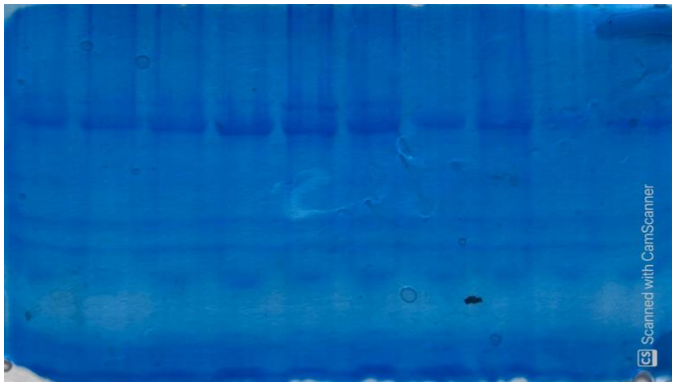

Fig. 1. Representative gel of samples from Japanese quail (*C. japonica*) obtained from Teaching and Research Farm, AAUA, Ondo State.

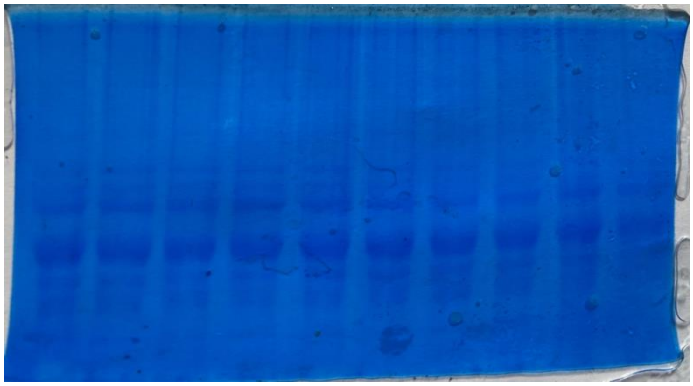

Fig. 2. Representative gel of samples from African Bush Fowl (*P. bicalcaratus*) caught from the wild at Akure North Local government, Ondo State.

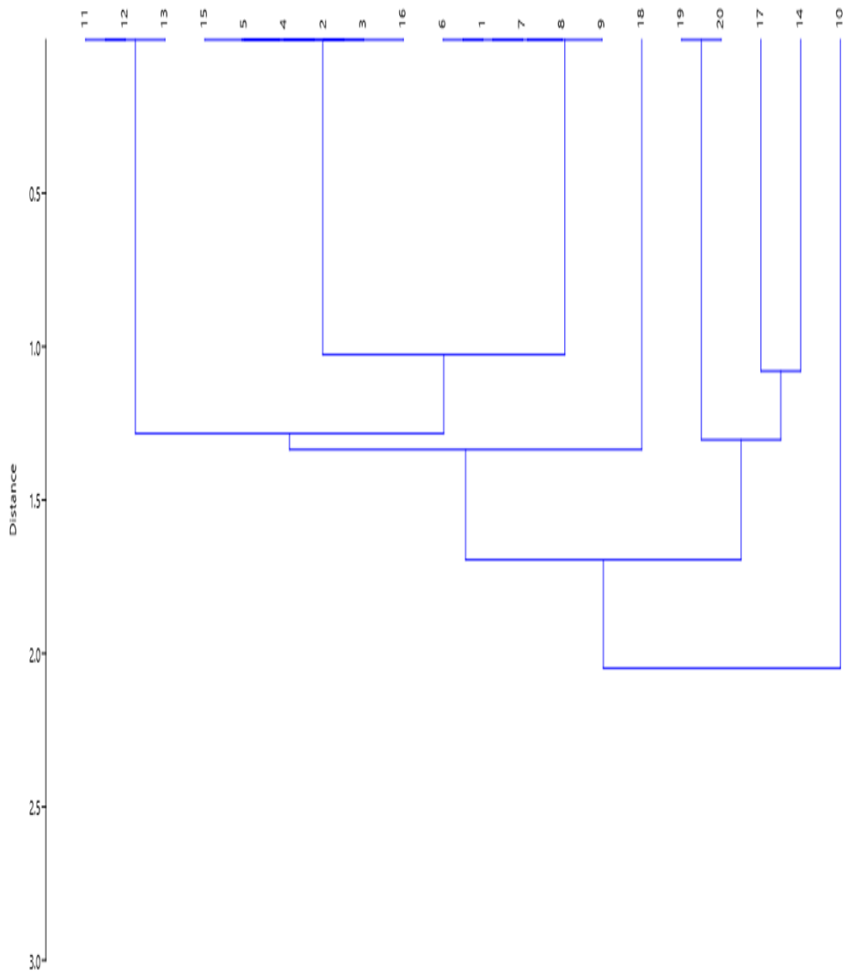

Fig. 3. Dendrogram showing genetic distance between populations of Quail (*C. japonica*) and African Bush Fowl (*P. bicalcaratus*)

The dendrogram for cluster analysis between *C. japonica* and *P. bicalcaratus* species ultimately revealed genetic distance of 2.0% (0.02). Two major clusters at 1.7% (0.017) were indicated; with the first major cluster having four sub clusters at 1.35% (0.0135); the first cluster has three samples (11, 12, 13) while the second cluster has six samples (15, 5, 4, 2, 3, 10), the third cluster also have five samples (6, 1, 7, 8, 9) leaving the fourth with just one sample (18). The second major cluster also has four sub-clusters at 1.35% (0.0135); the first major cluster having two samples (19, 20) while the second to fourth cluster has just one sample each (17),(14) and (10) respectively.

Discussion

The cluster analysis from the result that generated the dendrogram showed there was low coefficient of genetic variation of genetic distance at 2.0% (0.02) (that is 98% (0.98) similarity) between *P. bicalcaratus* and *C. japonica*. This showed proximity between these two genera and signaled for possible hybridization between the two genera; as one of the pointers of hybridization (Diyaware *et al.,* 2011). The 2% (0.02) genetic variation could be pointer to non-distant phylogenetic relationship (Hoffmann *et al.* 2004; Pym *et al.,* 2006; Banerjee, 2012; (Olaniyi *et al.,* 2018). Notable, as reported by Diyaware *et al.,* (2011) that such level of similarity could be good indicator for successful hybridization. In essence, hybridization will be a good solution to increase the population of these species.

Conclusion

In conclusion, both Japanese quail and African Bush Fowl are known for their high quality of meat and for their good quality eggs, rich in proteins and vitamins. As known, quail eggs have many therapeutic virtues. Since both species are exposed to hunting that has several negative effects including reducing the populations, the results obtained through this research leads to several solutions to increase these two populations, hybridization can be done due to the genetic similarity between them, or an enhancing breeding program.

Author's contributions

Conceptualization: W.A. OLANIYI; Methodology: W.A. OLANIYI, F.T. Azeez, J.O. Muhammad, D.O. Olayinka; Investigation: F.T. Azeez, J.O. Muhammad; Data curation: F.T. Azeez, O.S. ABE ; Writing: W.A. OLANIYI, O.S. ABE; Original draft preparation: W.A. OLANIYI, B.H. Ajao; Review and editing: B.H. Ajao, A.C. ADEOLA, O.G. OMITOGUN; Project administration: W.A. OLANIYI, O.S. ABE.

All authors have read and agreed to the published version of the manuscript.

Ethics

All procedures for this study were approved by the Ethical committee of Department of Animal Science, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

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