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Analysis of Genetic Diversity of Nigeria Indigenous Muscovy Duck Ecotypes using RAPD Marker

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Abstract
Genetic characterization of Muscovy ducks collected from two agro- ecological zones of Nigeria (guinea savannah and rain forest) was carried out using Random amplified polymorphic DNA method. Seven random amplified polymorphic DNA primers were employed using blood sample from 50 birds from the two populations. The RAPD bands were scored for their presence (1) or absence (0). The genetic distance between the populations was calculated based on band sharing between the pooled sample profiles, 19 polymorphic bands ranging from 220 to 1660 basepairs. High similarity was obtained between the populations (0.86%), genetic distance was small 0.14, suggesting that they have common ancestor and evolved little adaptive variation as a result of distribution.
Keywords*; Muscovy duck; RAPD; ecotype, diversity, population.*

Introduction

Polymorphism on the DNA level are frequently used as genetic marker for herd studies on domestic animals. Genetic markers and the variations detected at theses loci reflect the level of variation in the entire genome. Using genetic markers to introgresss genes can shift specific characteristics of a population (Notter *et al*., 2007). The utilization of this tool in genetic studies have been reported by many authors Chen *et al*. (2001) in chicken, Sharma *et al.* (2004) in buffalo etc. This helps in conservation and preservation of indigenous genetic resource. Conservation of indigenous animal resources have been proposed as a method for slowing down the loss in diversity in livestock breeds through extinction. Apart from preventing extinction, conservation of indigenous breeds is also important for the future health of the animal industry globally as they could be a resource for novel genes that can permit sustained genetic improvement as well as enable adaptation to changing breeding objectives and environments (Notter, 1999).

Muscovy duck is an indigenous bird that is found in all the agro ecological zones of Nigeria among the rural communities. The bird have served as source of food and income to the rural populace and have received little or no attention in terms of improvement, conservation and development. The evaluation of some performance traits of this bird in Nigeria were earlier undertaking by (Ogah *et al*., 2011a; Ogah *et al.*, 2011b and Ola, 2000). Morphological differentiation was what was used in discrimination among the

local populations of Nigeria indigenous Muscovy duck (Ogah *et al.,* 2009). Morphological traits have some limitations in discriminating between close populations thus molecular markers which are assumed to be neutral to the selection forces are appropriate in the study of genetic relationship between breeds and ecotypes (Parker *et al.*, 1998). Classification using molecular markers provides a large unbiased basis for the estimates of average breed/ecotype similarities and/or differences. The rapid development in modern molecular genetics has given rise to many new nucleic acid fingerprinting techniques. Molecular markers such as restriction fragment length polymorphism (RFLP) and DNA fingerprinting (DFP) have so far proved to be useful in establishing genetic relationship among the livestock populations including poultry (Hillel *et al*., 1992). Arbitrary amplification of polymorphic DNA sequences, termed random amplification of polymorphic DNA (RAPD) analysis or Arbitrarily Primed PCR (AP-PCR) typing (Welsh and McClelland, 1990; Williams *et al*., 1990) as with other molecular markers, it was demonstrated that they lead to new approaches for genetic analyses of livestock species, and has been used for estimating genetic relatedness in livestock animals (Kemp and Teale, 1994; Appa *et al*., 1996) and avian populations (Zhang *et al*., 1995; Smith *et al*., 1996).

However, RAPD analysis presents some practical problems including lack of reproducibility, thus requiring stringent protocols to be set and optimized, and it follows co-dominant inheritance. To lay the ground for further studies, this study was designed with the specific objective of assessing genetic diversity of Nigeria indigenous Muscovy duck from two agro ecological zones using RAPD marker

Materials and Methods

Fifty Muscovy ducks from two agro-ecological zones of Nigeria, rain forest and guinea savannah zones which were managed under semi intensive system were used for the experiment. Blood sample of about 10ml was collected from the brachial vein of 25 individual birds of either sex from each of the two ecotypes. Genomic DNA was extracted by the use of phenol chloroform extraction method (Kwon *et al*., 1995).

Determination of genomic DNA concentration and DNA purity

The concentration and purity of individual genomic DNA samples were determine by using a spectrophotometer. The optical density (OD) at 260 and 280 nm were measured. The purity of DNA was determined by absorbance ratio A260/280. The DNA concentration was calculated from the absorbance value at 260nm by the following formula:

$$\text{DNA concentration } (\mu g/\mu l) = \frac{A260 \times \text{dilution factor} \times 50}{1000}$$

Agarose gel electrophoresis was used to determine the quantity of genomic DNA sample. Agarose gel (0.8%) was prepared by using 0.8g of agarose powder mixed with

100ml x TBE buffer and boiling until dissolved, then cooled down at room temperature and poured into electrophoresis chamber set. The genomic DNA was mixed with 6 x loading dye, 25% glycerol 60 nm EDTA, 0.25% bromophenol blue and loaded into the gel. Gel electrophoresis was performed at 80 volt for 1hour.
Synthetic primers used for the RAPD analysis in this study were purchased from TAG Copenhagen, Fruebjervel DK-2100 Copenhagen.

Table1. The sequence of the primers used and their annealing temperature

Primer	Sequence 5^1 - 3^1	(G + C)% At 0^o	
Oligo 1	TCA CGA AGCC	60	28.9
Oligo 2	TGG ACC GGTG	70	33
Oligo 3	GAC CGC TTGT	60	28.9
Oligo 4	AAC GCG TCGG	70	33
Oligo 5	GAA CGG ACTC	60	28.9
Oligo 6	GTG AGG CGTC	70	33
Oligo 7	AAA GCT GCGG	60	28.9

The RAPD bands were scored for their presence (1) or absence (0) .The index for similarity between ecotypes and within ecotype was calculated using the formula developed by (Lynch, 1990).

Bab= 2Nab/Na+Nb

Where Nab= number of fragments observed in individuals a and b
Na and Nb = total number of fragments scored in a and b

The genetic distance between the populations was calculated based on band sharing between the pooled sample profiles. The genetic distance between ecotypes was calculated as described by Chatterjee *et al*. (2007) using the POPGENE program (Population Genetic Analysis) version 1.31 (Yeh *et al*., 1999)

Dab=1/N.1- (Nab/Na+Nb-Nab)

Where Nab= number of common bands between ecotypes
Na= number of common bands in ecotype a
Nb=number of common bands in ecotype b
N= number of primers

Results and Discussion

While 7 random primers were screened, out of them 5 primers produced clear and distinct patterns across all samples (Table 2, Fig. 1). PCR amplification with each of these 5 primers was done twice before scoring them. These primers generated a total of 113 bands ranging in size from roughly 300 bp to 2000 bp whereas the range with individual primers was 2–5 bands. To ensure that the amplified DNA bands originated from genomic DNA and not primer artifacts, negative control was carried out for each primer /ecotype combination. No amplification was detected in the control reaction. All amplification products were reproducible when reactions were repeated using the same reaction condition. Five of the primers (71.4%) were successfully amplified, polymorphic bands among the two ecotypes as shown in (Table 2 and Fig 1). The total numbers of amplified bands were 59 and 54 for the guinea and rainforest ecotypes, respectively. There were more number of bands (polymorphic and monomorphic) in the guinea savannah than the rainforest ecotype. The primer oligo 1 gave the highest bands in both ecotypes. Table 3 gave the genetic variability between the two ecotypes, and the similarity between the two ecotypes was 0.86 while the genetic distance between the populations was 0.14

Table 2. Number of RAPD bands for each primer using agarose gel in the two muscovy duck ecotypes

Primer	Guinea savannah			Rainforest		
	NAB	NPB	NMB	NAB	NPB	NMB
Oligo 1	15	5	10	14	4	10
Oligo 2	9	3	6	9	3	6
Oligo 4	13	4	9	13	4	9
Oligo 6	10	3	7	8	3	5
Oligo 7	12	4	8	10	2	8
Total	59	19	40	54	16	38

NAB=number of amplified bands, NPB = number of polymorphic bands, NMB = number of monomorphic bands, RAPD = Random amplified polymorphic DNA

Table 3. Genetic variability between the two ecotypes

	Guinea savannah	RainForest
Guinea Savannah	0	0.86[1]
RainForest	0.14[2]	0

1= Genetic similarity 2=Genetic distance

Genetic variability between the two populations is presented in Table 3, similarity between the two populations was 86 percent while genetic distance was lower 14 percent substantiating the genetic similarity of the two populations.

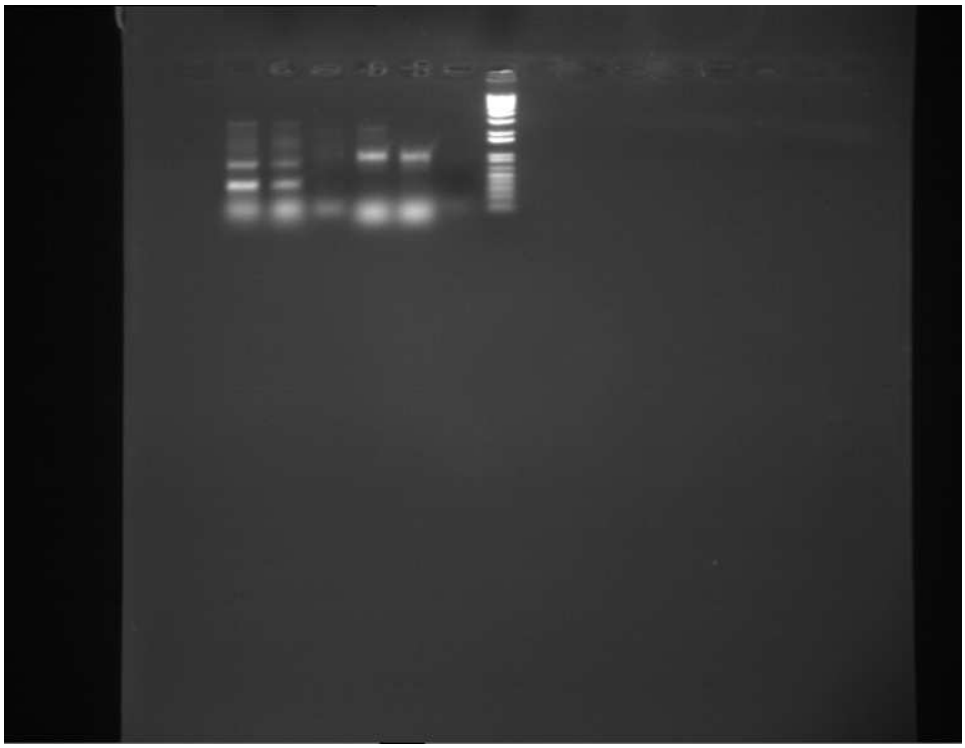

Figure 1. RAPD Profile of individual sample generated by the primers

Random amplified polymorphic DNA (RAPD) developed by Welsh and McCelland 1990 and Williams *et al*.(1999*)* proved to be a powerful tool in different genetic analyses. This approach detects DNA polymorphisms based on amplification using a single primer of arbitrary nucleotide sequence of genomic DNA fragments. RAPD markers are attractive because they are specific and quick, nanograms of DNA are required, automation is feasible, there is no requirement for previous DNA sequence information, modest coast and ability to detect relatively small amounts of genetic variation (Welsh and McClelland, 1990).

The similarity or band sharing between ecotypes obtained in this study was 0.86 is similar to the findings of El-Gendy *et al*. (2005), who reported 0.83 for Muscovy duck populations in Egypt. These values are however higher than what was obtained for other breeds like White Pekin, Khaki Campbell and Dametti ducks with 0.68, 0.74 and 0.68. respectively as reported by same author in Egypt. This is an indication of closeness of the populations.

Genetic distance between the ecotypes was as low as 0.14. This aids in understanding the genetic relationship and divergence between the ecotypes. The value obtained here is however lower than what El-Gendy *et al*. (2005) obtained for cairina species (0.40) when they used five primers. Similarly, higher values were obtained by Xiao *et al*. (2005) for Fugian local duck populations in different ecological zones (67.97%). The higher similarity obtained in this study for the ecotypes of Nigerian indigenous Muscovy duck supports the low distance between them, suggesting possibility of little genetic divergence between the populations, thus indicating that they have common ancestors and have little genetic differentiation despite ecological isolation. This can also be explain the ecological differentiation do not impart much on the ducks despite differences in environment.

Conclusion

The findings of this study implies that the identified RAPD marker can be successfully employed in evaluating and identifying the DNA polymorphism of Nigerian indigenous Muscovy duck, especially as a specific DNA marker for the Muscovy ducks. If this marker can be linked with a quantitative trait loci for economically important traits, it would be

successfully employed in marker-assisted programs for Muscovy duck breeding.

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