MOLECULAR GENETIC ANALYSIS OF DIVERSITY IN VILLAGE CHICKENS IN NIGERIA

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ABSTRACT

Indigenous chickens obtained from the central and northern parts of Nigeria were evaluated for incidence and frequencies of endogenous viral, ev, genes 1, 3, 15 and 21 using blood samples collected by branchial venipuncture from free ranging adult birds of both sexes. Results indicated that 60.7% of the birds do not carry any of the four ev elements and that none of the birds carried genes for ev 1. The frequencies ev 1, 3, 15 and 21 genes were 0.00, 0.07, 0.07 and 0.24. Significantly ($P < 0.05$) higher frequencies of birds carrying the homozygous negative genes for each of the four ev elements are expected among the local chicken. Significantly ($P < 0.05$) higher frequency of birds carrying the heterozygous than the homozygous positive forms for ev 3, 15 and 21 are also expected. The obtained negative genotypes agree closely with the expected, although slightly lower frequencies were obtained for ev 3 and 15 and higher frequency for ev 21 than expected. However, there were no birds carrying the homozygous positive forms for ev 3 and ev 15 and slightly lower frequency for ev 21 than expected. The frequencies for the heterozygotes, though slightly lower for ev 21 and higher for ev 3 and 15, agree closely with the expected. Analysis of the DNA finger print, DFP, bands from RFLP studies indicated that the level of genetic diversity in the local chicken in Nigeria is only about 52%. The study suggests that there may be

true genetic differences in the Nigerian local chicken population and hence the slightly high variation in genetic differences could be exploited for genetic improvement.

Keywords: Indigenous Chickens, ev genes genetic diversity

INTRODUCTION

A number of molecular genetic technologies can now be used to effectively characterize genetic stocks. Such studies can lead to discovery of genes or germ plasm that need to be conserved or those affecting certain production traits and which can be introduced into the present commercial stocks. One of such technologies is the study of the distribution and frequency of the endogenous viral (ev) elements which has been reported to be a measure of genetic homogeneity in a population. Other methods for molecular qualification of genetic diversity include deoxyribonucleic acid (DNA) sequencing, DNA hybridization, electrophoretic analysis of protein variation, immunologic methods and restriction fragment length polymorphisms (RFLP) (Chambers and Bayless, 1983, Nei, 1987, Sharp, 1987). Out of these methods, the RFLP technology is the most reliable as it reveals a greater level of genetic variation. Endogenous viral genes have been studied extensively in poultry (Smith, 1987, Crittenden, 1991) and their frequencies vary among populations (Bumstead *et al.*, 1987, Lamont *et al.*, 1992), ecological zones (Smith and Ray, Unpublished) and Production

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(Kuhnlein *et al.*, 1989). However these studies have been limited to commercial or improved

stocks. The viral elements have been shown to influence a number of performance traits in poultry including egg production and egg weight (Gavora *et al.*, 1991), body weight and feed efficiency (Aarts *et al.*, 1991, Aarts and Leenstra, 1995, Grunder *et al.*, 1995, Smith *et al.*, 1995) and disease resistance (Astrin, 1978, Crittenden *et al.*, 1984, Kuhnlein *et al.*, 1989, Smith, 1987, Tereba *et al.*, 1979).

Most developing countries have livestock species of unknown genotype and of undetermined potential. Perhaps the largest of these species is the local or indigenous poultry which has served as a major contributor of animal protein (egg and meat) in most of these countries. For instance, Akinwunmi *et al.* (1979) reported that the local chickens accounted for 61.03% and 19.5% of the 1977-1978 internal supply of poultry meat and egg, respectively, in Nigeria. It has also been reported that almost every family in the rural area of Nigeria keeps some local chickens (Ayorinde, 1986, 1990) as sources of family egg and occasional meat.

In order to improve performance of chickens in developing countries NRC (1993) recommended that a study of the level of genetic diversity in different populations is the first step. FAO (1992) announced the initiation of program to conserve and develop the livestock and poultry genetic resources of developing countries (Cunningham, 1992). Such an understanding can lead to discovery of genes that can be used in the breeding and genetic improvement of chickens indigenous to developing countries.

An earlier work using the indigenous Tanzanian chickens (Smith and Ray, unpublished) revealed variations within regions suggesting need to thoroughly evaluate populations from other countries. The present study was therefore undertaken to determine the frequencies of endogenous viral genes 1, 3, 15 and 21 and evaluate the extent of genetic

diversity in the local chicken in Nigeria from RFLP analysis.

MATERIALS AND METHODS

Sources of blood samples

Blood was collected by brachial venipuncture into microtubes containing 0.5M EDTA from 120 adult local chickens (64 hens and 56 cocks) from the central (Ilorin) and northern (Zaria) areas of Nigeria. The isolation, enzymatic digestion and probing of the DNA samples were carried out on the blood samples transported under cold storage to the Department of Agricultural Sciences, Tuskegee University, Alabama, U.S.A. for the DNA and DFP preparation.

Genomic DNA from each bird was purified from 500 μ l of blood using the salting out procedure. This consisted of adding 500 μ l Lysis buffer (10 mM Tris HCL, 400 mM NaCl and 2 mM EDTA, pH 8.2), 20 μ l of 10% SDS and 50 μ l of 50 μ g/ μ l proteinase K solution to the blood sample and digesting overnight at 70°C with gentle shaking. About 500 μ l of 6M NaCl was then added and the mixture properly homogenized, centrifuged at 13,000 rpm for 15-20 minutes and the supernatant removed. DNA was precipitated by adding two volumes of 100% ethanol to the supernatant at room temperature. The DNA was then rinsed with 400 μ l of 70% ethanol, air dried and dissolved in 200 μ l of distilled water.

Genotyping for endogenous viral genes

Birds were genotyped for ev 1, 3, 15 and 21 by the Polymerase Chain Reaction (PCR) using a modified method of Benkel *et al.* (1992). The amplifications were carried out in 50 μ l reaction mixtures containing 10 μ l of the template (100 μ g of DNA), 5 μ l of 10 x PCR buffer, 3 μ l 25mM MgCl₂, 1 μ l of 10 mM dNTP, 0.25 μ l Taq DNA polymerase, primers and double distilled water added to make up the volume. The PCR primers used are as reported by Smith *et al.* (1994). The reaction mixtures were overlaid with sterile mineral oil and loaded into the thermal cycler using the

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modified 'touchdown' procedure of Don *et al.* (1991). This consisted of two cycles at 96°C

for 1 min, 65°C for 1 min. and 72°C for 2 mins., 10 cycles at 96°C for 1 min., 59°C for 2 mins. And 72°C for 2 mins. and a 10 mins. final extension at 72°C to minimize secondary priming. Fifteen microliters of each amplification product was analysed on a 1.2% agarose gel stained with ethidium bromide.

RFLP Analysis

Equal amounts of DNA from each of five birds of each sex within each region were mixed together. Each mixed sample was digested with Pst 1 following a modification of the manufacturer's recommendation. To each mixed sample was added 4 ul of 10x buffer, 4 ul spermidine and 4 ul of the enzyme before incubating at 37°C. The digested samples were electrophoresed using 0.8% agarose gel stained with ethidium bromide. The materials were then transferred to a nylon membrane (Amersham, Arlington Heights, IL.) overnight using the Southern transfer. The membranes were washed briefly with constant shaking in 5X SSC (sodium chloride and sodium citrate), blotted and fixed at 1200 Joules by autocrosslink in a uv Stratalink (Stratagene, San Diego, CA). The membranes were labelled to P32 using the random labelling technique and then hybridized to Bg 32 probe using the protocols of Sambrook *et al.* (1989). The membrane exposed to X-ray films (Fuji) at 80°C for 7 days. After autoradiographing, the DNA fingerprints (DFPs) were analysed by scoring for the bands present in the various lanes by three of the researchers and the average and standard deviation found. To assess genetic similarity between the two regions, the scorable bands from the DFP samples from each region were used to calculate band sharing (BS) using the formula of Jeffrey *et al.* (1986):

$$BS = 2(Nab) / (Na + Nb).$$

where BS = level of band sharing between regions a and b, Nab = number of bands shared

by regions a and b, Na = total number of bands for a and Nb = total number of bands for b.

Statistical analysis

The number of either the negative or positive (homozygous) and both (heterozygous) forms of cv genes on individual birds from each zone was counted and the gene (ev 1, 3, 15 and 21 – or +) and genotype (homozygous positive and negative and heterozygous) frequencies were computed using the HardyWeinberg principle, $(P + q)^2 = 1$ (Falconer, 1981) the cv genes and genotype frequencies were compared by the chi-squared tests (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The frequencies of the four endogenous viral genes and the expected and observed genotypes are shown in Table 1. All the birds carry ev 1 negative genes and significantly ($P < 0.05$) higher number carry the ev 3, 15 and 21 negative genes. The low incidence of the ev genes agree with the reports of Aarts *et al.* (1991) and Sabour *et al.* (1992) in improved birds and in the local chickens in Tanzania (Smith and Ray, unpublished). Significantly ($P < 0.05$) higher incidence of birds with homozygous negative endogenous viral genes than homozygous positive and heterozygotes are expected. Also the frequencies of heterozygosity should be significantly ($P < 0.05$) higher for ev 3, 15 and 21 negative genes. The low incidence of the ev genes agree with the reports of Aarts *et al.* (1991) and Sabour *et al.* (1992) in improved birds and in the local chickens in Tanzania (Smith and Ray, unpublished). Significantly ($P < 0.05$) higher incidence of birds with homozygous negative endogenous viral genes than homozygous positive and heterozygotes are expected. Also the frequencies of heterozygosity should be significantly ($P < 0.05$) higher for ev 3, 15 and 21 than the homozygous positive.

For this work, DFP lanes were compared only if they were in close proximity on a gel. Comparison of distant lanes of an autoradiogram or lanes on different

TABLE 1: FREQUENCIES OF EV 1, 3, 15 AND 21 GENES AND OF EXPECTED AND OBSERVED GENOTYPES IN NIGERIAN LOCAL CHICKEN

Gene	Frequency	Genotype	Expected Freq.	Observed Freq.
ev 1-	1.00 ^a	ev 1-ev 1-	1.0000 ^a	1.0000 ^a
ev 1+	0.00 ^b	ev 1+ ev 1+	0.0000 ^b	0.0000 ^b
		ev 1+ ev 1-	0.0000 ^b	0.0000 ^b
ev 3-	0.93 ^a	ev 3- ev 3-	0.8649 ^b	0.7946 ^a
ev 3+	0.07 ^a	ev 3+ ev 3+	0.0049 ^b	0.0000 ^b
		ev 3+ ev 3-	0.1302 ^c	0.2054 ^c
ev 15-	0.93 ^a	ev 15- ev 15-	0.8649 ^a	0.8661 ^a
ev 15+	0.07 ^b	ev 15+ ev 15+	0.0049 ^b	0.0000 ^b
		ev 15+ ev 15-	0.1302 ^b	0.1339 ^c
ev 21-	0.76 ^a	ev 21- ev 21-	0.5776 ^a	0.6875 ^a
ev 21+	0.24 ^b	ev 21+ ev 21+	0.0576 ^b	0.0375 ^b
		ev 21+ ev 21-	0.3648 ^c	0.2768 ^c

Frequencies within each gene or genotype with no common superscripts differ significantly ($P < 0.05$).

autoradiograms were not accurate and thus, were not used. DFP bands were classified as absent or present at the same position in other lanes.

The observed frequencies of the various genotypes showed that all the birds had the genotype ev 1-ev. Also, there were significantly ($P < 0.05$) higher frequencies of homozygous negative birds for the other three ev genes. This higher frequency of the negative genotypes agree with the expected frequency. However significantly ($P < 0.05$) more birds than expected carried the ev 21 – ev 21 – genotype.

The amplified products of the ev genes analysed on a 1.2% agarose gel stained with ethidium bromide revealed that birds with ev 1- ev 1 – genotype have only one band with fragment size of 510 base pairs. Birds homozygous negative for ev 3 have single bands of 270 bp fragments while those with ev 3+ ev 3- genotype have both bands with sizes 190 and 270 base pairs. Birds with the genotype

ev 15-ev 15- have bands of 181bp only while those with ev 15 + ev 15 – have both 181 and 461 bp fragments. As expected, birds with ev 21 – ev 21 – genotype have single bands of 396 fragment size. Those having ev 21 + ev 21 + have 341 bp only while the heterozygotes have both 341 and 396 bp products.

Most endogenous viral elements are associated with endogenous virus production (Smith and Crittenden, 1986) which predispose birds to avian leucosis virus infection and viremia (Crittenden *et al.* 1982). Although there is some evidence of deleterious (Crittenden *et al.* 1982) or advantageous (Robinson *et al.* 1981) effects of ev genes on the fowl, they are assumed to be neutral under normal conditions (Bumstead *et al.* 1987). Under the extensive system in which the local chickens are raised, it is likely that all the birds with ev 1 + ev 1+, ev 3 + ev 3+ and ev 15 + ev 15+ genotypes and most of those with ev 21 + ev 21+ (table 1) died before reaching the age at which the blood samples were collected. It can be inferred therefore that

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natural selection is probably responsible for the difference in the expected and observed genotypic frequencies.

From the PstI digested mixes, the number of scorable bands ranged from 5-16 with an average of 9.97 and a standard deviation of 2.04. The females carried an average of 10.33 bands while the males had on the average 9.56 bands. The average number of bands from the central area was 10.42 (11.00 for females and 9.74 for males) while that from the north was 7.5 (6.33 for females and 8.67 for males). The estimation of relatedness as reflected in the degree of similarity between the DNA fingerprints of the analysed groups indicated a band sharing level of 0.4833 which implies genetic similarity of about 48%. There is thus about 51.62% difference in the genotype of birds from the two regions based on DNA mixes digested with PstI.

A probable reason for the low level of genetic diversity in the genotypes of birds within each region might be as a result of the management system employed in keeping the birds. Mukherjee (1990) observed that the practise of raising chickens on the free range allows unrestricted matings between more closely related birds leading to increased inbreeding and homogeneity. The relatively similar DFP might also be due to the way the local birds, especially from the north, are marketed. Frequently these birds are transported southwards where there is more market and since the birds in the north are generally bigger than those found in the south, utilization of the bigger cocks from the north for free range mating, which is a common practise, would have introduced the blood of chickens from the north to settlements along the trade routes.

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