GENETIC VARIABILITY OF GRASSCUTTER (*Thyronymus swinderianus L) IN IMO STATE* USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS

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ABSTRACT
Genetic variability of grasscutter (*Thyronymus swinderianus L*) was investigated using random amplified polymorphic DNA markers in Imo state. A total of four primers of arbitrary sequence were used and all of them generated reproducible, scorable and polymorphic bands. The highest percentage polymorphic band was recognized for primer OPA19 (100%) while the lowest percentage of polymorphic band was recognized for the primers OPA14 (50%). The observed number of alleles (Ne) had values ranging between 1.50±0.52 – 2.00±0.00 for OPA14 and OPA19 respectively. Similar trend was observed for Ne, H, and I with values ranging between 1.39±0.42 for OPA14 to 1.81±0.14 for OPA19; 0.22±0.23 for OPA14 to 0.44±0.05 for OPA19 and 0.31±0.33 for OPA14 to 0.64±0.05for OPA19 respectively considering the genetic diversity indices for combined loci. Similarly, considering the genetic diversity indices between the grasscutter population/strain, the light strain had the higher value for all the diversity indices; Na (1.71), Ne (1.56), H (0.25) and I (0.44) than the dark strain; Na (1.61), Ne (1.39), H (0.25) and I (0.34). The study therefore suggested that RAPD can be successfully utilized for detecting genetic variability among the studied grasscutter population and there could be selection within and between the studied populations for genetic improvement.
Keywords: Grasscutter, RAPD markers, genetic variability, strain, heterosis.

INTRODUCTION
Asibey and Adel (2000) reported that grasscutter is the most preferred and most expensive bush meat in West Africa (Nigeria, Togo, Benin, Ghana and Cote d'ivoire) and contributes to both local and export earning of most of these countries. The meat is a delicacy (Adu and Yeboah, 2000; Addo, 2002) eaten by all classes of people with no religious prohibition (Opara, 2010b), and is also exported to continental Europe and the United states, where it is sold mainly to West Africans living in these regions (Adu, 1999).

According to Akinloye (2005), Grasscutters show slight phenotypic variations especially in the colour of their fur so it is important to analyze their genetic variability in order to identify populations and individuals of particular merit.

Nowadays, due to advancement in molecular genetics and affordability of DNA analysis, genetic diversity can be measured directly on the DNA itself and it accurately presents the genetic variations between breeds, within breeds, and within half and full-sib groups. Hence the study intends to identify the genetic variability of grasscutter in Owerri, Imo State, using RAPD markers; determine allelic diversity of the populations and the genetic distance between the two studied strains of grasscutter (*thryonymus swinderianus),* to help understand the evolutionary process that affect it.

MATERIALS AND METHODS
The research was conducted in Owerri and Okigwe, Imo State Nigeria. Owerri is one of the largest and the populous cities in Southern Nigeria. It is the capital city of Imo State with coordinates; 5°28'34.7160" N and 7°1'33.0708" E. and Okigwe has the coordinates; 5°49'0" N and 7°21'0" E. Annual rainfall in Imo State usually varies from 1990mm to 2200mm. The mean annual temperature is above 20° c with an average annual relative humidity of 75%. Five to ten hair samples were taken from a total of 30 grasscutters (15 grasscutter from each colour variants) selected from two different locations; the wild (by hunters from bush meat market, Okigwe) and from Imo State Zoo, Owerri, both in Imo State between November - December, 2018 during the survey stage of the study. The hair samples were preserved and transported to African Bioscienecs Laboratory, Ibadan, Oyo State, for molecular analysis. Out of the 30

hair samples collected, only 24 samples lysed while 6 samples were unable to lyse. Therefore, DNA extraction was carried out with 24 samples that were able to lyse. The quantity and quality of DNA was measured using NanoDrop spectrophotometer (Thermo Scientific, USA). The DNA was then stored at -30^{O}C until ready for use. The extracted DNA was amplified by Polymerase Chain Reaction (PCR), and the amplified products were size-fractionated in a 1.2% agarose gel containing ethidium bromide in Tris-borate EDTA buffer and visualized under UV transillumination. The PCR amplified bands were scored visually on the basis of their presence (1) or absence (0). The scores obtained were then pooled for constructing a single data matrix, which was used for estimating the genetic diversity indices using GenAlex software.

RESULTS AND DISCUSSIONS

The result on table1 reveals the genetic diversity indices for all loci combined. The displayed diversity indices were; observed number of allele (Na), effective number of alleles (Ne), Nei's heterozygosity (H) and Shannon's index (I). The observed number of alleles (Na) had values ranging between 1.50±0.52 – 2.00±0.00 for OPA14 and OPA19 respectively. Similar trend was observed for Ne, H and I with values ranging between 1.39±0.42 for OPA14 to 1.81±0.14 for OPA19; 0.22±0.23 for OPA14 to 0.44±0.05 for OPA19 and 0.31±0.33 for OPA14 to 0.64±0.05 for OPA19 respectively. There is no previous information about using RAPD marker to investigate genetic diversity of grasscutter. However, the range values obtained for the diversity indices are compared with other rodents such as rabbits and laboratory rats, guinea pig and mice. Shannon index value obtained in the study was lower than that reported by Kumar *et al*., (2014). The Shannon Index of Nigerian grasscutter breed in the study revealed low species richness and evenness since all the indices were below 3.5, the mark set for high species evenness and richness (Krebs, 1989). The low average Shannon's Index (I) obtained in the study could be attributed to close descent. The value of effective number (Ne) of alleles obtained in the study was lower than the findings of El- Sayed *et al*., (2018) and Mohammed and Abdelfattal (2018). The lower value of (Ne)could be attributed to effective sample size. This agrees with the findings of de Vicente, (2004) who opined that effective number of allele (Ne) can be used with codominant marker data and its calculation may be affected by sample size. Heterozygosis deficiency (low genetic variability) recorded in the study may have also resulted from one or more of these following factors; presence of null alleles, small sample size, Wahlund effect and/or the decrease in heterozygosity due to increased consanquinity (inbreeding) (Kumar *et al.,* 2006).

Table 1: **Genetic diversity indices for all the loci combined**

Markers	Na	Ne	H	I
OPA08	1.92 (0.29)	1.72 (0.33)	0.32 (0.16)	0.56 (0.21)
OPA11	1.92 (0.29)	1.58 (0.31)	0.34 (0.14)	0.51 (0.19)
OPA14	1.50 (0.52)	1.39 (0.42)	0.22 (0.23)	0.31 (0.33)
OPA19	2.00 (0.00)	1.81 (0.14)	0.44 (0.05)	0.64 (0.05)

Na – Observed number of allele, Ne – Effective number of allele, H – Nei's heterozygosity or gene diversity, I – Shannon's Information Index, OPA08 – primer 1, OPA11 – primer 2, OPA14 – primer 3, OPA19 – primer 4.

The result of the genetic diversity indices within the grasscutter population is shown in table 2. Diversity indices values for the light strain population were; Na (1.71), Ne (1.56), H (0.31) and I (0.44). While indices for the dark strain were; Na (1.61), Ne (1.39), H (0.25) and I (0.34). the values for the light strain were generally higher than those for the dark strain/population.

Table 2: Genetic Diversity Indices between the Grasscutter Population/Strain

Population/strain	Na	Ne	H	I
Light	1.71	1.56	0.31	0.44
Dark	1.61	1.39	0.25	0.34

Na – Observed number of allele, Ne – Effective number of allele, H – Nei's heterozygosity or gene diversity, I – Shannon's Information Index.

Nei's original measure of genetic similarity and Genetic distance were used to assess the genetic similarity (above diagonal) and genetic distance (below diagonal) across the two colour variants population of grasscutter in Imo state as shown in Table 3. The genetic similarity obtained in the result was 0.7904 (79.04%), while the genetic distance was 0.2353 (23.53%). Genetic distance is a measure of the genetic divergence between species or between populations within a species, whether the distance measures time from common ancestors or degree of differentiation (Nei, 1987). However, the genetic similarity of the grasscutter populations studied in Imo state was very high while genetic distance in the grasscutter populations was very low. This is an indication that the population/strains are closely related and have common ancestors. Hence, this will give an insight on the level at which the species is endangered as suggested by Ruane (1999).

Table 3: Measures of Genetic Similarity and Genetic Distance (Nei's 1972)

Population ID	Dark	Light
Dark	…….	0.7904
Light	0.2353	……

Above diagonal, genetic similarity; below diagonal, genetic difference

CONCLUSION

As far as genetics of grasscutter is concerned, this is the first time RAPD markers have been used to study the population in Nigeria and of course the first of its kind in the species range. These results will form a baseline from which inferences can be made for comparison in future variability, and diversity studies on the grasscutter. Since variations exist within and between the studied populations, selection of superior individuals within and between the populations/strains could be done for outcrossing. The diversity indices revealed how informative the primers are in assessing heterogeneity in the population and serves as an index that reflects genetic variation of RAPD loci. Hence, the various values of the diversity indices showed that assertions should not be made only with an index. There is need to combine the various information from the indices before assertions could be made.

RECOMMENDATIONS

The study therefore recommends that the light population should be given more attention for selection and improvement, since it harbors more diversity in the gene, as this will help the population to adapt to changing environmental conditions. However, superior individuals within the different populations/strains could be selected, cross bred within the populations (inbreeding) and crossed between the populations/strain (outcrossing) for improvement and heterosis respectively. Further molecular genetic

studies should be conducted on grasscutter in Nigeria, especially one involving the use of other marker tools and sequencing of the DNA to infer accurately and precisely, if the colour variations that exist within this breed could have a genetic potential needed for breeding, other than mere phenotypic variation.

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