

GENETIC DIFFERENTIATION BETWEEN BLACK-SKINNED AND WHITE-SKINNED ECTOTYPES OF GIANT AFRICAN LAND SNAILS (*Archachatina marginata*) IN CALABAR, NIGERIA

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ABSTRACT

This noble research provides information on the genetic differentiation between black-skinned and white-skinned ectotypes of giant African land snails (*Archachatina marginata*). Ten (10) snails consisting of five (5) black-skinned and five (5) white-skinned ectotypes were examined by using the random amplified polymorphic DNA (RAPD) technique. Five (5) primers (OPAD-09, OPAE-04, OPAE-05, OPAF-07 and OPAF-09) were screened and selected to amplify DNA from the ten (10) samples of snails. A total of 31 bands were generated from the two snail types, out of which 14 bands were generated from the black-skinned ectotype, while 17 bands were from the white-skinned ectotype. The DNA banding between the two ectotypes showed no statistical difference ($P > 0.05$) between the black-skinned and the white-skinned ectotypes of *Archachatina marginata*. There were however, relative high genetic differences in numerical number of bands between the two ectotypes. This implied that the genetic similarities are relatively low. The high genetic differences between the two ectotypes of *Archachatina marginata* snails studied signaled high level of genetic diversity and heterogeneity among the giant African land snails (GALS).

KEYWORDS: DNA, Banding, Differentiation, Black-skinned, White-skinned.

INTRODUCTION

Giant African land snails consist of black-skinned and white-skinned ectotypes belonging to the family *Achatinidae* (Akinnusi, 2002, 2004; Venette and Larson, 2004; Okon and Ibom, 2012). Classification within the group is based on conchological features (Bequart, 1950), phenotypic traits (Okon and Ibom, 2012), and the highly variable reproductive tract (Mead, 1991). Molluscs like other invertebrates are lesser known because relationships between them are not clear while their taxonomic inferences are often hindered by lack of morphological diversification between different lineages, as it occurs in different cryptic species showing overlapping variability (Elejalde *et al.*, 2008). Genetic differences exist among snails of a given breed (type) partly because of strain differences in the form of foot (skin) pigmentation and partly because of differences in their places of origin. Hence, the popular formula, $P = G + E$, linking the trait(s) with the genotype and environment, where P is phenotype, G is the genotype and E is the environment (Okon and Ibom, 2012). Most economically-important traits of snails are influenced to some degree by heredity. The degree to which heredity (genetics) affects performance depends on the particular

trait concerned (Okon and Ibom, 2012).

Recent advancement in molecular biological techniques, such as polymerase chain reaction (PCR) and DNA automated sequencing, nucleic acid data are becoming more and more important in biology (Hillis *et al.*, 1996). One of the modern marker techniques for studying genetic variability is Random Amplified Polymorphic DNA (RAPD) (Williams *et al.*, 1990). The techniques require no prior knowledge of the genome and it needs only a small technique and polymorphism can be detected in closely related organism.

MATERIALS AND METHODS

Experimental site

The research was carried out in the Biotechnology Laboratory, Federal University of Agriculture, Abeokuta, Nigeria.

Sample collection

Ten (10) *Archachatina marginata* snails, consisting of five (5) black-skinned and five (5) white-skinned from the snail sanctuary of the Department of Animal Science, University of Calabar, Calabar, Nigeria were used for the genetic differentiation between the black-skinned (Plate

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1) and white-skinned (Plate 2) ectotypes of giant African land snails (*A. marginata*).

DNA extraction and RAPD

DNA was extracted based on the CTAB method of extraction described by Rolfs *et al.* (1992). The quality of DNA was measured by obtaining the absorbance reading at 260nm and the purity of DNA was estimated by calculating the ratio of absorbance reading at 260nm and 280nm. Five (5) RAPD primers (OPAD-09, OPAE-04, OPAE-05, OPAF-07 and OPAE-09) (Table 1) were screened. Primers that have the basic of sharpness, clarity of the profile and the existence of polymorphism were chosen for further study (D'amato and Corach, 1997). The total reaction mixture containing 2.5ng of snail's DNA was used with the final concentration containing 30 x reaction buffer - 2.5u.l, magnesium chloride 50mM, TagDNA polymerase 2.0ul, 2.0ul dNTPs, DMSO of 1.0u.l, and 1.0ul primer. The DNA was amplified by using a Master Cycle Gradient (Eppendorf). The amplification was programmed at 45cycles for 30 seconds of denaturation at 94°C, 30 seconds of annealing at 72°C and final extension of 2 minutes at 72°C. PCR product was electrophoresed on 1.5%(w/v) agarose gel in 1 x TBE buffer at 55V for 1 to 2 hours depending on the size of amplified fragment from each primer. The gel was stained in 15ul of ethidium bromide for 20 to 30 minutes after which a photomicrograph was taken where different band patterns were seen (Plate 3).

Data analysis

The data collected based on the DNA banding patterns were counted based on the individual snail sample and then were subjected to t-test analysis according to Steel and Torrie (1990).

RESULTS AND DISCUSSION

The results of the genetic differentiation between the black-skinned and the white-skinned ectotypes of giant African land snails (*Archachatina marginata*) (Plate 3) revealed a total number of 31 bands generated from the two (2) snail types, and out of these numbers, 14 bands were generated from the black-skinned ectotype, while 17 bands were from the white-skinned ectotype (Table 1). The DNA banding of the two snail ectotypes (Table 1) indicated that the white-skinned ectotype had the highest number of DNA bands (17), while the black-skinned ectotype had the lowest number of DNA bands (14). The differences that existed between the black-skinned and the white-skinned snail ectotypes may be due to the presence or absence of pigmentation on the skin and also suggested that land snails are prone to effects of population differentiation with reduced gene exchanges between them, leading presumably to strong local, differentiation (Schilthuizen and Lombaerts, 1994).



Plate 1: Black-skinned Ectotype



Plate 2: White-skinned Ectotype

Table 1: List of Primers and Products Generated through Amplification

S/N	Primer	Sequence (5 to 3)	Total number of bands	Number of bands generated based on snail sample	
				Sample 1	Sample 2
1	OPAD-09	TCGCTTCTCC	3	1	2
2	OPAE-04	CCAGCACTTC	9	4	5
3	OPAE-05	CCTGTCAGTG	8	3	5
4	OPAF-07	GGAAAGCGTC	5	3	3
5	OPAF-09	CCCCTCAGAA	6	3	3
Total			31	14	17

Sample 1 = Black-skinned snail ectotype, Sample 2 = White-skinned snail ectotype.

Table 2: T-Test Analysis Between Black-Skinned and White-Skinned Ectotypes in Terms of Number of Bands.

S/N	DNA Bands for WS	DNA Bands for BS
1	2	1
2	5	4
3	5	3
4	2	3
5	3	3
\bar{X}	3.40	2.80

WS = White-skinned snail ectotype, BS = Black-skinned snail ectotype,
 NS = Non-significant ($P > 0.05$), $t_{tab} = 2.306$, $t_{cal} = 0.542^{NS}$

Though there were high genetic differences between the black-skinned and the white-skinned ectotypes in terms of number of bands (Table 1), there was no statistical difference ($P > 0.05$) between the two ectotypes with respect to the bands number (Table 2). The high genetic differences between the two ectotypes of *Archachatina marginata* studied, signals high level of genetic diversity, low genetic similarity and heterogeneity among the giant

African land snails (GALS). The genetic differences between the two ectotypes in terms of banding pattern have been established for the black-skinned and white-skinned ectotypes of giant African land snails (*Archachatina marginata*). It is therefore recommended that researchers should work towards determining the gene(s) that are responsible for the differences amongst these giant African land snails (GALS).

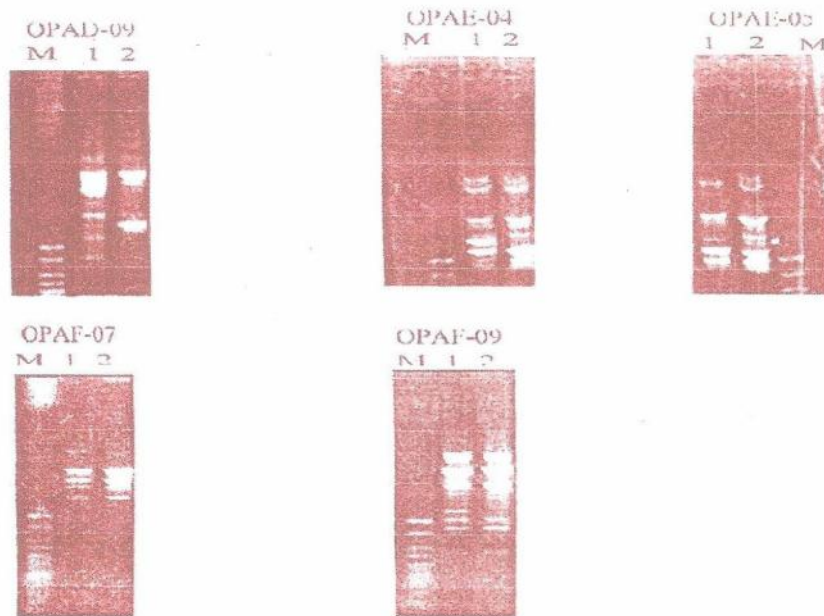


Plate 3: Electrophoresis gel pictures for the RAPD primers

CONCLUSION

The results of this noble research showed that the number of bands between the black-skinned and the white-skinned ectotypes of giant African land snails (*Archachatina marginata*) were not statistically ($P > 0.05$) different, but differed numerically.

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