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Evaluation of Mutation Type Associated with SNPs of Black Skinned and White Skinned Snails (*Archachatina marginata*) in the Rainforest Zone of Nigeria

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Abstract:- This study evaluated the type of mutation associated with SNPs of black skinned and white skinned snails (*Archachatina marginata*) in the rainforest zone of Nigeria. Fourty eight (48) *Archachatina marginata* snails were assigned to two treatments using completely randomized design. The two treatments consisted of twenty four (24) white skinned ecotypes and twenty four(24) black skinned ecotypes with 12 replicates each. These snails were taken to laboratory to conduct DNA analysis to evaluate the mutation type that is associated with the SNPs of the two snail ectotypes. The quality of DNA was assessed using gel electrophoresis. Data collected was subjected to statistical analysis using Mega 7.0, DNAsp version 5 and GENPOP software packages. Mutation result obtained in this study revealed that both types undergo both substitution and deletion with deletion being predominant in the black and substitution in the white skinned *A. marginata.* It was also observed that the codons mostly affected by deletion are the nucleotides coding for amino acids phenyl alanine and alanine. These amino acids are responsible for growth, immunity and skin colouration. Since the immune-related genes are absent in black people, the high rate of deletion that black experience may be the cause of their high mortality rate. Self-fertilization, a high degree of inbreeding, and restricted gene flow all contribute to the high rate of deletion. Thus, it would be beneficial to promote the crossbreeding of *A. marginata* snails with black and white skin.

Keywords:- *Mutation, Evaluation, Substitution, Deletion.*

I. INTRODUCTION

In several coastal settlements in Nigeria alongside other areas of Africa, the meat of the massive African land snails, also known as gals, is highly valued as a delicacy and a major source of animal-derived protein. They are an unconventional source of protein (Omole et al., 2017; Fatai 2018). The decreased ability of the traditional protein sources—goats, sheep, cattle, and chickens—to satisfy demand and address the issue of protein insufficiency is the reason behind the rise in the use of this non-conventional protein. According to Cagiltayet al. (2011), the incapacity to meet demand is brought on by illness, a protracted drought, high feed costs, antiquated methods of animal husbandry, low productivity of native breeds, as well as an increase in the number of people and their standard of living, all of which put significant strain on the traditional sources of animal protein.

According to Danladi et al. (2020), meat from snail is tasty, healthy, and extremely with vital amino acids such as isoleucine, lysine, phenylalanineand leucine. It is low in fat, cholesterol, and sodium and high in minerals like iron, calcium, and phosphorus. It has linoleic and linolenic acids, which are important fatty acids (Danladi et al., 2020).

In major regions of Africa, the sample snails are used extensively in traditional medicine to prepare treatments for conditions like whooping cough, diabetes, and asthma (Offiong et al., 2013). Because of their comparatively low cholesterol levels, snails have also been suggested as a treatment for anemia, asthma, and certain related conditions (Wosu, 2003).

A snail excretes a large amount of slimy material called snail slime along its route(Nyoagbe et al., 2016; Jeff et al., 2017). According to Jeff et al. (2017), this slime helps the snail move more smoothly, prevents liquid from vaporizing, repairs its impaired shell and body, and protects its internal structure from harm from impact.According to Nyoagbe et al. (2016), snail slime's animal protein content is expected to be a significant substance in the curing bleeding and suppression of inflammation. While the shell is used to make jewelry and ornaments, it is also used in the manufacturing of drugs by pharmaceutical and cosmetic companies (Nyoagbeet al., 2016).Achatina fulica, Achatina achatina, and Archachatina marginata are some of the prevalent land snails in Western region of Africa (Ejidike 2002, Smith and Fowler 2003). Okonet al. (2012) state that the most common and second-largest breed of snail kept or raised in Nigeria is the Archachatina marginata. According to Akinnusi (2004), Ejidike (2002), Ibom (2009), Okon et al. (2008), Okon and Ibom (2012), A. marginata may have black or white skin. In many African nations and beyond, snails are widely

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recognized and valued (Akinnusi, 2004; Okon and Ibom, 2012; Ibom, 2009).

Despite the many advantages of A. marginata snails, they are still harvested excessively and indiscriminately within the wild; along deforestation, has significantly decreased the number of African giant land snails (AGLS). Accordingly, there is a risk of extinction for the species (Adikwu 2012; Ojaking and ogar 2002; Okon et al., 2008; Etukudo 2017; Okon and Ibom 2012).

Puurtinen et al. (2004) also pointed out that small and isolated populations may be more vulnerable to extinction due to genetic threats that coincide with their small size. It is imperative that a quick study be conducted to obtain genetic information on GALS (A. marginata), since there are no current genetic studies about this breed. The goal of this study is to calculate the genetic separation between and within GALS Archachatina marginata individuals with white and black skin. Genetic difference is an estimation of inherited diversity between groups of individuals within a species, regardless of whether the distance is expressed as a matter of duration from a shared ancestor or extent of diversity.

It is critical to comprehend the genetic makeup of farm animals in order to improve genetic diversity and subsequently use this diversity to reduce food insecurity in developing nations (Etukudo et al., 2018). This is based on the assertion that their assessments of variation yield foundational information that guides the selection of parent strains for a crossing program and locates genetic material for enhancement and cultivation of animals (Duran et al., 2009; Askariet al., 2011). This study's objective is to assess the kind of mutation linked to SNPs in the black- and white-skinned Archachatina marginata collected from South-South, Nigeria.

II. MATERIALS AND METHODS

In order to perform the DNA analysis, 48 grower snails from Edo, Bayelsa, and Akwa-Ibom state were randomly selected, two from each replicate, for each of the two treatments. The snails were then brought to the laboratory. The snails were killed by cracking open their shells, removing the edible flesh from the fluid and shell, and then chopping off a piece of their muscular foot to extract their DNA. In accordance with the manufacturer's instructions, tissue was pounded into a smooth, blended form and combined with the Zymo Research DNA Extraction kit to extract DNA. The Department of Animal Science, University of Port Harcourt, Port Harcourt, Genomic Laboratory stored DNA extractions at -120C.

To achieve the best results, 500 µl of user-supplied beta-mercaptoethanol was introduced to the Genomic Lysis Buffer at a final dissolution of 0.5% (v/v), or 100 ml.

- A specimen (or specimens) was placed in a ZR Bashing BeadTM Lysis bottle (2.0 mm). The tube was filled with 750l Bashing BeadTM Buffer and tightly sealed. (In general, no more than 50mg of tissue was sampled because larger samples would exceed the spin column's DNA binding capacity). A total of 400 l of whole blood cells suspended in 200 l PBS was sampled.
- After that, it was placed in a bead beater with a 2 ml tube holder assembly (such as the Disruptor GenieTM) and processed for 10 minutes at maximum speed. Processing time is dependent on the bead beater and sample input. The use of high-speed cell disrupters (FastPrep® -24) was carried out within five minutes.
- A micro centrifuge was used to centrifuge the ZR Bashing BeadTM Lysis bottle (2.0 mm) for 60s at a force of ≥10,000 x g.
- The supernatant (about 400µl) harmonized at 8,000 x g for 60s after being transferred to a Zymo-SpinTM III-F Filter in a receiving bottle.
- The filtrate from Step 4, make up to 1,200µl of Buffer used for lysing and thoroughly mixed.
- After transferring 800µl of the mixture from Step 5 in a Zymo-SpinTM IICR Column1 in a Collection Tube, it was centrifuged for one minute at 10,000 x g.
- After discarding the flow through from the Collection Tube, Step 6 was carried out once more.
- After adding the DNA (200µl) Pre-Wash Buffer to the Zymo-SpinTM IICR Column in a fresh Collection Tube, the tube was centrifuged for one minute at 10,000 x g.
- Actual Zymo-SpinTM IICR Column was then filled with 500µl Wash Bufferof g-DNA, and it was centrifuged for one minute at 10,000 x g.
- The Zymo-SpinTM IICR Column was then moved to a sterile 1.5 ml microcentrifuge tube, and 100 µl of DNA elution buffer (a minimum of 35 µl can be used) introduced straight to the column matrix. After that, the DNA was extracted by centrifuging it for 30 seconds at 10,000 x g.

Using a DNA monogram, the amount and quality of DNA were evaluated; samples containing less than 5 µml were discarded. The quantified DNA was further examined by gel electrophoresis about an hour, utilizing a portable gel hood with a built-in Blue LED (470nm) by Royal Biotech/Biolympics (www.royalbiotech.com). They were photographed in the ultraviolet light and made visible using Ethidium bromide staining. The ladder that is being used is a thermo scientific 100 base pair ladder.

➢ *Cytochrome B Primers*

Cytb F 5'- CCATCCAACATCTCAGCATGATGAAA -3'
Cytb R 5'- CCCCTCAGAATGATATTTGTCCTCA-3'

The subsequent cocktail mixture was used to precondition the DNA for PCR.

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Table 1. Polymerase Chain Reaction Cocktail Mix

Materials	Proportion
10× PCR buffer	2.50
25mM $MgCl_2$	1.00
5pMol forward primer	1.00
5pMol reverse primer	1.00
DMSO	1.00
2.5Mm DNTPs	2.00
Taq 5u/µl	0.10
10ng/µl DNA	3.00
H_2O	13.40
Total volume	25µL

- *TOUCH DOWN PCR CONDITION*

Table 2 Conditions for Touch Down PCR

	9 Cycle			35 Cycles				
Initial den.	**Den.**	**Ann. Tempt**	**Extension**	**Den.**	**Ann. Tempt**	**Extension**	**Final extension**	**Hold tempt**
94˚C	94˚C	65˚C	72˚C	94˚C	55˚C	72˚C	72˚C	10˚c
5min	15sec	20sec	30sec	15sec	20sec	30sec	7min	∞

Den = Denaturation; Ann = Annealing; Tempt = Temperature

For about an hour, the amplicons from the aforementioned reaction were loaded onto 1.5% agarosegel at a steady voltage and 1X TBE. They were photographed in the ultraviolet light and made visible using Ethidium bromide staining. The ladder that is being used is a thermo scientific 50 base pair ladder. Using the following primers, the Mitochondria DNA (Cytochrome B) gene was amplified:
F 5'- CCATCCAACATCTCAGCATGATGAAA -3'Cytb
R 5'- CCCCTCAGAATGATATTTGTCCTCA-3'Cytb

Each Polymerase Chain Reaction (PCR) microtube had 25µl volume and was included 5µl of Nuclease-free water, Quick-Load 2x Master mix One Taq* with standard buffer, 10.0µl of PCR premix, 8µl of genomic DNA, and 1.0µl of MC4R forward and reverse primers, respectively. A touch-down PCR condition was applied, comprising of initial denaturation at 94˚C in five minutesand nine cycles of denaturation at about 94˚C for fifteen seconds each, an annealing at 62˚C for 20s, and thirty seconds of extension at 72˚C. The next 35 cycles were: 15 seconds at 94˚Cof denaturation, 20 seconds at 58˚Cfor annealing, 30 seconds at 72˚Cof extension, and 7 minutes at 72˚Cof final extension. The results for the Polymerase Chain Reaction (PCR) were electrophoresed for 40 minutes on a 1% agarose gel with IX TBE and a constant voltage. They were photographed in the ultraviolet light and made visible using Ethidium bromide staining. The International Institute for Tropical Agriculture (IITA), located in Ibadan, Nigeria, received the qualitative Polymerase Chain Reaction (PCR) products for sequencing. Mega 6.0 was used to align DNA sequences (Tamura et al., 2013). DNAsp version 5, Mega 7.0, and the GENEPOP software package were used to determine single nucleotide polymorphism and other sequence variation parameters (Rousset, 2008).Finch TV 1.4.0 and Mega 7.0 software were used to perform the statistical analysis of the aligned sequences.

III. RESULTS AND DISCUSSION

The result on plate 1 and Table 3 show the mutations in the sequences of black and white snails used in this study. Plate 1 shows the nucleotide. From the results, it was observed that there are two mutations types associated with white skinned and black skinned *A.marginata* snails which is deletions and substitutions. In cyt 42 deletions were observed in the first nucleotide in the 6^{th} , 19^{th} and 30^{th} , codons likewise for cyt. 14, deletions were observed in the 1^{st}, 4^{th}, 10^{th}, 13^{th}, 16^{th}, 21^{st} , 26^{th} , and 39^{th} codons meanwhile for cyt 11 deletions were observed in the 1^{st} , 4^{th} , 5^{th} , 6^{th} , 10^{th} , 13^{th} , 16^{th} , 21^{st} and 26^{th} codon. Majority of the deletions are found to affect the amino acid alanine and phenyl alanine. These finding are in tandem with the findings of Libiger et al., 2009 who discovered that phenylalanine enable the production of melanin pigmentation which provides the skin, hair and eye colouration, while alanine helps to breakdown vitamin B6 and tryptophan an animo acid responsible for growth performance.

Table 3 showed the InDels (Insertion – Deletion) polymorphism among the snails used in this study. There were 180 sites for white snails and 181 for black snails overall (not counting sites with gaps in the data). Additionally, 109 InDel sites with black snails and 110 with white snails were examined overall. Average InDel length event was 24.692 in black and 19.231 in white snails. In addition, Number of InDel Haplotypes were 7 in black snails and 10 in white snails. However, InDel Diversity, k (i) was 3.905 in black snails and 3.222 in white snails. In black snails, the sequence in Theta was 5.306, while in white snails, it was 4.595. For black

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snails, Tajima's D value was -1.446, and for white snails, it was -1.359. Kimura (1983) tested the neutral theory of molecular evolution using Tajima's test, also known as the D test statistic (Tajima, 1989). In other words, the great majority of molecular variations resulting from spontaneous mutations do not affect an individual's fitness. Genetic drift, gene flow and natural selection, are the mechanisms that causes changes in frequencies of allele over time in a population, and they may be responsible for the variation in these values between black and white snails (Andrews, 2010).

The result also revealed that the black skinned (BS) snails are more affected by deletion than the white skinned (WS) snails. This makes the black snail more polymorphic unlike the white snails that are more conserved. Mutations are modifications in the DNA sequence of the genome of an individual (Beadle & Tatum1941). Mutations are changes to an individual's genome's DNA sequence (Beadle & Tatum, 1941). Codon errors brought about by variations in nucleotide bases are known as mutations. Not all mutations will have a significant impact. The genetic code is degenerate, so even if the codon GAA changes to the codon GAG, it will still code for the amino acid glutamate. Silent mutations are those that have no effect. On the other hand, certain mutations can significantly impact the way that amino acid coding is produced, which can alter the proteins that are made and ultimately impact how cells and organisms function. The findings of this study could potentially be explained by random genetic drift or the pressure of directed selection, which frequently leads to new, active genetic mutations spreading over extended periods of time as a result of "survival of the fittest" (Wade, 2008).

Removals or substitutions are two types of mutations. A mutation known as substitution occurs when one base pair is swapped out for another base pair. The substitution of one amino acid for another in a protein is also referred to by this term. The term "substitution" describes the process of changing one amino acid in a protein for another or one nucleotide in DNA or RNA for another.

Generally speaking, substitutions always result in one of two outcomes: either a polymorphism, or a difference between an individual and other individuals within a population, or a particular type of polymorphism known as a mutation. One kind of mutation that involves the loss of genetic material is called deletion. In actuality, deletion indicates a missing component. And when a geneticist talks about deletion, what they mean is that a piece of genetic material is missing. This piece of missing genetic material could be small, like a single base pair, or it could be larger, like a gene or its entirety, or even larger, like a chromosome. Gonzalez (2020) has suggested that natural selection, mutation, gene flow, and random genetic drift are the causes of this variation. In essence, mutations are changes in an allele brought about by evolutionary processes. Allele frequencies may change as a result of the interaction and opposition between these evolutionary processes, which may occur concurrently (Svensson, 2017).

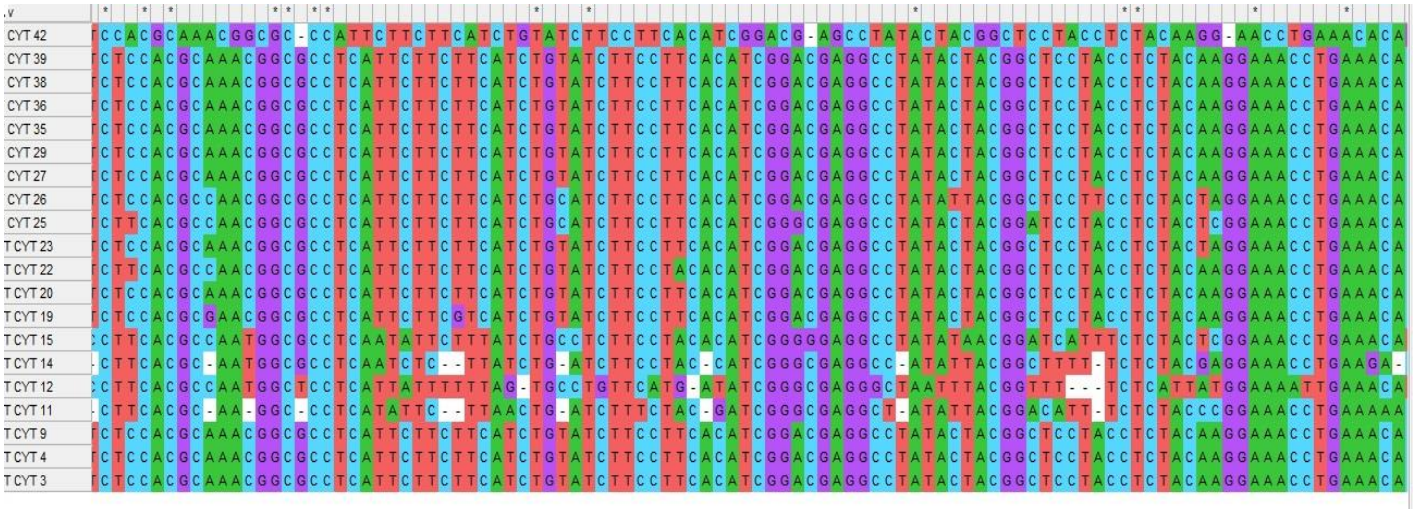

Plate 1: Aligned Nucleotide sequences of 20 snail samples showing mutations – deletions and substitutions. * Shows uniformity of nucleotide in all snail sequences

Key: White 3, 4, 9, 20, 25, 27, 35, 36, 38, 42 Black 11, 12, 14, 15, 19, 22, 23, 26, 29, 39
Dash (-) means delition,

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Table 3 InDels (Insertion – Deletion) polymorphism

Parameters	Black	White
Total number of sites (excluding sites with gaps/missing data)	181	180
Total number of InDel sites analysed	109	110
Average InDel length event	24.692	19.231
Number of InDel Haplotypes	7	10
InDel Diversity, k(i)	3.905	3.222
Theta (per sequence) from I, Theta(i)-W	5.306	4.595
Tajima's D	-1.446	-1.359
Statistical significance	Not significant, P > 0.10	Not significant, P > 0.10

IV. CONCLUSION

The findings show that the snail population is associated with two types of mutations (Substitution and Deletion) related to SNPs. The black population is more impacted by deletion, which can be further amplified by self-fertilization, inbreeding, and decreased gene flow, ultimately resulting in low survivability and high mortality. Additionally, it was found that the majority of the deleted codons contain nucleotides that code for the amino acids' alanine and phenylalanine. While alanine aids in the breakdown of tryptophan and vitamin B6, phenyl alanine is applicable for the secretion of melanin, a natural pigment that produces coloration of the skin, hair, and eyes. Tryptophan enhances muscle protein synthesis, lowers stress levels, controls insulin, and enhances the quality of meat in animals. It also aids in the body's utilization of sugar and provides energy for the central nervous system and muscles. Poor growth and high mortality have been observed in the black snails as a result of the significant deletion of these amino acids. Therefore, it is advised that upgrading and crossbreeding be done to lower the black snails' rate of deletion.

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