academicJournals

Vol. 12(32), pp. 5035-5039, 7 August, 2013
DOI: 10.5897/AJB2012.2949
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Genetic differentiation between the black skinned and white skinned snails *(Archachatina marginata)* using random amplified polymorphic DNAs

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Accepted 31 July, 2013

The study investigates the genetic differentiation between the black skinned and white skinned ectotypes of the giant African land snails *(Archachatina marginata)* from Cross River State in Niger Delta region of Nigeria. The random amplified polymorphic DNA (RAPD) technique was employed in this study. Five (5) oligonucleotide primers (OPAD-09, OPAE-04, OPAE-05, OPAF-07 and OPAF-09) were used to amplify DNA from five samples of *A. marginata* constituting black skinned and white skinned ectotypes. A total of 58 RAPD bands with 24 polymorphic bands (40.18%) with size range of 150 to 5,500 bp, were scored from the populations. The black skinned ectotype had mean percentage polymorphism of 34.50%, while the white skinned ectotype recorded mean percentage polymorphism of 41.40%. Genetic similarity coefficient ranged from 60 to 63%, while the genetic distance ranged from 0.37 to 0.40. The genetic similarity between the two ectotypes of *A. marginata* from Cross River State is high and depicted low genetic differences. This reveals that the genetic variability of the species *(A. marginata)* from Cross River State is gradually eroding. Efforts should be made to conserve the genetic pool of this species, as the erosion of genetic variability is dangerous and could continue unnoticed till extinction is imminent.

Key words: Genetic, differentiation, snail, ectotypes, Niger Delta.

INTRODUCTION

Archachatina marginata is one of the species of African land snails known as Giant African land snails (GALS) (Akinnusi, 2002; 2004; Okon et al., 2008; Okon and Ibom, 2012). It belongs to the phylum Mollusca and the class gastropoda, it could be black- or white- skinned (Ejidike, 2002; Akinnusi, 2002, 2004; Okon et al., 2008; Ibom, 2009; Okon and Ibom, 2012). The snail is popular and highly appreciated as a valuable source of animal protein in many countries of Africa and beyond (Akinnusi, 2002, 2004; Ibom, 2009; Okon and Ibom, 2012). In Nigeria, *A. marginata* constitutes an important component of the food of numerous rural dwellers, especially in the rain-forest zones (Akinnusi, 2004).

A. marginata offers an opportunity to complement the supply of animal protein from conventional and regular sources such as beef, pork, chevon, mutton, among others. This will to a great extent reduce the problem of animal protein inadequacy, as the per capita protein consumption of an average Nigerian is still low (World Bank, 2001). However, several workers (Ojating, 2001; Ojating and Ogar, 2002; Okon et al., 2008; Okon et al., 2009) have reported a drastic depletion of populations of GALS owing to excessive and indiscriminate gathering of snails (both juvenile and adult) from the wild; coupled with deforestation, bush burning and other human activities. Consequently, the genetic diversity of GALS

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Table 1. Primers and size of fragments generated.

Primer	Size of fragment (bp)
OPAD-09	200 - 1500
OPAE-04	250 - 2500
OPAE-05	150 - 2500
OPAF-07	250 - 3000
OPAF-09	200 - 5,500

(A. marginata) is gradually eroding thus threatened with extinction. Puurtinen et al. (2004) also noted that genetic threats associated with small population size may contribute to the elevated extinction risk of small and isolated populations. This necessitates a swift study to provide genetic informations on GALS (*A. marginata*) as there is presently little or no information on genetics differentiation and breeding of GALS.

The study of the structure and function of genes at molecular level in a breeding population can help to determine the genetic differentiation and similarity of such population (Lee et al., 2011). One of the modern marker techniques for such study is random amplified polymorphic DNAs (RAPD) (Williams et al., 1998). The technique is simple and does not require prior knowledge of the genome. Also, it requires small amount of DNA (Hadrys et al., 1992). The technique also enables detection of polymorphism in closely related organisms. The objective of this study was to genetically differentiate between the black skinned and the white skinned ectotypes of *A. marginata* using RAPD.

MATERIALS AND METHODS

Collection of samples

A total of 60 snails (*A. marginata*) comprising of 40 black skinned and 20 white skinned ectotypes were randomly picked from the three agro-ecological zones (Southern agro-ecological zone, Central agro-ecological zone and Northern agro-ecological zone) of Cross River State, Nigeria.

DNA extraction and RAPD

Live snails were transferred to the molecular laboratory of Federal University of Agriculture, Abeokuta (FUNAAB) for genotyping. DNA was extracted following a modified phenol chloroform extraction and ethanol precipitation as described by Sambrook et al. (1989). About 10 µl of each DNA was taken into eppendorf tube with 90 µl sterile distilled water to obtain 20 - 50 ng/µl. The total reaction volume of 20 µl was used with final concentration containing 20 ng of genomic DNA, 10x reaction buffer (2.0 µl), 25 mM magnesium chloride (1.6 µl), 5% Tween 20 (2.0 µl) water (8.2 µl). The amplification was programmed at 45 cycles for 20 s of denaturation at 94°C, 20 s of annealing temperature at 37°C, 40 s of primers extension at 72°C and final extension of 7 min at 72°C. This process was repeated four times to ensure accuracy. Five RAPD primers (Table 1) from Operon Kit A (with 60% G + C content) were used. PCR product was electrophoresed on 1.4% agarose gel in 1 x TAE buffer at 150 V and 0.5 miniamp for 2 h. The gel was stained in 2.5 mg/L ethidium bromide for 3 min and further destained for 10 min in sterile water and then viewed under ultra violet light and the picture was taken.

Data analysis

The RAPDs banding profiles were visually scored for all the DNA samples and for each primer. The recording of the data was according to the presence/absence criterion. Clear and visible bands were marked as present (1) or absent (0). Bands that were faintly stained were not considered in the data. These bands were considered as polymorphic when they were absent in some samples in frequency greater than 1% (Jorde, 1995) and change in band intensity was not considered as polymorphism. Similarity coefficients were calculated across all the possible pair wise comparisons of snail samples among populations, using the formula below:

$$Sxy = \frac{2nxy}{nx + ny}$$

Where, nxy is the number of common bands shown in both individuals x and y, and nx and ny are the total numbers of bands observed in individuals x and y respectively (Nei and Li, 1979). As means of providing a visual representation of genetic relationships, a dendrogram was constructed based on the similarity coefficient values (1 - Sxy) between pairs of snail samples. The dendrogram was constructed using the unweighted pair group method of Arithmetic averging (UPGMA) employing the sequential, agglomerative, hierarchical and nested clustering (SAHN) from NTSYS-PC program (Rohlf and Marcus, 1993).

RESULTS AND DISCUSSION

Generation of RAPD pattern of Genomic DNA

Five primers from the Operon Kit A (OPAD-09, OPAE-04, OPAE-05, OPAF-07, OPAF-09) with 60% G+C content were used in this study. These primers were used to generate RAPD pattern of genomic DNA for samples of snails (*A. marginata*) from Cross River State, Nigeria. The results showed that the largest fragment was 5,500 bp, while the smallest fragment was about 150 bp in size (Table 1).

The results of the RAPD DNA fragment showed that different primers generated different numbers of fragments and lengths of DNA amplification products (Table 2). From the five samples, a total of 58 bands were generated using the five primers. Primers OPAD-09 and OPAE-04 each generated 13 fragments; primer OPAE-05 generated 10 fragments, OPAF-07 generated seven fragments, while OPA-09 generated 15 fragments (Figure 1).

The numbers of RAPDs bands generated by the five primers and in each of the two ectotypes of *A. marginata* studied are shown in Table 3. The total numberof polymorphic bands were 24 out of 58 reproducible bands

Table 2. Code sequence, nucleotide lengths and G+C content of primers used in the RAPD analysis.

Primer name	Primer sequence (5' to 3')	Nucleotide length	G+C content (%)
OPAD-09	TCGCTTCTCC	10-mers	60
OPAE-04	CCAGCACTTC	10-mers	60
OPAE-05	CCTGTCAGTG	10-mers	60
OPAF-07	GGAAAAGCGTC	10-mers	60
OPAF-09	CCCCTCAGAA	10-mers	60

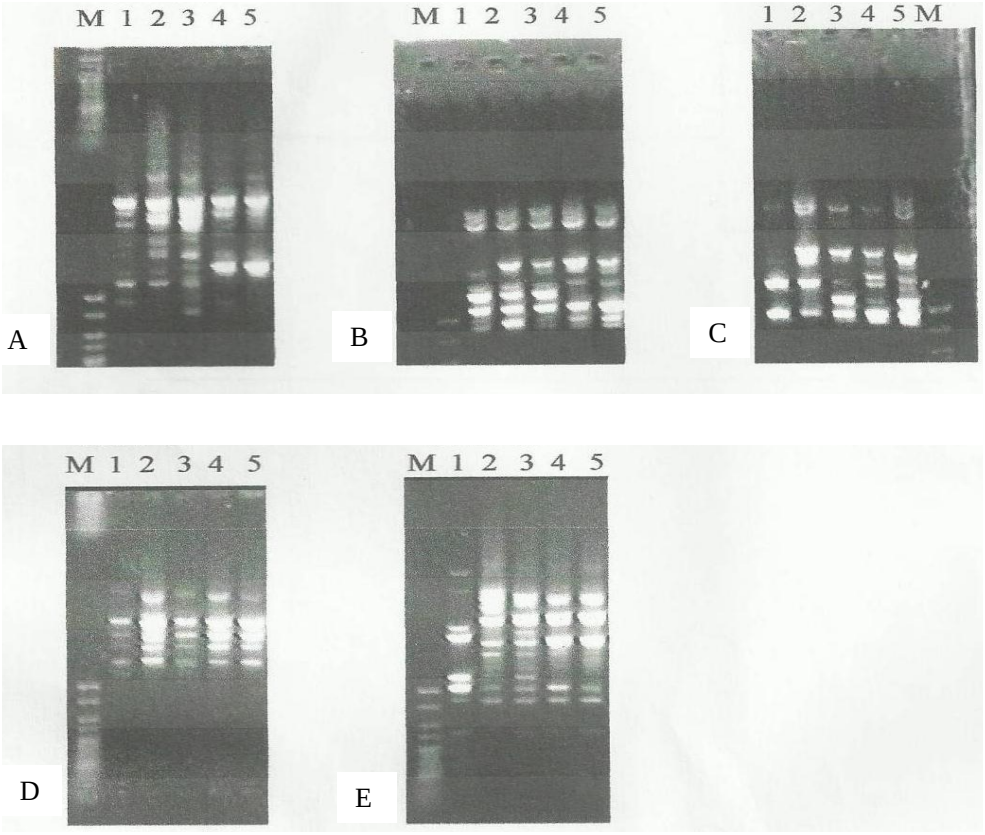

Figure 1. Electrophoresis gel pictures for the RAPD primers. A, OPAD-09; B, OPAE-04; C, OPAE-05; OPAF-07; E, OPAF-09.

Table 3. The number of RAPD fragments and polymorphic fragments generated by five primers in black skinned and white skinned *A. marginata*.

Primer	Total reproducible band	Total polymorphic band	Number of polymorphic bands according to ectotypes		Percentage polymorphic bands (%)
			*BSS	*WSS	
OPAD-09	13	8	7	8	61.54
OPAE-04	13	4	4	4	30.77
OPAE-05	10	4	3	4	40.00
OPAF-07	7	2	1	2	28.57
OPAF-09	15	6	5	6	40.00
Total	58	24	20	24	40.18
Percentage polymorphism (%)			34.50	41.40	

*BSS, Black skinned ectotype; *WSS, white skinned ectotype.

Table 4. Similarity coefficient between black and white skinned snails (*A. marginata)*.

Sample	1	2	3	4	5
1	-				
2	60	-			
3	72	60	-		
4	60	63	60	-	
5	60	63	60	83	-

generated. Primer OPAD-09 generated the highest percentage of polymorphic bands (61.54%), followed by primers OPAE-05 and OPAF-09 (40.00%), while primer OPA-07 recorded the least percentage of polymorphic bands (28.57%). Meanwhile the white skinned snail recorded the highest percentage of polymorphic bands of 41.40% against the black skinned snails with 34.50% (Table 3).

This high polymorphism in white skinned ectotype of *A. marginata* could point towards a moderately higher population of white skinned ectotype and thus higher diversity than black skinned ectotype in the state (Puurtinen et al., 2004). According to Uboh et al. (2010), there is a cultural discrimination against consumption of white skinned snails in some parts of Cross River State, Nigeria. This implies that more of the black skinned ectotype is removed from the wild than the white skinned ectotype, thereby explaining the higher population of white skinned snails in the state.

The similarity coefficient between the white skinned and black skinned snails ranged from 60 to 83% (Table 4) which are quite high. This showed that the genetic differences between the two snail ectotypes is moderately low. This is in agreement with the findings of Wan et al. (2009) who noted that high genetic similarity indicates low genetic differences or variability between snail species. These low genetic differences between the white skinned and black skinned *A. marginata* could be attributed to a relatively small population size of these snails in the state of study, which might have resulted in high level of inbreeding.

This confirms the findings of Ojating (2001), Ojating and Ogar (2002), Okon et al. (2008) and Okon and Ibom (2012) that the excessive and indiscriminate gathering of GALS from the wild, couple with deforestation, bush burning have led to depletion of GALS population, thus subjecting the genetic diversity of the snail to threat of extinction. The low genetic variability or high genetic similarity could also be as a result of high rate of self-fertilization in these snails (Charlesworth et al., 1993; Worth et al., 1997). Furthermore, low genetic variability is reported to arise from reduction in effective population size which then allow further accumulation of deleterious mutations, thus reducing the fitness of the population (Puurtinen et al., 2004).

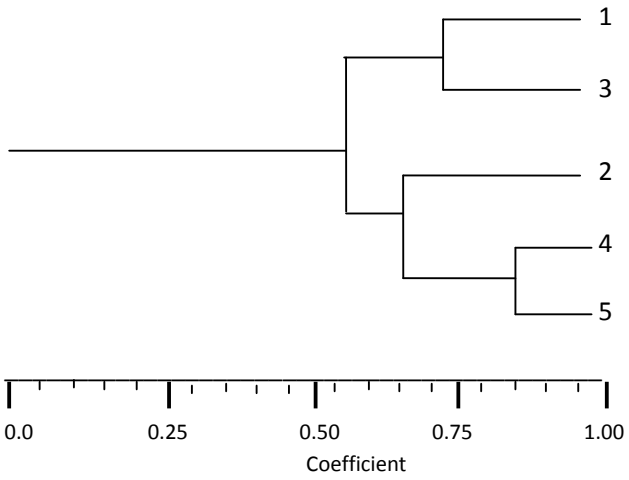

Figure 2. Neigbouring-joining dendrogram showing the genetic relationship between populations of white skinned and black skinned *A. marginata* in Cross River State of Nigeria.

The dendrogram (Figure 2) revealed that the snail samples were not just grouped based on spatial distribution but also based on the genetic closeness. This is indicated in the second cluster where the white skinned sample (5) snail is grouped along the black skinned snails' samples (2) and (4). This implies that the black skinned and white skinned *A. marginata* are of the same genetic background, otherwise there would have been distinct cluster for the white skinned snail.

Thus, the genetic differences here could be due to environmental factors such as random drift, mutation and possibly, selection (Weiner, 1994; Akinnusi, 2004; Puurtinen et al., 2004; Okon et al., 2008; Okon and Ibom, 2012). The genetic distance estimates between black skinned and white skinned *A. marginata* ranged from 0.37 to 0.40.

This small distance estimate may indicate population substructure like subpopulations in which there is random mating, but between which there is reduced amount of gene flow (Brent, 1996; Wan, 2009).

Conclusion

The genetic differentiation between samples of black skinned and white skinned snails *(A. marginata)* from Cross River State is low. This is depicted in the high genetic similarity (60 to 83%). Thus, it can be inferred that the genetic differences between these snails is mostly based on the influence of the environment (random drift, mutation and selection), since the snails are of the same genetic background. The results also demonstrated that loss of genetic variability or low genetic differences between these snails is associated with drastic decrease in population size, high level of inbreeding, high level of self-fertilization and thus limited

gene flow. The gradual erosion of genetic variability is dangerous, because it may go unnoticed, especially since the population is regulated by ecological factors, until the reproduction rate of the population is so low that extinction is unavoidable. Hence, more research should be carried out towards the conservation of genetic pool of GALS.

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