

Full Length Research Paper

Genetic and phenotypic variations in egg shell colour of two ectotypes of Giant African Land Snail (*Archachatina marginata* var. *saturalis*)

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Data from the snail farm of the University of Calabar, Nigeria were collected and utilized to investigate the distribution and gene frequencies of egg shell colour of two ectotypes of Giant African Land Snails (*Archachatina marginata* var. *saturalis*), black skinned (BS) × black skinned mating group, and white skinned (WS) × white skinned mating group. The eggs collected were scored for the presence of yellow (Y_p), light yellow (L_p) and milky white (M_p) egg shell colour. The egg shell colour distributions between the two ectotypes (BS × BS and WS × WS) were 46.50 vs. 3.77%, 42.68 vs. 48.11% and 10.83 vs. 48.11% for yellow, light yellow and milky white, respectively. In both ectotypes, the dominant gene for yellow, light yellow and milky white egg shell colour segregated at low frequencies (0.26 vs. 0.02, 0.24 vs. 0.31 and 0.06 vs. 0.31). The lowest value being yellow shell of the white skinned × white skinned mating group with frequency of 0.02, followed by milky white shell of the black skinned × black skinned mating group with frequency of 0.06. These values were much lower than the Mendelian value of 0.75. This is an indication that snails have not been purified through artificial breeding. Estimate of genetic distance between the two ectotypes were 0.060, 0.005 and 0.063 at yellow, light yellow and milky white loci, respectively. This shows that the ectotypes are closely related at the egg shell colour loci.

Key words: Genotype, phenotype, snail egg, shell colour, variations.

INTRODUCTION

Snail farming in Nigeria has received numerous attentions in the past decades (Akinnusi, 2002). Snail meat is widely consumed all over the world by both the rich and the poor (Murphy, 2001; Ebenso, 2003). The flesh of the Giant African Land Snail is of remarkable nutritive value, with high iron content (Ogbeide, 1974),

and a protein content of 37.00 to 51.30 g/100 g dry matter (Udedibie, 1989). It is possible that snail eggs might also be of high nutritive value, though it is not consumed by humans in Nigeria at the present. Okon et al. (2013) reported that snail eggs are good sources of protein and basic minerals (K^+ , Na^+ , Ca^{++} , Fe^{++} , Mg^{++} and

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Zn⁺⁺) that compare favourably with the flesh and chicken eggs. With growing awareness of the role of cholesterol in various heart and arterial diseases, the demand for low cholesterol meat like snails and by extension snail eggs will become more acute. Characterising snail eggs will help consumers' choice and/or preference, just as does chicken eggs colour.

Among the most common land snails in West Africa are *Achatina achatina*, *Achatina fulica*, *Archachatina marginata* and *Limicolaria aurora* (Ejidike, 2002; Smith and Fowler, 2003). According to Smith and Fowler (2003), among the most common snails, *A. marginata* and *A. fulica* are truly Giant African Land Snails.

A. marginata is the second largest snail and most popular breed of snail kept and reared in Nigeria (Venette and Larson, 2004; Okon et al., 2012b). *A. marginata* produces a peristome with a reflected lip and reaches maturity 2 to 4 months later than *A. fulica* (Raut and Barker, 2002). The columella and parietal callus of *A. marginata* are either white or red in colour (Venette and Larson, 2004).

The physiological adaptability to the environment and genetic variation among and within breeds has a marked effect on the performance and productivity of snails than other factors (Okon et al., 2012a). The diversity of gene pool, natural selection and free mating among individuals has given rise to different population of snails. In genetic analysis, knowledge of relatedness or variation is used to estimate the genetic parameters such as heritability and genetic correlations (Falconer and Mackay, 1996). In artificial selection, estimation of breeding values relies on the knowledge of relatedness of individuals (Lynch and Walsh, 1998).

Characterisation of breed is the first approach to sustainable use of animal genetic resources. Studies on diversity and variability between and within breeds of Giant African Land Snails (GALS) on the basis of quantitative and qualitative variables have become inevitable (Okon et al., 2011; Ugwu et al., 2011; Okon et al., 2012b; Ibom et al., 2012). Information on the egg shell colour of this animal species is not available in the literature. The present study is aimed at providing information on the variation of egg shell colour among eggs produced from the mating between black skinned × black skinned and white skinned × white skinned ecotypes of *A. marginata var. saturalis* snails.

MATERIALS AND METHODS

The study was carried out at the Botanical Garden, University of Calabar, Calabar, Nigeria. Calabar is located within the geographical area of longitude 8°17' and 10°43' E of the Greenwich meridian, and latitude 4°58' and 15°39' N of the equator. The annual rainfall and temperature ranges between 1260 and 1280 mm and 25 to 30°C, respectively. The botanical garden is described in Okon et al. (2009a,b).

Eighty (80) adult snails comprising of 40 black skinned and 40 white skinned ecotypes of *A. marginata* snails were used for the study. The snails were purchased from a local market in Calabar.

They were allowed to acclimatize for 35 days. This was to allow them shed the eggs they came with from the wild. They were allotted into two mating groups (black skinned × black skinned) and (white skinned × white skinned) on the basis of skin (foot) colour. There were two snails per cell and replicated 20 times to ensure that any egg emerging from the cell is a product of mating(s) between the two. The snails were housed in wooden cells measuring 40 × 40 cm by 30 cm within a hutch compartments.

The cells were filled with treated soil up to 15 cm depth from the bottom. Eggs were collected within 24 h of lay as the soil is turned and moistened. Moistening the soil helped maintain the humidity and moisture content.

The snails were fed a combination of concentrate and pawpaw leaves. The diet was formulated to contain 24% crude protein and 2580.36 Kcal/kg.

Eggs collected from the mating groups were classified according to their colours. The white skinned × white skinned mating group had a total of 106 eggs, comprising of 4 yellow shelled eggs, 51 light yellow shelled eggs and 51 milky white shelled eggs. The black skinned × black skinned mating group recorded a total of 157 eggs in the order of 73: 67:17 (yellow shelled eggs: light yellow shelled eggs: milky white shelled eggs), respectively.

Statistical analysis

The distribution of the various colours, yellow shelled eggs (Y), light yellow shelled eggs (L) and milky white shelled eggs (M) were expressed in percentages (Table 2). The frequencies of the recessive alleles (Y_a for recessive yellow colour, L_a for recessive light yellow colour and M_a for recessive milky white colour) were estimated using Hardy-Weinberg equilibrium (Falconer and Mackay, 1996) as follows:

$$q = \sqrt{N/M}$$

That is $q = \sqrt{M - d/M}$, where $N = M - d$

The frequency of the dominant allele (Y_p for dominant yellow colour, L_p for dominant light yellow colour and M_p for dominant milky white colour) were calculated as follows:

$$p = q - 1$$

Where q is the frequency of the recessive gene, N is the observed number of eggs exhibiting the particular recessive trait, d is the dominance or dominant gene observed, M is the total number of eggs collected, and p is the frequency of a particular dominant allele.

The observed frequencies were then tested against the Mendelian ratio of 3:1 corresponding to the value of 0.75 for dominant allele and 0.25 for the recessive allele using Pearson's Chi-square test. Pearson's Chi-square test for goodness of fit is as follows:

$$X^2 = \sum (\text{Observed} - \text{Expected})^2 / \text{Expected}$$

The level of significance of the test was examined at $P < 0.05$.

Genetic distance between the black skinned and black skinned ecotypes were estimated at the different egg colour locus using their respective gene frequencies. The method of Bodmer and Cavalli-Sforza (1976) was adopted as follows:

$$d^2 = (p_1 - p_2)^2$$

Where d^2 is the genetic distance estimate between the two populations, p_1 and p_2 are genetic frequencies of population one and population two, respectively.

Table 1. Distribution of eggs shell colours in percentages.

Trait	Mating group							
	Black skinned × Black skinned				White skinned × White skinned			
	No. of eggs	Alleles	No. of eggs	%	No. of eggs	Alleles	No. of eggs	%
Yellow eggs shell (Y)	157	Y _P	73	46.50	106	Y _P	4	3.77
		Y _a	84	53.50		Y _a	102	96.23
Light yellow eggs shell (L)	157	L _P	67	42.68	106	L _P	51	48.11
		L _a	90	57.32		L _a	55	51.98
Milky white eggs shell (M)	157	M _P	17	10.83	106	M _P	51	48.11
		M _a	140	89.17		M _a	55	51.98

Table 2. Gene frequencies and Chi-square (χ^2).

Trait	Alleles	Mating group							
		Black skinned × Black skinned				White skinned × White skinned			
		Expected	Observed	G. Freq.	X ² test	Expected	Observed	G. Freq.	X ² Test
Yellow eggs shell (Y)	Y _P	117.75	73	0.26	17.01 ^c	79.50	4	0.02	71.70 ^a
	Y _a	39.25	84	0.74		26.50	102	0.98	
Light yellow eggs shell (L)	L _P	117.75	67	0.24	21.57 ^b	79.50	51	0.31	10.22 ^b
	L _a	39.25	90	0.76		26.50	55	0.69	
Milky white eggs shell (M)	M _P	117.75	17	0.06	86.20 ^a	79.50	51	0.31	10.22 ^b
	M _a	39.25	140	0.94		26.50	55	0.69	

Y_P, L_P and M_P = Dominant allele for yellow, light yellow and milky white egg shell colours, respectively. Y_a, L_a and M_a = Recessive allele for yellow, light yellow and milky white egg shell colours, respectively. ^{abc}Means with different superscript along the same column are significantly different (P<0.05).

RESULTS AND DISCUSSION

Table 1 shows the distribution of the shell colour in percentages. The black skinned × black skinned mating group recorded 46.50% over white skinned × white skinned mating group with 3.77% for yellow egg shell colour. The same percentages were recorded by black skinned × black skinned mating group for both light yellow egg shell colour (48.11%) and milky white egg shell colour (48.11%). The black skinned × black skinned mating group recorded lower percentages in light yellow egg shell colour (42.68%) and milky white egg shell colour (10.83%). This apparent wide variation in egg shell colour is an indication that the population has not been purified through impeccable selective breeding (Yakubu et al., 2010).

The gene frequencies of the two ectotypes are presented in Table 2. The frequencies of the dominant alleles for both black skinned and white skinned ectotypes were 0.26 (Y_P) vs. 0.02 (Y_P), 0.24 (L_P) vs. 0.31 (L_P) and 0.06 (M_P) vs. 0.31 (M_P) (Table 2). These

Table 3. Genetic distances between Black Skinned and White Skinned Snail Ectotypes.

Trait	White skinned vs. black skinned
Yellow egg shell colour	0.060
Light yellow egg shell colour	0.005
Milky white egg shell colour	0.063

values were quite lower than the expected Mendelian value of 0.75. At the recessive allele, higher frequencies were observed for both black skinned and white skinned ectotypes; 0.74 (Y_a) vs. 0.98 (Y_a), 0.76 (L_a) vs. 0.69 (L_a) and 0.94 (M_a) vs. 0.69 (M_a), respectively.

Table 3 shows the genetic distance between the black skinned and the white skinned ectotypes. The genetic distance between the black skinned and the white skinned ectotypes of *A. marginata* var. *saturalis* studied were 0.06, 0.005 and 0.063 at the yellow, light yellow and

milky white egg shell colour loci, respectively. Genetic distance makes it possible to evaluate the degree of genetic similarity between two populations by measuring the probability of one or more characters appearing in one population but not in the other (Sournia, 1991). The smaller value (0.005) obtained at the light yellow locus is an indication of phylogenetic relationship between the two ectotypes, while the higher values at the yellow and milky white loci are indicative of genetic differentiation which could be used to classify the two ectotypes into distinct population (Yakubu et al., 2010).

Conclusion

This study has shown that the ectotypes of snail influenced the gene frequencies of yellow, light yellow and milky white egg shell colours. The dominant alleles in both ectotypes were found to segregate at lower frequencies. Estimate of genetic distance showed the relatedness of white skinned and black skinned ectotypes. Efforts should be geared towards constructing genetic study that will find the genes associated with these morphological differences.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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