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Aestivation and arousal interaction in the determination of reproduction in giant African land snails: 2. Preliminary morphometric study of the female organs

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ABSTRACT: The morphometric changes of the female reproductive organs of giant African land snails during aestivation and arousal were taken on 84 snails comprising 42 *Archachatina marginata* and 42 *Achatina achatina* and analysed. The aim was to provide baseline information on the role of the annual cycle of snails (aestivation-awake phases) on the reproductive ability of the giant African land snails. The weight, length and width of the organs were measured during a 6-week aestivation and 6-week post-aestivation periods. Significant decrease ($P < 0.05$) in the parameters of common hermaphrodite ducts, little hermaphrodite ducts and albumen glands were recorded. The rate of degeneration was higher ($P < 0.05$) in *A. achatina* than in *A. marginata* in almost all the organs studied. Morphological recovery was within 2 weeks of post-aestivation in both species.

Keywords: Aestivation, arousal, morphometric, female reproduction.

Introduction

Land snails are well known for their abilities to survive adverse environmental conditions, especially the lack of water and food. In one oft cited case, first reported in the 19th century (Stearns, 1877), one specimen of the Middle Eastern species *Eremina desertorum* survived almost 4 years attached to a display case in the British Museum.
This phenomenon, known as aestivation is naturally programmed in snails. Reports (Omoyakhi *et al*., 2008a,b) have shown that apart from the adaptability of snails, aestivation liken to sleep or rest has biochemical and physiological benefits (Omoyakhi and Osinowo, 2010). Behavioural study of snails (Omoyakhi, 2007) had shown that the provision of water and feed to *Archachatina marginata* and *Achatina achatina* during the dormancy phase of the annual cycle gave 'forced awakening' which obviously would impose stress. Reproduction is known to be the first process to be altered by stress.

Generally, land snails are hermaphrodites, that is, possess both male and female reproductive organs, producing spermatozoa and ova (Odaibo, 1997). Akinnusi (1998) stated that though land snails are hermaphrodites, they must mate before they can fertilise their eggs. Base on this development, our research took special detailed studies of the sex organs apart.

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In order to achieve a maximum domestication and breeding programmes, there is a need to understand the specific biology of the snails. The aim of this study therefore was to compare aestivation and arousal by comparing the size of the female reproductive organs through gross observation and statistical analysis.

Materials and Methods

The study was conducted at the Snail Research Unit of the College of Animal Science and Livestock Production (COLANIM), University of Agriculture, Abeokuta. Abeokuta lies within the Rain Forest vegetation zone of Western Nigeria at latitude 7^o 13' 49.46"N, longitude 3^o 26' 11.98"E (Google Earth, 2009) and altitude 76 m above sea level. The climate is humid with a mean annual rainfall of 1,037 mm, an average temperature of 34.7^oC and an average relative humidity of 82 % throughout the year (60 % in January and 94 % in July to September.

Materials used in this experiment included a total of 84 apparently healthy snails (42 *A. marginata* and 42 *A. achatina*) of 150 – 200 g liveweight, 42 well ventilated plastic basket cages with covers, 42 each of shallow feeders and drinkers, humus soil, sensitive electronic weighing scale, marker for proper identification, dried pawpaw leaf meal, layer's mash and water.

The experiment was laid out in a 2 x 7 factorial arrangement (species x duration) in a completely randomized design with 6 replicates. The cages were prepared and filled with sun-dried humus soil up to a depth of 5 cm and moistened with 300 ml of water. Each cage was assigned a drinker and a feeder. The snails were weighed using sensitive electronic balance. They were randomly allocated to the treatments with a snail per basket. The treatments were balanced for snail liveweight. The snails were fed a mixture of layer's mash and dried pawpaw leaf meal (1:1, w/w) and water provided *ad libitum*. At the end of a 2–week adjustment period, the liveweights of the snails in all treatment groups were taken, feed and water were withdrawn. Prior to withdrawal of feed and water, snails in the control group were dissected. Subsequently, snails from the respective treatment groups were dissected after 2, 4 and 6 weeks of aestivation. At the end of the 6th week, post-aestivation treatment groups were hydrated and fed (arousal) continuously. They were also dissected after 2, 4 and 6 weeks of arousal. Snails were dissected according to the procedures outlined by Segun (1975). Various organs were extracted according to the identification of Segun (1975). The weight, length and width of the female reproductive organs were collected. Data obtained were statistically analysed using the Systat Analytical Computer Package, Version 5.0 (Systat Inc., 1992). Tukey's highest significant difference (HSD) was used to separate the means where significant differences existed.

Results

The least square means of the effects of duration of aestivation and reverse arousal on the weight, length and width of vagina of *A. marginata* and *A. achatina* are shown in Table 1. *A. achatina* had a significantly heavier ($P < 0.01$) and wider ($P < 0.001$) vagina than *A. marginata* on the overall averages. Length of aestivation and post-aestivation did not significantly ($P > 0.05$) change the weight and length of the organ in both species. Species differences existed in the oviduct length (1.06 ± 0.05 vs 0.81 ± 0.05 cm; $P < 0.01$) and width (0.66 ± 0.02 vs 0.60 ± 0.02 cm; $P < 0.05$) of *A. marginata* and *A. achatina* respectively. Duration of aestivation did not significantly ($P > 0.05$) affect the weight and width in both species. However, aestivation stretched the length of oviduct by 56.4% ($P < 0.01$) in *A. marginata* but not significantly ($P > 0.05$) in *A. achatina* at 6 weeks. Post-aestivation returned the oviduct length of *A. marginata* to the pre-aestivation value while *A. achatina* was not significantly affected (Table 2).

Table 3 summarizes the effects of aestivation and reverse arousal on the weight, length and width of common hermaphrodite duct of *A. marginata* and *A. achatina*. Significant species effects were observed in the weight (2.78 ± 0.18 vs 1.06 ± 0.18 g; $P < 0.001$), length (4.28 ± 0.19 vs 3.05 ± 0.19 cm; $P < 0.001$) and width (0.94 ± 0.03 vs 0.61 ± 0.03 cm; $P < 0.001$) in *A. marginata* and *A. achatina* respectively. Weight significantly decreased ($P < 0.05$) after 6 weeks of aestivation in both species. Hydration and feeding for 6 weeks returned the common hermaphrodite weight to the pre-aestivation value in *A. marginata* but not in *A. achatina*. While width followed a similar trend, length was not significantly altered by aestivation and post-aestivation treatment.

Table 1. Effects of duration of aestivation and reverse arousal on the sizes of the vagina of *A. marginata* and *A. achatina*

PARAMETER	SPECIES	LEAST SQUARE MEANS							SEM
		LENGTH OF AESTIVATION (WKS)				POST-AESTIVATION (WKS)			
		0	2	4	6	2	4	6	
Weight (g)	*A. marginata*	0.52	0.54	0.32	0.43	0.65	0.67	0.79	0.13
	A. achatina	0.68	0.81	0.76	0.71	0.72	0.85	0.92	0.13
Length (cm)	*A. marginata*	0.73	0.78	0.71	0.90	0.99	0.97	1.07	0.14
	A. achatina	1.25	0.74	1.21	0.83	0.89	1.10	1.00	0.14
Width (cm)	*A. marginata*	0.78^{b}	0.97^{ab}	0.76^{a}	0.73^{b}	1.10^{ab}	1.03^{ab}	0.96^{ab}	0.08
	A. achatina	0.88^{b}	1.08^{ab}	1.08^{ab}	0.94^{ab}	1.08^{ab}	1.19^{a}	0.96^{ab}	0.08

Values are least square means (± sem), n = 5

[ab] Means with different superscripts within the same parametric row differ significantly ($P < 0.05$)

Table 2. Effects of duration of aestivation and reverse arousal on the sizes of the oviduct of *A. marginata* and *A. achatina*

PARAMETER	SPECIES	LEAST SQUARE MEANS						SEM
		LENGTH OF AESTIVATION (WKS)			POST-AESTIVATION (WKS)			
		0	2	4	6	2	4	6	
Weight (g)	*A. marginata*	0.47	0.36	0.31	0.26	0.29	0.30	0.31	0.07
	A. achatina	0.34	0.26	0.24	0.21	0.34	0.42	0.47	0.07
Length (cm)	*A. marginata*	0.78^{b}	1.04^{ab}	1.06^{ab}	1.22^{a}	0.99^{ab}	1.44^{a}	0.88^{ab}	0.12
	A. achatina	0.61^{b}	0.61^{b}	0.81^{b}	0.64^{b}	0.86^{b}	1.11^{ab}	1.04^{ab}	0.12
Width (cm)	*A. marginata*	0.67	0.63	0.60	0.60	0.64	0.63	0.82	0.06
	A. achatina	0.63	0.43	0.60	0.61	0.65	0.65	0.64	0.06

Values are least square means (± sem), n = 5

[ab] Means with different superscripts within the same parametric row differ significantly ($P < 0.05$)

Table 3. Effects of duration of aestivation and reverse arousal on the sizes of the common hermaphrodite duct of *A. marginata* and *A. achatina*

PARAMETER	SPECIES	LEAST SQUARE MEANS							SEM
		LENGTH OF AESTIVATION (WKS)				POST-AESTIVATION (WKS)			
		0	2	4	6	2	4	6	
Weight (g)	*A. marginata*	3.54^{a}	2.62^{ab}	1.74^{b}	1.39^{b}	3.16^{a}	3.52^{a}	3.50^{a}	0.33
	A. achatina	2.12^{a}	1.60^{b}	0.81^{b}	0.75^{b}	0.99^{b}	0.84^{b}	1.00^{b}	0.33
Length (cm)	*A. marginata*	3.41	5.27	2.85	4.21	4.66	4.98	4.56	0.50
	A. achatina	2.90	3.57	3.64	2.35	2.84	3.02	3.03	0.50
Width (cm)	*A. marginata*	0.81^{b}	0.93^{ab}	0.87^{b}	0.82^{b}	0.98^{ab}	1.08^{a}	1.05^{a}	0.09
	A. achatina	0.64^{bc}	0.61^{bc}	0.60^{bc}	0.58^{c}	0.59^{c}	0.56^{c}	0.67^{bc}	0.09

Values are least square means (± sem), n = 5

^{abc} Means with different superscripts within the same parametric row differ significantly ($P < 0.05$)

Table 4. Effects of duration of aestivation and reverse arousal on the sizes of the little hermaphrodite duct of *A. marginata* and *A. achatina*

PARAMETER	SPECIES	LEAST SQUARE MEANS							SEM
		LENGTH OF AESTIVATION (WKS)				POST-AESTIVATION (WKS)			
		0	2	4	6	2	4	6	
Weight (g)	*A. marginata*	0.26^{a}	0.19^{ab}	0.14^{b}	0.13^{b}	0.13^{b}	0.15^{b}	0.17^{ab}	0.02
	A. achatina	0.06^{c}	0.05^{c}	0.03^{cd}	0.02^{d}	0.04^{cd}	0.05^{c}	0.07^{c}	0.02
Length (cm)	*A. marginata*	2.15^{b}	3.00^{a}	3.13^{a}	1.95^{b}	2.76^{ab}	2.79^{ab}	2.97^{a}	0.28
	A. achatina	2.01^{bc}	2.02^{bc}	1.88^{c}	1.11^{c}	1.48^{c}	2.04^{bc}	2.37^{bc}	0.28
Width (cm)	*A. marginata*	0.24^{ab}	0.34^{a}	0.36^{a}	0.30^{a}	0.29^{ab}	0.29^{ab}	0.31^{ab}	0.03
	A. achatina	0.41^{a}	0.21^{b}	0.23^{b}	0.17^{b}	0.18^{b}	0.24^{ab}	0.23^{ab}	0.03

Values are least square means (± sem), n = 5

^{abcd} Means with different superscripts within the same parametric row differ significantly ($P < 0.05$)

Table 5. Effects of duration of aestivation and reverse arousal on the sizes of the albumen gland of *A. marginata* and *A. achatina*

PARAMETER	SPECIES	LEAST SQUARE MEANS							SEM
		LENGTH OF AESTIVATION (WKS)				POST-AESTIVATION (WKS)			
		0	2	4	6	2	4	6	
Weight (g)	*A. marginata*	7.78^{a}	5.66^{ab}	4.87^{b}	3.21^{bc}	4.20^{b}	6.58^{a}	7.88^{a}	1.38
	A. achatina	4.01^{b}	2.12^{bc}	1.02^{c}	1.05^{c}	2.53^{bc}	2.12^{bc}	2.11^{bc}	1.38
Length (cm)	*A. marginata*	4.63^{ab}	5.91^{a}	3.06^{b}	3.20^{b}	3.77^{ab}	4.49^{ab}	3.97^{ab}	0.78
	A. achatina	3.15^{b}	3.00^{bc}	2.48^{c}	2.75^{c}	3.26^{b}	3.20^{b}	3.52^{b}	0.78
Width (cm)	*A. marginata*	2.05^{a}	1.63^{ab}	1.34^{ab}	0.88^{bc}	1.07^{ab}	1.41^{ab}	1.29^{ab}	0.45
	A. achatina	1.90^{ab}	0.81^{bc}	0.75^{bc}	0.68^{c}	1.08^{ab}	1.00^{ab}	0.99^{bc}	0.45

Values are least square means (± sem), n = 3

^{abc} Means with different superscripts within the same parametric row differ significantly ($P < 0.05$)

A. marginata had heavier ($P < 0.001$), longer ($P < 0.001$) and wider (0.01) little hermaphrodite duct than *A. achatina*. The weights of both species were significantly reduced ($P < 0.001$) following 6 weeks of aestivation and fully compensated when rehydrated and fed for 6 weeks. Length followed a similar trend in *A. marginata* but unaffected significantly ($P > 0.05$) in *A. achatina*. Consequently, the width of *A. marginata* did not significantly ($P > 0.05$) change with duration of aestivation and post-aestivation but significantly depressed ($P < 0.05$) and recovered ($P < 0.05$) in *A. achatina* under the same treatment conditions (Table 4).

Albumen gland weight ($P < 0.001$), length ($P < 0.05$) and width ($P < 0.01$) were significantly affected by species. *A. marginata* showed higher values for the three parameters than *A. achatina*. Six weeks of aestivation significantly ($P < 0.001$) depressed albumen weight in both species which significantly ($P < 0.001$) recovered to the pre-aestivation values after 6 weeks of post-aestivation in *A. marginata*. Both length and width followed a similar trend in both species (Table 5).

Discussion

Unlike several other organs, the vagina weight, length and width of *A. achatina* are heavier, longer and wider than that of *A. marginata*. This agrees with an earlier observation of Abiona (2005) who described the vagina of *A. marginata* as tubular and short while that of *A. achatina* as tubular but long. The anatomical reason for this modification is not fully understood since *A. marginata* are known to lay larger eggs than *A. achatina* (Omoyakhi, 2007). The weight and length were not significantly affected by length of aestivation and post-aestivation in this study. Rosiji (2005) however recorded higher vagina weight during the dry season in both species.

The oviduct length and width were higher in *A. marginata* than in *A. achatina*. This may be correlated with the larger egg size laid by *A. marginata*. The length of the oviduct also stretched during aestivation but further increased during arousal. This is similar to the report of Rosiji (2005) that oviduct length showed higher values during wet season than the dry season.

The influence of species on common hermaphrodite duct (weight, length and width) has been observed to be an indication that *A. marginata* has its reproductive tract more developed than that of *A. achatina* (Abiona, 2005). Weight and width depressions during aestivation and consequent involutions when aroused are indications of the dynamics of reproduction as influenced by seasons.

Similarly, little hermaphrodite duct (weight, length and width) and albumen gland (weigh, length and width) were significantly higher in *A. marginata* than in *A. achatina*. Weights, lengths and widths were significantly decreased during aestivation. Albumen gland sizes in *A. achatina* did not return to the pre-aestivation values when aroused. Rosiji (2005) confirmed the influenced of season and moisture level on the spermatheca weight and length. The effect of aestivation on the albumen gland may have been drastic because of its reproductive activity in active snails. Besides, the secretion granules within the cells of the albumen gland are usually broken down by crinophagy during starvation (Marijike, 1973). Wijsman, (1989) however noted that glycogen and galactogen are the primary storage polysaccharides in pulmonate snails. Both polysaccharides are found in secretory cells of the albumen gland. If present in the albumen gland of giant African land snails, may play important role in energy process during and after aestivation.

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