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Aestivation and arousal interaction in the determination of reproduction in giant African land snails: 1. Preliminary morphometric study of the male organs

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ABSTRACT: A preliminary investigation was conducted to evaluate the effects of aestivation on the reproductive efficiency of two giant African land snails, *Archachatina marginata and Achatina achatina*. A morphometric study of the male reproductive organs of 42 *A. marginata* and 42 *A. achatina* monitored under 6 weeks of aestivation and 6 weeks of post aestivation was undertaken. The results indicated significant ($P < 0.05$) atrophy of the organs with increasing length of aestivation period. *A. marginata* showed higher resistance to morphometric degeneration to the adverse condition than *A. achatina*. In both species, the sizes of the organs returned to and sometimes over their pre-aestivation states after 2-4 weeks of hydration and feeding. This indicates that aestivation may be a restful period in preparation for the next breeding season.

Keywords: Aestivation, arousal, morphometric, male reproduction.

Introduction

Snails belong to the phylum Mollusca which is the second largest group of phyla following Insecta. Snail is the common name of almost all members of the molluscan class gastropoda that have coiled shells in the adult stage. Land snails are therefore any of the many species of snails that live on land, as opposed to those that live in water.

The domestication and captive rearing of rodents as well as other species of manageable sizes that are tractable, prolific and widely accepted for public consumption have been described as one of the surest ways of ameliorating the shortfall of animal protein in Nigeria (FAO, 1970; Ajayi, 1971; and Mbah, 1989, Onadeko, 2002). Availability of captive bred snails among other things will also augment the exploitation of bushmeat from the wild with the attendant reduction of hunting pressure in wild game.

Presently, the rearing of snail in captivity is attracting the keen interest of both scientists and farmers, suggesting the potential of this species as farm animal of the future in the West Africa sub-region. Our previous studies have shown that aestivation is a naturally programmed phenomenon in snail (Omoyakhi *et al*., 2008a,b) with a number of physiological benefits (Omoyakhi and Osinowo, 2010 a,b). This phenomenon has been completely eliminated in the captive propagations of snails and may constitute a long term major setback in the domestication.

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In a series of systematic investigations set out to provide adequate scientific information on the aestivation (Omoyakhi *et al*., 2008a,b; Omoyakhi and Osinowo, 2010a,b), biochemical activities, constitutive and adaptive changes of giant African land snails, *Archachatina marginata* and *Achatina achatina* were taken into serious consideration in order to initiate knowledge of the physiology of aestivation.

As part of the preliminary investigations of the physiological implications of aestivation and arousal, measurable parameters such as weight, length and width of the male reproductive organs for assessing reproductive soundness and therefore fertility were monitor during and post-aestivation in the two common giant African land snails *A. marginata* and *A. achatina*.

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^{o}C 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 (arousal) and fed 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 male reproductive organs were collected. Data obtained were analysed statistically 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 weight, length and width of the penis of *A. marginata* and *A. achatina* are shown in Table 1. There were no significant differences ($P > 0.05$) in the penis weight and width between the species. The penis of *A. marginata* (3.21 ± 0.12 g) was however longer ($P < 0.01$) than that of *A. achatina* (2.65 ± 0.12 g). The penis weight was not significantly affected ($P > 0.05$) by the duration of aestivation and post-aestivation. Only the length of the penis of *A. achatina* was significantly ($P < 0.05$) decreased by length of aestivation.

For the weight and width of the penile sheath, *A. marginata* was significantly ($P < 0.001$ and $P < 0.01$) heavier (0.58 ± 0.04 g vs 0.30 ± 0.04 g) and wider (0.88 ± 0.02 cm vs 0.76 ± 0.02 cm) than those of *A. achatina* respectively, while there was no species difference in the length of the penile sheath. Similarly, aestivation caused weight and width of the penile sheath to decrease but not significantly ($P > 0.05$) within species as shown in Table 2.

Table 3 presents the weight, length and width of vas deferens of *A. marginata* and *A. achatina*. There were no species differences ($P > 0.05$) in the weight and width of the vas deferens while *A. marginata* had a significantly longer ($P < 0.001$) vas deferens (6.66 ± 0.22 cm) than that of *A. achatina* (4.30 ± 0.22 cm). Length of aestivation and post-aestivation did not significantly alter ($P > 0.05$) the weight, length and width of vas deferens of both species.

Table 1. Effects of duration of aestivation and reverse arousal on the sizes of the penis 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*	1.30	0.95	0.83	0.74	1.14	1.02	1.15	0.16
	A. achatina	1.13	0.94	0.87	0.71	0.90	0.94	0.75	0.16
Length (cm)	*A. marginata*	2.95^{ab}	3.39^{ab}	3.16^{ab}	3.07^{ab}	3.23^{ab}	3.42^{ab}	3.24^{ab}	0.31
	A. achatina	3.40^{ab}	3.97^{a}	2.18^{b}	2.05^{b}	2.41^{ab}	2.35^{b}	2.20^{b}	0.31
Width (cm)	*A. marginata*	0.57^{ab}	0.63^{ab}	0.58^{ab}	0.66^{ab}	0.73^{ab}	0.72^{ab}	0.88^{a}	0.06
	A. achatina	0.63^{ab}	0.50^{b}	0.73^{ab}	0.64^{ab}	0.78^{ab}	0.74^{ab}	0.67^{ab}	0.06

Values are least square means (± sem), n = 6

^{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 penile sheath 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*	0.75^{a}	0.42^{ab}	0.45^{ab}	0.39^{ab}	0.63^{a}	0.66^{a}	0.74^{a}	0.09
	A. achatina	0.31^{ab}	0.25^{b}	0.28^{b}	0.24^{b}	0.32^{ab}	0.33^{ab}	0.40^{ab}	0.09
Length (cm)	*A. marginata*	1.01	1.47	1.24	1.16	1.58	1.67	1.78	0.22
	A. achatina	1.99	1.08	1.16	1.27	1.07	1.39	1.81	0.22
Width (cm)	*A. marginata*	0.70^{b}	1.00^{a}	0.75^{b}	0.82^{ab}	0.90^{ab}	0.87^{ab}	1.13^{a}	0.06
	A. achatina	0.61^{bc}	0.54^{c}	0.89^{ab}	0.66^{bc}	0.92^{ab}	0.85^{ab}	0.84^{ab}	0.06

Values are least square means (± sem), n = 6

^{abc} 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 vas deferens 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*	0.12	0.12	0.10	0.15	0.18	0.21	0.24	0.03
	A. achatina	0.11	0.16	0.11	0.16	0.14	0.17	0.19	0.03
Length (cm)	*A. marginata*	6.39	6.70	5.53	6.97	7.81	7.15	6.07	0.58
	A. achatina	4.33	4.31	4.27	4.85	4.14	4.18	3.99	0.58
Width (cm)	*A. marginata*	0.19	0.20	0.21	0.19	0.24	0.21	0.22	0.02
	A. achatina	0.29	0.22	0.21	0.14	0.25	0.23	0.26	0.02

Values are least square means (± sem), n = 6

Table 4. Effects of duration of aestivation and reverse arousal on the sizes of the penile retractor muscle 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*	0.13^{a}	0.06^{b}	0.06^{b}	0.06^{b}	0.10^{ab}	0.11^{ab}	0.13^{a}	0.01
	A. achatina	0.05^{b}	0.06^{b}	0.06^{b}	0.06^{b}	0.07^{ab}	0.10^{ab}	0.10^{ab}	0.01
Length (cm)	*A. marginata*	1.44^{b}	2.21^{b}	2.05^{b}	2.07^{b}	2.41^{b}	3.05^{a}	3.69^{a}	0.21
	A. achatina	1.42^{b}	1.71^{b}	2.40^{b}	1.38^{b}	1.97^{b}	2.07^{b}	1.61^{b}	0.21
Width (cm)	*A. marginata*	0.38	0.28	0.39	0.35	0.39	0.35	0.40	0.03
	A. achatina	0.32	0.39	0.36	0.35	0.36	0.39	0.34	0.03

Values are least square means (± sem), n = 6

[ab] 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 spermatheca 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*	0.08	0.06	0.08	0.09	0.16	0.16	0.12	0.02
	A. achatina	0.06	0.08	0.09	0.05	0.07	0.06	0.07	0.02
Length (cm)	*A. marginata*	1.02	1.19	1.11	1.18	0.82	1.33	0.93	0.18
	A. achatina	0.79	0.83	1.06	0.78	1.12	0.72	0.65	0.18
Width (cm)	*A. marginata*	0.48	0.57	0.47	0.44	0.55	0.62	0.59	0.07
	A. achatina	0.40	0.39	0.48	0.37	0.63	0.46	0.44	0.07

Values are least square means (± sem), n = 6

Significant species differences were observed in penile retractor muscle weight (0.09 ± 0.01 and 0.07 ± 0.01 g; $P < 0.05$) and length (2.42 ± 0.08 and 1.79 ± 0.08 cm; $P < 0.001$) in *A. marginata* and *A. achatina* respectively. Length of aestivation significantly depressed ($P < 0.01$) the weight of the retractor muscle by over 50% in *A. marginata* which also significantly returned to the pre-aestivation weight after 6 weeks of rehydration and feeding. *A. achatina* was not significantly affected ($P > 0.05$) in both cases (Table 4). Duration of aestivation did not significantly ($P > 0.05$) affect the length of the penile retractor muscle in both species but at rehydration, the muscle was significantly ($P < 0.001$) stretched beyond the pre-aestivation value in *A. marginata*. Species and duration of aestivation and post-aestivation did not have significant ($P > 0.05$) change on the width of the penile retractor muscle.

The overall averages of the spermatheca weights (0.11 ± 0.01 vs 0.06 g; $P < 0.001$), lengths (1.09 ± 0.07 vs 0.85 ± 0.07 cm; $P < 0.05$) and widths (0.53 ± 0.03 vs 0.45 ± 0.03 cm; $P < 0.05$) between *A. marginata* and *A. achatina* were significantly different respectively. Duration of aestivation and reverse arousal did not significantly ($P > 0.05$) affect the weight, length and width in the two species (Table 5).

Discussion

This study showed that the species differences did not significantly affect the weight and width of the penis. Penile sheath recorded similar observation. However, penis length and the penile sheath weight and diameter were significantly larger in *A. marginata* than in *A. achatina*. This is similar to observations made by Abiona (2005) and Rosiji (2005). Abiona (2005) noted that *A. marginata* of equal weight with *A. achatina* has its reproductive tract more developed. The penis length and width and the penile sheath width were significantly influenced by the duration of aestivation and reverse arousal. This agrees with the observation of Odiete (1999) that the reproductive organs undergo atrophy during aestivation. Rosiji (2005) showed that the values of penis length were higher in snails placed on high soil moisture condition. This may not be unconnected with the functional reproductive activity of snails in high moisture condition of the rainy season which coincides with the post-aestivation treatment in this study.

The weight and width of the vas deferens were not significantly affected by species but *A. marginata* had longer vas deferens than *A. achatina*. However, Abiona (2005) recorded differences between the species for the length and width of vas deferens and suggested that they were part of the militating factors against successful interbreeding. The lengths of the vas deferens were stretched in both species as the snails entered into aestivation and eventually returned to the pre-dormancy values when aroused. Though not significantly, this result is similar to the findings of Rosiji (2005) who observed that the values of vas deferens length were higher during the hot wet and cold dry seasons which indicate that the vas deferens length is subjected to the balance system between the ambient temperature and humidity.

Similarly, penile retractor muscle length followed the trend of the vas deferens length during aestivation. The same reason as above may also be adduced. However, when aroused, penile retractor muscle continued to elongate. The reason for this is quite unclear. The weight depressed with length of aestivation and recovered as the snail aroused. This may be in preparation for the reproductive phase of the annual cycle as observed in the wild (Cobbinah. 1992).

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