Differences in the spermatozoa and ova of two species of giant African land snails (*Archachatina marginata* and *Achatina achatina*)

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Abstract

A comparative study of the gametes of two species of Giant African Land Snails; *Archachatina marginata* and *Achatina achatina* was carried out. Three groups of snails weighing 80-100, 101-150 and 151-250 g were used in a 3x2 factorial design, with 5 replicates, involving a total of 30 snails. Albumen gland, little hermaphrodite duct spermatheca and ovo-testis were examined for the presence of ova and spermatozoa. The morphology of the spermatozoa and ova of both species was studied. Ova were found in the albumen gland and ovo-testis. Spermatozoa were only found in the little hermaphrodite duct. Spermatozoa head and tail lengths were not significantly ($P>0.05$) different between species, while the mid-piece length was significantly ($P<0.05$) different. Liveweight group 101-150 g had the highest number of spermatozoa and ova count in both species. Morphologically, spermatozoa head, collar and mid-piece crest differed between species. *Archachatina marginata* spermatozoa had pointed head, longer collar and highest number of mid-piece crests compared to *Achatina achatina*. Ova seen in the ovo-testis of *A. marginata* were larger compared to those seen in *A. achatina*. It was concluded from the study that there are differences in the morphology and dimensions of their germ cells.

Keywords: Sperm dimension, sperm morphology, sperm count, ova, ovo-testis

Introduction

Spermatozoa dimension and shapes differ from one species of livestock to another. However, there may be some observed differences in the spermatozoa dimension even among closely related species or different breeds of animals. Many scientific studies showed that each individual differed in the frequency of occurrence of spermatozoa with morphological changes (Kondracki *et al.*, 2002., Pinart *et al.*, 1998). The journey from the point of release of spermatozoa in the female tract to the point where the ova is

fertilized is determined by several factors, some of these factors include: female factor (such as vagina fluid, pH, reproductive tract contraction which support spermatozoa movement) and male factor (sperm motility, concentration and ratio of normal to abnormal spermatozoa together with spermatozoa morphological make-up for egg adaptation in different species). In micro livestock, especially giant African land snails that is known to be hermaphrodite, much is not known about the spermatozoa morphology of this animal. Detailed knowledge of this aspect in the

animal may broaden advanced study, which can serve as a useful tool in the manipulation of production of this animal which may open door to boosting mass production. The aim of this study was to determine morphological and morphometric differences in the germ cells of giant African land snails.

Materials and Methods

Experimental Area.

The study was carried out at the Physiology Laboratory, College of Animal Science and Livestock Production (COLANIM), University of Agriculture, Abeokuta, Nigeria. The location lies within the rain forest belt of Western Nigeria, latitude 7° 10' N, longitude 3° 2' E and altitude 76 masl. The climate is humid with a mean annual rainfall of 1,037 mm, mean temperature of 34.7°C and mean relative humidity of 82 % (OORBDA, 2003)

Experimental animals and their management.

Thirty snails (15 *Archachatina marginata* and 15 *Achatina achatina*) used for this study were obtained from the COLANIM Snail Unit of University of Agriculture, Abeokuta, Nigeria. The snails were fed 75 g (group feeding) of a mixture of layer's mash and dried pawpaw leaves (1:1, w/w) per day, while water was provided *ad libitum* in plastic troughs. Feed and water troughs were washed daily, while the snail in each cage was changed every two weeks. The dimension of the cage in which the animals were kept is 34 cm x 22 cm x 25 cm.

Procedure of experiment.

The snails were weighed (using sensitive scale) and grouped based on their weight as; small (80-100 g), medium (101-150 g) and large (151-250 g). After dissection, albumen gland, little hermaphrodite duct, structure containing ova-testis and spermotheca were isolated.

Dissection to show the genital ducts and ova-testis

The mantle cavity was opened by cutting its roof transversely just behind the collar and longitudinally along the right side of the rectum. A mid-dorsal longitudinal incision through the floor of the mantle cavity was made starting just behind the point where the collar had previously been cut, moving forward along the mid-dorsal line to the tip of the mouth and backwards. This exposed the reproductive organs and alimentary system (Segun, 1975). The reproductive organs were carefully separated from the alimentary canal (Plate 1).

Determination of presence of spermatozoa and ova

In order to determine the presence of spermatozoa and/or ova, the ova-testis, spermotheca, albumen gland, and little hermaphrodite duct were separately homogenized in both species. These parts were cut into small bits using a pointed, sharp pair of scissors in a Petri-dish. About 150 – 300 µl of normal saline was added before homogenization. A smear of the homogenate fluid was made on a glass slide and stained with eosin. The slide was then air-dried.

Slide examination, reading and Photomicrographs

Each of the slides was examined under the microscope at 40, 100 and 400 x magnification for the presence of spermatozoa and ova. After examination, the slides were kept in a slide box. Slides made from two replicate per group with spermatozoa and ova were selected for both species, length and diameter ocular reading were taken. Photomicrographs of the slides were taken (Reichert 1762Y).

Plate 1. Dissection to show both reproductive and digestive organs

Statistical analysis

The analysis of variability of spermatozoa morphometric traits was carried out according to the following mathematical model:

$$Y_{ijk} = \mu + a_i + b_j + ab_{ij} + e_{ijk}$$

Where: Y_{ijk} = tested trait value; μ = population average; a_i = effect of species; b_j = effect of liveweight; ab_{ij} = effect of cooperation of control factors; e_{ijk} = residual error.

Significant treatment means were separated using Duncan multiple range test (Gomez and Gomez, 1984).

Results

Spermatozoa were only found in the little hermaphrodite duct (Plates 3 and 4). None was found in the ovo-testis, albumen gland and spermatheca. The relative abundance of spermatozoa in *A. marginata* and *A. achatina* at three liveweight ranges is presented in Table 1. The least square means of the dimensions of spermatozoa are presented in Table 2. Spermatozoa total length, head and tail length

were not significantly ($P > 0.05$) different for both species. However, mid-piece length was significantly ($P < 0.05$) different. Table 3 and plate 2 show morphological differences between spermatozoa of *A. marginata* and *A. achatina*. Both species have oval shaped head but different tips. *A. marginata* had pointed head tip while that of *A. achatina* was blunt. So also, *A. marginata* had longer collar compared to *A. achatina* with short collar. Similarly, mid-piece crest number was also higher in *A. marginata* (8-12) compared to *A. achatina* (5-8).

Ova were found in the ovo-testis and albumen gland. None was found in the little hermaphrodite duct and spermatheca. The relative abundance of ova in *A. marginata* and *A. achatina* at three liveweight is presented in Table 4.

The summary of the least squares means showing the effect of species on ova dimension is presented in Table 5. There was a highly significant ($P < 0.001$) difference in the size of ova seen in ovo-testis of both species. In contrast, the size of ova seen in the albumen gland was

Spermatozoa and ova of two species of snails

Table 1. Sperm count in the little hermaphrodite duct of *A. marginata* and *A.achatina* of different liveweights

Liveweight group	Species	
	<i>A. marginata</i>	<i>A. achatina</i>
80-100 g	+++	+
101-150 g	+++	+++
151-250 g	+	+

Relative quantity are indicated by number of +

Very high = ++++

High = +++

Few = +

Plate 2: Comparison of sperm head shape in *A. marginata* (a&b) and *A. achatina* (c&d)

Table 2. Least square means for whole length, head, midpiece and tail length of spermatozoa from *A. marginata* and *A. achatina*

Species	Parameter			
	Whole length (µm)	Head length (µm)	Midpiece (µm)	Tail length (µm)
<i>Achatina marginata</i>	584.29± 14.19	6.58± 0.39	172.63± 17.94 ^a	405.08± 24.31
<i>Achatina achatina</i>	578.46± 14.19	5.67± 0.39	107.88± 17.94 ^b	464.92± 24.31

Values in the same column with different superscripts differ significantly (P<0.05)

Table 3. Morphological differences between *A. marginata* and *A. achatina* spermatozoa

Spermatozoa part	<i>Archachatina marginata</i>	<i>Achatina achatina</i>
Head	Oval with pointed tip	Oval with blunt tip
Collar	long	short
No of mid-piece crest	8-12	5-8

Plate 3. Photomicrograph of spermatozoa found in the little hermaphrodite duct of *Archachatina marginata* (X 400)

Spermatozoa and ova of two species of snails

Table 4: Ova counts in ovo-testis and Albumen gland

Liveweight group	Ovo-testis		Albumen gland	
	<i>A. marginata</i>	<i>A. achatina</i>	<i>A. marginata</i>	<i>A. achatina</i>
80 -100 g	++++	+++	++	++
101-150 g	++++	++++	++	++
151 – 250 g	+++	++	+	+

Relative quantity are indicated by number of +

Very high = ++++

High = +++

Moderate = ++

Few = +

Table 5. Least square means for ova in ovo-testis and albumen gland

Species	Parameter	
	Ovo-testis (µm)	Albumen gland (µm)
<i>Archachatina marginata</i>	17.21± 0.78 ^a	17.92± 1.21
<i>Achatina achatina</i>	10.38± 0.78 ^b	14.60± 1.21

^{ab}Means in the same column with different superscripts differ significantly (P<0.05)

not significantly (P>0.05) different for both species. Plates 5 and 6 show the photomicrographs of ova seen in both ovo-testis and albumen gland at high power (x 400 magnification).

Discussion

Yoloye (1994) attributed the production of both eggs and spermatozoa to ovo-testis. Cuezso (1995) reported the presence of spermatozoa in

the little hermaphrodite duct of *Scutalus tupacii* (d'orbigny), a stylommatophoran. In this study, spermatozoa were only seen in little hermaphrodite duct of *A. marginata* and *A. achatina*. The higher sperm numbers found in the medium group (101-150 g) of each species compared to other liveweight groups, is an indication that the medium liveweight groups in both species are more sexually active than other liveweight groups. Spermatozoa lengths for

Plate 4. Spermatozoa found in the little hermaphrodite duct of *Achatina achatina* X 400

Plate 5. Ova found in ovo-testis of *Achatina achatina* (a) and *Archachatina marginata* (b) mag X 400

Plate 6. Ova seen in albumen gland of *Achatina achatina* (a) and *Archachatina marginata* (b) (X 400)

several species of slug were listed by Maxwell (1983) as: *Deroceras reticulatum* 140 μm , *Tandonia sowerbyi* 246 μm , *Arion hortensis* 300 μm and *Ariolimax columbianus* 265 μm . In this study, the two species were not significantly different in length. The spermatozoa length for *Archachatina marginata* and *Achatina achatina* were measured to be $584.29 \pm 14.19 \mu\text{m}$ and $578.46 \pm 14.19 \mu\text{m}$ respectively. The close values recorded may be due to the same family (Achatinidae) in which they belong.

So also, long mid-piece crest of *Archachatina marginata* (8-12) as compared to *Achatina achatina* (5-8) may be an adaptation for ATP production needed for motility (Bohnensack and Halangk, 1986; Ford and Rees, 1990; Mita *et al.*,

1991; Hofmann *et al.*, 1992) since the reproductive tract of *Archachatina marginata* have been confirmed to be longer, wider and heavier compared to that of *Achatina achatina* (Abiona, 2005). Similarly, pointed tip of the head together with longer collar of *Archachatina marginata* observed during this study may be an adaptation for cleaving to egg while the blunt tip and short collar of *Achatina achatina* spermatozoa head may similarly play a role in egg cleavage.

The result of this study shows that ova are localised in ovo-testis and albumen gland of both species. Ovo-testis is known for egg production (Yoloye, 1994); similarly, the function of the albumen gland is the production of albumen or perivitelline fluid for the egg (Bayne, 1967),

hence, the presence of ova in these two organs confirm the reports from previous studies. Across the three liveweights, it was obvious that for spermatozoa and ova production, liveweight range 101-150 g had the highest number compared to other liveweight ranges. The reason for this observation may be an indication that this weight range has the highest capacity in term of production.

Conclusion

It can be concluded from this study that there were differences in the sperm morphology of both species. Snail of liveweight 101-150 g has greater capacity for production compared to other liveweight ranges studied.

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