The structure of the ovotestis of the common African land snails found in Abeokuta, South Western, Nigeria

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Abstract

A comparative study of the structure of the ovotestis of African land snails found in Abeokuta was investigated. Of all the five snails species found and dissected, *Archachatina marginata*, *Achatina achatina*, *A. fulica*, *Helix pomatia* and *Limcolaria aurora*, only the ovotestis of *H. pomatia* could not be observed. The positioning, shape and arrangement of the ovotestis was the same for all the other four species. The ovotestis is embedded in the digestive gland at the anterior region of the coiled posterior end of the visceral mass. It is cream coloured and made up of sac-like lobes arranged on a single plane. It is differentiated into an ovarian and a testicular region. The number and size of the lobes of the ovotestis differ significantly ($P < 0.05$) in all the snails. Each lobe is made of several follicles and the number of which varied in the four species. Statistical analysis showed that live weight, shell length, shell width and shell circumference of the snails had no significant influence on the size of their ovotestis. Meiotic metaphase spreads of the ovotestis tissues revealed chromosome numbers $2n=56$, $2n=44$, $2n=54$ and $2n=28$ for *A. marginata*, *A. achatina*, *A. fulica*, and *L. aurora*.

Keywords: Snail, ovotestis, chromosome, follicles

Introduction

The species of African land snails, *A. marginata*, *A. achatina*, *A. fulica*, *H. pomatia* and *L. aurora* are consumed in many countries of the world (Amusan et al, 1999). They are highly favoured in Nigeria and Africa where they constitute the most conspicuous terrestrial molluscs (Odaibo, 1997). The potential for their domestication and commercialization has not been fully exploited although many studies have shown that snail farming could be a highly profitable and productive business (Amusan and Omidiji, 1998). Snail meat is a high quality food that is rich in protein, low in fats, and a source of iron (Akinnusi, 2002). According to Imevbore and Ademosun (1988), snail meat contains 88.37% crude protein, a value that compared favorably with conventional ani-

mal protein sources whose values ranged from 82.42% (pork) to 92.75% (beef). Apart from its high nutritive value, it has been used traditionally in Nigeria for treating ailments such as diabetes, hypertension, epilepsy, measles, small pox, chicken pox, headache and infertility (Amusan et al 1999, Akinnusi, 2002). The bluish liquid produced when the bottom shell is carefully cracked is used to stop bleeding during circumcision and inscription of tribal marks (Amusan and Omidiji, 1998).

The ovotestis is the reproductive organ of snails. A typical snail is an hermaphrodite, that has both male and female sex organ which produce eggs and sperms. Idokogi and Osinowo, (1998) reported that the reproductive system of *A.*

marginata has a high correlation with the live weight of the snail. That is as the snail matures, the reproductive organ also increases in size. Cytological studies have been carried out on about fifty species of aquatic pulmonate snails (Burch, 1960) and majority of the species have haploid chromosomal number ($n=18$). In the land snails, the haploid numbers of about eighty species range from $n=17$ to $n=34$ (Burch and Heard, 1962) and there was not a single form that could be interpreted as a polyploidy in the group. Fagbuaro *et al* (2002) reported haploid chromosome numbers of $n=28$ and $n=22$ of individual cells for *A. marginata* and *A. achatina*, respectively, although no sex chromosomes were observed. Despite their importance and the growing interest in their cultivation (Fagbuaro *et al* 2002), not much has been done on their biology.

Information on the biology of African land snail species is still scarce. There is little or no information on the structure of the ovotestis in all the African land snails especially those found in Abeokuta, South West Nigeria.

Materials and Methods

Twenty mature African land snail specimens of the different species were collected from different locations in Abeokuta. Morphometric data were collected using vernier caliper and live weight of the snails were taken using the sensitive electronic weighing scale (Mettler DM II-K).

The snails were killed by carefully breaking the shells with a hard substance carefully so as not to damage the digestive gland. The digestive glands were dissected and the ovotestis was removed with a pair of forceps onto slides. The lobes of the ovotestis were counted and the size of each lobe was measured in situ for all the species used. The length and width of the ovotestis were measured using a vernier caliper. The ovotestis was subsequently weighed using the sensitive electronic weighing scale (Mettler DM II-K). The ovotestes were removed and fixed in labelled bottles containing 10% formalin. The

fixed specimens were dehydrated and embedded in paraffin wax (melting point 58-60%). Sections were cut at 10 μ m and stained with haematoxylin and eosin. Stained slides were observed under the microscope and pictures were taken.

The ovotestes of the snails were removed and dropped in specimen bottles containing freshly prepared fixative (Methanol: Acetic acid; 3:1) solution and left for 24 hours.

Small strands of the ovotestis were cut with sharp blade and dropped into two drops of acetic acid in a mortar. These strands were ground until a homogenous solution was formed. 3-5 drops of acetic acid were added to enhance suspension. Using a micropipette, the solution was dropped on prewarmed slides and allowed to dry. The slides were stained in FLP orcein for about 75 minutes, and then covered with cover slips. The slides were then observed under $\times 40$ and $\times 100$ objectives and pictures of metaphase spreads were taken. Data collected were analyzed using simple mean and correlation analysis to determine relationship between the measured parameters.

Results

Morphometrics

Measurements of the shell parameters as well as the body weights of *A. marginata*, *A. achatina*, *A. fulica*, *H. pomatia* and *L. aurora* are presented in Table 1. Mean body weight, shell length, shell width, and shell circumference followed the trend *A. marginata* > *A. achatina* > *H. pomatia* > *A. fulica* > *L. aurora*. Dissection revealed that the ovotestis of *A. marginata*, *A. achatina*, *A. fulica*, *L. aurora* is embedded in the digestive gland at the anterior region of the coiled posterior end of the visceral mass (Plate 1&2). The ovotestis of *Helix pomatia* could not be located after dissection. The ovotestis is a small structure relative to the digestive gland in which it is lodged. It is cream coloured which makes it to stand out from the dark brown digestive gland. It is visible through the thin and transparent

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covering mantle lying on the surface of the digestive gland. Observation showed that the

ovotestis is made up of lobes, which are of different numbers and sizes in the various species (Table 2).

Table 1: Body Parameters of the different land snails.

SNAIL SPECIES	Mean weight (g)	Mean Shell Parameter (cm)		
		Body Length	Width	Circumference
<i>Archachatina marginata</i>	133.56 ± 6.67 ^a	10.98 ± 0.13 ^a	6.16 ± 0.16 ^a	18.84 ± 0.37 ^a
<i>Achatina achatina</i>	127.22 ± 7.38 ^a	10.91 ± 0.24 ^a	6.13 ± 0.12 ^a	18.17 ± 0.31 ^a
<i>Achatina fulica</i>	19.9 ± 0.62 ^c	7.17 ± 0.12 ^b	3.5 ± 0.06 ^b	10.32 ± 0.16 ^b
<i>Helix pomatia</i>	32.2 ± 2.54 ^b	3.15 ± 0.17 ^c	4.0 ± 0.12 ^b	12.21 ± 0.55 ^b
<i>Limicolaria aurora</i>	3.5 ± 0.42 ^d	3.39 ± 0.13 ^c	1.58 ± 0.05 ^c	5.31 ± 0.11 ^c

Values (mean ± SD) within column followed by different superscripts were significantly different (P < 0.05)

The lobes are arranged on a single plane in all the species observed. Generally, the lobes are sac-like in shape and each lobe is made of several follicles (Figure 1 & 2). The follicles are attached to a central dividing follicular duct. The follicular ducts from inside each lobe merge into a single larger duct that connects the ovotestis to the little hermaphrodite duct (Figure 3).

The number of follicles per lobe is highest in *A. achatina* (39) followed by *A. marginata* (27) while *A. fulica* and *L. aurora* had 19 and 18, respectively. The mean weight, length

and width of the ovotestis are presented in Table 2. *A. marginata* had the highest size while *A. fulica* had the lowest. Unlike the trend observed in the measurements of body parameters, mean weight, length and width of the ovotestis followed the trend *A. marginata* > *A. achatina* > *L. aurora* > *A. fulica* (Table 2). There was a significant difference (p < 0.05) in the mean weight, length and width of the ovotestis across the species. The result of correlation analysis showed that the live weight, shell length, shell width and shell circumference of snails had no significant influence on the size of the ovotestis (Table 3).

Table 2 : Measurements of ovotestis of the different land snails.

SNAIL SPECIES	Mean Ovotestis Parameters			Mean lobe number	Lobe Size (cm)		Mean follicle Number per lobe
	Weight (g)	Length (cm)	weight (cm)		Length	Width	
<i>Archachatina marginata</i>	0.34 ± 0.02 ^a	2.29 ± 0.11 ^a	0.94 ± 0.04 ^a	4	0.7	0.5	27
<i>Achatina achatina</i>	0.31 ± 0.03 ^a	1.86 ± 0.15 ^b	0.59 ± 0.04 ^b	6	0.4	0.3	39
<i>Achatina fulica</i>	0.12 ± 0.02 ^c	1.15 ± 0.11 ^c	0.42 ± 0.05 ^c	3	0.2	0.1	19
<i>Limicolaria aurora</i>	0.24 ± 0.02 ^b	1.41 ± 0.07 ^c	0.24 ± 0.04 ^d	4	0.3	0.2	18

Values (mean ± SD) within column followed by different superscripts were significantly Different (P < 0.05)

Plate 1 : A scanned picture of the ovotestis of *Archachatina marginata* embedded in the digestive gland

Plate 2 : A scanned picture of the ovotestis of *Achatina achatina* embedded in the Digestive Gland

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Table 3: Summary of correlation analysis of ovotestis length and body parameters.

Body Parameters	<i>Archachatina marginata</i>	<i>Achatina achatina</i> R value	<i>Achatina fulica</i>	<i>Limicolaria aurora</i>
Live Weight	0.19	0.41	-0.15	0.18
Shell Length	0.33	0.40	-0.33	0.21
Shell Width	0.49	0.45	0.3	0.13
Shell Circumference	0.26	0.26	-0.41	-0.004

Not significant at ($P < 0.05$)

Sectioning showed that the ovotestis consists of an ovarian and defined testicular region as shown in Plate 3. The two regions are connected by connective tissue. This structure is similar in all the four snail species observed. The ovarian portion consists of follicles, which contains a primary oocytes with a spherical nuclei displaced to one side. The testicular region is made up of seminif-

erous tubules (Plate 4). Each tubule is made up of an outer layer of connective tissue and an inner thin basement membrane. The tubule is filled with stratified epithelium consisting of sertoli cells. These cells are ovoid shaped with nuclei that occupy almost half of the cell. They extend from the basement membrane to the lumen of the seminiferous tubule.

Plate 3: Transverse Section of the ovotestis of *A. marginata* showing Ovarian and Testicular tissues
(ot: ovarian tissues tt: testicular tissues pf: Primordial follicle Arrow: Sertoli cells)

Chromosome numbers of experimental snails

The metaphase of meiosis in *A. marginata* showed a haploid number of $n=28$ which establishes a diploid number of $2n=56$. The bivalents were homologous and rod like. No special sex chromosomes were observed. The meiotic metaphase of *A. achatina* showed a diploid number of $2n=44$. The bivalents observed were homologous, rod like and acrocentric. No sex chromosomes were observed. The meiotic metaphase of *A. fulica* showed a haploid chromosome number of $n=27$ establishing a diploid number of $2n=54$. The Chromosomes were acrocentric and rod like. The metaphase of meiosis in *L. aurora* showed a haploid number of $n=14$ establishing a diploid number of $2n=28$. The chromosomes were acrocentric and rod like.

Discussion

Of the entire snail species dissected, only the ovotestis of *H. pomatia* could not be observed despite the fact that they matured ones. It is possible that the removal of *H. pomatia* from its aquatic environment probably halted development of its ovotestis. Boyle and Yoshino (1999) in a related study observed that when water source was changed in *B. glabrata*, the functioning of the ovotestis was halted.

The present study revealed that each of the ovotestis of *A. marginata*, *A. achatina*, *A. fulica* and *L. aurora* is embedded in the digestive gland at the apex of the visceral hump. This observation agrees with the findings of Segun (1975). The ovotestis in all the four snail species was made up of lobes

Plate 4: Transverse Section of the ovotestis of *A. achatina* showing testicular regions with seminiferous tubules
(se: stratified epithelium ct: connective tissue)

that were generally sac-like in shape and cream in colour. This shape and colour observed in the study are comparable to that observed by Segun (1975) for *A. marginata*.

The lobes of the ovotestis are arranged on a single plane. However, the lobe number and size differ in the four snail species. *A. achatina* had the highest mean lobe number followed by *A. marginata* and *L. aurora* while *A. fulica* recorded the lowest mean lobe number. The study further revealed that each of the lobes of the ovotestis was made up of several follicles which is in accordance with the findings of Chase (2000), Segun (1975), and Whitworth et al (1999). The number of the follicles per lobe differs in all the snail species. *A. achatina* had the highest follicle number per lobe compared to that of other species studied.

The relationship between lobe number and follicle number could not be ascertained. From the study *A. marginata* and *L. aurora* with significant weight differences had the same lobe number (4) but different follicle numbers of 27 and 18, respectively.

There is a probable relationship between number of follicles and number of eggs produced in each species. *A. achatina* with a mean lobe number (6) and follicle number per lobe (39), lays 100-300 eggs 1-2 times each growing season while *A. marginata* with a mean lobe number (4) and follicle number (27), lays 7-10 eggs 4-8 times each growing season. (Akinnusi, 2002). Interestingly, correlation analysis showed that live weight, shell length, shell width, shell length and shell circumference had no significant influence on the size of the ovotestis. Histological studies showed that the ovotestis contains a morphologically ovarian component and a testicular region as observed by Whitworth et al (1999). The ovarian portion contains normal follicles while the testicular portion consists of seminiferous tubules.

The results of chromosomal examination showed haploid chromosome numbers $n=28$ for

A. marginata, $n=22$ for *A. achatina*, $n=27$ for *A. fulica* and $n=14$ for *L. aurora*. This observation in *A. marginata* and *A. achatina* agrees with the report of Tegbuaru et al (2002). The observed chromosome numbers were consistent in all the slides observed and there was a consistent uniformity in the morphology of the chromosomes. No special sex chromosomes were observed. This observation is in agreement with the report of White (1977).

Conclusion

The findings of this study have revealed that the location, shape and structure of the ovotestis are similar in all the species observed. However, lobe and follicle number differs in the four species. Also live weight shell length, width and circumference have no influence on the ovotestis size.

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