

INFECTED FRESHWATER SNAILS, *BIOMPHALARIA ALEXANDRINA*, THE INTERMEDIATE HOST OF *SCHISTOSOMA MANSONI*

BY

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Abstract

The objective was to study the chromosomes of *Biomphalaria alexandrina* snails (class Gastropoda) in control and infected state which could be helpful in understanding how host-parasite relationships in feasible and effective control measures. The chromosomal changes were studied using the air-drying method. The results showed that *B. alexandrina* had a diploid chromosome number, $2n = 36$. Also, the meiotic stages were detected as early-leptotene and late leptotene, zygotene, diplotene, metaphase I. The result also revealed the presence of a primitive sperm, with a conical head and a very long, uni-flagellate tail. A comparative meiotic chromosome analysis between the control and infected ones showed some significant differences, as pachytene and diplotene were more condensed.

Key words: *Biomphalaria alexandrina*, Infected, Non-infected, Chromosomes.

Introduction

Biomphalaria spp. is of medical relevance as this Neotropical gastropod contributes as intermediate host of *S. mansoni* that causes one of the neglected tropical disease (Adema *et al*, 2017). Globally, *S. mansoni* infects about 83.31 million people (Park and Yong, 2014). *B. alexandrina* is a species of an aquatic gastropod in the family Planorbidae. Their native distributions include Africa and Middle East (Ibrahim and Abdalla, 2017). The study of the genome at chromosomal level can be used to differentiate one species from another (Bakry and Garhy, 2011). Modern cytogenetic techniques have been used for studies of Gastropoda since mid-1990s. The subfamily *Biomphalarinae* is a conservative group which has haploid chromosome numbers 18 (Park and Yong, 2014). Most species of the *Biomphalaria* have been investigated; *B. glabrata*, *B. pfeifferi*, *B. madagascariensis*, *B. sudanica*, *B. tanganyicensis*, *B. alexandrina*, and *Bulinus truncates*; they have a haploid chromosome number of 18 and these chromosomes were relatively small and monomorphic (Park and Yong, 2014). Burch (1962) utilized preparations from the ovo-testes and reported a haploid complement of 18, where, these cells were in the meiotic stage and stated that it was difficult to identify each and every

chromosome. The cytological methods were developed especially after using of hypotonic treatment of tissue samples and pre-treatment with colchicine which is known as air drying technique (Thiriot-Quévieux, 2003). Abdel-Haleem (2013) revealed that the diploid chromosome number ($2n$) of *B. glabrata* and *B. alexandrina* each was 36 and stated that, ovo-testes of these snails provided a good opportunity to study the karyotype and chromosomal aberrations. The morphology of mitotic metaphase chromosomes has been reported in 10 species of the subfamily *Biomphalarinae* with $n=18$ and $2n=36$ (Park and Yong, 2014).

This study aimed to characterize chromosomes in infected and non-infected Egyptian *Biomphalaria alexandrina* snails.

Materials and Methods

1. Experimental animals (snails):

Clean laboratory-bred *B. alexandrina* snails (8-10mm) were obtained from Medical Malacology Laboratory, Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Snails were kept in plastic aquaria (16 x 23 x 9cm). The aquaria were provided with dechlorinated aerated tap water (10snails/L) and covered with glass plates. Oven dried lettuce leaves and blue green algae (*Nostoc muscorum*) were used for feeding and water in the aquaria was changed weekly. Two

groups of *B. alexandrina* snails, each of 30 snails were used. First group was a control group used without exposure to miracidia. Second group was exposed to freshly hatched *S. mansoni* miracidia at a dose of 10 miracidia/snail and left for 24 hours under illumination source. After that, the snails of each experimental group were washed thoroughly with water and transferred to another clean plastic aquarium containing one liter of dechlorinated water and daily supplied with oven dried lettuce leaves till examination for cercarial shedding in multi-dishes under artificial light for 3 hours and 2ml dechlorinated water/snail (Meuleman, 1971). The positive *B. alexandrina* that shed cercariae were used for chromosomal studies.

Schistosome miracidia: *Schistosoma mansoni* ova used were obtained from Medical Malacology Laboratory, from intestine of infected albino mice (*Mus musculus*). Ova were allowed to hatch to miracidia in a small amount of dechlorinated water under direct light from a desk lamp. Then, the hatched miracidia were used in the experimental tests.

Chromosome preparation: It was done on hermaphrodite glands of the specimens by the usual air-drying method (Park, 2011). Snails (about 10 snails for each group) were placed directly in 0.1% colchicine at room temperature, for one day. Ovo-testes samples were cut into small pieces, squashed, and then mixed with 0.48% KCl as hypotonic solution, at room temperature. After discarding all large tissue pieces, 15ml of the cell sediments were transferred to a centrifuge tube and incubated for 25–35 min. KCl was discarded from the supernatant after another centrifugation at 2500 rpm for 10 minutes. The cell pellet was fixed in freshly prepared mixture of absolute methanol and glacial acetic acid (3:1) for 15 minutes, and then centrifuged at 2500rpm with three changes of 15 minutes duration, after which the supernatant was discarded. The fixation was repeated until the supernatant was clear. Finally, 1-2 ml of freshly prepared fixative

were added to cell pellet and 3-5 drops of cell suspension were dropped on clean wet glass slides(previously kept at 4°C in 70% ethanol) which is flame-dried.

Chromosome staining: Conventional staining was done using 4% Giemsa's solution for 30- 45 minutes and examined under a high power microscope with an oil immersion and photographs were taken.

Compliance with Ethical Standards: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Results

In the prophase of meiosis I, the chromosomes formed 18 bivalents, and their number confirmed the diploid number of $2n = 36$ chromosomes as a characteristic of this species. Some cells showed the polyploidy, where; there were incidences of polyploid nuclei (Fig. 1a, b).

The first meiotic division begins with a long prophase (Fig. 2 A), which was subdivided into four stages (Figs. 2 B- H); leptotene, zygotene, pachytene, and diplotene. During the leptotene there are two phases early and late (initiation of chromosome shrinking (Fig. 2B& C), the chromatin of the chromatids was stretched out very thinly, and it is difficult to follow each single chromosome throughout its entire length i.e. the chromosomes were densely stained granules. At the zygotene (initiation of chromosome synapsis), the chromosomes were much shorter, more contracted and quite clearly visible (Fig.2 D). Zygotene chromosomes were contracted and darkly stained than the leptotene chromosomes. Pairing of homologous strands is completed in zygotene forming pachytene chromosomes of double thickness and haploid in number. At the pachytene stage (completion of chromosome synapsis), chromosomes were more condensed (Fig. 2E). During the diplotene, the chromosomes were much shorter, more contracted and quite clearly visible (Fig. 2F), where, at the beginning of diplotene, the homologous began to repel

one another, causing the chromosomes to separate. Distinction between diplotene and diakinesis was not a clear one and metaphase chromosomes appeared (Fig.2 G & H).

In the infected *B. alexandrina*, the meiotic cell division included prophase I (meiosis I), with distinctly observable interphase (chromatin network (Fig. 3A, B, C, D & F), leptotene, zygotene and pachytene, and the condensation of the chromosomes was variable in each cell.

The meiotic cycle is completed when the four haploid nuclei were formed, giving rise to four cells. All four cells usually develop into gametes. In the meiotic chromosomal slides, spermatozoa were also observed, which consisted of a spherical head, a short mid piece and a long tail (flagellum) (Fig. 4).

Discussion

Pulmonata species of family Planorbidae were interesting with their large morphological diversity (Szabelska *et al.*, 2015). Cytogenetic studies contribute useful information supplementary to the morphological, biochemical and other characters used for systematic analysis of freshwater snails (Nakamura, 1986; Tohamy and Mohamed, 2006).

The present results revealed that the diploid chromosome number of *B. alexandrina* is 36. This agrees with the observations of (Abdel-Haleem, 2013; Bakry and Garhy, 2011). Also, (Park and Yong, 2014) most species of the *Biomphalaria* were investigated and have a haploid chromosome complement of 18. The chromosomes are relatively small and monomorphic (Goldman, 1984) and often difficult to identify each and every chromosome (Burch, 1967). The present study showed the presence of the polyploidy in *B. alexandrina* snails and the occurrences of polyploid nuclei. This agreed with Tohamy and Mohamed (2006) who observed polyploidy in the digestive gland of *Cleopatra bulimoides* and a *Bithynia* spp. (class Gastropoda and subclass Prosobranchia respectively). Da Silva and Brown

(1982) found the polyploidy in the gill and digestive gland of *Bullia* snails.

In the present study, described various meiotic stages. These were early-leptotene and late leptotene, zygotene, diplotene, metaphase I, without recognizable anaphase I owing to its short duration. They followed a normal course of meiosis in *C. bulimoides* and *Bithynia* spp. (Tohamy and Mohamed, 2006) as well as the land snail *Macrochlamys indica* (El-Alfy *et al.*, 1994). In the present study, the diakinesis stage (formation of bivalent chromosomes) could not be observed. Also, this stage was seldom observed on the chromosomes of the Egyptian freshwater *Melanoides tuberculata* (Yaseen, 1996).

Szabelska *et al.* (2015) reported that in chromosomal sections of gonads, the relatively rare mitotic divisions of the oogonia or spermatogonia were observed, but was relatively easy to obtain and visualize the snails' meiotic chromosomes forming bivalents.

In the present study, sperm (a primitive sperm) was composed of a conical head and a very long, uni-flagellate tail. It had a head, a short mid piece and a long tail (Franzén, 1983). This structure is similar to the spermatozoon of *Siphonaria algesirae* (Azevedo and Corral, 1985). Sperm morphology was adapted in molluscs systematics and phylogeny (Drozdo *et al.*, 2012).

The present meiotic chromosome analysis of control and infected *B. alexandrina* revealed some significant differences, as pachytene and diplotene were more condensed. Shalaby *et al.* (2011) showed that there is a genetic variation between susceptible and resistant strains to parasite infection within *B. glabrata* and *B. alexandrina* snails. Silva *et al.* (2007) showed the absence of the protein expressed in the nucleus during cell division of infected *B. glabrata* with *Schistosoma mansoni*.

Conclusions

No doubt, studying the Cytogenetic parameters of *B. alexandrina* chromosomes

number and morphology are of great importance to understand how parasite affects its hosts. Also, it gives valuable clues to phylogeny evolution and taxonomic relationships.

These results have spotlights on meiotic chromosomes and the spermatozoa of *B. alexandrina* control and infected. Insightful observation of meiosis may in the long-run perspective allow the recording of disturbances in this process among snails, caused by water pollution.

Conflict of interest: The authors declared that they neither had any conflict of interest, nor received fund.

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Explanation of figures

Fig. 1: Chromosomal spread in control snail. a- showed number of chromosomes $n=18$. b- showed polyploidy in *Biomphalaria alexandrina*.
 Fig. 2: Giemsa - stained chromosomes from ovotestes of *Biomphalaria alexandrina* snails; (A) A group of cell in early prophase stage. (B, C) in early and late leptotene stage. (D) Zygotene stage. (E) Pachytene stage. (F) in diplotene stage. (G, H) Metaphase stage.
 Fig. 3: Giemsa - stained chromosomes from ovotestes of infected *B. alexandrina* (A) A group of cell in early prophase stage. (B) A group of in early leptotene stage. (C) Zygotene stage. (D) Pachytene stage. (E) A group of in diplotene stage. (F) Metaphase stage.
 Fig. 4: Spermatids formation (H: Head, arrow: refers to tail).

