

SHORT COMMUNICATION

SPERMATOGENESIS AND DESICCATION IN *Biomphalaria tenagophila* (ORBIGNY, 1835) (GASTROPODA, PLANORBIDAE)

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ABSTRACT

The results show the effects of prolonged periods of desiccation on the progress of spermatogenesis of *Biomphalaria tenagophila*. A technique of cell suspension was adapted for the purpose of chromosome analysis, yielding very good results.

INTRODUCTION

In the present paper results with a new technique for preparations of meiotic chromosomes in Molluscs are presented.

Species of the genus *Biomphalaria* show an adaptative answer to dry environmental conditions. Desiccation is an adaptative phenomenon through which the animals are able to withdraw within the shell during prolonged absence of water. Some species are able to survive in these conditions for several months (Barbosa and Dobbin, 1952; Barbosa, 1953). The metabolic activity of desiccated animals is reduced to basal levels.

Studies with desiccated animals in species which are hosts of *Schistosoma mansoni* have shown that the intermediate stages of the parasite can also survive within the snail through the periods of desiccation (Barbosa and Coelho, 1955).

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Our group has a special interest in the study of desiccated animals in relation to the functions of the ovotestis. In the present paper which is a pilot experiment to our studies, the behavior of meiotic chromosomes in desiccated snails as seen in the light microscope was one of the objects of study.

MATERIAL AND METHODS

The experiment was carried out with *Biomphalaria tenagophila*, a species which is commonly encountered from southeastern Brazil to Northern Argentina (Paraense, 1981). The snails were collected in ponds of the Cidade Universitária Armando de Salles Oliveira - USP - São Paulo - Brazil, and raised under laboratory conditions during four years.

The animals are kept in stock aquaria containing 20 liters of water, periodically supplemented with CaCO_3 and fed twice a week with either fresh or dried lettuce leaves. Stock aquaria were always kept at room temperature approximately 22°C .

Only sexually mature animals were used. This was attained by collecting animals with shell diameter greater than 7 mm.

For the desiccation experiment adult snails withdrawn from the stock aquaria were placed in mini-aquaria which contained a layer of wet earth on the bottom.

After desiccation periods of 5, 7, 9, 10, 15, 20 and 28 days these animals were sacrificed.

Control adult snails were sacrificed just after being removed from the stock aquaria.

From each of the desiccated and control snails the shells were gently broken between two glass plates thus exposing the soft parts; the ovotestis were then removed by dissection and prepared according to the procedures described below:

1. after removing the ovotestis immerse it in hypotonic solution of 3 parts 0.075 M KCl:1 part distilled water. Maintain the ovotestis in that solution for 40 min. at 37°C ;
2. resuspend solution with a Pasteur pipette and centrifuge at 1000 rpm for 5 min.;
3. replace test tube solution with 3:1 methanol-acetic acid;
4. repeat step 2;
5. without disturbing the tissue at the bottom of the test tube remove with a Pasteur pipette 90% of the fixative;
6. repeat steps 2 through 5 twice;
7. add 50% acetic-acid and repeat step 2;
8. without disturbing the tissue in the test tube remove some of the solution making sure to leave enough to distribute through the slides;

9. resuspend and using a Pasteur pipette drip 3 drops of the solution on clean slides heated to 40°C;
10. after 1 min. pipette off drops from slides;
11. bathe (immerse) the slides for 8 min. in 1N HCl at 60°C;
12. stain the slides in 2% Giemsa (phosphate buffer pH 6.8) for 8 min.

RESULTS AND DISCUSSION

The spermatogenic stages of *Biomphalaria tenagophila* obtained through the technique described here are shown in Figure 1. Figures 1D, 1E, 1F, 1G and 1H show $n = 18$ chromosomes.

Differently sized poliploid nuclei were found in the ovotestis cells; although not measured cytophotometrically these obviously present a rather large variability in ploidy (Tuan *et al.*, 1984; Tuan and Simões, 1986). Probably these nuclei characterize the differentiated nutritional cells from the ovotestis and like some blastomeres of *Biomphalaria glabrata* may reach poliploidy by endomitosis (Schreiber and Camey, 1966).

The results in Figure 1 show more meiotic phases than those described by other authors (Burch, 1960; Narang, 1976; Giacomozzi *et al.*, 1979). That was made possible due to the technique here described, allied to desiccation. The meiotic process is slowed down when desiccation takes place rendering available for analysis stages which in normal development would be ephemeral, thus very difficult to find in cytological preparations.

While in control animals diplotene, metaphases I and II are rarely found, apparently due to its short duration, in desiccated animals these phases are common.

Animals analyzed from the 6th to the 10th day of desiccation present a large number of diplotenes and metaphases I and II; animals desiccated from the 15th to the 20th day exhibited pre-pachitenes, pachitenes and spermatozoa. The longer the desiccation the larger the number of spermatozoa encountered while the meiotic phases observed diminish.

This observation suggests that the meiotic process once initiated will proceed even under duress but at a slower pace. The spermatozoa then produced are stored.

Preliminary results obtained in our laboratory indicate that the spermatozoa produced before desiccation may remain available at least for a month under desiccated conditions (Dias, personal communication).

The results presented above, besides introducing a promising technique for chromosome analysis in Molluscs is also important since it casts some light on this important adaptative phenomenon of aestivation.

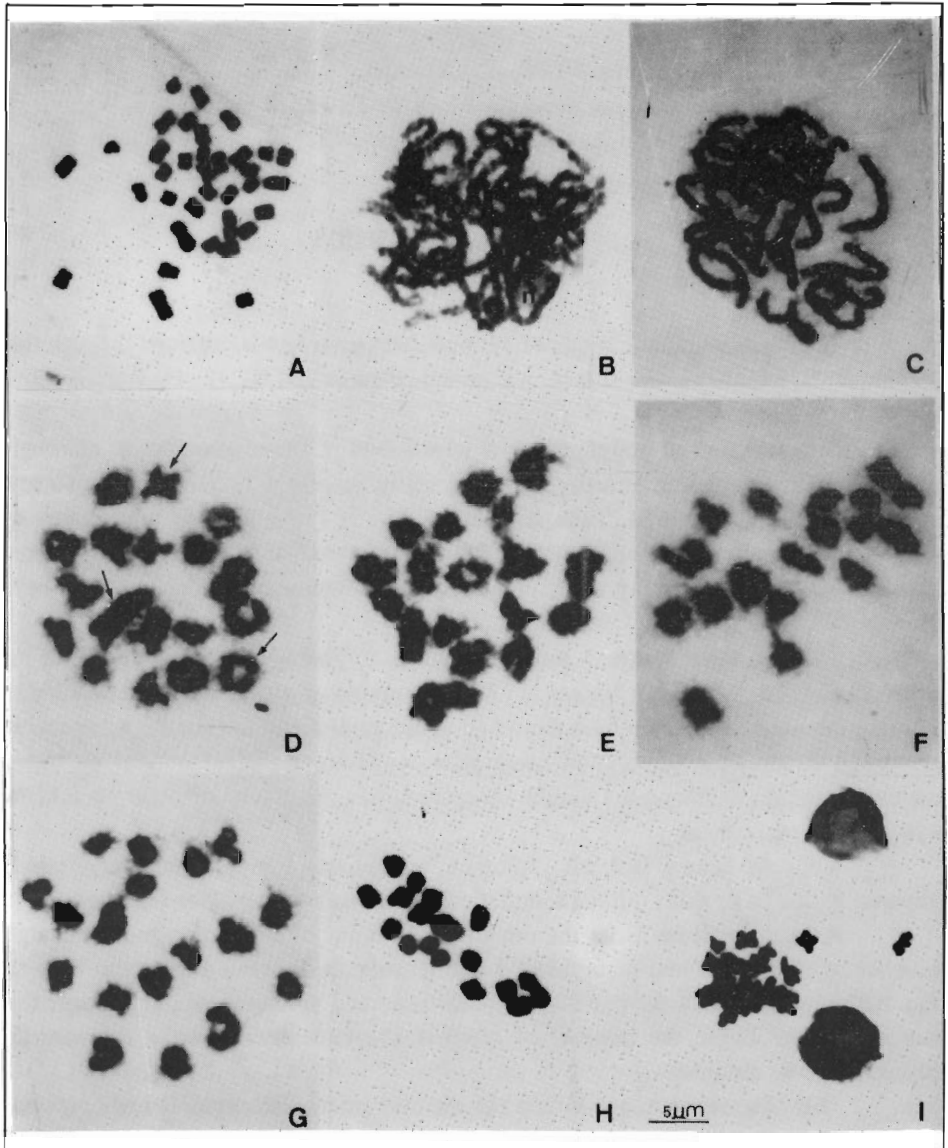


Figure 1 - Spermatogenic stages of *Biomphalaria tenagophila*. A, spermatogonial metaphase with $2n = 36$; B, zygotene; C, pachytene with nucleolus (n); D diplotene with meiotic configurations (arrows); E-F, diakinesis; G, early metaphase I; H, metaphase I; I, metaphase II.

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RESUMO

Os resultados mostram o efeito de períodos prolongados de dessecação sobre a espermatogênese em *Biomphalaria tenagophila*. Para tanto adaptou-se ao ovotestis, a técnica de suspensão celular, o que possibilitou a obtenção de ótimas preparações cromossômicas.

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