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Analysis of Synaptonemal Complex and Nucleolus Organizer Regions in the Spermatocytes of Tilapia (*Oreochromis mossambicus*) adopting Surface-spread and Silver Staining Technique

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The behaviour of synaptonemal complex (SC) and nucleolus organizer (NOR) activities in the spermatocytes of *Oreochromis mossambicus* were investigated by surface-spread silver stained technique. Results indicate that the number of synaptonemal complex is 22 with no atypical bivalents, and most of the pachytene nuclei have two pairs of NOR-bearing chromosomes.

Key Words: *Oreochromis mossambicus*, **Synaptonemal complexes, Nucleolus organizer regions, Surface-spreads**

Introduction

Surface-spread silver stained method has proved useful for studying the meiotic behaviour at light microscopic level (Dresser & Moses 1979, Fletcher 1979), and this is why it is being increasingly applied in meiotic studies of different mammalian species. In this study, a slightly modified protocol of this technique has been used for the testicular preparation of *Oreochromis mossambicus*, a fish species belonging to the family Cichlidae, to examine its feasibility for staining the Synaptonemal Complexes (SCs), Nucleolus Organizer regions (NORs) and Kinetochore in the pachytene nuclei during early stages of prophase. On the other hand, with the help of this technique, we describe the behaviour of synaptonemal complexes (SCs) and nucleolus organizer regions (NORs) in the early stages of prophase of this fish.

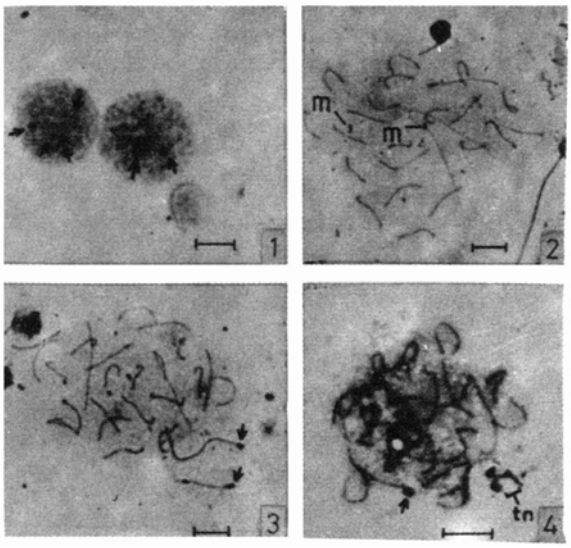
Material and Methods

Mature fish were collected from the local market. In the laboratory, the fish were kept in an aquarium at 20–23°C. Surface-spread silver staining technique of Fletcher (1979) was used with slight modification. The testes were dissected out and squeezed in fresh 0.075 M KCl solution to remove excess sperms. It was then teased and suspended in the same solution for hypotonic treatment for 15–20 min. After treatment, two or three drops of the suspension were put on the slides, and 0.2 M sucrose added to it. After a few seconds, the cells were fixed by 4% paraformaldehyde dissolved in 3.4% sucrose (*p*H 8.0–8.5) for 30 min. or more and rinsed in 0.4% photoflo (*p*H 8.5). Staining was carried out by 50% $AgNO_3$ solution at 65°C until the slides became brownish yellow. The slides were washed with distilled water, air-dried and mounted in DPX.

Results and Discussion

Previous reports (Prasad & Manna 1976, Majumder 1984) and our observations show that diploid chromosome number of *Oreochromis mossambicus* is 44 with no heteromorphic chromosome pair.

SCs were well contrasted in the majority of pachytene nuclei (105), and numbered 22 in each. No evidence of atypical nature of bivalents (figures 2, 3) was found, thus confirming diploid number of chromosomes in this species to be 44, with no sex elements (Dresser & Moses 1979, Quack & Noel 1977, Moses 1977a, b). The number of NOR-bearing chromosomes in diploid complement was found to be four (our unpublished data). 66.6% of pachytene nuclei had two NOR-bearing bivalents (figure 3), while 76.8% of preleptotene nuclei possessed four NORs (figure 1). These results confirm our previous data about NOR-bearing chromosomes in this species. In a considerable number



Figures 1–4 Silver stained pre-leptotene and pachytene nuclei showing synaptonemal complexes, nucleolus organizer regions and kinetochore of *Oreochromis mossambicus*. **1,** Two pre-leptotene nuclei. Small arrow indicates Ag-stained nucleolus organizer regions. Large arrow indicates fused NORs; **2,** Pachytene nucleus showing 22 distinct synaptonemal complexes (**m** = micronuclei); **3,** Pachytene cell shows 22 synaptonemal complexes with two nucleolus organizer regions (arrow); **4,** Synaptonemal complexes with heteromorphic nucleolus organizer regions. One NOR is triplicated in the synaptonemal complex and shows a tripartite structure (tn). Arrow indicates NOR; Bar represents 5 μm

of nuclei, the NORs got fused and formed either a single or large Ag-block (figure 1) or several small dark bodies located on the SCs or outside the complex (figure 2). These small dark bodies may either be artefacts or may represent micronucleoli as reported in various stages of spermatogenesis in many vertebrate species including *Branchiostoma* (Hofgartner et al. 1979, Schmid et al. 1982) and in human oocytes (Stahl et al. 1975, Wohlgmuth-Jarashow et al. 1977). Heteromorphic nature of the NORs was frequently observed in the species studied (figure 4), probably due to tandem duplication or triplication in one of the two NORs. The position of kinetochore in each SC was difficult to make out by the present technique. It is hoped that the technique deployed in present preparation, would help in studying the behaviour of meiotic prophase in variety of cases.

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