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35. ON THE CHROMOSOMES OF A BIVALVE, *ANADARA RHOMBEA*

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ABSTRACT

Study of chromosomes of bivalves is technically difficult. Spermatogonia and spermatocytes, oocytes, and cleavage stages of the fertilized eggs are more favourable than the somatic tissues of the adultss for the study of chromasomes. Hypotonic treatment, prior to fixation, is an essential pre-treatment for obtaining well-spread chromosomes. 1he study of the chromosomes of a bivalve, *Anadara rhombea,* from the Porto Novo waters, has been the first of its kind in an Indian bivalve. Oiakinet'C and first m^taphase stages from 'squash' preparations of testis show that In male 2n •= 28 in this species; the sex chromosomes, if any, are not distinguishable.

INTRODUCTION

A survey of the literature on the chromosomal cytology of the molluscs available upto 1978 (when the study reported here was undertaken) will reveal the relative paucity of information on the chromosomes of this group despite the fact that molluscs constitute the third large phylum in the animal kingdom (Fretter & Graham 1962); among molluscs, very few bivalves have been studied cytologically. Lillle's (1901) study on the egg of i//?/© is the earliest known on the chromosomes of a bivalve, based on its maturation, fertilization and cleavage. Other works on the chromosomes of bivalves include those of; Ahmed and Sparks (1967 a) on oysters, clams and mussels, Ahmed and Sparks (1967 b) on *Ostrea* and *Crossostrea,* Ahmed and Sparks (1970) on two spp of *MytiJus;* leyama (1975J on three spp of Pteriomorpha: leyama and Inaba (1974) on ten spp of Pteriomorpha; Keyl (1956) on the mussel, *Sphaerium;* Kobayashi (1954) on two spp of *Ostrea:* Longwell et al (1967) on *Crossostraa;* Menzel (1988) on nine families of marine bivalves; Menzel and Menzel (1965) on two spp of *Mercenaria* and their hybrids, and Ropes (1972) on the surf *clam. Sp/'su/a.* It will be obvious from this list that there was no work on Indian bivalves till 1978.

MATERIAL AND METHODS

The specimens of *Anadar* rhombea* were kindly provided by Dr. George John who was then working in the Centre of Advanced Study in Marine Biology, Porto Novo, on the Biology *oi Anadara spp.* The specimens were obtained from Vellar estuary. Somatic tissues from the ctenidia and midgut gland, and the germinal material from the testis were used for this study. Testis proved to be the most favourable material for the study of chromosomes. Fragments of live testis were subjected, for 30 minutes, to hypotonic treatment prior to fixation; filtered estuarine water was diluted with an equal quantity of distilled water and this was used as the hypotonic fluid. The fragents were then fixed in Clarke's fluid or Newcomer's fluid, and stained in lacto-propiono-orcein or acetic-orcein. The chromosomes were studied from 'squash- preparations which were dehydrated in ethanol and mountedin euparal.

The slides were examined in an Olympus trinocular research microscope under oil immersion objective of N. A. 1.3 and with Kohler illumination; camera lucida sketches were drawn with a table top magnification of 3200 dia.

OBSERVATION

Th6 chromosomes were studied from spermatocytes undergoing first meiotic division; cells in diakinesis and I metaphase provided satisfactory preparations. The hapioid number of chromosomes in *A. rhombee* was found to be 14 in males (Fig 1). The chromosomes are metacentric and form 1^ bivalents. There is no localisation of chiasmata, there being proximal, interstitial and distal chiasmata. The meiotic behaviour of the chromosomes does not show any peculiarity. It was not possible to distinguish any sex chromosomes based on the morphology and behaviour of the chromosomes,

Fig. 1. Chromosomes of *Anadar, rhombea.* Squ«h preparations of testes fixed, after hypotonic treatment, [„ Clarl<e's fluid and stained in lacto-proplono-orcein. Cameia lucida sicetches; magnification: 3200 X.
Bivaients of a ceiin diakinesis; n = i4.
^- ^'^"""" °' • "" '" ' "'«"Ph"e; n = u,

DISCUSSION

"^^^ dearth of information orr the chromo- ^°"^^^ °^ bivalves is in all probability due to the technical difficulty of obtaining good preparations of well-spread chromosomes; molluscs, '" S^"^''^" ^nd the bivalves, in particular, have their chromosomes (which are in some spp large in number) in the relatively small volume of their nuclei. The conventional methods of 'Chromosome preparations, employed mthe ^^^^y of most other invertebrates, are not adequate for the investigation of the bivalve chromosomes; it is absolutely necessary to subject the tissues to hypotonic treatment in order to swell the nuclei and to disperse the chromosomes so that they are spread well while squashing, without an overlapping of the chromosomes. Besides diluted natural medium, 1 % aqueous solution of sodium acetate or 0.4% aqueous solution of potassium chloride maybe used for hypotonic treatment of the material; 20 to 30 minutes in the hypotonic fluid will suffice; the tissues may then be fixed and stained; squashes can be prepared from such material and they will show a good number of well-spread chromosomes for observation.

Regarding the choice of favourable material for the study of chromosomes, in the experience of the present author, it is better to choose germinal material from the males or females during active gametogenesis. The most favourable materials for chromosomal studies are the spermatogonia and spermatocytes in the males, and the maturation stages of the oocytes in the females; cleavage stages of the fertilized eggs provide good mitotic chromosomes. Somatic tissues of the adults, such as the ctenidia or midgutglands, do provide mitotic stages, but are not as good as the gametocytes or the early stages of cleavages. For instance, Alagarswami & Sreenivasan (MS) found that the oocytes of pearl oysters were the most favourable for a study of the chromosomes; more than even the testis, the oocytes provided excellent stages of maturation in which the chromosomes were distinctly discernible. Cleavage stages in the oysters were not very useful in their studies

Patterson (1969) had reviewed the chromosome numbers of the bivalves and stated that the haploid numbers ranged from 10-23. Later work has indicated that the lower haploid number is 7. Among the bivalves cytologically investigated, the family Ostreidae have been the most irivestigated; except in tvvo spp of *Ostrea,in* all other spp *ofOstrea* and *Crossostrea* which belong to this fam ly, the haploid number has been recorded to be 10. In every family of the bivalves, the haploid number is constant for all the genera. Thus, the haploid number for the family Arcidae has been reported (Ieyama 1975) to be 14. In *Anadara rhombea,* which belonges to Arcidae, the haploid number has been found by the present author to be 14 Thus, the present observation confirms the constancy of the familial haploid number.

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