

(Fig. 1A) and then stained in 1% aceto-carmine, whole EMCs were certainly isolated. Meiotic chromosome configurations were clearly observed when the isolated EMCs were softly squashed (Fig. 1B, C).

The present clearing-staining-squash procedure is summarized as follows:

1. Fix young buds (3.5 to 4.5 mm long) of *Allium tuberosum* in Newcomer's fluid (Newcomer 1953) below 4°C for at least one day. Wash in three changes of 70% ethanol.
2. Take the ovules out of ovary and dehydrate in 90 and 99% ethanol for 3 min each.
3. Clear in BB-4½ fluid (mixture of lactic acid, chloral hydrate, phenol, clove oil, xylene, and benzyl benzoate, 2:2:2:2:1:1 by weight; cf. Herr 1982) overnight.
4. Mount several ovules at a time on a slide with a well (1 mm deep) filled with BB-4½ fluid. Select the ovules at the definite meiotic stage by observing with Nomarski differential interference-contrast optics.
5. Transfer the selected ovules to vials, wash in 99% ethanol, and hydrate in 90, 70, 50, 30, 15% ethanol, 3 min each, then in distilled water for at least 3 min.
6. Mount each selected ovule to a clean slide, add a drop of 1 N HCl, and macerate by heating the slide for 1 min on a hot plate maintained at 50 to 60°C. Remove the ovular tissue with the aid of a pair of fine needles.
7. Replace 1 N HCl with a drop of 1% aceto-carmine. Stain for about 10 min at room temperature, supplying iron by soaking an iron needle.
8. Replace the stainer with a drop of 45% acetic acid. Remove the inner integument.
9. Apply a cover-slip and tap it gently until EMC pops out of the nucellar cap.
10. Place blotting paper on the cover-slip and squash EMC softly.

The present clearing-staining-squash method will contribute to understand the quantitative and dynamic aspects of diplospory in *A. tuberosum*. This method seems to be suited also to other *Allium* species and other genera, irrespective of persistency of nucellar cap.

Acknowledgements: We thank Dr. Y. Nagato, Tohoku University, for his valuable suggestions and Dr. J.N. de Vries, Centre for Plant Breeding Research CPO, The Netherlands, for his criticism.

References

- Esau, K. 1953. *In Plant Anatomy*. John Wiley & Sons, New York. Gohil, R.N. & Kaul, R. 1980. *Chromosoma* 77: 123-127. Gohil, R.N. & Kaul, R. 1981. *Chromosoma* 82: 735-739. Herr, J.M., Jr. 1982. *Stain Tech.* 57: 161-169. Hillary, B.B. 1940. *Bot. Gaz.* 102: 225-235. Newcomer, E.H. 1953. *Science* 118: 161. Ved Brat, S. 1966. *Chromosomes Today* 1: 31-40.

¹⁾Laboratory of Plant Breeding, Faculty of Agriculture, Tohoku University, Sendai 981, and ²⁾Biological Laboratory, Osaka Gakuin University, Suita, Osaka 564, Japan

Goswami, U.: Karyotype details of *Crassostrea gryphoides* (Rivalvia: Ostreidae) from central West Coast of India

Based on the reports of the earlier workers Longwell (1976) embodied the information that all the species of *Crassostrea* and *Ostrea* had the same number and type of chromosomes. The species of *Crassostrea* included were *C. virginica*; *C. gigas*; *C. angulata*; the Portuguese oyster; *C. rhizophorae* the

Puerto Rico Oyster; *C. rivularis* from Karachi; *C. iredalei* from the Philippines; *C. cucullata*; *C. commercialis* from Australia, and *C. glomerata* and *C. gryphoides* from West Pakistan (Ahmed and Sparks, 1967; Longwell *et al.*, 1967; Menzel, 1968; & Ahmed, 1973). Nakamura (1985) had recently reviewed cytogenetic data on *C. corteziensis* from Mexico. Ahmed (1973) had restricted his descriptions of *C. gryphoides* chromosomes only to the diploid number (20) without incorporating any drawings/microphotographs and the other karyotypic details. In India, *C. gryphoides* was an economically important species. To undertake applied genetic aspects for improvement of stocks, it was essential to know the basic karyotypic details. The present report was the first of its kind from the Indian waters.

Twenty specimens of *Crassostrea gryphoides* were collected from Siolim, Goa, in the month of January, 1990. The oysters were acclimatized to the tank conditions for 24 hours and stripped. The male & female specimens were separated and the eggs were artificially fertilized. The samples of fertilized eggs and the embryonic stages were collected. These were treated with 0.025% Colchicine and 50% seawater for 30 minutes each. The material was fixed in 3:1 acetic alcohol. The yolk extraction with 1:1 chloroform and ethanol mixture was tried for 150 min. The slides were prepared by the conventional air-dried technique, stained in Giemsa stain (6.4 pH) and were screened for chromosomal analysis.

The diploid chromosome number was encountered from more than 300 larval cells at the stages of prophase, prometaphase and metaphase. There were variations in the size of the chromosomes amongst different celled stages. The karyotypic data given in Table 1 was based on the ten metaphase plates from 16-32 celled stage. The haploid chromosome number was encountered during meiosis. The other details for the analysis of the karyotype were followed after Levan *et al.* (1964).

The studies revealed that *Crassostrea gryphoides* from Goa, central west coast of India, had a diploid number of 20 and haploid number of 10 chromosomes (Figs. 1A, B & C). The karyotype formulae for the species was 20 M. All the chromosomes were metacentric with 40 fundamental number of arms with the arm ratio between 1.07-1.18. The size of the different homologous pairs varied between 2.68-6.12 μm (Table 1). The only pairs which were distinctly separate in size were the number 1 and 10 (Figs. 2A & B). Amongst others the sizes decreased very gradually. The homologous pair number 10 was found acrocentric in 0.5% of the cases (Fig. 1A & Fig. 2A). In 99.5% cells it was metacentric.

Table 1. Karyotypic data of the oyster *Crassostrea gryphoides*

Homologous chromosome pair No.	Total length (μm)		Arm ratio (L/S)		Chromosome type
	Mean	SD	Mean	SD	
1	6.12	0.55	1.18	0.24	M
2	5.60	0.55	1.18	0.24	M
3	5.40	0.42	1.12	0.07	M
4	5.20	0.41	1.07	0.12	M
5	4.97	0.38	1.17	0.25	M
6	4.77	0.35	1.13	0.12	M
7	4.57	0.25	1.11	0.12	M
8	4.14	0.26	1.16	0.05	M
9	3.47	0.54	1.10	0.12	M
10	2.68	0.72	1.09	0.13	M

M = metacentric



Fig. 1. A & B: plates showing 20 somatic metaphase chromosomes.
Fig. 1. C: meiotic metaphase-I, showing 10 bivalents.

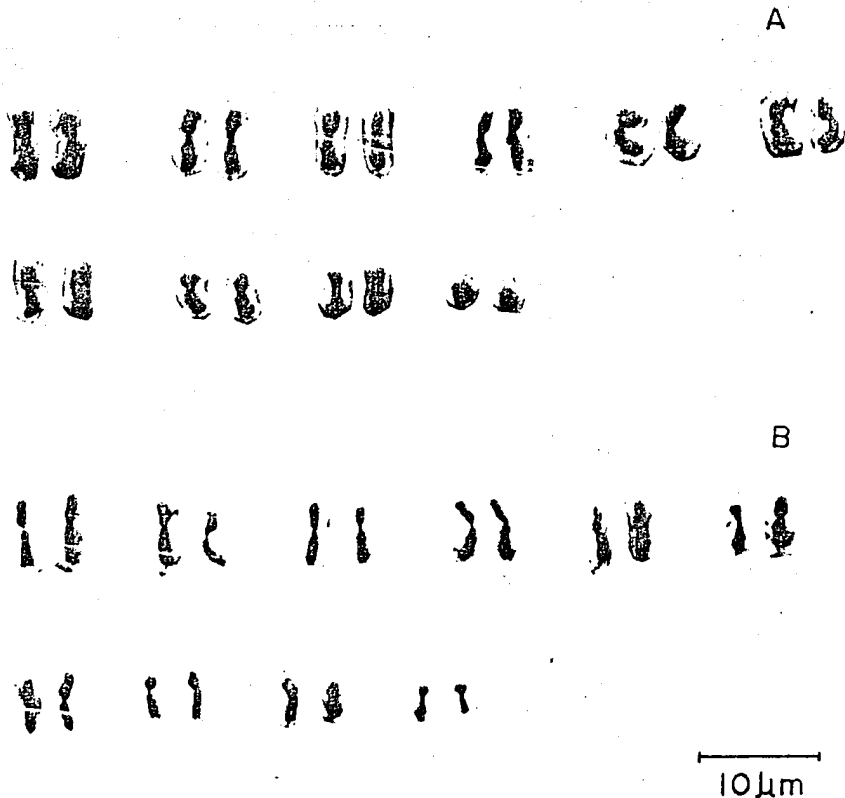


Fig. 2. A & B : Karyograms prepared from the metaphase plates shown in Fig. 1. A & B respectively.

Acknowledgements : The author is thankful to Dr. B.N. Desai, Director, NIO and to Dr. A.H. Perulekar, Head, BOD for the laboratory facilities and the encouragement.

References

- Ahmed, M. 1973. *Cytologia*, 38 : 337-346. Ahmed, M. & A.K. Sparks, 1967. *J. Fish. Res. Bd. Can.* 24 : 2155-2159. Levan, A., Fredga, K., and Sandberg, A.A. 1964. *Hereditas*, 52 : 201-220. Longwell, A.C., Stiles, S.S. & Smith, D.G. 1967. *Can. J. Genet. Cytol.* 9 : 845-856. Menzel, R.W. 1968. *Proc. Symp. on Mollusca, Part 1* : 75-84.

National Institute of Oceanography Dona Paula, Goa-403 004, India

4. Manna, G.K. & Sadhukhan, A. : A search for genotoxic active principle within spore components of the fungus, *Fusarium oxysporum* tested in mice

In an earlier communication the genotoxic potential in mice treated with the isolated spores of plant pathogenic fungus, *Fusarium oxysporum* recorded by a battery of tests like chromosome aberrations in bone marrow cells and male meiotic cells, elevated micronucleated erythrocytes and sperm head abnormal-