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A Squash Method for Chromosomes of Ascidians (Tunicata)

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A SQUASH METHOD FOR CHROMOSOMES OF ASCIDIANS (TUNICATA) *

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The information, sometimes in question (TAYLOR 1967; COLOMBERA 1968, 1969), about the chromosomes of Ascidians, has been limited almost entirely to the haploid or diploid number for a few species (MINGANTI 1956; COLOMBERA 1963, 1967; MILKMAN 1965).

With the technique here described for spacing out and staining the chromosomes as well as for fixing and preserving the material before squeezing, chromosomes preparations of many Ascidians have been made succesfully.

MATERIAL AND METHODS

Schedule for quickly-made preparations.

1 The material is washed in 50% acetic-acid for a few minutes, in order to eliminate the sea water which would induce flocculation of orcein during the staining.

2. The material is kept in acetic-orcein, where the acetic acid is at a concentration of 50%, for 5-15 minutes (DARLINGTON and LA COUR 1960).

3. Treatment in 50% acetic acid under microscope control washes away the excess of orcein (STEFANI 1963) and causes the tissues to swell and soften.

4. The material becomes squeezed between the slide and its cover first with a pin and then with the thumbs.

5. The edges of the slides, after allowing the excess of acetic acid to become absorbed, are sealed with an ordinary pen soaked in melted paraffin.

These quickly made preparations are now ready for examination under a phase contrast microscope, paying attention to the fact that immersion oil melt the paraffin.

These preparations (Fig. 1) last only few weeks.

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Schedule for permanent preparations.

1. The slide and its cover, cleaned from the paraffin and kept in alcohol 75% are separated with the aid of a razor-blade.

2. Both slide and cover are stained with ferric haematoxylin Weigert following the quick method described by H. C. BURCH (1969) or with Gallocyanin (PEARSE 1960) or are not stained at all, according to the case.

3. The two glasses mounted in Canada balsam are ready for examination.

Schedule for fixing and preserving the material before squeezing.

3 parts of 90% etanol, or better 90% metanol and 1 part of glacial acetic proved to be the best and simplest medium wherby to fix and keep the material in good condition for squeezing even after the lapse of a month or more.

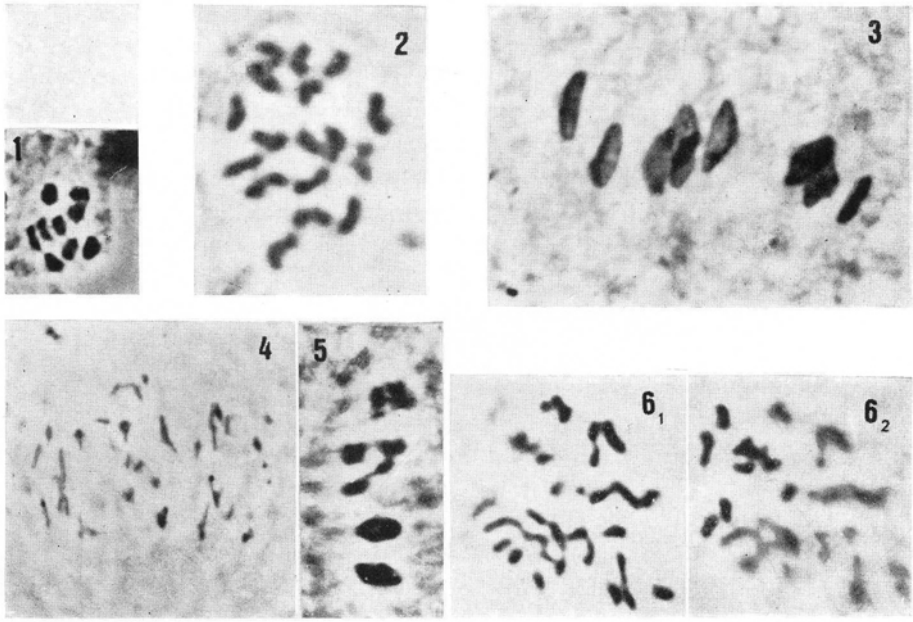

Fig. 1. — 9 bivalents in testicles of *Clavelina lepadiformis* (Mueller). Squash preparation made on fresh material without any staining (x 1400).
Fig. 2. — 16 bivalents in testicles of *Botrylloides leachi* (Savigny). Squash preparation made on fresh material with staining in gallocyanin (x 2300).
Fig. 3. — 9 bivalents in egg of *Ascidia mentula* (Mueller) Squash preparation made on fresh material with staining in ferric haematoxylin (x 1400).
Fig. 4. — Anaphase in dividing egg of *Ciona intestinalis* (Linneaus). Squash preparation made on fresh material, stained with orcein only (x 1400).
Fig. 5. — Bivalents in egg of *Ascidia virginea* (Müller) from the coasts of Norway. Squash preparation made on material preserved for about 50 days (x 1400).
Figs. 6_1 - 6_2. — The same photo focused on two different planes. 32 metaphase chromosomes of *Botryllus schlosseri* (Pallas) from Woods Hole (USA). Squash preparation made on material preserved for about 40 days. (x 1400).

Squeezing must be avoided immediately after fixation because the material first hardens and then slowly softens.

The method of preparing chromosomes from fresh or preserved material is nearly the same: with preserved material the time of permanence in acetic acid must be somewhat longer.

RESULTS

With these techniques it has been possible to start the examination of the chromosomes of *Clavelina lepadiformis* (Fig. 1), *Botrylloides leachi* (Fig. 2), *Ascidia mentula* (Fig. 3), *Ciona intestinalis* (Fig. 4), *Ascidia virginea* (Fig. 5), *Ascidiella aspersa* and others.

It is interesting to note that none of the hypotonic solutions employed, warm or cold (such as diluted sea water, distilled and doubledistilled water, etc.), in order to swell the nuclei and space out the chromosomes gave any perceptible results, which on the contrary have been obtained using acetic acid 45 %-50 %.

Sometimes the chromosomes of preserved material show certain alterations, such as, for instance, relaxation of coiling.

The time of fixation, modality of squeezing, handling of the material depends on the species investigated and are, to some extent, a matter of personal preference.

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SUMMARY

A squash method for chromosomes from living and preserved Ascidians (Tunicata) has been established.