It is clear that the results described in this paper do not permit conclusions regarding the size of the shearing forces acting during homogenization, or the number of attached cross bridges necessary to prevent the complete disintegration of myofibrils into single filaments. However, mechanical experiments with 5 m*M* Mg-AMPPNP[13] suggest that a reduction of the dynamic stiffness to about 80% is enough to allow the formation of the single filaments demonstrated in Figure 1[14].

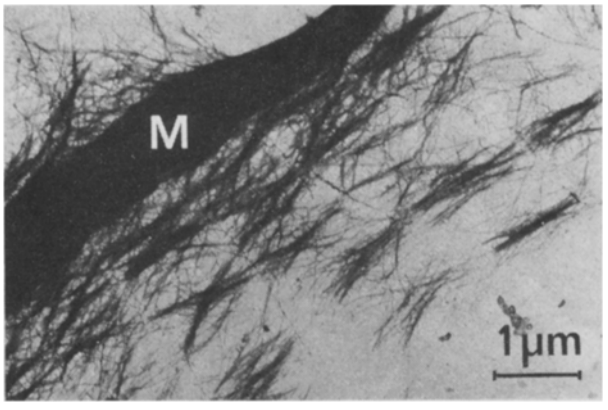

Fig. 3. Frog sartorius muscle. Preparation of myofilaments. Myofibrils have been homogenized in relaxing solution containing 5 m*M* Mg-PP. The myofibril (M) crossing the micrograph seems to disintegrate into bundles of myofilaments.

Zusammenfassung. Myofibrillen, die in einer Erschlaffungslösung mit Mg-ATP oder Mg-β, γ-imino-ATP homogenisiert werden, zerfallen in einzelne Myofilamente. Im Gegensatz dazu scheinen Myofibrillen bei einer Verwendung von Mg-Pyrophosphat (Mg-PP) als Weichmacher allenfalls zu Bündeln von Myofilamenten zerschlagen zu werden, wobei die Filamente noch durch Querbrücken verbunden bleiben. Hieraus wird auf eine unvollständige Dissoziation des Aktomyosin-Komplexes (bzw. die Bildung eines Gleichgewichtes zwischen losgelösten und angehefteten Querbrücken) in Gegenwart von Mg-PP geschlossen.

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10 September 1973.

[13] J. BARRINGTON LEIGH, K. C. HOLMES, H. G. MANNHERZ, G. ROSENBAUM, F. ECKSTEIN and R. GOODY, Cold Spring Harbor Symp. quant. Biol. *37*, 443 (1973).

[14] Acknowledgment. We are greatly indebted to Prof. Dr. J. C. RÜEGG and Dr. H. J. KUHN for many helpful discussions and to Dr. D. J. MILLER for checking the English.

Chromosomes of *Molgula manhattensis* de Kay (Ascidiacea)[1]

Since the family Molgulidae, suborder Stolidobranchiata, is one of the least known at a karyological level in the whole class Ascidiacea, the only cytogenetic studies being those regarding the chromosome number of *Molgula manhattensis*[2,3], the present study is intended to give a preliminary description of both mitotic and meiotic chromosomes of *Molgula manhattensis* in order to reduce the gap in our knowlegde of the chromosomes of ascidians.

Material and methods. The present study deals with the chromosomes in male gonads, unfertilized and cleaving eggs of *Molgula manhattensis* from the Lagoon of Venice. Fixation, squash preparations and observations were made according to the methods described elsewhere[4,5].

Meiotic chromosomes. At metaphase-I oocyte bivalents (Figure 1) appear as 2 deeply stained, more or less closely connected roundish bodies; heterotypic elements are not present and only 1 bivalent can be recognized by its greater size. Although these bivalents show the tendency to join randomly together, a count of 15 metaphase-I plates has confirmed the haploid number of 16.

At early pachitene (Figure 2) each spermatocyte bivalents consists of a rod-shape homogeneously stained body in which some segments appear to be thicker and more condensed than others; although heterotypic chromosomes are not present, the small chromosomes are often slightly more condensed than the long ones. The homologous are not distinguishable from each other and neither kinetochore nor terminal zones are differentiated. One chromosome can be distinguished from the others by its distinctly greater length.

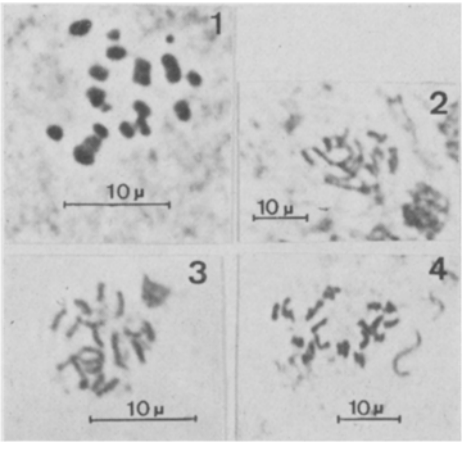
At late pachitene (Figure 3) spermatocyte bivalents are thick rod-shaped and homogeneously stained bodies in which neither the homologues nor differentiated zones are distinguishable. Measurement of pachitene chromosomes from 4 plates indicates that the relative chromosome lengths vary from one plate to another. Although the presence of some random connections between chromosomes, the haploid number 16 was consistently determined on pachitene chromosomes.

At diakinesis, the bivalents (Figure 4) are very short and deeply stained. The analysis of 30 diakinetic plates indicates that there are only 2 types of bivalents: rod shape and cross shape. The rod-shaped bivalents often show thicker roundish ends and from their morphology it is not possible to determine whether they bear chiasmata. The cross-shaped bivalents probably possess unterminalized chiasmata but it might be, at least in some cases, that the cross-shape outline be due to the precocious separation of daughter kinetochores. The diakinetic bivalents are randomly dispersed throughout the nucleus and a random association often occurs between these. The haploid number 16 was determined in most of the 30 plates examined.

Mitotic chromosomes. Although the number of observations was rather limited, it was established that a precocious separation of daughter kinetochores occurs from prometaphase till metaphase in cleaving eggs of *M. manhattensis* (Figure 5). At prometaphase the chromosomes appear as homogeneously stained rods in which no differentiated zones are present. The position of kine-

[1] This research was supported by CNR grant No. 72.01030/04 115.0542 from the Institute of Marine Biology, CNR, Venice.
[2] H. E. CRAMPTON, Am. Naturalist. *32*, 126 (1898).
[3] D. COLOMBERA, Marine Biol., in press.
[4] D. COLOMBERA, Caryologia *23*, 113 (1970).
[5] D. COLOMBERA and M. SALA, Caryologia *25*, 409 (1972).

tochores, as individuated by the distancing of daughter chromatids, varies from a distal to a medial location. The somatic parallel pairing of homologous chromosomes was recognizable only in some plates. The chromosome counts confirm the expected diploid number of 32.



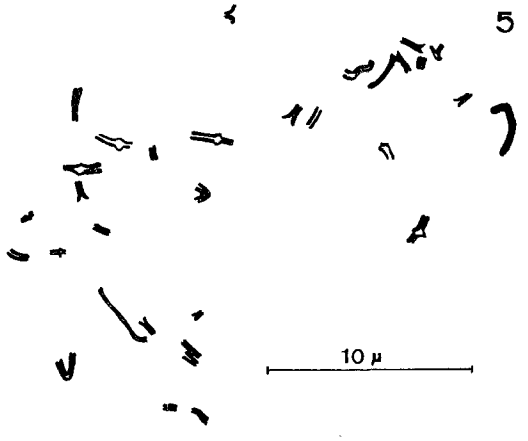


Fig. 1. Oocyte bivalents at metaphase-I.
Fig. 2. Spermatocyte bivalents at early pachitene.
Fig. 3. Spermatocyte bivalents at late pachitene.
Fig. 4. Spermatocyte bivalents at diakinesis.
Fig. 5. Mitotic chromosomes at prometaphase in cleaving eggs.

Discussion and conclusion. The haploid number 16 and the diploid number 32 have been confirmed without doubt for the population of *M. manhattensis* from the lagoon of Venice[3]. Although it has not been possible to examine populations of *M. manhattensis* from the Woods Hole area, I suspect that the haploid number of 18 given by CRAMPTON[2] for animals from that zone is probably erroneous due to the rather questionable technique employed by the author.

With respect to chromosome behaviour, precocious separation of daughter kinetochores[6] and distant somatic pairing of homologous[7] has once again been noted for cleaving eggs.

Considering chromosome morphology, *M. manhattensis* possess both telocentric, acrocentric, submetacentric and metacentric chromosomes and roundish deeply stained oocyte bivalents, as *Botryllus schlosseri*[8], the only peculiarity being the presence of 1 couple of chromosomes clearly differenciated from the others by virtue of its greather length and the aspect of spermatocyte bivalents at late diakinesis.

Because the karyology of *M. manhattensis* shows more peculiarities than that of *Botryllus schlosseri*, I feel inclined to support the view, at present based on comparison between gross morphology[9,10], that Molgulidae are less primitive than Styelidae.

Riassunto. Nell'uovo in segmentazione di *Molgula manhattensis* i cromosomi presentano una precoce separazione dei cinetocori fratelli ed un appaiamento a distanza dei cromosomi omologhi. *M. manhattensis* si distingue dagli altri Stolidobranchiati per la morfologia dei bivalenti spermatocitari e per la presenza di una coppia di omologhi nettamente più grande degli altri cromosomi.

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7 September 1973.

[6] D. COLOMBERA, Caryologia *26*, 35 (1973).
[7] D. COLOMBERA, Caryologia *26*, 27 (1973).
[8] D. COLOMBERA, Caryologia *22*, 339 (1969).
[9] N. J. BERRILL, Proc. R. Soc., London (1950).
[10] R. H. MILLAR, Marine Sci. *1966*, 519.

Male Chromosomes in two Populations of *Branchiostoma lanceolatum*

The chromosomes of only 3 members of the cephalochordate genus *Branchiostoma* are known, the particulars so far reported being: the haploid chromosome numbers 10 and 12 for *B. lanceolatum*[1–4]; the haploid number 16, confirmed by the diploid number 32, for *B. belcheri*[5,6] and the haploid number 19 together with the diploid number 38 for *B. floridae*[7]. Sex-chromosomes were individuated by their end-to-end association and by their size in *B. belcheri*[6], in which all chromosomes are considered to be telocentric or subtelocentric, owing to their rod-shaped appearance. On the contrary, neither *X* nor *Y* chromosomes were found in *B. floridae* and, although the majority of the chromosomes were described as having terminal or subterminal centromeres, a few metacentric or submetacentric chromosomes were noted[7].

Material and techniques. 10 male individuals of *Branchiostoma lanceolatum* from the Devon coast and 5 male individuals from the Gulf of Naples were examined Squash preparations of the tested were executed using the technique of COLOMBERA and SALA[8]. Observations, drawing and photos were made employing a Zeiss phase contrast microscope. The measuring of chromosomes were performed as elsewhere described[8].

Branchiostoma lanceolatum from the coast of Devon. At pachitene (Figure 1) all the chromosomes are nearly

[1] O. VAN DER STRICHT, Bull. Acad. R. Belg., Ser. *3*, *30*, 539 (1895).
[2] O. VAN DER STRICHT, Archs. Biol. *14*, 1 (1896).
[3] J. SOBOTTA, Arch. mikrosk. Anat. *50*, 15 (1897).
[4] P. CERFONTAINE, Arch. Biol. *22*, 229 (1905).
[5] S. NOGUSA, Mem. Hyogo Univ. Agr. *3*, 1 (1960).
[6] S. NOGUSA, Annotnes. zool. jap. *30*, 42 (1957).
[7] W. M. HOWELL and H. T. BOSCHUNG, Experientia *27*, 1495 (1971).
[8] D. COLOMBERA and M. SALA, Caryologia *25*, 409 (1972).