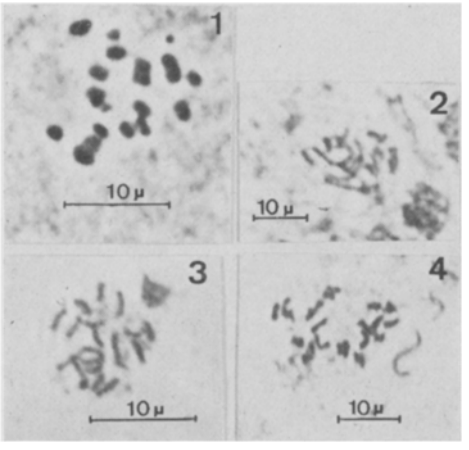
tochores, as individuated by the distancing of daughter chromatids, varies from a distal to a medial location. The somatic parallel pairing of homologous chromosomes was recognizable only in some plates. The chromosome counts confirm the expected diploid number of 32.



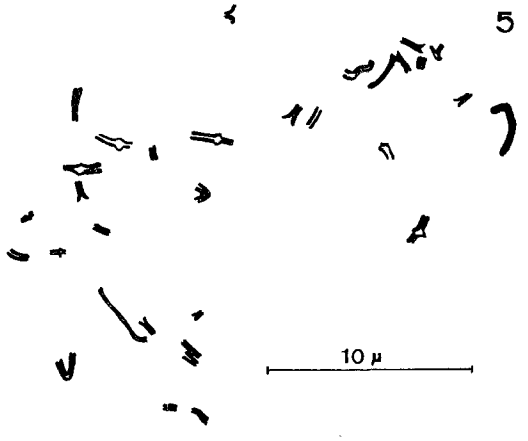


Fig. 1. Oocyte bivalents at metaphase-I.
Fig. 2. Spermatocyte bivalents at early pachitene.
Fig. 3. Spermatocyte bivalents at late pachitene.
Fig. 4. Spermatocyte bivalents at diakinesis.
Fig. 5. Mitotic chromosomes at prometaphase in cleaving eggs.

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*

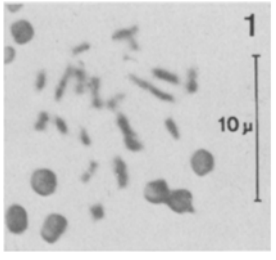
The chromosomes of only 3 members of the cephalochordate genus *Branchiostoma* are known, the particulars so far reported being: the haploid chromosome numbers 10 and 12 for *B. lanceolatum*[1–4]; the haploid number 16, confirmed by the diploid number 32, for *B. belcheri*[5,6] and the haploid number 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].

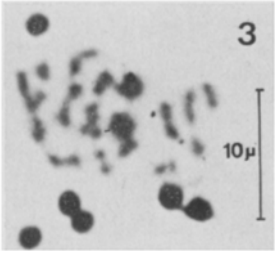
Material and techniques. 10 male individuals of *Branchiostoma lanceolatum* from the Devon coast and 5 male individuals from the Gulf of Naples were examined Squash preparations of the tested were executed using the technique of COLOMBERA and SALA[8]. Observations, drawing and photos were made employing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

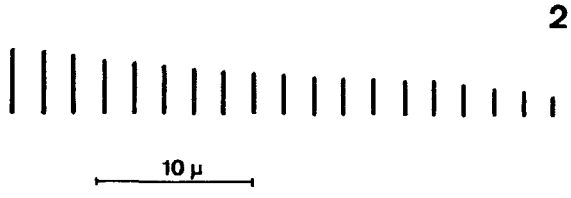
Branchiostoma lanceolatum from the coast of Devon. At pachitene (Figure 1) all the chromosomes are nearly

[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).

homogeneously stained, so that differentiated zones are not distinguishable; the homologues are so tightly paired that they cannot be recognized. Heterotypic chromosomes are not demonstrable among the various chromosomes which can be distinguished only by their lengths. There are often random end-to-end connections between the randomly arranged chromosomes. The chromosomes from 14 pachitene plates were counted, measured (Table) and arranged according to their mean lengths (Figure 2). The relatively high values for some of the larger chromosomes are due to the fact that during pachitene there is a disproportionate condensation of these as compared to the smaller ones.







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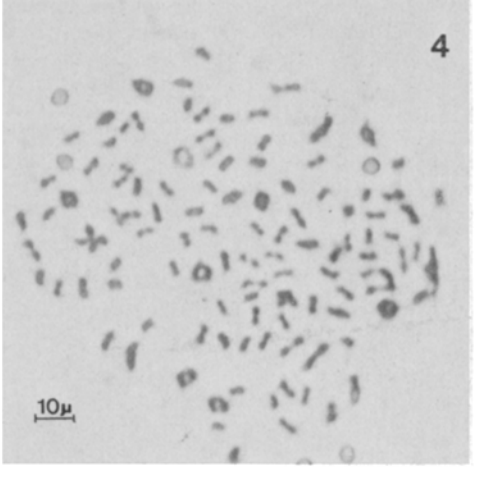

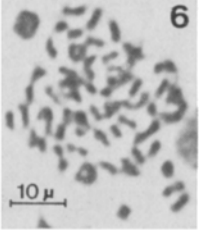

Fig. 1. Pachitene chromosomes in male gonads of *Branchiostoma lanceolatum* from the Devon coast.
Fig. 2. Idiogram constructed on male pachitene chromosomes.
Fig. 3. Diakinetic bivalents in male gonads of *B. lanceolatum* from the Devon coast.
Fig. 4. Polyploid cells in male gonads; diakinetic bivalents.
Fig. 5. Pachitene chromosomes in male gonads of *B. lanceolatum* from the Gulf of Naples.
Fig. 6. Polyploid cells in male gonads of *B. lanceolatum* from the Gulf of Naples: diakinetic bivalents.

The analysis of 50 diakinetic plates (Figure 3) confirms the haploid number of 19, previously estimated from pachitene chromosomes, provided the numerous polyploid metaphase-I plates are not considered. (Figure 4). At diakinesis 3 main types of bivalents occur fairly regularly: 1. ring-shape bivalents, normally the largest ones, numbering 2 or 3 per cell, suggesting the presence of 2 terminalized chiasmata and a medial or submedial centromere; 2. cross-shape bivalents, indicating a medial or submedial centromere and non-terminalized chiasmata; 3. rod-shape bivalents, by far the most frequent, characterized by a thin medial zone connecting 2 thicker roundish ends. These bivalents may be either achiasmatic or, more probably, possess a terminal centromere with one-end terminalized chiasmata.

Branchiostoma lanceolatum from the Gulf of Naples. Pachitene (Figure 5) and diakinetic chromosomes (Figure 6) are much the same as those found in the population of the Devon coast. The same haploid number of 19 was determined, although with some reservation, due to the small number of plates available for study. Heterocyclic chromosomes, which might be interpreted as sex-chromosomes, were also absent in the testes of amphioxi from the Gulf of Naples and this material contained the same type of bivalents as were found in the population from the coast of Devon.

Discussion and conclusion. A major incongruity that develops from a karyological analysis within the genus *Branchiostoma* is certainly that of the seeming absence of correlation between chromosomal characteristics and the rather homogeneous morphological similarity at other levels. But, after a close examination of the data available in the literature, I am inclined to believe that the earlier reports concerning *B. lanceolatum* and *B. belcheri* chromosomes are questionable and that some of the differences found between male bivalents of *B. floridae* and *B. lanceolatum* are simply due to the use of colchicine in the study of the former species. It follows

Chromosome length reckoned from 14 pachitene plates

Chromosomes	Chromosome length (µm)	Standard deviation
1	1.19	± 0.1
2	1.47	± 0.07
3	1.71	± 0.08
4	1.95	± 0.07
5	2.14	± 0.09
6	2.19	± 0.07
7	2.28	± 0.07
8	2.33	± 0.07
9	2.38	± 0.07
10	2.47	± 0.06
11	2.61	± 0.09
12	2.71	± 0.07
13	2.85	± 0.1
14	3.09	± 0.14
15	3.19	± 0.11
16	3.33	± 0.04
17	3.52	± 0.13
18	3.90	± 0.13
19	3.95	± 0.14

All values are means ± standard deviation.

that the high morphological uniformity found in the genus *Branchiostoma* exists also at a karyological level, if the older doubtful results are not considered.

In particular the genus *Branchiostoma* seems to be characterized by chromosomes which are all autosomes, regularly graded in size, mainly telocentric, with only 2 metacentric pairs; the chromosomes numbers are 19 (n) and 38 ($2n$) respectively and the content of DNA is very low, in comparison with that of vertebrates[9]. Spermatogenesis is chiasmatic.

A comparison of chromosome numbers, morphology and content of chromosomal DNA – allowing that the results obtained within the genus *Branchiostoma* can be extrapolated to include other amphioxi – indicates a closer relationship between amphioxi and some ascidians from the order Enterogona, rather than to pelagic tunicates[10] and a considerable separation of lower chordates from the vertebrates[7,9,11].

Riassunto. In entrambe le popolazioni di *Branchiostoma lanceolatum* del Golfo di Napoli e delle coste del Devonshire, Inghilterra, é stato trovato il numero aploide 19. Si sono individuati sia cromosomi metacentrici che telocentrici ma non cromosomi sessuali differenziati. È stata notata una certa somiglianza tra i cromosomi dei cefalocordati e di alcuni ascidiacei.

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[9] N. B. ATKIN and S. OHNO, Chromosoma *23*, 10 (1967).
[10] D. COLOMBERA, in press.
[11] K. M. TAYLOR, Chromosoma *21*, 181 (1967).
[12] This research was supported by grant No. 72.01034/04 115.0542 from the Institute of Marine Biology, C.N.R., Venice, to the Institute of Comparative Anatomy, University of Padova, Italy.
[13] Acknowledgments: I would like to thank Dr. W. J. CANZONIER for correcting the manuscript and Dr. M. TENCA for the drawing of the idiogram.

Critical Thermal Maximum: Ecotypic Variation Between Montane and Piedmont Chorus Frogs (*Pseudacris triseriata*, Hylidae)

The critical thermal maximum (CTM) of ectothermic vertebrates provides a convenient index to adaptations to the thermal environment that have occurred at the subcellular level of organization[1]. Whereas most prior attempts to correlate CTM of amphibians with variations in the thermal environment have relied upon interspecific comparisons[2-7], few investigators have reported ecotypic variation of this character within a single species[6-9]. We here report differences in CTM of montane and piedmont chorus frogs (Hylidae: *Pseudacris triseriata*) that correspond with important differences in ambient temperatures of the respective environments[10].

Male chorus frogs were captured from breeding congregations in Larimer County in northcentral Colorado in May and June, 1973. Three populations were sampled both in the piedmont (elevation 1542–1631 m) and in the Front Range of the Rocky Mountains (elevation 2763–3040 m). Five animals from each population were acclimated in darkness at constant temperatures of 5 °C and 20 °C for 4–12 days prior to determining CTM by the method of DUNLAP[5]. Experiments were performed between 05.00 and 11.00 Mountain Daylight Time in order to minimize the effect of circadian variations in CTM[11-13]. All frogs were held at 5 °C until beginning acclimation; and the interval between capture and

[1] G. N. SOMERO, Am. Nat. *103*, 517 (1969).
[2] B. H. BRATTSTROM, Comp. Biochem. Physiol. *24*, 93 (1968).
[3] J. S. FROST and E. W. MARTIN, Copeia *1971*, 750 (1971).
[4] D. L. CLAUSSEN, Comp. Biochem. Physiol. *44A*, 137 (1973).
[5] D. G. DUNLAP, Physiol. Zool. *41*, 432 (1968).
[6] H. HEATWOLE, N. MERCADO and E. ORTIZ, Physiol. Zool. *38*, 1 (1965).
[7] V. H. HUTCHISON, Physiol. Zool. *34*, 92 (1961).
[8] P. V. CUPP, JR. and E. D. BRODIE, JR., Am. Zool. *12*, 689 (1972).
[9] J. R. SPOTILA, Ecol. Monogr. *42*, 95 (1972).
[10] The average air temperature for June–August is about 12 °C for the montane localities and 19 °C for the piedmont habitats. Colorado State Planning Commission, Water Conservation Board, and State Engineer, *Water Resources Survey, Climatological Data of Colorado* (Denver 1938).
[11] J. J. MAHONEY and V. H. HUTCHISON, Oecologia *2*, 143 (1969).
[12] C. R. JOHNSON, Comp. Biochem. Physiol. *41A*, 727 (1972).
[13] D. G. DUNLAP, Copeia *1969*, 852 (1969).

Table I. Critical thermal maxima of montane and piedmont chorus frogs following acclimation at 5 °C or 20 °C

Elevation		Acclimation temperature 5 °C	20 °C
Piedmont	Population 1	37.0 ± 0.051	38.7 ± 0.037
	Population 2	37.0 ± 0.037	38.6 ± 0.040
	Population 3	37.0 ± 0.066	38.6 ± 0.049
	$\bar{\bar{X}}$	37.0 °C	38.6 °C
Montane	Population 1	35.4 ± 0.073	36.9 ± 0.060
	Population 2	35.5 ± 0.051	38.1 ± 0.037
	Population 3	35.3 ± 0.037	38.1 ± 0.058
	$\bar{\bar{X}}$	35.4 °C	37.7 °C

Each mean (± the standard error) is based upon measurements of 5 different animals.