Chromosomes of the Lancelet, *Branchiostoma floridae* (Order Amphioxi)

Although lancelets hold considerable phylogenetic interest to vertebrate evolutionists, their cytotaxonomy has been largely ignored. Earlier workers only vaguely alluded to lancelet chromosomes in their descriptions of ovogenesis or of egg development in *Branchiostoma lanceolatum*[1-5]. NOGUSA[6,7] is the only researcher to study lancelet chromosomes from a cytotaxonomic viewpoint.

Chromosome numbers have not been determined for any species of western Atlantic lancelet; thus, the purpose of this paper is to describe the chromosomes of the lancelet, *Branchiostoma floridae* Hubbs. This species is found in the Gulf of Mexico along the west coast of Florida to the mouth of the Mississippi River. Taxonomic accounts are given by BOSCHUNG and GUNTER[8,9].

Twenty individuals of *B. floridae* were selected from a large series of specimens collected off Petit Bois Island in Mississipi Sound on 26 July 1970. Slides of gonadal tissue, gill epithelium, and fin epithelium were made using the technique of HOWELL and DENTON[10]. Mitotic and meiotic divison figures were abundant in male gonadal tissue but none were found in female gonads. Mitotic division figures were absent in gill and fin epithelia. Even the treatment of specimens with colchicine failed to accumulate mitotic metaphase figures. The reason for the lack of somatic mitotic activity is not clear; however, we believe it to be correlated with our observation that adult lancelets held in captivity tend to undergo tissue degeneration with much sloughing away of tissue, especially from the head and/or tail regions. It seems that when tissue degeneration sets in, the capacity to repair sloughed tissue by mitosis is lost. Consequently, the data presented in this paper was obtained from slides of gonadal tissue from eight adult males.

Eighty well-spread meiotic figures were counted, one of which is shown in Figure 1. Table I shows the distribution of meiotic counts. Although counts ranged from 18 to 20, a strong modal haploid number of 19 was obtained. 14 mitotic figures were counted, one of which may be seen in Figure 2. Table II shows that the distribution of mitotic counts ranged from 37 to 40 with a strong modal diploid count of 38.

The position of the centromere appeared subterminal to terminal in 15 to 17 pairs of meiotic chromosomes while being median to submedian in at least 2 to 4 pairs. However, no accurate pairing and karyotyping were attempted as the chromosomes were extremely small, ranging from 0.3 μm to 3.0 μm in length.

Early workers[1-5] reported diploid numbers ranging from 20 to 24 for *Branchiostoma lanceolatum*; however, the accuracy of their counts has been questioned[6,7]. Using more advanced cytological techniques, NOGUSA[6,7] studied male gonadal tissue of the Japanese lancelet, *Branchiostoma belcheri* Gray, and found a haploid number of 16 and a diploid number of 32. Thus, his counts are in closer

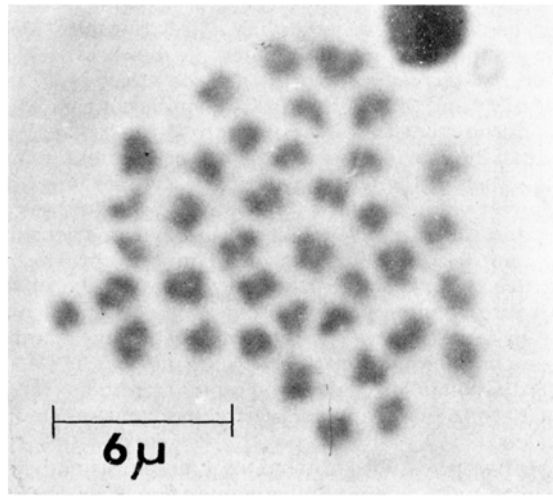

Fig. 2. Mitotic metaphase chromosomes from gonadal tissue of male *Branchiostoma floridae*, $2n = 38$.

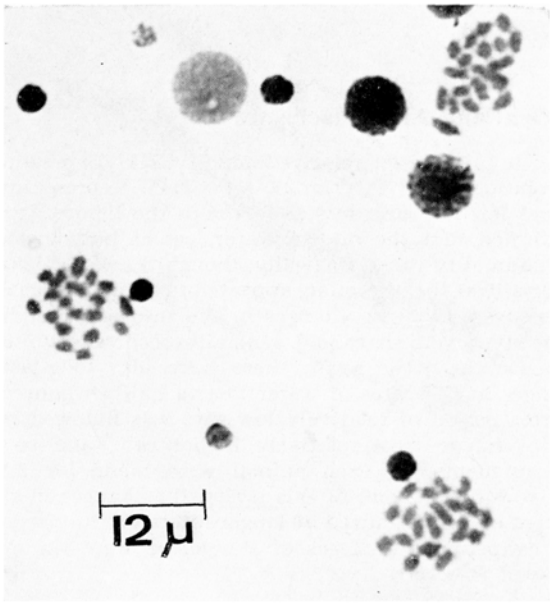

Fig. 1. Three different metaphase figures showing paired chromosomes of the first meiotic division from the gonadal tissue of male *Branchiostoma floridae*, $n = 19$.

Table I. Distribution of counts for the meiotic (haploid) chromosome number of *Branchiostoma floridae*

Meiotic (haploid) numbers	18	19	20
No. of counts	11	65	4

Table II. Distribution of counts for the mitotic (diploid) chromosome number of *Branchiostoma floridae*

Mitotic (diploid) numbers	37	38	39	40
No. of counts	2	10	1	1

[1] O. VAN DER STRICHT, Bull. Acad. R. Belg. Ser. *3*, 30 (1895).
[2] O. VAN DER STRICHT, Arch. Biol. *14* (1896).
[3] J. SOBOTTA, Arch. Mikroskop. Anat. *50*, 15 (1897).
[4] P. CERFONTAINE, Acad. R. Belg. Sci. (1905).
[5] P. CERFONTAINE, Arch. Biol. *22* (1905).
[6] S. NOGUSA, Mem. Hyogo Univ. Agr. *3*, 1 (1960).
[7] S. NOGUSA, Annotnes. zool. jap. *30*, 1 (1957).
[8] H. T. BOSCHUNG JR. and G. GUNTER, Tulane Stud. Zool. *9*, 245 (1962).
[9] H. T. BOSCHUNG JR. and G. GUNTER, Copeia *3*, 485 (1966).
[10] W. M. HOWELL and T. E. DENTON, Copeia *2*, 393 (1969).

agreement with our counts of 19 (n) and 38 ($2n$) for *B. floridae* than those reported by the earler workers for B. *lanceolatum*. The sizes of the chromosomes reported for the Japanese lancelet are similar to those in *B. floridae*. However, NOGUSA[6,7] reported that the chromosomes in *B. belcheri* are rod-shaped, indicating subterminal to terminal centromeres. He did not mention any median to submedian centromeres as found by us in *B. floridae*. In addition, he found a remarkable size difference in 2 chromosomes which he interpreted as being an XY-pair of sex chromosomes. We did not see any such chromosomes in *B. floridae*.

There is much uncertainty regarding the phylogenetic position of lower chordates of the subphyla Cephalochordata (lancelets) and Urochordata (tunicates). In addition, there has been much speculation on the relationships of these lower chordates to the most primitive of vertebrates, the agnathans. Recent studies on the chromosomes of tunicates[11] and the relative cellular DNA content of tunicates, lancelets, and agnathans[12], have given us a better understanding of relationships among these groups. The diploid chromosome numbers of 32 and 38 for *B. lanceolatum* and *B. floridae* are very close to those of 28 and 32 reported for two species of tunicates[11]. In addition, the size of the tunicate chromosomes[11] was less than 3μm as in *Branchiostoma*. Furthermore, relative DNA values of the lancelet and tunicate were lower than the lowest value obtained for any vertebrate and seem to indicate a somewhat close lancelet-tunicate relationship[12].

A comparison of lancelet and lamprey chromosomes indicates that their only similarity seems to be in their small size. Lamprey chromosomes are generally under 3μm in length[10,13–16], except in Australian lampreys, whose chromosomes are up to 6 μm in length[13,14]. The very dissimilar diploid chromosome numbers ranging from 70 to 168 in lampreys[16] easily distinguish them from lancelets.

The hagfishes with diploid chromosome numbers of 48 and 52 are closer numberwise to lancelets than are lampreys; however, hagfish chromosomes are generally larger, some being up to 5 μm in length[11].

A comparison of the DNA values relative to the human leucocyte value has shown that the lancelet with 17% is closer to the lamprey with 38% than to the hagfish with 78%[12].

Our cytological findings of a small diploid number and small-sized chromosomes in *B. floridae* are consistent with the idea that vertebrates evolved from an organism with a very small amount of DNA, and that an increase in DNA by gene duplications and polyploidization began to occur before the jawed fishes evolved[12].

Résumé. Une étude des chromosomes de *Branchiostoma floridae* Hubbs, une espèce de lancelet de l'Atlantique occidentale, révéla la présence d'haplocaryons et diplocaryons au nombre de 19 et de 38 respectivement. Ces chromosomes furent comparés à ceux de 2 espèces de lancelets du Vieux Monde, *Branchiostoma lanceolatum* et *B. belcheri*. On résume les études concernant les chromosomes des Urochordata et des Agnatha et on émet des hypothèses sur las relations possibles entre les Protochordata et Agnatha.

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[11] K. M. TAYLOR, Chromosoma *21*, 181 (1967).
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[14] E. S. ROBINSON and I. C. POTTER, Copeia *4*, 824 (1969).
[15] I. C. POTTER and B. ROTHWELL, Experientia *26*, 429 (1970).
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Evaporation in Terrestrial Isopods is Determined by Oral and Anal Discharge

The evaporation rates of the terrestrial isopods *Porcellio scaber* Latr. and *Armadillidium vulgare* (Latr.) are known to vary cyclically in dry air[1]. The average length of time from one maximum to the next was found to be about 1 h. This cycle was also shown to affect the orientation of these animals in a humidity gradient[1]. I have repeated some of these evaporation experiments using a finer technique, and as a result I now introduce a new theory concerning the regulation of water loss in terrestrial isopods. In short, the evaporation cycle appears to be the result of an oral and anal discharge of fluid; this fluid then spreads over the cuticle and contributes to the overall rate of evaporation of water from the body. One implication of this is that the water permeability data published (cf. reviews by EDNEY[2] and LINDQVIST[1]) may be too high.

The weight changes of *Porcellio scaber* were measured using a Cahn RG Electrobalance connected to a 1 mV recorder. The readings were made to the nearest 0.01 mg. The animal was kept in a small brass cage on the stirrup. The humidity of the weighing chamber was controlled by Drierite (for 5 ± 2% relative humidity, RH) or a saturated solution of KH_2PO_4 (for 95 ± 1% RH). Representative curves for the water loss are given in the Figure. It was confirmed that the rate of water loss at both low and high humidity varies cyclically, though the period length, especially at the beginning, appeared to be highly variable. There were stepwise changes in the rate of water loss; these steps were sharp and generally occurred in 1 to 3 min. On the other hand, there were also long-lasting changes in the rates of water loss; a half an hour or a shorter period of relatively low rate was followed by a sharp change to a relatively higher rate, and so on. Measurements for each animal were made for 3.5 h and towards the end of this period the changes in rates leveled off and occurred at longer intervals. In principle, the evaporation patterns of *A. vulgare* were similar to those of *P. scaber*.

[1] O. V. LINDQVIST, Ann. Zool. Fenn. *5*, 279 (1968).
[2] E. B. EDNEY, Am. Zool. *8*, 309 (1968).