species of Anura[11] – that the nucleolus organizer region is localized in this position. The fluorochrome staining permits the conclusion that the heterochromatic regions in the karyotype of *G. riobambae* differ greatly with regard to their DNA-base composition. The quinacrine-bright heterochromatin is AT-rich, and the heterochromatin with a mithramycin-bright fluorescence consists of GC-rich DNA-sequences[11,12]. The comparison of the quinacrine- and mithramycin-stained karyotypes reveals complementary banding patterns (fig. 2,a and b).

The specific staining of the nucleolus organizer regions according to the $AgNO_3$-technique[9,14] confirms the result obtained with mithramycin fluorescence. The only nucleolus organizer is localized in the secondary constriction of the short arm of the X chromosome (fig. 2,c). Since nucleolus organizers are located neither on the Y chromosome nor on any autosome, there is a dosage ratio of 1:2 between male and female animals with regard to the number of 18s and 28s ribosomal RNA genes. Such a sex-specific number of nucleolus organizer regions is very rare among vertebrates[13,14].

In the diakinetic stages of male meiosis of *G. riobambae* all autosomal bivalents possess 2 terminal chiasmata, which gives them a ring-like configuration (fig. 2,d). This exclusive occurrence of terminal chiasmata in diakinetic bivalents is characteristic of male meiosis in the highly evolved families of the Anura[2,15]. In contrast to the autosomal bivalents, the heteromorphic XY sex chromosomes of *G. riobambae* exhibit an end-to-end arrangement in the diakinetic stage (fig. 2,d). This is the first species found in the Anura in which the existence of a sex bivalent can be demonstrated, as in the male meiosis of mammals. The conclusion from this is that there is almost no homology any longer between the heteromorphic XY sex chromosomes of *G. riobambae*.

There are several reasons why the XY sex chromosomes of *G. riobambae* are attractive for cytogenetic studies: 1. The Y chromosome is larger than the X chromosome; this is the first example of a vertebrate species whose Y chromosome is larger than the X chromosome. 2. An X-linked nucleolus organizer region (NOR), and, consequently, a sex-specific difference in the number of NORs (females 2 NORs: males 1 NOR). 3. An unequivocal XY sex bivalent in male meiosis. A comparative cytogenetic investigation on the 32 further species of the genus *Gastrotheca*[16] is necessary in order to determine whether these unusual XY sex chromosomes are an exception or whether they are present in other species.

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2 Morescalchi, A., in: Cytotaxonomy and vertebrate evolution, p. 233. Eds A. B. Chiarelli and E. Capanna. Academic Press, London/New York 1973.
3 Morescalchi, A., in: Evolutionary biology, vol. 8, p. 338. Eds T. Dobzhansky, M. K. Hecht and W. C. Steere. Plenum Press, New York 1975.
4 Morescalchi, A., Genetica *50* (1979) 179.
5 Schmid, M., in: Cytogenetics of vertebrates, p. 4. Ed. H. Müller. Birkhäuser, Basel/Boston/Stuttgart 1980.
6 Schmid, M., Differentiation (1983) in press.
7 Noble, G. K., The biology of the Amphibia. McGraw-Hill Book, New York 1931.
8 Duellman, W. E., Occ. Pap. Mus. natl Hist. Univ. Kansas *22* (1974) 1.
9 Schmid, M., Chromosoma *66* (1978) 361.
10 Schmid, M., Chromosoma *68* (1978) 131.
11 Schmid, M., Chromosoma *77* (1980) 83.
12 Schweizer, D., Chromosoma *58* (1976) 307.
13 Hsu, T. C., Spirito, S. E., and Pardue, M. L., Chromosoma *53* (1975) 25.
14 Goodpasture, C., and Bloom, S. E., Chromosoma *53* (1975) 37.
15 Schmid, M., Chromosoma *80* (1980) 69.
16 Duellman, W. E., in: Das Tierreich, vol. 95, p. 12. Eds R. Mertens and W. Hennig. Walter de Gruyter, Berlin/New York 1977.

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A Feulgen technique for identification of cucumber chromosomes

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Summary. A Feulgen procedure was followed for identifying the somatic chromosomes of cucumber (*C. sativus* L.). The chromosomes were differentiated into dark and light banded regions facilitating the precise karyotyping of somatic complement. Pre-treatment temperature was found to be not critical for producing Feulgen bands in cucumber.

One of the pre-requisites of assigning genes to specific chromosomes is the unmistakable and easy identification of the individual chromosomes. The introduction of the banding techniques has to a great extent helped gene mapping even in organisms where controlled breeding is not possible. While these techniques have been extensively used in animal cytology, their success in plant cytogenetics has been very limited. Plants with small chromosomes pose further difficulties in responding to these new techniques, hampering even identification of individual chromosomes. In cucumber (*Cucumis sativus* L.), even though 83 genes have been identified[2] it has not been possible to assign them to different chromosomes which are not easily distinguishable. In view of this, attempts were made to improve on the staining technique to overcome this difficulty. The present paper communicates a simple staining method which has yielded better clarity and easier identification of chromosomes compared to the previous report[3].

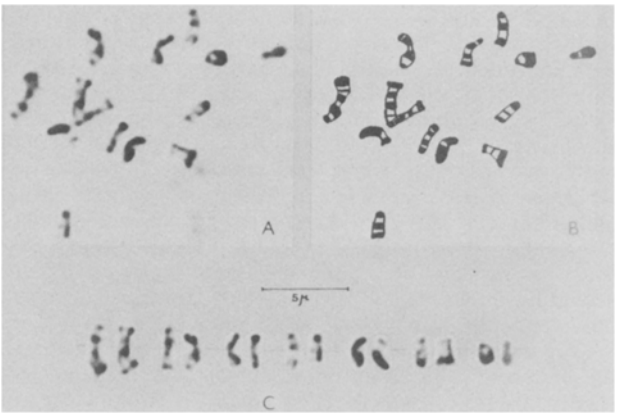
For the present investigation, root tips of *Cucumis sativus* L. variety Japanese Long Green were pretreated with 0.002 M 8-hydroxyquinoline for 2 h and fixed in Carnoy's II fluid (6 alcohol : 3 chloroform : 1 acetic acid) for 48 h. The root tips were then hydrolyzed in 1 N HCl at 60 °C for 15 min and stained with leuco-basic fuchsin for 10–15 min. For softening, the stained root tips were macerated in 2.5% pectinase + 2.5% cellulase solution for 30 min at 20 °C. The root tips were washed in water, and squashed in 0.5% acetocarmine.

Following this technique the chromosome differentiated into distinct dark and light banded regions facilitating easy

Length, arm ratio, classification and banding patterns of cucumber chromosomes

Chromosome No.	Length	Arm ratio L/S	Classification*	Number of bands Long arm	Short arm
1	2.72	1.58	SM**	3	2
2	2.52	1.39	SM**	2	2
3	2.30	1.00	M	2	2
4	1.69	1.26	SM**	1	2
5	1.48	1.76	ST	1	1
6	1.48	1.10	M	2	1
7	1.25	1.50	SM	1	1

*Giorgi and Bozzini[9]. **Chromosomes with satellite. M, Metacentric; SM, submetacentric; ST, subtelocentric.



A Somatic chromosome complement of cucumber (2n = 14) exhibiting Feulgen banding. *B* Interpretive drawing of figure A. *C* Karyotype based on length, arm ratio and banding patterns.

identification of individual chromosomes. It was possible to study in detail the karyotype following this technique. The banding patterns of chromsosomes are presented in figure A and the interpretive drawing in figure B. The conspectus of the cucumber chromosomes on the basis of length, arm ratio and banding pattern listed in the table are used in karyotyping the complement (fig. C). The specific chromosome identity produced by this technique may pave the way for further cytogenetic and evolutionary studies in cucumbers.

Feulgen banding has already been reported in animal[4-6] and plant systems[7,8]. Several mechanisms have also been postulated by these investigators to explain the chromosome banding following Feulgen staining in conjunction with different pre-treatments. Even though Schlarbaum and Tsuchiya[7] have attributed success of Feulgen banding technique to specific temperature pre-treatments, such pre-treatment is not required in cucumbers.

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2 Robinson, R.W., in: Conference on the biology and taxonomy of Cucurbitaceae, Cornell U•iv., Ithaca (USA), abstracts.
3 Sekioka, T.T., Mok, M.C., and Mok, D.W.S., Microsc. Acta *80* (1978) 277.
4 Ennis, T.J., Can. J. Genet. Cytol. *17* (1975) 75.
5 Rodman, T.C., Science *184* (1974) 171.
6 Rodman, T.C., and Tahiliani, S., Chromosoma *42* (1973) 37.
7 Schlarbaum, S.E., and Tsuchiya, T., J. Hered. *72* (1981) 62.
8 Vosa, C.G., and Marchi, P., in: Chromosomes Today, p.433. Eds J. Wahrman and K.R. Lewis. Halsted (Wiley), New York 1973.
9 Giorgi, B., and Bozzini, A., Caryologia *22* (1969) 249.

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Somatostatin-like immunoreactive neurons in a protochordate

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Summary. Peptidergic neurons have been localized in the cerebral ganglion of the ascidian *Styela plicata.* Using an antiserum to mammalian somatostatin, these cells display an intense immunoreactivity. The probable function of somatostatin-like peptides in the nervous system of protochordates is discussed.

Only recently have peptidergic neurons been demonstrated in protochordates. In particular, ACTH-like[1], LH-RH-like[2] and somatostatin-, substance P-, calcitonin-like[3] immunoreactivity has been localized in the nervous system of the phlebobranchiate ascidian *Ciona intestinalis.* Furthermore, CCK/gastrin-like immunoreactive neurons and nerve fibers have been detected in the cerebral ganglion of the ascidians *Styela clava* and *Ascidiella aspersa*[4].

The aim of the present work is the localization, by means of immunocytochemical methods, of somatostatin-like peptides in the cerebral ganglion of the ascidian *Styela plicata,* belonging to the suborder Stolidobranchiata.

Materials and methods. Specimens of the ascidian *Styela plicata* (Protochordata) were collected in the water inside the port of Genova. After dissection, the neural complex was fixed with Bouin's fluid in sea water for 4 h. Then, it was embedded in paraplast and serially sectioned. The sections (4 µm) were placed on albumin-coated glass slides and used for the cytochemical and immunocytochemical assays. In order to verify the presence of neurosecretory cells, the following cytochemical methods were used: Gomori chromohaematoxylin, Alcian B GX 3%-phloxine, Gomori aldehyde fuchsin, Bodian silver impregnation. The indirect immunofluorescence method[5] was applied to dewaxed and rehydrated sections. After incubation for 12–14 h in a moist chamber at 4 °C with rabbit antiserum to cyclic somatostatin conjugated to thyroglobulin (batch No. R-1801; Milab, Sweden) (diluted 1:1600 in 0.04 M