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Some bryozoan karyotypes and chromosome numbers

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SUMMARY

A technique for obtaining bryozoan chromosome spreads is described. Karyotypes were prepared for the following phylactolaemate species: *Plumatella emarginata*, $2n = 14$; *Hyalinella punctata*, $2n = 14$; *Fredericella sultana*, $2n = 14$; and *Pectinatella magnifica*, $2n = 18$. Approximate numbers were obtained for *Lophopodella carteri*, $2n = 18$–22, and three ctenostomes, *Pottsiella erecta*, $2n = 22$ or 24; *Amathia semiconvoluta*, $2n$ about 30; and *Paludicella articulata*, $2n$ about 20. Differences between the bryozoan karyotypes seen in this study correlate with taxonomy.

1. INTRODUCTION

Despite there being nearly 4000 living species in the phylum (Ryland, 1970), there has been little work published on bryozoan chromosomes. What has appeared are counts from gametogenesis studies when particularly favourable specimens became available. No karyotype has been previously prepared.

Makino (1951) stated that $n = 6$ or 7 for *Plumatella fungosa* in secondary spermatocytes but gave the mitotic $2n$ as 5, citing Braem (1897), who, however, disclaimed (p. 8) having determined a chromosome number for this species. Makino's numbers may have been derived from Braem's figures. Bonnevie (1907) reported $n = 11$ 'small' chromosomes for *Membranipora* (*Electra*) *pilosa*, based on an oocyte preparation. Grellet (1957) found a $2n$ of approximately 18 in *Alcyonidium gelatinosum* from spermatogenesis studies.

2. MATERIALS AND METHODS

Plumatella emarginata (Allman), *Hyalinella punctata* (Hancock), *Fredericella sultana* (Blumenbach), *Pectinatella magnifica* (Leidy), *Pottsiella erecta* (Potts), and *Paludicella articulata* (Ehrenberg) were collected 1975–6 from various localities in the vicinity of Washington, D.C. *Amathia semiconvoluta* (Lamouroux) was taken by trawl from a tidal channel at Wallops Island, Virginia, September 1975. *Lophopodella carteri* (Hyatt) was germinated within the laboratory from statoblasts kindly provided by Professor Timothy S. Wood (Wright State University, Dayton, Ohio).

Propanocarmine was prepared by boiling 45 ml propanoic acid, 55 ml distilled water, and slowly adding 1 g carmine. After most of the dye had dissolved, the hot liquid was aspirated through filter paper and 2 drops 4 M-$FeCl_3$ added. The

stain required 7–10 days refrigeration before use. Acidified propanocarmine, consisting of 1 part concentrated HCl to 2 parts propanocarmine, was used for prestain fixation and softening.

Specimens were used within 24 h of collection (36 for *Amathia*). For *Plumatella emarginata* and *F. sultana* terminal polypides with buds were dissected from the zooecium; for *H. punctata* and *Pectinatella magnifica* clusters of zooids were cut from colonies and used whole. For *L. carteri* smallest polypides were dissected from the colony. Terminal zooids of *Paludicella articulata* and smaller individuals of *Pottsiella erecta* were detached from the substrate, and part of the zooecium peeled away to expose the polypide. For *A. semiconvoluta* colony ends were detached. Samples were immersed 30–40 min in 2–3 ml 0·1 % colchicine in tap-water (sea water for *Amathia*).

Specimens were fixed 10 min in 3–4 drops acidified propanocarmine in a concavity microscope slide. At this time smaller polypides of *Pectinatella* and *Hyalinella* were dissected out and the residue discarded. There followed three 10 min changes of propanocarmine stain.

A 5 × 10 mm piece of thin transparent plastic film (Saran Wrap) was placed for 1 min on an albuminized microscope slide, transferred albuminized side down to a clean slide, and covered with Scotch Tape. Preparations with air bubbles between tape and wrap were discarded.

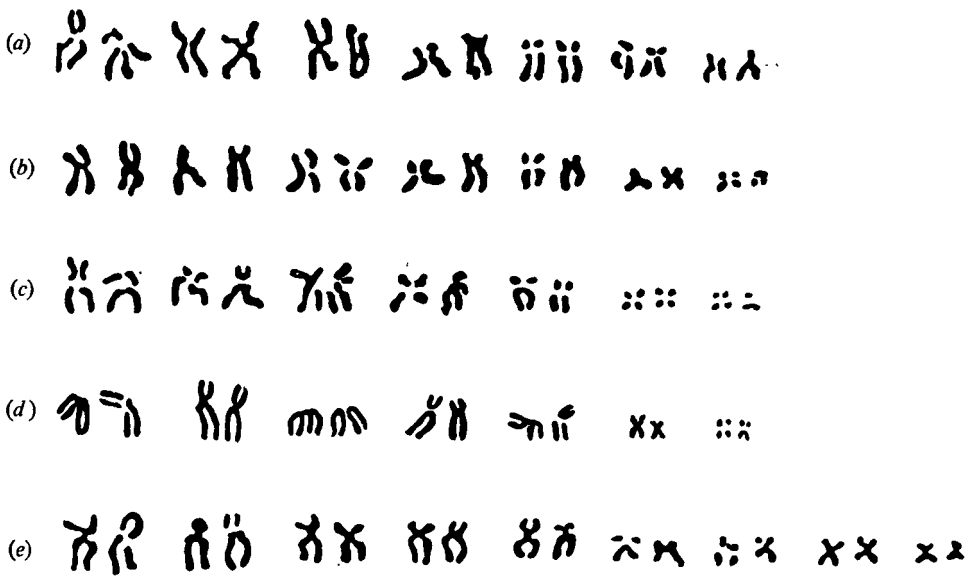
A single stained polypide (except for *Amathia*, where a stolon with buds was used) with enough fluid to cover it was placed on a 24 × 40 mm no. 1 coverslip on a slide and immediately covered with albuminized film under tape. Air bubbles were pressed out carefully. Preparations were covered with paper and squashed. For viewing, the coverslip was turned over. A drop of immersion oil on the slide kept the coverslip in place.

3. RESULTS AND DISCUSSION

Phylactolaemate preparations showed many cells in colchicine-induced metaphase arrest; most of these were poorly spread. Those karyotypes which could be determined with any certainty are presented in Text-fig. 1. Good representative phylactolaemate spreads are shown in Plate 1. The ctenostomes yielded fewer spreads, their smaller size made them more difficult to work with, and they were not so readily available. The best ctenostome spreads are given in Plate 2.

Identical karyotypes were obtained from two forms of *Plumatella emarginata* (Text-fig. 1*b*, *c*). The first form (Plate 1, fig. 2), which had keeled zooecial tubes attached for almost their entire length to the substrate, only the zooecial lips rising slightly above it, was clearly recognizable as *P. emarginata* as described by Rogick (1935) and Prenant & Bobin (1956). The second form lacked keels or furrows, and was part of a tangled mass growing from the substrate. Both had approximately 36 tentacles, and both were collected from rapidly flowing water. Professor Timothy S. Wood examined the second, and concluded from the presence of frequent septa that this was also probably *P. emarginata*. The karyotype has $2n = 14$ (7 spreads).

Hyalinella punctata (Text-fig. 1*d*) was identifiable from its swollen, non-encrusted ectocyst, fitting the description given by Rogick (1935). The karyotype, with $2n = 14$, was taken from a single spread in which the chromosomes appeared larger than those of *Plumatella emarginata*. This was probably due to unavoidable variations in preparation, and should not be considered significant; relative lengths are the same. The karyotypes of these two species are then extremely similar or identical, suggesting that some of the other *Plumatella* forms may be likewise. Braem's figures 20–22 (1897), without indicating centromere positions, suggest the chromosomes of *Plumatella fungosa* have about the same proportional sizes to one another as do those of *P. emarginata* and *H. punctata*.



Text-fig. 1. Karyotypes of: (*a*) *Fredericella sultana*; (*b*) *Plumatella emarginata* (keeled type); (*c*) *Plumatella emarginata* (tangled type); (*d*) *Hyalinella punctata*; and (*e*) *Pectinatella magnifica*. All approx. × 700, except for *H. punctata*, × 475.

Fredericella sultana (Text-fig. 1*a*; Plate 1, fig. 1), with its antler-like branches and circular lophophore, was as described by Rogick (1935). It also has $2n = 14$, clearly seen in three spreads; this number is in agreement with independent work done by R. Potter (personal communication). However, there are noticeable differences between its karyotype and those of *P. emarginata* and *H. punctata*. For example, chromosome number 2 is more metacentric for *F. sultana*, and its two smallest sets are larger than those of the other two species.

Pectinatella magnifica (Text-fig. 1*e*), identified from its massive gelatinous colonies and characteristic spinoblasts (Rogick, 1935), has $2n = 18$, based on three spreads, in agreement with an independent determination by R. Potter (personal communication). Arm ratios in the number 2 sets, and the sizes of the two smallest, 8 and 9 of *Pectinatella*, 6 and 7 of the previously discussed species,

indicate a closer relationship of this species to *Plumatella emarginata* and *H. punctata* than to *F. sultana*. This agrees with morphological evidence.

The chromosomes of *Lophopodella carteri* (spinoblasts were as described by Rogick, 1935) consistently stained poorly, although *F. sultana* processed at the time in the same solutions yielded good results. One of the least unsatisfactory spreads indicated $2n = 18–22$. When seen, larger chromosomes had arm ratios between 2:1 and 3:1, similar to those of *P. magnifica*.

Pottsiella erecta (Plate 2, fig. 1) has $2n = 22$ or 24 (3 spreads seen). Colonies consisted of erect individuals arising from stolons, as described by Pennak (1953). Orifices tended to be square for younger zooids, pentagonal for the older ones. The specimens were collected from slowly flowing water.

Amathia semiconvoluta (Plate 2, fig. 2) has $2n$ about 30 (photographs of 3 spreads). Groups of helically arranged double rows of zooids were arising from branching stolons, as described by Prenant & Bobin (1956). This species and *Pottsiella erecta* gave spreads with predominately small metacentrics, indicating karyotypes different from those of the phylactolaemates; chromosome numbers are higher, and there is a striking uniformity in size.

Paludicella articulata (Plate 2, fig. 3) has $2n$ about 20 (2 spreads seen). Colonies were composed of a series of recumbent club-shaped zooecia, arranged end to end, as described by Pennak (1953). The chromosomes, some possibly acrocentric, are smaller than those of any of the other species seen in this study; this may be related to small cell size (White, 1973).

4. CONCLUSIONS

Among phylactolaemates, *Plumatella emarginata* and *Hyalinella punctata* seem closely related on the basis of very similar or identical karyotypes. *Fredericella sultana*, *Pectinatella magnifica* and probably *Lophopodella carteri* are distinct, although *Pectinatella magnifica* seems more closely related to *Plumatella emarginata* and *H. punctata* than to *F. sultana*. The ctenostomes *Amathia semiconvoluta* and *Pottsiella erecta* seem more distantly related to these phylactolaemates than either the former or latter are to each other. *Paludicella articulata*, with its small chromosomes, seems more closely related to the other two ctenostomes in this study than to any of the phylactolaemates. For this limited sample, therefore, there is agreement between karyotypic and morphological evidence as to taxonomical relationships.

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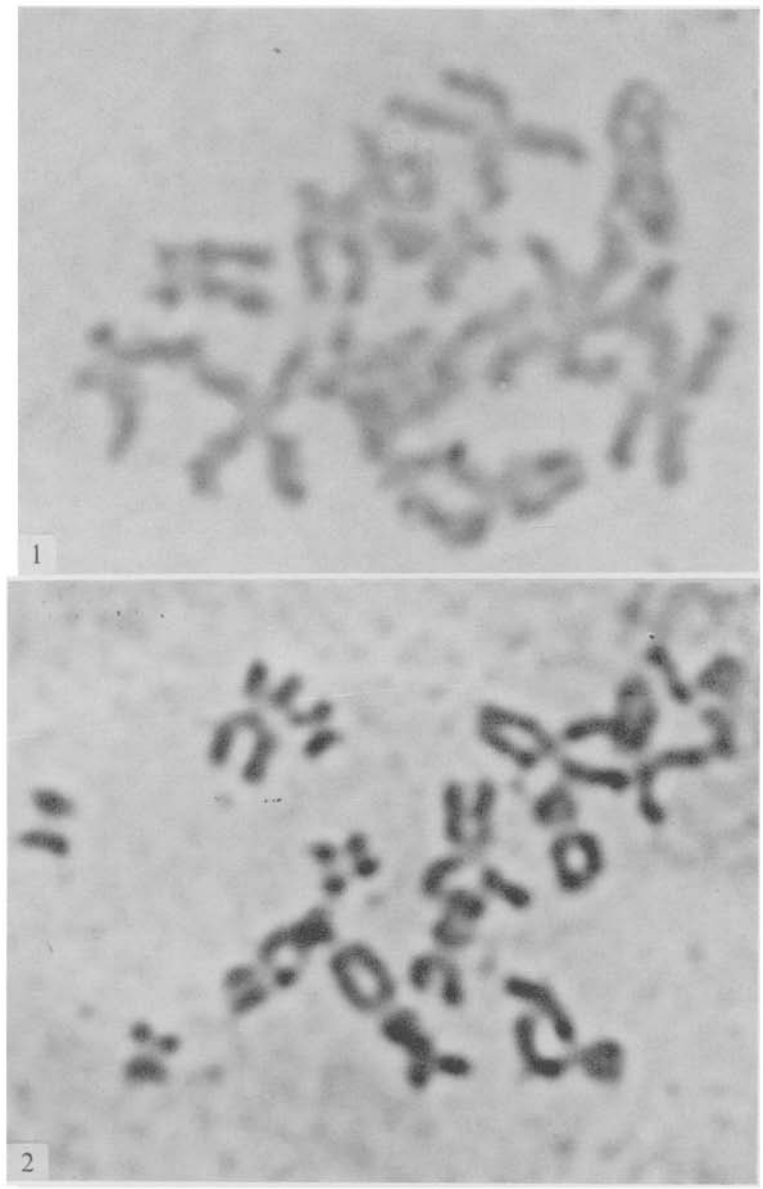

Fig. 1. Chromosome spread from *Fredericella sultana* (Blumenbach) indicating $2n = 14$. × 2200 approx.

Fig. 2. Spread from *Plumatella emarginata* (Allman), keeled and furrowed type with $2n = 14$. × 2200 approx.

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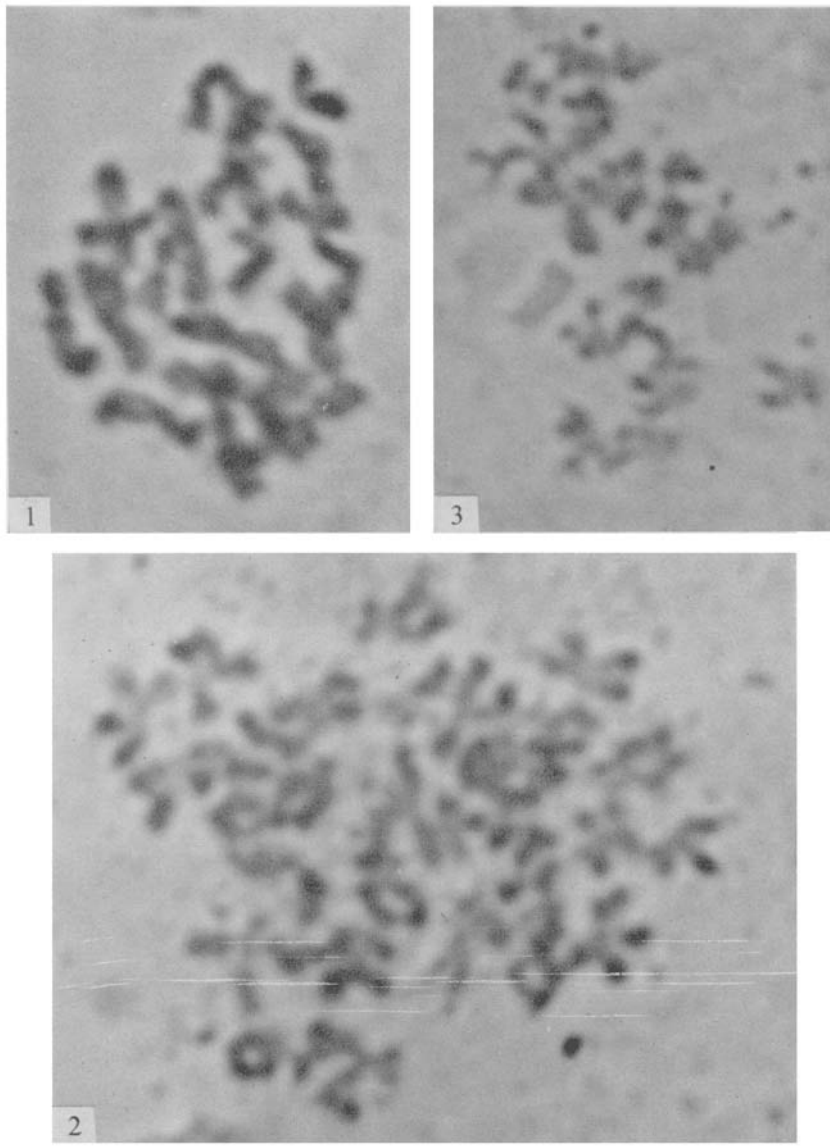

Fig. 1. Chromosome spread from *Pottsiella erecta* (Potts) indicating $2n = 22$ or 24. × 2200 approx.

Fig. 2. Chromosome spread from *Amathia semiconvoluta* (Lamouroux) showing $2n$ about 30 × 2200 approx.

Fig. 3. Spread from *Paludicella articulata* (Ehrenberg) showing a $2n$ of about 20, with chromosomes generally smaller than those of any of the other species seen in this study. × 2200. approx.

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