approach to identify such factors would be to characterize the experimental conditions which perturb the symbiont:host ratio.

1 The hydra and *E. viridis* were provided by M.H. Christopher and J.R. Turner. I thank Professor D.C. Smith, Dr J.B. Searle and Dr T.A.V. Rees for the valuable comments on drafts of this paper. This work was supported by research grants from S.E.R.C.
2 Muscatine, L., and Pool, R.R., Proc. R. Soc. Lond. (B) *204* (1979) 131.
3 McAuley, P.J., Experientia *37* (1981) 346.
4 Collins, C.R., and Farrar, J.F., New Phytol. *81* (1978) 71.
5 Douglas, A.E., and Smith, D.C., in: Endocytobiology, endosymbiosis and cell biology, vol.2, p.633. Eds W. Schwemmler and H.E.A. Schenk. Walter de Gruyter & Co., Berlin 1983.
6 Colley, N.J., and Trench, R.K., Proc. R. Soc. Lond. (B) *219* (1983) 61.
7 Cobb, A.H., Protoplasma *92* (1977) 137.
8 Muscatine, L., and Lenhoff, H.M., Biol. Bull. *128* (1965) 415.
9 Shephard, D.C., Levin, W.B., and Bidwell, R.G.S., Biochem. biophys. Res. Commun. *32* (1968) 413.
10 Holden, M., in: Chemistry and biochemistry of plant pigments, p.461. Ed. T.W. Goodwin. Academic Press, London 1965.
11 Strickland, J.D.H., and Parsons, T.H., Bull. Fish. Res. Bd Can. *167* (1972).
12 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
13 Douglas, A.E., J. mar. biol. Ass. U.K. *63* (1983) 437.
14 Peterson, G.L., Analyt. Biochem. *83* (1977) 346.
15 Douglas, A.E., Hydrobiologia *109* (1984) 207.
16 Smith, D.C., in: Endocytobiology, endosymbiosis and cell biology, vol.1, p.317. Eds W. Schwemmler and H.E.A. Schenk. Walter de Gruyter & Co., Berlin 1980.
17 Doonan, S.A., Ph. D. thesis, University of Aberdeen, 1979.
18 Healey, F.P., Crit. Rev. Microbiol. *3* (1973) 69.
19 Meeks, J.C., in: Algal physiology and biochemistry, p.161. Ed. W.D.P. Stewart. Blackwell Scientific Publications, Oxford 1974.
20 Douglas, A.E., and Smith, D.C., Proc. R. Soc. Lond. (B) *221* (1984) 291.
21 Svoboda, A., and Porrman, T., in: Nutrition in the lower metazoa, p.87. Eds D.C. Smith and Y. Tiffon. Pergamon Press, Oxford 1980.
22 Holligan, P.M., and Gooday, G.W., Symp. Soc. exp. Biol. *29* (1975) 205.
23 Muscatine, L., and Lenhoff, H.M., Biol. Bull. *129* (1965) 316.

0014-4754/85/020280-03$1.50 + 0.20/0

A simple method for the preparation of hydra chromosome spreads: introducing chromosome counts into hydra taxonomy[1]

A. Rahat, M. Rahat and J.B. Searle

Department of Genetics, The Hebrew University of Jerusalem, Jerusalem (Israel), Department of Zoology, The Hebrew University of Jerusalem, Jerusalem (Israel), and Department of Agricultural Science, University of Oxford, Oxford (England),
6 February 1984

Summary. A simple method to prepare chromosome spreads of hydra is described. Chromosome counts for a non-symbiotic 'brown hydra', *Hydra vulgaris attenuata,* and the Swiss strain of a symbiotic 'green hydra' indicated a diploid number of about 2n = 30 in each case. It is suggested that chromosome number may be used to define hydra species more precisely.
Key words. Hydra; chromosomes; taxonomy.

The small freshwater coelenterate *Hydra* has been used for many years in research on animal behavior, cell biology, morphogenesis and symbiosis[2].

Hydra are commonly divided into two groups: non-symbiotic 'brown hydra' and hydra containing symbiotic green algae ('green hydra'). Morphological characteristics, e.g. size, and structure of nematocytes and embryotheca have been used to distinguish between different species within each group[3,4]. However, these criteria are not entirely satisfactory (e.g. because of variation with environmental conditions) and as a consequence of this taxonomic ill-definition, published data are frequently difficult to interpret.

In the following communication we describe a quick and simple method to prepare hydra chromosomes for counts, so that hydra can be defined more precisely.

Our chromosome counts are consistent with previous results for a brown hydra, *H. vulgaris*[5,6], but differ from published chromosome numbers of a green hydra, *H. viridis*[7].

Materials and methods. Organisms. A brown hydra identified by R.C. Campbell as *H. vulgaris attenuata* and the Swiss strain of green hydra were used in our studies. Stock cultures were grown in M solution[8], but without TRIS, at 15 °C in a 12/12 h light/dark regime. The hydra were fed three times a week with freshly hatched nauplii of *Artemia* sp.

Procedure. For chromosome analysis, hydra were fed and placed for 18 h in growth medium containing 0.1–5.0 μg colcemid ml^{-1} (*H. vulgaris*) or 0.2 μg colcemid ml^{-1} (green hydra). (There was no indication that colcemid concentration influenced the results over the range tested.) The hydra were then rinsed, cut into several small pieces and incubated in distilled water. After about 20 min the hydra pieces were fixed in 3:1 methanol:acetic acid for 3–5 min. Hydra may be left in this fixative, in a refrigerator, for several days.

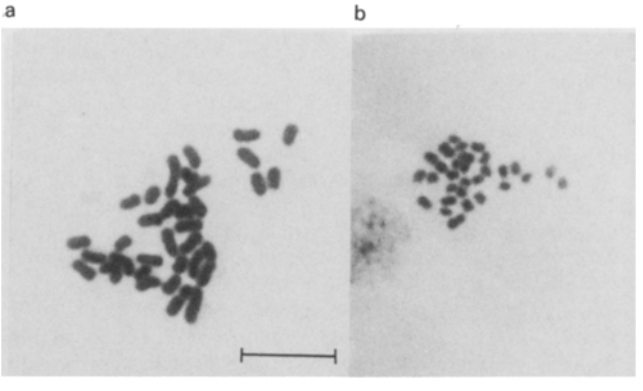

Figure 1. Representative photomicrographs of metaphase spreads of *H. vulgaris* (a) and green hydra cells (b). The chromosome counts for these cells were 33–34 and 30–32, respectively. Note that in our preparation the total chromosome area tends to be smaller in preparations of green hydra cells. Scale = 10 μm.

To prepare slides for microscopic examination, the method described by Meredith[9] for mammalian tissue was used. The pieces of fixed hydra tissue were transferred into a Dreyer tube containing 0.5 ml 60% acetic acid. Within 5 min the tissue loses its coherence, and a cell suspension could be formed by aspiration with a finely-drawn pasteur pipette. A drop of cell suspension was then transferred onto a microscope slide on a hot plate at about 60 °C and immediately withdrawn. The same drop of cell suspension was applied to the slide in this manner 5–10 times before discarding. The procedure was repeated with further drops of suspension until all the fixed material was applied onto the slide. The slides were than placed vertically for 10 min in a 5% aqueous solution of Giemsa stain, rinsed with tap water and air-dried.

The preparations were scanned for metaphases under the microscope. Chromosome counts were made for all metaphase spreads in which the chromosomes showed little or no overlap. We applied a preparation of three hydra per slide and could observe on average 12 metaphase spreads, of which an average of 2–3 spreads could be used for analysis.

Results. Chromosome counts were made from 31 spreads of *H. vulgaris* cells and 24 spreads of green hydra cells. Representative photomicrographs of spreads of each species are given in figure 1. For both species a range of chromosome counts were obtained with a maximum of 34 chromosomes per spread, a minimum of 26, and most counts around 30 (fig. 2). The most likely explanations for the variation in chromosome counts between spreads are observational error and artifactual loss of chromosomes during preparation.

Discussion. A search of the literature shows that very few attempts have been made to develop a method for the examination of hydra chromosomes[5, 6, 10–13] and those methods that have been developed involve cumbersome sectioning or squash procedures. The method described here should enable the researcher working with hydra to make chromosome counts easily and quickly. In this manner the species of hydra may be defined more precisely.

Previous reports on the chromosome number of *H. vulgaris* show 2n = 32 (Niiyama[5]; Datta[6]). Our counts are consistent with this number for the subspecies we used *(attenuata)*. For green hydra no attempts to count the chromosome number has been reported since Dowing[10, 11], who recorded 2n = 12. Our counts of about 30 chromosomes per cell differ markedly from this number. It would be of much interest to verify whether the chromosome number does vary between species or strains of green hydra.

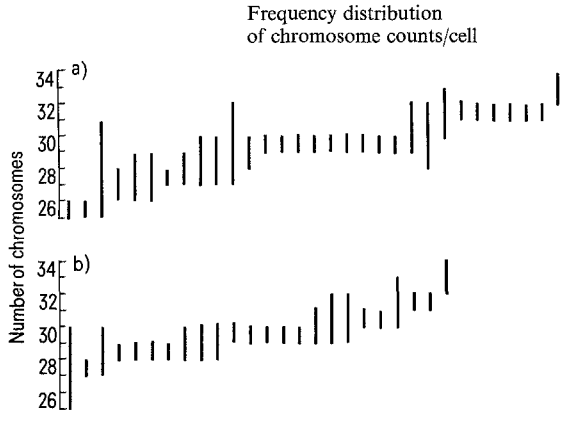

Figure 2. Chromosome counts based on spreads of *H. vulgaris* cells (a) and green hydra cells (b). Each bar represents one spread. For some spreads more than one interpretation in terms of chromosome number was possible.

1 This work was done while A.R. and M.R. were on leave in Oxford. We thank Prof. D.C. Smith F.R.S. for providing laboratory facilities. We wish to thank Prof. Smith and Dr A.E. Douglas for reading earlier drafts of this paper.

2 Lenhoff, H.M., ed. Hydra: Research Methods. Plenum Press, New York 1983.
3 Campbell, R.C., in Hydra: Research Methods, p. 19. Ed. H.M. Lenhoff. Plenum Press, New York 1983.
4 McAuley, P.J., Biol. J. Linn. Soc., in press.
5 Niiyama, H., Cytologia *13* (1944) 204.
6 Datta, M., The Nucleus *13* (1970) 132.
7 Makino, S., A review of the chromosome number in animals. Hokuryukan, Tokyo 1956.
8 Lenhoff, H.M., and Brown, R.D., Lab. Anim. *4* (1970) 139.
9 Meredith, R., Chromosoma *26* (1969) 254.
10 Dowing, E.R., Zool. Jb. Abt. Anat. *21* (1905) 379.
11 Dowing, E.R., Zool. Jb. Abt. Anat. *28* (1908) 295.
12 McConnel, C.H., Z. mikr. Anat. Forsch. *28* (1932) 192.
13 McConnel, C.H., Roux. Arch. EntwMech. Org. *135* (1936) 202.

0014-4754/85/020282-02$1.50 + 0.20/0

Announcements

USA

Plant flavonoids in biology and medicine: biochemical, pharmacological, and structure-activity relationships

Buffalo, N.Y., 22–26 July 1985

This interdisciplinary symposium will review the role of flavonoids in plants and animals and their effects in numerous mammalian cells systems. Invited lecturers will review recent advances in our understanding of their significance in physiology and function and explore their potential therapeutic uses. For further information contact Dr Elliott Middleton, Jr, Departement of Medicine, Buffalo General Hospital, Buffalo, N.Y. 14203/USA.

France

10th European symposium on hormones and cell regulation

St.-Odile, Strasbourg, 30 September – 3 October 1985

Information can be obtained from Dr. R.J.B. King, Imperial Cancer Research Fund Laboratories, Hormone Biochemistry Department, P.O.Box 123, Lincoln's Inn Fields, London WC2A 3PX, England.