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A New Alga/Hydra Symbiosis: *Hydra magnipapillata* of the "Nonsymbiotic" Vulgaris Group Hosts a *Chlorococcum*-Like Alga

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Abstract

Only one group of symbiotic hydra has been described to date i.e. the various strains of the green *Hydra viridis* that host unicellular algae of the genus *Chlorella* in their digestive cells. These strains are classified as a separate group within the genus *Hydra*. The brown hydra such as *H. vulgaris*, *H. attenuata* and others have been claimed to be nonsymbiotic and unable to form symbioses with algae. We report here a new, yet undescribed, stable algal endosymbiosis in the japanese *Hydra magnipapillata*, a brown hydra belonging to the nonsymbiotic *vulgaris* group. The endosymbiont in this hydra is a *Chlorococcum*-like alga not previously reported in the known *H. viridis* endosymbioses. The finding of this symbiosis introduces yet unknown cosymbionts and thus opens new pathways for the comparative study of host/symbiont interrelations. We suggest that further host/symbiont variants of algal endosymbioses can occur in the genus *Hydra* as a result of suitable algal/hydra encounters in nature and in the laboratory.

Keywords: *Hydra magnipapillata*, *Chlorococcum*, symbiosis.

1. Introduction

Two major groups are recognized in the genus *Hydra*: the green "viridissima" hydra that host endosymbiotic algae in their digestive cells and the brown nonsymbiotic hydra such as *H. vulgaris* or *H. attenuata* (Campbell, 1983).

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The *Chlorella* endosymbiosis in the green hydra has been studied extensively over the last 20 years (Park et al., 1967; Pool, 1979; Jolley and Smith, 1980; Meints and Pardy, 1980; McNeil et al., 1981; McAuley and Smith, 1982). As a result of these and similar studies it has been claimed that only chlorellae can form stable endosymbioses in hydra; that the green hydra "recognize" their endosymbiotic chlorellae; that free living chlorellae cannot infect those hydra and that the brown nonsymbiotic hydra cannot form stable endosymbioses with algae.

However, we have recently shown that aposymbiotic *H. viridis* can be infected with a variety of free living chlorellae to form stable symbioses (Rahat and Reich, 1984; 1985). We suggested that it is preadaptation rather than recognition that enables the formation of algal/hydra symbioses (Rahat, 1985a,b).

Here we report a new alga/hydra symbiosis described for the first time in a "nonsymbiotic" brown hydra: *Hydra magnipapillata* (Sugiyama and Fujisawa, 1977) hosts in its cells a *Chlorococcum*-like alga (Starr, 1955).

It appears that the range of stable endosymbiotic associations between hydra and green algae is broader than previously suggested.

2. Materials and Methods

The hydra obtained from Dr. T. Sugiyama, National Institute of Genetics, Mishima Japan, were cultured in M solution, a buffered mixture of salts resembling pond water (Lenhoff and Brown, 1970), under continuous illumination of $\sim$ 2500 lux at 20°C, and fed thrice weekly with freshly hatched larvae of *Artemia* sp. By the addition of phenol red to this medium we could visually monitor the pH in the cultures and verify optimal growth conditions for the hydra (Rahat and Reich, 1983).

For microscopic examination of their cells, hydra were macerated according to David (1983). Photomicrography (Figs. 2 and 3) was done by Nomarski Differential Interference Microscopy on Technical Pan film.

3. Results

As shown in Fig. 1, *H. magnipapillata* is larger than the Swiss and European strains of *H. viridis*, and resembles in size the nonsymbiotic *H. vulgaris*. Table 1 summarizes the similarities and differences between the well known symbiosis in *H. viridis* and the new symbiosis in *H. magnipapillata*.

Figure 1. Symbiotic and nonsymbiotic hydra: A and B, Swiss and European strains of *Hydra viridis*. C. Green *H. magnipapillata*. D. Nonsymbiotic *H. vulgaris*. Scale, 1cm.

Fig. 2 clearly shows that the endosymbiotic algae in the cells of the hydra are located both proximal and distal to the cell's nucleus, and Fig. 3 shows the non-chlorellean nature of these algae.

4. Discussion

Hydra magnipapillata, a species common in Japan (Sugiyama and Fujisawa, 1977), has been described to belong to the "vulgaris" group together with *H. littoralis* and *H. attenuata* "all very similar species which can only be easily distinguished on the basis of their geographical origin" (Campbell, 1983). A detailed genetic study of *Hydra magnipapillata* has been carried out by Sugiyama and Fujisawa (cited in Sugiyama, 1983), but there are no references in the literature to their green color and algal endosymbionts.

The marked differences between the well studied symbiosis in *H. viridis* and the one in *H. magnipapillata* (Table 1, Figs. 1,2), are primarily due to

Table 1. Properties of the algal endosymbioses in *H. viridis* and *H. magnipapillata*

Properties common to both symbioses

- Symbiosis hereditary, algal endosymbionts passed on to vegetative offsprings.
- Algae hosted in gastrodermal digestive cells.
- Algae dividing in host cells.

Properties of H. viridis	*Corresponding properties of H. magnipapillata*
Even distribution of algae in polyps.	Highest concentration of algae in hypotome, very few in tentacles.
Complete greening of hydra.	Some polyps with few algae only.
Algae retained in the dark.	Algae lost in the dark.
More than 15 algae/host cell.	Except for the hypostome, less than 10 algae/host cell.
Most algae located proximal to nucleus of host cell.	Many algae located distal to nucleus of host cell.
Algal symbiont a *Chlorella* sp.	Algal symbiont a *Chlorococcum*-like alga.
Native symbiotic algae cannot be cultured *in vitro*.	Symbiotic algae can be cultured *in vitro*.

the two different cosymbionts partaking in the respective symbioses. The two hydrae are different species and the algae belong to different genera (Fig. 3). As the new symbiosis is further studied more differences will certainly be found.

The exact taxonomic classification of the endosymbiotic *Chlorococcum*-like alga (Star, 1955), is not known yet. It differs from *Chlorella* by its numerous small starch grains, and mode of cell reproduction which takes place by complete cell cleavage (Fig. 3). We do not know of similar algae that have been described as endosymbionts, and we are now trying to culture them *in vitro*.

To date, all studies on algal/hydra symbioses, have been on European and North American strains of *H. viridis* and geographical separation is known

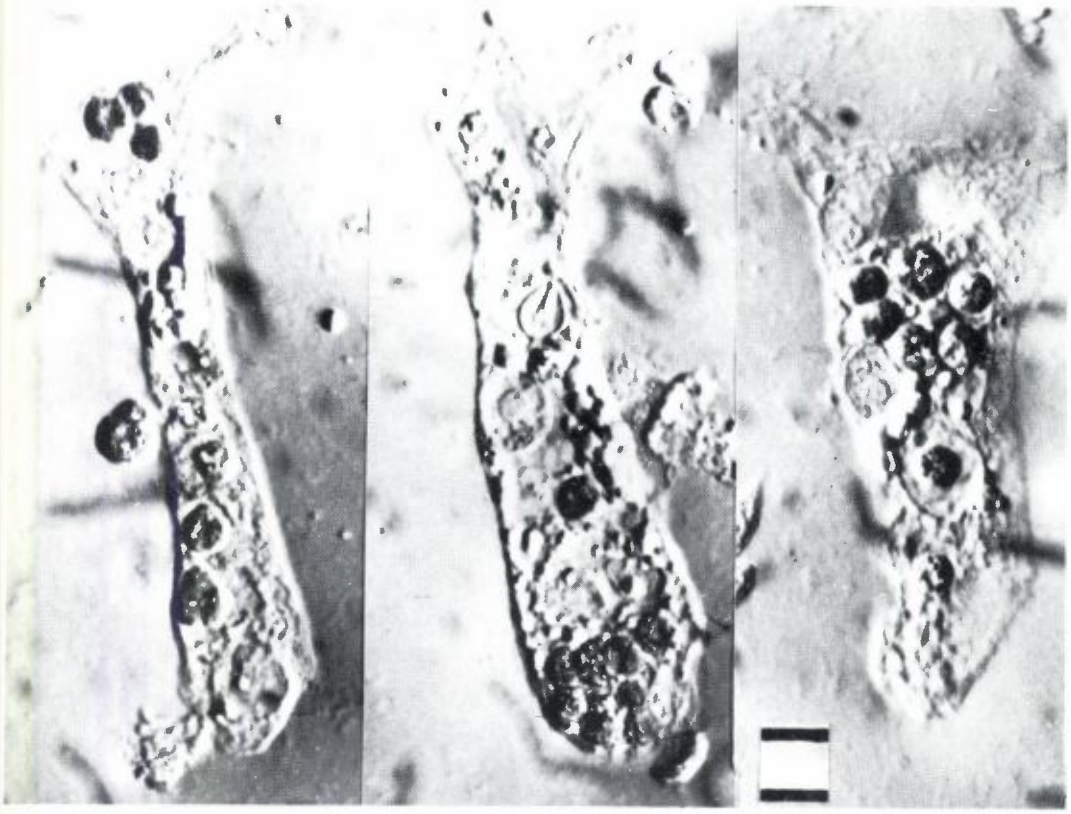

Figure 2. *Chlorococcum*-like algae in the cells of macerated *H. magnipapillata*. Scale, 10μm between bars.

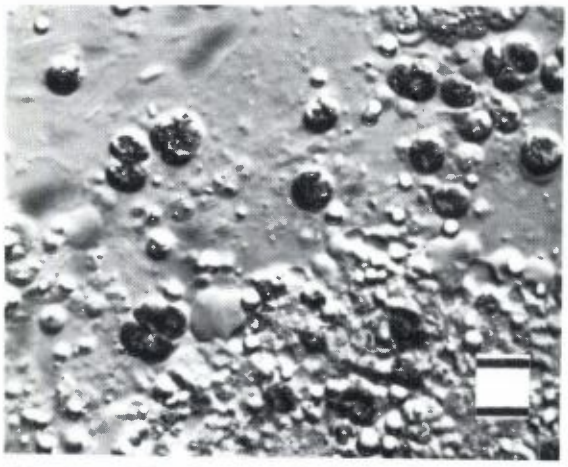

Figure 3. *Chlorococcum*-like algae squeezed from *H. magnipapillata*. Note two and four-cell stages of dividing algae. Scale, 10μm between bars.

to have enabled many parallel evolutionary trends. *H. magnipapillata* originates from Japan, has not been described from Europe or North America, and differs from the "western" related nonsymbiotic species by hosting endosymbiotic algae. Moreover, these algae are of a genus not found in the green "western" hydra. We may thus assume that "western" hydra have not been subjected to infections with the endosymbiotic algae of the Japanese hydra. The Japanese *Chlorococcum*-like algae might thus be able to form symbioses with some of the brown "western" hydra that are claimed to be nonsymbiotic. Similarly, the Japanese hydra might be able to host the 'western' hydra-infecting chlorellae. Results of experiments examining these questions will be reported separately.

In nature, hydra feed on invertebrate prey such as small crustaceans filter-feeding on unicellular algae. These crustaceans serve as a vector that brings about the infection of hydra with various unicellular algae (Rahat and Reich, 1984). We have claimed before that the algal/hydra endosymbioses are the result of chance encounters of ingested algae preadapted to live in an organic-nutrient enriched environment prevailing in the host cells, in preadapted hydra that apparently cannot rid themselves of the infecting algae (Rahat and Reich, 1985). The ensuing infections are then subjected to host-symbiont coevolution that results in the present day symbioses (Rahat, 1985a,b). The algal/hydra symbiosis described here, comprising two "new" cosymbionts corroborates our hypothesis. We further suggest that like this new symbiosis, other "new" algal/hydra symbioses might be found that have not been described before.

REFERENCES

Campbell, R.D. 1983. Identifying hydra species. In: *Hydra: Research Methods.* H.M. Lenhoff, ed. Plenum Press, New York, NY, pp. 19–28.

David, C.N. 1973. A quantitative method for the maceration of hydra tissue. *Wilhelm Roux Arch. Entw. Mech. Org.* **171:** 259–268.

Fott, B. and Novakova, M. 1969. A monograph of of the genus *Chlorella* the fresh water species. In: *Studies in Phycology.* B. Fott, ed. Sweizerbartsche Buchverhandlung and Czechoslovak Acad. Sci., Stuttgart, pp. 10–74.

Hohman, T.C., McNeil, P.L., and Muscatine, L. 1982. Phagosome-lysosome fusion inhibited by algal symbiont of *Hydra viridis. J. Cell Biol.* **94:** 56–63.

Jolley, E. and Smith, D. C. 1980. The green hydra symbiosis. II. The biology of the establishment of the association. *Proc. R. Soc. London. B.* **207:** 311–333.

Lenhoff, H.M. and Brown, R.D. 1970. Mass culture of hydra: an improved method and its application to other aquatic invertebrates. *Lab. Anim.* **4:** 139–154.

McAuley, P.J. and Smith, D.C. 1982. The green hydra symbiosis. VII. Conservation of the cell habitat by the symbiotic algae. *Proc. R. Soc. Lond. B.* **216:** 415–426.

McNeil, P.L., Hohman, T., and Muscatine, L. 1981. Mechanisms of nutritive endocytosis. II. The effect of charged agents on phagocytic recognition of digestive cells. *J. Cell. Sci.* **52:** 243–269.

Meints, R.H. and Pardy, R.L. 1980. Quantitative demonstration of cell surface involvement in a plant-animal symbiosis: Lectin inhibition of reassociation. *J. Cell Sci.* **43:** 239–251.

Muscatine, L., Cook, C.B., Pardy, R.L., and Pool, R.R. 1975. Uptake recognition and maintenance of symbiotic chlorellae by *Hydra viridis*. *Symp. Soc. exp. Biol.* **29:** 175–203.

Park, H.D., Greenblatt, C.L., Mattern, C.F.T., and Merril, C.R. 1967. Some relationships between *Chlorohydra*, its endosymbionts and some other chlorophyllous forms. *J. Exp. Zool.* **164:** 141–162.

Pool, R.R. 1979. The role of antigenic determinants in the recognition of potential symbionts by cells of *Chlorohydra*. *J. Cell Sci.* **35:** 367–379.

Rahat, M. and Reich, V. 1983. Visual monitoring of pH with phenol-red. In: *Hydra*: Research Methods. H.M. Lenhoff, ed. Plenum Press, New York, NY, pp. 35–37.

Rahat, M. and Reich, V. 1984. Intracellular infection of aposymbiotic *Hydra viridis* by a free-living *Chlorella* sp.: Initiation of a stable symbiosis. *J. Cell Sci.* **65:** 265–277.

Rahat, M. and Reich, V. 1985. Correlations between characteristics of some free-living *Chlorella* sp. and their ability to form stable symbioses with *Hydra viridis*. *J. Cell Sci.* **74:** 257–266.

Rahat, M. 1985a. Competition between chlorellae in chimeric infections of *Hydra viridis*: the evolution of a stable symbiosis. *J. Cell Sci.* (in press).

Rahat, M. 1985b. Origin and evolution of the green hydra symbiosis. *Arch. Sci. Geneve* (in press).

Starr, R.C. 1955. A comparative study of *Chlorococcum meneghini* and other spherical zoosphore producing genera of the *Chlorococcum*. *Indiana Univ. Pub. Sci. Ser.* **20**.

Sugiyama, T. and Fujisawa, T. 1977. Genetic analysis of developmental mechanisms in hydra I. Sexual reproduction of *Hydra magnipapillata* and isolation of mutants. *Develop. Growth and Differ.* **19:** 187–200.

Sugiyama, T. 1983. Isolating hydra mutants by sexual inbreeding. In: *Hydra*: Research Methods. H.M. Lenhoff, ed. Plenum Press, New York, NY, pp. 211-221.