Symbiosis, 12 (1992) 75–81
Balaban, Philadelphia/Rehovot

Minireview

The Green Hydra Symbiosis: Most of the History is Recent

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Received April 10, 1991; Accepted July 16, 1991

> C'est la plus petite que j'ai connus la premiere. Les polypes de cette espece sont d'un assez beau verd. It was the smallest that I knew first. The polyps of this species are of a rather beautiful green.

Thus wrote Abraham Trembley in his *Memoires* (1744) describing what is probably the first published description of the green hydra. Trembley observed the hydra clinging to floating plants and his initial reaction was that the polyps, being green, were probably plant parasites. Lyonet's illustration in the *Memoires* (Fig. 1), shows the animals attached to a rootlet of a duckweed plant. Despite the fact that Trembley observed the hydra to move and their tentacles to contract, he admitted that their plant-like shape and green color kept him from fully accepting his growing suspicion that they were animals. He thought that they might be some sort of sensitive plant. Painstaking observations finally convinced Trembley that indeed, the green polyps were animals.

The green hydra is a remarkable animal to study. Not only does it possess all the typical hydra features making this hydrozoan such a superb experimental organism, it also harbors green algae as endocellular symbionts. This combination, the green hydra symbiosis, is the model *sine qua non* for experimental studies of animal-algal symbiosis. While Trembley described the green

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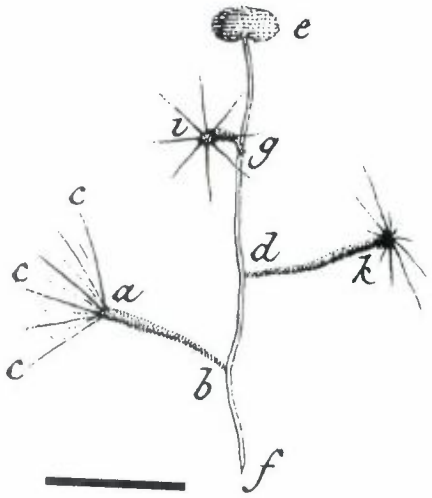

Figure 1. Reproduction of Lyonet's drawing of green hydra attached to the rootlet of *Lemna*. The drawing appears on Plate 1 of Trembley's *Memoirs*. Bar = 1 centimeter.

hydra, he did not probe the nature of the green color. Paradoxically, having decided that the little polyps were animals, Trembly never appreciated that the little green ones were in fact, almost half plant! We now know that the algal endosymbionts are photosynthetically active and release large quantities of carbohydrates (mainly maltose) to the surrounding host tissue. We suspect that the reproduction of the algae is regulated in some manner so that the size of the standing population of symbionts remains relatively constant. Contemporary experiments suggest the possibility of a recognition system that prevents the host from indiscriminately associating with non-symbiotic algal strains.

Our purpose here is not to present a comprehensive review of the status of green hydra research, but rather to present a historical perspective on how we come to know what we do about this symbiosis. We assessed the literature for the development and extent of symbiosis research on the green hydra since Trembley's first report. We were struck by the long lag between Trembley's first description and the first attempts to describe the symbiosis. Moreover, our simple analysis showed us that there were two major periods of relatively intense research on the topic: we are currently in the second of those periods.

We surveyed the literature from 1744 to the present and noted publications dealing specifically with the symbiosis or some aspect of the symbiosis. Figure 2 is a histogram showing the number of publications (book chapters, abstracts,

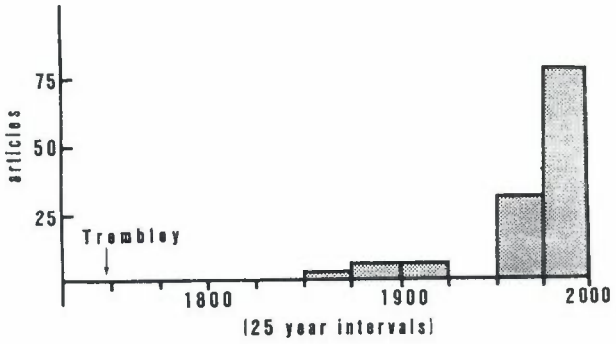

Figure 2. Distribution of published articles dealing with hydra-algae symbiosis in 25 year intervals from 1700 to the present. Arrow indicates the publication of Trembley's *Memoirs*.

journal articles) appearing in 25 year intervals from 1744 to the present. The histogram shows that published reports concerned with the nature of the green hydra did not appear until the late 1800s. These dealt mainly with the identity of the elements giving rise to the green color and were set in the context of an ongoing argument concerning the existence or non-existence of animal chlorophyll. Ultimately the color was shown to be due to the presence of algae in the animal's tissues (Brandt, 1882). While these early studies were not focused on symbiosis biology, they were important because they established the organismic (plant) basis of the green hydra's color. This finding provided the necessary foundation for later studies dealing with the biology of the hydra-algae association.

The 1900's opened with a pair of fascinating studies by Whitney (1907a,b) describing the technical and cellular aspects of the glycerine induced elimination of symbiotic algae from green hydra. This work was a landmark in that it appears to be the first study dealing with the symbiotic relationship. Two important and enduring aspects of Whitney's work are the development of a technique for producing aposymbiotic (algae-free) animals from green hydra, and the demonstration that aposymbiotic hydra survive without their algal symbionts providing the animals are fed regularly. Experimentally produced aposymbiotic hydra were thus destined to be the controls for virtually all experiments concerning the biology of the symbiosis. The importance of Whitney's pioneering observations and technique cannot be overstated.

In the short span 1924–26, Goetsch (1924, 1926) and Haffner (1925) published observations based on a comprehensive series of experiments dealing with the green hydra symbiosis. These scientists performed prototype experiments that were to be repeated with greater sophistication decades later

yielding essentially the same results. For example, Goetsch (1924) performed re-infection experiments using a variety of potential symbionts. Not only was he able to cause successful re-establishment of the symbiosis, he also showed that native symbionts would replace symbionts cultured *in vitro* and was the first to argue that the algae were related to *Chlorella vulgaris*, a common, ubiquitous chlorophyte.

Following Goetsch and Haffner there was an apparent hiatus in hydra symbiosis research that lasted over 20 years (Fig. 2). For some reason, interest in the symbiosis waned. Perhaps biologists considered the subject closed, if they considered it at all. However, in the late 1950's there began a relative explosion of hydra symbiosis research as shown in Fig. 3. From our survey of

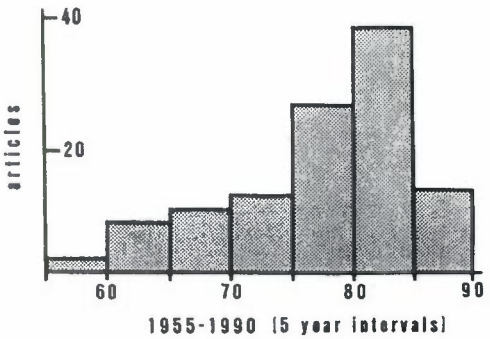

Figure 3. Distribution of published articles dealing with hydra-algae symbiosis in 5 year intervals from 1955 to the present

the literature we have identified at least 130 abstracts, journal articles, and book chapters appearing from 1959 to the end of 1988. What rekindled this interest in hydra symbiosis research? What has sustained it?

The ignition of research fervor seems to have begun with the publication of an abstract by Lenhoff and Zimmerman in 1959 concerning the uptake of carbon-14 by symbiotic algae in green hydra. In their experiments Lenhoff and Zimmerman incubated green hydra in a medium containing $^{14}CO_2$. Following this incubation they separated the animal's ectodermal and endodermal layers. Each layer was then analyzed separately for the presence of radioactivity. They found that while the endoderm had considerable radioactivity (this is where the symbionts reside) the ectoderm was also labeled, suggesting a movement of photosynthetically fixed ^{14}C from symbiont to endoderm to ectoderm.

The importance of the Lenhoff-Zimmerman experiments is threefold. First, the experiments pointed to the utility of using $^{14}CO_2$ in algal symbiosis studies. Use of this tracer has been crucial to describing the nature and extent

of the symbionts' photosynthetic contribution to the association. Secondly, these experiments demonstrated, for the very first time, a metabolic interconnectin between host and algal symbiont. The nature and extent of this interconnection remains a major research focus of scientists studying hydra symbiosis. Thirdly, the published abstract describing the experiments caught the attention of a young symbiosis worker, Leonard Muscatine. Subsequent to graduating from Berkeley, Muscatine went as a postdoctoral student to study green hydra symbiosis with Lenhoff in Miami. This collaboration was to set the course of green hydra symbiosis research for the next three decades. Virtually every contemporary hydra symbiosis worker has been a graduate student, postdoctoral student, or collaborator associated with either Lenhoff or Muscatine. Those few researchers who have not interacted directly with either Lenhoff or Muscatine, invariably have been associated with someone who has.

If the Lenhoff-Zimmerman (1959) abstract marks the beginning of the modern era of hydra symbiosis research, what are the factors responsible for promoting and sustaining research interest for the last three decades? It is our contention that expansion of research in the field resulted from the sequential development of a few key techniques and the phenomena revealed by the techniques.

From the contemporary literature (1959–1988) we have identified five techniques which seem to have played a significant role in the progress of hydra-algae symbiosis research. These are: the experimental production of aposymbiotic hydra; the production of large numbers of green and aposymbiotic hydra through mass culturing; the experimental separation of the symbiotic partners; the experimental re-association of the symbiosis; the dissociation of intact cells from hydra tissues.

Production of algae-free hydra: The production of aposymbiotic hydra by treating green hydra with glycerine (Whitney, 1907) has already been mentioned. Rediscovery of this technique can be credited to Muscatine and Lenhoff (1965). At least 13% of the published work concerns analyses involving aposymbiotic hydra as either experimental organisms or as controls.

Mass culturing of green hydra: While procedures for mass culturing non-symbiotic hydra were developed by Loomis (1953) for non-symbiotic hydra, Muscatine and Lenhoff (1965) formulated a culture medium (M solution) and regimen specific for green hydra. Virtually every published work concerning experiments on green hydra was performed on animals cultured according to Muscatine and Lenhoff (1965).

Physical separation of the symbiotic partners: Muscatine (1965) described the preparation of intact, viable algal symbionts from homogenates of whole

green hydra. Centrifugation of these homogenates yielded an animal fraction which could be analyzed biochemically and a pellet of living algal symbionts that could be used in a wide variety of *in vitro*, biochemical, physiological, and cell biological studies. About 13% of contemporary research utilized this technique either to harvest living symbionts or to prepare algal-free suspensions of animal tissue. With this technique Muscatine (1965) showed that green hydra symbionts translocate the disaccharide maltose. Approximately 10% of the contemporary published research involved some aspect of the translocation phenomenon.

Resynthesis of the symbiosis: Pardy and Muscatine (1973) described a technique for the controlled re-introduction of algal symbionts into aposymbiotic green hydra. By microinjecting dense slurries of algae previously harvested from green hydra directly into the coelenteron of recipient hydra, these workers were able to bring about a resynthesis of the symbiosis. The technique and resultant description of how the symbiosis is re-established opened up another research avenue. About 8% of the contemporary work has utilized the injection technique.

Preparation of isolated cells from hydra tissue: If green hydra are placed in a maceration fluid developed by David (1973) for the study of non-symbiotic hydra and agitated, the animals "dissolve" into their individual cells. Suspensions of cells prepared in this manner are examined with light microscopy. Under the microscope the symbiont bearing cells are readily identified and the number of algae in a given cell is easily determined. This important technique has been adapted by symbiosis workers for analyzing and evaluating a range of experiments dealing with the endocellular disposition of the symbionts. The power of this elegant technique cannot be overstated. Over 15% of the contemporary published studies utilized this technique. Used in conjunction with the microinjection of algae, the maceration technique led directly to the "recognition paradigm" of symbiotic resynthesis. Approximately 10% of the contemporary studies have dealt with this aspect of the symbiosis.

Each of the above techniques was reported in the context of experimentation analyzing some biological aspect of the symbiosis. The synergism between the specialized technical methods and the experimental data (discoveries) was significant in driving the field and keeping up the interest of the researchers. The discoveries led to more questions and provided a growing conceptual basis for further research; the techniques provided workers the means for continued exploration and analyses, but it was the fruitful Lenhoff-Muscatine collaboration that rekindled interest in the green hydra symbiosis. This collaboration provided the foundation for the current period of hydra symbiosis research

to which virtually all contemporary hydra symbiosis investigators (numbering about 34) can trace their roots.

Acknowledgements

The authors are grateful to Clare Royce for her critical reading of the manuscript and helpful suggestions. Part of this work was supported by funds from the Research Council of the University of Nebraska-Lincoln.

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