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# ORIGIN AND EVOLUTION OF SYMBIOSIS IN GREEN HYDRA

BY

### M. RAHAT 1

# INTRODUCTION

When Abraham Trembley (1744) first described the green "insect" he found on a fresh-water plant, he was not sure whether he had in his hands a plant or an animal. Trembley proved that it was an animal, but never knew that the color of the hydra he discovered was green because it contained symbiotic algae within its cells. This hydra currently is referred to by the name of *Hydra viridis*, given it by Linnaeus (1767). Campbell (1983), however, points out that *Hydra viridissima* is the more correct name according to the International Code of Zoological Nomenclature. It was through the studies of Siebold in 1849, Schultze in 1851 and Brandt in 1883 (see Kanaev, 1952), that we learned about the symbiotic nature of the green hydra, where endodermal cells of the animal host unicellular algae of the genus *Chlorella*.

In 1907, Whitney showed that the algae can be "artificially removed" from the green hydra, and the aposymbiotic "white hydra" can be cultured without their symbionts. Though this work of Whitney provided an excellent tool to study separately the biology of one cosymbiont, it was utilized for this purpose only many years later.

The renaissance of research on the biology of the green hydra symbiosis began after Loomis and Lenhoff (1956) developed a simple method to culture hydra en masse in the laboratory. Later, Muscatine and Lenhoff (1965) showed that symbiotic hydra resist starvation better than aposymbiotic polyps, and concluded that algal metabolites augment the growth of the green hydra. These results were confirmed later (Kelty & Cook, 1976; Rahat and Reich, 1980), and we know now that among other mutual effects there is also a continuous transfer of nutrients between the hydra and its symbiotic algae (Thorington and Margulis, 1981).

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### SPECIFICITY OF ALGAL SYMBIOSES WITH H. VIRIDIS

Early attempts to infect aposymbiotic *H. viridis* with nonsymbiotic free living chlorellae failed (Park et al., 1967), but aposymbiotic polyps could be reinfected with chlorellae isolated from *H. viridis* or from the ciliate *Paramecium bursaria* (Pardy and Muscatine, 1973; Muscatine et al., 1975; Jolley and Smith, 1980). At the same time it has been shown that brown hydra do not engulf algae.

A question raised about the above findings was: what are the factors behind the specificity of the former and the "refusal" of the latter?

We have yet no information as to the factors that prevent uptake of chlorellae by brown hydra, but many studies have been done on the specificity of chlorellae uptake by *H. viridis*.

The acquisition of algae by hydra has been described to involve several "phases". These are: contact between the algae in the coelenteron of the hydra and the surface of the digestive cells, "recognition" of the algae by the latter, phagocytosis, and migration of the engulfed chlorellae from the apex of the digestive cells toward its base, where presumably no digestive processes take place (Pardy and Muscatine, 1973; McAuley and Smith, 1982a).

This ability of some chlorellae to be taken up by hydra, to be recognized and sorted out to become stable endosymbionts, has been attributed to several factors. Among the latter are the release of maltose by the chlorellae (Cernichiary et al., 1969; Mews and Smith, 1982), the possession of specific antigenic determinants (Pool, 1979; Meints and Pardy, 1980), having suitable cell-surface charges (McNeil et al., 1981) and the ability to prevent phagosome-lysosome fusion in the digestive cells that would bring about their digestion (O'Brien, 1982; Hohman et al., 1982).

The above characteristics were claimed to be missing in free-living chlorellae, presumably unable therefore to form stable symbioses with *H. viridis*.

# COLONIZATION OF HYDRA CELLS BY FREE-LIVING CHLORELLAE

It was Smith (1980) who suggested "symbiosis is essentially an ecological concept... it is crucial that algal symbionts should be able to survive on the nutrients available to them within their host".

This was a new approach to the study of symbiosis. The question asked before, "How do hydra recognize and select their algal symbionts?" can now be asked in another way. "What preadaptations are required of *Chlorella* and *Hydra* that enable the one to colonize the endocellular vacuoles of the other?"

How does one study such a question?

As it happens in science, an incident in the laboratory supplied the tools to investigate this problem.

For many years now we have been culturing in our laboratory a Swiss strain of symbiotic *H. viridis* (Ssh) and an aposymbiotic strain (Sah) derived from it (Rahat et al., 1979).

In 1982 we were surprised to find that some of the supposed aposymbiotic hydra had green patches. Microscopical examination showed us that these hydra were infected with a green algae identified to be a *Chlorella* sp. (Fott and Novakova, 1969). This chlorella appeared to be different in its pattern of infection from that of the native symbionts (Figures 1 and 2). We isolated the infected hydra and cultured them separately.

One distinct characteristic of this "new" symbiosis was that the algae (coined by us Fs—Foreign symbionts vs. Ns—Native symbionts) reproduced at such a rate inside the cells of hydra that these cells, hosting sometimes more than 100 chlorellae (Figure 2), expelled the surplus algae into the coelenteron of the hydra (Figure 3). The algae were then eventually evacuated into the culture dish, covering its bottom with a green layer.

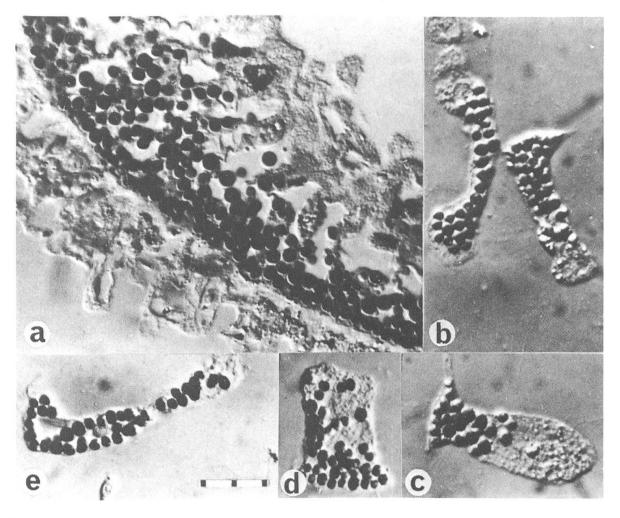


Fig. 1. — Photomicrographs of: a. Thick mid-section of *H. viridis* showing regular distribution of native zoochlorellae in endoderm; b-e. Native zoochlorellae in endodermal cells of macerated hydra.

Scale: 10 μm between bars.

This green layer suggested that these algae might be cultured *in vitro*, providing us with an eventual tool to study them both *in vivo* in the cells of hydra and *in vitro* in nutrient media. As the Ns cannot be cultured *in vitro*, such cultures would be a breakthrough in the study of the hydra cosymbionts.

We had first to comply with Koch's postulates, and verify the identity of the infecting chlorellae with those growing *in vitro*. We prepared bacteria-free Fs-infected hydra (Rahat and Reich, 1983) and isolated Fs from them into nutrient media. The Fs were subcultured several times and then reinfected into aposymbiotic hydra. All subsequent experiments were done with progeny of these hydra (Rahat and Reich, 1984).

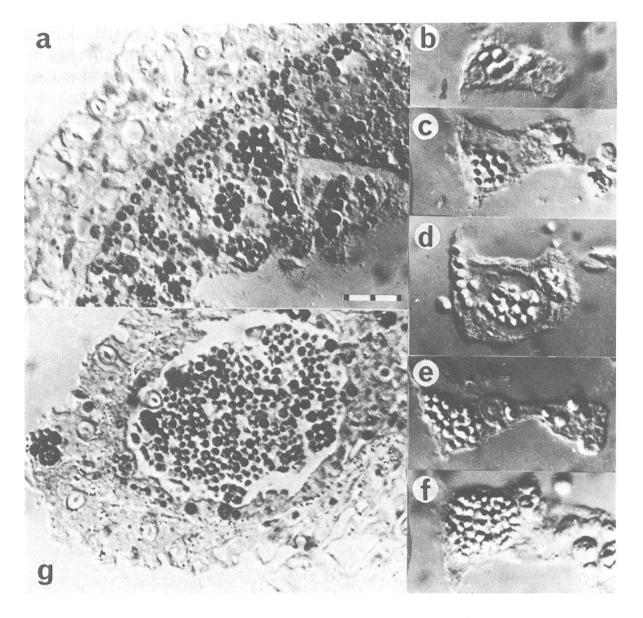


Fig. 2. — Photomicrographs of: a. Thick mid-section of *H. viridis* infected with Fs. Note irregular distribution of algae in endoderm. b-f. Numerous Fs in vacuoles of endodermal cells from macerated hydra. g. Thick oblique section through tentacle showing Fs in ectoderm and coelentric space. Scale: 10 µm between bars.

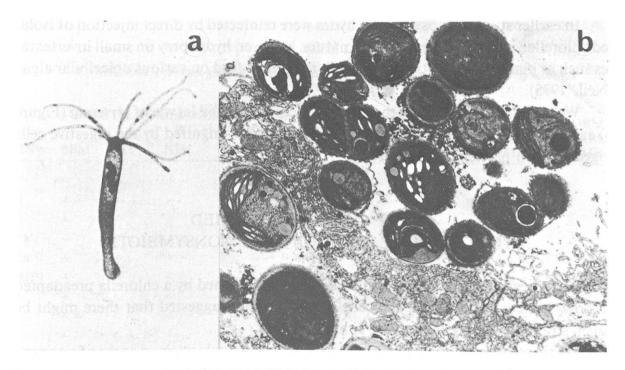


Fig. 3. — Photomicrograph of whole *H. viridis* infected with Fs. Note a. Clusters of algae in coelenteron of stem and tentacles b. Electron micrograph of Fs in endoderm and coelenteron of Fs infected *H. viridis*.

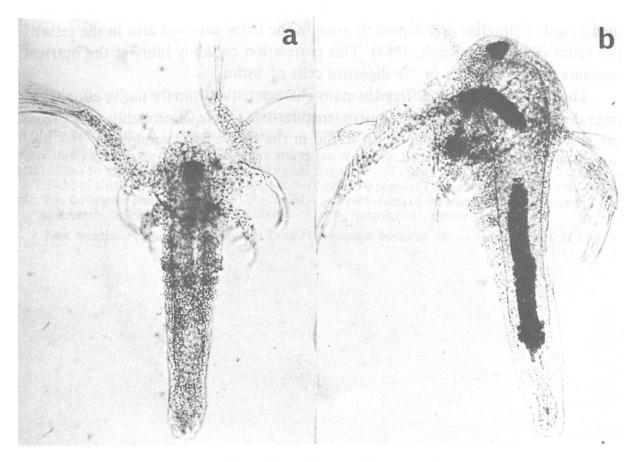


Fig. 4. — Larvae of Artemia sp. (a), containing chlorellae in its gut (b).

In earlier studies, aposymbiotic hydra were reinfected by direct injection of isolated chlorellae into the coelenteron. In nature, however, hydra prey on small invertebrates such as planktonic crustaceans, that in turn filter-feed on various unicellular algae (Neil, 1975).

We preferred to imitate nature and used chlorellae-fed larvae of *Artemia* (Figure 4) as a vector to infect our hydra. The algae were thus engulfed by the digestive cells together with particles of the prey.

# "NEW" SYMBIOSES FORMED WITH CHLORELLAE ORIGINALLY NONSYMBIOTIC

The "new" Fs/hydra symbiosis was obviously formed by a chlorella preadapted to grow both *in vitro* and in the cells of hydra. This suggested that there might be other strains of chlorellae having similar traits.

We therefore examined 16 more strains, some of symbiotic and some of nonsymbiotic origin (Table 1). Of these, six formed stable symbioses with hydra, one formed a transient infection that lasted several weeks only and nine strains were cleared out of the hydra within one-two days. We found a clear-cut correlation between the ability of the chlorellae to form stable symbioses and their ability to grow in nutrient-enriched media; only chlorellae preadapted to grow in the latter survived also in the cells of the hydra (Rahat and Reich, 1985). This correlation certainly hints at the nutrient contents of the vacuoles in the digestive cells of hydra.

The "new" symbioses differed in many characteristics from the native established one; their qualitative and quantitative irregularities (Table 2) probably result from the absence of a long coadaptation found in the native Ssh symbiosis.

Table 1.

Correlations between growth of chlorellae in vitro and their formation of symbioses with Hydra viridis

GROWTH OF CHLORELLAE IN BOLDS BASAL MEDIUM (BBM) WITH VARIOUS ADDITIONS (b)							FORM STABLE			
STRAIN(a)	BBM	VIT	NH <sub>4</sub>	UREA	G	P	PLY	GPLY		ALGAE(c)
211/6	_	_	<u> </u>	<u> </u>	<u> </u>	<u>, , , , </u>	<u> </u>	~ ~ ~	<u> </u>	Pb
211/7a		<u> </u>			_	<u> </u>	<u> </u>	<u> </u>	<u> </u>	NS
211/8p	<u></u>	<u></u>	<u></u>	<u></u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>		NS
211/11a	_	-			-	<u> </u>	<u> </u>	<u> </u>	<u></u>	NS
211/11n	_	<u></u>		<u>'</u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u></u>	NS
NC64A(P)		<u> </u>			7 7 7 7	<u> </u>	<u> </u>	<u> </u>		PB
Fs	_		_	_	-	<u> </u>	<u> </u>	<u> </u>	<u> </u>	NS
211/8b		1 1		<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	NS
211/1e		<u> </u>		<u> </u>	<u> </u>	_	_	_	_	NS
211/8k	<u>/</u>			<u>'</u>		_	_	_	_	NS
211/9a			<u></u>	<u> </u>	<u> </u>	_	_	_	-	NS
211/11c			<u> </u>	<u> </u>	<u> </u>	-	-	_	_	NS
211/21	<u> </u>	<u></u>		<u> </u>	<u> </u>	_	_	_	1 3	NS
NC64A(M)		<u> </u>	<u> </u>	<u> </u>	<u> </u>	_	_	_	_	Pb
Utex		<u> </u>		<u> </u>	<u> </u>	_	_	_	_	NS
CE/76	_			<u> </u>	<u> </u>	_	_	_	_	Hv
2		<u> </u>	<u></u>	<u> </u>	<u> </u>	<u> </u>	·	_	_	PB

a. All strains designated 211/ are described in the List of Strains, Culture Centre of Algae and Protozoa, 1976, Cambridge. Origin of the other strains is shown in Rahat & Reich, 1985.

b. Abbreviations used for the different media and estimation of growth: BBM – Bolds Basal Medium (Bischoff & Bold, 1963). Additions to BBM: Vit – 1  $\mu$ g/ml and 10  $\mu$ g/ml respectively of vitamins B<sub>1</sub> and B<sub>12</sub>; NH<sub>4</sub> – 0.01 M of NH<sub>4</sub>Cl; Urea – 0.01 M of Urea; G – 0.5% glucose; P – 0.5% of proteose peptone (Difco No. 3); L – 0.01% of Liver infusion (Oxoid); Y – 0.1% yeast extract (NBC); GPLY – BBM  $\neq$  a combination of the above. Estimated growth: –, no growth;  $\neq$  –, growth barely visible;  $\neq$  to  $\neq$   $\neq$   $\neq$   $\neq$  , degrees of color intensity of cultures in test tubes.

<sup>&</sup>lt;sup>c.</sup> NS - Non symbiotic origin. Pb - Isolated from Paramecium bursaria. Hv — Isolated from H. viridis

#### TABLE 2.

Properties of native and "new" symbioses in H. viridis

Properties common to native and "new" symbioses
Algae persistant in polyps
Algae dividing inside host cells

Properties of native symbioses absent from "new" ones	Correlated properties of "New" Symbioses
Algae growing in host cells only	Algae growing also in nutrient media
Uniform distribution of algae in appropriate cells and tissue	Irregular distribution and number of algae in host cells and tissue
All digestive cells occupied by algal endosymbionts	Many digestive cells free of chlorellae
Algae residing in individual vacuoles empty of electron-dense substances	Single or clusters of algae in vacuoles with electron-dense substances
Regulation of algal reproduction by host	Uncontrolled algal reproduction, excess algae expelled into coelenteron
Algae not digested by host	Some algae digested by host
Complete greening of hydra	Irregular green patches in hydra
Buds are completely green	Some buds just slightly infected
Symbiosis preserved in host and in population	Symbiosis might be lost in population
Algae enhance growth of host when underfed	Algae retard growth of host even when well fed

## ONTOGENESIS OF THE CHLORELLA/HYDRA SYMBIOSES

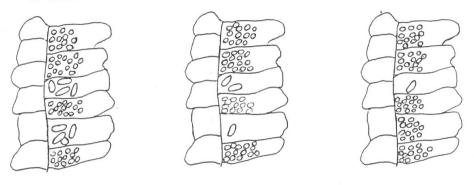
A distinction should be made between the establishment of a cellular stable infection, a stable infection in a polyp, and a stable symbiosis formed in a population that has been passed on to subsequent generations. Chlorellae infecting a hydra compete for their survival at these three levels (Figure 5).

(1) Intracellular competition of chlorellae: From our experiments (Rahat, 1985; Rahat and Reich, 1985), we learned that through an initial engulfment of different chlorellae hydra get infected with more than one strain of Chlorella. This brings about chimeric infections with hydra hosting more than one strain in their cells (Figure 6). Ensuing intracellular interalgal competition results in the survival of chlorellae more adapted to reproduce and live in the intracellular habitat (Table 3). These experiments show also that chlorellae already infecting a cell protect their territory against new invaders (see also McAuley and Smith, 1982b).

### 1 INTRACELLULAR COMPETITION BETWEEN CHLORELLAE



2 INTERCELLULAR COMPETITION BETWEEN THE INFECTED CELLS OF HYDRA



3 COMPETITION BETWEEN INFECTED POLYPS

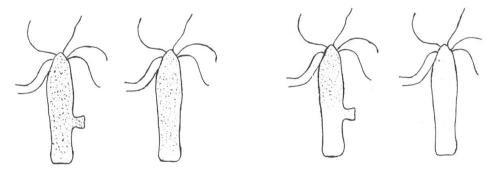


Fig. 5. — Three levels of competition in hydra, towards the establishment of a stable symbiosis.

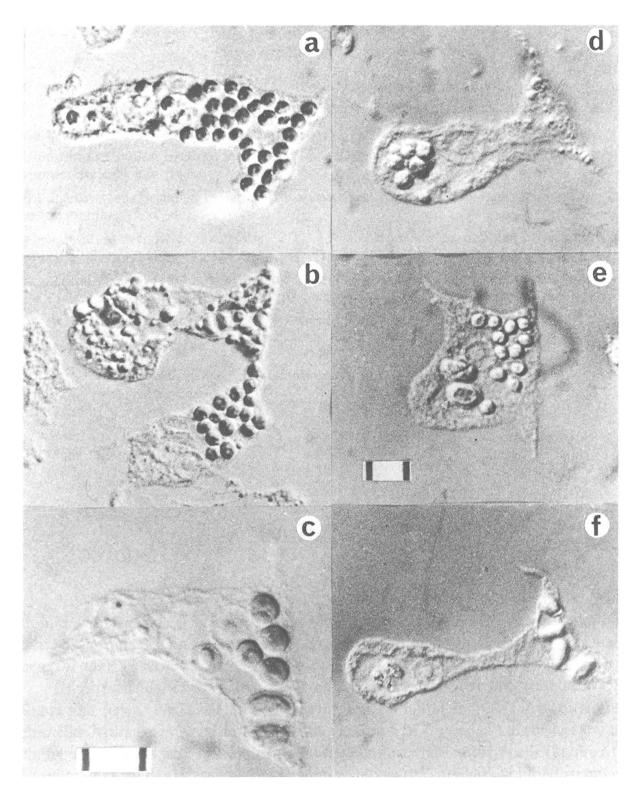


Fig. 6. — Cells of macerated H. viridis infected with one or two different strains of chlorellae. a. Cell with native symbionts. b. Two adjacent cells from the same hydra hosting different chlorellae. c. Two different chlorellae in the same cell. d. Cell hosting Fs in its apex. e. Two different chlorellae in the same cell. f. Cell hosting 211/8p chlorellae. Scale 10  $\mu$ m between bars. (Note different scale for c.).

Competition of chimeric symbioses in H. viridis TABLE 3.

Resulting symbioses 2-3 months after infection		Ssh	SFsh	Ssh	Ssh	$S211/8p\rangle\rangle\rangle Fsh(d)$	SFsh
Quantitative estimation of the different strains of chlorellae in cells of macerated hydra 2-3 weeks after infection(c)		$Ns\rangle\rangle\langle211/8p\rangle\rangle Ns & 211/8p$	Fs>>>211/8p	Ns only	Ns>>>211/8p>>Ns & 211/8p	211/8p>>>Fs>>211/8p & Fs	Fs>>>211/8p>>Fs & 211/8p
<i>a</i> sp. 211/8p	John	7	7	7			7
Strains of <i>Chlorella</i> sp. used for infection Ns Fs 211.		-1	7		7	7	
Digestive cells free of		all	all	none	some	some	some
Type of host hydra(a)		Sah	Sah	Ssh	S211/8ph	S211/8ph	SFsh
Exp.		1	2	3	4	5	9

Sah - Aposymbiotic hydra of the Swiss strain. Ssh - Swiss symbiotic hydra hosting the native symbionts, Ns. S211/8ph - Sah previously infected with 211/8p and hosting this strain only. SFsh - Sah previously infected with Fs and hosting this strain only. a)

In Sah all digestive cells are available for algal infections. In Ssh all digestive cells are occupied by the native Ns symbionts. In hydra hosting Fs or 211/8p, only some of the cells are infected, and many digestive cells are still available for new infections. (q

Ns>>>211/8p>>Ns & 211/8p - The majority of the cells contain Ns only, some contain 211/8p, and few contain chlorellae of both strains. G G

S211/8p>>>Fsh - Hydra hosting both 211/8p and Fs, the former being by far more abundant than the latter.

(2) Intercellular competition of hydra cells: Hydra are known to replace their cells every two-three weeks (Campbell, 1967), new cells being produced by cell proliferation in the polyp and old cells being shed off at the hydra's extremities.

In hydra infected with originally nonsymbiotic chlorellae not all cells host algae. Many cells are available for further infections. As a result of subsequent infections with different chlorellae, chimeric polyps would be obtained having dissimilar infected cells.

In chimeric infected polyps, an intercellular competition would thus decide which strains of chlorellae will finally remain as the sole symbiont in the polyp (Table 3). We can only guess that the effect of the algal symbiont on the reproduction rates of the respective host cells would affect such competition.

Thus, even chlorellae that survived the interalgal intracellular competition might finally be lost, and a less vigorous growing strain that enhances hydra-cell proliferation would remain in the polyp.

(3) Competition between polyps: As a result of the above described competitions, a population of hydra might comprise polyps which host respectively different strains of chlorellae, and another competition would ensue, involving both polyps and their respective symbionts.

The distribution pattern of various hydra-infecting chlorellae at or above the budding zone of the hydra are different (Figure 7). Some chlorellae are found all along the column of the polyp while others infect mainly the upper part above the budding zone. As chlorellae placed above the budding zone are not passed on to subsequent generations, hydra that are "green" above this zone only might form buds that are just slightly infected.

All hydra hosting "new" symbioses had a budding rate less than that of the Ssh hosting Ns chlorellae, and some had a budding rate less than the others. This inhibition of budding is apparently the result of an algal effect, details of which we do not know yet.

The distribution pattern of the infecting algae and their effect on the reproduction rates of the polyps are two factors that influence the final results deciding which polyps will survive in future generations with their respective "new" symbionts.

# ORIGIN AND EVOLUTION OF THE CHLORELLA/HYDRA SYMBIOSIS: A SCENARIO

On the basis of our data we may now try to reconstruct the formation and evolution of the present day stable green hydra symbiosis as it happened in nature.

Preying on filter feeding crustaceans, digestive cells of various hydra became infected with several species of free-living algae. Of the different species of hydra, some were preadapted to host chlorellae and some strains of chlorellae were preadap-

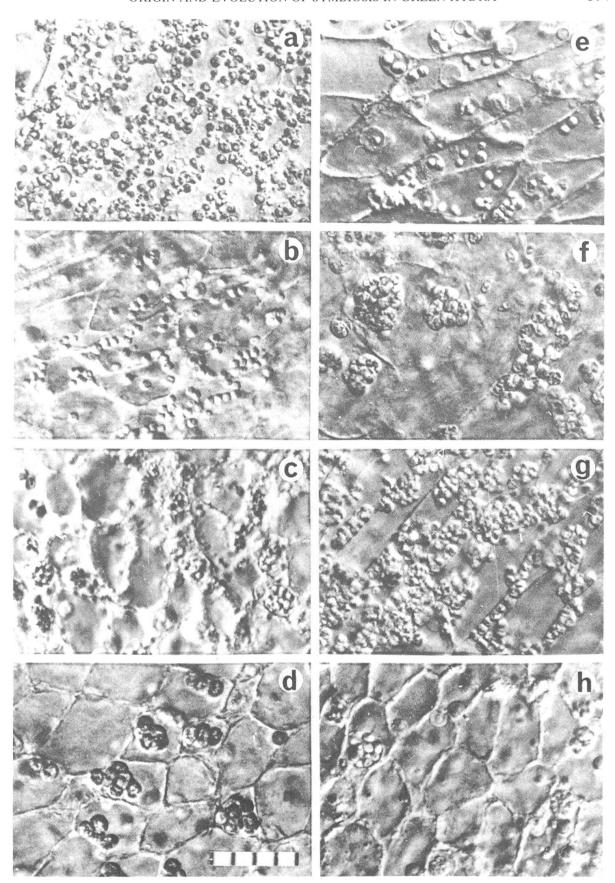


Fig. 7. — Chlorellae in digestive cells of the budding zone in live *H. viridis*. Photomicrograph of hydra flattened under a coverslip. Note the different number and diverse distribution pattern of the different chlorellae:

a. — Ns. b. —  $\frac{211}{6}$ . c —  $\frac{211}{7a}$ . d —  $\frac{211}{8p}$ . e. —  $\frac{211}{11a}$ . f. —  $\frac{211}{11n}$ . g. — NC64A(P). h. — FS.

ted to live in a nutrient rich environment. When the latter infected the former, prolonged infections ensued. Through intracellular interalgal competition in the digestive cells of hydra, competition between such dissimilar infected cells and competition between differently infected polyps, one strain of *Chlorella* survived with its host.

In time, through coevolution, the present day stable symbiosis was formed, which became obligatory for the *Chlorella*. As in the coadapted symbioses all cells available for colonization already host symbionts, further infections are constantly cleared out of the hydra.

It was this highly evolved green hydra symbiosis that Abraham Trembley encountered almost 250 years ago in the green polyps he found and studied.

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