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REGULATION OF INTRACELLULAR ALGAE BY VARIOUS STRAINS OF THE SYMBIOTIC *HYDRA VIRIDISSIMA*

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SUMMARY

In observations on three strains of green hydra, the host and the algal mitotic index is closely coordinated only for the smallest. As the hydra strain size increases the coordination of host and algal mitosis progressively breaks down, first in timing for a medium-sized strain and then in rate for a large strain. Despite disparities between host and algal mitotic index, the number of algae per host cell remains constant in all strains during the interval measured. To account for this constancy we suggest that the hydra may either prolong the duration of the algal tetraspore stage or cull excess algae.

INTRODUCTION

A primary requirement for stable endosymbiosis, such as that of *Hydra viridissima* and *Chlorella* spp., is that there be a mechanism providing balanced growth of the two symbionts. For any given set of conditions a hydra endodermal cell will harbour a remarkably constant number of algae (Pardy & Muscatine, 1973; Pardy, 1974; Muscatine & Pool, 1979; McAuley, 1980, 1981*a*; Muscatine & Neckelmann, 1981; McAuley & Smith, 1982*a*; Bossert & Slobodkin, 1983). This constancy may be due to the inhibition of algal growth in the host environment, since the free algae are reported to have a growth rate some 32 times that in the symbiotic environment (Jolley & Smith, 1978).

Alternatively, the stability of the association might be due to the capacity of green hydra to digest or to exocytose excess endosymbiotic algae. Green hydra do have the capacity to digest or exocytose and expel endosymbiotic algae, but these types of behaviour have been observed only under experimentally contrived conditions (Pardy, 1976; McAuley, 1981*a*; Muscatine & Neckelmann, 1981; Steele & Smith, 1981; Hohman *et al.* 1982; McAuley & Smith, 1982*b*; O'Brien, 1982; Neckelmann & Muscatine, 1983; McNeil & McAuley, 1984).

In the absence of evidence for digestion or expulsion as natural mechanisms of regulation and on the basis of a striking correspondence in the timing of mitotic activity between host and algae (McAuley, 1981*b*, 1982), the regulation of algal density in host tissue is believed to be achieved through inhibition of algal mitosis except during host cell division (Douglas & Smith, 1984).

Key words: *Hydra viridissima*, symbiosis, *Chlorella*.

This regulatory mechanism may not occur in all hydra strains to the same degree, however. A tight correlation between host and algal mitotic index has been observed only in the European strain (McAuley, 1982). Further, given that the size of a hydra is determined by the concentration of at least two hormones (Schaller *et al.* 1977), both of which have mitogenic effects (Schaller, 1976*a*,*b*; Bossert, unpublished data), it is likely that the relationship between host and algal mitosis is different in hydra strains of different size.

In green hydra any mechanism that regulates algal density by means of the inhibition of algal mitosis is further complicated by the fact that the *Chlorella* endosymbionts produce not two but four daughter cells with each division. Thus synchronous host and algal cell replication in hydra will result in a doubling of algal density with each round of host cell mitosis.

In the European strain of *H. viridissima*, McAuley (1982) obtained a mean mitotic index of approximately 1·3% for host endodermal cells and 1·1% for the algae for the 24-h period following feeding (means calculated from data estimated from graphs). Applying McAuley's data to the equation of McDuff & Chisholm (1982) and allowing for the production of tetraspores by algae, we find:

$$\text{for the algae: } \mu_t = 0{\cdot}0325/t_{d,\text{algae}}$$

and

$$\text{for the host: } \mu_t = 0{\cdot}0129/t_{d,\text{hydra}},$$

where μ_t is specific growth rate and t_d is the duration of the indexed mitotic state, a value that has not been determined for either symbiont.

If algal densities in host tissue remain constant during the experimental interval we must accept one of two conclusions. First, the recognizable tetraspore stage used by McAuley to index algal division may last some two and a half times as long as the condensed chromosome state used to index host division. Alternatively, if host and algal mitosis are of similar duration, algal growth may exceed host growth. In this alternative case, in order for algal density to remain constant during the experimental interval, some mechanism of eliminating excess algae must be acting.

Without data on numbers of algae per host cell we cannot be sure that the density of endosymbionts was not, in fact, changing during the period of McAuley's study. As a preliminary step to distinguishing between the two alternative interpretations of McAuley's data outlined above we monitored the density of algae in host tissue as well as the mitotic index of each partner during the experimental interval. As we suspected that the relationship between host and algal mitosis might be dependent on the size of the host strain, we included hydra strains of three distinct sizes.

MATERIALS AND METHODS

Hydra maintenance

Hydra used in these studies were maintained in a controlled temperature chamber at 20°C in constant light in M solution (Muscatine & Lenhoff, 1965*a*). We have provisionally designated all our green hydra, *H. viridissima*, giving strains a name based on their origin. Thus, Carolina strain was originally purchased from the Carolina Biological Supply Co., Frome strain was originally

collected by P. J. McAuley from the River Frome near Bristol in England, and Texas strain was collected by Bassett Maguire, Jr from the Colorado River in Austin, Texas. All strains had been cultured in our laboratory for at least one year prior to the onset of these experiments.

All stock hydra are fed to repletion every Monday, Wednesday and Friday with freshly hatched *Artemia salina* nauplii.

Controlled feeding

To establish 'steady state' hydra (Otto & Campbell, 1977) 20 healthy animals from each strain were selected and maintained, five animals to a bowl. Each animal of each strain was fed a single *Artemia* nauplius with a micropipet every Monday, Wednesday and Friday for 10 consecutive feedings. Animals that failed to ingest the prescribed amount were not included in the experiment.

At intervals (1·5, 3·0, 6·0, 12·0 and 24·0 h after the last feeding) individual animals from each strain were macerated on a slide (David, 1973).

Preparation of slides

Individual animals were macerated directly on uncoated glass slides by placing them in a drop of water/glycerol/glacial acetic acid (1:1:14, by vol.) (David, 1973). After several minutes, gently tapping or teasing with a fine needle caused the cells to separate.

Slides were air dried and stored at room temperature until stained with the DNA-specific dye 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) in order to visualize mitotic figures (Muscatine & Neckelmann, 1981). Immediately after staining, cells were examined using a Leitz epifluorescent microscope.

Determination of host and algal mitotic index

Host cell mitotic index is the percentage of host cells in mitosis. Normally, 400 algal-bearing host cells per slide, made from a single animal, are assayed for the presence of mitotic figures. On some slides fewer than 400 cells could be scored. For these, the percentage of cells in mitosis was based on a lower number of cells assayed (always more than 200 cells per slide).

Algal mitotic index is the percentage of algal tetraspores visible in a sample of 1000 algal cells from a single animal.

Determination of number of algae per host cell

For each slide prepared, the number of algae in 30 host cells was counted using phase-contrast optics at ×1000.

Determination of hydra size

Size of hydra was determined by measuring the maximum observed length of 10 individuals each bearing one newly formed bud with a Graf/Pen digitizing pen interfaced to an Olivetti P6060 microcomputer.

RESULTS

The results are presented in Figs 1–4 and summarized in Table 1.

In order to verify the size differences among the three hydra strains we measured the length of relaxed specimens of hydra bearing one bud (see Fig. 4). The Texas strain is significantly longer than the Carolina strain ($P < 0{\cdot}05$) and the Carolina strain is significantly longer than the Frome strain ($P < 0{\cdot}01$, according to a test for the equality of means with heteroscedastic samples; Sokal & Rohlf, 1981).

Host and algal mitotic index were approximately equal and synchronous in Frome, our smallest strain. In the intermediate-sized Carolina strain, however, the co-incident pattern of host and algal mitosis is lost. In the large Texas strain not only

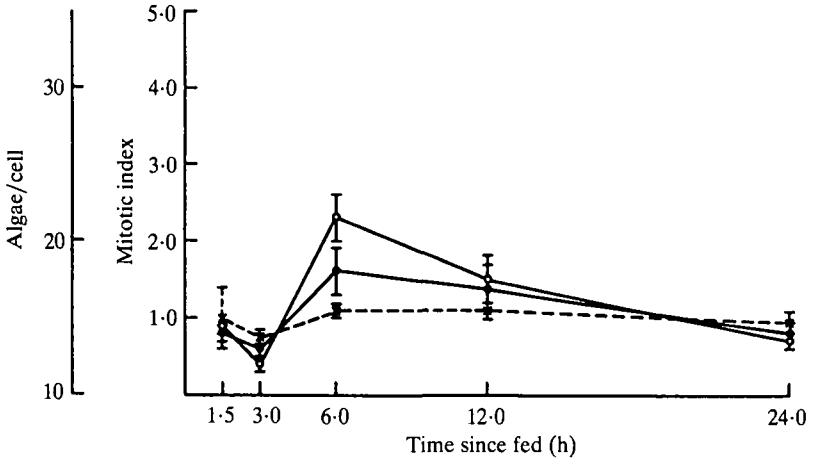

Fig. 1. Frome strain: mitotic indices of hydra gastric cells (●), algal cells (○), and number of algae per host cell (×) in the 24-h period after ingestion of a single *A. salina* nauplius.

does algal mitosis precede host mitosis but the average mitotic index of the algae also exceeds that of the host by a factor of three during the experimental interval.

It is noteworthy that the numbers of algae per host cell are fairly stable in all strains and seemingly independent of whether host or algal mitosis predominates during the preceding time period. The small strain, Frome, experiences no disruption in number of algae per cell, while the medium strain, Carolina, experiences a temporary increase that is corrected within 6 h. The largest strain, Texas, experiences a protracted increase in algae per cell that lasts between 12 and 24 h.

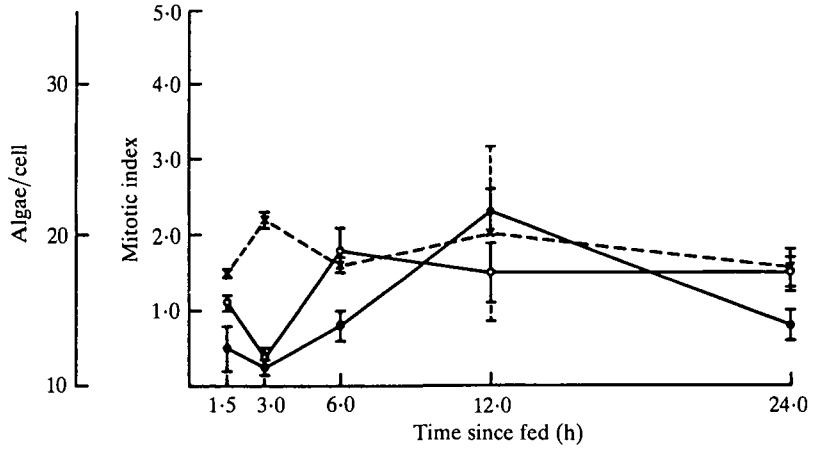

Fig. 2. Carolina strain: mitotic indices of hydra gastric cells (●), algal cells (○), and number of algae per host cell (×) in the 24-h period after ingestion of a single *A. salina* nauplius.

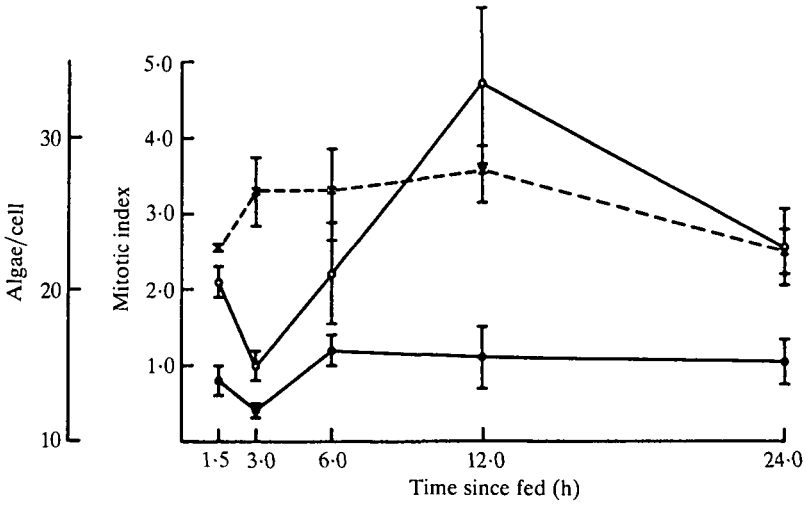

Fig. 3. Texas strain: mitotic indices of hydra gastric cells (●), algal cells (○), and number of algae per host cell (×) in the 24-h period after ingestion of a single *A. salina* nauplius.

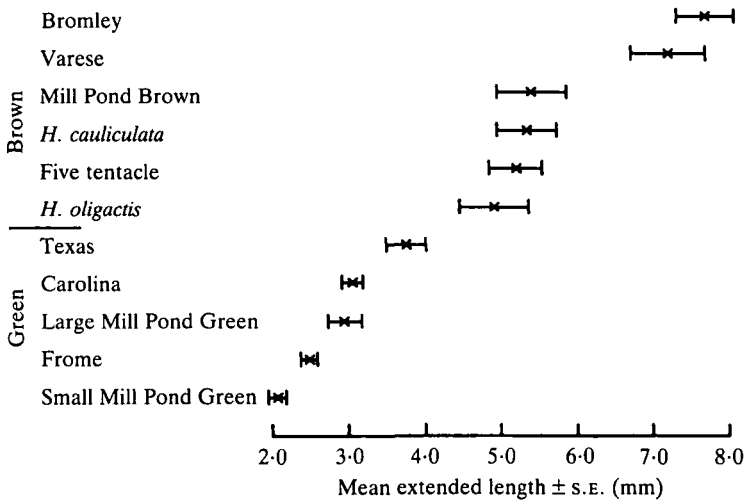

Fig. 4. Maximum extended lengths of the various strains of hydra maintained under identical conditions in our laboratory.

DISCUSSION

The benefit provided by the algal endosymbionts to the animal partner in the *H. viridissima* symbiosis has been repeatedly demonstrated. Green hydra survive starvation better than either aposymbiotic hydra (hydra cloned from individuals whose algae have been removed) or non-symbiotic hydra (Muscatine & Lenhoff, 1965*b*; Kelty & Cook, 1976; Rahat & Reich, 1980; Cook & Kelty, 1982). The algae have not been demonstrated to harm the animal in any measurable way in what might

Table 1. *Summary of results*

Strain	Mean mitotic index (%)		$t_{d,a}/t_{d,h}$*	Mean extended length (mm ± S.E.)
	Algae	Host		
Texas	3·10	0·96	9·32	3·75 ± 0·25
Carolina	1·44	1·34	3·17	3·06 ± 0·13
Frome	1·32	1·07	3·62	2·49 ± 0·11

* Assuming equivalent specific growth rates for host and algae.

be called normal circumstances, although the algae may be detrimental to the hydra during protracted darkness (Mews & Smith, 1982), in the presence of high concentrations of certain ions (Muscatine & Neckelmann, 1981) and during regeneration of hypostome and tentacles (Bossert & Slobodkin, 1983).

Although aposymbiotic specimens of normally green species of hydra have never been recorded in nature, a fact taken as evidence of the desirability of endosymbiotic algae in natural conditions (Smith *et al.* 1969), many species of hydra are non-symbiotic. This fact raises the question of why, if symbiosis is beneficial to hydra, are not all hydra symbiotic? We believe a clue to the answer to this question lies in our observation that brown hydra are, for any given maintenance regime, larger than green hydra (see Fig. 4). The question then becomes why are large hydra not symbiotic? Our results suggest that a developmental constraint may operate in hydra, which prevents the evolution of large symbiotic hydra.

We find that host and algal mitotic index is closely coordinated only for our smallest strain (Frome, Fig. 1). As the hydra strain size increases, the coordination of host and algal mitosis progressively breaks down: first, in timing, for a medium-sized strain (Carolina, Fig. 2); and then, in magnitude, for a large strain (Texas, Fig. 3). Only in the smallest strain, Frome, do we find the closely coordinated growth patterns reported by McAuley (1982) in the European strain, itself a small hydra (K. W. Dunn, personal observation). We also find that the disruption in numbers of algae per cell, absent in the smallest strain, increases with strain size.

These size-specific changes in mitotic coordination suggest that the morphogens thought to determine the size of a hydra may affect the mechanisms of algal density regulation. We suggest that the low ratio of head activator to head inhibitor necessary for the attainment of large size (Schaller *et al.* 1977) may undermine a large strain's ability to maintain the sort of coordinated host and algal growth seen in smaller strains.

Uncoordinated host and algal mitosis does not lead to substantial or permanent changes in algal density, however. The large Texas strain maintains algal densities nearly as constant as those of smaller strains, despite host and algal mitotic indexes that are much more disparate. This observation can be explained in one of two ways. First, to the extent that the durations of the algal tetraspore stage or host gastric cell mitosis are flexible, the Texas strain may be prolonging or shortening them, respectively, relative to the smaller strains.

Alternatively, these stages of cell division may have fixed durations for all strains. If so, host and algal t_d values that give comparable host and algal growth rates in the small strains yield a predicted algal growth rate three times that of the host in the Texas strain. Consider also that for any of the strains studied here the average number of algae per cell is remarkably constant over time, even during and following intervals of exceptionally high levels of algal mitosis. These observations are difficult to explain without invoking a mechanism for eliminating excess algae.

Although no data in support of a mechanism of culling excess algae have been reported under normal conditions, Dunn (unpublished data) has found both temporary and permanent decline in the size of the total *in situ* algal population during the 48-h period following feeding in the Carolina strain. As only a small fraction of these missing cells has been found in the surrounding medium, it appears that algae are disappearing inside the hydra *via* host digestion or autolysis. Further evidence for the destruction of algae is given by the fact that in these same experiments an increasing endosymbiont population had a lower average mitotic index than did a declining population. If we entertain the possibility that the changes in endosymbiont population size are functions of both birth and culling processes in the Carolina strain, then data given here suggest that in the Texas strain these culling processes, while still effective, are more heavily taxed.

We have never, despite some effort, found green hydra larger than the Texas strain. This may be because the Texas strain is near the upper size limit possible for a green hydra. On one hand, the Texas strain may be near the limit of a hydra's ability to hasten mitosis of its own cells or retard the maturation of endosymbiont algal tetraspores. On the other hand, hydra larger than the Texas strain may be unable to digest excess algae as fast as they are being produced.

Although in the laboratory green hydra out-compete large brown hydra when provided with small prey (Slobodkin, 1964), large brown hydra probably persist in the environment because they are able to exploit large prey types more efficiently (LeGuyader & Slobodkin, unpublished data). A large body size would be advantageous in at least some settings. We suggest that the reason for the apparent inability of green hydra to evolve to exploit these opportunities (or, for that matter, for the apparent inability of large hydra to exploit endosymbiosis) lies in a developmental constraint that prevents the evolution of large green hydra (see also Slobodkin *et al.* 1986). The factors determining the size of hydra may interact with the regulation of endosymbiotic algal density in such a way that stable symbiosis is impossible in a large hydra.

The mechanisms of regulation that we suggest may be determining the upper size limit of green hydra are not yet clear, but at present we are investigating the hypotheses presented here.

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