

A review of some physiological and evolutionary aspects of body size and bud size of *Hydra*

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Abstract

and reproductive rates depend on food particle size. see M55

Green hydra with endosymbionts are smaller than brown asymbiotic ones. Regeneration experiments, mitotic index studies on algal and hydra tissue, and evidence for consumption and expulsion of algae are reviewed and it is suggested that larger green hydra have more difficulty controlling algal increase than smaller ones and that hydra have an upper size limit for maintenance of stable symbioses. A mathematical model is discussed which starts with simple physiological assumptions about hydra and generates field testable conclusions about how body and bud size, quantity and temporal distribution. Unlike most analytic ecological-evolutionary models, this one integrates physiology, ecology and evolution without needing simplifying assumptions.

Introduction

For all species examined, *Hydra* body size is smaller at lower feeding rates. However, individual hydra at constant feeding rates are larger at lower temperatures (Hecker & Slobodkin, 1976). Furthermore, there is also a genetic component in size determination.

For example, when 11 independently collected clonal stocks were maintained in our laboratory, under identical feeding, light and temperature, régimes for many months, or even years, size differences persisted. Briefly, 10 mature individuals, each with one bud, from each clone were measured. Five clones were brown *Hydra oligactis*: one from North Carolina (USA); one from Lake Varese (Italy); two from different locations on Long Island (USA); and one sent by Dr P.J. McAuley from a location in the UK. There was also a clone of *H. cauliculata* from California

(USA). The smallest brown hydra was the Carolina strain which had a mean extended length of 3.8 ± 0.2 mm (SE). The green strains of *H. viridissima* were also from widespread locations: one from the Colorado river in Austin, Texas (USA); one from N. Carolina; two from the same mill pond in Stony Brook, one only 2.1 ± 0.1 mm long when extended, the other 3.0 ± 0.2 mm (From the same pond (on Long Island) came a *H. oligactis* strain 5.4 ± 0.3 mm long); and one from the Frome River (UK), also from Dr McAuley, which was intermediate in size between the two green 'Mill Pond' strains. The other strain sizes, and their other characteristics, as well as detailed information in culture procedures, specific identity and measurement procedures are presented in Bossert & Dunn (1986).

What is significant here is that no strain of green hydra which was consistently larger than any strain of asymbiotic brown hydra has been

found, although well nourished green hydra can be larger than starved brown ones. Which prey can be consumed by hydra depends in part on whether or not the prey are susceptible to nematocysts. Some zooplankters seem immune to the nematocysts of hydra (Schwartz *et al.*, 1983). For example, even neonate *Simocephalus* are undamaged by hydra while much larger *Daphnia* in the same dish are eaten (Slobodkin, unpubl.). Nevertheless, size in hydra has clear consequences for their capacity to capture food. Large strains of hydra can be maintained in the laboratory on large food organisms, such as *Daphnia magna*, while small hydra can only take smaller prey. Some prey organisms are simply too large for hydra to catch and consume (Hershey & Dodson, 1987).

From the size reduction of starving hydra and the inability to consume excessively large prey we infer that, in nature, there is a possible danger of a hydra starving to such a small size that no small enough food would be available. Because starving hydra subsist on their reserves, including phagocytosis of their own endodermal cells (Bosch & David, 1984) we infer that large hydra can endure a longer period of starvation before reaching this minimum critical size than can smaller hydra. Starved hydra can be restored to full reproductive size by increasing their food supply. If an adequate supply is not restored soon enough, the hydra may become so small that they cannot attack and consume their prey. This accounts for the oscillation of experimental populations of green hydra fed on brine shrimp nauplii (Slobodkin, 1964).

Assuming that brown hydra are generally larger than green, we consider two aspects of hydra size. Why aren't large hydra green, and how can we account for size differences among brown hydra strains among green hydra strains?

Why are large hydra not green or what prevents green hydra from becoming large?

The ability of green hydra to endure starvation without excessive reduction in size is enormously

increased by their symbionts under illumination (Kelty & Cook, 1976). We have starved green hydra for as long as four months, as have R.L. Pardy (pers. comm.) and others. In addition to consuming their own endodermal cells, green hydra also digest some of their algal symbionts, and do so at an enhanced rate when starved (Dunn, 1987). Darkness is deleterious for green hydra. Small hydra had best be green, for, if a shortage of animal food should occur, they can be nourished by their symbionts and can even consume the symbionts directly. This may delay their shrinking to so small a size as to make them unable to feed when prey become available again. However, an alternative question which denies the generality of this kind of adaptationist answer is: If being green is so advantageous, why aren't big hydra also green?

The general similarities in shape in hydra of different body size, and the essential constancy of cell sizes and types make it unlikely that the absence of large greens is explicable by generalized size-related arguments, for example, that there is somehow a difference in nutrients cost-benefit ratio between larger and smaller green hydra which sets a size limit. There is no evidence for such a size dependent difference. Also notice the occurrence of symbionts in some much larger cnidarians such as hermatypic corals and anemones. Our suggested explanation will be based on more specific properties of hydra biology.

Since budding in hydra involves an assignment of resources and tissue which might otherwise become part of the mother's body wall, the size of an adult hydra is partially determined by the axial position at which buds form. If buds occur closer to the existing head, the hydra will be smaller than if bud formation occurs further down the body stalk. The cell differentiation that is prerequisite to bud formation has been attributed to the relative amounts of head activator and head inhibitor to which an interstitial stem cell is exposed (Berking, 1977, 1979; Schaller *et al.*, 1977; for review of relevant literature see Schaller, 1983). We assume therefore that increased amounts of head activator produce smaller hydra.

Mitoses in hydra gastric cells and their symbiotic algae can be temporally correlated in green hydra (McAuley, 1982; Bossert & Dunn, 1986; Dunn, 1987; Bossert, 1988). Bossert & Dunn (1986) measured mitotic indices of green hydra cells and their algal symbionts in 3 different sized strains of *H. viridissima*. The algal mitotic indices are higher than the mitotic indices of the hydra cells in all 3 strains with the greatest disparity in the largest strain. If one assumes the duration of mitotic stages to be comparable in algal and hydra cells and recalling that the algae from 4 cells, rather than 2, at each mitotic division, then the algae are reproducing about 9.3 times as fast as the hydra cells in the 3.75 ± 0.25 mm long 'Texas' strain, but only 3.2 times as fast as in the 3.06 ± 0.13 mm 'N. Carolina' strain and 3.6 times as fast as in the 2.49 ± 0.11 mm 'Frome' strain. In short, the largest green strain has a clearly greater excess of algal reproduction than the 2 smaller green strains. Despite the greater reproductive rate of the algal cells all the strains maintain constant algal density and a stable symbiosis.

Bossert (1988) measured mitotic indices of both gastric cells and algal symbionts in several green *H. viridissima* strains in a variety of nutritional states, various times after feeding, with and without purified head activator and crude aquatic extracts of hydra tissue. The interactions are complex. A sample experiment is summarized in Table 1, which demonstrates that the larger of the 2 'Mill Pond' strains showed a consistently higher excess of algal reproduction than the smaller and that the discrepancy was reduced in both strains by head activator, but only in the smaller strain with head activator added did the ratio even approach unity. There are differences in time relations between algal and hydra mitoses that cloud simple interpretation. The results are, however, all compatible with the assertion that head activator increased the mitotic activity of the hydra cells, and did not seriously alter the mitotic rate of the algae. That is, the cells in small hydra, i.e., hydra with higher levels of head activator, are reproducing more rapidly relative to the reproductive rate of their algal symbionts, than are cells of larger hydra with less head activator.

Table 1. Cell division rate of gastric cells, divided by algal cell division rate (calculated as the hydra mitotic index divided by $2 \times$ the algal mitotic index to take account of tetrad formation in the algae) for a Large (L) and a Small (S) green hydra strain both from Stony Brook Mill Pond, in M solution with (A), and without (C) head activator added (calculated from data in Bossert, 1988)

Hours after feeding	L		S	
	A	C	A	C
6	2.00	2.00	0.78	1.71
12	2.00	3.83	1.25	2.15
24	5.00	5.00	1.47	1.50

Bossert & Slobodkin (1983) found that starved decapitated green hydra regenerated better in the dark than in light. Failure to regenerate in the light occurred more often in the larger of the 2 green strains studied and was accompanied by an increase in the number of algae per hydra cell. In these experiments the 'tight link' between host and algal mitosis reported by McAuley (1981, 1982) could not be found.

One conceivable explanation for these size dependent differences might be that the apparent greater mitotic rate of algae is illusory, perhaps related to a longer duration of algal mitoses in larger hosts. There is no evidence for this. A more plausible explanation for these results is that larger green hydra must be removing algae from their gastric cells at a greater rate. Evidence for digestion and elimination of algal cells is presented by Dunn (1987). In his studies the cell of the large N. Carolina strain of *H. viridissima* contained algal debris twice as frequently as those of a small European strain of the same species.

As noted by Sugiyama & Fujisawa (1979), budding rate, developmental rate and polyp size 'which in theory can be all regulated by independent mechanisms, show strong correlations with each other. This suggests that the underlying mechanisms regulating these characters must be closely related to each other.' Jolley & Smith (1978) report the potential division rate of algae as being considerably higher than that of their host cells. We are suggesting that the ease with which

algal increase is controlled varies with hydra body size, being more difficult for larger strains. Obviously the interaction between hydra gastric cells and their symbionts involves many more elements than we have considered here, but these few aspects of the relation suggest a tentative answer to our initial question. Since the problem of excess algae seems significantly more severe for the largest green strains, the lack of occurrence of even larger green hydra may be due to an incompatibility between the mechanisms of algal control and those of bud inhibition that permit large body size.

If the same mechanism that permits larger size turns algae into a real danger rather than a potential benefit we might expect larger hydra to be selected for resistance to invasion by symbionts. Rahat & Reich (1985a) have examined the relation between the large brown hydra, *H. magnipapillata* and an alga of unidentified species (not *Chlorella*). Despite the possibility of invasion in this case, green specimens of *H. magnipapillata* are extremely rare in nature constituting not more than 2% of the population and *H. magnipapillata* in the laboratory can only achieve a green color temporarily under strong light conditions. Green animals die (T. Sugiyama, pers. comm.). That is, *H. magnipapillata* is not an exception to the general rule that stable symbioses are limited generally to smaller hydra.

If, as suggested by Rahat & Reich (1985b, 1986) and Douglas & Huss (1986), a variety of free-living *Chlorellae* can become stable symbionts with aposymbiotic hydra, we would not expect hydra symbiosis to be a strong selective force on algal evolution. Presumably the fraction of the population of any given algal species that is resident in hydra is so small as to make any selective pressures associated with symbiosis evolutionarily trivial.

What sets body and bud size?

If size is so flexible within each genotype (Slobodkin, 1964; Hecker & Slobodkin, 1976), what is the significance of size differences among

genotypes, particularly for brown hydra in which symbiotic interactions are not an issue? We expect that the size of available prey, the length of periods of starvation, and food abundance, will be of major importance in selecting for different genetic clones in nature. These environmental properties have been incorporated into an analytical model of the evolution of different hydra body sizes, budding rates and bud sizes, as a function of varying quantities of available food. The model is primarily applicable to non-symbiotic hydra. The mathematical details are given by Gatto *et al.* (1989) and biological arguments are presented here.

The model assumes that, if food is absent, hydra lose resources to metabolism at a fixed rate while growing smaller. Also, each hydra is assumed to have a genetically determined 'allotment policy' for dividing incoming resources between the maintenance and growth of its own body and reproduction. For example, a policy might be to not reproduce at all until food is so abundant that the hydra can reach some specific size and then allotting half of any food resources above the maintenance cost at this size to reproduction and the remainder to further increase of body size. Another policy might be to always reproduce, even if food is in short supply and body size of the mother must be correspondingly reduced. Each organism also has a 'reproductive policy' for determining how much of the resources allotted to reproduction shall be allotted to each bud. For example, it might produce very few relatively large buds or an abundance of small ones from the same pool of reproductive resources. (The physiological mechanisms in this process relate to such things as head activators and inhibitors but these physiological properties are assumed to be flexible enough to respond to selection and are not explicitly modeled).

It was assumed that hydra of a particular size have a range of food particle sizes that they can eat, with the efficiency of catching and using the food particles being size dependent. Death occurs either from long term starvation, making the hydra too small to feed, or from density independent factors. Food particles are assumed to have some

statistical distribution in time and space so that food is characterized by its particle size; average abundance and the variance around that level.

All the animals therefore may encounter situations of extra food, no food, low food levels, or chemically unsuitable environments. Food above maintenance needs will either be used to increase body size or the store of material for reproduction, or both. Any animal which grows too small for the particular food particle size available will be unable to recover from an excessively long interval of starvation. Also, even if food is present it may occur in such low concentration that organisms will be unable to eat enough to balance their metabolic needs, and therefore shrink, and perhaps die. This produces a death rate based on food level, food distribution and the policies of the organisms.

The theory generates a reproductive rate dependent on the amount of resource available for young and the policy as to its subdivision. New individuals must be large enough to be able to feed and grow, at least under suitable food conditions. The size of new bud should therefore not be less than the minimum viable size of an organism that has shrunk to the limit which permits recovery, which depends on actual environmental circumstances of food distribution and particle size.

Organisms with different 'policies' for allocating resources between budding and maternal body growth and maintenance can be permitted to compete in computer environments characterized by the size and distribution of food particles. Also, optimum policies which are evolutionarily stable strategies for particular environments can be determined analytically in the rigorous sense of Hastings (1978).

The analysis by Gatto *et al.* (1989) produced the following conclusions. Environments and types of organisms are correlated. While there are intergrades, we can distinguish 3 types of policies corresponding to 3 types of organisms living in 3 easily characterized types of environment. Each of these will out-compete all organisms with alternative policies in these types of environments only. These are what we call 'Spendthrifts', 'Savers' and 'Investors'. As indicated below,

actual hydra have properties that seem similar to those of the 'Savers', but the other 2 are theoretically possible under some environmental conditions. That they do not exist is itself of possible interest.

'Spendthrifts' allot all but the amount of resources required to maintain a very small body size to reproduction. The buds produced are essentially the same size as their parents. Neither the young buds nor their parents can survive even short periods of starvation. The 'Spendthrifts' evolve if food shortages are rare or even absent, but, if and when they occur are of such long duration the animals starve to death.

The 'Saver' uses all of its resources above its maintenance costs for its own growth below some body size significantly larger than its birth size and then allots all of the energy above that required to maintain its size equilibrium to the production of buds, each of which is considerably smaller than itself. These animals evolve if there are frequent, intermediate length, food shortages.

Environments in which the 'Spendthrift' strategy is optimal are those in which the product of the mean waiting time between starvation incidents and duration is ≥ 1 , while if this product is < 1 the 'Savers' have the advantage. As expected, as the border is approached the body size and bud size of the 'Savers' tend to decrease.

Animals living in the same environment as the 'Savers' but that do not lose feeding efficiency with increasing body size can invest some fraction of their food income above that required for reproductive size maintenance in further increasing their own body size, thereby increasing the duration of starvation episodes they are capable of surviving. This is the strategy of the 'Investor.'

From this analysis it seems likely that larger strains of hydra should occur in environments containing larger prey species, bud size should be the smallest body size from which recovery from starvation is possible under natural food conditions, and the difference between bud size and adult size should depend on the range of prey sizes available and the length and frequency of periods when food is absent. The overall results should hold for both brown and green hydra, with

the important distinction that the green hydra should be generally smaller since they have a supplemental source of nutrition increasing the time they can survive the absence of animal prey. While these conclusions have not yet been tested in the field, they are testable by comparing the properties of hydra in ponds that differ in the properties of their zooplankton populations. It would be expected, for example, that hydra bud sizes should be smaller when smaller zooplankton are present.

We tentatively conclude, subject to field and laboratory testing, that excessively large green hydra cannot exist because of incompatibility between body size-controlling mechanisms and the mechanisms limiting algal reproduction within the gastric cells. Also a model based on the size, abundance, and spatial and temporal distribution of food particles suggests that these may be selective forces controlling the evolution of body and bud size of brown hydra in nature.

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References

- Berking, S., 1977. Bud formation in hydra: inhibition by an endogenous morphogen. *Wilhelm Roux Arch. dev. Biol.* 181: 215-225.
- Berking, S., 1979. Control of nerve cell formation from multipotent stem cells in hydra. *J. Cell Sci.* 40: 193-205.
- Bosch, T. C. G. & C. N. David, 1984. Growth regulation in hydra: relationship between epithelial cell cycle length and growth rate. *Dev. Biol.* 104: 161-171.
- Bossert, P., 1988. The effect of hydra size on growth of endosymbiotic algae. Ph.D. thesis, State University of New York.
- Bossert, P. & K. Dunn, 1986. Regulation of intracellular algae by various strains of the symbiotic *Hydra viridissima*. *J. Cell Sci.* 85: 187-195.
- Bossert, P. & L. B. Slobodkin, 1983. The effect of fast and regeneration in light vs. dark on regulation in hydra-algal symbiosis. *Biol. Bull.* 164: 396-405.
- Douglas, A. E. & V. A. R. Huss, 1986. On the characteristics and taxonomic position of symbiotic *Chlorella*. *Archs Microbiol.* 145: 80-84.
- Dunn, K., 1987. Growth of endosymbiotic algae in the green hydra, *Hydra viridissima*. *J. Cell Sci.* 88: 571-578.
- Gatto, M., C. Matessi & L. B. Slobodkin, 1989. Physiological profiles and demographic rates in relation to food quantity and predictability: an optimization approach. *Evol. Ecol.* 3: 1-30.
- Hastings, A., 1978. Evolutionarily stable strategies and the evolution of life history strategies. I. Density dependent models. *J. theor. Biol.* 75: 527-536.
- Hecker, B. & L. B. Slobodkin, 1976. Responses of *Hydra oligactis* to temperature and feeding rate. In G. O. Mackie (ed.), *Coelenterate Ecology and Behavior*. Plenum Press, N.Y.: 175-186.
- Hershey, A. & S. Dodson, 1987. Predator avoidance by *Cricotopus*: cyclomorphosis and the importance of being big and hairy. *Ecology*, Brooklyn 68: 913-920.
- Jolley, E. & D. C. Smith, 1978. Isolation, culture and characteristics of the *Chlorella* symbiont of 'European' *Hydra viridis*. *New Phytol.* 81: 637-645.
- Kelty, M. O. & C. B. Cook, 1976. Survival during starvation of symbiotic, aposymbiotic and nonsymbiotic hydra. In G. O. Mackie (ed.), *Coelenterate Ecology and Behavior*. Plenum Press, N.Y.: 409-414.
- McAuley, P. J., 1981. Control of cell division of the intracellular *Chlorella* symbionts in green hydra. *J. Cell Sci.* 47: 197-206.
- McAuley, P. J., 1982. Temporal relationships of host cell and algal mitoses in the green hydra symbiosis. *J. Cell Sci.* 58: 423-431.
- Rahat, M. & V. Reich, 1985a. A new alga-hydra symbiosis: *Hydra magnipappillata* of the 'non-symbiotic' *vulgaris* group hosts a *Chlorococcum*-like alga. *Symbiosis* 1: 177-184.
- Rahat, M. & V. Reich, 1985b. Correlations between characteristics of some free-living *Chlorella* spp. and their ability to form symbioses with *Hydra viridis*. *J. Cell Sci.* 74: 257-66.
- Rahat, M. & V. Reich, 1986. Algal endosymbiosis in brown hydra: host/symbiont specificity. *J. Cell Sci.* 86: 273-86.
- Schaller, H. C., 1983. Hormonal regulation of regeneration in *Hydra*. In J. L. Baker & J. F. McKelvy (eds), *Current Methods in Cellular Neurobiology*, Vol. IV. Model Systems. Wiley and Sons, N.Y.: 1-14.
- Schaller, H. C., T. Schmidt, E. Flick & C. J. P. Grimmelikhuijzen, 1977. Analysis of morphogenetic mutants of hydra. III. Maxi and mini. *Wilhelm Roux Arch. dev. Biol.* 183: 215-222.
- Schwartz, B., C. Hann & D. N. Hebert, 1983. The feeding ecology of *Hydra* and possible implications in the structuring of pond plankton communities. *Biol. Bull.* 164: 136-142.
- Slobodkin, L. B., 1964. Experimental populations of hydrida. *J. anim. Ecol.* 32(suppl.): 131-148.
- Sugiyama, T. & T. Fujisawa, 1979. Genetic analysis of developmental mechanisms in hydra. VII. Statistical analyses of developmental-morphological characters and cellular compositions. *Dev. Growth Differ.* 21: 361-375.