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THE *MÉMOIRES* OF ABRAHAM TREMBLEY: III. HIS DISCOVERIES ON HYDRA AND HIS APPROACHES TO BIOLOGY

BY

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INTRODUCTION

Most references to Abraham Trembley describe him as the discoverer of regeneration. Few authors, however, with the late John R. Baker (1952) being one of the exceptions, are aware of the range of discoveries made by Trembley using hydra as reported in his *Mémoires, pour servir à l'histoire d'un genre de polypes d'eau douce, à bras en forme de cornes* (1744).

Among the many discoveries and experiments published there are the demonstrations that: a) complete animals can regenerate from small cut pieces of those animals; b) animals can reproduce asexually by budding; c) tissue sections from two different animals of the same species can be grafted to each other; d) the materials oozing out of the edges of cut tissue have properties that fit the definition of protoplasm as described by Dujardin one hundred years later; e) living tissues can be stained, and those stained tissues can be used in experiments; and f) "eyeless" animals can exhibit a behavioral response to light.

These discoveries and many more were made in four short years while Trembley worked in relative isolation as the tutor of the two young sons of Count William Bentinck on his estate near The Hague in Holland. What kind of scientist was this unknown who burst upon the scene with such startling discoveries and received the prestigious Copley Medal of the Royal Society of London in 1743? What makes his discoveries and approach to science dynamic and interesting today much as they were 240 years ago?

To try to answer these questions, in this article we: a) look briefly at the range of discoveries and observations that he reported in his *Mémoires;* b) propose a number of ways that we might categorize the approaches to biology that were taken by Abraham Trembley; and c) examine in detail his approaches to quantitative studies and to organismic biology, as an operationalist and cautious interpreter of results.

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MAJOR SCIENTIFIC CONTRIBUTIONS DESCRIBED IN HIS *MÉMOIRES*

In Table 1, we list nearly 60 original contributions made by Trembley as he worked with hydra, including discoveries that add much to our knowledge of the animal. From the headings, it can be seen that those discoveries cover a broad range of disciplines. We have placed asterisks next to Trembley's major findings with hydra, most of which were subsequently determined to be significant in regard to other animals. In addition, we have also placed asterisks next to important tenets of the scientific philosophy (category A.) that Trembley espoused in the *Mémoires,* and next to some of the unique and or generally applicable techniques (category J.) that he described for the first time. All of these findings and techniques were developed using hydra, except those discussed in category H.

As described in the Introduction [items a), b), c) on regeneration, budding and grafting], Trembley is probably recognized most for his basic discoveries in developmental biology. See Table 1, category E for the extent of his discoveries in this area. In the field of animal behavior, however, Trembley has more recently been accorded greater recognition (Bodemer, 1967) as the first to observe and to devise experiments demonstrating that animals which do not possess eyes are attracted to and move towards light (F.5). One fascinating brief observation on animal behavior mentioned by Trembley in the *Mémoires* has not been noted previously. This is his description of habituation, i.e. the process whereby an organism stops responding to a stimulation once that stimulation has been applied a number of times in succession. We refer to the legend of Figure 1, Plate X of Memoir III. The figure shows "an aquatic Caddis Worm . . . swimming with eight long-armed Polyps attached to its case by their posterior ends." In the legend Trembley notes that he has "seen a number of [these] Polyps which were not induced to contract by the [swimming] motion of the Caddis Worm any more than those shown in this figure." Thus, Trembley, with his eye for the unusual, observed and first recorded an instance of habituation. Previously he had noted many times that hydra invariably contract in response to mechanical stimulation (F.2). Apparently those hydra attached to the case of the swimming Caddis Worm eventually habituated to the repeated mechanical stimuli resulting from those motions. Such habituation was eventually described in hydra in greater detail over two hundred years later by Rushforth *et al.* (1963).

Even a casual glance at Table 1 will indicate the breadth of Trembley's discoveries, most of which were made on the hydra.

TREMBLEY'S APPROACHES TO BIOLOGY

To our minds Trembley is outstanding among eighteenth century biologists in a number of spheres. For example, he may be praised for his excellence as 1) an *observer,* who was quick to notice the unusual, and to report his findings with great accuracy and

TABLE 1. SCIENTIFIC CONTRIBUTIONS TO BE FOUND IN THE MEMOIRS

A. Scientific Philosophy

*1. Distrust general rules
*2. Operationalism - give detailed methods
*3. Exercise caution -- withhold judgement
*4. Do more and varied experiments
*5. Repeat experiments frequently
*6. Repeat under natural conditions
*7. Let the organism guide the direction of your research

B. Natural History

1. Observing under natural conditions
2. Finding hydras in nature
3. Noticing seasonal variation in distribution and number of hydra

C. Taxonomy

*1. Characterized morphological differences among three kinds of hydra
2. Described behavioral characteristics of different species
3. Observed differences in pigmentation among the species

D. Morphology

1. General structure of hydra
2. Number and distribution of arms
3. Structure of body
4. Structure of foot
5. Structure of buds
6. Pigmentation of hydras
7. Structure of area around mouth
8. Description of structures shown later to be spermaries and eggs
9. Description of clusters of granules in tentacles shown 100 years later to be nematocysts

E. Developmental Biology

*1. Development of buds
*2. Regeneration experiments
*3. Budding experiments; rate of budding
*4. Inversion (reversal) experiments
*5. Grafting experiments
*6. Relationship of amount of food consumed and temperature to budding rate
7. Developmental abnormalities, naturally occurring and induced
8. Polarity of regeneration
9. Multiplication by transverse fission
10. Continuing development of bud when half of parent removed
11. Healing of wounds of cut hydra

F. Behavior

1. Activities of parts of body
2. Stimuli for contractions
3. Different kinds of locomotion
4. Feeding behavior
*5. Propensity for light
6. Mechanisms for suspending from surfaces of water
7. Separation of buds
*8. Observation of habituation to contractions stimulated by mechanical agitations
9. Development of feeding behavior of developing bud
10. Observation that well-fed animals do not readily eat
*11. Experiment indicating hydra have memory of light (see Josephson, 1985)

G. Physiology

1. Factors affecting contraction and elongation
2. Effect of temperature on many body processes
3. Uptake of pigments and nutrients
4. Digestion and egestion
*5. Properties of viscous material (protoplasm)
6. Adhesion to surfaces
7. Transfer of food from gut to buds and to arms
*8. Ability of hydra to recognize different or same species in grafting experiments and attempts at forced cannibalism

H. Research on Other Organisms

1. Various prey animals, such as Daphnia and Tubifex
2. Body lice (Kerona) on hydra
*3. Anatomy of Lophopus (a bryozoan)
*4. Budding by Lophopus
*5. Budding by Stylaria (an annelid)

I. Ecology

1. Hydra move to sites where food is most abundant
2. Some types of waters support growth of hydra, whereas others kill the animals
3. Hydras in nature vary in numbers depending upon the season
4. Hydra attached to snails advance more rapidly

J. Techniques and Methodology

1. Methods for finding hydras
2. Best waters for culturing hydra
*3. Use of vital stains to study uptake of nutrients, and paths of distribution of food
4. Method for observing an extended arm
5. Methods for measuring budding rate
*6. Method for inverting hydra
*7. Method for distinguishing between attraction to light or temperature or air
*8. Method for making many headed "Hydras"
9. Methods for collecting live food for hydra

detail; 2) a *naturalist,* who discovered a number of new species; 3) an *investigator of processes,* such as asexual reproduction by budding, when his contemporaries were mostly concerned with descriptions and with philosophical problems of biology; 4) an *experimentalist* who was not content until he could prove his findings in a number of ways; 5) a *technician par excellence* who carried out complex and delicate operations, many of them in a drop of water held in the palm of his hand using hardly more than a scissors and a boar's hair; 6) a *quantitative biologist* who backed many of his experiments with numbers and who repeated those experiments a sufficient number of times until he was convinced of their veracity; 7) an *organismic biologist* who investigated many phases of the life history of one animal; and 8) an *operationalist* who believed that an experiment had no lasting value unless complete directions are given regarding the methods by which both the experiments were carried out and the results observed. He has been called by some "the father of experimental zoology" (see Baker, 1952, pp. 171-172).

TREMBLEY AS A STUDENT OF QUANTITATIVE PROCESSES

Trembley came close to developing a form of quantitative biological inquiry. One of the better examples can be taken from examining his data in the *Mémoires* on the budding rate of individual hydra (pp. 164-167). He presents a table which lists the number and order in which buds both emanated and then detached from a single hydra over a two month period. From this experiment and others, he concluded that a single polyp produces an average of about 20 buds per summer month, some animals producing more and some less. So precise were his records that we were able to plot the data and obtain the graph of the rate at which buds appeared on and separated from a single hydra (Fig. 1). The graph is virtually the same as any biologist would obtain today were

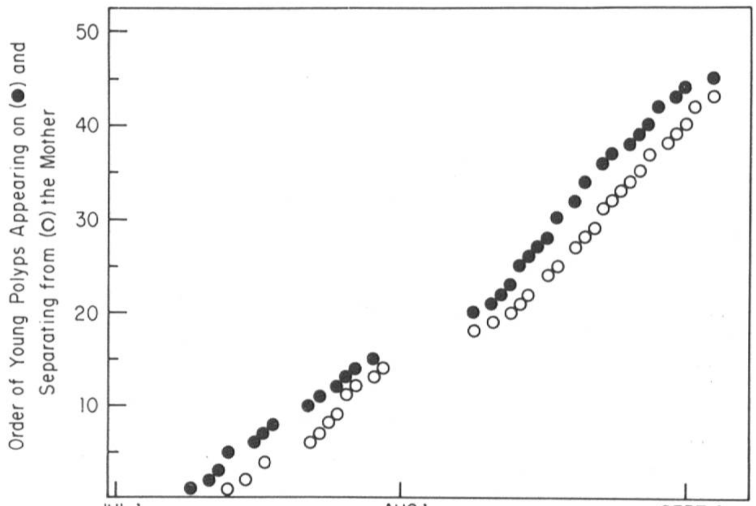

FIG. 1. — Rate at which buds appeared on (●) and separated from (○) a single hydra.

he or she to conduct the same experiment. Interestingly, the graph shows that whereas the budding rate during July was about 20 buds per month, the rate during August was closer to 30 per month. Possibly the latter rate reflected the higher temperature of August, a factor which Trembley said would increase the rate at which buds are produced.

Trembley also calculated that it takes about five days for a well-fed newly detached bud to begin reproducing by budding itself. Hence, he concludes, "Taking all of this into consideration, it is easy to understand at the end of two months that the number of descendants from a single polyp can be prodigious." Trembley was so right. Our colleague Dr. Richard Campbell, using a computer, took Trembley's two assumptions, one, that an animal can produce 20 buds a month, and, two, that a new bud can initiate its own budding within five days, and came up with Table 2.

TABLE 2

Number of progeny developing from a single newly detached bud

Days since experiment began	Number of possible detached progeny
1	1
15	14
30	349
60	250,000
90	185,000,000
120	134,000,000,000

The projections expressed in Table 2 show that given Trembley's assumptions and given ideal experimental conditions, an enormous number of hydra can be produced by budding in a very short time. Thus, although Trembley's expression "prodigious" is not precise, it certainly is appropriate. This estimate of Trembley's has a slight air of prophecy hinting at the exponential pattern of the multiplication of animals. As Loomis first demonstrated in 1954, cultures of hydra grown in the laboratory can double every 2 days, yielding Trembley's prodigious numbers (see Lenhoff and Brown, 1970, and Lenhoff, 1983).

Other places in the *Mémoires* hint at Trembley's regard for quantification. For example, he tells the reader that he has repeated a particular experiment a certain number of times and with what results. When looking for the opening between a dissected portion of the parent and the bud, he wrote, "I was not satisfied with doing this experiment once; I tried it on seven occasions, and succeeded on five."

A little further in the *Mémoires* he states that he has seen hydra divide on their own by a sort of transverse fission, but he adds the qualifying statement, "Although I have studied a considerable number of Polyps over a period of three years, I have not seen more than twelve divide [by transverse fission] on their own."

Trembley provides other quantitative data throughout the *Mémoires.* Examples can be found in his discussions of the effect of temperature on both the rate and amount of food consumed, on the initiating of budding, on the detachment of the buds, and on the hydra's general ability to respond to stimuli. Also, from a well-kept journal of his experiments and observations, he provides excerpts that often include dates, the time of day of the observation or operation, and the number of animals studied.

TREMBLEY, AN ORGANISMIC BIOLOGIST

Trembley shows himself to be a model example of an "organismic biologist." For our purposes, we define an organismic biologist as one whose research is focused primarily on a single organism, and who investigates virtually the whole of nature as lived by that organism. We contrast an organismic biologist with a problem-oriented biologist, that is one who uses an organism, or a group of organisms, in order to investigate a particular question. Once an organismic biologist starts to conduct research on an animal, that individual may be led on to investigate one phenomenon after another with no immediately apparent connection between them and without regard to the discipline of biology in which he or she is trained. That is, at one point the subject under investigation may be behavior; at another instance, developmental biology; at another, physiology; at still another, ecology; and then maybe back to behavior, or again to physiology. Or, as Trembley wrote in his preface, "I was swept along, as it were, from one observation to another with barely the time to make notes in my journal."

If we focus only on Trembley's experimentation, i.e. not his descriptive and procedural work, we can follow the path of his experiments and see how one separate line of research grows out of a seemingly entirely unrelated one (see Fig. 2).

Trembley's observation that hydra had a propensity toward light was the stimulus that got him to start investigating the animal seriously. Once he began to give hydra all of his attention, he observed that the animals did not have an equal number of arms. Hence, he thought of sectioning the hydra into two parts just to check once again the vague possibility in his mind that the hydra might be a plant. This experiment led him to his elegant series of experiments on regeneration.

Trembley noticed when he cut the hydra into pieces that many granules from the body wall ("skin") of the animal were released into the surrounding solution. It was his recollection of this observation, in fact, when he was concerned with the color of the hydra, that led him literally from one observation to another. By examining the granules, he noticed that they were held together by "viscous material" that today we would call protoplasm. By further examination of the color of the granules he got his ideas about how hydra assimilated food along with the colored material from the prey.

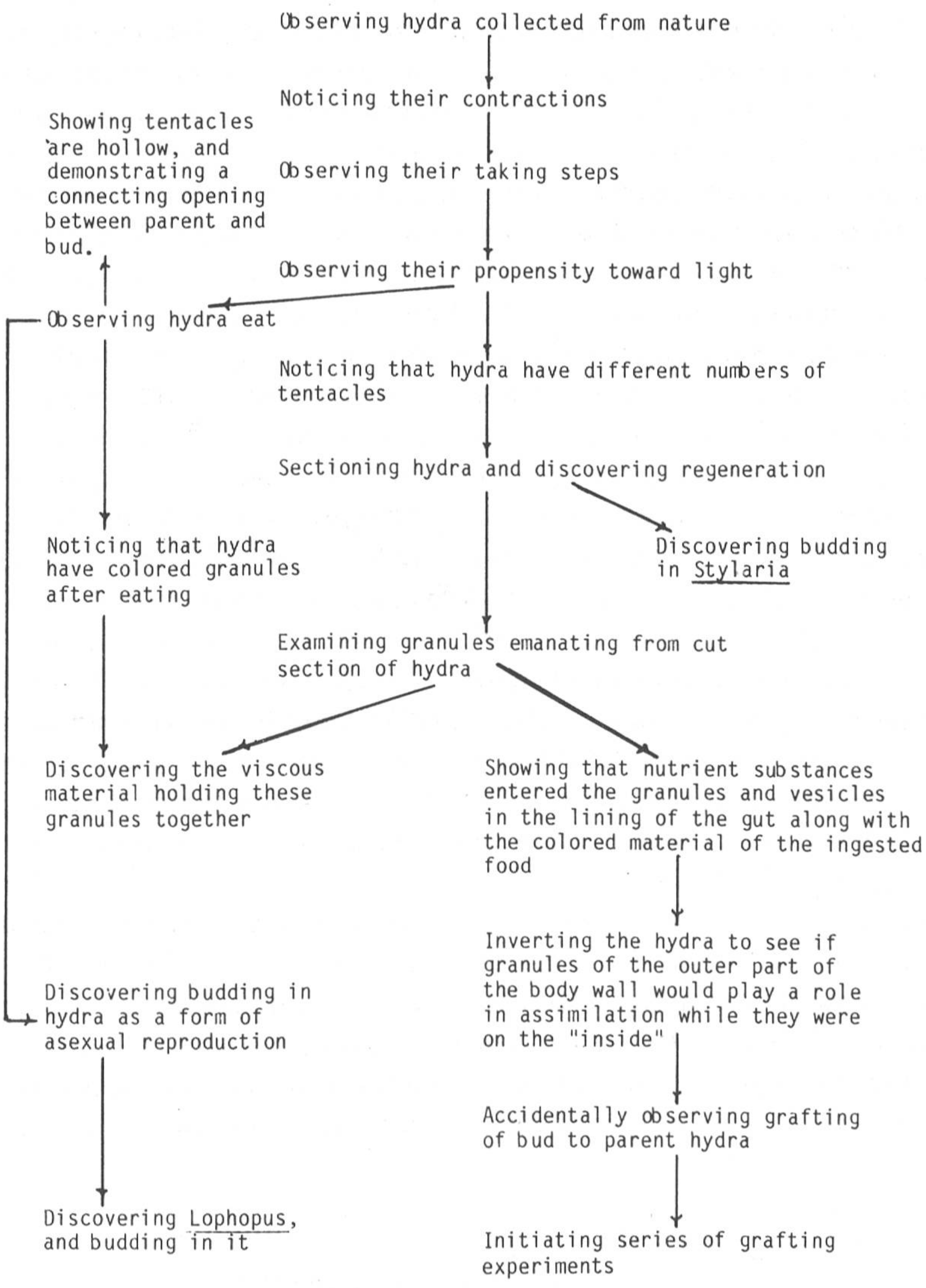

Fig. 2 A flow chart of this organismic chain of experiments and observations.

Because he noticed this role of the colored granules and vesicles in the lining of the stomach in taking up food, he devised a number of experiments in which he sought to nourish a hydra by placing it in a solution of nutrients. In his final experiment, he "thought of inverting them so that the external surface of their skin would form the walls of their stomach." Once Trembley had succeeded in inverting the hydra, he observed in one instance that a bud had grafted on to the parent. From that point on he continued to devise a series of experiments showing conclusively that it was possible to graft pieces from two different hydra together (H.M. Lenhoff and S.G. Lenhoff, 1984).

It would be disingenuous of us not to mention that the organismic approach which we ascribe to Abraham Trembley is the same one that has been used by one of us (H.M.L.) for the past 25 years in his research on hydra. For example, he has investigated such problems as culture of hydra in the laboratory (Lenhoff and Brown, 1970), migration of cnidoblasts (Lenhoff, 1959), composition of nematocyst capsules and toxins (Lenhoff *et al.*, 1957; Blanquet and Lenhoff, 1966; Hessinger and Lenhoff, 1976), chemical control of feeding behavior (Lenhoff, 1969; Lenhoff, 1981), mechanism of protein digestion (Lenhoff, 1961), chemical nature of endosymbiosis (Muscatine and Lenhoff, 1961), induction of budding in developmental mutants (Lenhoff, 1965; Novak and Lenhoff, 1981), composition and role of hydra's, acellular mesolamellae (Barzansky and Lenhoff, 1974), hydra's pigments (Krinsky and Lenhoff, 1965), control of differentiation of gonads (Rutherford *et al.*, 1983), use of hydra as a biological control for mosquito larvae (Lenhoff, 1978), and now the history of how research on hydra began (Lenhoff, 1980; Lenhoff and Lenhoff, 1984). Just as with Trembley, many of the discoveries of H.M.L. and his co-workers followed the serendipitous path of organismic biology.

Trembley recognized that chain of organismic experimentation which led to his famous discovery of regeneration and modestly commented with regard to it: "Because of its nature, that finding was to be not the fruit of long patience and great wisdom, but a gift of chance." If there is a lesson in Trembley's organismic approach for today's aspiring biologist, it might be: Do not overspecialize. Get a good background in experimental techniques, start to observe and investigate your organism, and let it — not your preconceived ideas — be your guide.

TREMBLEY, AN OPERATIONALIST

> It is not enough to say . . . that one has seen such and such a thing . . . unless at the same time, the observer indicates how it was seen, and unless he puts his readers in a position to evaluate the manner in which the reported facts were observed . . . . Insofar as I am able, I shall bring the reader into my study, have him follow my observations, and demonstrate before his eyes the methods I used to make them.

These simple words, taken from the first two paragraphs of Trembley's first Memoir, state a most important aspect of his scientific philosophy. Today we might call this philosophy "operationalism," suggesting a version of the scientific method which in essence states that the lasting truth of an investigation resides in an accurate description of the results, the methods by which the experiments were carried out, and the means by which the results were observed. All else is considered conjecture and might not stand the test of time as new means for investigating the same phenomena are developed and new facts are uncovered.

Trembley insists that to judge the validity of an observation or experiment, one must know how it was performed and under what conditions. Hence, the *Mémoires* are full of experimental detail. The figures at the close of each Memoir, to which Trembley refers heavily, are prominently displayed and feature meticulous labeling of fine points of animal structure. They are preceded in each case by pages of explanatory notes. The descriptions of his methods in the text are extremely clear and well written, leaving nothing to the imagination for others who wish to repeat his experiments.

Trembley emphasized the importance of observing living organisms under natural conditions and of differentiating between experimental results obtained in the study and events in the animal's natural habitat. For example, in Memoir III, Trembley recounts his excitement upon discovering in one of the ditches at Sorgvliet at a certain season huge branches completely covered with polyps. Off he dashed with a sample to put it safely away in his study. He then hastened back to the ditch just as quickly to set a plank out over the water on which he could lie to observe this striking new abundance of polyps in their natural setting. As another example, Trembley shows sensitivity to the important differences that may exist in artificial as opposed to natural settings when he points out differences in the numbers of hydra tentacles he has seen in nature versus those of animals kept in his study. He comments also on the effect on the polyp's fecundity deriving from the quantity of food material available to the animals kept in his jars versus the lesser amount generally available under natural conditions.

Not only is it important to observe the animal repeatedly and in its natural environment, Trembley warns, but also under comparable conditions. For example, he indicated that he needed to repeat observations made in nature a year later so that he might carry them out under "identical circumstances."

TREMBLEY AS A CAUTIOUS INTERPRETER OF RESULTS

When Trembley reported his results, many of which were revolutionary, he couched them in cautious terms. We provide but a few examples of his caution, one or two from each of the four Memoirs making up his classic volume (1744). In Memoir, I, when explaining the possible adhesion of the polyp by a combination of the meshing of the skin with an irregular surface, and the involvement of a viscous substance, Trembley adds, "I would not wish to allege, nonetheless, that no other causes may be involved." On this point, Trembley admitted the limits on discovery imposed by the nature of the organism and by the state of eighteenth century technology. He gave up on trying to discover how the hydra's adhesion to surfaces was controlled. "The Polyp is too small an animal to permit experiments to be made that would answer this question conclusively." Similarly, Trembley points out that though he finds only one canal in the polyp, "It may be that there are some others . . . which may be so small that they have escaped my scrutiny."

In Memoir II, after Trembley has proven that hydra move toward light, he admits that he cannot find "any part which, by its location or by its structure, gave me reason to suspect that it was an eye." But does he conclude that they "have no means of perceiving light on the objects it renders visible"? No, Trembley concludes, "When facts are lacking in such research, it is more appropriate to suspend judgment rather than make decisions which almost always are based on the presumption that Nature is as limited as the faculties of those who study her."

In a choice passage in the third Memoir as Trembley describes his experiment on the possible existence of a connecting opening in the Polyps between mother and young, he admonishes his reader regarding the value of repeating experiments, urging that one not "become disheartened by want of success, but . . . try anew whatever has failed. It is even good to repeat successful experiments a number of times. All that it is possible to see is not discovered, and often cannot be discovered, the first time."

On this same subject, in order to show that a bud actually developed from an evagination of the parent hydra body wall, Trembley cut out that portion of the wall and saw clearly the hole connecting the gut of the parent hydra and its bud. But Trembley remained cautious in drawing final conclusions, because, as he said, "It was still possible, however, that at the place where the two Polyps joined there could be a . . . [transparent membrane] . . . which separated the two stomachs." He then proceeded to prove that there was no blocking membrane by observing colored food go from the stomach of the parent into that of the bud, and vice versa.

Further on in this same Memoir, when Trembley described how he attempted to determine if the bud received some sort of "reproductive factor" from its parent hydra by means of the external environment, and was unable to find any such interaction at all, he nonetheless, concluded that "what I am attempting to discover, supposing something of the kind ever existed, was either imperceptible or at the least very difficult to see".

Or, in Memoir IV, after he found that virtually every part of a hydra could regenerate a complete animal, except pieces of isolated tentacles, he wrote, "The experiment did not succeed. I would not wish to conclude, however, that successful regeneration from a single arm is impossible." Further along in Memoir IV, when he was unsuccessful in attempts to graft pieces of different species of hydra together, he decided his experiments were done "neither with sufficient care nor with sufficient frequency, however, to assert that it cannot succeed."

In the examples just given, we see Trembley practicing a science of limited conclusions; he voices the need for such an approach repeatedly in the *Mémoires.* For example, Trembley was willing to accept that in many cases he would find no satisfactory answers despite long and laborious efforts. His attempt to understand how the hydra digested their food is an example. "I have never flattered myself that I have acquired very precise ideas on the subject," he says. As to how the nutritious elements of the food are finally absorbed in the body walls of the hydra, he cautions, "I will not promise satisfactory answers . . . . I am simply going to set forth some observations." Finally, regarding how the nutritive material spreads from "the granules" into which it has passed, to other parts of the body, "I find myself completely unable to answer that question."

Conclusions

We feel that these very features of operationalism and cautious interpretation which make Trembley so unusual among the early biologists, at the same time contributed somewhat to his fading into relative obscurity during the past two centuries. They are the same features, however, which are prompting fresh interest in Trembley's work among biologists today.

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