Effect of food stress on *Obelia geniculata* zooid differentiation

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Abstract

Colonial hydrozoans are well known for their phenotypic plasticity. Colonies of the *Obelia geniculata* (Cnidaria:Hydrozoa) maintain distinctly different zooids that are specialized for different tasks. It is not known, however, whether *O. geniculata* is capable of modulating the relative numbers of these structures in response to environmental conditions. In this study I subject *O. geniculata* to feeding regimes designed to simulate starvation, food-limited, and abundant-food conditions, and measure the numbers of each zooid type to see if they respond to these treatments.

1. Introduction

1.1 Context

Establishing the link between genotype and phenotype is one of the overarching goals pursued by biologists (Schwenk et al. 2009). Such an achievement would advance our understanding on a broad front of biological topics, from the origin and progression of genetic disease to patterns of past evolution and constraints in the future. Phenotype can be influenced by epigenetics, environmental cues, development, transcriptional and translational regulation, and many other factors (Aubin-Horth and Renn 2009). An organism or a trait is considered to be phenotypically plastic when a single genotype can produce more than one phenotype. While the proximate mechanisms of phenotypic plasticity remain relatively poorly understood, there is robust investigation of patterns and evolution of the phenomena.

Among the most dramatic demonstrations of phenotypic plasticity can be observed among colonial hydrozoans. During their polyp life stage, these animals form

networks of asexually produced individuals that remain physiologically connected via gastrovascular structures known as stolons. Each individual forms a distinct structure known as a zooid, which, depending on the species, can be specialized for one of several tasks – commonly feeding and reproduction. Some species (e.g. Portuguese person-of-war, *Physalia physalis*) exhibit such wildly polymorphic and integrated zooids that they were initially thought to be entirely separate organisms and begin to push the boundaries of what defines an individual (Wilson 1999). Each of these highly specialized zooids is produced from the same genotype, making colonial hydrozoans a propitious system and under-utilized in which to study phenotypic plasticity. It is especially appealing because the phenotypic plasticity is inter-individual, raising the additional question of what influences plasticity on such a scale.

The tradeoff between feeding (survival) and reproduction is a ubiquitous challenge faced by organisms (Stearns 1989). An organism's response to poor conditions depends on its life history strategy – it may invest heavily in survival in order to reproduce in the future, or it may produce dispersing offspring that may encounter more favorable conditions. The colonial hydroid *Hydractinia* is capable of rearranging colony morphology in response to experimental manipulation of its gastrovascular structures, demonstrating its ability to alter investment in vascular tissue versus ramet tissue (Dudgeon and Buss 1996). If hydrozoans are capable of altering investment in this manner, they may do the same for the different types of ramet tissue (zooid polymorphs) by adjusting the proportions of zooid polymorphs in response to prevailing environmental conditions. This phenotypic plasticity would allow them to make distinct tradeoffs between reproductive structures and feeding structures.

1.2 Study System

The colonial hydroid *Obelia geniculata* Linnaeus, 1758 (Cnidaria: Hydrozoa: Leptomedusae: Campanulariidae) is well suited to a study concerned with the questions raised above (Figure 1). It is abundant, sessile, and is simple relative to some other hydrozoans (e.g. siphonophores), typically producing only two easily distinguished zooid polymorphs, termed gonangia and hydranths (Crowell 1953). These zooids are responsible for producing free-swimming medusae and feeding, respectively. *O. geniculata* is able to produce zooids of either type, resorb them, and then redifferentiate the zooid into the other type (Berrill 1949). The proximate mechanisms governing this process are not well known and are not the subject of this study, but conditions that induce such activity and reveal active adjustment of investment in zooid differentiation are ripe for investigation.

As a sessile suspension feeder, *O. geniculata* has limited options when confronted with poor nutrient conditions. It can produce more hydranth feeding polyps, send out runners and raise more stalks in close proximity to the original colony, or it can invest in medusa-producing gonangia, which effect long-distance dispersal (Dudgeon and Buss 1996). If it is capable of adjusting investment in zooid proportions in response to food conditions I hypothesize that under food-limited conditions it will increase investment in gonangia. Such an investment would produce more clonal medusae that would be able to disperse and potentially arrive at a location with more concentrated resources. The aim of this study is to determine whether *Obelia geniculata* actively adjusts its investment in reproduction and feeding zooids in response to food stress. I will subject *O. geniculata*

colonies to different feeding regimes to simulate starvation, limited, and abundant food, and I will measure the change in the proportion of zooid polymorphs.

2. Materials and Methods

2.1 Specimen Collection and Preparation

I collected two large *Obelia geniculata* from algae attached to two tires hanging from the landward side of the Friday Harbor Labs dock. I placed the colonies, still attached to their seaweed substrate, into sea tables for 2 days prior to experimental set up.

I separated 18 individual stalks of *O. geniculata* from their colonies (9 stalks from each) by cutting the algae around their bases and scraping off any remaining stalks or polyps, thereby ensuring that a single isolated stalk remained. I then trimmed all stalks to approximately 1.5 cm to control for the impact that colony size may have on redifferentiation of zooids. After trimming stalks, I counted and recorded the number of each zooid type. I then separated the stalks into 9 pairs – one stalk from each colony – and used gel-type superglue to attach each pair of stalks to its own ceramic tile, separated by three centimeters.

2.2 Experimental Setup

I set up a flow system in an outdoor sea table, fed by a header tank, to house the *O. geniculata* over the course of my experiment. A plastic terrarium (header tank) was placed on top of cinderblocks at a height of approximately 0.3 meters above the floor of the sea table. An overflow hole ensured that water remained at a constant level thereby maintaining constant flow. To eliminate most particulates from the seawater, and thereby

ensure that my starved animals received no appreciable food, I placed a 1.0μm filter bag beneath the spigot filling the header tank. To avoid fouling and maintain constant flow, I changed the filter bag approximately every eight hours. I attached five equal-length pieces of plastic tubing to nozzles at the base of the header tank and subsequently split each tube into two flows using T-junctions and more (smaller diameter) plastic tubing for a total of 10 separate flows of equal velocity.

I placed each ceramic tile containing *O. geniculata* stalks in its own 10x10x10 plastic container. I used laundry clips to attach the small diameter tubing to the side of each plastic container, ensuring that in each container the tubing was in the same position relative to the *O. geniculata* colonies. In a tenth container I placed the *O. geniculata* colonies that remained after sample preparation, which I used to gauge whether or not the starved individuals in my experiment were dying.

2.3 Feeding

I subjected colonies to one of three feeding treatments: starvation, meager, and satiation, wherein colonies were not fed, fed once every other day, and fed twice a day, respectively. Each treatment contained six individual stalks, contained in three separate containers. For food I used Tetra Marine flakey fish food, which I ground into fine particulate with a mortar and pestle. I mixed 0.25 grams of this with 3mL of filtered seawater to form a paste, which I then injected into the outflow of the plastic tubing in each container. Beginning in the evening, I fed colonies at 12-hour intervals and ran the experiment for eight days, resulting in a total number of feeding events of 14 for satiation individuals and four for meager individuals.

2.4 Statistical Analysis

All statistical analysis was carried out in R. I began by coding my data according to treatment, time stage, and proportion of gonangia to the total number of zooids. Colonies that detached during the experiment were not included in the analysis. I then defined a linear model wherein the zooid counts before and after the experiment were defined as response variables to treatment group. I performed a Shapiro-Wilk normality test on the before and after data blocks and normalized the data using a fourth root transformation in R. I then tested for heteroscedasticity using Levene's test. Once I determined that all the conditions were satisfied, I performed a one-way repeated measures ANOVA to determine whether the proportion of gonangia to total zooids responded significantly to feeding regime.

3. Results

I did not detect any significant response of *O. geniculata* to feeding regime in terms of proportion of zooid polymorphs with this experiment. Three of the 18 colonies detached for some reason over the duration of the experiment – one from the starvation treatment and two from the satiated treatment. My zooid count data were not normal, but were normalized following a fourth root transformation. Post-transformation, data met conditions for heteroscedasticity. Test statistics are summarized in Table 1.

The one-way repeated ANOVA revealed that the proportion of gonangia to total zooid number did indeed change significantly ($p=0.0421$, Table 2), but that the change could not be attributed to the different feeding regimes ($p=0.2040$, Table 2).

4. Discussion

In this study, I investigate the phenotypic plasticity of *O. geniculata* by subjecting colonies to three feeding regimes and measuring their response in terms of investment in reproduction versus feeding. I was unable to reject my null hypothesis: that the proportion of gonangia (reproductive zooids) to total zooid number would change in response to feeding regime.

My initial hypothesis was based on the supposition that if *O. geniculata* are able to alter their investment in different polymorphs under food-stressed conditions, they would shunt resources toward producing a higher proportion of gonangia so as to increase the probability of dispersal to locales with more resources. There are several reasons why this may have been unsupported by my study. It is conceivable that total reproductive output of medusae does not depend as much on the number of gonangia as it does on the rate of output from each given zooid. While this has not been reported in any literature on *O. geniculata* life history (Berrill 1949, Crowell 1953), it could be tested in a relatively straightforward manner by subjecting *O. geniculata* to environmental stresses and tracking individual gonangia to determine whether the rate of medusa production increases. It is also possible that I did not create the stressful conditions in the lab that I intended. Hydrozoans are preyed upon in the wild by a wide variety of organisms, and have developed an array of defensive measures (Stachowicz and Lindquist 2000). By isolating them in the lab, I may have removed stressors that resulted in a change in reproductive investment unrelated to feeding regime.

There are several improvements that could be made to this experiment. First, tracking individual zooids with vital dyes would allow me to determine if zooids are being resorbed prior to redifferentiation or are redifferentiated in situ. Second, if zooids could be counted daily without dramatic disturbance, it would provide better temporal resolution for the progression of zooid changes.

If future work demonstrates the ability of *O. geniculata* or other hydrozoans to actively modulate their investment in zooid polymorphs, an interesting avenue of study would be to determine how these environmental cues are proximal tied to the phenotypic plasticity exhibited by these animals.

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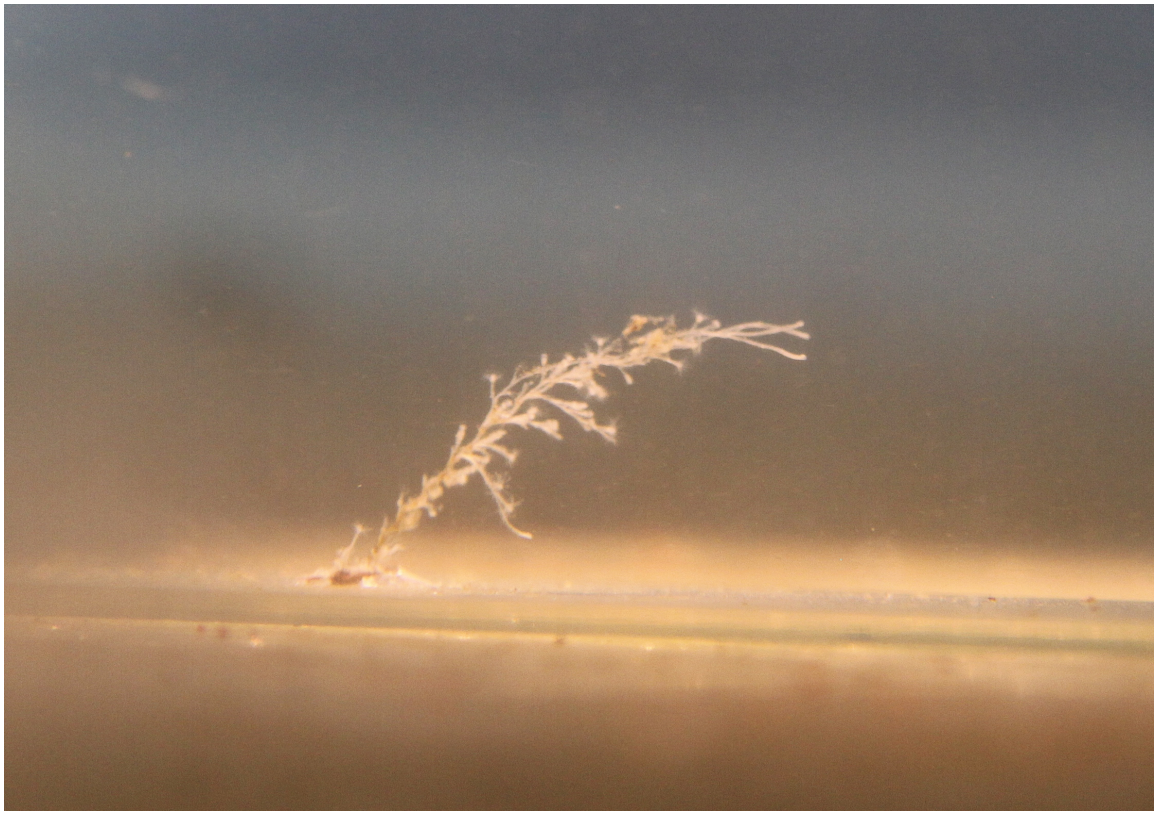

Figure 1. *Obelia geniculata*. Photograph of *O. geniculata* stalk separated from the larger colony, but still attached to a small piece of algae substrate. Photo credit: Andrea Rummel.

	Shaprio-Wilk Test (pre-transform)	Shapiro-Wilk Test (post-transform)	Levene's Test
Before	0.6328	0.2608	0.4383
After	0.0381	0.6473	0.5265

Table 1. Test statistics of zooid count data. Shapiro-Wilk test were performed on both data blocks to determine normality of the data. Data was normalized using a fourth root transformation and heteroskedasticity was tested using Levene's test. All analyses were performed in R.

	Change gonangia/total zooid over repeated measure	Change due to treatment
Significance value	p = 0.0421	p = 0.2040

Table 2. One-way repeated measures ANOVA. This test reveals that the there was a significant change in the proportion of gonangia zooids to the number of total zooids but that change cannot be attributed to treatment.

* indicates significance.