Effect of Cloning Rate on Fitness-Related Traits in Two Marine Hydroids

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Abstract. Hydractinia symbiolongicarpus and Podo-coryna carnea are colonial marine hydroids capable of reproducing both sexually and asexually. Asexual reproduction, by colony fragmentation, produces a genetic clone of the parent colony. This study examines the effect of very different cloning rates on colony growth rate, oxygen uptake rate, and colony morphology. Colonies of one clone of each species were maintained for an extended time in two treatments: in a state of constant vegetative growth by repeated cloning, and in a state restricted from vegetative growth (no cloning). For both species, tissue explants taken from the growing colonies grew more slowly than similar explants taken from the restricted colonies. For one species, tissue explants from the growing colonies used oxygen at a higher rate than similar explants from restricted colonies; for the other species, no difference was detected, although the sample size was small. For both species, tissue explants from restricted colonies formed more circular, “sheet-like” shapes, whereas those from their growing counterparts formed more irregular, “runner-like” shapes. After these experiments, in the third winter of treatment, all colonies experienced a severe tissue regression. Within 6 months after this event, the colonies had regrown to their former sizes. A growth assay at this point revealed no difference in growth rate, possibly suggesting an epigenetic basis for these results. Changes in clonal growth rates and morphology correlated with variation in fragmentation rate might affect the ecology of these and other clonal organisms.

Introduction

Clonal (or “modular”) organisms differ from unitary, sexually reproducing ones in that asexual reproduction produces identical genetic copies of the parent rather than genetically unique offspring. In many organisms, episodes of clonal reproduction may be intercalated with periods of gamete production. This clonal life cycle has obviously been successful; two-thirds of metazoan phyla contain clonal species (Bell, 1982), and most of the earth’s sessile biotic covering is composed of clonal life forms (Jackson et al., 1985). During the past few decades, studies in evolutionary biology have delineated various differences between clonal and unitary organisms. In terms of ecology, for instance, clonal organisms typically outcompete asexual ones in marine hard substratum environments, where space is commonly a limiting resource (Jackson, 1977; Larwood and Rosen, 1979). Because its modules are functionally independent, a colony has great regenerative powers and can recover from a substantial colony mortality (Hughes and Cancino, 1985). In addition, a comparison of life histories between the two reproductive modes reveals some inherent and fundamental differences. Fecundity of clones or colonies is indeterminate, because iteration of vegetatively produced modules can yield an indefinite number of reproductive units. This contrasts with unitary organisms, whose fecundity typically levels off or declines with age (Hall and Hughes, 1996). Consequently senescence, a derived property of the unitary soma (Medawar, 1952), may be negligible in clonal genets (Hughes, 1989), which may be very large and comprise a number of unconnected ramets (genetically identical but physiologically separate units).

Despite this considerable attention, one attribute of clonal organisms that has not been studied is the effect on fitness-related traits of the rate of cloning—that is, the number of episodes of asexual reproduction prior to the sexual phase of the life cycle. The rates might differ, for example, between corals inhabiting a turbulent shallow-water habitat and those occupying a deeper, more physically stable environment (e.g., Wulff, 1985). How might

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variability in this attribute affect the functioning and fitness of an organism?

This question was addressed experimentally using marine hydroids as a model system. This is an appropriate use, since hydractinid hydroids are colonial organisms commonly used in laboratory manipulations. The species used here, *Hydractinia symbiolongicarpus* and *Podocoryna (=Podocoryne carnea)*, reproduce both asexually (by colony fragmentation) and sexually (Brusca and Brusca, 1990). The rate of cloning can be precisely controlled in the laboratory, since a colony fragment can be surgically excised from a parent colony and cultured as an independent, yet genetically identical, colony. For a genotype of each of these species, rate of cloning was varied, and growth rate and colony morphology were measured. These are fitness-related traits (Larwood and Rosen, 1979; McFadden et al., 1984; Jackson et al., 1985; Yund, 1991; Brazeau and Lasker, 1992). Fitness has been defined as the expected contribution of a phenotype, genotype, or allele to future generations, relative to other organisms and genes in the environment; and thus it may be measured as numerical dominance over time (Stearns, 1992). For hydroids, which typically inhabit space-limited habitats, the fitness advantage of a high relative growth rate is manifest. Colony morphology is another factor that may affect competitive ability, and therefore it also is important to the success of a clone in a particular environment (Larwood and Rosen, 1979; Jackson et al., 1985). Although laboratory experiments such as these cannot measure actual fitness in nature, we attempt to gain insight into what might happen when similar colony fragments, one from a rapidly fragmenting clone and one from a relatively unfragmented clone, meet in the same natural environment. Do such fragments grow at different rates and thus have different fitnesses? A difference in growth rate could be correlated with a difference in metabolic efficiency. After finding a difference in colony growth rate between these treatments, we measured oxygen consumption rate as an indicator of overall metabolic rate. The implications of these results are discussed in the general context of clonal biology.

**Materials and Methods**

*Study species*

Hydractinid hydroids (phylum Cnidaria) are marine animals that live as encrusting colonies consisting of repeated modular units (polyps) specialized for feeding or reproduction. The polyps are interconnected by tubular stolons that house gastrovascular canals, forming a net-like structure. Each colony thus comprises a single integrated physiological unit. A colony grows onto suitable available substratum by extending peripheral stolons and developing erect feeding polyps at intervals along them. Colony growth ceases when space is no longer available; for instance, in *H. symbiolongicarpus*, which typically encrusts the shells of hermit crabs, colony growth is limited by the size of the shell. *P. carnea* also grows on hermit crab shells, but is found on other hard substrata as well (Edwards, 1972). The two species differ further in that *H. symbiolongicarpus* forms a relatively dense mat of stolonal tissue as the colony enlarges, but *P. carnea* does not—its stolons are separated by areas free of tissue. Clonal reproduction occurs when a fragment that is separated from a parent colony (e.g., by physical abrasion) is situated on a surface suitable for attachment and growth (Jackson et al., 1985). An entire new colony, genetically identical to the parent colony, can grow, limited in size by available space. Sexual reproduction is accomplished by gamete formation and release into the surrounding seawater where syngamy may occur, leading to the development of a motile planula larva. (*H. symbiolongicarpus* produces gametes directly from specialized reproductive polyps; the life cycle of *P. carnea* includes a motile gametogenic medusa stage.) The planula larva may then attach to a hermit crab shell or other hard substratum suitable for growth, where a genetically novel colony develops.

*Culture methods*

Colonies of both *H. symbiolongicarpus* and *P. carnea* were collected from the shells of hermit crabs near the Yale Peabody Museum Field Station in Connecticut in 1994. Explants, consisting of a small portion of a colony made up of a few feeding polyps along with interconnecting stolons, were surgically removed from the field-collected colonies and secured with nylon thread to rectangular (3 in × 1 in) glass microscope slides to create stock colonies from which samples could be removed. The slides were then suspended from floating racks in 120-l aquaria filled with Reef Crystals artificial seawater (salinity 35‰), and maintained at a temperature of 20.5° ± 0.5°C. The aquaria used undergravel filtration, and 50% of the water was changed each week. Ammonia, nitrites, and nitrates were maintained below detectable levels (Aquarium Systems test kits). The colonies were fed brine shrimp nauplii 3 times per week. No attempt was made to control the amount of food ingested; observation indicates that generally all polyps in all colonies feed to repletion. Thus, colonies with more or larger polyps are capable of consuming more food. An artificial light cycle of 14:10, L:D, was provided, supplemented by natural light coming in through windows. The colonies were allowed to grow over the slides until large enough to permit removal of a sufficient number of small explants for use as experimental replicates. All replicates used in the experiment were maintained in the same conditions as the stock colonies.

*Experimental manipulations*

For each hydroid species, 25 clonal replicates from a single parent colony were created on 12-mm round glass
coverslips by surgical explanting from the stock colonies. New explants were secured to the coverslips with nylon thread. Within about one day, the colony attaches itself to the glass of the coverslip. Five of these (for each species) were treated in the following way: a colony was allowed to grow until it had either nearly covered the coverslip or until it began to produce reproductive polylys (in preparation for gamete production). Then a small piece of the colony, consisting of two feeding polylys together with the interconnecting stolon, was explanted onto a fresh coverslip. The new colony was cultured as before, and the old colony was discarded. In this way, the growing replicates were maintained in a state of constant growth and purely clonal reproduction; these colonies are herein referred to as “growing.” The remaining 20 replicates (for each species) were allowed to grow completely over the 12-mm coverslips and to produce gametes or medusae in an unrestricted manner. They were left undisturbed for the duration of the experiment, except for removal of explants for the purpose of the various assays. These colonies are referred to as “restricted.” The restricted replicates were maintained in higher numbers because of the impossibility of regenerating them (without cloning) in the event of colony mortality. The number of colonies produced ensured that sufficient colony tissue was available for the assays.

Measures of growth rate

Explants consisting of exactly two intermediate-sized feeding polyps and a minimal amount of interconnecting stolon were taken from the experimental colonies, with multiple explants from the same replicate kept to a minimum; that is, an effort was made to take suitably sized fragments from all of the 5 growing replicate colonies and as many of the 20 restricted colonies as possible to obtain the 12 replicates of each treatment for the growth assays. These were then attached synchronously to fresh 12-mm coverslips. Although 12 replicates per treatment per species were initiated, some explants failed to attach to the coverslips, so actual sample sizes per treatment were smaller. Explants were allowed to grow for a period of 3 weeks; none exhausted the available space during the assays. None of the colonies assayed entered a gamete- or medusa-producing phase, so all polyps present during the assays were feeding polylys. Colony size was measured as number of polyps produced and, in two of the three growth assays performed, by total protein content of the colony. For the 3-week polyp counts and total protein measures, between-treatment comparisons were made for each species using analysis of variance.

To ensure that the experimental colonies did not inadvertently get replaced by any vagrant colonies (of different genotype) that might have found their way into the aquarium, clonal identity was tested. This was also done to support the assumption that significant genetic divergence was not occurring in the colonies during the experiments. To test clonal identity, explants were made from all five of the growing colonies for each species onto clean microscope slides (one slide per explant). One explant from a randomly selected restricted colony was then placed on each slide, and the pair of colonies was allowed to grow until stolonial contact was made. When meeting in this way, colonies from the same clone will merge to produce a single physiological entity having interconnected stolons (Hughes, 1989; Mokady and Buss, 1996). When unrelated clones meet, tissue rejection rather than fusion occurs.

Measures of total protein

Subsequent to polyp counts, the colony to be measured was first macerated in ultrapure water (200–450 μl, depending on colony size) using a Teflon pestle driven by an electric drill. Then a small sample of the resulting fluid was assayed with the Bio-Rad protein assay kit #500-001, which uses a bovine gamma globulin protein standard and the Bradford method of protein staining (with Coomassie brilliant blue G-250 dye). Binding of the dye to proteins causes a maximum absorbance shift from 465 nm to 595 nm. Absorbance at this wavelength was measured in a Beckman DU-64 spectrophotometer and compared to a standard curve to determine protein amounts. Although questions arise in using a bovine standard for assays of cnidarians (Zamer et al., 1989), these concerns are mitigated in this case because the same genotypes are being compared, and thus relative, not absolute, comparisons are sufficient.

Measures of oxygen uptake rate

Colonies to be assayed were obtained by explanting two-polyly fragments from all five growing colonies and several restricted colonies onto fresh coverslips. These were allowed to grow until they nearly reached the edge of the coverslip. All assays were performed 24 h after feeding: at that time polyps are generally not contracting (Dudgeon et al., 1999), and the colonies could be considered to be in a resting state. Measures of oxygen uptake made at these times can be used as an indication of standard metabolic rate (Schmidt-Nielsen, 1997; Lowell and Spiegelman, 2000). For each assay, a colony of each treatment type was selected, matched as closely as possible in size to minimize any size effects. The two colonies were then assayed sequentially. The assays were done in this pairwise fashion so that any ambient conditions that might affect oxygen uptake rate (variation in atmospheric pressure, etc.) would not introduce a sampling bias into the data for either treatment.

Colonies were assayed for rate of oxygen uptake with a Strathkelvin Instruments oxygen meter, model 781. The temperature of the oxygen measurement chamber was controlled with a Neslab Instruments model RTE-100D exter-
nal circulation waterbath at 20.5° ± 0.02°C. The colony was attached, with a small amount of grease, to a 12-mm glass coverslip to which a small stir bar had been affixed. After instrument calibration, the measurement chamber was loaded with 1.0 ml of seawater filtered to 0.2 μm and saturated with oxygen by stirring. Oxygen uptake was measured every 3 min for a period of at least 30 min with stirring. Shortly after each individual assay was begun, the rate of oxygen uptake by the sample colony stabilized and remained linear for the entire 30-min period. The rate thus obtained from each sample provided an observation to be used in the data analysis. Data from the oxygen uptake rate assays were analyzed using analysis of covariance, beginning initially with a test of heterogeneity of slopes. When the slopes were found to not differ, between-treatment differences in elevation were compared.

Characterization of colony morphology

A hydroid colony can be described as tending towards having a more “sheet-like” or “runner-like” morphology (McFadden et al., 1984). Sheet-like colonies, typical of *H. symbiolongicarpus*, are characterized by a relatively circular central stolonal area whose periphery has few projecting stolons with free ends. Runner-like colonies, characteristic of *P. carnea*, have a relatively large number of projecting free-ended stolons and a small enclosed central stolonal area. A size-free shape measure that may be used to compare colony morphologies is given by (colony perimeter)/√(colony area) (Blackstone and Buss, 1991). A minimum value of 2√π describes a circular colony with no projecting peripheral stolons; this is the quintessential sheet. As the value of the metric increases, the colony appears more runner-like. Using this shape metric, colonies of both species were tested for a treatment effect. Colonies to be analyzed were explanted onto fresh 12-mm glass coverslips, then performing image analysis with OPTIMAS 5.0 software (Media Cybernetics) for the Windows operating system. Data gathered in this way were analyzed using analysis of covariance, beginning with a test of heterogeneity of slopes. Colony perimeters and areas were quantified by first imaging the coverslip, at which time shape analysis was begun. Colony analyzed were explanted onto fresh 12-mm glass coverslips two months after initial explants. At this same time, oxygen uptake assays of *P. carnea* were done. In November 1998, oxygen uptake assays of *H. symbiolongicarpus* were begun. Shortly after this time, when six pairs of *H. symbiolongicarpus* colonies had been assayed for oxygen uptake, all of the colonies in the experiment underwent a severe tissue regression. This event truncated the *H. symbiolongicarpus* oxygen uptake assays and precluded a planned second growth assay for *P. carnea*. Similar midwinter regressions generally occur in field-collected hydroid colonies exposed to natural light (pers. obs.). In the case of the manipulated colonies, this regression was especially severe, with all colonies experiencing almost complete tissue death. However, enough living tissue remained in the colonies so that within 6 months they had regained their previous size. A final growth assay was done for *P. carnea* after 32 months from the initiation of the experiment (beginning of May), and for *H. symbiolongicarpus* after 35 months (beginning of July). Also after 35 months, fusion tests between restricted and growing colonies of each species were begun.

Results

Measures of growth rate

The first growth-rate assay was performed about 12 months after the initial explants of the experimental colonies were made. For both species, the growing colonies grew more slowly than the restricted ones (Fig. 1; ANOVA of log-transformed 3-week polyp counts; *H. symbiolongicarpus*, *F* = 9.27, df = 1, 20, *P* < 0.007; *P. carnea*, *F* = 13.21, df = 1, 22, *P* < 0.002). A second growth assay was begun for *H. symbiolongicarpus* after 24 months, entailing polyp counts as well as measures of total protein. Again, the restricted colonies grew at a faster rate than their growing counterparts, this time to a more pronounced degree (Fig. 2; ANOVA of log-transformed polyp counts, *F* = 65.02, df = 1, 17, *P* ≪ 0.001; ANOVA of log-transformed total pro-

![Figure 1. Growth rate comparisons of growing and restricted Hydractinia symbiolongicarpus and Podocoryna carnea colonies from the assay performed after 12 months of experimental treatment. Means and standard errors of the number of polyps in a colony are represented.](image-url)
tein, $F = 227.70, \text{df} = 1, 17, P \ll 0.001$). Note that colony size, measured as number of polyps, and total colony protein are highly correlated (Fig. 3). A second growth assay for the P. carnea colonies was precluded by the widespread midwinter tissue regression that occurred in early 1999.

The fusion tests resulted in the colonies fusing, suggesting that significant genetic divergence, at least at histocompatibility loci, had not occurred. This result also strongly supports the assumption that experimental colonies were not replaced by other genotypes during the experiments.

** Measures of oxygen uptake rate

At all sizes, growing colonies of P. carnea consumed oxygen at a higher rate than did restricted colonies (Fig. 4a).

Although no significant difference in oxygen consumption rate was found between treatments for H. symbiolongicarpus (Fig. 4b), a trend may be discerned in the data that would indicate agreement with the result found for P. carnea. The sample size is too small to render this trend statistically significant, however.

** Characterization of colony morphology

Growing colonies of both species had a more runner-like morphology than their restricted counterparts (Fig. 5: H. symbiolongicarpus, $F = 12.56, \text{df} = 1, 20, P < 0.002$; P. carnea, $F = 6.16, \text{df} = 1, 22, P < 0.0212$).

** Growth rate after regression

A growth assay was performed 4 to 6 months after the pronounced winter regression. At this time, no significant
difference was detected between treatments in either species for growth as measured by total colony polyp counts (Fig. 6a; H. symbiolongicarpus, $F = 0.06$, df = 1, 16, $P > 0.806$; P. carnea, $F = 0.44$, df = 1, 18, $P > 0.516$, data for both analyses log-transformed) or by total colony protein (Fig. 6b; H. symbiolongicarpus, $F = 0.17$, df = 1, 16, $P > 0.689$; P. carnea, $F = 1.04$, df = 1, 18, $P > 0.321$; data for both analyses log-transformed).

**Discussion**

Two experimental treatments were used in this study of hydroid colonies. One group of replicates was allowed to completely overgrow and remain undisturbed on 12-mm coverslips ("restricted" colonies); a second group was repeatedly cloned as vegetative growth continued, without being allowed to enter into a gamete-producing sexual phase ("growing" colonies). A clear difference in growth rate was found between treatments in both species studied, with restricted colonies exceeding growing colonies in growth rate during controlled assays. Since only one clone was used per species, this result is not replicated at the level of the species. Nevertheless, at a higher level (i.e., species within family), the two clones provide replication of this primary result.

Assays of the oxygen uptake rate between treatments revealed that the growing colonies of Podocoryna carnea exceeded the restricted ones in oxygen consumption. Although no significant statistical difference was found for Hydractinia symbiolongicarpus, the sample size was small, and a trend seems to be discernible in the data that would suggest agreement with the result for P. carnea. Such a result may seem counterintuitive; the colony that uses more oxygen might also be expected to grow faster. On the other hand, higher oxygen uptake may be correlated with lower growth rate if the former indicates greater metabolic expenditure on, for instance, somatic maintenance. Such a hypothesis is not entirely implausible. These hydroid colonies are ecologically space-limited, typically inhabiting small hermit crab shells. It is likely that selection favors rapid sequestration of available space to prevent the settlement of competitors; colonies may maximally allocate energy resources to growth until the available space is covered. Under such conditions of intense metabolic demand, cellular metabolism may generate high levels of reactive oxygen species (Allen, 1996; Chiueh, 2000). These reactive species can cause various defects in macromolecules, so continuously growing colonies might experience defects in the mechanisms of oxidative phosphorylation or allocate greater resources to production of anti-oxidant enzymes (e.g., Blackstone, 2001). Thus the data are consistent with the hypothesis that growing colonies expend more energy on functions other than somatic growth, although further study of this issue is needed. Our interpretation of these results is that the restricted colonies are metabolically more efficient and so can allocate more energy to growth (Lowell and Spiegelman, 2000).
The widespread tissue regression that occurred apparently reset to zero the growth rate difference that had been entrained by the experimental treatments. By this view, the physiological basis of the difference prior to regression was transmitted to the clonal fragments of the growing colonies, becoming enhanced over time as shown by the decreasing colony growth rate. This may suggest an epigenetic basis for the phenomenon, wherein a particular state of gene activity underlies the increased rate of oxygen consumption coupled with the reduced growth rate. During the regression event, all colonies lost most of their living tissue, effecting a cell population bottleneck. The elimination of the growth rate difference could perhaps be due to sampling error in the cells that escaped death during the regression, or to some dedifferentiation process involving a return to a metabolic ground state. In any case, cells of similar condition and gene activity seem to have survived the regression. Periodic regressions of this kind have been observed in some clonal taxa and are possibly related to senescence (Bayer and Todd, 1997; Gardner and Mangel, 1997). The life span of the modules (polyps) that make up a colony may be extended through cycles of degeneration and regeneration (Hughes, 1989).

Comparing absolute growth rates of colonies undergoing both treatments early in the experiment (Fig. 1) with those measured some two years later (Fig. 3) reveals a consistent decline. Furthermore, the growth rate equalization after regression occurred not by the growing colonies recovering a rapid growth rate but by the faster growing restricted ones assuming a similarly diminished rate. This reduction in growth rate over time may be considered to be a manifestation of colony senescence (Bell, 1988). By this criterion, growing colonies senesced more rapidly than restricted ones prior to the tissue regression event, suggesting that a high cloning rate accelerates colony senescence relative to uncloned colonies. After regression, the degree of clonal senescence (measured by growth rate) became equalized.

Hydractinid hydroid colonies fragment to produce potentially viable clonal modules, thus enlarging and dispersing the genet asexually (Cerrano et al., 1998). The colony fragmentation rate (equivalent to the cloning rate considered in this study) presumably could vary with the physical environment in which the hydroids are found. In aquaria, Cerrano et al. (1998) found that clonal colonies arising from fragments of Podocoryna exigua colonies can grow on a sandy-bottom substratum and that hermit crabs with naked shells placed into this environment were colonized within a few days. If such a process occurs naturally in P. exigua and other hydractinid hydroids, such as the species used in this study, a genet might extend itself naturally by fragmentation. Clonal lineages may vary in fragmentation rate and growth rate of colonial ramets. This study shows that cloning rate could possibly affect the growth rate of a ramet within a lineage through negative feedback, since variation in growth rate may be passed on through some epigenetic mechanism such as cytosine methylation (but see Tweedie and Bird, 2000; and Amedeo et al., 2000). Nevertheless, histocompatibility data (Grosberg et al., 1996; Mokady and Buss, 1996) suggest that in at least some populations of H. symbiolicarpus the rate of fragmentation is low relative to the rate of sexual recruitment.

The alteration in morphology with variation in cloning rate might have a bearing on the ecological functioning of a hydroid colony (McFadden et al., 1984; Yund, 1991; Brazeau and Lasker, 1992). Intraspecific competition is common between Hydractinia colonies (Buss and Blackstone, 1991). The present study has shown that a high cloning rate can produce a more runner-like colony morphology, thus tending towards a form associated with a “guerilla” ecological strategy (Jackson et al., 1985). Such a clone might have more limited direct competitive ability, but might also be dispersed to more locations due to its greater rate of fragmentation.

Asexual reproduction is an essential part of the life history of all clonal organisms and is thus an important factor in their evolution and ecology. In some taxa, fragmentation rate depends on morphological characters, which are at least in part genetic and thus subject to selection. The fragmentation rate of clones of branching coral reef demosponges was found to depend on branch thickness (Wulff, 1985). A coral of the genus Plexaura has evidently evolved morphological characters that make fragmentation more common in this species than in its congeners and produce some populations in which more than 90% of the individuals are clonemates (Lasker, 1990). A possible difference in growth rate dependent on cloning rate would have to be taken into account when considering the demographic impact of fragmentation.

The effects of the two experimental treatments on the clonal replicates of both hydroid species indicate that frequently fragmenting colonies exhibit reduced colony growth rates, hence diminished reproductive potential and compromised competitive ability in the space-limited habitats in which they are typically found. Moreover, a within-species difference in colony morphology was found between unfragmented colonies and those maintained in a constant state of vegetative growth by repeated cloning (fragmenting); this difference could affect the ecological functioning of the colonies in nature. However, these discrepancies may disappear if a large-scale regression of colony tissue occurs. Regardless of the specific physiological mechanisms producing these differential effects, fragmentation rate can be important to various aspects of the biology of clonal organisms.
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