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Maintenance of Variation and Adaptive Consequences of Encrusting Growth Forms in the Clonal Hydroid Genus Hydractinia

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MAINTENANCE OF VARIATION AND ADAPTIVE CONSEQUENCES OF ENCRUSTING GROWTH FORMS IN THE CLONAL HYDROID GENUS *HYDRACTINIA*

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To my daughter Emily

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ABSTRACT

Traits with close ties to fitness are central in understanding the process of adaptation and whether, and in what contexts, selection acts to erode genetically based phenotypic variation. The persistence and even predominance of apparently unfit genotypes in natural populations is particularly problematic. Genetically based growth form variation in the clonal hydrozoan genus *Hydractinia* epitomizes this problem. A rich literature exists in this model system, indicating that frequent and intense intraspecific competition occurs, primarily between juveniles, and ends in the elimination of subordinates. Despite this strong source of directional selection, extreme variation in competitive ability, mediated by early ontogenetic growth form, is known to exist in all species studied to date. While a genetic trade-off between size at first reproduction and competitive ability has been proposed and discussed in the literature, no published data address this or other explanations for the conspicuous morphological variation observed in natural populations. Here I show that (1) competitively inferior phenotypes are most abundant in 2 of 3 species found in the northwestern Atlantic and northern Gulf of Mexico, (2) shifts in the distribution of predominant growth forms among species reflect, at least in part, interspecific differences in the strength of selection imposed by intraspecific competition, (3) an intrinsic genetic trade-off with life history components or growth does not account for widely variable competitive abilities within species, but (4) superior competitors suffer greater costs in terms of survival and early growth in the context of dense hermit crab populations, suggesting that competitively inferior phenotypes are more physically robust and better withstand mechanical disturbance imposed by hermit crab interactions, and (5) evidence from natural populations on spatial structure and temporal variation is consistent with the hypothesis that environmental heterogeneity in symbiotic host density, as a result of host species and size variation, create spatial and temporal variability in disturbance regimes, favoring competitively dominant growth forms at low density and physically robust growth forms at high density.

INTRODUCTION

Variation in adaptive traits abounds in nature. Traits with close ties to fitness (e.g., life history traits) are often quantitative and polygenic (Charlesworth 1987, Charlesworth and Hughes 2000). Gaining insight into mechanisms underlying the genetic basis of extant variation in adaptive traits and its persistence in natural populations remains a formidable but fundamental challenge faced by contemporary evolutionary biologists.

Directional selection forces that favor one end of a quantitative phenotypic spectrum are commonly identified (Rieseberg et al. 2002) yet genotypes conferring these most fit phenotypes do not necessarily become fixed in natural populations and may be present only in low abundance. Limits to directional selection operating within the range of extant phenotypic variation typically require conflicting selective pressures but other possibilities exist, including a lack of sufficient additive genetic variance, effects of other evolutionary processes such as gene flow (e.g., mussel hybrids, Gilg and Hilbish 2003; flower color, Schemske and Bierzychudek 2001), or an absence of fit intermediates leading to an optimal phenotype (Barton and Partridge 2000).

Empirical systems in which balancing selection is thought to play a key role in the maintenance of adaptive genetic polymorphisms typically involve trade-offs. That is, no single morph exhibits the greatest overall fitness at all times, in all environments, or at all levels of selection. Trade-offs occur when one or more fitness traits exhibit a negative genetic correlation with the variable focal trait. Genetic trade-offs can be achieved through antagonistic pleiotropy (single gene effects), tight physical linkage among trait loci, or linkage disequilibrium (Lynch and Walsh 1998). The extent to which discrete character models, often assuming a single diallelic locus, apply to polygenic, quantitative characters is unknown; yet mechanisms originally proposed for maintaining genetic variation in discrete characters, such as spatial or temporally variable selection (e.g., flower color, Schemske and Bierzychudek 2001, Frey 2004; alternative male mating strategies, Sinervo and Zamudio 2001), are commonly extended to continuous, quantitative characters (e.g., egg size, Svensson and Sinervo 2004). However, key trade-offs among fitness traits can be elusive, and life history theory is in desperate search of a

general explanation for why empirical life history studies frequently fail to identify trade-offs (Reznick et al. 2000 and references therein).

An ideal system for examining alternative models that act to prevent trait fixation by limiting the effects of directional selection should include a clearly adaptive quantitative trait, persistent extant phenotypic variation, and evident directional selective pressure. Despite apparently strong directional selection (e.g., Yund 1991) and a significant genetic component to colony growth form (Buss et al. 1984, Buss and Grosberg 1990, Yund 1991, Ferrell 2004a, Müller 2004 et al.), extensive variation in this quantitative trait exists in natural populations of the marine colonial hydroid genus *Hydractinia*. Comparable variation occurs in natural populations of at least three species (Buss et al. 1984, Buss and Grosberg 1990, Yund 1991, Ferrell 2004a). Growth patterns yield "runner-like" (= guerilla) or "sheet-like" (= phalanx) forms, growth strategies common in many clonal animal and plant taxa (Harper 1977, Buss 1979, Jackson 1979a, Harper 1985). Sheet-like forms typically outcompete runner-like forms (e.g., Buss 1976, 1979; Jackson 1979a). In *Hydractinia*, mat-like phenotypes correspond to sheet-like growth, and highly stoloniferous phenotypes have been equated with runner-like morphologies. Importantly, however, the typical relationship between growth form and competitive ability appears to be reversed in *Hydractinia* in which highly stoloniferous growth forms are better able to mount a species-specific inducible defense used to overgrow conspecifics. Growth form is an important morphological trait and even has been considered a life history trait by some authors in both plants (e.g, Fischer and van Kleunen 2001) and animals (e.g., Blackstone and Buss 1991). Its impact on competitive ability in *Hydractinia* owes to a highly specific inducible response in which tissues in the region of inter-genotypic contact raise from the substratum and accrue toxic stinging organelles (i.e., nematocysts), ultimately killing and overgrowing less stoloniferous phenotypes (Buss and Grosberg 1990). Intraspecific competition has received much attention (e.g., Buss and Grosberg 1990; Yund 1991; Van Winkle and Blackstone 2002; Ferrell 2004a, 2005), and frequent intraspecific competition between small juvenile animals (routinely imposing mortality on losers) provides a potent directional selective force in this genus.

The vast majority of prior experimental work in this system has been conducted in a setting far removed from nature. Often, asexually derived 'recruits' are artificially propagated from mature animals and, in the absence of obligate symbiotic host species, manually attached to

artificial substrata that provide an unusually large colonizable area. However, in nature, sexually-derived planulae recruit to a variety of (typically small) gastropod shells, which are occupied by hermit crabs that commonly reside in dense, shell-limited populations in which crabs likely disturb epibiont species frequently (e.g., during shell switching negotiations). Yund (1987, dissertation ms.) attributed maintenance of growth form variation in *Hydractinia* to a trade-off between competitive ability and size at first reproduction based on a game theory model and a phenotypic correlation between size at first reproduction and growth form among male colonies in a common garden laboratory setting; however, substratum size strongly influences the onset of reproduction in *Hydractinia* (e.g., Haüenschild 1954), and colonies were grown on artificial substrata providing a surface area greatly exceeding that most frequently encountered in nature. Although a genetic trade-off between size at first reproduction and competitive ability (Yund 1987) appears plausible as an explanation for the maintenance of growth form variation, no published data address this hypothesis despite the fact that (1) the *Hydractinia* system has developed into a model system in several subdisciplines (Frank et al. 2001), (2) growth form is fundamental to colony growth and development and may be closely tied to many colony traits of adaptive significance in addition to size at first reproduction (Blackstone and Buss 1991, Buss and Blackstone 1991), and (3) genetically based growth form variation is conspicuous and extensive in all species studied to date (Buss et al. 1984, Buss and Grosberg 1990, Yund 1991, Ferrell 2004a).

Here I undertake the problem of the maintenance of growth form variation in this genus using a variety of empirical tools, including experimental, observational, field and laboratory approaches using both sexually and asexually derived organisms. First, I quantitatively characterize the growth form variation at issue in three of the most well studied *Hydractinia* species and gauge how the strength of selection favoring highly competitive growth forms may vary among species. Then I consider the notion that competitive ability trades off with one or more life history traits. My field experimental results demonstrate that no genetic trade-offs exist between competitive ability and survival, fecundity, size and age of first reproduction, or growth despite the fact that strong positive genetic correlations (all $r \geq 0.80$ and $P < 0.01$ at 56 days) were detected among all life history components and between life history and growth. I then present direct experimental evidence that in the context of dense host hermit crab populations, experienced widely in nature, competitively inferior growth forms better withstand

severe growth costs and mortality imposed by crab-mediated mechanical disturbance, suggesting that they are physically more robust than stoloniferous phenotypes. Natural host densities vary considerably in space and time as a result of among-site variation and species-specific differences in host demography and behavior as well as a strong seasonal component to host activity patterns and aggregative behavior. Many models of environmental heterogeneity suggest that several conditions should be met to maintain adaptive trait variation, including significant genetic structure among populations and sufficiently strong conflicting selective pressures among populations and/or between seasons. The combination of spatial and temporal variation in selective regimes, as proposed here, is much stronger mechanistically than either source of variation acting alone. I propose that *Hydractinia* growth form variation is maintained by spatial and temporal variation in selective regimes favoring competitively dominant phenotypes in populations and seasons with low host density and physically robust phenotypes in those with high host density.

CHAPTER 1

DIFFERENCES IN ENVIRONMENTAL PREDICTABILITY UNDERLIE DIVERGENT COMPETITIVE ABILITIES IN THREE CONGENERIC HYDROIDS

Abstract

Species in which individuals experience predictable and uniform environments should be most finely adapted to their environment. Many hydrozoan species in the genus *Hydractinia* simultaneously occupy similar microhabitats (gastropod shells inhabited by hermit crabs) yet experience considerable differences in their immediate environment (size and species of shells and hosts). Here I show that hydroid species experience differences in environmental predictability and traits that mediate competitive ability (growth form and growth rate). Inferred competitive ability was directly proportional to the extent to which the gastropod environment promotes interactions between small, juvenile colonies, which always end in competitive elimination. Extensive intraspecific variation in competitive ability was explained primarily by crab host species or site. Dense host populations impose more severe disturbance regimes that favor competitively inferior, but physically robust, phenotypes. Interplay between different types of variation (gastropods and hermit crabs) provides a possible mechanism for the maintenance of intraspecific growth form variation.

Introduction

Although unpredictable environments sometimes may serve as a selective pressure, as in the evolution of bet-hedging life history (e.g., Boyce and Perrins 1987) or risk avoidance behavioral (e.g., Real 1980) strategies, environmental unpredictability may reduce the strength or direction of selection on adaptive traits as a result of inconsistencies in selective environments experienced among individuals. Consequently, organismal adaptations shaped by directional selection may be expected to be most refined in those species in which individuals most consistently experience predictable and uniform environments.

As a direct result of their spatial restriction as adults, individuals of sessile species may appear most likely to experience a single, uniform spatial environment. However, depending on the season, size, and homogeneity of the habitat chosen for settlement, this may or may not be

true. Botanists have long recognized that many clonal plants exhibit patterns of asexual lateral propagation, often termed 'foraging' growth strategies, that allow individual genets to experience local environments varying widely in light regimes, resource availability, or abiotic stress (e.g., Lovett-Doust 1981, Salzman 1985, Sutherland and Stillman 1988, deKroon et al. 1994, Stuefer et al. 1994, Stuefer et al. 1996). Similarly, other laterally propagating clonal taxa – including multicellular algae, most fungi, and several marine invertebrate phyla (Blackstone and Buss 1991) – also exhibit growth indeterminacy and patterns of growth that promote the occupancy of multiple spatial environments by individual genets (e.g., Jackson 1977, Buss 1979, Jackson 1979a). From this perspective, it may appear that consistency among individuals with respect to environments experienced is unlikely in clonal taxa. Yet individuals of indeterminate clonal species can be restricted to small, discrete patches of suitable habitat, thereby limiting variation experienced by an individual genet. In species consistently settling on extremely similar discrete substrata, either as a result of highly specific habitat selection/requirements (Buss 1979) or limited diversity of suitable substrata provided by the community or abiotic environment, variability experienced among individual genets is minimized. Thus, discrete habitats may be ideal systems in which to investigate the effects of inter-individual environmental variability (or, equivalently, environmental (un)predictability experienced at the species level) on adaptive trait evolution in clonal taxa.

Discrete habitats abound in the marine environment. Varied plants, invertebrate animals, and cobble serve as suitable discrete habitat patches for a wealth of sessile clonal species. In seagrass meadows, epiphytic algae and colonial invertebrates (including sponges, hydroids, bryozoans and ascidians) utilize individual seagrass blades as ephemeral habitats (e.g., Trautman and Borowitzka 1999). Submerged roots of mangrove trees in the tropics harbor rich sessile epifauna that may differ greatly in community composition even at small spatial scales (e.g., Bingham 1992). In reef environments, encrusting algae and animals (sponges, bryozoans, and ascidians) commonly compete for space on the undersurface of coral skeletons (e.g., Jackson 1979b), and coral rubble provide substrata for diverse epibionts, such as coralline algae and zoanthid cnidarians (e.g., Karlson 1983). Over 550 invertebrate species, many of these clonal, are known to associate with hermit crabs and the gastropod shells they inhabit (Williams and McDermott 2004). Invertebrate associates may encrust the external or internal shell surface, penetrate the shell material, reside inside the shell, or associate directly with the crab host. In

each of these varied ecological contexts, sessile organisms experience a limited amount of habitable space, due to the discrete nature of the spatial resource. Consequently, competition for space is frequent in these marine encrusting communities, and constituent species of discrete environments, especially those that are obligate inhabitants, often possess morphological adaptations that enhance spatial competitive ability (Buss 1979, Jackson 1979a).

In the hydrozoan genus *Hydractinia*, individuals exhibit genotype-specific growth forms that are directly related to competitive ability (Buss and Grosberg 1990). Although other hydroid species are common earlier settlers that are easily outcompeted by subsequent community colonizers (Boero 1984), *Hydractinia* species are capable of both exploiting adjacent open space when made available and subsequently resisting overgrowth (Sutherland and Karlson 1977, Karlson 1978). Colonies are sometimes found on alternative substrata, such as rocks and pilings, where they may persist perennially, but they typically occupy gastropod shells inhabited by hermit crabs, which serve as discrete microhabitats. Although colonies may encounter other species, especially on larger gastropod shells (Karlson and Shenk 1983, Shenk and Karlson 1986) and artificial substrata (Sutherland and Karlson 1977, Karlson 1978), intraspecific competition occurs frequently within at least three species: *H.* GM (Ferrell 2004b, 2005), *H. symbiolongicarpus* (Yund et al. 1987, Buss and Yund 1988, Hart and Grosberg 1999), and *H. polyclina* (Yund and Parker 1989, Yund 1991). GM is the designation given to an undescribed *Hydractinia* species found only in the northern Gulf of Mexico (Cunningham et al. 1991). During initial post-recruitment growth, colonies expand asexually and exhibit variable growth forms (Figure 1). In pairwise competitive encounters between small, juvenile colonies, more stoloniferous colonies overgrow and quickly kill less stoloniferous and stolonless colonies (Buss and Grosberg 1990, Ferrell 2004a). Competition between larger colonies, in contrast, may or may not result in rapid competitive exclusion, depending on the growth form of the colonies involved (Ferrell 2004a; 2005). In natural *H.* GM populations, interactions between sexually mature colonies occur regularly at some sites and appear to be related to gastropod shell architecture and size (Ferrell 2004b).

Here I characterize variation in the traits that mediate competitive ability, growth form and growth rate, in three shell-encrusting hydroid congeners, and further explore its relationship to the immediate environment. An intimate symbiotic relationship with several host hermit crab species subjects them to widely variable microenvironments, including species-specific

differences in crab size and behavior and an array of colonized gastropod shells that differ in size, species, and morphology. Among-site differences also exist with respect to each of these hydroid microenvironmental variables. I will address the following specific questions: (1) What is the extent of genetically-based growth form variation and does it differ among species? (2) Do host species identity and/or shell characteristics (size, species, morphology) influence hydroid growth form? (3) In particular, do more stoloniferous colonies predominate on gastropod species with a well-developed siphonal canal? This expectation is derived from the finding that post-recruitment survival is known to vary with gastropod morphology (Yund et al. 1987). Shells with a siphonal canal may be more likely to recruit two spatially distant conspecifics, thereby setting the stage for colony encounters later in development (and therefore at larger size), which result in either competitive standoffs (Ferrell 2005) or prolonged overgrowth interactions, ultimately favoring highly stoloniferous colonies. On shells encrusted fully by only one colony, only the latter scenario, in which multiple recruitment followed by (prolonged) overgrowth, is possible. (4) Lastly, do hydroid species experience different levels of microenvironmental predictability, and if so, does competitive ability vary accordingly among species?

Study system

Hydractinia species (Family Hydractiniidae) characteristically encrust the surface of gastropod shells occupied by pagurid hermit crabs (Cunningham et al. 1991, McDermott 2001). *H.* GM, an undescribed species, is found only in the northern Gulf of Mexico whereas *H. symbiolongicarpus* and *H. polyclina* are distributed in the northwestern Atlantic with limited geographical overlap between species (Buss and Yund 1989). Two hermit crab hosts, *Pagurus longicarpus* and *P. pollicaris*, are broadly distributed in the northwestern Atlantic and northern Gulf of Mexico (Williams 1984). *H.* GM commonly associates with both of these host species (Ferrell 2004b, 2005). *H. symbiolongicarpus* typically associates with *P. longicarpus* but sometimes can be found with small *P. pollicaris* individuals (Buss and Yund 1989), and *H. polyclina* associates with *P. longicarpus* and *P. acadianus*, the latter of which is restricted to coasts north of Cape Cod (Buss and Yund 1989, Cunningham et al. 1991). *P. longicarpus* is a relatively small hermit crab species, reaching adult sizes less than half that of *P. acadianus* and *P. pollicaris*, and consequently occupies relatively small gastropod shells, such as *Cantharus cancellarius, Ilyanassa obsoleta, Littoraria irrorata, Littorina littorea, Nassarius* spp., and

Urosalpinx spp. (Wilber and Herrnkind 1982, 1984; Buss and Yund 1988; Buss and Yund 1989; McDermott 2001; Ferrell 2004b) *P. acadianus* occupies primarily large *Littorina littorea* shells (Buss and Yund 1989, Yund and Parker 1989, Folino 1993) as well as that of whelks, such as *Thais lapillosa* and *Buccinum undatum*, and the moon snail *Polinices duplicatus* at some sites (Yund and Parker 1989, Grant 1963, Grant and Ulmer 1974). *P. pollicaris* adults occupy a much larger range of gastropod shell species including large conchs and whelks, such as *Busycon* spp., *Fasciolaria* spp., *Melongena corona*, *Phyllonotus pomum* and *Polinices duplicatus* (e.g., Karlson and Shenk 1983, Shenk and Karlson 1986, Ferrell 2004b).

Hydractinia colonies are gonochoric and polymorphic, possessing specialized reproductive polyps, or gonozooids, on which the gametes are produced. Sexual reproduction occurs during broadcast spawning events in which gametes are released in response to light cues (Bunting 1894, Ballard 1942, Levitan and Grosberg 1993). Zygotes develop into crawling planula larvae that recruit to hermit crab-occupied shells (Yund et al. 1987). Upon successful recruitment, planulae metamorphose and grow into colonies by asexually producing repeated structural units (polyps) connected by intervening stolons. When two or more colonies recruit to the same shell, the colonies most likely will grow into contact and compete, unless one colony dies first. Contact between unrelated conspecifics usually elicits a well-characterized agonistic response (Ivker 1972, Buss et al. 1984). The ability to mount an agonistic attack (i.e., competitive ability) depends directly on colony growth form (Buss and Grosberg 1990; Figure 1). Some individuals grow solely by expansion of continuous, unbranching tissue (mat phenotypes) whereas others grow primarily by proliferating thin branches of tissue, or stolons (stoloniferous phenotypes). Colonies exhibit the full range of variation between these two morphological extremes. Ectoderm of stolons gradually becomes fused to form an ectodermal mat such that all colonies, regardless of growth form during colony development, converge on the mat phenotype and consist of nearly entirely mat tissue when mature (Frank et al. 2001). Growth form evidently bears a significant genetic basis, as indicated by significant clonal repeatability, in each of the three focal species: *H.* GM (Ferrell 2004a), *H. symbiolongicarpus* (Buss et al. 1984, Buss and Grosberg 1990), and *H. polyclina* (Yund 1991). In agonistic interactions, existing stolons swell due to the recruitment of nematocytes (cells bearing the nematocysts) and discharge of nematocysts (highly specialized stinging organelles) (Buss et al. 1984). These "hyperplastic stolons" (Ivker 1972) discharge nematocysts in an attempt to

competitively exclude the opposing colony via tissue destruction and subsequent overgrowth (Buss and Grosberg 1990). Colonies with greater stolon proliferation (stoloniferous phenotypes) have an increased capacity to mount an agonistic response. Clearly, growth form is a good indicator of competitive ability in laboratory contests between small colonies grown on artificial, flat surfaces (Buss and Grosberg 1990, Ferrell 2004a), and intraspecific competition occurs frequently in the field (Yund et al. 1987; Buss and Yund 1988; Yund and Parker 1989; Yund 1991; Hart and Grosberg 1999; Ferrell 2004b, 2005). To the extent that small-colony interactions predominate in nature and laboratory contests accurately portray competitive encounters, growth form may be under intense directional selective pressure favoring highly competitive growth forms.

Materials and methods

At 13 field sites in the northern Gulf of Mexico and northwestern Atlantic (Table 1), I hand-collected *Hydractinia* colonies while snorkeling or wading in shallow water (0.5–2 m deep) during summer 2003. Colonies were identified to one of three species (*H.* GM, *H. polyclina*, *H. symbiolongicarpus*) by morphometric characters (Buss & Yund 1989) and comparisons of species ranges (Cunningham et al. 1991, Folino & Yund 1998) with site of collection. I selectively sampled only hard substrata, typically gastropod shells occupied by pagurid hermit crabs, with hydroid growth over at least 80% of the external shell surface.

In the laboratory, each shell was isolated in a small fingerbowl dish (radius = 5 cm). I selected shells occupied by only a single adult colony, as indicated by the continuity of colony tissue along the encrusted surface. Sexually mature, adult colonies were distinguished from juvenile colonies by the presence of ripe gonophores containing either sperm or eggs. For each encrusted shell, I obtained the maximum shell length and width using a segment of monofilament thread (8-lb. test) to trace the contour of the curved external surface. Estimated external shell surface area then was obtained by calculating the ellipsoid area (= π * length/2 * width/2). Focal colonies occupied at least 80%, but typically 100% (or nearly so), of the external shell surface. Gastropod shell and host species, if present, were recorded.

Colonies and host crabs were maintained in aerated wet tables for up to 5 days and fed 2-day-old *Artemia* brine shrimp nauplii (Ocean Star International, Inc., Pro 100) daily. During this

period, typically within 24 hours, I obtained tissue samples to determine colony growth form. Maintaining field-collected colonies, along with their crab hosts, on a diet of *Artemia* nauplii in the laboratory for extended periods of time (>> 5 days) does not alter growth form estimation (simple linear regression fit through origin: growth form estimate *within 24 hours of field collection* = 1.02*growth form estimate *after lab diet*, $F_{1,10} = 62.6$, $P < 0.001$). For each colony, I used a microscalpel to excise five, small portions of colony tissue, each including 5-10 feeding zooids. Thus, tissue explants were standardized for colony size. I then used (8-lb. test) monofilament thread to secure each tissue explant individually to a plain glass microscope slide. Slides were transferred to standard plastic slide boxes (from which the tops and bottoms had been removed to permit water movement) and suspended in an aquarium containing re-circulated 1 μm-filtered seawater (temperature ~18°C, salinity ~28 ppt). Explanted colonies were fed 2-day-old brine shrimp nauplii daily. After seven days of growth, colonies were removed, rinsed thoroughly in 70% ethanol and air-dried. I later counted the number of peripheral stolon tips (Figure 1), an indicator of colony growth form (Ferrell 2005).

A secondary determinant of competitive ability is biomass growth rate, or the rate of production of mat and stolon tissue (Ferrell 2004a). Biomass growth rate was estimated from the surface area covered by new tissue growth of explanted colonies. The mat and total stolon surface area (defined as the surface area generated by connecting peripheral stolon tips, which includes inter-stolon gaps) was obtained for each colony using SigmaScan Pro 4 software and a Nikon camera (60 mm lens) interfaced to a computer. I then estimated stolon biomass surface area, which does not include inter-stolon gaps [stolon biomass SA = 0.12*(stolon tips) + 0.07*(total stolon SA); $F_{2,32} = 124.5$; $R^2 = 88.6\%$; $P < 0.001$], and calculated total biomass growth rate by summing the mat and stolon biomass surface areas standardized by growth period (i.e., seven days).

Additional shells and colonies were collected while at each field site to determine the frequency with which juvenile or sexually mature colonies co-occurred on single shells. To estimate the frequency of co-occurring juvenile colonies, I haphazardly collected at least 100 hermit crab-occupied shells per site that did not exhibit extensive hydroid coverage. Because extremely small colonies, likely new recruits, can be overlooked very easily, each shell was closely scrutinized under a dissecting microscope. I noted the presence of one or more colonies per shell and the reproductive status (juvenile or mature) of each colony. Shells with hydroid

growth on > 80% of the external surface, which sometimes consisted of two or more mature colonies separated by a distinct inter-clonal boundary (Ferrell 2005), also were considered in the estimation of the frequency of co-occurring mature colonies. The number of these mature colonies was recorded and, along with other available information on the incidence of mature colonies gained from the haphazard sampling of hermit crabs without regard to hydroid coverage, used to calculate the proportion of shells harboring two juvenile colonies, two mature colonies, or one juvenile and one mature colony. A contingency χ^2 test was performed to test whether the frequency with which each of these types of co-occurrences was observed depended on species. Then, for each species, a goodness-of-fit χ^2 test was used to test whether co-occurrences between colonies of alike sexual status were observed more frequently than expected by chance. Null expectations for the frequency of the three possible types of co-occurrence – juvenile-juvenile, juvenile-mature, and mature-mature – were based on the overall frequency of juvenile (p) and mature (q) colonies on doubly-colonized shells. Expected frequencies were as follows: juvenile-juvenile = p^2, juvenile-mature = 2pq, mature-mature = q^2.

One-way analysis of variance (ANOVA) was performed to examine interspecific differences in mean growth form. The overall relationship between growth form and external shell area was examined using species as a categorical variable in an analysis of covariance (ANCOVA). A similar analysis between growth form and external shell area was conducted on a subset of the data in which no variation in gastropod and crab species existed. This subset consisted of only those *H. symbiolongicarpus* and *H. polyclina* individuals found encrusting *Littorina littorea* shells inhabited by *Pagurus longicarpus* hermit crabs. Thus, this second test eliminated any influence of shell and hermit crab species, which co-vary with external shell area. Then, for each of the three hydroid species, an individual ANCOVA was conducted on the four main effects (external shell area, gastropod species, host hermit crab species, site of collection). Interaction terms were investigated, when possible, but excluded if $P > 0.25$ (Sokal and Rohlf 1995). If a significant effect of gastropod species was detected, all pairwise comparisons between gastropod species were conducted using a Bonferroni correction. In all cases, an a priori contrast comparing gastropod species with and without a siphonal canal was performed. Square-root transformations were performed when necessary to meet model assumptions. All statistical tests were performed using SAS, Version 9.1 software.

Results

The three species each exhibited considerable growth form variation among genotypes (Figure 2). Variation in the mean number of peripheral stolon tips differed among species (Bartlett's test statistic = 12.9, P = 0.002), and was least in *H.* GM (variance = 39.2, range = 0-32.0), intermediate in *H. symbiolongicarpus* (variance = 66.6, range = 0-37.2), and greatest in *H. polyclina* (variance = 81.1, range = 0.5-51.2). A square-root transformation was performed to achieve homoscedasticity, and significant differences in mean growth form were detected among species ($F_{2, 334} = 36.7$, $P < 0.001$); all Bonferroni-corrected pairwise comparisons among the three species were statistically significant. Mat-like growth forms predominated in *H.* GM and were least abundant in *H. polyclina.* Conversely, highly stoloniferous colonies were most and least abundant in *H. polyclina* and *H.* GM, respectively. *H. symbiolongicarpus* exhibited a more even distribution of mat-like and more stoloniferous morphologies. *H.* GM, *H. symbiolongicarpus*, and *H. polyclina* exhibited overall means of 7.6, 10.9, and 17.1 stolon tips, respectively. Broad-sense heritability estimates of growth form, as indicated by the mean number of peripheral stolon tips, were 23.5% (*H.* GM), 21.1% (*H. symbiolongicarpus*), and 21.4% (*H. polyclina*). Growth form frequency distributions appeared to be multi-modal in all three species (Figure 2). To exclude the possibility that distinct abundance peaks represented different cryptic species, standardized experimental matings between *H.* GM colonies of known parental growth form were conducted. Results provided no evidence of differential reproductive success in matings between dissimilar growth forms relative to matings between alike growth forms (see Appendix 1).

Host species included pagurid hermit crabs – *P. acadianus, P. longicarpus,* and *P. pollicaris* (Figure 3). In addition to those colonies reported in Figure 2, eight *H. polyclina* colonies were found encrusting other substrata at Glen Cove, ME (7 rocks, 1 live *Littorina littorea* snail). Some colonies were found encrusting cobble or shells that were uninhabited at the time of collection. *H. symbiolongicarpus* associates primarily with *P. longicarpus* (Buss and Yund 1989) and occasionally with small *P. pollicaris* individuals; in this study only two of the 143 H. *symbiolongicarpus* colonies occupied *P. pollicaris*-inhabited shells. Mean external shell area of *P. longicarpus*-inhabited shells varied between sites, ranging from approximately 300-600 mm^2. Substantial variation in external shell area of colonized shells was observed in *H. symbiolongicarpus* ($\sigma^2 = 111957$, range = 85-1545 mm^2). *P. longicarpus* hosts also inhabited the

vast majority of *H. polyclina*-colonized shells collected in this study. Mean external shell area of colonies among sites with this host species ranged from approximately 900-1200 mm^2. At increasingly northern latitudes, *H. polyclina* commonly associates with *P. acadianus* (Buss and Yund 1989, Folino and Yund 1998). This crab species hosted hydroid colonies at Glen Cove, ME, the most northern site included in the present study (Table 1). *P. acadianus*-inhabited shells provided notably less external shell area than *P. longicarpus* shells found at Glen Cove as well as all other *H. polyclina* sites. Variance in gastropod size observed in *H. polyclina* (σ^2 = 52854, range = 248-1863 mm^2) was less than that observed in *H. symbiolongicarpus*. In contrast to *H. symbiolongicarpus* and *H. polyclina, H.* GM exhibited much greater variation in shell size: σ^2 = 2709316, range = 103-7064 mm^2. *H.* GM colonies commonly associated with both *P. longicarpus* and *P. pollicaris*, although the occurrence of host species varied among sites. Turkey Point, FL (TP) yielded a preponderance of *P. longicarpus* hosts whereas *P. pollicaris* hosts dominated at St. Joseph Bay, FL (SJB). Only *P. pollicaris* was observed at St. Andrew's Bay, FL (SAB). Mean external shell area reflected differences in crab host size. Mean external shell area for *P. longicarpus*-inhabited shells with *H.* GM was similar to that of shells colonized by the other two hydroid species. Shells inhabited by the much larger flat-clawed crab, *P. pollicaris*, were much larger than all other colonized shells. Overall, significant differences in the external shell area of colonized shells were detected among hydroid species ($F_{2,338}$ = 83.7, $P < 0.001$).

The three species overlapped in external shell area over the range of 0-2000 mm^2 (Figure 4). Within this size range, all species exhibited a positive relationship between the mean number of peripheral stolon tips (growth form) and external shell area. Although the strength of the positive association, as indicated by slope, did not differ among species, colony growth form differed significantly among species (Table 2, Figure 4). The external area of shells observed in *H.* GM extended beyond this overlapping range up to approximately 7000 mm^2. The similar analysis conducted on a subset of these data in which no variation in gastropod shell or crab host species existed yielded different results. Whereas differences in growth form between species remained detectable, no relationship between growth form and external shell area was detected (Table 3). Thus, the overall positive relationship between external shell area and growth form is likely attributable to other co-varying factors.

When analyzing individual species, statistical significance of the main effects of gastropod species, crab host species, and collection site varied among hydroid species. In *H.* GM, all three main effects were significant (Table 4). Pairwise comparisons (Table 4a) among gastropod shell species revealed that growth form of colonies found on the crown conch *Melongena corona* were significantly more stoloniferous than that found on two other shell species, *Cantharus cancellarius* and *Urosalpinx perrugata* (Bonferroni adjusted $\alpha = 0.0006$). *H.* GM colonies associated with *P. pollicaris* encrusted larger shells (Figure 3) and were significantly more stoloniferous than those encrusting *P. longicarpus*-inhabited shells (Table 4, Figure 5), and growth form also varied significantly among sites (Table 4) that varied widely in shell composition (Figure 6). *H.* GM specimens encrusted a total of 13 different gastropod species. In *H. symbiolongicarpus*, an effect of host crab species was not investigated because all but two individuals associated with a single host species, *P. longicarpus*. The two colonies associated with *P. pollicaris* were excluded from analyses. Growth form and shell composition (Table 5, Figure 7) varied significantly among sites in this species as well, but evidence of differences among gastropod shell species was marginally non-significant (Table 5). *H. symbiolongicarpus* specimens colonized a total of 5 different gastropod species. Analysis of *H. polyclina* revealed a significant association between growth form and host crab species but not site or gastropod species (Table 6). Colonies associated with *P. acadianus* inhabited smaller shells (Figure 3) than conspecifics at the same and other sites, and exhibited less stoloniferous growth forms than those associated with *P. longicarpus* (Figure 9). *H. polyclina* specimens encrusted only 2 different gastropod species (Figure 8), and one species, *Littorina littorea* predominated (> 90% of colonized shells) at 3 of 4 sites. In each of the single species analyses, none of the a priori contrasts comparing growth form of colonies on shells with and without a siphonal canal were significant (all $P > 0.05$).

One-way ANOVA detected overall differences among hydroid species in the external shell area of colonized shells ($F_{2,313} = 50.4$; $P < 0.0001$). Pairwise comparisons (Bonferroni adjusted $\alpha = 0.0167$) showed that all three species differed from each other. *H.* GM encrusted the largest (mean = 1691 mm^2) and most variable shells (range: 6961 mm^2). *H. symbiolongicarpus* and *H. polyclina* exhibited a similar range of variation (1460 vs. 1421 mm^2), but *H. symbiolongicarpus* encrusted significantly smaller shells (mean = 454 mm^2) than *H. polyclina* (mean = 1066 mm^2). ANOVA also indicated that the three hydroid species differed

with respect to mean gastropod species richness per site (*H.* GM = 7.7, *H. polyclina* = 1.75, *H. symbiolongicarpus* = 2.5; $F_{2,10} = 7.5$, P = 0.01). Pairwise comparisons (Bonferroni adjusted α = 0.0167) detected differences between *H.* GM and both Atlantic species but not between *H. polyclina* and *H. symbiolongicarpus*.

Interspecific differences in mean biomass growth rate differed also were detected (Table 7). Respectively, *H.* GM, *H. symbiolongicarpus*, and *H. polyclina* exhibited mean total biomass growth rates of 1.23, 1.70, and 2.54 mm^2/d. Growth rates of the two components of total biomass, mat and stolon tissue, also differed among species when considered individually. When considering mat tissue growth only, *H.* GM grew significantly slower than the other species, and when exclusively considering stolon tissue, *H. polyclina* exhibited a significantly higher growth rate. Thus, *H. symbiolongicarpus* exceeded *H.* GM in mat tissue growth but fell short of *H. polyclina* in stolon tissue growth.

Observed frequencies with which an individual gastropod shell was encrusted by two colonies of the same (both juvenile or both mature) or different (one juvenile, one mature) sexual status differed among species (Contingency $\chi^2_{\text{calc, df=4}} = 19.4$, P < 0.001). Co-occurring juvenile colonies were observed most frequently in *H. polyclina* and least frequently in *H.* GM whereas co-occurring mature colonies were observed most frequently in *H.* GM and least frequently in *H. polyclina* (Figure 10). Both juvenile and mature co-occurrences were observed with intermediate frequency in *H. symbiolongicarpus*. Goodness-of-fit χ^2 tests performed within each species revealed that co-occurrences in *H.* GM and *H. symbiolongicarpus* differed from random expectations (Table 8). Co-occurrence of colonies of different sexual status was observed less frequently than expected. That is, an overabundance of co-occurring mature and co-occurring juvenile colonies was observed in each of these species. However, whereas co-occurring mature and co-occurring juvenile colonies occurred with nearly equal frequency in *H. symbiolongicarpus*, the number of shells with co-occurring mature colonies far exceeded the number of shells with co-occurring juvenile colonies in *H.* GM. Observed co-occurrences did not differ from random expectations in *H. polyclina*.

Discussion

Environmental predictability and interspecific differences in competitive ability

The immediate ecological context in which intraspecific competition occurs in *Hydractinia* includes its hermit crab host and gastropod shell species, each of which may influence the persistence of hydroid growth forms differentially within species. However, the probability that individual recruits experience similar or widely variable contexts varied among the three hydroid species investigated. Species experiencing more predictable microenvironments in which gastropod shells consistently promote interactions between small, juvenile colonies experience greater uniformity in the conditions under which competition ensues (i.e., whether it involves juvenile, typically smaller, colonies or mature, typically larger, colonies). Shell size and morphology-specific recruitment patterns (e.g., Yund et al. 1987) – and not gastropod species diversity, per se – underlie the consistency of the competitive environment. Differences in relative microenvironmental uniformity corresponded to interspecific differences in predominant growth forms. Table 9 summarizes the observed interspecific differences in environmental heterogeneity (gastropods and hermit crabs), frequency of co-occurrence of two juvenile or two mature colonies on single shells, and competitive ability. Hydroid species experiencing greater gastropod species uniformity and predominance of shell species promoting interactions between small, juvenile colonies, such as *H. polyclina* and to a lesser extent *H. symbiolongicarpus*, exhibited competitively superior, more stoloniferous growth forms and higher growth rates. Conversely, *H.* GM colonized a diverse array of gastropod shells varying widely in terms of species, size, and morphology, fostering interactions between hydroid colonies ranging widely in size: this hydroid species exhibited less stoloniferous growth forms and lower growth rates.

Of the three species, *H. polyclina* experienced the most strikingly consistent gastropod environment, colonizing primarily *L. littorea* shells. At 3 out of 4 sites this gastropod species accounted for more than 90% of colonized shells (Figure 8). Other sites at which *H. polyclina* is common reveal similar trends. Folino (1993) reported that 97% of *H. polyclina*-encrusted shells at the Isle of Shoals were *L. littorea*, Yund and Parker (1989) reported that *L. littorea* accounted for 80-95% hydroid-encrusted shells at all but one (65%) of eight sites in the Gulf of Maine, and Buss and Yund (1989) found 100% *L. littorea* across four *H. polyclina* sites, ranging from

Nahant, MA northward to Stardbord, ME. Thus, the predominance of a single gastropod shell, *L. littorea*, is widespread in *H. polyclina* populations. Hermit crab-inhabited *L. littorea* shells accrue multiple hydroid recruits (Yund and Parker 1989; Yund 1991; this study, Figure 10) but provide only a single site for successful larval recruitment (Yund et al. 1987, Buss and Yund 1988, Yund and Parker 1989). This microenvironmental context sets the stage for overgrowth competition between juvenile conspecifics and strong directional selection on competitive ability. Fittingly, single shells bearing two juvenile colonies were observed most frequently in *H. polyclina* (Figure 10), and this species displayed a preponderance of highly stoloniferous growth forms relative to the other two species. A more detailed inspection of growth rates according to tissue type (mat vs. stolon) shows that *H. polyclina* allocates more resources to stolon growth relative to *H.* GM and *H. symbiolongicarpus* and exhibits the greatest growth rate in terms of stolon (but not mat) tissue (Table 7).

In contrast to *H. polyclina*, *H.* GM experienced little gastropod uniformity in terms of species as well as shell size and morphology. *H.* GM encrusted a total of 13 different gastropod species and more than three times as many species per site, on average, than both *H. symbiolongicarpus* and *H. polyclina* (Table 9). Of these three species, *H.* GM exhibits the highest incidence of competitive standoffs between adult colonies (Ferrell 2005). Accordingly, in the present study, mature conspecifics co-occurred more frequently in *H.* GM than in *H. symbiolongicarpus* and *H. polyclina*, while co-occurring juvenile colonies were observed least frequently in this species (Figure 10). Colonies encrusting a single shell will encounter one another as each proliferates asexually, unless one colony dies prior to contact. Colonies may be most likely to die prior to contact on larger shells, simply as a result of the potentially greater distance separating colonies. Thus, the low co-occurrence rate observed between juvenile, typically smaller, colonies may overestimate the number of co-occurring colony pairs that will engage in future competition in *H.* GM, which encrusts much larger gastropod species (in addition to small species). As a result, directional selection on competitive ability, stemming from known competitive outcomes between small, similarly sized colonies (Buss and Grosberg 1990, Ferrell 2004a), may be relaxed in many *H.* GM populations. Moreover, the potential for size differences between competing colonies, as a result of temporally distinct recruitment events, is magnified on larger shells. A size advantage often enables inferior competitors to withstand overgrowth from hydroid competitors (McFadden 1986), and thus may further obscure

directional selection on competitive ability in species colonizing larger gastropod substrata. Consistent with this ecological characterization and its consequences for the relative strength of selection on competitive ability, competitively inferior growth forms were most abundant in *H.* GM (Figure 2).

Growth form of single colonies on shells of variable size/morphology

Larger shells provide larger targets for planular recruitment and may be more likely to accrue multiple recruits. As a result, a positive relationship between shell size and hydroid competitive ability (growth form) may be expected because single colonies found on larger shells are more likely to be survivors of prior competitive bouts. Although shell size indeed exhibited an overall positive relationship with competitive ability in all three hydroid species (Figure 4), ANCOVA analyses within each species showed that this relationship was attributable to other factors: site of collection (*H.* GM and *H. symbiolongicarpus*) and host crab species (*H.* GM and *H. polyclina*). (Gastropod shell species was also a significant factor in *H.* GM, although pairwise comparisons showed that this was driven by a single gastropod species, *Melongena corona*, which exhibited more stoloniferous hydroids when compared to 2 of 12 other gastropod species.) Competitively superior growth forms also may be expected to predominate on gastropod species with shell morphologies bearing two distant sites for successful hydroid recruitment, provided that competitive standoffs (Ferrell 2005) do not result. However, contrasts between gastropod species with shell morphologies providing one vs. two sites for successful hydroid recruitment revealed no differences in growth form between these two morphological classes of gastropods. Temporally distinct recruitment events may allow competitively inferior colonies to persist on shells with 2 recruitment sites in those instances in which inferior morphs recruit first and attain a sufficiently large size advantage (McFadden 1986). Regardless, these combined results provide no evidence for a within-species relationship between shell characteristics that may foster multiple recruitment (target size, shell morphology) and growth form of constituent hydroids, and instead suggest that differences between sites and host hermit crabs best explain the observed intraspecific variation in growth form.

Directional selection on competitive ability and morphological divergence among species

Directional selection on competitive ability likely exists in *Hydractinia* species, based on the results of field observations, laboratory experiments, and genetic studies. Intraspecific contacts between juvenile colonies, which end in the death of less stoloniferous individuals (Buss and Grosberg 1990, Ferrell 2004a), are known to occur commonly in the species studied here (Yund et al. 1987; Buss and Yund 1988; Yund and Parker 1989; Yund 1991; Hart and Grosberg 1999; Ferrell 2004b, 2005; this study, Figure 10). The little that is known regarding the genetics underlying the expression of growth form also suggests that competitive ability is under strong selection. Additive genetic variance for growth form in a small *H. symbiolongicarpus* laboratory population was present but low relative to that generally observed in morphological traits (Blackstone and Buss 1991). This may be due to growth form's close genetic ties to life history traits, which are typically exposed to stronger selection and consequently exhibit low levels of additive genetic variation (Mousseau and Roff 1987, Roff and Mousseau 1987, Roff and Emerson 2006; but see Houle 1992). Directional selection can cause morphological divergence (Rieseberg et al. 2002), and the results presented here are consistent with different strengths of selection among species driven by interspecific differences in the commonness of juvenile conspecific interactions and consistency of the gastropod microenvironmental context in which competition ensues (Table 9).

Interspecific differences in gastropod environment modulate only the strength of selection on competitive ability, not its direction. Thus, gastropod environment does not explain what is maintaining less stoloniferous growth forms in natural populations. Mat-like phenotypes were always present, and in 2 of the 3 species studied here, these individuals were most abundant (Figure 2). Interspecific differences in the amount of growth form variation maintained in natural populations were due solely to the occurrence of increasingly stoloniferous growth forms in some species, and were proportional to the relative strength of selection on competitive ability, which may extend the range of variation to include increasingly stoloniferous, in addition to mat-like, individuals. In a common garden laboratory study with colonies grown individually on large artificial surfaces, Yund (1987) observed a positive phenotypic correlation between size at first reproduction and competitive ability (i.e., inferior competitors mature at smaller size), and suggested that this trade-off maintains growth form variation in *H. polyclina*. This association was observed only in male colonies, as females did not mature sexually within the timeframe of the study. At this point, the sex ratio of colonies that had attained sexual maturity was 79:5

(male:female), suggesting that males mature more quickly in this laboratory environment. However, field experiments with isolated hermit crab hosts and small gastropod shells (representative of those most frequently encountered in nature) provide no evidence for a genetic trade-off between competitive ability and a suite of life history characters, including size at first reproduction (Chapter 2). In the laboratory (e.g., Ferrell 2004a), positive genetic correlations between competitive ability and reproductive investment or growth rate are commonly observed. Rather than serving as an explanation for the maintenance of growth form variation, the absence of genetic correlations in the field and significant, but positive, genetic correlations in the laboratory makes its explanation more problematic.

Hermit crab hosts and the maintenance of growth form variation within species

Hermit crab hosts significantly influence growth form variation. In this study, host species were strongly associated with different growth forms (Table 4, Figure 5; Table 6, Figure 9). Smaller hosts harbored primarily mat-like growth forms whereas larger hosts associated primarily with more stoloniferous morphs. Foremost among ecological and behavioral differences between these hosts is the natural densities at which they occur, which may influence the persistence of mat-like and stoloniferous hydroids differentially through divergent disturbance regimes.

Several aspects of the behavioral and population ecology of hermit crabs, especially *P. longicarpus*, likely inflict significant disturbance to shell epibionts, including hydroids. Hermit crabs depend on shells, and some epibionts, for protection, primarily from predators (e.g., Ross 1971, Wright 1973, Hazlett 1981, Scully 1983a, Brooks and Mariscal 1985, Buckley and Ebersole 1994, Weissberger 1995), and individuals must acquire new, larger shells as they grow and molt. Prior to entering a new shell, hermit crabs typically exhibit detailed inspections involving manipulation of the shell and insertion of appendages in and around the shell aperture (e.g., Scully 1986). Populations are universally shell-limited, either in terms of shell quantity or quality, and therefore alternate shells are in demand but already occupied by other crabs (Hazlett 1981, Scully 1983b). Most individuals in hermit crab populations consist of small crabs in shells larger than required, thus the great majority of shell exchanges involve “shell fights” initiated by larger crabs (reviewed in Hazlett 1981). Although shell exchange is a common outcome, encounters can be prolonged and end in no shell exchange as a non-initiating crab is unlikely to

vacate its shell unless it also would gain in shell adequacy from the exchange (Hazlett 1978, 1981; Scully 1983a,b). In addition to those occurring during shell exchange events, agonistic interactions commonly ensue over limited detrital food resources (Ramsay et al. 1997), and male hermit crabs aggressively compete for access to females (Hazlett 1981); in both of these cases, larger crabs generally dominate. In each of these cases (procuring and defense of shells, competition for food, and competition for female mates), physical disturbance is imposed on shell epibionts and smaller crabs generally experience more frequent and prolonged crab-mediated disturbance. Moreover, the frequency of aggressive physical encounters increases with crab density (e.g., Hazlett 1968, Ramsay et al. 1997), although acclimation to neighboring crabs, and decreased aggression, may occur in groups in which particular individuals repeatedly interact (Hazlett 1981, Scully 1983).

Mechanical disturbance experienced in hermit crab populations can influence growth of encrusting hydroid symbionts and thereby influence the duration (and sometimes outcome) of interspecific competitive encounters (Van Winkle et al. 2000). However, crab-mediated disturbance may exert its greatest impact on hydroid population structure and growth form abundance by influencing colony survival. Adult hydroids encrusting hermit crab-inhabited shells generate a tough chitinous skeleton through which ridged spines project, thereby protecting the underlying ectodermal mat tissue and zooids (when fully retracted). Thus, abrasion of the shell surface by the rigid exoskeleton or occupied shell of interacting hermit crabs from the general population may damage individual zooids or portions of tissue but most likely does not impact survival of the colony as a whole. However, high population densities of hermit crabs coincide with peak *Hydractinia* recruitment (Buss & Yund 1988, Yund & Parker 1989), and strong effects on hydroid recruitment and early growth and development should be especially important in determining *Hydractinia* abundance patterns in natural populations. Unlike adult colonies, which exhibit a chitinous spiny skeleton in addition to soft tissue, new hydroid recruits exhibit easily damaged soft tissue only that remains exposed until the colony becomes well established and produces substantial skeletal material. Small juvenile recruits represent a critical life stage which must pass through a mortality bottleneck mediated through two primary factors: (1) physical disturbance, intensified in dense hermit crab populations, and (2) competition with closely settling conspecifics, which occurs with variable frequency among hydroid species.

High host crab density reduces the number of feeding zooids borne by a colony and results in less stoloniferous phenotypes in *Hydractinia* (see Chapter 2). Van Winkle et al. (2000) similarly found this to be the case in a study of two hydractiniid hydroid species, *Podocoryna carnea* and *H. symbiolongicarpus*, when colonies were grown on *L. littorea* shells, although different results were obtained when grown on glass slides exposed to hermit crabs. Impacts on colony size and growth form may be due to several factors. Hermit crabs may mechanically disturb hydroid colonies by inadvertent removal of tissue during aggressive interactions between crabs or while scavenging on food items garnered by the hydroid that may be too large for individual zooids to ingest. *P. longicarpus* uses a variety of feeding methods to opportunistically utilize diverse resources (reviewed in Whitman et al. 2001) and does not rely entirely on detritus as its sole source of nutrition. Direct predation on hydroid tissue by hermit crabs occurs occasionally (Wright 1973), and likely occurs most frequently in food-limited, dense host populations. Other hydroid predators, such as hydroid specialist nudibranchs, may be locally abundant at particular *H. polyclina* sites (Rivest 1978; Folino 1993, 1997) but were absent from all sites in the current study, and has been reported absent from most *H. polyclina* sites (Yund 1991). Effects of physical disturbance and predators have been dismissed as unimportant in maintaining diversity in growth form, and therefore competitive ability, in *Hydractinia* (Yund 1991 but see Folino 1985). However, mat-like tissue is more robust than delicate stolon tissue (Van Winkle et al. 2000) and may be crucial in allowing colonies, especially new recruits, to remain attached to the shell surface in the face of disturbance or predation by host crabs.

Hydroid species and genotypes that proliferate primarily using delicate stolon tissue may suffer greater disturbance-mediated mortality in dense hermit crab populations. Other hydractiniid hydroids, such as *P. carnea* and *P. selena,* also encrust hermit crab-inhabited shells but lack mat tissue and grow by means of stolons only. *Podocoryna* larvae are significantly smaller and stolons even more delicate and less robust than that of *Hydractinia*. This may explain why *Podocoryna* colonies only rarely associate with hermit crabs but can be extremely common in live snail populations, such as *Cantharus cancellarius* (Wells 1969, Mills 1976a, D.L. Ferrell, unpublished data). Moreover, *Podocoryna* can be collected consistently in every month of the year except August and September in the northern Gulf of Mexico (Mills 1976a) when hermit crab breeding aggregations peak, and increases in abundance in winter when hermit crab population densities are lowest, at least in the northern Gulf of Mexico (D.L. Ferrell,

unpublished data). Hermit crab species and size classes occur at different characteristic densities. Populations of *P. longicarpus*, a small hermit crab species, can reach extremely high densities, up to 200 crabs/m^2 (Hart and Grosberg 1999) at some sites at which *H. symbiolongicarpus* is common and *H. carnea* is rare (Mills 1976a), and small juveniles of larger crab species, including *P. acadianus* and *P. pollicaris* (Grant 1963, Grant and Ulmer 1974), reside in dense populations whereas larger adults are widely spaced. Although always rare compared to mat-producing *Hydractinia* species (McFadden 1986; McDermott 2001; Mills 1976a,b), *P. selena* is more abundant on *P. pollicaris*-occupied shells compared to that of *P. longicarpus* (Mills 1976b). In the current study, less stoloniferous *H.* GM colonies were found disproportionately in association with the small *P. longicarpus* species (Figure 5). While *P. acadianus*-associated shells were colonized by less stoloniferous phenotypes than those associated with *P. longicarpus* at one *H. polyclina* site (Figure 9), these *P. acadianus* individuals represented extremely small size classes that inhabited smaller shells than sympatric *P. longicarpus* (Figure 3). In contrast to the small individuals encountered in this study, adult *P. acadianus* are comparable in size to *P. pollicaris* and occupy shells of large whelks and moon snails (Grant and Ulmer 1974). My findings suggest that *H. polyclina* encrusting shells occupied by larger *P. acadianus* individuals are more stoloniferous than the colonies surveyed here. Similarly, other *Hydractinia* species that associate primarily with large hermit crab species (e.g, *H. symbiopollicaris* on *P. pollicaris*-inhabited shells or *H. echinata* on *P. bernhardus*-inhabited shells) are likely predominated by highly stoloniferous growth forms.

Disturbance by hermit crabs also may alter competitive outcomes. Interspecific dominance relationships between *H. symbiolongicarpus* and the co-occurring hydroid, *P. carnea,* can be reversed in the presence of hermit crabs (Van Winkle et al. 2000). *Podocoryna* grows very rapidly, and consistently eliminates *Hydractinia* colonies in size-symmetric competitive pairings in the absence of hermit crabs (McFadden 1986); however, in the presence of hermit crabs, interactions are significantly prolonged. Under certain conditions, hindered stolon growth did not allow *Podocoryna* to surround and overgrow *Hydractinia,* and the more physically robust *Hydractinia* survived the competitive onslaught. This reversal was only observed in one *H. symbiolongicarpus* genotype (of unknown growth form) and only with small hermit crab hosts. Similarly, reversals in competitive outcome in *Hydractinia* intraspecific competitive encounters may occur. Competition is costly even to competitively dominant, stoloniferous growth forms

(Ferrell 2004a) and mat-like, competitively inferior phenotypes may be more likely to survive disturbance as a result of a more robust morphology and decreased energetic costs involved in mounting the hyperplastic agonistic response. The likelihood that competing colonies will be subject to significant disturbance by neighboring crabs increases with the duration of competitive encounters. Consequently, effects of disturbance may be less likely to impact competitive outcomes in species with rapid growth rates, such as *H. polyclina*, which likely resolve competitive contests quickly. In general, natural variation in crab population densities within species may create interplay between selection favoring stoloniferous phenotypes in the absence of crab-mediated disturbance and selection favoring robust, mat-like phenotypes at high crab densities. Significant intraspecific growth form variation among sites was observed, which may reflect between-site differences in host density and size structure.

Selection for robustness of colony form should be stronger in hydroid species associating exclusively with small vs. large host hermit crab species. Whereas *H.* GM associates with *both* small and large crab hosts, *H. symbiolongicarpus* and *H. polyclina* show host specificity (Buss and Yund 1989) and associate with small and large hosts, respectively. In the current study, *H. symbiolongicarpus* associates with smaller hosts occupying smaller shells and indeed consists of more mat-like growth forms relative to *H. polyclina* (Table 9, Figure 3). In addition to associating with larger hosts occupying larger shells, *H. polyclina* experiences greater predictability in its gastropod environment (Table 9), and predominantly colonizes a single gastropod species (*L. littorea*) that enhances selection on competitive ability and likely further increases the differences in growth form between these species. Ultimately, the interspecific differences in growth form observed here and their relationship to the relative strengths of selection on physical robustness and competitive ability among these hydroid species are correlative in nature and must await corroboration through rigorous experiments.

Studies linking diversity and physical disturbance in sessile, hard substratum communities and populations have a long history (Dayton 1971; Connell 1978; Sousa 1979a,b; Sebens 1982; Sousa 1984; Sebens and Thorne 1985; Aronson and Precht 1995; Connell et al. 1997). Building upon Darwin's (1859) original notion that grazing maintains diverse plant communities, ecologists have long recognized the importance of abiotic disturbance and, relatedly, predation (e.g., Paine 1966) in reducing competition and generating colonizable space for new recruits, including competitive subordinates. Although most typically invoked to

explain patterns of species diversity, disturbance can strongly influence genotypic diversity as well (e.g., Sebens 1982b). Disturbance-based explanations for maintaining diversity are most convincing when disturbance or predation selectively impacts competitively dominant individuals most severely, as is the case here in which I hypothesize that hermit crab-mediated disturbance inflicts greater mortality on competitively dominant hydroids. The explanation proposed here for the maintenance of diversity in growth form within these epibiotic hydrozoans builds upon the rich disturbance literature and offers a rare system in which an agent of disturbance also serves as a mutualistic partner.

Summary

Environmental predictability significantly influences growth form variation within and among *Hydractinia* species symbiotic with pagurid hermit crabs. Species-specific differences in the extent to which the gastropod environment promotes interactions between small, juvenile colonies provide a measure of the likelihood that competitive interactions favoring highly stoloniferous growth forms will occur whereas variability in host hermit crab species, population density, and size structure often may favor less stoloniferous, competitively inferior morphs. Interplay between these two different types of microenvironmental variation provides a possible mechanism for understanding the maintenance of growth form variation in this system.

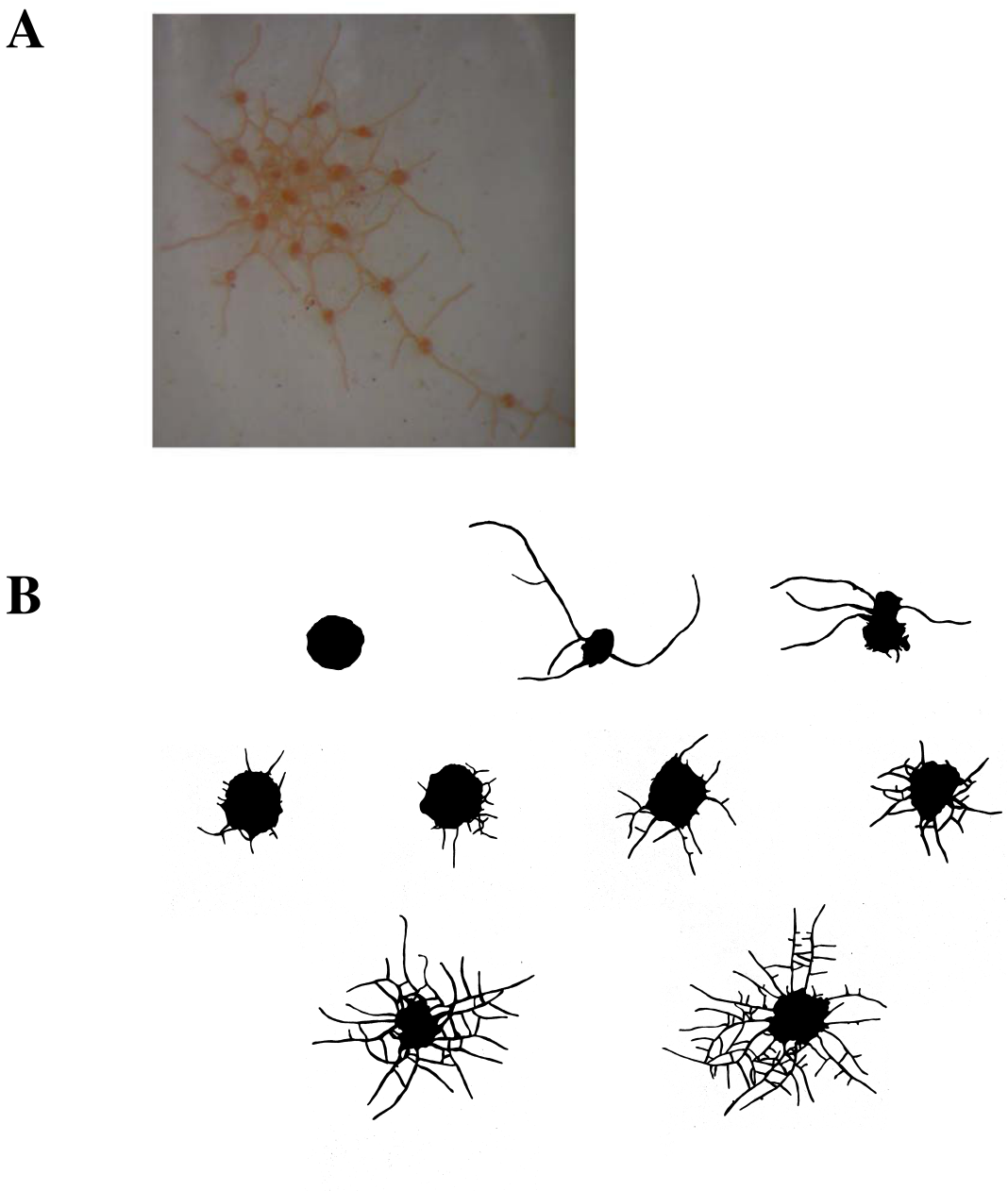

Figure 1. Intraspecific early ontogenetic growth form variation in *Hydractinia*. (A) Photograph of a 31-day-old *H.* GM colony generated by inducing sexually produced planulae to settle on an artificial substrate maintained in the laboratory. (B) Representative tracings of colonies of variable growth form. Upon metamorphosis, each colony initially produces a single primary polyp. Outward stolonal growth then ensues, and genotypes exhibit different patterns of stolon elongation and branching, which account for the variation in growth form observed among individuals in natural populations. The ectoderm of stolons fuses to form ectodermal mat tissue, beginning centrally and then radiating outward, within which an internal stolon network remains imbedded. At sexual maturity, a continuous sheet of ectodermal mat tissue underlies all colonies regardless of early growth form.

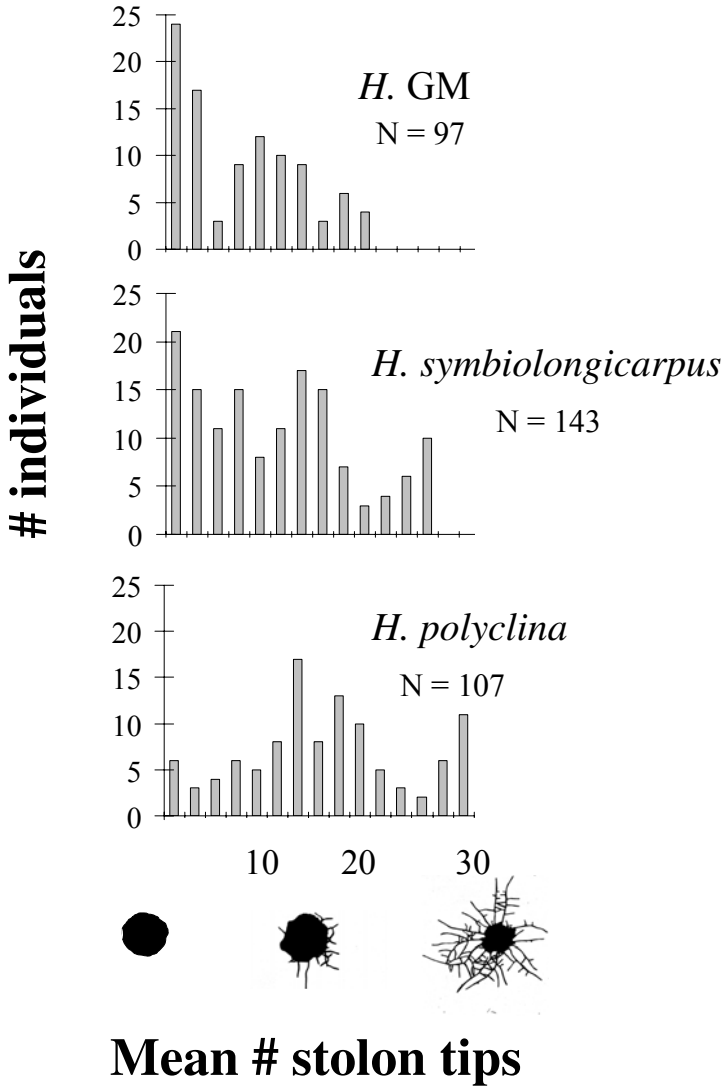

Figure 2. Interspecific growth form variation in three *Hydractinia* species. Growth form estimates represent the mean number of peripheral stolon tips (N = 5 per genotype) exhibited after 7 days of growth in standardized laboratory conditions (Ferrell 2005). *H symbiolongicarpus* and *H. polyclina* are northwestern Atlantic species whereas *H.* GM is found only in the northern Gulf of Mexico. Significant differences in mean growth form exist among all 3 species ($F_{2, 334} = 36.7$, $P < 0.001$). Overall mean number of stolon tips in *H.* GM, *H. symbiolongicarpus*, and *H. polyclina* was 7.6, 10.9, and 17.1 stolon tips, respectively.

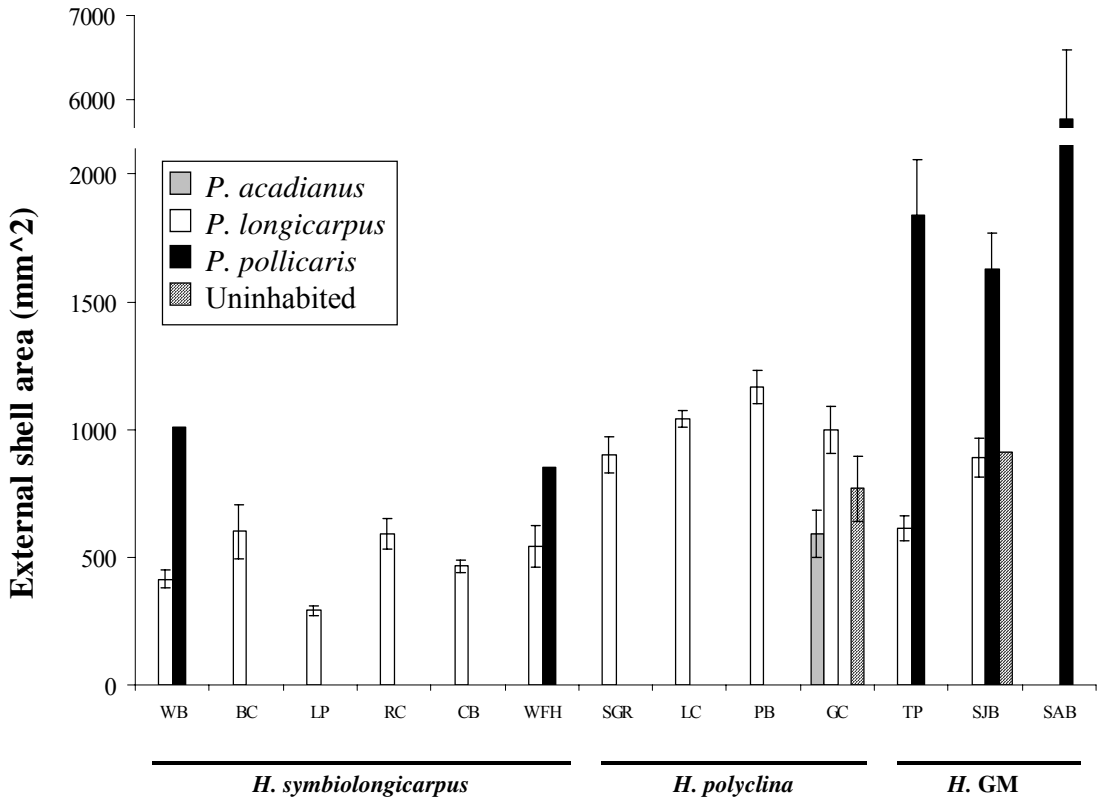

Figure 3. Mean (+/- SE) external surface area of gastropod shells encrusted by by field-collected hydroids among sites of collection. Legend indicates the hermit crab species (*Pagurus* spp.) inhabiting the shell at time of collection, if present. Sample size per species total 143, 99, and 98, respectively, for *H. symbiolongicarpus, H. polyclina,* and *H.* GM. Site abbreviations are given in Table 1. Note break in scale.

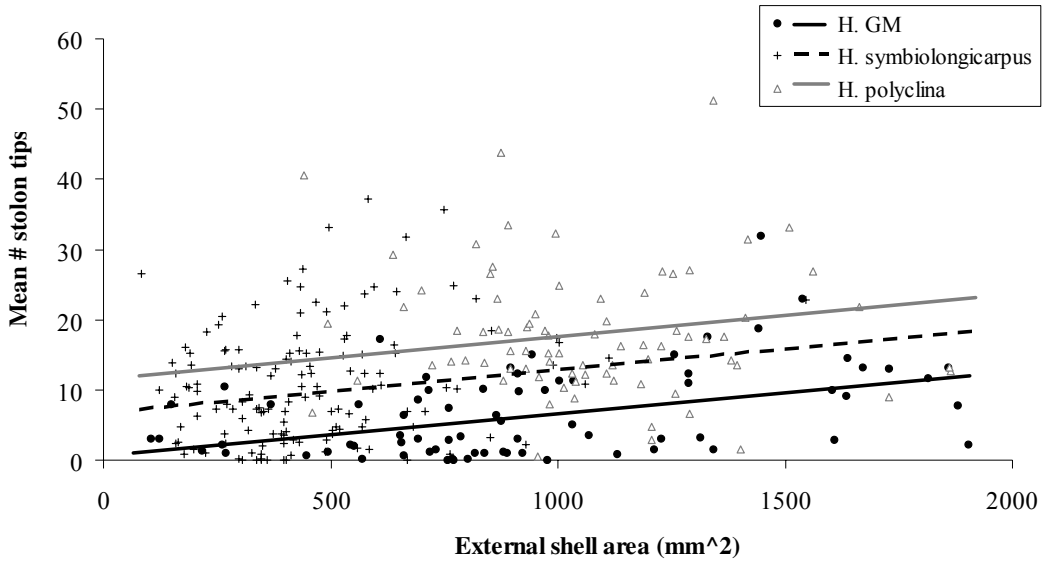

Figure 4. Relationship between colony growth form and external shell area including all individuals from each species. Plotted lines represent ANCOVA results (Table 2) for all three species over the 0-2000 mm^2 range.

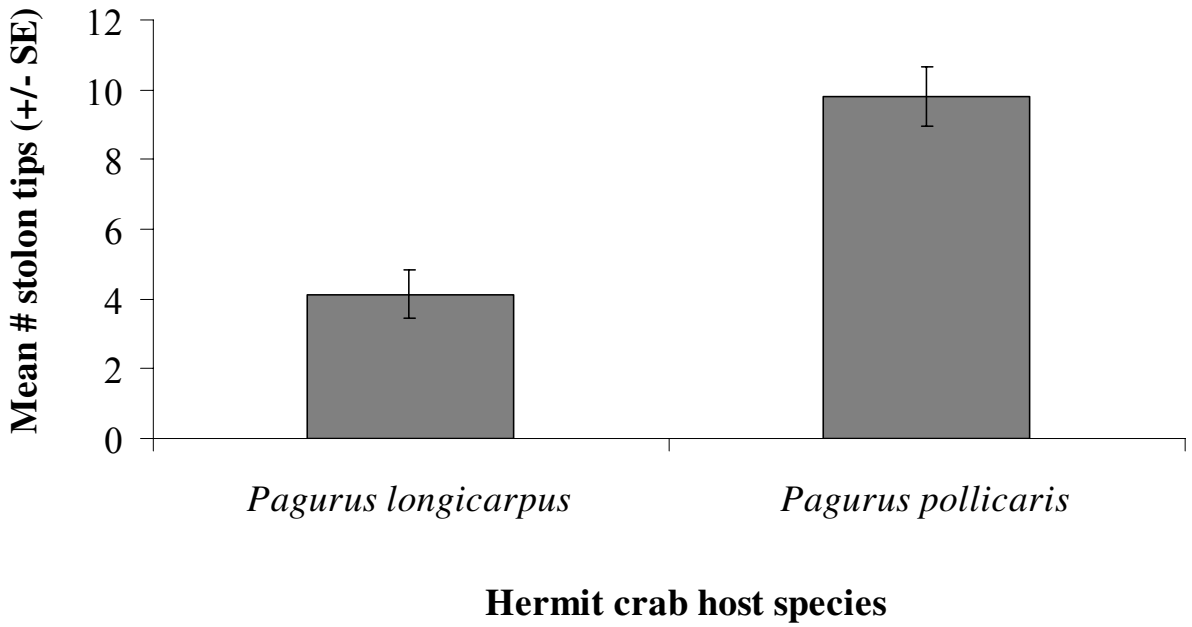

Figure 5. Growth form of *H.* GM colonies associated with different pagurid hosts, the long-clawed hermit crab *Pagurus longicarpus* and the flat-clawed hermit crab *Pagurus pollicaris*.

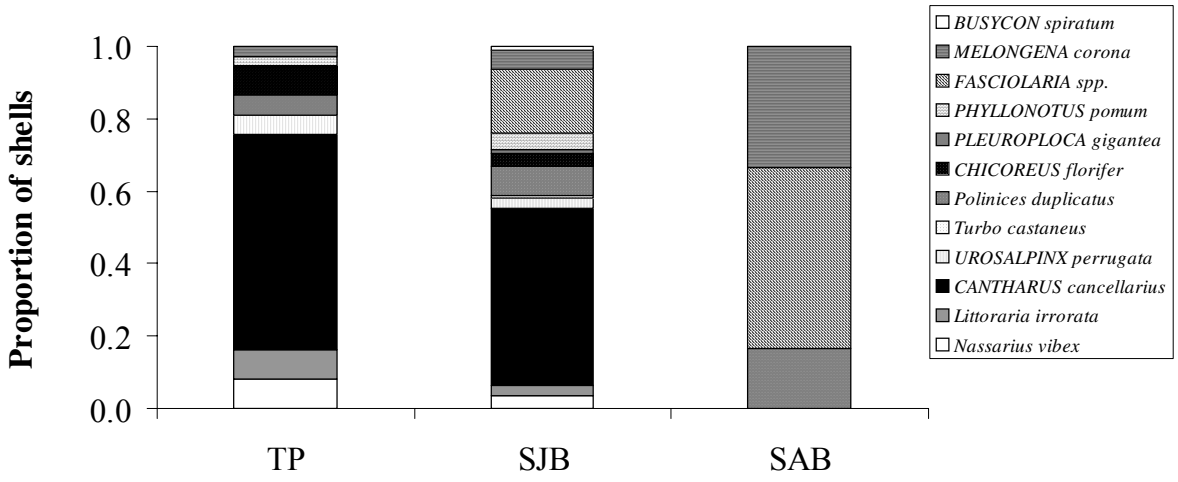

Figure 6. Gastropod shell composition per site of *H.* GM collection. Shell species in legend are listed bottom to top from those smallest to largest in external shell area. Capitalized genera indicate shells that provide two spatially segregated regions of high juvenile survivorship, as indicated by shell morphology. *Fasciolaria* spp. includes *F. lilium hunteria* and *F. tulipa*. TP = Turkey Point, FL; SJB = St. Joseph Bay State Park, FL; St. Andrew Bay, FL. Latitude and longitude are given in Table 1.

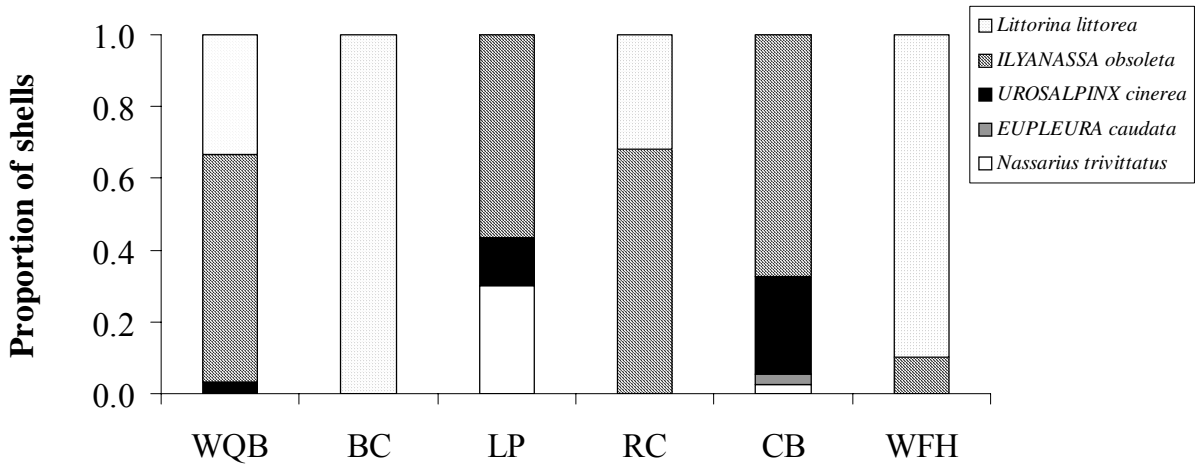

Figure 7. Gastropod shell composition per site of *H. symbiolongicarpus* collection. Shell species in legend are listed bottom to top from those smallest to largest in external shell area. Capitalized genera indicate shells that provide two spatially segregated regions of high juvenile survivorship, as indicated by shell morphology. WQB = Waquoit Bay, MA; BC = Brenton Cove, RI; LP = Lighthouse Point, CT; RC = Round Cove, MA; CB = Cotuit Bay, MA; WFH = West Falmouth Harbor, MA. Latitude and longitude are given in Table 1.

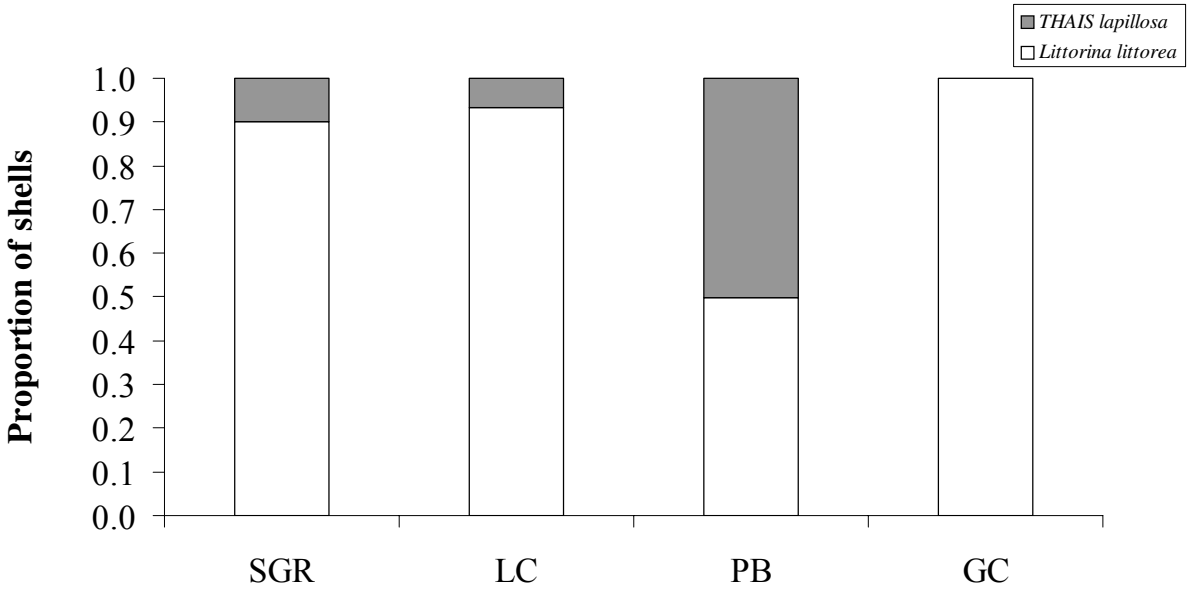

Figure 8. Gastropod shell composition per site of *H. polyclina* collection. Shell species in legend are listed bottom to top from those smallest to largest in external shell area. Capitalized genera indicate shells that provide two spatially segregated regions of high juvenile survivorship, as indicated by shell morphology. SGR = St. George River, ME; LC = Lowe's Cove, ME; PB = Pemaquid Beach, ME; GC = Glen Cove, ME. Latitude and longitude are given in Table 1.

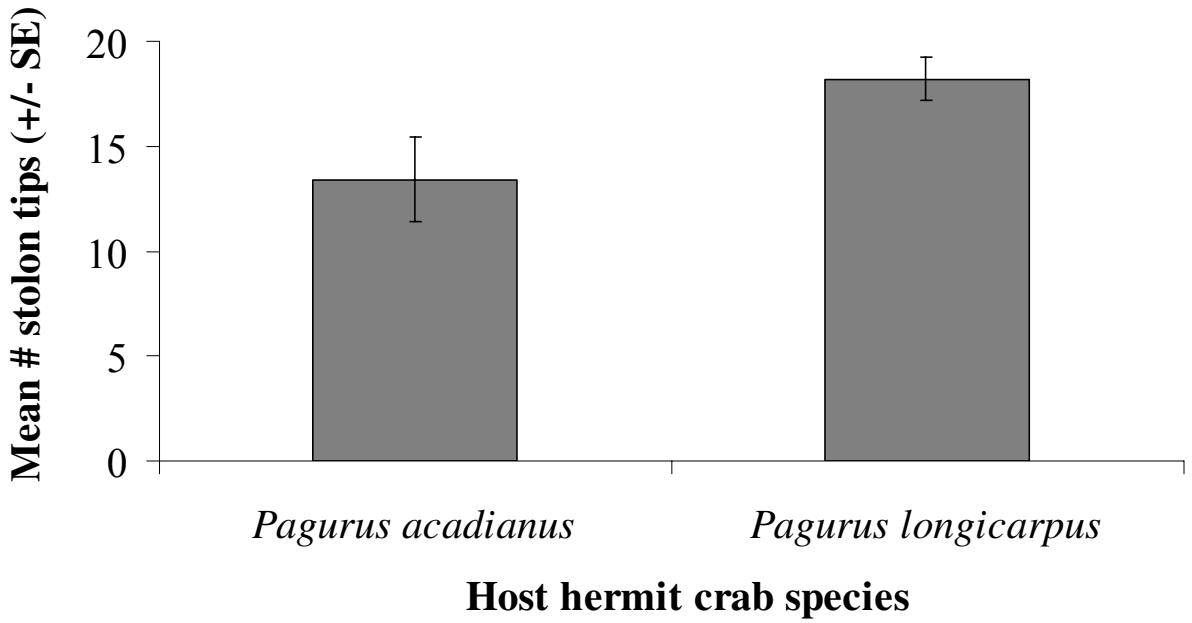

Figure 9. Growth form of *H. polyclina* colonies associated with different pagurid hosts, the acadian hermit crab *Pagurus acadianus* and the long-clawed hermit crab *Pagurus longicarpus*.

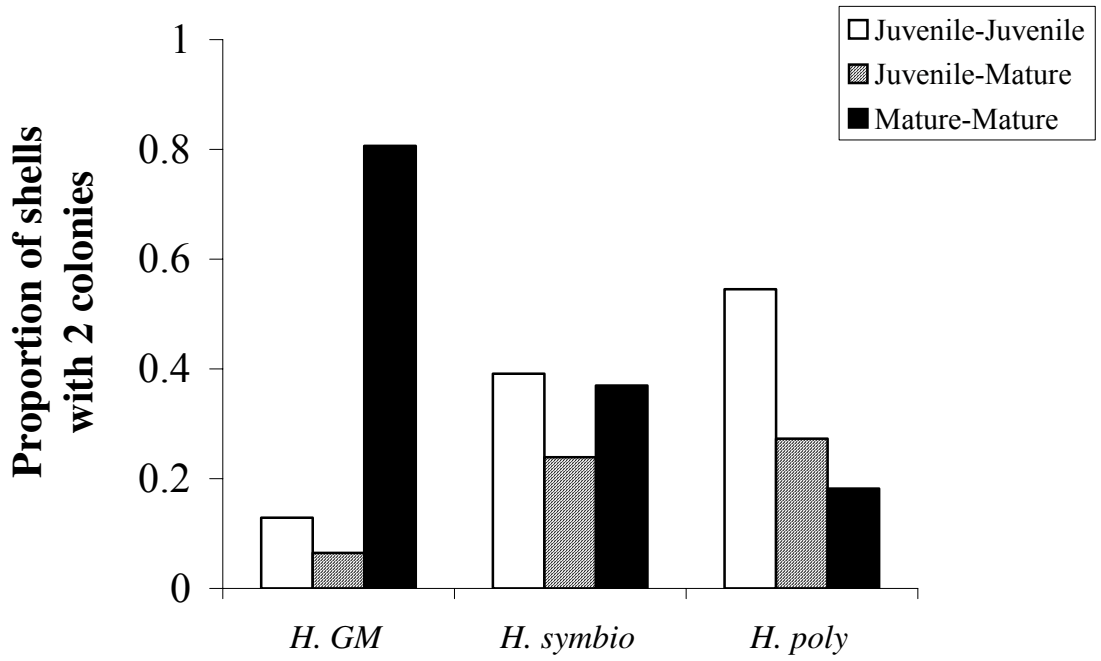

Figure 10. Interspecific differences in the proportion of doubly-colonized shells involving juvenile and mature colonies. '*H. symbio*' and '*H. poly*' refer to *H. symbiolongicarpus* and *H. polyclina*, respectively. The relative occurrence of each type of double colonization depended on species (Contingency $\chi^2_{\text{calc, df=4}} = 19.4$, $P < 0.001$).

Table 1. *Hydractinia* species field collection sites.

Species	*Site*	*Latitude/Longitude*
H. GM[a]	FSUCML[c], Turkey Point, FL (TP)	29°55'N/84°19'W
	NOAA[d], St. Andrew Bay, FL (SAB)	30°'10'N/85°44'W
	St. Joseph Bay State Park, FL (SJB)	29°47'N/85°23'W
H. symbiolongicarpus[b]	Brenton Cove, RI (BC)	41°30'N/71°20'W
	Cotuit Bay, MA (CB)	41°40'N/70°25'W
	Lighthouse Point, CT (LP)	41°13'N/85°23'W
	Round Cove, MA (RC)	41°43'N/70°00'W
	WBNERR[e], Waquoit Bay, MA (WQB)	41°33'N/70°31'W
	West Falmouth Harbor, MA (WFH)	41°'36'N/70°39'W
H. polyclina[b]	Glen Cove, ME (GC)	44°10'N/69°5'W
	Lowe's Cove, ME (LC)	43°55'N/69°35'W
	Pemaquid Beach, ME (PB)	43°47'N/69°30'W
	St. George River, ME (SGR)	44°0'N/69°15'W

[a]Collected May-July, 2003; GM is the designation given by Cunningham et al. (1991) to an undescribed *Hydractinia* species found in the northern Gulf of Mexico

[b]Collected August, 2003

[c]Florida State University Coastal and Marine Laboratory

[d]National Oceanic and Atmospheric Administration facility

[e]Waquoit Bay National Estuarine Research Reserve

Table 2. ANCOVA results examining the relationship between colony growth form and external shell area in three species. Growth form was estimated by calculating the mean number of peripheral stolon tips for five replicates per genotype. *Hydractinia* GM colonies greater than 2000 mm^2 in colony area were excluded due to lack of data overlap with *H. symbiolongicarpus* and *H. polyclina*. The interaction term of the more inclusive model, an indicator slope heterogeneity, was not statistically significant and therefore omitted ($F_{2,309} = 1.6$; $P = 0.21$). *** = $P < 0.001$

Source	df	MS	F-ratio
Shell area	1	747.4	11.9***
Species	2	2176.2	34.7***
Error	311	62.8	

Table 3. ANCOVA results examining the relationship between hydroid growth form and external shell area in a subset of *H. symbiolongicarpus* and *H. polyclina* colonies in which no variation exists in host hermit crab or gastropod shell species. All colonies encrusted *Pagurus longicarpus*-inhabited, *Littorina littorea* shells.
* = $P < 0.05$

Source	df	MS	F-ratio
Shell area	1	5.3	0.1
Species	1	396.9	5.5*
Error	95	72.5	

Table 4. ANCOVA results examining the relationship between *H.* GM growth form and external shell area, host hermit crab species, gastropod shell species and site of collection. Uninhabited shells were excluded. Statistical tests were performed (a) including all gastropod shell species and (b) excluding gastropod shell species represented by only a single datum. † = P < 0.10, * = P < 0.05, ** = P < 0.01

(a)

Source	df	MS	F-ratio
Shell area	1	73.9	3.3†
Host crab	1	113.3	5.0*
Gastropod	12	61.1	2.7**
Site	2	99.9	4.4*
Area*site	2	44.1	1.9
Error	75	22.8	

(b)

Source	df	MS	F-ratio
Shell area	1	26.8	1.3
Host crab	1	83.2	3.9*
Gastropod	9	67.4	3.2**
Site	2	82.1	3.9*
Area*host	1	43.8	2.1
Area*gastropod	9	35.2	1.7
Area*site	2	32.8	1.5
Error	65	21.2	

Table 5. ANCOVA results examining the relationship between *H. symbiolongicarpus* growth form and external shell area, gastropod shell species and site of collection. *Pagurus pollicaris*-inhabited shells were excluded due to small sample size (N = 2). Statistical tests were performed (a) including all gastropod shell species and (b) excluding gastropod shell species represented by only a single datum. † = P < 0.10, *** = P< 0.001

(a)

Source	df	MS	F-ratio
Shell area	1	0.8	0.02
Gastropod	6	79.9	1.5
Site	5	288.1	5.5***
Error	128	52.8	

(b)

Source	df	MS	F-ratio
Shell area	1	8.5	0.2
Gastropod	4	111.8	2.2†
Site	5	289.4	5.6***
Error	129	52.1	

Table 6. ANCOVA results examining the relationship between *H. polyclina* growth form and external shell area, host hermit crab species, gastropod shell species and site of collection. Uninhabited shells were excluded. Statistical tests were performed (a) including all gastropod shell species and (b) excluding gastropod shell species represented by only a single datum. * = $P < 0.05$

(a)

Source	df	MS	F-ratio
Shell area	1	24.4	0.3
Host crab	1	340.7	4.3*
Gastropod	2	90.3	1.1
Site	3	91.7	1.2
Error	79	78.9	

(b)

Source	df	MS	F-ratio
Shell area	1	24.4	0.3
Host crab	1	340.7	4.3*
Gastropod	1	0.3	0.0
Site	3	91.7	1.2
Error	79	78.9	

Table 7. Interspecific differences in mean biomass growth rate (mm^2/d). The percentage of total biomass growth accounted for by mat and stolon tissue is given in parentheses. For each of total, mat, and stolon biomass growth rates, 1-way ANOVA results are given, and different letters indicate statistical significance with correction for multiple comparisons (Bonferroni adjusted $\alpha = 0.0167$). *** = $P < 0.001$

	Species			
	H. GM	*H. symbiolongicarpus*	*H. polyclina*	$F_{2,174}$
Total	1.23^a	1.70^b	2.54^c	31.4***
Mat	0.31^a (26%)	0.44^b (26%)	0.42^b (17%)	18.5***
Stolon	0.92^a (74%)	1.26^a (74%)	2.12^b (83%)	29.0***

Table 8. Observed (and expected) number of shells colonized by two juvenile, two mature, or one juvenile and one mature colony. Shells colonized by > 2 colonies were not included. Expected frequencies were calculated using the overall frequency of juvenile (p) and mature (q) colonies found on these doubly-colonized shells (juvenile-juvenile = p^2, juvenile-mature = 2pq, mature-mature = q^2). Goodness-of-fit χ^2 tests showed that *H.* GM ($\chi^2 = 18.0$, $P < 0.001$) and *H. symbiolongicarpus* ($\chi^2 = 12.5$, $P < 0.001$) differed from random expectations whereas *H. polyclina* did not ($\chi^2 = 1.5$, $P > 0.20$).

Species	Juvenile-Juvenile	Juvenile-Mature	Mature-Mature
H. GM	4	2	25
	(0.8)	(8.4)	(21.8)
H. symbiolongcarpus	18	11	17
	(12.0)	(23.0)	(11.0)
H. polyclina	6	3	2
	(5.1)	(4.7)	(1.1)

Table 9. Summary of relationship between gastropod diversity and size, the frequency with which two juvenile *or* two mature colonies co-occur on a single shell, relative size of host hermit crab species, growth rate, and growth form. Size refers to the mean and variance (range) in external shell area (mm^2). All nominal differences – low, interm (intermediate), high – between species were statistically significant. For the mean number and size of gastropod species encrusted per site, different letters denote statistically significant differences.

	Gastropod shells			Prop. shells with 2 colonies				
		Mean # species		Juvenile-	Mature-	Adult host	Biomass growth rate	Growth form
Species	# species	per site	Size	Juvenile	Mature	size	(mm^2/d)	(# st. tips)
H. GM	13	7.7a	1691_a $\sigma^2 = 2632506$ (103-7064)	low	high	small/large	low	low
H. symbiolongicarpus	6	2.5b	454_b $\sigma^2 = 111957$ (85-1545)	interm	interm	small	interm	interm
H. polyclina	2	1.8b	1066_c $\sigma^2 = 50266$ (442-1863)	high	low	large	high	high

CHAPTER 2

DISTURBANCE-MEDIATED MORPHOLOGICAL DIVERSITY IN A CLASSIC MUTUALISM: SELECTION ON HYDROID GROWTH FORM DEPENDS ON HOST HERMIT CRAB DENSITY

Abstract

Maintenance of high levels of standing variation in adaptive traits poses a fundamental paradox in evolutionary biology. Adaptive traits are, by definition, under strong selection that should quickly purge individuals with unfit phenotypes from natural populations. Genetically based variation in early ontogenetic growth form in the clonal hydroid *Hydractinia* epitomizes this problem. Competitive ability is transitively hierarchical and predictable based on growth form, and intraspecific competition between small recruits is known to be particularly important in this system, always ending in overgrowth of subordinate phenotypes. As in other clonal taxa, growth form influences not only competitive ability but also may be intricately tied to life history; however, my field experimental results with isolated hermit crab hosts show that intrinsic genetic trade-offs between life history and competitive ability are entirely absent. No significant association was detected between growth form and field survival, fecundity, size and age of first reproduction, or growth rate. Yet nearly all genetic correlations among life history traits and between life history and growth were significant and positive, indicating that low statistical power does not underlie the absence of significant correlations with growth form. I highlight an additional adaptive consequence of growth form: physical robustness. *Hydractinia* is an obligate symbiont of hermit crabs that often interact frequently in dense populations, conditions that impose physical disturbance on epibionts of crab-inhabited shells. The recruit stage is particularly vulnerable to mechanical abrasion as a result of small size and exposure of its delicate soft tissue. Here I provide direct experimental evidence that stoloniferous growth forms suffer reduced growth and complete mortality in the presence of dense hermit crabs, suggesting that mat-like phenotypes are more robust to physical disturbance, and propose that early ontogenetic growth form has evolved in response to the competing demands of frequent competition with conspecifics and disturbance from host hermit crabs. Evidence from natural populations suggests that selection may vary strongly in space and time, and that stoloniferous

growth forms predominate only in populations and seasons experiencing low host hermit crab density. Thus, combined with a competitive ability-robustness trade-off, spatial and temporal variability in disturbance regimes – itself determined by hermit crab host species/size and, ultimately, density – may maintain the extreme variation in natural populations.

Introduction

Disturbance is a pervasive structural force in nature, although its source, frequency, and magnitude may vary considerably among natural communities and populations. Defined as, "a discrete, punctuated killing, displacement, or damaging of one or more individuals (or colonies) that directly or indirectly creates an opportunity for new individuals (or colonies) to become established" (Sousa 1984), disturbance can prevent resource monopolies by competitively dominant species or genotypes (Darwin 1859; Paine 1966; Dayton 1971; Connell 1978; Sousa 1979a,b; Connell et al. 2004). Whether abiotic (e.g., fire, wave action, storm effects) or biotic (e.g., grazing, predation), disturbance agents may exert strong impacts on community composition and species diversity, providing competitive subordinates with resource access needed to sustain coexistence in systems that might otherwise exclude competitive subordinates if allowed to reach competitive equilibrium. Living space is often the primary limiting resource in marine hard substratum environments (e.g., Connell 1961, Buss 1979, Jackson 1979), and disturbance is thought to play a particularly important structural role in these habitats.

Physical disturbance also may influence genotypic diversity in clonal organisms (e.g., Sebens 1982, Reusch 2006), but this possibility has received far less attention. As an extreme example, the clonal aggregating anemone *Anthopleura elegantissima*, a common inhabitant of northeast Pacific rocky intertidal and subtidal communities, exhibits divergent patterns of clonal diversity on exposed and unexposed coasts, which differ dramatically in disturbance regimes; exposed coasts consistently experience much more frequent and severe disturbance as a result of wave action than sheltered coasts. *A. elegantissima* can be a highly aggressive space competitor, and exhibits wide variation in allocation to aggressive structures and, hence, competitive ability (Ayre and Grosberg 1995, 1996; Ferrell 2005). Successful genotypes may persist for decades (Sebens 1982, 1983) and form expansive clonal aggregations, consisting of hundreds of individuals through asexual fission over time. In the absence of frequent disturbance, competitively dominant clones can nearly exclusively occupy areas up to 100 m^2 whereas highly

disturbed regions of comparable size may support up to 25 different clones (Sebens 1982). In contrast to its effects on community composition, disturbance has immediate selective consequences. In evolutionary terms, the prevention of a competitive monopoly ultimately may translate into increased genotypic diversity and a more even distribution of net fitness among genotypes.

Although not prerequisite for providing competitive refuge, selective mortality or damage of dominant individuals ("compensatory mortality;" Sousa 1984), may be most effective in maintaining subordinate competitors (species or genotypes) in nature (Valdivia et al. 2005). Biotic sources of disturbance, such as predation or herbivory, may be most likely to inflict selective mortality (e.g., Paine 1966, Lubchenko 1978), but abiotic disturbance may as well (e.g, Connell 1978, Sousa 1979b, Paine and Levin 1981). Larger, perhaps older, inhabitants of rocky intertidal (Sousa 1984) or mangrove root (Bingham and Young 1995) communities are more vulnerable to removal by wave action and strong currents. Morphological traits in addition to size may also be important. For example, colonial invertebrates display an enormous variety of growth forms (Jackson 1979), some of which resist physical disturbance better than others. Erect branching sponges (e.g., Wulff 1991) and corals (e.g., Connell 1978) may be more likely to suffer from wave damage, e.g., from hurricanes, than others exhibiting compact, mound-like growth. Encrusting organisms exhibit similar morphological variation, but along two-dimensional axes. Growth form variation in encrusters is known to have strong adaptive consequences, although the relationship with disturbance resistance is less well demonstrated (but see Wulff 2006c). Highly compact (phalanx, or sheet-like) growth forms typically withstand competitive challenges better than more diffuse (guerilla, or runner-like) morphologies, and may actively exclude adjacent competitors (e.g., via overgrowth) in some cases. The less robust architecture of guerilla morphologies may make them more susceptible to (at least partial) mortality imposed by local disturbance, in addition to competitive exclusion, but guerillas may be more likely to survive local disturbance at the genet level as a result of their wider spatial cover. Phalanx-guerilla distinctions are often made between species (Blackstone and Buss 1991), but phalanx-guerilla variation may occur among genets within species, and depending on the disturbance regime and scale, growth form-dependent vulnerabilities among genets may promote intraspecific morphological variation.

The clonal hydrozoan genus *Hydractinia* exhibits great inter-genotypic variation in growth form that varies in a phalanx-guerilla fashion. Early in colony ontogeny, highly stoloniferous phenotypes asexually proliferate primarily via thin branches of tissue whereas mat-like morphologies produce few outwardly radiating peripheral stolons and instead grow primarily by centralized ectodermal mat tissue (Chapter 1: Figure 1). Most animals exhibit growth forms intermediate between these two extremes (Chapter 1: Figure 2). Maintenance of growth form variation is not well understood in this system despite the fact that (1) the *Hydractinia* system is a model system in several subdisciplines (Frank et al. 2001), (2) early ontogenetic growth form may be strongly tied to life history, and other adaptive, traits, (3) extensive genetically-based growth form variation is present in all *Hydractinia* species studied to date (Buss et al. 1984, Buss and Grosberg 1990, Blackstone and Buss 1991, Yund 1991, Ferrell 2004a), and (4) highly stoloniferous phenotypes invariably win intraspecific competitive bouts (Buss and Grosberg 1990, Ferrell 2004a), which occur commonly in nature (Yund et al. 1987; Buss and Yund 1988; Yund and Parker 1989; Yund 1991; Hart and Grosberg 1999; Ferrell 2004b, 2005), generating strong directional selection on growth form that should quickly lead to the loss of mat-like forms. Differences in indicators of the strength of selection on competitive ability are associated with shifts in the distribution of growth forms (mean/mode) that can be observed at the population (Yund 1991) and species (Chapter 1) levels, but wide variation persists within populations and species.

Early ontogenetic morphological variation in this system represents a classic paradox in evolutionary biology: what prevents fixation in genetically based, adaptive traits? Extant levels of genetically based variability in growth form are extreme (Chapter 1: Figure 2), and selection on competitive ability, although variable in strength (Yund 1991, Chapter 1), is universally present and unidirectional (Buss and Grosberg 1990, Yund 1991, Ferrell 2004a, Chapter 1), making it unlikely that mutation-selection balance entirely accounts for the standing variation. Negative genetic correlations – driven by antagonistic pleiotropy, physical gene linkage, or linkage disequilibrium – may underlie fitness trait variation in some systems (Roff and Fairbairn 2007), but evidence for genetic trade-offs between life history and growth form in *Hydractinia* are entirely lacking. A disproportionate emphasis on intraspecific competition has constrained a more comprehensive understanding of growth form variation in this well studied system and led to a neglect of balancing selection explanations. Yund's (1991) statement that, "No ecological

factor other than intraspecific competition is known to differentially affect colony morphology in this genus," reflects a longstanding and commonly held view. The role of disturbance in maintaining high growth form diversity has been largely discounted despite the fact that these animals obligately associate with symbiotic hosts that impose frequent physical damage (Van Winkle et al. 2000). Here I present evidence that trade-offs between between life history, or growth, and competitive ability in *Hydractinia* are context dependent, and are only observed in the context of hermit crab populations. I propose that spatial and temporal variability in disturbance regime maintains extant growth form variation, and test the specific prediction that highly stoloniferous hydroid recruits are more susceptible, in terms of mortality and early growth, to hermit crab-mediated disturbance in dense hermit crab populations.

Study system

Hydractinia species in the northern Gulf of Mexico and North Atlantic encrust the surface of gastropod shells occupied by pagurid hermit crabs (Cunningham et al. 1991, McDermott 2001). Colonies exhibit zooid polymorphism, typically producing two primary types of polyps: gastrozooids (for feeding purposes), and gonozooids (sexual reproduction). Other zooid types are sometimes present, such as tentaculozooids (for colony defense) and spiralzooids (unknown function). Unlike many other hydroids, *Hydractinia* does not have a medusa stage. Mature gametes are produced by and held directly on the gonozooid within gonophores located along gonozooid stalks. Individual gonophores may vary with respect to the number of gametes present at any given time, but female gonophores hold 3-8 eggs (Buss and Yund 1989). Colonies spawn their gametes in response to light cues, and are capable of participating in broadcast spawning events on a daily or semi-daily basis (Bunting 1894, Ballard 1942, Levitan and Grosberg 1993). Upon fertilization, a crawling planula develops and recruits to a gastropod shell inhabited by the host hermit crab species (Yund et al. 1987). Successful recruits metamorphose, initially forming a primary polyp (i.e., small gastrozooid) and then asexually proliferating along the colonized substratum using stolons, which may form a dense network that soon forms a continuous sheet of ectodermal mat tissue through stolonal fusion (mat-like phenotypes) or may expand more rapidly over the substrate by increased stolon proliferation (stoloniferous phenotypes). Stoloniferous morphs may exhibit fewer, longer stolons with greater inter-stolon spacing, or densely packed, short stolons with many stolonal anastomoses. In all *Hydractinia*

species studied to date, growth form bears a significant genetic basis, as indicated by breeding (Blackstone and Buss 1991) and clonal repeatability studies (Buss et al. 1984; Buss and Grosberg 1990; Yund 1991; Ferrell 2004a; Chapter 1). Ultimately, regardless of early ontogenetic growth form, in all mature colonies the ectoderm of stolons becomes fused to form an ectodermal mat (Frank et al. 2001). Gastrozooids form primarily on fused ectodermal mat but are found on individual stolons in some stoloniferous individuals. Gonozooids typically are produced later in colony development and form only on fused ectodermal mat (Haüenschild 1954). The number of gastrozooids is a good indicator of colony size and allocation to somatic growth, especially during the initial stages of colonization.

Two or more planulae often recruit to a single gastropod shell (Yund et al. 1987; Buss and Yund 1988; Yund and Parker 1989; Yund 1991; Hart and Grosberg 1999; Ferrell 2004b, 2005; Chapter 1). In this case, colonies may compete subsequently, unless one or both colonies die prior to contact. Conspecific contact typically induces an agonistic response mounted by one or both interacting colonies (Ivker 1972, Buss et al. 1984), in which existing stolons swell due to the recruitment of nematocytes and subsequent discharge of potent nematocysts carried within these cells. Each colony's "hyperplastic" (Ivker 1972) stolons project off the substratum in an attempt to overgrow its competitor, and dense stolon masses can result between competing colonies. Laboratory experiments on artificial substrata show that more highly stoloniferous phenotypes always win (Buss and Grosberg 1990, Ferrell 2004a), unless colonies are sufficiently large or similar in competitive ability, in which case competitive standoffs may result (Ferrell 2004a,b; 2005). Although frequency varies among species (Chapter 1), competition often occurs between small, juvenile colonies and ends in exclusion of the inferior competitor (i.e., the individual exhibiting a less stoloniferous early ontogenetic growth form).

Materials and methods

Asexually derived colonies and the estimation of early ontogenetic growth

This experiment examines the often implicit, but untested, assumption that growth patterns exhibited by asexually derived, juvenile colonies (established from tissue portions of mature animals) accurately reflect the pattern of growth exhibited in true early ontogeny from its sexually derived state. After collecting 6 *H.* GM colonies (3 male, 3 female) and their hermit

crab hosts from shallow (< 1 m) sand-mud flats at FSUCML, I obtained sexually derived colonies from 3 haphazardly paired parental combinations using modified *H. symbiolongicarpus* mating protocols (Levitan and Grosberg 1993, Grosberg et al. 1996, Hart and Grosberg 1999). A male-female pair was transferred to a single dish, and maintained in dark conditions for 24-48 hours, which was immediately followed with direct exposure to a light source for 1 hour. After 48 hours, individual planulae were transferred to small petri dishes containing 10 ml of 80 mM CsCl-seawater solution (1:1 mix). Temporary exposure to CsCl causes an ionic imbalance in planulae, which results in planular contraction, reduced crawling movements, and ultimately metamorphosis, when returned to normal seawater (Müller 1973). Just prior to metamorphosis, planulae no longer appear elongate but instead assume a roughly teardrop shape. The length of exposure to CsCl needed to attain this characteristic pre-metamorphic state varies considerably among clutches (D.L. Ferrell, unpublished data), but typically takes 3-4 hours. CsCl-treated planulae were transferred to unoccupied *Littoraria irrorata* gastropod shells. Size-standardized *L. irrorata* shells (18-20 mm in length) were positioned in individual Falcon tray wells filled with 1 μm-filtered seawater. I used a fine-tipped drill bit to place a single shallow depression on each shell near the aperture where the outer body whorl meets the spire, a site associated with high hydroid recruitment success (Yund et al. 1987, Buss and Yund 1988, Yund and Parker 1989). Upon larval transfer, I used a sterile micropipette to place a single planula in the drilled shell depression. Successful metamorphs (N = 35) subsequently were fastened to plain glass slides and transferred to modified slide racks, in which the covers had been removed to permit water movement. Shells were secured to glass slides using cable ties. All slides were maintained in a single aquarium containing re-circulated 1 μm-filtered seawater (temperature ~18°C, salinity ~28 ppt). Colonies were fed 2-4 day-old brine shrimp nauplii daily. After 33 days, I estimated growth form (peripheral number of stolon tips; Ferrell 2005) for each colony. Experimental colonies on shells temporarily were returned to the aquarium environment, and then transferred to the field after 4 days. *Pagurus longicarpus* hermit crabs, also collected from shallow waters at FSUCML, were removed from their original field-collected shells and introduced to the experimental *L. irrorata* shells, each with a focal hydroid recruit. Each of the 35 hydroid/shell/hermit crab units was isolated in a small field cage placed in shallow subtidal waters at the FSUCML original site of collection. Each cage, constructed of 40 mm^2 (1/4") mesh hardware cloth, provided a roughly circular 50 cm^2 area for crab movement over the sand and

mud sediment. The diet of field experimental animals was not supplemented, as significant organic debris and meiofauna likely moved freely in and out of the cages, thereby providing sufficient food for hermit crabs and hydroid symbionts. Pilot studies had demonstrated previously that colonies initiated at very small size (5-7 zooids) consistently grew and attained sexual reproductive maturity under these conditions (D.L. Ferrell, unpublished data). Dead or absent host hermit crabs were replaced as necessary.

After 95 days in the field, 23 colonies had died (see Results). The remaining colonies subsequently were maintained individually in isolated containers in the laboratory until all colonies had attained sexual maturity. (One additional colony died prior to transfer to the laboratory.) Plastic containers (10 cm X 10 cm X 70 cm deep) contained 500 ml of 1 μm-filtered seawater and were lined with 1-2 cm of mud and sand sediment collected from the same FSUCML field site. At that time, 5 asexually-derived explants of colony tissue per genotype, each containing 5-10 gastrozooids, were secured to individual plain glass microscope slides using (8-lb. test) monofilament thread and transferred to modified slide racks maintained in a single common garden aquarium environment, as described above. All animals were fed a standard diet of 2 to 4-day-old brine shrimp nauplii daily for 7 days, after which the slides washed thoroughly in 70% ethanol and air-dried. Mean number of stolon tips (N = 5) was calculated for each of the 11 genotypes to estimate genotype-specific growth form (Ferrell 2005).

One-way analysis of variance (ANOVA) was used to test whether the mean growth form exhibited early in ontogeny differed among colonies that, after 95 days, (1) died before reaching maturity, (2) remained alive but juvenile, or (3) remained alive and had attained sexual maturity. An additional contrast adjusted for Bonferroni comparisons was performed comparing the mean growth form between all surviving (juvenile and mature) and non-surviving animals. T-tests were used to test for differences in growth rate (surface area growth rate) and size (number of gastrozooids) between juvenile and mature colonies. Simple linear regression analysis was used to examine the relationship between asexually derived estimates of growth form and that exhibited during the true early ontogeny of each genotype from its sexually derived state.

Life history, growth rate, and growth form: a common garden field experiment

From shallow (< 1 m) sand-mud flats at FSUCML, I collected 55 hermit crabs occupying shells with external surfaces fully colonized by mature *H.* GM colonies, and characterized the

growth form of each colony according to established methods using asexually derived colony replicates (Ferrell 2005; Chapter 1; this study: "Asexually derived colonies and the estimation of early ontogenetic growth" subsection). Within 24 hours of field collection, 5 explants of colony tissue, each containing 5-10 gastrozooids, were secured to individual plain glass microscope slides using (8-lb. test) monofilament thread (total of 275 asexually derived colonies). Hermit crabs and hydroid symbionts were maintained on wet tables with running seawater (~28 ppt), and fed 2 to 4-day-old brine shrimp nauplii daily. After obtaining growth form estimates for all colonies, I then partitioned the observed range of growth forms into 5 categories and selected 2 genotypes per category for which colonies exhibited similar growth form estimates (total of 10 genotypes). Mean number of stolon tips (N = 5 per genotype) for selected colonies in each of 5 growth form categories were: 0.2, 1.0 (extremely mat-like); 4.2, 4.4 (intermediate mat-like); 10.0, 10.2 (intermediate); 16.25, 16.75 (intermediate stoloniferous); 21.4, 23.0 (highly stoloniferous). Hereafter, these 5 growth form categories are referred to simply as 0, 4, 10, 16, and 22, in reference to the mean number of stolon tips exhibited by experimental genotypes in these categories.

For each of the 10 selected genotypes, 10 asexual replicates were established individually on unoccupied *L. irrorata* shells (length = 18-20 mm) by tying a small explant of colony tissue, consisting of 3-5 gastrozooids, to each shell near the aperture where the outer body whorl meets the spire. Shells were secured to plain glass slides, and maintained in modified slide racks in a single aquarium containing re-circulated 1 μm-filtered seawater (temperature ~18°C, salinity ~28 ppt). Colonies were maintained in these conditions for 21 days, at which point all colonies had attached to the shell through new tissue growth and produced at least 5 new gastrozooid polyps. I then removed the monofilament line and tissue explant, introduced a naked *P. longicarpus* hermit crab to each shell, and isolated each hydroid/shell/crab unit in a small field cage placed in shallow subtidal waters at FSUCML, the original site of collection. Field cage dimensions and maintenance were identical to that described above. At 21, 56, 77, 133, and 175 days after transferring colonies to the field, I recorded colony survival, reproductive status (juvenile or mature), number of mature gonozooids, surface area (SA) growth rate, and number of gastrozooids. SA growth refers to asexual growth of somatic tissue (peripheral stolons or ectodermal mat) via lateral propagation over the shell substrate. Maximum colony length and width (parallel and perpendicular to the shell columella axis) was measured by using

monofilament line to closely trace the contours of the colonized shell surface, and SA growth rate estimated as follows: π*(length/2)*(width/2)/time. Dead or absent host hermit crabs were replaced as necessary.

Pearson product moment correlations were calculated to test whether genetic correlations existed between estimated growth form (mean number of stolon tips; N = 5 per genotype) and life history (field survival, number of mature gonozooids, size at first reproduction in terms of surface area covered and number of gastrozooids, and age of first reproduction) or growth (surface area growth rate, number of gastrozooids) for the 10 experimental genotypes. Age of first reproduction was estimated as the proportion of clonal replicates per genotype that had attained sexual maturity at the earliest time at which mature colonies were observed. Genotypic means (N = 10 clonal replicates) were used for each of the quantitative life history and growth characters. Genetic correlations between life history traits and between life history and growth were investigated as well. Differences among the five growth form categories in surface area growth rate, number of gastrozooids, and number of mature gonozooids were examined using a one-way ANOVA with Bonferroni pairwise comparisons treating growth form category as a fixed factor. A goodness-of-fit χ^2 test was used to test whether survival differed among the five growth form categories.

Effect of host hermit crab density on colony survival, early growth, and growth form expression

Six *H.* GM colonies (3 male, 3 female) and their *P. longicarpus* hermit crab hosts, collected from shallow subtidal waters at the Florida State University Coastal and Marine Laboratory, were maintained in the laboratory isolated in small fingerbowl dishes for 8 days. Colonies were saturated with *Artemia* brine shrimp nauplii daily (Brine Shrimp Direct, Premium Grade), after which the 1 μm -filtered seawater was changed. Three mating pairs of colonies were chosen haphazardly, and each pair transferred to individual small glass fingerbowl dishes. *Hydractinia* spawns in response to light cues simulating the onset of dawn (Bunting 1894, Ballard 1942). I induced spawning by maintaining colonies in dark conditions for 24 hours, and then exposing them to a light source for 1 hour (similar to the methods of Levitan and Grosberg 1993 and Grosberg et al. 1996). After 48 hours, I transferred individual planulae to small petri dishes containing 5 ml of 1 μm-filtered seawater and 5 ml of 80 mM CsCl. Temporary exposure to CsCl induces planulae contraction and the onset of developmental events involved in

metamorphosis (Müller 1973). Untreated planulae may continue to crawl for several days post-fertilization (Yund et al. 1987). CsCl-treated planulae attain a characteristic teardrop shape, which is a visual indication that they will soon metamorphose; planulae crawl very little after attaining this shape. CsCl-treated planulae were monitored hourly for up to 12 hours, and transferred in equal numbers to one of two substrates (1 cm x 1 cm x 2 mm thick, mother-of-pearl tiles or unoccupied *Littoraria irrorata* gastropod shells) when larvae had reached the characteristic teardrop shape. Size-standardized *L. irrorata* shells (18-20 mm in length) were stabilized by placement in individual Falcon tray wells filled with 1 μm-filtered seawater. Tiles similarly were maintained in individual seawater-filled Falcon tray wells. Using a fine-tipped drill bit, I placed a single shallow depression on each shell, as above (see "Asexually derived colonies and the estimation of early ontogenetic growth" subsection). At the time of transfer, I placed a single planula per tile or shell either at the center of the tile or in the drilled shell depression. Tiles and shells containing successful metamorphs subsequently were secured to plain glass slides and transferred to modified slide racks, in which tops and bottoms had been removed to permit water movement. Shells were secured to glass slides using cable ties, while tiles were suspended from slides by monofilament line threaded through the tile. All slides were maintained in a single aquarium containing re-circulated 1 μm-filtered seawater (temperature ~18°C, salinity ~28 ppt). Colonies were fed 2-4 day-old brine shrimp nauplii daily. After 9 days, 16X-magnifiied digital images of all surviving colonies on tiles and shells were obtained, and colony size (number of gastrozooids) and growth form (number of peripheral stolon tips; Ferrell 2005) was estimated.

Based on the distribution of variable growth forms exhibited by colonies from the matings, equal numbers of colonies from each of four growth form classes (1-4, 5-8, 9-12, and 13-17 stolon tips; see 'Results') were randomly assigned to one of two hermit crab densities. Number of experimental animals in each of four growth form classes was 10, 20, 6, and 6, respectively. At this time, naked *P. longicarpus* hermit crabs were introduced to empty *L. irrorata* shells encrusted by focal hydroid recruits, and each crab/shell/hydroid unit was transferred to low and high hermit crab density experimental units. Low crab density experimental units contained only a host hermit crab occupying a shell with a focal hydroid whereas high crab density units contained the focal host crab/shell/hydroid as well as 6 additional *P. longicarpus* hermit crabs collected from local sites near Bald Point, FL. Additional

hermit crabs, none of which occupied shells encrusted by hydroids, consisted of individuals composing three size classes: small (N = 3; maximum cheliped length < 3.5 mm), medium (N = 2; maximum cheliped length = 5-7.5 mm), and large (N = 1; maximum cheliped length > 10 mm), representative of natural abundances of size classes in natural populations (Hazlett 1981). 'Large' and 'medium' hermit crabs primarily inhabited *Polinices duplicatus* gastropod shells. 'Small' crabs occupied a variety of gastropod shells, including *Cantharus cancellarius, L. irrorata, Nassarius vibex, Urosalpinx perrugata*, and small *P. duplicatus* shells. Low and high crab density replicates were maintained in small plastic containers (10 cm X 10 cm X 70 cm deep), containing 500 ml of 1 μm-filtered seawater and lined with 1-2 cm of mud and sand sediment collected from the same FSUCML at which parental hydroids were collected originally. Experimental colonies were saturated with 2-4 day-old brine shrimp nauplii daily, and crabs were fed a portion of shrimp abdomen (~1 cm^2) once weekly. Dead hermit crabs were replaced as necessary.

Each focal colony was viewed under a dissecting microscope daily to monitor survival. After 10 days, for all surviving colonies, I again measured colony size (number of gastrozooids) and growth form (number of peripheral stolon tips). The number of gastrozooids subsequently was counted every 2-3 days for the remainder of the experiment, which was terminated after 42 days after all colonies had reached a sufficiently large size that mortality was unlikely. Throughout the experiment, colonies were dyed using a mixture of methylene blue and seawater when necessary to enable accurate measurements.

Analyses of covariance (ANCOVA) tests were performed to examine the effect of growth form (covariate) among the three treatments (lab tile, low density, high density) as fixed factors on colony growth (SAS, Version 9.1 software). A log-likelihood ratio test was performed to test the significance of the interaction between early ontogenetic growth form (continuous independent variable) and density treatment by comparing the relative likelihoods of binary logistic regression analyses including and excluding the interaction term (Sokal and Rohlff 1995).

Growth form variation in natural populations

H. GM colonies were hand-collected during summer, 2003 while snorkeling or wading in shallow water at two sites in the northern Gulf of Mexico: Florida State University Coastal and

Marine Laboratory, Turkey Point, FL and St. Joseph Bay, FL (see Chapter 1: Table 1). Each colony was sexually mature and encrusted > 80% of the external shell surface of a gastropod shell inhabited by one of two host species of pagurid hermit crab: the long-clawed hermit crab *Pagurus longicarpus* or flat-clawed hermit crab *P. pollicaris*. These hermit crab species reside in populations with different characteristic densities. *P. longicarpus* is relatively small, reaching adult sizes less than half that of *P. pollicaris*, and resides in populations that can reach extremely high densities, up to 200 crabs/m^2 (Hart and Grosberg 1999). In contrast, adults of larger species, such as *P. pollicaris,* are widely spaced (Grant 1963, Grant and Ulmer 1974). If mat-like phenotypes better withstand mechanical disturbance encountered in dense host populations, a positive association between the predominance of *P. longicarpus* hosts and mat-like hydroids is expected.

For each field-collected colony (genotype), I used asexually derived replicates to obtain an estimate colony growth form, according to established methods (Ferrell 2004a, 2005; Chapter 1; this study: "Asexually derived colonies and the estimation of early ontogenetic growth" subsection). A t-test was performed to investigate differences between sites in mean growth form of hydroids. Mean growth form per genotype was square root-transformed to meet model assumptions. A 2 x 2 contingency χ^2 test was used to examine whether the abundance of the two species of host hermit crab depended on site.

Results

Asexually derived colonies and the estimation of early ontogenetic growth

Six colonies, all female, were sexually mature after 95 days. These colonies exhibited early ontogenetic growth forms that were significantly more stoloniferous than (1) the early growth forms exhibited by surviving, but still juvenile, colonies, and (2) the early growth forms exhibited by those colonies that died in the field prior to reaching sexual maturity (Table 10, Figure 11). An additional contrast comparing the early growth forms of dead vs. all surviving (juvenile + mature) colonies also was statistically significant, indicating that more stoloniferous colonies displayed overall increased survival in the field. Early-maturing colonies were larger than juvenile colonies, both in terms of areal coverage and number of gastrozooids. At day 95, mature colonies occupied 131.9 mm^2 and bore 197.2 gastrozooids, on average, compared to 71.6

mm^2 coverage and 92.3 gastrozooids held by juvenile colonies (area: T = 2.64, df = 10, P = 0.025; gastrozooids: T = 2.71, df = 9, P = 0.024).

Asexually derived growth form estimates per genotype was not strongly associated with the growth form actually exhibited at the recruit stage early in ontogeny (Figure 12, slope = +1.4, $F_{1,9} = 0.9$, $R^2 = 0.09$, P = 0.37). The 11 colonies for which asexually derived growth form estimates were obtained consisted of 5 males and 6 females.

Life history, growth rate, and growth form: a common garden field experiment

Approximately one-third of experimental colonies died within 3 weeks of transfer to field conditions, but very little mortality occurred over the next 5 weeks. At approximately week 10, an anoxic event occurred at the field site; significant mortality was observed among experimental colonies when monitored at 11 weeks, likely as a result of the anoxic disturbance. Nearly all shells still were occupied by live hermit crab hosts upon collection. Contingency χ^2 analysis comparing pre- and post-anoxic survival (8 vs. 11 weeks) did not detect any difference in mortality among growth form categories ($\chi^2 = 3.4$, df = 4, P = 0.50). Until the termination of the experiment at 25 weeks, only approximately 10% additional mortality was observed.

At day 56 (8 weeks), prior to the field anoxic event, differences in fitness components among growth form categories were detected. Significant differences in field survival were observed among growth form categories (Contingency $\chi^2 = 9.5$, df = 4, P = 0.015, Figure 13A). Colonies in 2 of the intermediate categories (4 and 16 stolon tips) exhibited decreased survivorship relative to the other 3 categories. Similarly, categories 4 and 16 generally showed decreased surface area growth rate (Figure 13B), number of gastrozooids (Figure 13C), and number of mature gonozooids (Figure 13D). Although ANOVA detected significant overall differences with respect to each of these fitness components among the 5 growth form categories (Table 11), pairwise comparisons did not always detect significant deviations when comparing categories 4 and 16 to others (Figure 13B-D). Significant differences between categories 4 and 16 were never detected. With respect to surface area growth rate, categories 4 and 16 grew significantly slower than only the most highly stoloniferous category (22 stolon tips). The same pairwise differences were detected with respect to gastrozooid production, and in addition, category 4 exhibited significantly fewer gastrozooids also when compared to the most mat-like category (0 stolon tips). Fecundity, or number of mature gonozooids, offers perhaps the best

indicator of fitness measured in this study. Pairwise comparisons showed differences among categories identical to that observed in number of gastrozooids; that is, categories 4 and 16 were significantly different from category 22, and category 4 also was different from category 0. No significant pairwise differences among the three intermediate categories (4, 10, and 16) were observed in any of the fitness components.

Growth form estimates, obtained independently of the field experiment, of the ten genotypes were not significantly correlated with any of the field fitness components prior to the field anoxic event (day 56, Table 12). Yet strong positive genetic correlations were detected between life history components (survival, fecundity, size and age of first reproduction) and between life history and growth (SA growth rate, gastrozooids) (all $P < 0.01$), indicating that the experiment possessed sufficient power to detect genetic correlations, if they existed. A relationship between colony size at first reproduction and growth form, originally proposed by Yund (1987), was examined only prior to the anoxic event (day 56). Whether measured in terms of surface area covered or number of gastrozooids, size at first reproduction did not show a significant genetic correlation with growth form (surface area: $r = 0.37$, $P = 0.29$; gastrozooids: $r = 0.19$, $P = 0.60$). The proportion of clonal replicates per genotype that had attained maturity by day 56 (an estimate of the age of first reproduction) varied considerably among genotypes from 0.2 to 1.0, but no significant genetic correlation with growth form was detected ($r = 0.21$, $P = 0.55$). Size and age of first reproduction exhibited a significant positive genetic correlation, however, whether size was measured in terms of surface area covered ($r = 0.83$, $P = 0.003$) or number of gastrozooids ($r = 0.84$, $P = 0.002$). None of these coefficients indicate increased performance or fitness of mat-like colonies.

The field anoxic event likely had a large effect on experimental colonies. Immediately following the event (day 77), correlations between fitness components were initially less strong, and no colonies bore gonozooids; presumably, gonozooids were resorbed by the colony, but colonies are known to autotomize gastrozooids and gonozooids in response to stressful conditions (D.L. Ferrell, unpublished data) and this possibility cannot be ruled out. Post-anoxia, the genetic correlations between early ontogenetic growth form and life history (survival, fecundity) or growth (SA growth rate, gastrozooids) were not significant (Table 13A-C). Positive genetic correlations again were detected between life history and growth (survival, fecundity and SA growth rate, gastrozooids) (Table 13A-C). The most consistent correlation

occurred between survival and surface area growth rate, which was significant at all dates. Surface area growth rate also was tied closely with gastrozooid and mature gonozooid production (Table 13A,C), which were significant after 175 days in the field.

Effect of host hermit crab density on colony survival, early growth, and growth form expression

Survival

Experimental colonies in the three treatments showed different patterns of survival over time (Figure 14). All colonies maintained on artificial tile substrates in the laboratory survived. Significant mortality was observed in both crab treatments, primarily during the first 10 experimental days. After 10 days, no further mortality was observed in the low density treatment whereas mortality continued to gradually accrue in the high density treatment. Colonies experiencing high crab density suffered significantly greater overall mortality than those maintained at low crab density (Contingency $\chi^2 = 4.2$, df = 1, P = 0.04).

Early ontogenetic growth form of 9-day-old colonies ranged from 1-17 stolon tips. These colonies composed 4 morphological classes, including those bearing 1-4, 5-8, 9-12, and 13-17 stolon tips. Colonies experiencing low and high crab density were represented equally within each category (Figure 15). Differential mortality among growth forms varied between density treatments (Figure 15). At 42 days, 100% of the most highly stoloniferous colonies survived at low density whereas no highly stoloniferous colonies survived at high density. The only high density colonies remaining composed the 2 mat-like morphological categories. Colony survival depended significantly on density treatment (log-likelihood ratio statistic = 4.725, $P < 0.05$), showing significantly greater mortality of highly stoloniferous morphs at high crab density.

Growth

Most surviving colonies 10 days after the introduction of hermit crabs exhibited positive growth, in terms of the number of gastrozooids present relative to that exhibited just prior to crab introduction. A few colonies, primarily in the high density treatment, exhibited negative or zero growth (Figure 16). In the laboratory environment, increasingly stoloniferous colonies exhibited greater growth in terms of gastrozooid production in comparison to each of the crab treatments (Table 14, Figure 16). ANCOVA detected a significant interaction in the relationship between

gastrozooid growth and growth form at this early experimental stage when comparing low and high density treatments only. This was true whether including only surviving colonies (9 and 7 colonies for low and high density treatments, respectively; $F_{1,13} = 27.5$, $P = 0.044$) or all colonies, in which case all dead colonies, possessing zero gastrozooids, showed negative growth ($F_{1,38} = 115.1$, $P < 0.001$). The significant interaction indicates that more stoloniferous growth forms experienced growth costs at high, but not low, crab density only 10 days after crab introduction.

At the end of the experiment, 39 days after host crab introduction, disparities between low and high density treatments with respect to the relationship between gastrozooid production and growth form were magnified (Figure 17). At this point, only 3 high-density colonies remained; thus, statistical analysis was conducted on all colonies, alive or dead. Again, a significant difference in slopes between the two density treatments was detected (Table 15). The majority of low density hydroids exceeded 150 gastrozooids in size, and the largest colonies were also most stoloniferous. In contrast, high density hydroids did not exceed 20 gastrozooids, and highly stoloniferous growth forms experienced maximal growth costs.

Growth form

A positive relationship between growth form 10 days after host crab introduction and that just prior to crab introduction was observed in all treatments (Figure 18, Table 16). Colonies exposed to hermit crab treatments exhibited significantly less stolon proliferation than those maintained in the laboratory (low density: $F_{1,48} = 15.9$; high density: $F_{1,48} = 14.6$, both $P < 0.001$), but no differences between density treatments was observed ($F_{1,48} = 0.23$, $P = 0.63$). Thus, although hermit crabs influenced growth form, relative differences in growth form among colonies remained intact following exposure to hermit crabs.

Growth form variation in natural populations

Mean growth form of *H.* GM colonies varied between the FSUCML and SJB sites in the northern Gulf of Mexico ($T = 2.90$, $df = 141$, $P = 0.004$). Hydroids collected at FSUCML exhibited significantly more mat-like growth forms than those collected at SJB (Figure 19A). The abundance of host hermit crab species varied between sites (Contingency $\chi^2 = 27.5$, $df = 1$, $P < 0.001$). Approximately 72% of hydroid-colonized shells were inhabited by *P. longicarpus* at

FSUCML whereas 73% of hydroids at SJB encrusted shells inhabited by *P. pollicaris* (Figure 19B).

Discussion

Fitness of *Hydractinia* growth forms varies with host hermit crab density. Competitively inferior growth forms persist in the face of crab-mediated disturbance, suggesting that less stoloniferous colonies, while susceptible to overgrowth by conspecifics, are more physically robust in the face of disturbance. These results highlight the importance of environmental context (sensu Reznick et al. 2000) on the relationship between competitive ability, life history (survival), and growth rate; trade-offs heretofore undetectable in the laboratory (e.g., Ferrell 2004a) and in the absence of neighboring hermit crabs in the field (e.g., Table 12) were realized in the presence of dense hermit crab populations. The strong effects on growth rate (Figures 16 and 17) likely have huge impacts on fitness through other size-dependent sources of mortality, such as interspecific competition (McFadden 1986, Van Winkle et al. 2000) and subsequently expressed life history traits (e.g., timing of maturity). Relative inter-genotypic differences in growth form (and, hence, overgrowth competitive ability) remain intact in the presence of hermit crab disturbance (Figure 18); thus, there is no indication here of reversals in competitive ability. Importantly, however, reversals in intraspecific competitive outcome with increasing disturbance may still occur and indeed are likely, as the selective removal of highly stoloniferous phenotypes by disturbance is likely intensified when these individuals are attempting to mount the costly (Ferrell 2004a) hyperplastic response.

Field surveys of three *Hydractinia* species lend further support to the hypothesis that highly stoloniferous morphologies lack structural integrity needed to withstand frequent mechanical abrasion. Growth forms were not randomly distributed among hermit crab hosts in natural populations: mat-like *Hydractinia* colonies predominated on shells inhabited by crab species or size classes that likely experience more frequent epibiont disturbance (Chapter 1). Pagurid hermit crab populations can be extremely dense (up to 200 individuals/m^2; Hart and Grosberg 1999), and in this context, hermit crabs exhibit a suite of epibiont-disturbing behaviors (reviewed in Chapter 1). Species-specific and size-specific differences in aggressiveness and the frequency of conspecific interactions vary accordingly with natural densities (e.g., Grant and Ulmer 1974) with epibionts of smaller host species and size classes experiencing more frequent

and protracted bouts of disturbance. New recruits represent a critical stage in this hydroid's life cycle as a result of their small size, complete lack of protection for delicate soft tissue (unlike adults), and recruitment pattern, which seasonally peaks along with the formation of hermit crab breeding aggregations (Buss and Yund 1988, Yund and Parker 1989). These facts, combined with the experimental findings of the current study, point to the pivotal role that host crab-mediated disturbance plays in understanding early ontogenetic growth form variation in this system.

Trade-offs with competitive ability are context dependent

The relationship between competitive ability and life history traits or growth is positive in laboratory environments in the absence of hermit crabs (e.g., Ferrell 2004a; this study, Figure 16), non-significant in field conditions with isolated hermit crab hosts (Table 12), and negative in the context of dense hermit crab populations (Figure 17). Positive genetic correlations between fitness components are not surprising in artificial laboratory settings, which may promote this outcome as a result of idealized conditions free from natural stressors or introduction to a novel environment (Sgrò and Hoffman 2004). However, "field studies enable us to test evolutionary theory in a context in which all of the trade-offs associated with a trait are realized" (Reznick and Ghalambor 2005). Results of the current study represent a compelling case in which ecological realism of the experimental environment clearly influenced the detection of key trade-offs. Had this study been conducted in the laboratory (on artificial surfaces in the absence of hermit crabs), as is standard practice in this model system, a fundamentally different view of inter-trait relationships would have been emphasized, which may have made it more difficult to achieve an adaptive understanding of growth form.

Asexually derived organisms are often used to establish genotypic replicates in experiments with clonal taxa. This is true in the *Hydractinia* system, despite the fact that individuals are thought to recruit exclusively using sexually produced planula larvae (Frank et al. 2001). Many studies in this model system have relied on this previously untested assumption (e.g., Haüenschild 1954; Buss et al. 1984; McFadden 1986; Müller et al. 1987; Lange et al. 1989; Buss and Grosberg 1990; Blackstone and Buss 1991; Yund 1991; Van Winkle et al. 2000; Ferrell 2004a, 2005). Although positive, the relationship between asexually derived estimates of growth form, obtained by juvenilizing mature field-collected animals, and the actual true early

ontogenetic growth form was not significant (Figure 12). Thus, asexually derived estimates may not necessarily reflect relative inter-genotypic differences in early ontogenetic patterns of hydroid growth. Growth form, as determined using clonal replicates, clearly has a genetic basis (Buss et al. 1984; Buss and Grosberg 1990; Blackstone and Buss 1991; Yund 1991; Ferrell 2004a; Chapter 1), yet caution should be used when extrapolating to the adaptive consequences of early ontogenetic growth (Van Winkle and Blackstone 2002). Further exploration of the relationship, including greater genotypic sampling, would be useful, although considerable unpredictability in the relationship ($R^2 = 0.09$, Figure 12) exists regardless. Moreover, although inter-genotypic differences in stolon production are observed (Chapter 1: Figure 2; this study: Figures 12 and 13), both stoloniferous and mat-like phenotypes established using tissue explants from mature colonies generate significant ectodermal mat tissue immediately whereas initial growth of sexually derived recruits consists of stolons only, and stoloniferous phenotypes generate mat tissue more slowly than others. Thus, the trade-off between competitive ability and survival at high host crab density likely would not have been realized if asexually derived animals had been used. Asexually derived animals are useful and appropriate in many experimental contexts, but should be used cautiously in this system, especially when considering the physical robustness of new recruits.

As in many clonal plants (reviewed in Abramson 1980) and a variety of invertebrate animals (Braverman 1974; Yamaguchi 1975; Stebbing 1980; Harvell and Grosberg 1988; Harvell and Helling 1993; McKinney and Taylor 1997; Ferrell 2004a,b; Sakai 2005), timing of sexual maturity and colony size at maturity is highly plastic in *Hydractinia*, and particularly sensitive to the substratum size available for colonization (Haüenschild 1954, Müller 1964). Stolon production continues until further expansion is inhibited by substratum size whereupon ectodermal mat tissue, upon which gonozooids develop, then expands at an increasing rate (Buss et al. 1984, Blackstone and Yund 1989). Substratum-limited, mature colonies may possess as few as 2 gastrozooids for food acquisition whereas colonies given an essentially unlimited surface area for colonization may delay reproduction for several months (Haüenschild 1954). The use of ecologically realistic substrata, in terms of colonizable space, likely played a significant role in the results of the common garden field study.

Yund (1987) observed a phenotypic correlation between size at first reproduction and growth form in *H. polyclina*, in which mat-like male colonies matured at smaller colony size in a

laboratory common garden. It should be noted, however, that smaller size at first reproduction does not translate into an earlier age of first reproduction on small substrata (Table 12); thus, it is unlikely that smaller size at first reproduction would constitute a real benefit in the ecological context of small gastropod shells often encountered in the field (Yund et al. 1987; Buss and Yund 1988, 1989; Yund and Parker 1989; Yund 1991; Hart and Grosberg 1999; Ferrell 2004b; Chapter 1). Regardless, in the current study, animals were grown on small gastropod substrata (external surface area ~ 205 mm^2) whereas animals encrusted much larger substrata (plain glass slides > 4000 mm^2) in Yund's study. Highly stoloniferous growth forms first encrust all colonizable substrate before maturing whereas more mat-like colonies may mature before occupying the entire available surface (Ferrell 2004a and references therein). Differences between the current results (no genetic trade-off between size at maturity and growth form but an inverse relationship, i.e., no trade-off, between size and age at maturity) and that of Yund (mat-like males maturing first and at smaller size) likely reflect, to a large degree, interplay between the growth form-specific rules underlying the onset of sexual maturity, differential rates with which variable growth forms occupy uncolonized territory, and differences in the size of experimentally colonized substrata. If size at first reproduction indeed trades off with competitive ability, it would only be realized by colonies encrusting relatively large shells (e.g., Karlson and Shenk 1983, Shenk and Karlson 1986, Ferrell 2004b). This substratum size-dependent scenario should be investigated through controlled laboratory and field experiments, preferably using animals of sexual origin.

Rarity and low fitness of some intermediate growth forms

Field performance and fitness indicators observed in the common garden field experiment (Figure 13) closely correspond with patterns of commonness and rarity among growth forms observed in *H.* GM (Chapter 1: Figure 2). Intermediate growth forms representative of categories 4 and 16 were rarely observed in natural *H.* GM populations. Similar trends in the rarity of some intermediate growth forms were observed in two other *Hydractinia* species as well, *H. polyclina* and *H. symbiolongicarpus* (Chapter 1: Figure 2). Phalanx and guerilla, or sheet-like and runner-like morphologies in encrusting animals, respectively (Buss 1979, Jackson 1979a, Buss and Blackstone 1991), are characterized by highly compact or more diffuse, elongate colony-level morphologies. Sheet-like and runner-like

morphologies are often equated with mat-like and stoloniferous growth forms in *Hydractinia* and related species (e.g., Blackstone and Buss 1991, 1992, 1993; Buss and Blackstone 1991; Van Winkle and Blackstone 2002; Blackstone et al. 2004a,b), although highly stoloniferous, extremely mat-like, and some intermediate growth forms all may exhibit compact morphologies (Figure 20). Colonies growing exclusively by ectodermal mat (or nearly so) clearly constitute sheet-like morphologies, but the presence of mat tissue in not prerequisite for exhibiting sheet-like growth. *Podocoryna carnea* and *P. selena* co-occur sympatrically with *Hydractinia* species and sometimes encrust gastropod shells inhabited by the same hermit crab species with which *Hydractinia* associates (Mills 1976a,b). *Podocoryna*, however, exhibits only a stolon network with no true mat tissue (Blackstone and Buss 1993). Nevertheless, sheet-like morphologies can develop in this genus and are characterized by "closely packed polyps with short stolonal connections" (Blackstone and Buss 1991, 1992, 1993; Van Winkle and Blackstone 2002; Blackstone et al. 2004a,b). Highly stoloniferous *Hydractinia* growth forms fit this sheet-like description well, exhibiting high polyp densities and a highly branching network of stolons. Moreover, some intermediate morphologies also may exhibit sheet-like growth, albeit to a lesser degree than highly stoloniferous forms. In the intermediate case, colonies combine significant ectodermal mat growth with a limited, but highly branching, stolon network around the mat perimeter (e.g., Figure 20C). In *Hydractinia*, it is only some intermediate morphologies that exhibit the "widely spaced polyps and long stolonal connections" (Blackstone et al. 2004a). This can be accomplished in one of two ways: one or a few long stolons may proliferate (typically unidirectionally) from a central, compact mass of ectodermal mat (Figure 20D), or many widely interspersed, long stolons may extend out from the centralized ectodermal mat (Figure 20E).

Although useful as a morphological indicator of competitive ability (i.e., the capability of a given phenotype to mount an inducible hyperplastic response), 'number of peripheral stolon tips' borne by size-standardized colonies (Ferrell 2005) does not capture variability in colony-level compactness or elongation. Alternative measures of growth form, which reflect colony-level compactness or elongation, suggest that these two runner-like possibilities (Figure 20D,E) correspond to morphologies exhibiting reduced fitness in the field (categories 4 and 16 in Figure 13) and rarity in natural populations (Chapter 1, Figure 2). Runner-like morphologies may suffer fitness costs for at least 3 reasons: they are generally competitively inferior to compact morphologies (Buss 1979, Jackson 1979), (2) they likely require significantly greater energetic

investment to maintain adequate fluid pressure in the gastrovascular system (Blackstone and Buss 1992), which performs the important function of transporting food and nutrients throughout the colony, and (3) the benefits typically attributed to runner-like morphologies, e.g., exploitation of open habitats free of competitors (e.g., Buss 1979, Sutherland and Stillman 1988), are not realized in the *Hydractinia* system. *Hydractinia* colonies encrust discrete microhabitats in the form of hermit crab-occupied gastropod shells, which are typically very small and when competition occurs in this context, no competitive refuges exist. In contrast, sheet-like, or phalanx, 'strategies' are characterized by a high degree of commitment to the site of recruitment (reviewed in Buss and Blackstone 1991). Because only one site exists post-recruitment, selection for highly compact forms may be the rule. Many *Hydractinia* species are obligate hermit crab symbionts, generally possess robust, compact morphologies relative to other closely related hydroids (Van Winkle et al. 2000), and exhibit atypical growth strategies that include the abilities to both claim adjacent, uncolonized space (as opposed to 'foraging' for open space) and resist overgrowth by competitors (Sutherland and Karlson 1977, Karlson 1980). Growth forms exhibiting compact, radially symmetric growth may be best suited for both of these purposes in this system with such highly specific habitat requirements, and as a result of its reduced susceptibility to hermit crab-mediated disturbance, ectodermal mat tissue in particular may be a key adaptation to this unusual environment.

Conflicting selection in *Hydractinia*: physical robustness vs. competitive ability

Maintenance of adaptive trait variation remains a central paradox in evolutionary biology. I propose here that spatial and temporal variation in disturbance regime – driven by differences in host hermit crab species and size, and as a result demography and behavior – creates conflicting selective scenarios in nature, which contributes to maintaining extensive levels of early ontogenetic growth variation in *Hydractinia* (Chapter 1: Figure 2).

Other common explanations for adaptive trait variation, including intrinsic genetic trade-offs and phenotypic plasticity, do not hold in this system. Adaptive traits are molded by all selective regimes influencing the trait in question as well as genetically correlated traits experienced over the lifetime of the individual; they consequently may be expected to exhibit great genetic variability as a result. This argument is closely related to the notion of genetic trade-offs (e.g., antagonistic pleiotropy) as a causal mechanism underlying variation. That is, not

only may negative genetic correlations between fitness components occur, but also they may be realized at different points over the individual's lifespan. However, laboratory experiments on artificial substrata (e.g., Ferrell 2004a) and field experiments with isolated hermit crab hosts (this study) indicate that an intrinsic genetic trade-off between competitive ability and life history traits (including survival, fecundity, size and age at first reproduction) is not present (Table 12), and the absence of a growth form-life history relationship is consistent over time (Table 13). Life history-competitive ability genetic trade-offs (e.g., inferior competitors mature at smaller size; Yund 1987) do not account for growth form variation. It is well recognized that genetic trade-offs may only be realized under stressful conditions, e.g., food limitation (Reznick et al. 2000). The diet-supplemented experiments presented here were purposefully food-unlimited, and the rapid rates of growth of most colonies in the field suggest that food limitation was not operating in this environment as well. *Hydractinia* has a varied diet and feeds on diverse meiofaunal organisms found among sediment particles as well as organic debris made available during hermit crab feeding and foraging. In nature, food limitation (and the potential realization of trade-offs in this context) is unlikely in *Hydractinia*, at least during the late summer and fall in which hydroid recruitment and early growth peak. Phenotypic plasticity also may play a role in some systems and traits. If variation can be accommodated by plasticity, then genetic variation will be eroded by selection (Roff and Fairbairn 2007). Plasticity in growth form may vary in a species-specific fashion. For example, some *Caulerpa* species, a macroalga, show species-specific guerilla or phalanx growth forms whereas other species can generate either of these morphologies depending on photosynthetic environment (Collado-Vides and Robledo 1999). In *Hydractinia*, growth form clearly has a genetic component (Buss et al. 1984, Buss and Grosberg 1990, Blackstone and Buss 1991, Yund 1991, Ferrell 2004a), and although influenced by environment (Dudgeon and Buss 1996; Van Winkle et al. 2000; V. Paddy and D.L. Ferrell, unpublished data; this study, Figure 18), growth form expression does not exhibit a genotype-environment interaction in response to natural environmental cues. Thus, relative differences in growth form remain intact.

Hermit crab density determines the survival of alternate growth forms in the absence of competition (Figure 15); dense crab environments disfavor stoloniferous growth forms. However, intraspecific competition is extremely important in *Hydractinia* (Yund et al. 1987; Buss and Yund 1988; Yund and Parker 1989; Buss and Grosberg 1990; Yund 1991; Hart and

Grosberg 1999; Ferrell 2004b, 2005), and dense crab environments likely favor mat-like growth forms when in competition as well. Competitive overgrowth ability in *Hydractinia* is directly related to an individual's ability to mount the inducible hyperplastic response. Regardless of hermit crab densities, more stoloniferous individuals are better able to mount this response. Because relative differences in stolon proliferation among genotypes remain unchanged in the presence of hermit crab hosts (Figure 18), relative overgrowth competitive abilities also remain unchanged. Reversals in competitive outcomes are likely, however, because of the increased sensitivity of highly stoloniferous phenotypes to disturbance when also suffering the extreme growth costs that accompany the hyperplastic stolon agonistic response (Ferrell 2004a).

Variation in selective regimes in natural populations

Abundance of variable growth forms routinely differs significantly among sites of collection (*H.* GM and *H. symbiolongicarpus* – Chapter 1; *H. polyclina* – Yund 1991). However, it is often unclear (1) whether differences in growth form correspond to within or among-site spatial variation in hermit crab densities, and (2) to what degree gene flow is restricted among sites, if at all. Yund (1991) investigated *H. polyclina* growth form variation among 8 sites of collection in St. John's Bay, ME and showed, as evidence of selection on competitive ability in natural populations, that sites in which multiple recruitment to a single shell occurred most frequently (an estimate of the frequency of competition) were composed of more highly stoloniferous hydroids. To estimate the frequencies of multiple recruitments, hundreds of hermit crab-occupied shells were collected at each site, although sample size varied among sites (range = 186-397). In a closely related study in which these same 8 hermit crab populations were sampled during the same months, Yund and Parker (1989) reported that all hermit crabs within 2 m of a transect line were collected; thus, the differences in sample size among sites reflect differences in hermit crab density. A re-analysis of the data in this context shows that sites at which more crabs were collected exhibited significantly more mat-like growth forms. Whereas the originally investigated association between growth form and frequency of multiple recruitment was significant ($R^2 = 0.58$, $P < 0.03$), number of hermit crabs collected per site explained a greater proportion of the variation in growth form (Figure 21, $R^2 = 0.85$, $P < 0.001$). Moreover, multiple regression analysis including both the frequency of multiple recruitment and sample size explained only an increased 2 percent of the variation in morphology ($R^2 = 0.87$),

and sample size remained a statistically significant factor ($F_{1,5} = 3.62$, $P = 0.015$) whereas frequency of multiple recruitment was no longer significant ($F_{1,5} = 0.86$, $P = 0.42$). Although both intraspecific competition and hermit crab-mediated disturbance play prominent roles in growth form evolution, these results suggest that hermit crab density may better explain among-site growth form variation than a proxy of intraspecific competition in this species. Genetic structure among these 8 sites of collection (some < 1 km apart) is unknown, but Yund held that these constituted distinct populations, based on the presence of barriers to adult hermit crab movement (i.e., water depth and rocky substrate) among sites, and subsequent work (Folino and Yund 1998) also suggests that adult hermit crabs do not move freely over this spatial scale in the Gulf of Maine.

Relative differences in hermit crab densities experienced by *Hydractinia* species and populations can be inferred from differences in host species and size: among the pagurid hosts considered here, smaller species (and smaller individuals within species) reside in more dense populations than adults of larger species. That is, the small species *P. longicarpus* and small individuals of the larger species *P. acadianus* and *P. pollicaris* occur at high density whereas adults of the two large species occur at low density (Grant 1963, Grant and Ulmer 1974, Hart and Grosberg 1999, McDermott 2001). Surveys of morphological variation show that *H. symbiolongicarpus* exhibits more mat-like morphologies than *H. polyclina* (Chapter 1). Accordingly, *H. symbiolongicarpus* associates with *P. longicarpus* (Buss and Yund 1989) of all sizes whereas *H. polyclina* associates with *P. acadianus* (Buss and Yund 1989) and large *P. longicarpus* individuals at some sites (Folino and Yund 1998, Chapter 1). Similar inferences are possible at the population level in the current study. Two *H.* GM sites exhibit clear differences in hermit crab host density, as indicated by the predominance of either dense *P. longicarpus* or sparse *P. pollicaris* hermit crab hosts, tracked closely by site differences in hydroid growth form (Figure 19). Colonies at *P. longicarpus*-dominated sites exhibited mat-like phenotypes whereas those at *P. pollicaris*-dominated sites were more stoloniferous. In this case, evidence indeed exists that gene flow may be restricted between these sites. Experimental matings between colonies collected at different sites exhibit reduced reproductive success compared to within-site matings (Appendix 1). As a result of its reproductive ecology, *Hydractinia* possesses extremely limited larval dispersal ability (reviewed in Hart and Grosberg 1999), and therefore any population immigration or emigration must occur passively through adult hermit crab movement.

These two *H.* GM sites are separated by approximately 100 km of coastline, but unlike the *H. polyclina* populations in Yund (1991), no obvious physical barriers to movement of host hermit crabs are apparent. It is unknown to what extent reduced gene flow at this spatial scale is reflective of the broader geographical distribution of *H.* GM. At the least, these data provide an indication that significant genetic structure may exist at a relevant spatial scale in which differences in selective regime and trait means are evident.

In addition to spatially varying host densities, individual populations should experience strong seasonal fluctuations in selection as it is well known that hermit crab densities peak during the breeding season in late summer (up to 200/m^2; Hart and Grosberg 1999) and decline in winter when crab activity may cease altogether (Rebach 1974). Although data on intraspecific growth form variation among seasons is not available, strong seasonal trends in the abundance of the closely related hydroids *Podocoryna carnea* and *P. selena*, which co-occur with *H. symbiolongicarpus* and *H.* GM, respectively, are telling. *Podocoryna* grows exclusively by stolons and only rarely associates with hermit crabs (Wells 1969; Mills 1976a; D.L. Ferrell, unpublished data), is seasonally absent from hermit crab populations when crab densities peak (Mills 1976a) and most abundant in winter when crab activity and densities are lowest (D.L. Ferrell, unpublished data), and is more abundant on shells occupied by larger hermit crab species (Mills 1976b), which exist in less dense populations. Each of these observations is consistent with the hypothesis that stolons are less well equipped for intimate association with hermit crab hosts residing in dense populations.

Although many models consider the maintenance of polymorphism, their underlying mechanisms are commonly extended to variation in quantitative, polygenic traits (Grant and Price 1981, Sinervo and Zamudio 2001). Models evaluating the role of spatial and temporal heterogeneity often suggest that the conditions for maintaining variation in adaptive traits can be restrictive (e.g., Gillespie 1974, Slatkin and Lande 1976, Hedrick 1986). Strongly divergent selective regimes are required and, although not always necessary, increased genetic subdivision enhances the ability to maintain variation. In particular, the magnitude of the difference in the strength of selection relative to the amount of gene flow is typically thought to be critical (Grant and Price 1981). Taken together, the evidence from *H. polyclina* and *H.* GM suggests that restricted gene flow among sites experiencing variable hermit crab densities is not only possible, but is evident, at least between two sites. Despite model predictions that temporal variation

alone generally constitutes a much weaker mechanism for maintaining variation, especially in species with overlapping generations (but see Chesson 1985), good empirical examples of seasonal environmental variation maintaining adaptive trait variation within populations exist (e.g., Grosberg 1988), and moreover, temporal heterogeneity is especially powerful when occurring in combination with spatial heterogeneity (Svennson and Sinervo 2004). I conclude that spatially and temporally variable selection constitutes a viable model for explaining the maintenance of variation in genetically based variation in early ontogenetic growth patterns in *Hydractinia* populations.

Summary

Predominance of mat-like growth forms among *H. polyclina* and *H.* GM populations (Figures 19 and 21) and species (Chapter 1) experiencing high hermit crab host densities complement the experimental demonstration provided here in which stoloniferous *Hydractinia* growth forms thrived at low hermit crab density but exhibited 0% survival at high crab density. At low density, selection favors highly stoloniferous phenotypes as a result of their increased competitive ability whereas high host densities impose significant physical disturbance and favor physically robust, mat-like phenotypes. Significant genetic structure, often required by models incorporating only spatial heterogeneity, appears plausible in *H. polyclina* and is evident at the scale of approximately 100 km between at least two sites in *H.* GM. Individual populations likely experience strong temporal fluctuations in selection on growth form as pagurid hermit crab densities vary widely among seasons: hermit crab movement may cease altogether in winter whereas extremely active, dense assemblages (e.g, up to 200/m^2) form in late summer. Models suggest that conditions appear less restrictive and maintenance of variation is more likely when spatial and temporal environmental variation act jointly, as proposed here. Thus, extreme variation in early ontogenetic growth form in this system may be maintained not by a genetic trade-off between reproductive characters and competitive ability, but rather by spatio-temporal environmental variability in disturbance regimes and, hence, conflicting selection.

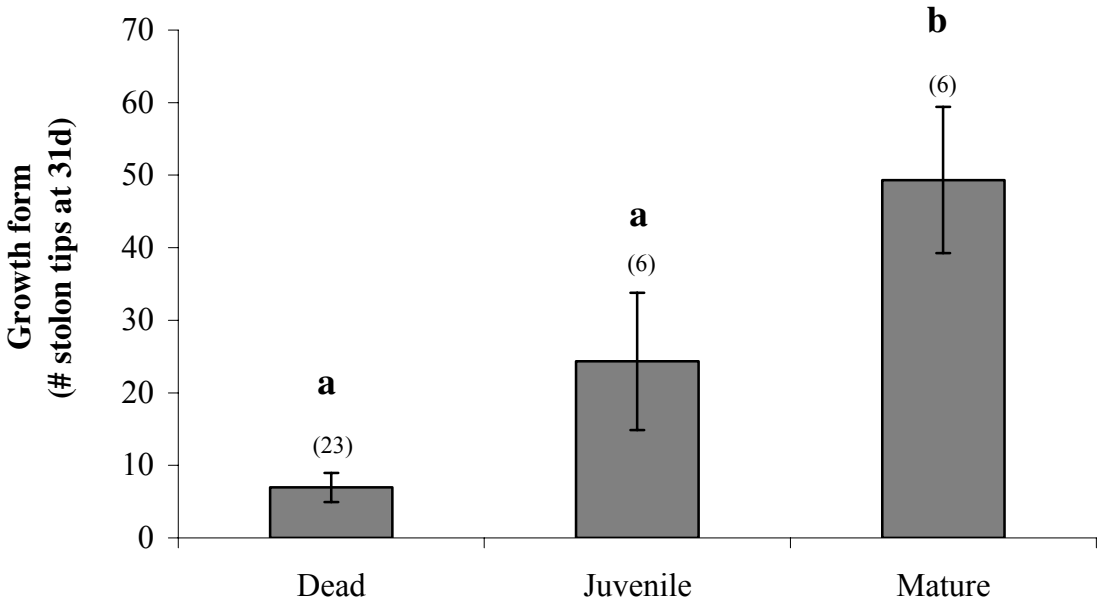

Figure 11. Mean growth form (number of stolon tips at 31 days) of dead and surviving (juvenile and mature) colonies at 95 days. Numbers in parentheses indicate sample size. Letters designate statistically significant pairwise comparisons, and an additional contrast comparing the mean growth form of all surviving colonies (juvenile + mature) to that of dead colonies also was statistically significant (Bonferroni corrected $\alpha = 0.0125$).

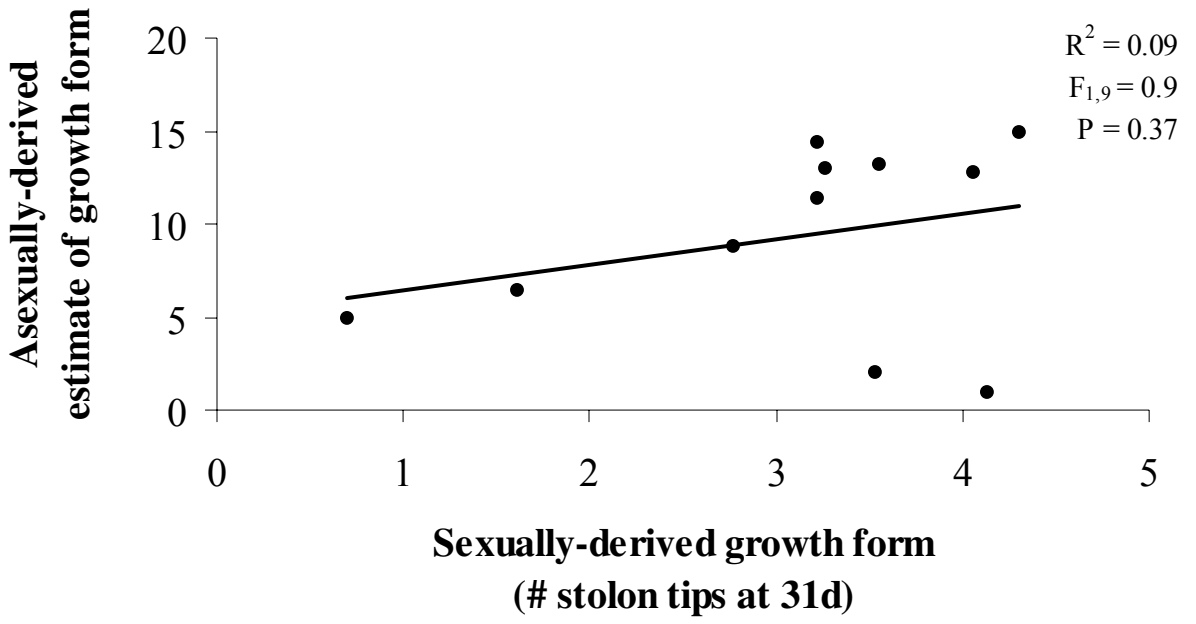

Figure 12. Relationship between asexually derived growth form estimates and growth form exhibited early in ontogeny by sexually derived, experimental recruits. Growth form initially was estimated for experimental 31-day-old recruits settled individually on *L. irrorata* shells. A host hermit crab subsequently was introduced to each shell, which then was maintained in isolation in a field cage for 95 days. Colonies were returned to the laboratory and fed brine shrimp nauplii until all colonies were sexually mature. Asexually derived estimates then were obtained according to established methods (Ferrell 2005). The x-axis was ln-transformed.

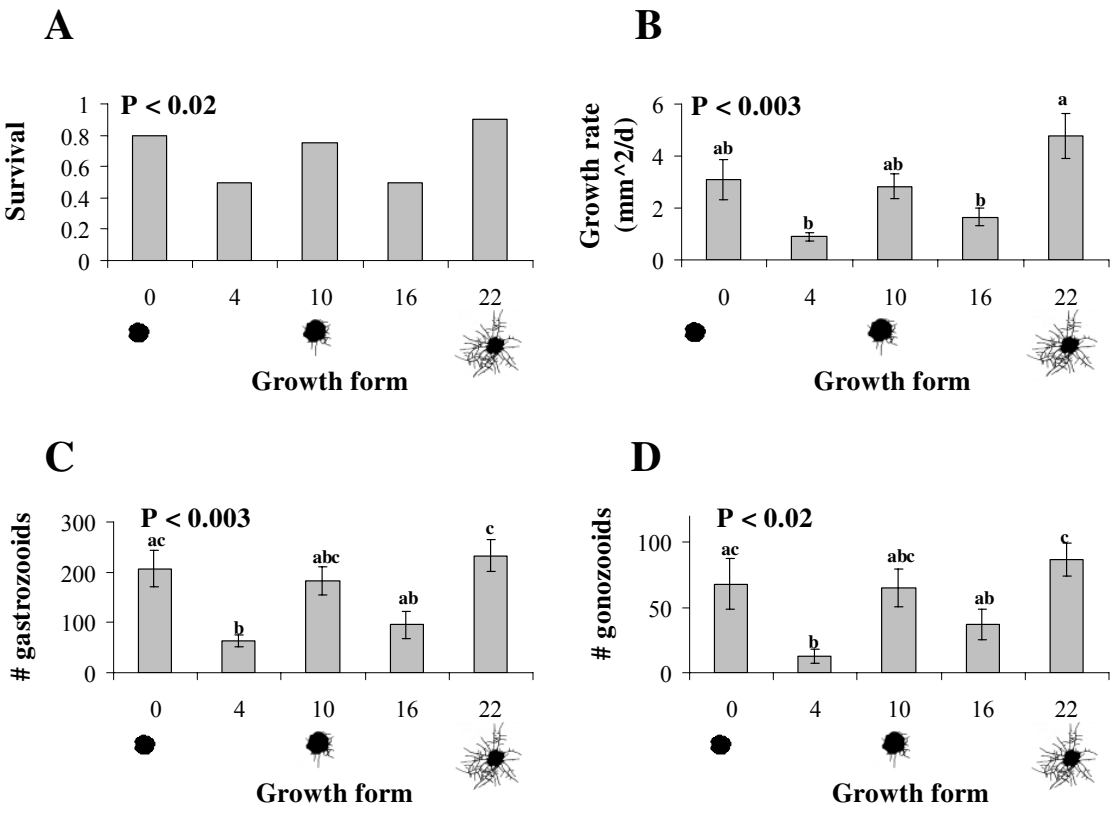

Figure 13. Fitness components of asexually-derived experimental colonies isolated in field cages at 56 days, prior to the anoxic event. (A) Colony survival depended on growth form category ($\chi^2 = 12.3$, df = 3, $P < 0.02$). For (B) surface area growth rate, (C) number of gastrozooids, and (D) number of mature gonozooids, letters designate statistically significant differences (Bonferroni corrected $\alpha = 0.005$).

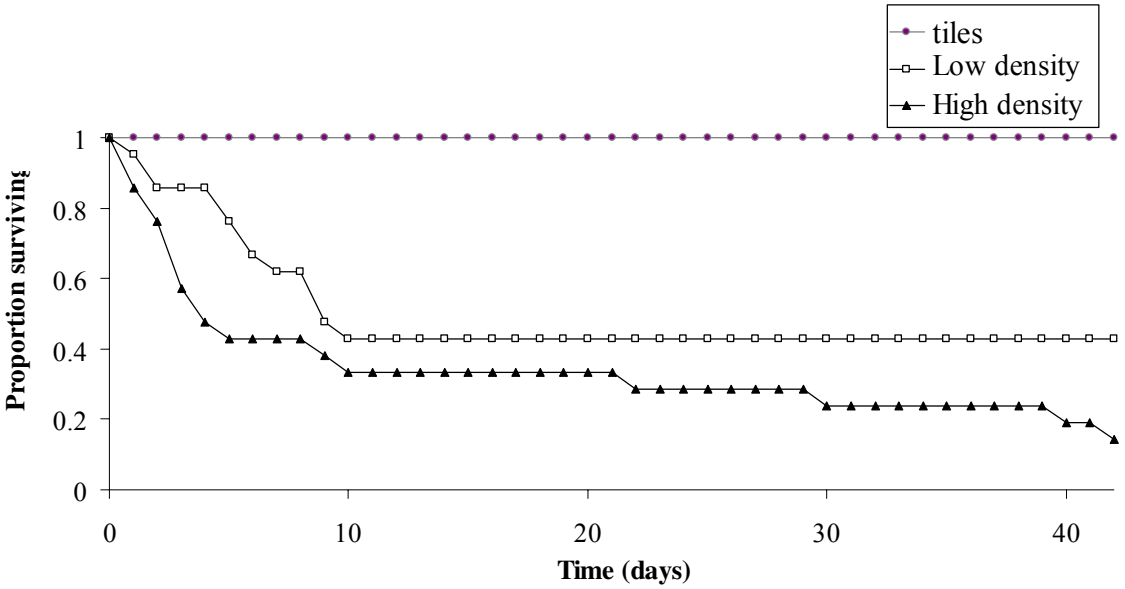

Figure 14. Colony survival over time. All colonies grown on artificial tiles and maintained in laboratory culture survived. Most mortality in the hermit crab treatments occurred within the first ten days of the experiment; however, significant mortality continued to accrue throughout the experiment in the high density treatment.

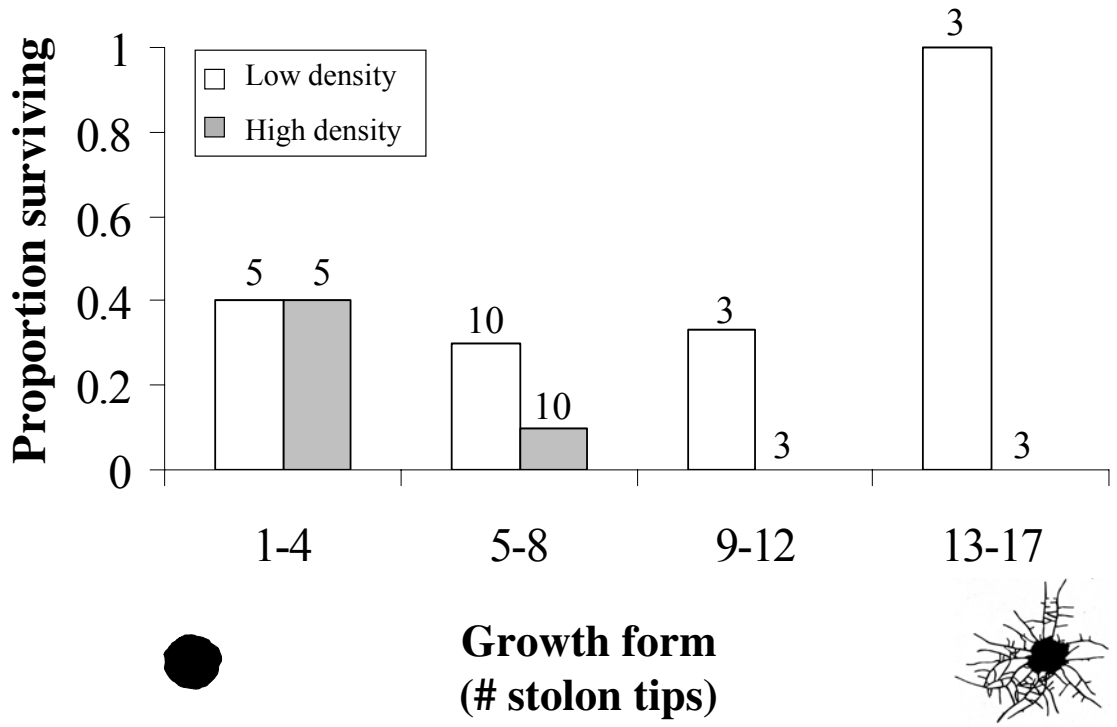

Figure 15. Colony survival among variable growth forms at termination of the experiment. Numbers above bars represent sample sizes. Experimental colonies were assigned low and high density treatments to provide equal representation of growth forms in each of four growth form categories (see 'Materials and Methods'). Growth form and density treatment exhibited a significant interaction ($P < 0.05$).

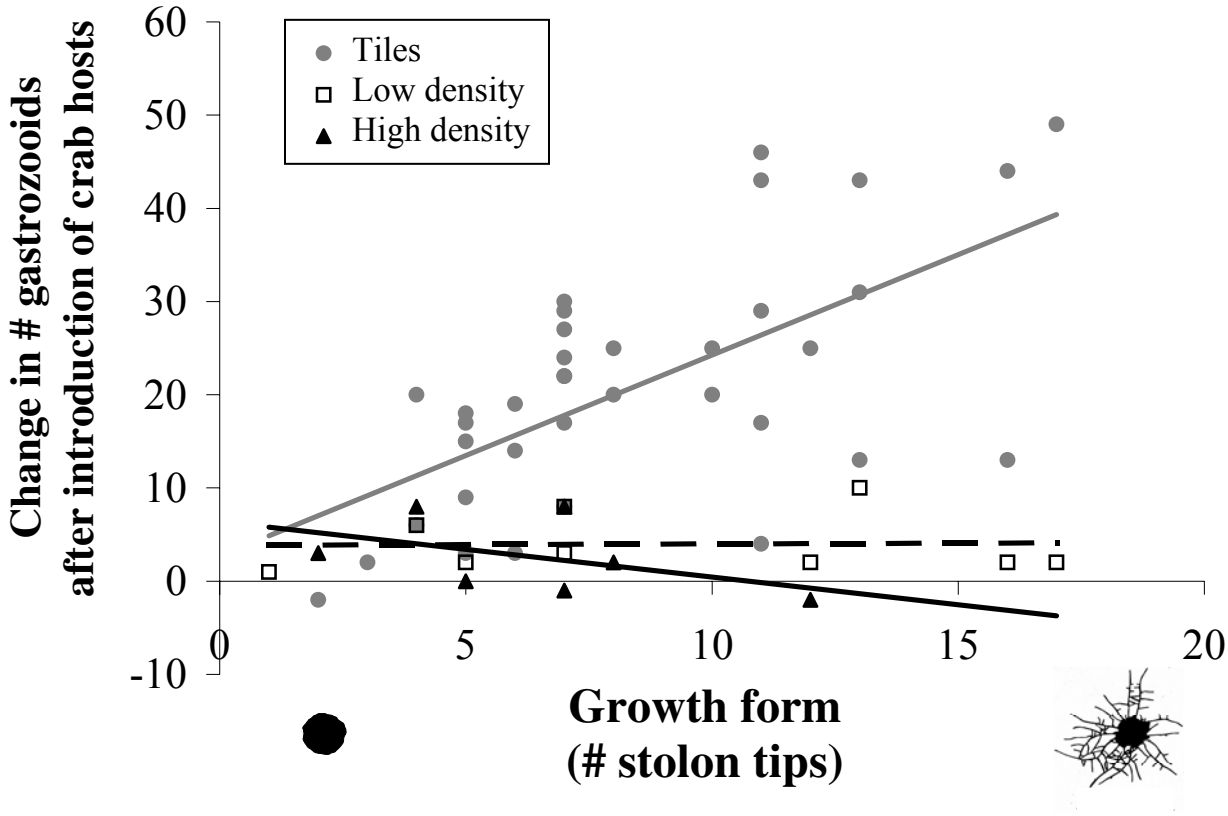

Figure 16. Colony growth as a function of growth form through first ten experimental days. Colonies grown on artificial tiles and maintained in laboratory culture exhibited a strong positive relationship between growth, in terms of gastrozooid production, and early ontogenetic growth form that was not observed at this early stage of the experiment in either of the hermit crab treatments. ANCOVA results revealed a significant interaction between treatments indicating that differences between the slopes of the laboratory tile data and each of the crab treatments but not between the two crab treatments (Table 14). ANCOVA including the two crab treatments only showed significant heterogeneity of slopes between low and high density treatments ($P < 0.04$; see text), indicating negative growth by more stoloniferous colonies experiencing high, but not low, hermit crab density. Lines represent simple linear regression best-fit lines.

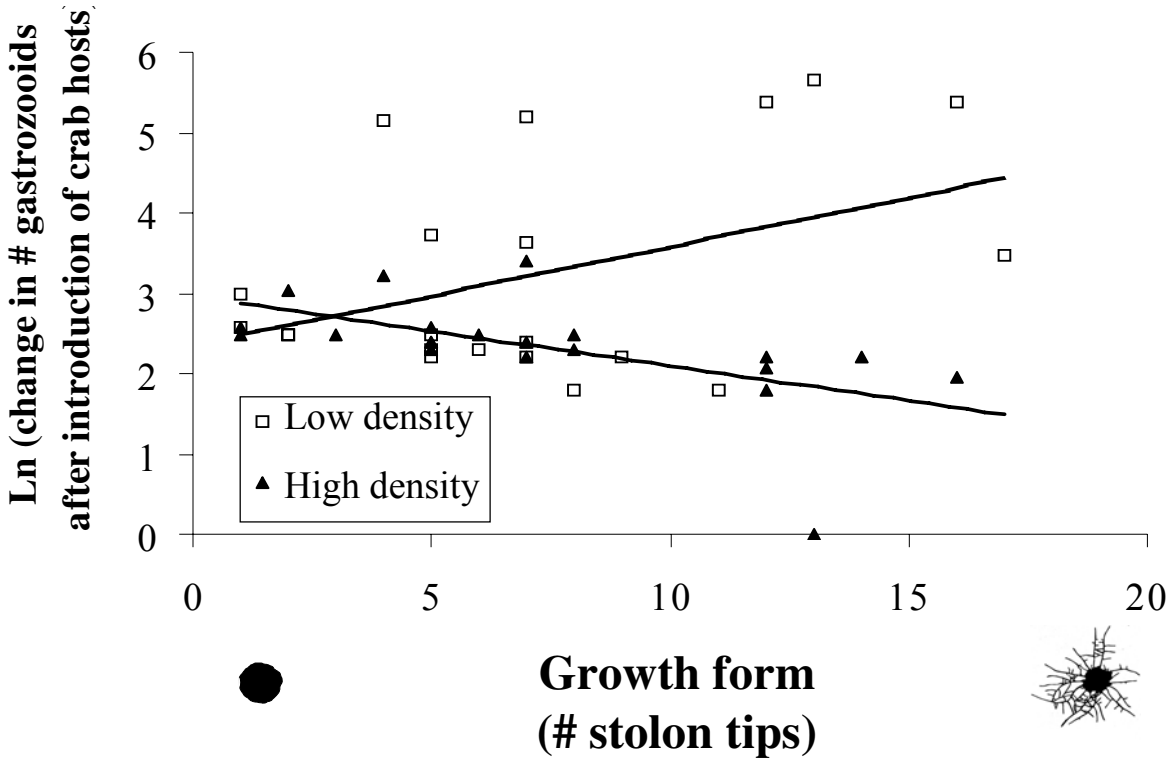

Figure 17. Colony growth as a function of growth form 39 days after the introduction of crab hosts. The y-axis was transformed to better enable comparisons between treatments, given colony size disparities between treatments. Most surviving 'low density' colonies exceeded 150 gastrozooids at 39 days whereas surviving 'high density' colonies did not exceed 20 gastrozooids. ANCOVA detected a significant interaction, indicating different slopes between treatments (Table 15). More stoloniferous growth forms grew most rapidly when maintained at low crab density but most slowly when maintained at high crab density. All colonies, whether surviving or dead, are included here (dead colonies = 0 gastrozooids) as only 3 'high density' colonies survived at the end of the experiment. Lines represent simple linear regression best-fit lines.

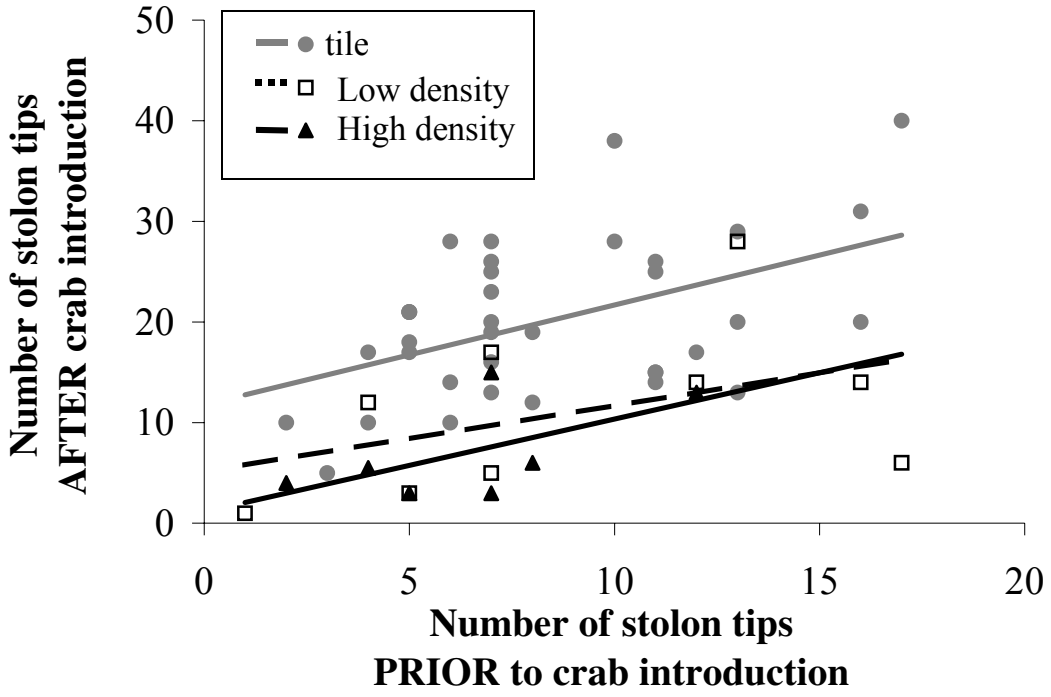

Figure 18. Effect of crab treatments on growth form. Growth form prior to crab introduction was measured 31 days post-metamorphosis. At this time, host hermit crabs were introduced to focal shells bearing experimental colonies and crab density treatments established. All colonies, regardless of treatment, exhibited a positive relationship between growth form prior to crab introduction and the same characterization performed after ten days. ANCOVA revealed no interaction, but detected significant differences in intercept between tiles and each of the crab treatments (Table 16). Lines represent simple linear regression best-fit lines.

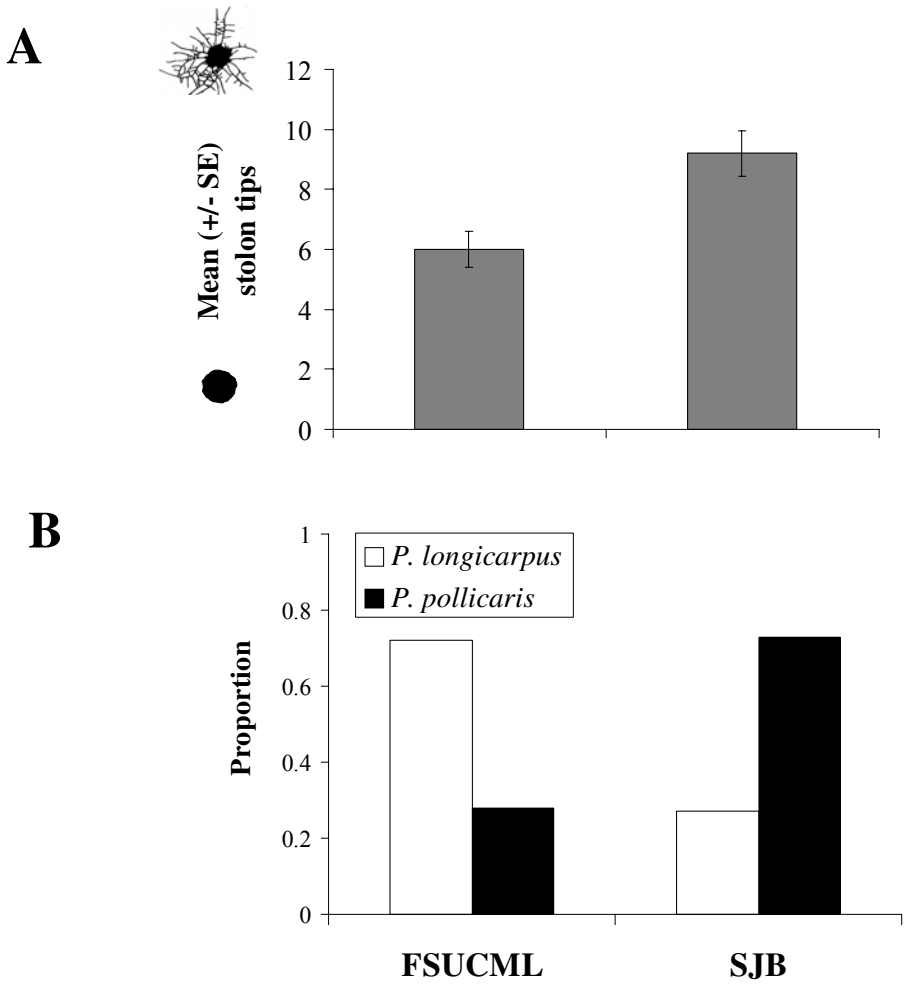

Figure 19. Variation in *H.* GM growth form and abundance of host hermit crab species between two sites in the northern Gulf of Mexico. *H.* GM associates with two hermit crab species, the long-clawed hermit crab *Pagurus longicarpus* and the flat-clawed hermit crab *Pagurus pollicaris*. *P. longicarpus* adults are relatively small and occur at characteristically higher densities than *P. pollicaris*; thus, inter-site differences in the abundance of hermit crab species reflect differences in host density. Hydroids collected at FSUCML (A) exhibited more mat-like growth forms ($T = 2.90$, $df = 141$, $P = 0.004$) and (B) associated predominantly with *P. longicarpus* (Contingency $\chi^2 = 27.5$, $df = 1$, $P < 0.001$). Standardized experimental matings between hydroids collected at different sites exhibit reduced reproductive success relative to within-site matings (Appendix 1), indicating significant reproductive isolation and reduced gene flow between these sites. FSUCML = Florida State University Coastal and Marine Laboratory, Turkey Point, FL; SJB = St. Joseph Bay, FL

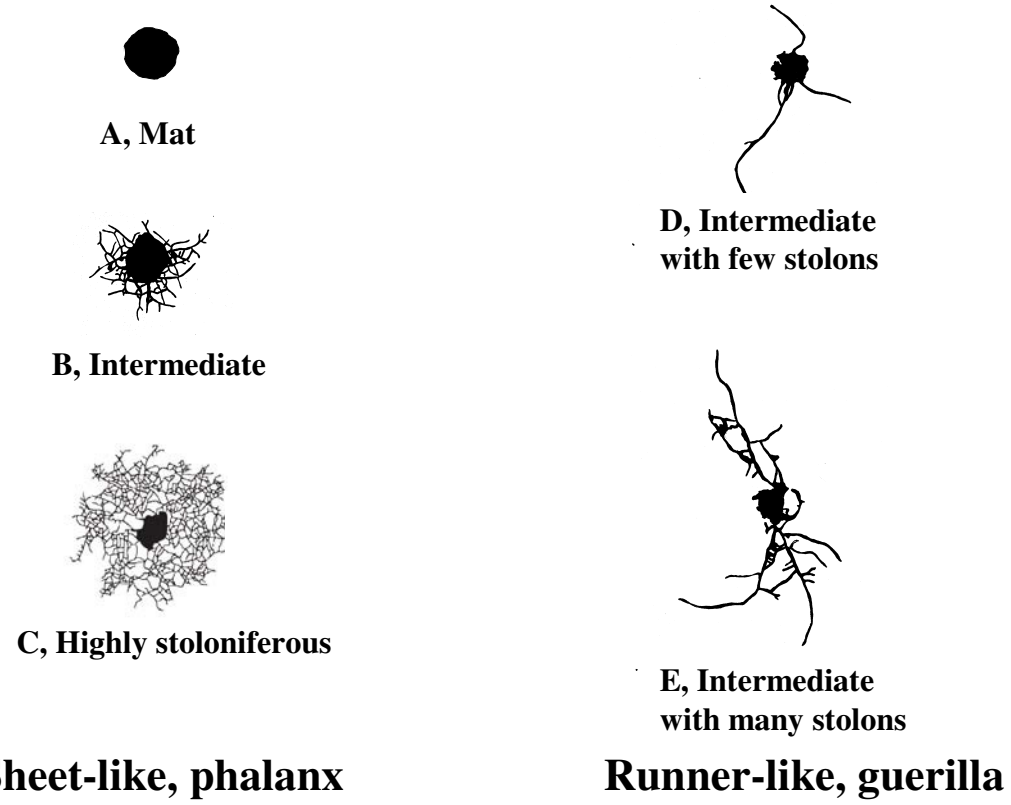

Figure 20. Relationship between mat-like, intermediate, and highly stoloniferous *Hydractinia* growth forms and the phalanx-guerilla, or sheet-runner, continuum. Entirely mat, or extremely mat-like, colonies clearly exhibit sheet-like growth. However, highly stoloniferous colonies are commonly equated with runner-like morphologies, despite the fact that they exhibit dense polyp spacing and short stolon connections. They are more accurately described as sheet-like phenotypes. Similarly, some intermediate growth forms may exhibit sheet-like morphologies through a combination of mat tissue and limited peripheral stolons with short branch lengths. At least two possibities exist for runner-like morphologies in *Hydractinia*. Both of these correspond to intermediate growth forms that result in relatively asymmetric, elongate growth forms.

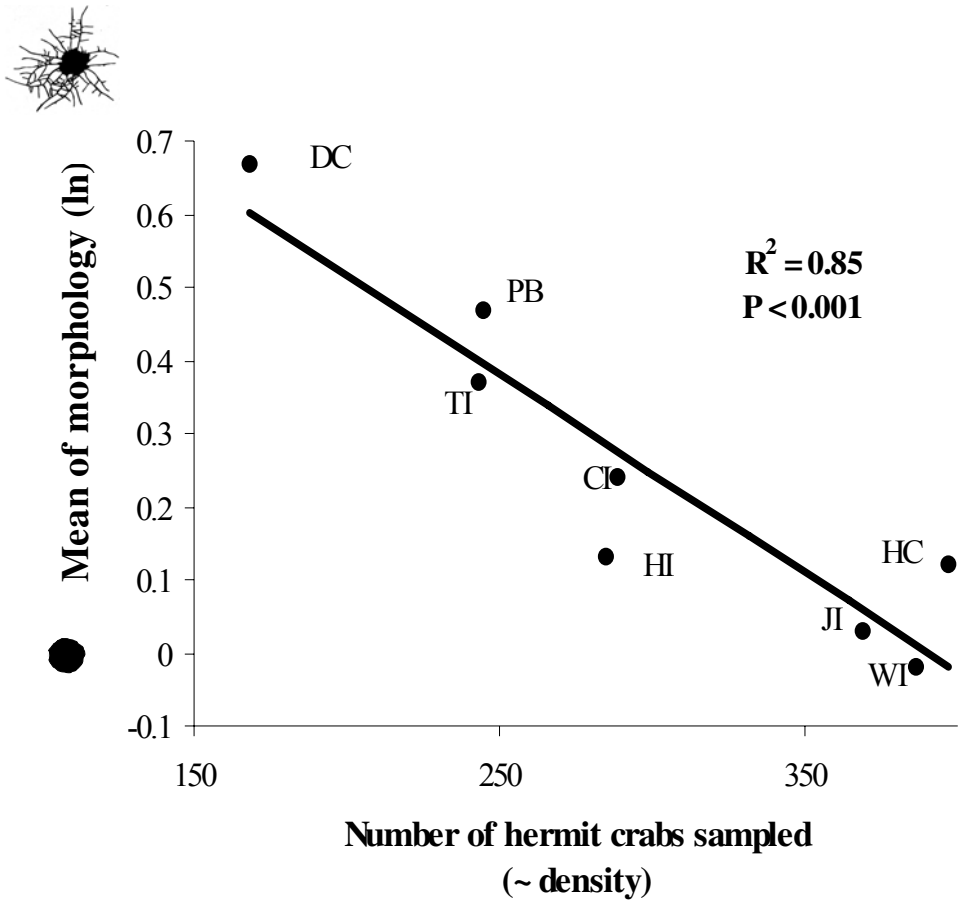

Figure 21. Mean growth form at each of 8 *H. polyclina* sites in the Gulf of Maine as a function of the number of hermit crabs sampled per site. This figure represents a re-analysis of data presented by Yund (1991). In this study, colony-level morphology (i.e., growth form) was estimated as a dimensionless ratio of stolon and mat tissue growth rates; higher values correspond to more stoloniferous phenotypes. Originally, a significant positive relationship was detected between mean growth form of hydroid colonies per site and an estimate of the frequency of competition ($R^2 = 0.58$, $P < 0.03$). According to the method of animal collection, reported in Yund and Parker (1989), hermit crab sample size increased with crab density among sites. Mean growth form is inversely related to hermit crab density ($R^2 = 0.85$, $P < 0.001$). In a multiple regression model incorporating both independent variables, 'frequency of competition' was not significant ($F_{1,5} = 0.86$, $P = 0.42$) whereas 'hermit crab density' remained significant ($F_{1,5} = 3.62$, $P = 0.015$). The proportion of the variance in growth form explained increased only minimally ($R^2 = 0.87$) from that explained by hermit crab density alone.

Table 10. ANOVA results testing for differences in mean growth form of dead, surviving (juvenile), and surviving (mature) field experimental colonies after 95 days. Pairwise comparisons indicated that mean number of peripheral stolon tips exhibited early in ontogeny (after 33 days) by colonies that attained sexual maturity in the field differed significantly from that of surviving juvenile colonies and animals that died before reaching maturity; an additional contrast showed that early growth form of dead colonies differed significantly from that of all surviving (juvenile + mature) field colonies (Bonferroni corrected $\alpha = 0.0125$). ***P < 0.001

Source	df	MS	F-ratio
Category	2	4457.0	18.9***
Error	32	236.4	

Table 11. ANOVA results at day 56 testing for differences in (A) surface area growth rate, (B) number of gastrozooids, and (C) number of mature gonozooids among five growth form categories, as shown in Figure 13B to D, respectively. All pairwise comparisons (Bonferroni corrected $\alpha = 0.005$) were performed and are indicated by different letters in Figure 18. * P < 0.05, **P < 0.01

A

Source	df	MS	F-ratio
Growth form	4	26.4	4.2**
Error	61	6.2	

B

Source	df	MS	F-ratio
Growth form	4	61623.4	4.7**
Error	61	13159.9	

C

Source	df	MS	F-ratio
Growth form	4	9553.9	3.2*
Error	61	3005.2	

Table 12. Genetic correlations among 10 genotypes in the field between growth form, life history – including survival, number of mature gonozooids (fecundity), size at first reproduction (SAFR) in terms of both areal coverage and number of gastrozooids, and the proportion of mature colonies at the earliest date of observed sexual maturity, an indicator of age of first reproduction (AOFR) – and overall growth in terms of both surface area covered (SA growth rate) and number of gastrozooids. Early ontogenetic growth form was characterized as the mean number of stolon tips of 5 age-standardized replicates per genotype whereas life history and growth traits were represented by the genotypic mean of 10 clonal replicates. Data were collected at 56 days, prior to the field anoxic event. Values represent Pearson product moment correlation coefficients, which are shown in bold if statistically different from zero. Shaded region represents genetic correlations between growth form and life history or growth. **P < 0.01, ***P < 0.001

	Growth form	Life history					Growth
	Stolon tips	Proportion surviving	Gonozooids	SAFR, areal coverage	SAFR, gastrozooids	AOFR, proportion mature	SA growth rate
Proportion surviving	0.33						
Gonozooids	0.32	**0.80****					
SAFR, areal coverage	0.37	**0.87*****	**0.79****				
SAFR, gastrozooids	0.19	**0.83****	**0.94****	**0.91*****			
AOFR, proportion mature	0.21	**0.80****	**0.81****	**0.83****	**0.84****		
SA growth rate	0.38	**0.89*****	**0.81****	**0.99*****	**0.89*****	**0.88*****	
Gastrozooids	0.21	**0.85****	**0.95****	**0.90*****	**0.99*****	**0.91*****	**0.91*****

Table 13. Genetic correlations among 10 genotypes in the field between growth form, life history, and growth *after* the field anoxic event. All traits were calculated as described in Table 12. A severe anoxic event occurred at the field site beginning approximately at day 70; all colonies regressed substantially and no colonies bore gonozooids immediately thereafter. Data were collected at (A) 77, (B) 133, and (C) 175 days. Values represent Pearson product moment correlation coefficients, which are shown in bold if statistically different from zero. Shaded region represents genetic correlations between growth form and life history or growth.
†P < 0.10, *P < 0.05, **P < 0.01, ***P < 0.001

		Growth form	Life history		Growth
		Stolon tips	Proportion surviving	Gonozooids	SA growth rate
A	Proportion surviving	0.30			
	Gonozooids	-	-		
	SA growth rate	0.47	**0.63***	-	
	Gastrozooids	0.14	0.22	-	**0.78***
B	Proportion surviving	-0.03			
	Gonozooids	-0.17	0.17		
	SA growth rate	0.54	**0.71***	-0.07	
	Gastrozooids	-0.08	0.61†	0.44	0.31
C	Proportion surviving	-0.17			
	Gonozooids	0.46	0.50		
	SA growth rate	-0.02	**0.81****	**0.67***	
	Gastrozooids	-0.21	**0.73****	0.64†	**0.90*****

Table 14. ANCOVA results examining the relationship between change in gastrozooid number and growth form 10 days after host crab introduction. All surviving colonies in each of the 3 treatments (tiles, low and high crab density) are included in this analysis. * = P < 0.05, *** = P < 0.001

Source	df	MS	F-ratio
Stolon tips	1	112.0	1.4
Treatment	2	1530.4	18.7***
Stolon tips*treatment	2	501.9	6.1*
Error	46	81.9	

Table 15. ANCOVA results examining the relationship between change in gastrozooid number and growth form 39 days after host crab introduction. All colonies were included in this analysis (dead colonies = 0 gastrozooids). Small sample size (N = 3 in high density treatment) precluded a similar analysis including surviving colonies at this point in the experiment. Analyses of surviving colonies only earlier in the experiment also detected a significant interaction (see text). * = P < 0.05, ** = P < 0.01

Source	df	MS	F-ratio
Stolon tips	1	10981	3.42**
Density	1	2183	0.68
Stolon tips*density	1	8220	2.56*
Error	38	3211	

Table 16. ANCOVA results examining the relationship between growth form estimates prior to and 10 days after exposure to hermit crab treatments. The intercepts of each of the hermit crab treatments differed from the laboratory tile treatment (low density: $F_{1,48} = 15.9$, $P < 0.001$; high density: $F_{1,48} = 14.6$, $P < 0.001$) whereas no intercept differences were detected between low and high crab density treatments ($F_{1,48} = 0.23$, $P = 0.63$). A more inclusive model showed that the interaction term was not statistically significant ($F_{2,46} = 0.20$, $P = 0.82$) and it therefore was excluded. *** = $P < 0.001$

Source	df	MS	F-ratio
Stolon tips	1	637.8	14.0***
Treatment	2	595.0	13.1***
Error	48	45.5	

CONCLUSION

Variable selection in space and time contributes to the maintenance of variation in genetically based variation in early ontogenetic growth patterns existing in natural *Hydractinia* populations. The frequency and severity of physical disturbance imposed by symbiotic host hermit crabs varies with host density, which is in turn determined by host species and size. When found in sparse host populations, competitively superior growth forms exhibit greatest survival and growth rate. In dense host populations, common in this system, competitively inferior growth forms exhibit greatest survival as well as early growth, which likely provides immediate benefits via escape from size-dependent sources of mortality and more distant benefits in terms of subsequently expressed life history traits, such as size and age at maturity. Evidence from natural populations of two hydroid species (*H. polyclina* and *H.* GM) suggests that spatial variation in host hermit crab density is tied closely to growth form with mat-like phenotypes predominating at dense sites. Although quantitative estimates of the strength of variable selection are not known, restricted gene flow among sites is plausible in *H. polyclina* and evident in *H.* GM. Selection on growth form likely varies within populations pagurid hermit crab densities exhibit a strong seasonal component: hermit crab movement may cease altogether in winter whereas extremely active, dense assemblages (e.g, up to 200/m^2) form in late summer. Models suggest that the joint action of spatial and temporal variation in selective regimes, as proposed here, represent a powerful mechanism for sustaining genetically-based trait variation. Atypical among hydroids, all *Hydractinia* colonies converge on a 'sheet' morphology, composed of continuous ectodermal mat tissue, as adults, but competitively inferior genotypes exhibit this morphology earlier in development. Ectodermal mat tissue may in fact represent an unusual morphological adaptation that has evolved in response to obligate and intimate association with hermit crab hosts.

APPENDIX A

REPRODUCTIVE COMPATIBILITY BETWEEN SITES AND AMONG VARIABLE GROWTH FORMS

Surveys of growth form variation among genotypes in natural populations revealed distinct peaks in abundance of variable growth forms in three *Hydractinia* species (Figure 2). Cryptic species are common in colonial invertebrates generally (Knowlton 1993, Hughes 2005) and have been identified in *Hydractinia* in the past (Buss and Yund 1989). Thus, it is possible that distinct abundance peaks actually represent unrecognized, but distinct species that differ in growth form. For this reason, I conducted standardized experimental matings between *H.* GM colonies of known parental growth form, including parents of mat-like and stoloniferous growth forms and within- and between-morph matings.

Materials and methods

In October and November 2005, I hand-collected *Hydractinia* GM colonies from sites TP and SJB and characterized growth form for each colony as described above. Hydroids of different sex were maintained in separate aquaria for at least four days, after which I conducted standardized laboratory matings (male x female) between colonies representing each of four cross types with respect to site of collection: TP x TP, TP x SJB, SJB x TP, and SJB x SJB. Using a microscalpel, tissue containing 20 ripe gonozooids from each parental colony was transferred to an opaque scintillation vial containing 15 ml of 1 μm-filtered seawater. Vials were loosely capped and maintained in the dark for 24 hours after which the caps were removed and vials were exposed to a bright light source for approximately 1 hour to induce spawning (Bunting 1984, Ballard 1942). After 48 hours, vial contents were examined, and the number of spawned but unfertilized eggs and planulae (i.e., fertilized eggs) were counted. I used an ANCOVA to examine the effects of parental site of collection (4 levels) and differences in parental growth form on reproductive success (proportion fertilized). Parental growth forms were categorized as mat (mean number of stolon tips < 10) or stoloniferous (mean number of stolon tips > 10). I then conducted three ANCOVAs, in which parental growth forms were defined with varying degrees of specificity. First, all parental growth form information was included (4 growth form levels: both parents mat; both parents stoloniferous; mat female X

stoloniferous male; stoloniferous female X mat male). Second, the between-morph crosses were pooled (3 levels). Third, both the within-morph and between-morph crosses were pooled (2 levels).

Reproductive compatibility

Site of animal collection significantly influenced reproductive success, defined as the proportion of eggs fertilized (Table 22). Experimental matings between *H.* GM colonies from sites TP and SJB exhibited significantly lower reproductive success in between-site crosses compared to within-site crosses, as indicated by linear contrasts (TP x TP, SJB x SJB versus TP x SJB, SJB x TP ($P < 0.05$ in each analysis, Table 17a-c). No significant difference in reproductive success was detected between crosses involving parental colonies of similar (both mat or both stoloniferous) or different (mat X stoloniferous) growth forms.

Conclusions

Matings between mat-like and stoloniferous growth forms did not exhibit reduced reproductive success relative to matings between alike growth forms. Thus, there is no evidence that extreme growth forms represent different species. If this were true, the central abundance peak in intermediate growth forms observed in natural populations (Figure 2) represents hybrids. However, laboratory matings within *H.* GM show no evidence that extreme growth forms exhibit reduced reproductive success or that field-collected colonies of intermediate growth form exhibit reduced fecundity or any degree of sterility (D.L. Ferrell, unpublished data). Also consistent with this result, molecular data show no evidence of genetic differentiation between extreme growth forms in *H. symbiolongicarpus*, the sister species of *H.* GM (R.K. Grosberg, personal communication). It seems unlikely that each of the three species exhibiting multi-modal frequency distributions (Figure 2) harbor cryptic species, and there is no evidence to date supporting such a scenario.

Table 17. ANCOVA results examining the effects of the difference between parental growth forms and site of animal collection (TP or SJB) on the proportion of spawned eggs fertilized. The results of three analyses are reported. (a) 4 growth form levels: both parents mat; both parents stoloniferous; mat female X stoloniferous male; stoloniferous female X mat male. (b) 3 growth form levels: both parents mat, both parents stoloniferous, between morphs. (c) 2 growth form levels: within morphs, between morphs. All three analyses included 4 levels for site effects: both parents TP, both parents SJB, TP (female) x SJB (male), SJB (male) x TP (female). *P < 0.05, **P < 0.01

(a)

Source	df	MS	F-ratio
Growth form	3	0.0533	0.7
Site	3	0.3508	4.3*
Error	32	0.0807	

(b)

Source	df	MS	F-ratio
Growth form	2	0.0757	1.0
Site	3	0.3499	4.5**
Error	33	0.0785	

(c)

Source	df	MS	F-ratio
Growth form	1	0.1441	1.9
Site	3	0.3564	4.7**
Error	34	0.0765	

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sice-blotched lizards. *American Naturalist* 163: 726-734.

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Yund, P.O., C.W. Cunningham, and L.W. Buss. 1987. Recruitment and postrecruitment interactions in a colonial hydroid. *Ecology* 68: 971-982.

BIOGRAPHICAL SKETCH

EDUCATION

Ph.D. Biology, Florida State University. 2007. GPA = 3.96

Dissertation: Maintenance of variation and adaptive significance of encrusting growth forms in the clonal hydroid genus *Hydractinia*.

M.S. Biology, Florida State University. 2002. GPA = 3.96

Thesis: Fitness consequences of allorecognition-mediated agonistic interactions in the colonial hydroid *Hydractinia* GM

B.A. Biology, Wabash College, Magna Cum Laude 1998. GPA = 3.89

TEACHING EXPERIENCE

Primary instructor, Florida State University, Department of Biological Science. Summer 2001-2004, 2006-2007.

- General Biology for non-majors, 6 semesters.

Assistant instructor. Florida State University, Department of Biological Science, Saturday-at-the-Sea Marine Education Program. 1999 – 2001.

Teaching Assistant, Florida State University, Department of Biological Science. 1998-present.

- Advanced Invertebrate Zoology, 1 semester.
- Animal Diversity, 1 semester.
- Biological Science II, 1 semester.

Teaching Assistant, Florida State University, Department of Biological Science. 1998-present. (continued)

- Biology of Higher Marine Invertebrates, 1 semester.
- Experimental Marine Ecology, 3 semesters.
- General Biology for non-majors, 4 semesters.
- Marine Biology, 4 semesters.

RESEARCH EXPERIENCE

Research technician and laboratory manager. Florida State University, Department of Biological Science. Fall 2004 – Summer 2005.

I assisted Dr. Don R. Levitan with the molecular components of his NSF funded sea urchin project. I have gained expertise in DNA extraction and quantification, PCR, microsatellite/paternity analysis of adults and larvae and sequencing and cloning adult DNA for the bindin gene. This research resulted in a publication in the journal *Science* (Levitan and Ferrell 2006). I have also used RAPD primers on *H.* GM for my own research, which Levitan originally had used for clonal identification and paternity analysis of *H. symbiolongicarpus*.

Research assistant. Florida State University, Department of Biological Science. Summer 2004.

I assisted Dr. Janie L. Wulff with her research program, which broadly entails the ecology and evolution of sponges and their symbionts, predators, and prey. My duties included laboratory management and laboratory work for studies of sponge life history and vicariance biogeography.

Research assistant. Bamfield Marine Sciences Centre. Summer 2001/2002.

I accompanied Dr. Don R. Levitan on his annual trip to Bamfield, Canada on the southwestern coast of Vancouver Island. There, I assisted him in conducting underwater field experiments (using SCUBA) and laboratory experiments dealing with the reproductive ecology of marine invertebrates, such as sea urchins and cup corals.

Graduate-level research. Florida State University, Department of Biological Science. 1998-present.

My interests lie in adaptive trait evolution of clonal organisms. I advocate a multi-faceted approach incorporating observational, experimental, field, and laboratory methods and using multiple lines of evidence to make inferences. Clonal taxa are pervasive features of terrestrial, aquatic and marine habitats. They are especially abundant in marine environments and can play a prominent role in marine communities. I am most interested in how ecological context drives the maintenance of variation in adaptive traits of clonal invertebrates, including those associated with recognition systems, competition, life history, and modes of vegetative growth. I successfully defended my M.S. thesis on January 23, 2002, under the supervision of Dr. Richard N. Mariscal, and my Ph.D. dissertation on June 29, 2007, under the supervision of Drs. Don R. Levitan and Janie L. Wulff.

Undergraduate-level research. Wabash College, Biology Department. 1997-1998.

Under the supervision of Dr. Eric J. Wetzel, I conducted research on the evolutionary ecology of internal parasites. During a Summer, 1997 internship, I sampled many freshwater snail populations in central Indiana for the occurrence of larval flatworm parasites. I subsequently designed and executed an independent research project with one of the species encountered in our sampling. The results of this research, along with a supplementary project by Nicholas J. Negovetich, were published in the February 2001 issue of the *Journal of Parasitology*.

Laboratory technician. Richard L. Roudebush V.A. Medical Center: Blood Bank, Hematology, and Coagulation Laboratories. Summer 1996/1997/1998.

Between spring and fall semesters of enrollment as an undergraduate or graduate student, I assisted laboratory technicians at a veterans' hospital in Indianapolis, Indiana. My duties included processing patient specimens, performing tests of blood coagulation properties on patient specimens, formalizing laboratory procedure records, developing forms for laboratory test results and data analysis, data entry, and evaluating patient treatment by hospital employees (e.g., nurses) as part of quality control assessment.

DIVING-RELATED CERTIFICATIONS

Research SCUBA diver. Academic Diving Program, Florida State University and Western Canadian Universities Marine Biological Consortium, Bamfield Marine Sciences Centre. April 2001.

SCUBA diver. National Association of Underwater Instructors. August 1999.

Boating Safety course. BOAT/US Foundation for Boating Safety. March 2000.

Standard First Aid. American Red Cross. January 2001.

Cardio-pulmonary resuscitation (CPR). American Heart Association. February 2001.

Oxygen administration. American Red Cross. February 2001.

Drysuit diver. Young Men's Club of America (YMCA) SCUBA. April 2001.

SCHOLARLY PUBLICATIONS

Levitan, D.R., and D.L. Ferrell. 2006. Selection on gamete recognition proteins depends on sex, density, and genotype frequency. *Science* 312: 267-269.

Ferrell, D.L. 2005. Competitive equivalence maintains persistent inter-clonal boundaries. *Oecologia* 142: 184-190.

Ferrell, D.L. 2004. Fitness consequences of allorecognition-mediated agonistic interactions in the colonial hydroid *Hydractinia* [GM]. *Biological Bulletin* 206: 173-187.

Ferrell, D.L. 2004. Gastropod shell size and morphology influence conspecific interactions in an encrusting hydroid. *Marine Ecology Progress Series* 275: 153-162.

Ferrell, D.L., N.J. Negovetich, and E.J. Wetzel. 2001. Effect of temperature on the infectivity of metacercariae of *Zygocotyle lunata* (Digenea: Paramphistomidae). *Journal of Parasitology* 87: 10-13.

AWARDS & HONORS

Graduate Student Publication Award. Florida State University. Department of Biological Science. 2006. $300.

Jack Winn Gramling Award in Marine Biology. Florida State University. Department of Biological Science. 2006. $2500.

Dissertation Research Grant. Florida State University. 2006. $500.

Thomas A. Cole Alumni Prize in Biology. Wabash College. 2006. $1000

Lerner-Gray Fund for Marine Research. American Museum of Natural History. 2005. $1050

Grand-in-Aid of Research. The Society for Integrative and Comparative Biology. 2004. $950

Robert B. Short Scholarship in Zoology. Florida State University. Department of Biological Science. 2002. $1500

Outstanding Teaching Assistant. Florida State University. Department of Biological Science. 2002.

Nomination for *Outstanding Teaching Assistant*. Florida State University. Department of Biological Science. 2001.

Completion of *Biology Teaching/Learning Workshop*. Florida State University. Department of Biological Science. 1998.

Phi Beta Kappa, Wabash College. 1998.

Dean's Honour List, Wabash College. 1994-'98.

Presidential Scholarship, Wabash College. 1994-'98.

PROFESSIONAL MEMBERSHIPS

American Society of Naturalists

Florida Oceanographic Society

Hydrozoan Society

National Marine Educators Association

Sigma Xi

The Marine Biological Association of the United Kingdom

The Society for Integrative and Comparative Biology