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Variation in Host-Symbiont Compatibility Among *Cassiopea*-Algal Symbioses

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Variation in Host-Symbiont Compatibility Among *Cassiopea*-Algal Symbioses

by

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Dedication

This dissertation is dedicated to the hundreds of thousands of *Cassiopea* that made this work possible. At times they were uncooperative but only because they had not yet recognized nor accepted their destiny….me!

Acknowledgements

How does one begin to acknowledge the fifty-two individuals, five organizations, three facilities, and two businesses that have provided support and assistance over the last six years while I pursued this dream? It's almost as difficult as writing the dissertation; nonetheless, I will list them all here so I can look them up later and remember that I owe them more than just my gratitude.

Because this has been an academic journey, I must undoubtedly begin with my 'pseudo-advisor', my colleague, and my friend, Tom Wilcox. Tom has been central to my research and training and, has been more kind and patient with me than I deserve. Many gray hairs later he has managed to teach me several important things: 1) be skeptical of your ideas, not yourself, 2)'way' good science 'absolutely' starts with a 'mad-gay' question, 3) there is such a thing as 'too many' polyps, 4) I monitor polyps for a living, and 5) islands are surrounded by water! I am forever indebted. Additionally, I would like to thank David Hillis, my primary advisor, who accepted me into his lab when he did not have to, and gave me the opportunity to follow through with my academic goals. Jim Bull (although he may not know it) was extremely compassionate and encouraging about my course in science and a big reason I continued. I would also like to acknowledge the other members of my thesis committee who have been supportive of my research goals: Ulrich Mueller, Mike Singer, and Harold Zakon.

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Investigators of endosymbiotic mutualisms are a fascinating bunch, and I am fortunate to have known and worked with the best. Bill Fitt helped with initial *Cassiopea* collections and graciously offered accommodations at his research facility, Key Largo Marine Research Laboratory. Ove Hoegh-Guldberg and Mary Alice Coffroth have both been a constant source of inspiration during the last few years.

Life as a graduate student can be a bumpy road, and I am thankful to those who have smoothed the ride through friendship and assistance. Lisa Meffert spent countless hours sitting through practice talks and reviewing proposals helping me improve my writing style and presentations. I owe a special thanks to my favorite Canadian, Franc Trampus. I don't know where to begin so, thank you $\times 10^{99}$ for laughs and commiseration! Laura Gutierrez has been a dedicated and attentive friend and spent many late nights assisting with analyses in the early days (a great shopping buddy too). A big thanks to Sandy Monahan, without whom I would have never survived paper work and politics! Also central to my research and training have been the graduate students and faculty who have come and gone over the years but have not been forgotten: Jennifer Reagan, Chris Caldow, Lori Hugg, Sara Hicks, Laurie Dries, Joel Sachs, Derrick Zwickl, Mark and Chris

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Variation in Host-Symbiont Compatibility Among *Cassiopea*-Algal Symbioses

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Abstract: Surprisingly few empirical studies have addressed the evolutionary ecology of mutualisms. In particular, there are few data available that address the following crucial questions: 1) what factors align the interests of symbiotic partners? 2) what is the degree of ecological and genetic variation in symbionts across multiple populations of a single host species? and, 3) what evolutionary mechanisms drive variation in host-symbiont compatibility where it exists? Although there is no general theory of mutualism, conventional wisdom suggests that mutualisms are best defined as reciprocal exploitations that provide net benefits to the partners involved. Contemporary theory regarding the evolution of virulence has identified several factors that help align host and symbiont interests. However, the extent to which natural systems conform to these theoretical

expectations and what factors are most responsible for maintaining cooperative symbioses remains unclear. I used *Cassiopea xamachana* to address what evolutionary and ecological factors influence endosymbiotic mutualisms. *Cassiopea*, like many marine invertebrates, harbors endosymbiotic algae within its tissues. Algal symbionts are acquired each generation via horizontal transmission. In chapter 3, I examined variation in host-symbiont compatibility by performing a series of cross-infection experiments using *Cassiopea* larvae and algal symbionts collected from a single medusa at ten sites in the Florida Keys. Results reveal significant differences among *Cassiopea*-algal combinations for both host survival and growth. In chapters 4 and 5, I quantify the observed variation by increasing the number of polyp lineages used per site. Results indicate that the observed variation among *Cassiopea*-algal combinations is geographically structured. Additionally, significant host-symbiont interaction effects suggest that the algal symbionts are locally adapted to jellyfish hosts within a given site. In chapter 5, I re-examine variation in host-symbiont compatibility by using seawater to infect *Cassiopea* hosts. The results roughly mimic the results obtained in chapters 3 and 4. In chapter 6, I investigate the population genetic structure of the algae inhabiting *Cassiopea* using RFLP and ISSR markers. Results indicate that the algal symbionts are members of the same species, *Symbiodinium microadriaticum*. Further, there is marked intraspecific

symbiont variation within this species. Overall, host-symbiont compatibility plays a vital role in the symbiotic outcome.

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VARIATION IN HOST SYMBIONT COMPATIBILITY AMONG *CASSIOPEA*-ALGAL SYMBIOSES

Chapter One: Introduction

PART 1: MUTUALISMS - A HISTORICAL RETROSPECTIVE

> Different kinds of organisms help each other out. This, in brief, is the idea of mutualism (Boucher, 1985).

Endosymbiosis is defined as an intimate association between individuals that are members of a different species for significant portions of the life cycle (De Bary, 1879). Mutualistic endosymbiotic associations involve a host and symbiont, and in some cases, multiple symbionts, that are beneficial to one another. Generally, the larger organism is considered the host and the smaller organism the symbiont. Symbiotic partners come from many different taxa and vary a great deal in the details of their relationship. The biological literature often treats mutualistic endosymbioses as exotic. In fact, until the last few decades, mutualisms were considered a fascinating biological topic, but unworthy of study because their ecological importance in populations was considered small (May, 1973a; 1973b). By the mid-70's it became clear that mutualisms were conspicuous and ecologically important factors in all ecosystems (May, 1976; Boucher, 1985; May and Seger, 1986). The idea that mutualisms had been seriously underestimated (Gilbert, 1975) was spurred by a flurry of papers describing both intimate and indirect mutualisms in a host of unexpected places: in mid-ocean diatom mats (Martinez *et al.* 1983), in toxic sulfur-rich waters in the

deep sea (Cavanaugh *et al.* 1981), in rice paddies (Joshi and Hollis, 1977), trees and epiphytes (Nadkarnia, 1981), and invertebrates and their epibionts (Bloom, 1975; Vance, 1978) to name a few.

Today it is clear that endosymbiotic mutualisms are ubiquitous geographically and evolutionarily, with mutualist partners found in all organismal kingdoms and in all ecosystems (Boucher, 1985). These associations are profoundly important at all levels of biological organization and often allow organisms to exploit novel ecological niches normally inaccessible to the free-living partners. Endosymbiotic mutualisms have been implicated in the evolution of organic diversity (Bermudes and Back, 1991), including the origin of eukaryotic cells, the development of metazoan diversity, the development of hydrothermal vent communities, and the origin of land plants (Lewis, 1991). Since the mid-70's, empirical studies have generated a sizable body of literature regarding the natural history of mutualisms, the benefits for the species involved in the association, and the conditions under which they operate. Unquestionably, mutualisms are ecologically important aspects of communities but, the increase in understanding of mutualisms has also generated a number of questions about how mutualistic interaction evolve and remain mutualistic. Said another way, what mechanisms ensure that the relationship between partners remains mutually beneficial and evolutionarily stable? Recently, theoretical techniques have been employed to address and explore these types of questions including: game theory and population dynamic models (Axelrod and Hamilton, 1981; Leimar, 1997; Doebeli and knowlton, 1998; Roberts and Sherratt, 1998; Killingback *et al.*, 1999;

Wahl and Nowak 1999a, 1999b), biological market models (Noe, 1990; Noe *et al.,* 1991; Noe and Hammerstein, 1994, 1995), and models of the evolution of virulence (Bremerman and Pickering, 1983; Ewald, 1987; Matsuda and Shimade, 1993; Yamamura, 1993, 1996; Lenski and May, 1994; Nowak and May, 1994; Tilman, 1994; Maynard-Smith and Szathmary, 1995; van Baalen and Sabelis, 1995, 2001; Genkai-Kato and Yamamura, 1999).

The rapid pace of theoretical developments has, so far, outstripped the pace of empirical research. Somehow, researchers must bridge the gap that exists between the empirical and theoretical work by testing theory with natural systems. This dissertation is concerned with empirically investigating host-symbiont compatibility and the mechanisms that drive cooperation versus virulence among mutualistic associates.

PART 2: THE EVOLUTIONARY PERSPECTIVE ON COOPERATION

> It is evident that we are on the threshold of further discoveries, and that a wide field of fruitful research is open to those who enter upon it….elucidation of the interesting biological problems that lie before us in the study of symbiosis and the allied subject of parasitism (Nuttall, 1923).

Recent theoretical attempts to explain the conditions necessary for the evolution of virulence (the harm a pathogen has on its host) have identified several factors that can help align the interests of symbiotic partners, permitting the evolution of cooperation (Ewald, 1987; Douglas, 1994, 1995; Maynard Smith and Szathmary, 1995; Maynard Smith, 1998; Herre *et al.*, 1999; Wilkinson, 2001). These factors are not independent but often interrelated. First, epidemiological models based solely on symbiont fitness reveal that cooperation

depends on the opportunities for transmission. In general, theory predicts that symbioses maintained by vertical transmission (transfer of symbionts from parent to offspring) will evolve benevolent symbionts because net transmission to new hosts is entirely determined by host reproductive success. Conversely, those endosymbioses maintained by horizontal transmission (transfer of symbionts between unrelated individuals) will be less likely to develop or maintain cooperation because symbiont fitness is no longer tied to the hosts' reproduction. The relationship between transmission mode and virulence has been empirically examined. For example, a theoretically well-behaved mutualistic system is the leaf cutter ant-fungal association (Currie *et al.*, 1999a, 1999b; Wilkinson 1999b). In this system researchers identified a mutualistic fungus and bacteria that were vertically transmitted and a parasitic fungus that was transmitted horizontally. Additionally, using a bacteriophage Bull *et al.* (1991) showed that increased opportunities for transmission resulted in higher degrees of virulence. Although some mutualistic endosymbioses are consistent with theoretical expectations, many do not conform to these textbook expectations. There are many associations that reassemble each generation via horizontal transmission and persist as cooperative complexes. It is regularly claimed that 80-90% of all land plants are mutualistically associated with mycorrhizal fungus, and that most vertebrate and invertebrate herbivores need mutualistic microbes to digest cellulose (Cohen, 1993), yet these ecologically crucial relationships depend on horizontal transmission of symbionts (Savage, 1977; Allen, 1991). Additionally, many marine symbionts are involved in mutually beneficial associations that reassemble

each generation via horizontal transmission and appear to be evolutionarily stable. Both geological and molecular systematic data indicate that reef-building corals have had a stable symbiosis with symbiotic algae since the late Triassic (Stanley, 1995; Wilcox, 1997). Similarly, legume-*Rhizobium* bacterial symbioses appear to have had a long stable history (Parker, 1999). Other examples of important horizontally transmitted mutualistic symbionts include the luminescent bacteria in fish and cephalopods (Genkai-Kato and Yamamura, 1999). Thus, vertical transmission is apparently not a necessary requirement for mutualistic cooperation.

A second factor that can help align the interests of mutualistic associates and facilitate the evolution of cooperation is genetic uniformity of symbionts within individual hosts. Genetic homogeneity of symbionts within a host reduces selection for traits that increase between-symbiont competitive ability to the detriment of the host's fitness. Vertical transmission (of benevolent symbionts) over many generations would facilitate the reduction of genetic diversity among symbionts by eliminating novel (selfish) inputs to the symbiont community and by providing a potential bottleneck at each generation (Douglas, 1996). In other words, vertical transmission can lead to symbiont sorting, hence genetic uniformity (Frank, 1996). Genetic uniformity of symbionts within the host has been considered vital to the stability of symbioses because a genetically homogeneous symbionts population would cost less to maintain than a heterogeneous complex of symbionts with variegated nutritional needs. For example, animals surrounding deep-sea vents house a single symbiotic

chemolithotrophic bacterial genotype (Madigan *et al*., 2000). Additionally, it has been suggested that heterogeneous symbiont populations would increase within host competition for resources resulting in the overexploitation of the host i.e. parasitism (Anderson and May, 1991; Law, 1991; Sigmond, 1993; Maynard Smith and Szathmary, 1995). In other words, selection should favor the symbiont that makes maximum use of the host before its competitors do so (Maynard Smith, 1998). Appealing as it may be, there are examples of mutualistic endosymbiotic associations in which hosts' house multispecies communities of symbionts that are acquired via horizontal transmission each generation but persist as cooperative complexes. For example, many species of mycorrhizal fungi can occupy the same plant root (Allen, 1991; Bruns, 1995). Additionally, many animals contain a plethora of microorganisms; for instance, a cow's rumen may contain 200-400 species of bacteria and 40-50 species of ciliate protests (Douglas, 1994). Finally, the reef-building coral *Montastraea* houses multispecies communities of algal symbionts. In this case, it has been suggested that hosting polymorphic symbiont communities increases host fitness when environmental heterogeneity is prevalent (Rowan, 1997; Carroll, 1998; Wilkinson, 1998). Therefore, it would appear that violation of within host genetic homogeneity does not preclude the absence of mutualistic associates.

Finally, spatial structure of populations leading to repeated interactions between would-be mutualist partners might also reduce potential conflict among symbiotic partners. Vertical transmission implies a continual interaction between host and symbiont lineages facilitating the evolution of complete dependence.

This in turn reduces the evolutionary viability of nonsymbiotic alternatives. In other words, restricted options outside the association for both partners are thought to promote cooperation and long-term stability; however, this does not require vertical transmission. For instance, although an intuitively attractive idea, there are horizontally maintained, obligate symbioses. Many marine invertebrates rely on algal symbionts to induce metamorphosis and complete their life cycle. Wilcox (1997), as a means of explaining the occurrence of obligate mutualistic endosymbioses, suggested that if the availability of uninfected hosts is low (i.e. low horizontal transmission opportunity), and if symbionts are restricted to a brief developmental 'window of opportunity' for infecting new hosts, then selection would favor symbionts that promote host viability. Thus, the probability of a symbiont successfully colonizing a juvenile host without killing it, and presumably itself, is much lower for selfish or malevolent symbionts.

Many recent reviews stress the importance of vertical transmission, genetic uniformity, and the spatial structure of populations to the evolutionary stability of mutualisms (Hoeksema and Bruna, 2000; Wilkinson and Sherratt, 2001; Van Baalen and Jansen, 2001). The framework of these arguments for factors that reduce conflict and support the evolution of cooperation are enticing and characterize many symbiotic systems yet, many of the most ecologically important mutualisms involve horizontal transmission and a diverse array of symbionts. Given these exceptions, it is important to determine the extent to which natural systems conform to these theoretical patterns and what factors are

most responsible for determining compatibility among symbiotic partners where it exists.

PART 3: GENERAL RESEARCH QUESTIONS

This dissertation broadly addresses variation in host-symbiont compatibility (chapter 3) but, more specifically, how variation is structured and maintained (chapters 4-6) and, most importantly, the evolutionary and ecological implications of the observed variation (Chapter 7). I have developed and used the *Cassiopea*-algal complex (Illustration 1.1) to examine the following general questions: 1) Are enodsymbionts equally benevolent across a single host species? 2) Does geography play a role in structuring variation in host-symbiont compatibility? and, 3) Does intraspecific symbiont variation drive host-symbiont interaction effects, thus dictating the symbiotic outcome?

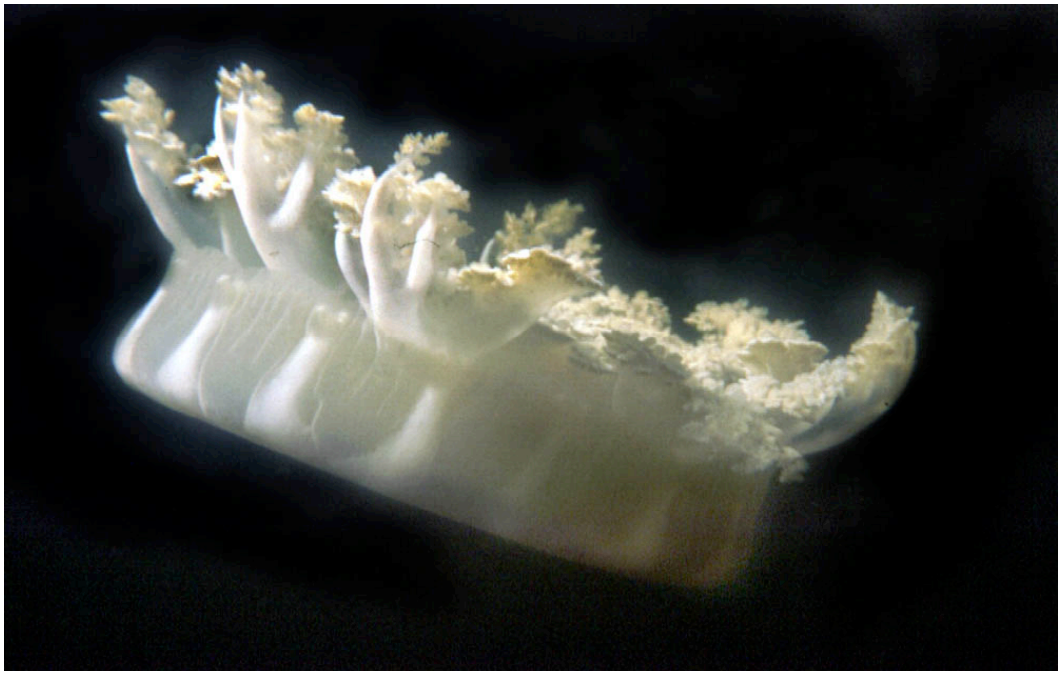


Illustration 1.1: *Cassiopea xamachana*, the mangrove or upside down jellyfish.

The brown color indicates the presence of marine endosymbiotic algae within the host's tissue. This is an obligate endosymbiotic association.

VARIATION IN HOST-SYMBIONT COMPATIBILITY AMONG *CASSIOPEA*-ALGAL SYMBIOSES

Chapter Two: Natural History of *Cassiopea xamachana* and it's algal symbiont, Symbiodinium microadriaticum

PART 1: ABSTRACT

The mangrove or upside down jellyfish, *Cassiopea xamachana*, harbors endosymbiotic algae within its tissues. The symbiotic algae provide the host with photosynthetically produced carbohydrates in exchange for inorganic and organic nutrients. By definition, this intimate complex is an endosymbiotic mutualism because both host and symbiont benefit from the association. *Cassiopea xamachana* is widespread in the Caribbean and often blankets the mangroves surrounding many Caribbean islands; however, little is known about the natural history of this species. Here I detail the life history of *Cassiopea xamachana*, general biology, physiology, and ecology. Additionally, I report methods for collecting and maintaining *Cassiopea xamachana* as well as its symbiotic algae, *Symbiodinium microadriaticum*, under standard laboratory conditions. The development of this system in the laboratory and field environment has made it a model system for examining evolutionary and ecological problems of endosymbiotic mutualisms.

PART 2: THE HOST - *CASSIOPEA XAMACHANA*

The mangrove jellyfish, *Cassiopea xamachana,* a member of the phylum Cnidaria, is a rhizostome scyphozoan. Unlike typical jellyfish, *Cassiopea* lack marginal tentacles and a central mouth. Instead, four pairs of oral arms arise from the manubrium (bell) and fuse to form numerous mouth openings or ostia (Aria, 1997). Commonly referred to as the 'upside down jellyfish', *Cassiopea* medusae rest on the sediment bottom with oral arms flowing upward and catching zooplankton in the water column.

Life cycle of *Cassiopea xamachana*

Cassiopea has a typical scyphozoan life cycle (Figure 2.1). During the life cycle, sexual medusae release aposymbiotic (algal-free) larvae that settle as scyphistomae (polyps) (Trench *et al.* 1981). Polyps attach to a wide variety of substrates within the mangrove habitat, including mangrove leaf debris (personal observation) and mangrove roots (W.K. Fitt, pers. com.). Symbiotic algae are then acquired from the environment by the polyps and only then undergo metamorphosis (strobilation) into free-swimming ephyra (see strobila Figure 2.1 – the orangish-brown circles within the host are indicative of algal infection). Prior to strobilation, scyphistomae may produce asexual buds from near the base of the polyp. These asexual buds will settle and develop into scyphistomae.

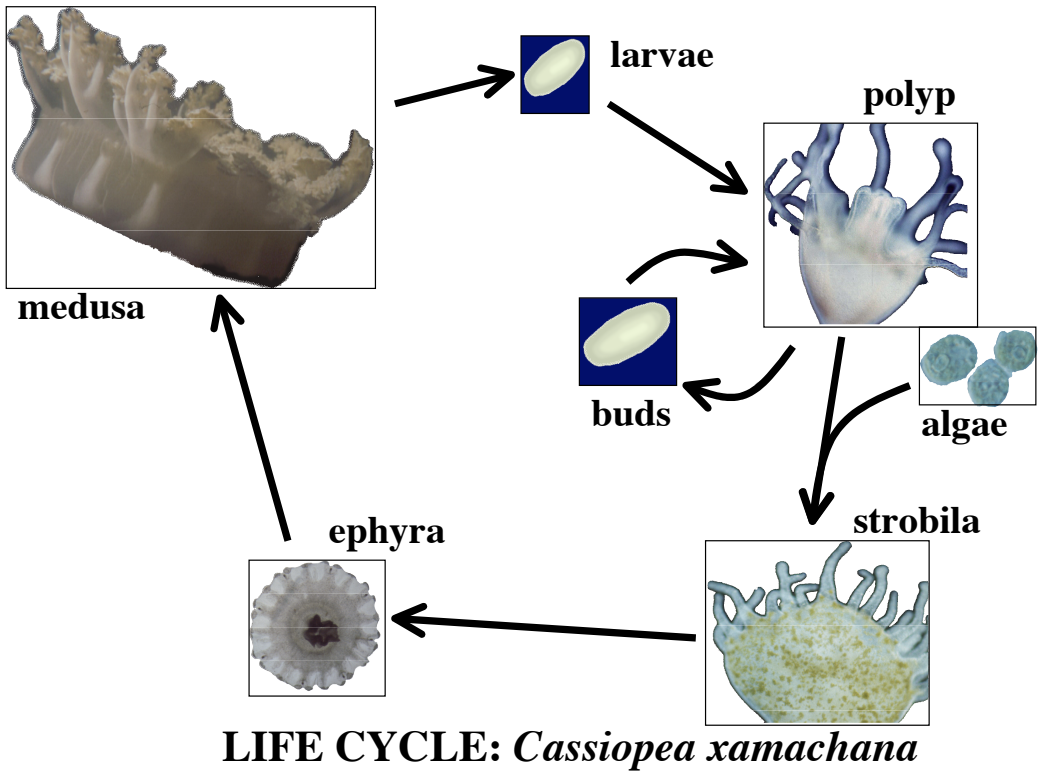


Figure 2.1: The life cycle of *Cassiopea xamachana*.

Adult *Cassiopea* medusa can reach 12-15 inches in diameter. The medusae sexually reproduce larvae, which settle on the substrate as aposymbiotic scyphistomae. Scyphistomae acquire symbiotic algae from the environment and begin metamorphosis to the free-swimming ephyra stage, which later become sexual medusa. The polyp life stage is much smaller and inconspicuous with sexually reproduced scyphistomae ranging in size from .1-.5mm in diameter and asexually reproduced scyphistomae ranging in size from 2-5mm in diameter.

Cassiopea xamachana is a gonochoristic species and is sexually dimorphic. *Cassiopea* medusae are sexually mature at approximately 5-7cm in

diameter (pers. obs.). Females have a distinct off-white circular brooding area where sexually reproduced eggs develop into planula or larvae. This brooding area is located where the oral arms converge. The larvae are eventually released by the medusa or actively swim out of the brooding area (pers. obs.). *Cassiopea xamachana* is continuously reproductive with peak reproduction occurring during the summer months when newly metamorphosed ephyra dominate shallow mangrove habitats. Newly settled polyps acquire symbiotic algae very quickly, (3-5 days) both in the field, and, in the laboratory (pers. obs.). Once the algal cells within the host become dense, polyps begin strobilation (pers. obs.). Strobilation is facilitated if the temperature of the water is greater than or equal to 25°C (Fitt, 1983). The ephyra then develop oral arms and turn into an adult medusa.

Host culturing

The sexually produced larvae of *Cassiopea* can be collected directly from the brooding area of gravid females by gentle pipetting. If maintained in filtered seawater (FSW) or artificial seawater (AWS - distilled water and 35 ppt Instant Ocean treated with NovAqua, Kordon, Inc.), the larvae will eventually settle as symbiont-free polyps.

The sexually produced larvae collected from female *Cassiopea* vary in the amount of time needed to settle to the polyp stage, depending upon the site they originate from and the time of year they are collected. I collected 200 larvae from three medusa at four sites in the Florida Keys: two northern sites and two southern sites separated by 160km (Figure 2.2). JJ, a bayside survey site, and CP, an Oceanside survey site, are located in the northern Florida Keys. BC and SL,

Oceanside survey sites, are located in the southern Florida Keys. The two most distant sites are separated by 160km. These sites are typical mangrove habitats ranging in depth from 2-3 meters. The salinity across the sites ranges from 37-40+ppt. At each site, larvae were collected from 3 similarly sized medusae. The average amount of time required for larvae from each site to settle as polyps was determined across four time intervals: May 2000, July 2000, May 2002, and July 2002. At each interval 600 larvae were collected from three medusa at each of the sites. An analysis of variance (ANOVA) showed that there is a significant interaction between larval origin and the month they were collected (Figure 2.3; $p < 0.0001$; $F = 27.0228$; D.F. = 3, 11) indicating differential development or reproduction across collection sites. For instance, larvae collected from southern sites take longer to settle as polyps in early summer (May); however, they take less time to settle as polyps in the late summer (July). Conversely, larvae collected from northern sites take less time to settle in the early summer and more time to settle in the late summer (Figure 2.3). There are no significant differences across the two years in this trend.

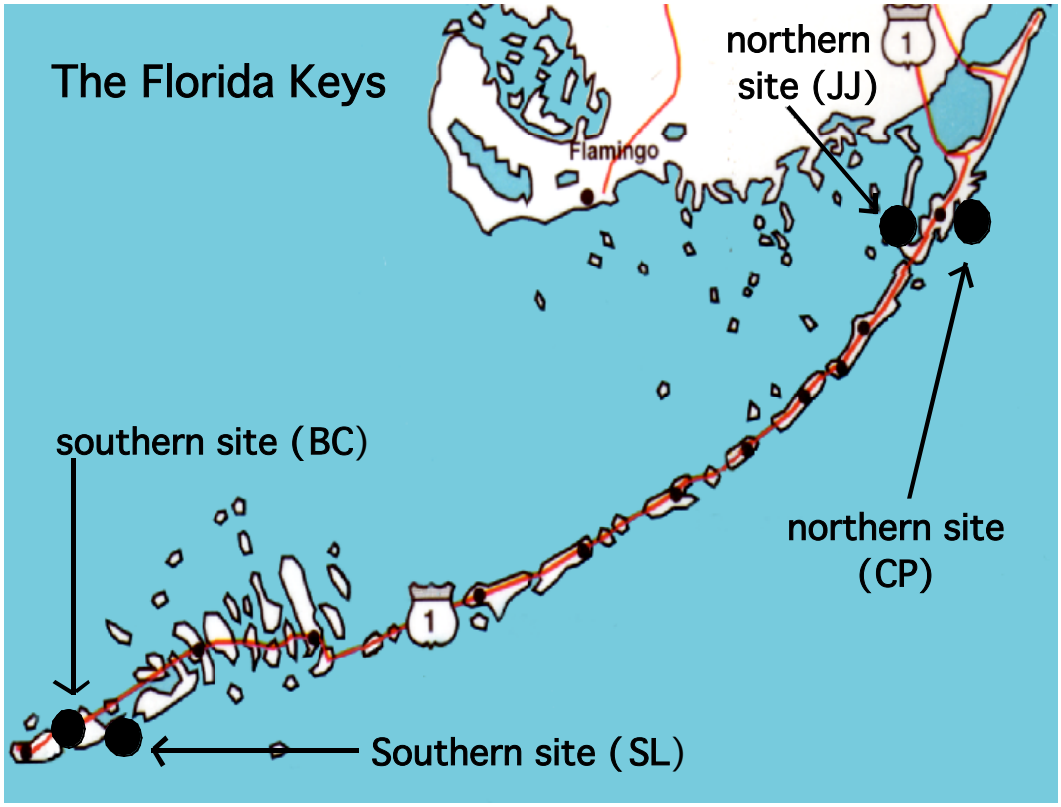


Figure 2.2: Map of the Florida Keys.

Surveys were conducted at the two northern sites: JJ (bayside) and CP (oceanside) and at a southern site: BC and SL (Oceanside southern sites). The two most distant sites are separated by approximately 160km.

Additionally, sexually produced larvae generally settle faster if maintained in 100μm-filtered seawater (FSW) from their site of origin. However, larvae will eventually settle as polyps in artificial seawater (ASW). For example, 600 sexually reproduced larvae were collected from a female *Cassiopea* medusa off of

Long Key in the Florida Keys (located in the middle Florida Keys; Figure 2.2). There was a significant difference in the time to settlement between larvae reared in ASW and larvae reared in FSW (Figure 2.4). By the last day (day 11), 51% of larvae maintained in FSW had settled as polyps compared to 9% settlement in those maintained in ASW ($p = 0.0025$; $F = 45.36$; D.F. = 1, 5).

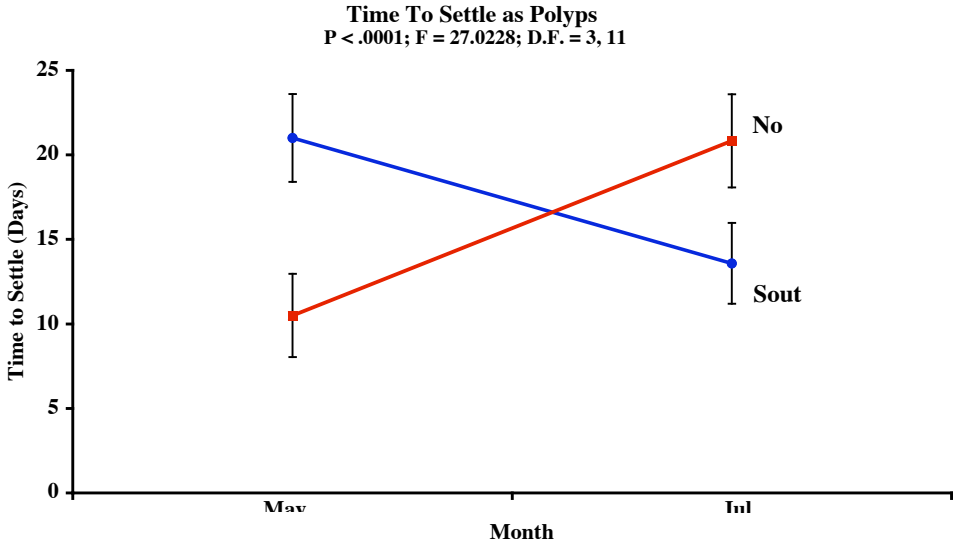


Figure 2.3: Time to settle as polyps.

A one-way ANOVA comparing northern and southern site larvae in their time to settle as polyps across two months. Approximately 600 larvae from each medusa were maintained in 500ml Erlenmeyer flasks filled with 200ml of ASW. The flasks were kept in the dark at room temperature (25^0C) and the ASW was changed every other day.

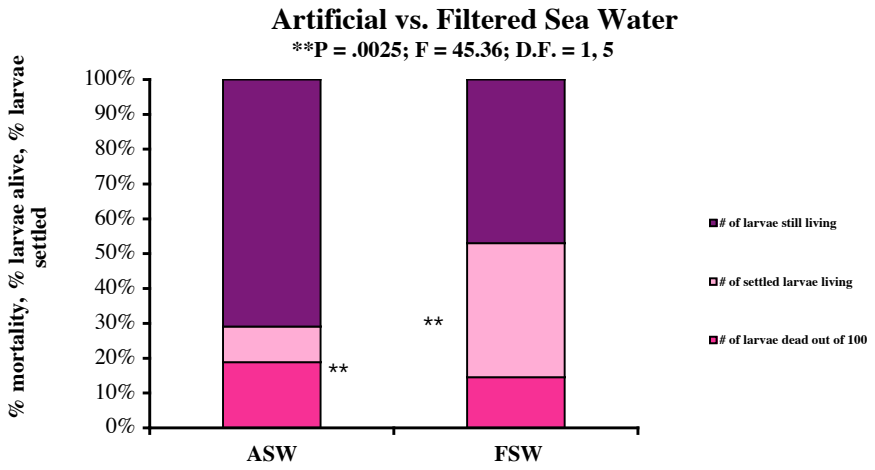


Figure 2.4: Artificial vs. Filtered Sea Water.

A one-way ANOVA comparing larvae reared in Artificial Sea Water (ASW) and larvae reared in Filtered Sea Water (FSW). One hundred larvae were placed in 200mls of ASW and one hundred larvae were placed in 200mls of FSW. There were three replicates per treatment maintained in 9.53cm glass changing bowls. All vessels were covered with aluminum foil and stored in the dark at room temperature (25^0C). The ASW and FSW were changed every other day during the course of the experiment. The number of larvae that settled as well as mortality was recorded every other day for eleven days. The number of living larvae, mortality, and the number of larvae settled to the polyp stage are reported.

Cassiopea xamachana Distribution and Abundance

Cassiopea xamachana is a Caribbean-wide species typically found in the shallow (.5-6.5m), stagnant water surrounding red mangroves (*Rhizophora mangle*). Mangroves are characterized by muddy bottoms with highly saline (37-40+ppt) low oxygen water.

When present, *Cassiopea xamachana* often blanket mangrove bottoms. Surveys conducted during the summer (2002) revealed marked differences in jellyfish cover across four sites in the Florida Keys: BC, SL, JJ, and CP (Figure 2.2). At each of these sites, five 50m transects separated by 3m were surveyed. Every five meters along each transect, the total number of *Cassiopea* were determined in a $1m^2$ area. SL was eliminated from the analysis because no *Cassiopea* were present during the survey. There were significant differences among the sites for population density ($p < 0.0001$; $F = 17.02$; D.F. = 2, 161). CP had the highest population densities of *Cassiopea* at the time of this survey, while BC had the lowest population density (Figure 2.5). However, *Cassiopea* population densities fluctuate over time (pers. obs.). For example, a cold snap (sustained air temperatures in the -1^0C-4^0C) in 1995 essentially wiped out *Cassiopea* in the northern most Florida Keys. Today their numbers have increased but, they are nowhere near the densities observed prior to the 1995 cold spell. Additionally, a *Cassiopea* population in the southern Florida Keys became absent of jellyfish after torrential rains in June 2002.

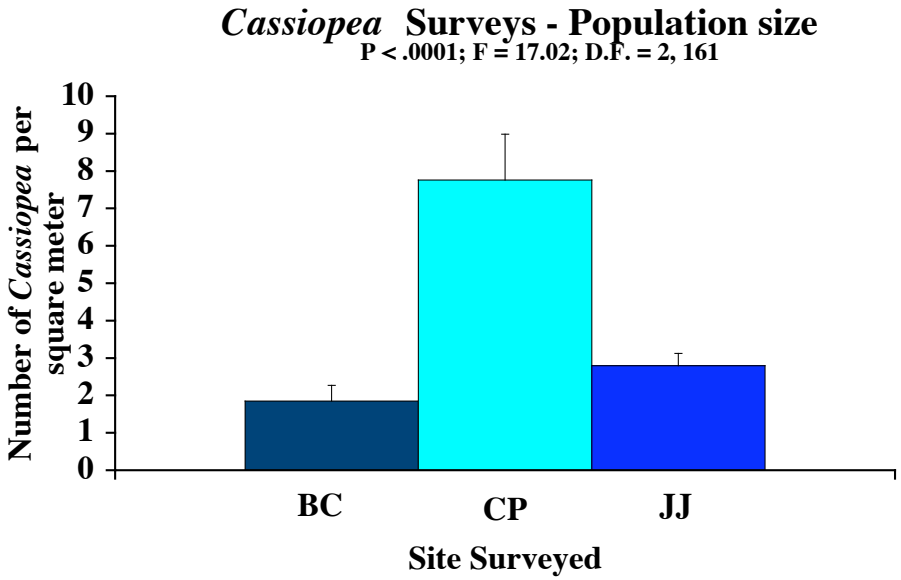


Figure 2.5: *Cassiopea* Surveys – Population size.

A one-way ANOVA Comparing the *Cassiopea xamachana* population size across three sites in the Florida Keys (see Figure 2.2). Large bars represent the average number of *Cassiopea* at each site. The lines above the bars are standard error bars. SL was eliminated from the analysis because no *Cassiopea* were present during the survey.

Thus, surveys should be conducted yearly over several decades in order to have a better understanding of population fluctuations through time and the environmental parameters that dictate those densities.

A survey conducted in summer (2002) across four sites in the Florida Keys: BC, SL, JJ, and CP (Figure 2.2) revealed that female *Cassiopea* medusae within a given location are often sparse. There was no significant difference in the sex ratio among the three surveyed sites ($p > .05$; D.F. = 2, 161; Figure 2.6). Overall, results indicate that these sites are extremely male biased with the proportion of females ranging from 27% - 33% across the three sites (Figure 2.6).

Additionally, the diameter of two female *Cassiopea* in each $1m^2$ plot along each transect was determined during these surveys. Results reveal significant differences among the three sites for size of female medusae (Figure 2.7). JJ (a northern site) had the smallest females while BC (a southern site) had the largest females ($p = 0.0004$; ChiSq = 15.65; D.F. = 2, 92). This too is likely to fluctuate depending upon the time of year surveys are conducted given the differential reproduction and development observed across northern and southern sites (Figure 2.3).

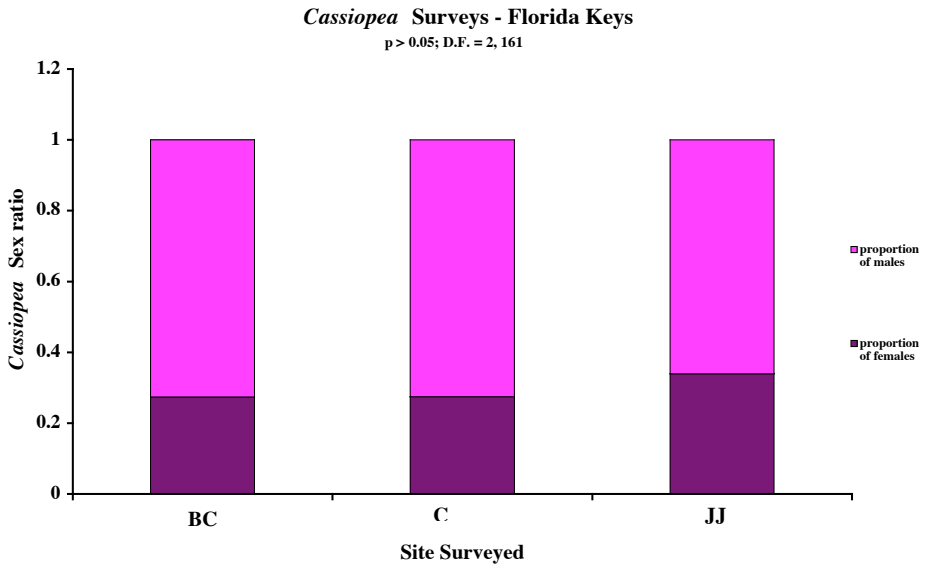


Figure 2.6: *Cassiopea* Surveys – Florida Keys.

A one-way ANOVA Comparing the *Cassiopea xamachana* sex ratio across three sites in the Florida Keys (see Figure 2.2). Each bar represents the average proportion of males (purple) and females (pink) at each site. SL was eliminated from the analysis because no *Cassiopea* were present during the survey. There was no significant difference in sex ratio across the three sites. Each site was significantly male biased.

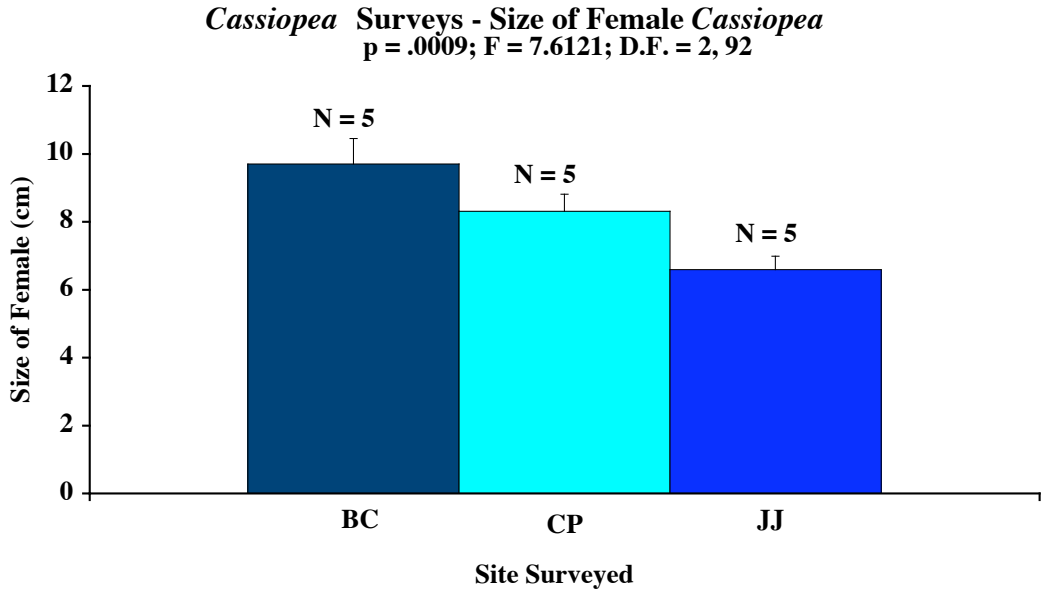


Figure 2.7: *Cassiopea* Surveys – Size of Female *Cassiopea*.

A one-way ANOVA Comparing female size at each of three sites in the Florida Keys (Figure 2.2). Each bar represents the average size of females at each site. The lines above the bars are standard error bars. SL was eliminated from the analysis because no *Cassiopea* were present during the survey.

PART 3: THE SYMBIONT – *SYMBIODINIUM MICROADRIATICUM*

The success of many marine invertebrates, such as reef corals, can largely be attributed to the presence of endosymbiotic dinoflagellates. Unicellular endosymbiotic dinoflagellates, colloquially referred to as zooxanthellae, are found in a wide variety of marine invertebrates, including single celled foraminifera, reef building corals, sponges, giant clams, and anemones to name a few.

Zooxanthellae are qualitatively more important than free-living benthic algae in nutrient-poor shallow tropical oceans, and they contribute significantly to the productivity in such systems (Ferguson, 1967). Zooxanthellae carry out photosynthesis and are sources of organic compounds for several scyphozoa including: *Linuche unguiculata, Cassiopea xamachana, Cassiopea frondosa, and Mastigias*. Within most cnidarian hosts, symbiotic algae are coccoid in form (approximately 8μm in diameter), with no flagella or surface grooves, and reproduce by mitotic division (Loeblich and Shirley, 1979). However, if cultured in a liquid algal media (ASP8-A or F/2 see appendix), zooxanthellae may develop flagella, surface grooves, and motility characteristic of dinoflagellates (Freudenthal, 1962; Loeblich and Shirley, 1979).

Culturing symbiotic algae

The symbiotic algae within *Cassiopea xamachana* have been classified as *Symbiodinium microadriaticum* (Freudenthal, 1962). The algae can be collected from *Cassiopea* medusa by clipping a small portion of the host's tentacle and isolating the algae from host tissue (Figure 2.8). The portion of tentacle collected from the medusa is placed in a changing bowl and the host tissue containing the highest concentration of algal cells is removed. The infected host tissue is rinsed with a squirt bottle containing ASW, placed in 25ml of ASW in a 50ml centrifuge tube, and ground using a tissue homogenizer. Homogenized samples are centrifuged for 3-5 minutes at 3500rpm. Visible host tissue is removed from centrifuged samples using a glass pasture pipette or water bottle containing ASW and the supernatant decanted. The remaining pellet is resuspended in 20ml FSW

and centrifuged again. This is usually repeated at least four times in order to reduce the amount of host tissue present in the pellet and to decrease the possibility of fungal contamination. The final algal pellet is re-suspended in 10ml of FSW. Each day for 5 days the algal samples are centrifuged and rinsed twice with 10ml of ASW. Once the algal isolates are clean, they can then be cultured in a liquid algal medium (F/2, Sigma Company) or used directly in infection experiments (Figure 2.8). The cultures are maintained at room temperature (25ºC) under fluorescent lights (40" F40DX full spectrum bulbs) on a 14hr light: 10hr dark cycle. Additionally, symbiotic algae can be cultured on F/2 agar plates. After 1-2 weeks of growth, individual algal colonies can be selected and grown in liquid algal media generating uniclonal algal lineages.

Isolating Algae and Infecting Polyps

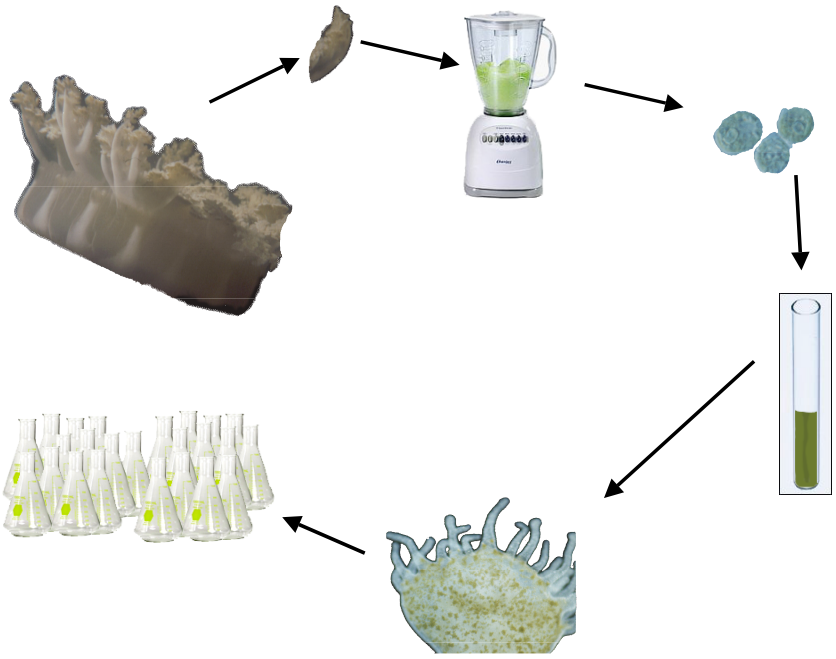


Figure 2.8: Isolating algae and infecting polyps.

How endosymbiotic algae can be isolated from host tissues and used to infect newly settled polyps. A small portion of the host's tentacle is clipped and macerated via tissue grinding. The algae are further isolated from host tissue by repeated centrifugation. Algae are then cultured in liquid F/2 media or used to infect polyps by adding algae to flasks containing newly settled polyps and artificial salt water (ASW).

Life within the host

In *Cassiopea xamachana* medusa, zooxanthellae are present in narrow bands beneath the exumbrellar and subumbrellar epithellia of the bell (Blanquet and Riordan, 1981; Blanquet and Phelan, 1987). Zooxanthellae, like free-living algae, photosynthesize and fix carbon dioxide into organic compounds, which is facilitated by their presence near actively metabolizing host tissues (Aria, 1997). A small *Cassiopea* medusa with its endosymbiotic algae can photosynthesize at a rate of at least 75μg photosynthetically fixed C/cm^2 host tissue surface area per hour in the light (Drew, 1972). Carbon dioxide is the primary substrate for photosynthetic carbon assimilation in zooxanthellae, which can fix metabolic CO_2 produced by the host (Aria, 1997). Symbiotic algae also require a source of nitrogen, phosphorus, and other elements. One source of nitrogen for symbiotic algae is the ammonium produced by *Cassiopea xamachana* protein metabolism. It has been shown that *Cassiopea* medusae excrete less ammonium into the surrounding seawater than aposymbiotic medusa (medusa devoid of symbiotic algae) (Cates and McLaughlin, 1976).

The degree of host specificity, particularly among algal-cnidarian symbioses, varies considerably. Some invertebrate hosts are capable of housing multi-species communities of symbionts from different clades (e.g. the coral *Montastria annularis*). Within the host, each algal species occupies a specific niche. For example, more sun-tolerant symbionts might occupy those areas of the host that are most exposed to the sun. Conversely, shade tolerant symbionts might occupy more protected areas of the host (i.e. the underside or sides of a coral

host). Other hosts are capable of hosting a variety of symbiont species, although, only a single species at a time (e.g. octocorals such as *Briareum*). Still other invertebrate hosts, such as *Cassiopea xamachana*, always house a single specific algal species (Chapter 6).

Taxonomy

The algal symbionts inhabiting many marine invertebrates were once believed to be a single pandemic species, *Symbiodinium microadriaticum.* However, over the past 20 years, physiological and genetic studies have revealed enormous, previously unexpected, taxonomic diversity among symbiotic dinoflagellates. At minimum, symbiotic species occur among seven genera in four orders (Freudenthal, 1962: Trench and Blank, 1987: Banaszak *et al.* 1993; Trench, 1993: McNally *et al.* 1994: Banaszak and Trench, 1995a,b). In the few symbiont species that have been studied extensively, considerable functional diversity appears to exist. For example, differences in diel patterns of motility and division have been documented between cultured strains of zooxanthellae (Fitt and Trench, 1981). Under laboratory conditions, zooxanthellae have been 'shuffled' between host species and recombined associations differ significantly from wild type associations in a number of parameters, including: zooxanthellae density, zooxanthellae growth rate, and host growth rate (Fitt, 1985; Trench, 1980). Zooxanthellae also differ in photoadaptive capabilities, susceptibility to UV damage, growth rate, nutrient uptake, kinetics, and lipid synthesis (Jokiel and York, 1982; Chang *et al.* 1983; Kinzie, 1979, 1984, 2001).

PART 4: THE ESTABLISHMENT OF THE *CASSIOPEA*-ALGAL SYMBIOSIS

> Don't compete! Combine – practice mutual aid! …the best guarantee of existence and progress (De Bary, 1879)

Cassiopea xamachana must acquire their symbiotic algae from the environment during the scyphistomae stage (horizontal transmission). Thus algae are absent in the eggs and planula larvae (Trench, Colley, and Fitt, 1985). In the laboratory, algal infection can occur by direct interaction via ingestion of prey containing algal symbionts or when motile algae directly enter the coelenteric cavity of *Cassiopea* (Aria, 1997). Infection is facilitated by responses of both the host and algae. For instance, the symbiotic algae are attracted to aposymbiotic scyphistomae as well as to fed symbiotic individuals. Empirical evidence indicates that ammonium may be the attractant since the seawater surrounding fed and aposymbiotic scyphistomae contain high levels of ammonium (Fitt, 1984). Sources of the algae infecting *Cassiopea* polyps in the wild are unknown. However, zooxanthellae released from neighboring hosts or by predators of those hosts are likely algal sources (Fitt, 1985; Trench, 1987). Scyphistomae ingest algae using responses similar to those in feeding. In the laboratory, the presence of algae, particularly motile forms, increases the frequency with which tentacles are moved into the mouth (reviewed in Aria, 1997). To date, little is known about the life cycle of symbiotic algae outside the host. Free-living *Symbiodinium microadriaticum* have rarely been found. This may be because, as noted in culture, motility is limited to short light periods and motile algae remain close to the sea bottom (Fitt *et al.* 1981). However, in summer 2001, I placed newly

settled aposymbiotic polyps at several collection sites and, all of the polyps that were retrieved four days later were infected with algal symbionts (Figure 2.9). In summer 2002, I repeated this experiment with laboratory reared asexually produced clones (from Wilcox, UT at Austin) since they are larger than sexually produced polyps. Again, every polyp that was retrieved was infected with symbiotic algae. These two pilot studies suggest that algae exist in the water column. Further support for free-living algae comes from an experiment conducted during the summer of 2002 (see also Chapter 5). Seawater collected from four sites in the Florida Keys (Figure 2.2) was used to initiate infection of aposymbiotic larvae collected from female medusa at the same four sites. Infection was apparent 4 days after the polyps were placed in the site water; thus, algae undoubtedly exist in the water column.

Establishment of the *Cassiopea*-algal symbiosis proceeds via phagocytosis. Once in the coelenteric cavity, the algae are endocytosed by the gastrodermal cells lining the cavity (Aria, 1997). Algae then trigger some unknown but necessary reaction invoking algal sequestration and persistence in appropriate positions in the host, all the while avoiding exocytosis or digestion by the host. In the laboratory, freshly isolated algae are phagocytosed at higher rates than those algae that have been cultured (Trench *et al.*1981). This may be because animal membranes are associated with the freshly isolated algae even after repeated washing, facilitating recognition by host cells. Eventually, algae migrate into the mesoglea to form 'amoebocytes', and then the endosymbiotic algae proliferate via mitotic division (reviewed in Aria, 1997).

Figure 2.9: Settled polyps in the field.

Tubes containing newly settled polyps placed in a mangrove in the southern keys. Rocks were glued to the tubes so that the tubes would be suspended in the water column (.5m below the surface and .75m off the bottom). Holes cut in the tubes were covered with mesh allowing symbiotic algae access to the aposymbiotic polyps. All polyps retrieved after four days were infected with algae.

Under favorable conditions, the population densities of zooxanthellae remain relatively constant (1×10^6) or decrease with the growth of the host medusa. To date there are no reported examples of symbiotic algae overgrowing their host (reviewed in Aria, 1997); however, during my experiments, I observed several algal isolates outgrowing and overgrowing their respective hosts (see Chapter 3).

PART 5: COSTS AND BENEFITS OF THE SYMBIOSIS – CONFLICT OF INTEREST?

> Every symbiosis is, in its degree, underlain with hostility, and only by proper regulation and often elaborate adjustment can the state of mutual benefit be maintained (Wells *et al.*, 1930).

Algal symbiosis is not widespread in scyphozoa, even in near surface water where light is present. It is not clear why some closely related putative species possess symbionts when others do not. It is also not known whether the complex association, when all effects are taken into consideration, is of benefit to only one of the partners or both the alga and host (reviewed in Aria, 1997). In fact, most investigators of endosymbiotic mutualisms agree that mutualism is best defined as reciprocal exploitations that nonetheless provide net benefits to the partners involved (Margulis, 1991; Douglas, 1994). Though the intimate physiological details of the *Cassiopea*-algal symbiosis remain largely unknown, there are some obvious costs and benefits to the associates. Photosynthetic carbon fixation by the algae can be of great importance to the host because excess photosynthate can be utilized for growth and reproduction. For example, photosynthetic activity by the algae is thought to induce strobilation in *Cassiopea*

polyps (Hofmann and Kremer, 1981). However, the molecular oxygen produced, as an obligatory byproduct of photosynthesis, is toxic and can be potentially damaging to the host (reviewed in Aria, 1997). Additionally, in order to carry out photosynthesis, algae must receive light, which is potentially damaging to both the host tissue and the algae (reviewed in Aria, 1997; Shick *et al*., 1995). Prolonged elevated temperature and harmful solar radiation will cause the association to decouple (Wilcox and Sloan pers. obs.). Finally, host behavioral modifications necessary to maintain light-dependent populations of symbionts can be costly in terms of host energetics. For instance, *Cassiopea* spend the majority of their time pulsing on the sediment bottom of shallow waters. This allows algal symbionts exposure to light but the pulsing modifies the currents bringing food particles to the host. Such modifications may not only represent energetic costs for maintenance but may also decrease efficiency of obtaining particulate food (reviewed in Aria, 1997; Shick *et al*., 1995).

There is still much to be learned regarding the entangled biochemical and physiological details of the *Cassiopea*-algal symbiosis. It would appear that the host bares the brunt of the costs associated with this endosymbiosis, but it is difficult to assess advantages and disadvantages when so many pieces of the puzzle remain missing. Several studies have documented that the net costs and benefits associated with mutualisms can vary for a variety of reasons (Thompson, 1994). Examples include variation in host densities that result in shifts in patterns of symbiont transmission (Bull *et al*. 1991), changes in the presence or abundance of influential third parties (Bronstein, 1994; Gaume *et al*., 1998), variation in

resource availability (Douglas, 1995; Bronstein, 1994), and/or variation in physical conditions (e.g. coral bleaching). Studies such as these invoke an interesting question concerning the degree of local adaptation in host and symbiont populations. For instance, do hosts benefit from local, presumably more highly adapted symbionts, or are the symbionts generalists?

PART 6: THE *CASSIOPEA*-ALGAL SYMBIOSIS - A MODEL SYSTEM

The *Cassiopea*-algal symbiosis is an excellent model system for examining a variety of evolutionary and ecological questions. First, it consists of relatively long-lived hosts with stable symbiont populations that are acquired by horizontal transmission every generation. This creates the potential for spatially heterogeneous selection if the genetic composition of partner populations differs among populations. Second, it is likely that the symbiont spends most if not all of its lifetime within the tissues of the host and symbiont reproduction within the host has a direct effect on host fitness. Third, thousands of larvae can be collected readily from *Cassiopea* medusa and maintained as algal-free polyps indefinitely. The algae are equally easy to acquire from *Cassiopea* medusa and can be used immediately in infection experiments or cultured for later use. Fourth, like corals, *Cassiopea* expel their algal symbionts (bleach) under stressful conditions, providing a unique opportunity to assess the stability of various *Cassiopea*-algal combinations under stress. Fifth, *Cassiopea*-algal symbioses are ubiquitous along 160km of coastline in the Florida Keys and multiple populations are readily accessible. Finally, varying levels of cooperation among *Cassiopea*-algal

combinations can be assessed in terms of symbiont reproduction (mitotic index within the host), host growth, and host longevity.

VARIATION IN HOST SYMBIONT COMPATIBILITY AMONG *CASSIOPEA*-ALGAL SYMBIOSES

Chapter Three: Variation among Algal Isolates in Their Effect on *Cassiopea* Growth and Survival

PART 1: ABSTRACT

It has become apparent that zooxanthellae taxonomic diversity of zooxanthellae is high and that the genetic diversity among zooxanthellae taxa correlates with their physiological and biochemical performance in some instances. However, virtually ignored in the literature is variation within a single symbiont species and how intraspecific genetic diversity correlates with functional diversity across multiple populations of a single host species. *Cassiopea xamachana* harbors endosymbiotic algae within its tissues. I used *Cassiopea* and its respective algal symbiont, *Symbiodinium microadriaticum,* in a series of manipulated interactions in order to examine the natural variation among symbionts across multiple populations in the Florida Keys. Results indicate that not all algal symbionts are equally benevolent across *Cassiopea xamachana* hosts. No algal isolate had the same effect on mortality and growth across *Cassiopea* hosts; moreover, no host lineage did equally well with all algal isolates. Some *Cassiopea*-algal combinations experienced 100% mortality, while others suffered little or no mortality ($p < 0.0001$). Further, some combinations grew, changed little in size, or shrunk during the course of the experiment ($p < 0.0001$). On average, maternal combinations grew more, and suffered less mortality, than

novel combinations. These results suggest that differential compatibility of host and symbionts are a significant factor influencing symbiotic outcomes.

PART 2: INTRODUCTION

The algal symbionts, or zooxanthellae, inhabiting marine invertebrates are not obviously different from each other. For this reason, symbionts from hard and soft corals, anemones, giant clams, and jellyfish were historically classified as a single pandemic species, *Symbiodinium microadriaticum* Freudenthal (Freudenthal, 1962; Kevin *et al.*, 1969; Taylor, 1974; Trench and Blank, 1987). However, physiological and genetic studies have revealed enormous, previously unexpected diversity with symbiotic algae spanning 5-6 clades. It is interesting, given the taxonomic diversity of algal symbionts, that most investigators of marine algal-invertebrate symbioses assume that all symbionts found within a particular host are equally benevolent across all hosts of that species. Empirical studies examining intraspecific symbiont variation among algal-invertebrate symbioses using multiple populations of the same host species are virtually nonexistent. This is surprising given that the presence of intraspecific symbiont variation could prove useful in interpreting patterns of variability observed during investigations of coral reef bleaching events. When algal-invertebrate endosymbioses are threatened by environmental fluctuations such as changes in temperature and/or irradiance, physiological responses resulting from stress can induce the loss of algal partners (e.g. coral bleaching). The loss of algal symbionts has a negative effect on host viability, often resulting in death. The extent of bleaching both within and between populations is temporally and spatially variable. In some instances, variation has been related to ecological gradients such

as depth (Lang, 1988; Cook *et al.* 1990; Edmunds, 1994); however, there are many instances where there is no clear correlate with bleaching.

Cassiopea xamachana, the upside down jellyfish, is a Caribbean-wide species typically found in the shallow (.5-7m), stagnant water surrounding mangroves. *Cassiopea* harbors symbiotic algae within its tissues, which provide it with photosynthetically produced carbohydrates. *Cassiopea xamachana* is believed to harbor only one symbiont species, *S. microadriaticum* (Fitt and Trench, 1981); however, no empirical studies have examined symbiont diversity extensively within this host species. *Cassiopea* has a typical scyphozoan life cycle, during which sexual medusa reproduce aposymbiotic (algal-free) larvae that settle as asexually reproducing scyphistomae (polyps) (Trench *et al.* 1981; Chapter 2). Symbiotic algae are then acquired from the environment by the polyps, then metamorphose (strobilate) into free-swimming ephyra, which develop into adult medusa. The sexually produce larvae of *Cassiopea* can be collected directly from female medusa. These larvae can be deprived of symbiotic algae and maintained in the laboratory until they settle as polyps. This provides an abundant source of uninfected hosts that can be used in carefully designed infection experiments.

Most investigators of endosymbiotic mutualisms assume that all symbionts are equally benevolent within the same host species. Thus, little attention has been paid to the natural variation among symbionts in terms of their effect on host fitness. Here I report a study in which I experimentally manipulated *Cassiopea*-algal associations to address the following question: Do different *Cassiopea*-algal combinations differ in performance?

PART 3: METHODS

Site Description

Planula larvae and symbiotic algae were collected from a single female at 10 sites. In order to maximize the potential genetic diversity of hosts and symbionts, and therefore maximize the potential for intraspecific variation in host-symbiont compatibility, a large range of sites spanning 160km of the Florida Keys, USA (Figure 3.1) were sampled.

Collection of *Cassiopea* larvae

Gravid female *Cassiopea* medusae (identified by a distinct discolored circular center where larvae are brooded) were collected by snorkeling and placed in a bucket containing site water. The diameter of each female medusa was measured prior to further handling and every effort was made to collect larvae and algae from similarly sized medusae (~15.24cm) across the ten sites. Larvae were carefully removed from the medusae using a glass pasture pipette and placed in a 50ml plastic centrifuge tube containing artificial seawater (ASW; 35%, Instant Ocean, Inc.). Additionally, a small portion of medusa tentacle was clipped and placed in ASW water in a separate 50ml plastic centrifuge tube. The female medusae were released after about 30 minutes of handling.

All collections were immediately returned to the lab, and approximately 1500 larvae per medusa (from the ≈2,000 originally collected) were then sorted and transferred into 300ml ASW. Larvae were then washed by swirling and transferred into 300ml of fresh ASW. The transfer and wash step was repeated three times to remove any algal cells that might have been transferred from the

original female medusa or site water. The larvae from each female were distributed among four 250ml Erlenmeyer flasks in 200ml of ASW and maintained 25ºC in the dark until they settled as polyps. As polyps began to settle, the cultures were fed brine shrimp, *Nauplii* sps., every other day followed by ASW changes. Polyps were allowed to feed approximately 2-3h prior to changing the ASW.

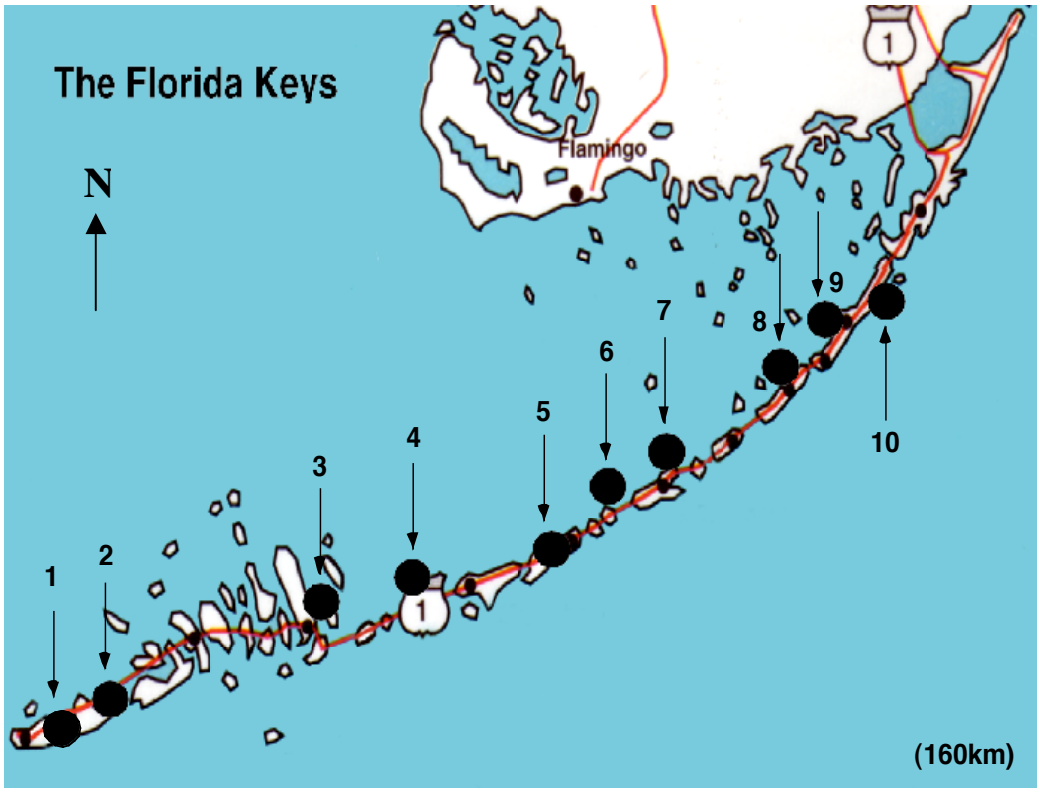


Figure 3.1: Map of the Florida Keys.

Ten collection sites used to collect *Cassiopea xamachana* larvae and symbiotic algae. These two sites are separated by ~160km. Site 5 is exactly the middle of the transect.

Algal Isolation

On the day of collecting, the portion of tentacle cut from each medusa was placed in a changing bowl and the host tissue containing the highest concentration of algal cells was removed using scissors. The infected host tissue was rinsed with a squirt bottle containing ASW, placed in 25ml of ASW in a 50ml centrifuge tube, and ground using a Tissue Tearer on high speed for one minute. Homogenized samples were then centrifuged for three minutes at 3500rpm. Visible host tissue was removed from centrifuged samples using a glass pasture pipette or water bottle containing ASW, and the supernatant was decanted. The remaining pellet was resuspended in 20ml FSW and centrifuged again. This was repeated at least four times in order to reduce the amount of host tissue present in the pellet and to decrease the possibility of fungal contamination. The final algal pellet was resuspended in 10ml of FSW. Each day for 5 days the algal samples were centrifuged and rinsed twice with 10ml of ASW. Clean algal cultures were then maintained at room temperature (25ºC) under fluorescent lights (40" F40DX full spectrum bulbs) on a 14hr light: 10hr dark cycle until they were used to infect polyps. Florescent lights were hung approximately 61cm above the clean algal cultures.

Experimental combinations

Polyps from each medusa were split into 10 groups (three replicates per group/30 polyps per replicate) and placed in 250ml Erlenmeyer flasks in 100ml of FSW (Figure 3.2). Ideally, each polyp group was infected with algae from one of the ten sites. However, this was not always possible due to mortality of the larvae

(white squares; Figure 3.2). The final combinations are presented in Figure 3.2 (white squares). Each replicate was infected with 10,000 algal cells/ml ASW (Fitt, 1983) and placed under florescent lights for 24 hours. Two uninfected control replicates per host lineage (30 polyps/replicate) were maintained at room temperature (25ºC) in the dark. These polyps remained devoid of symbiotic algae during the entire experiment. Overall, there were a total of 66 different *Cassiopea*-algal combinations (Figure 3.2).

After 24 hours of constant light, the experimental vessels were maintained under florescent lights (40" F40DX full spectrum bulbs) on a 14hr light:10hr dark cycle at room temperature (25ºC). The florescent lights were hung approximately two feet above the flasks. Additionally, the lab counter tops were covered with reflecting aluminum foil to enhance that amount of light each vessel received. All experimental and control vessels were fed every other day and the ASW changed 2-5 hours after feeding.

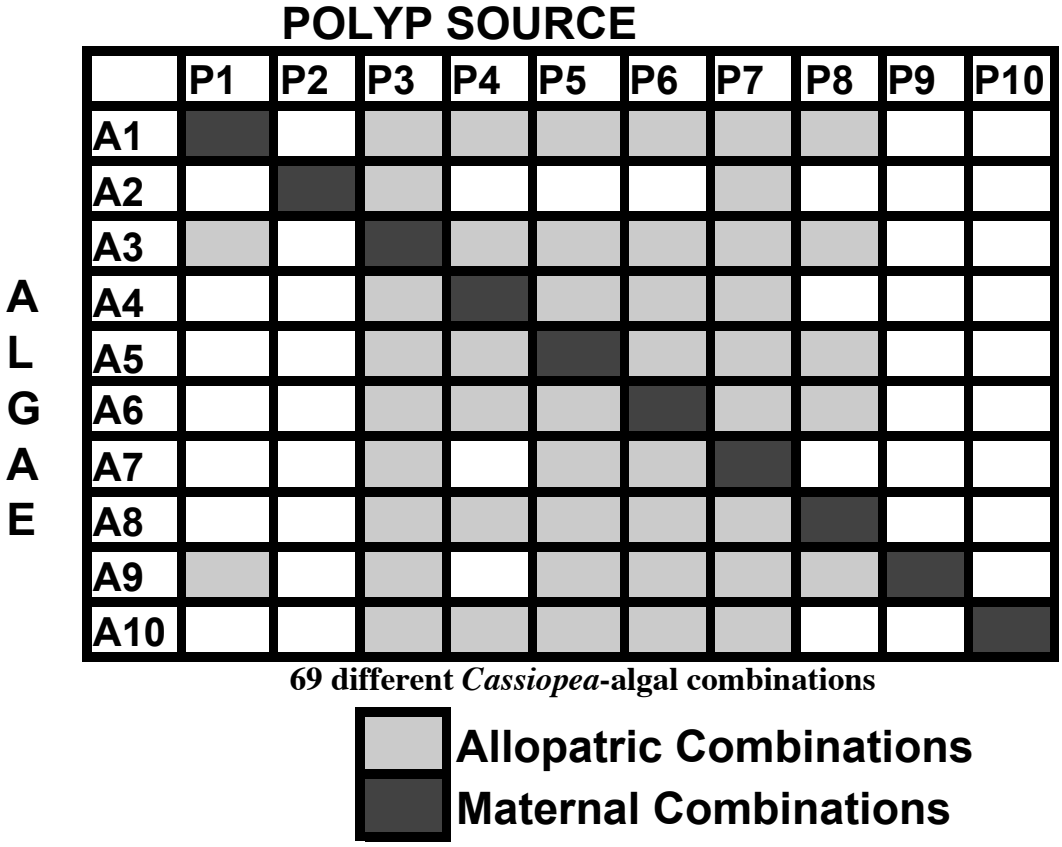


Figure 3.2: Experimental design.

Columns represent polyp source while rows represent algal source. White squares = combination not manipulated in experiment; diagonal (dark squares) = maternal combination; off diagonal (light squares) = novel combinations. There were 66 combinations, 3 replicates per combination. Each replicate contained 30 experimental polyps. There were 2 control replicates (polyps devoid of symbiotic algae) per polyp lineage.

Fitness measures

Host fitness was assessed in two ways during the course of the experiment: host survival and host growth. Mortality was assessed twice a week by recording the number of polyps remaining in each flask. I could not track individual polyps during the course of the experiment. Therefore, I assessed polyp growth rates by determining the change in the size distribution of polyps in each flask from the time when all polyps become noticeably infected to the end of the experiment. The size distribution of polyps in each flask was determined by measuring the diameter of the polyp crown (the widest part of the polyp 'head' excluding tentacles) for all polyps in a flask (Figure 3.3). Polyp crown measures were made using a compound microscope and a calibrated ocular micrometer. The experiment was terminated after 30 days.

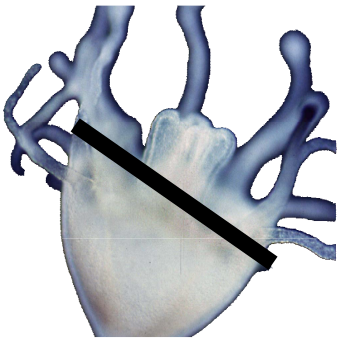


Figure 3.3: Measuring polyps.

The black line indicates the diameter of the polyp crown that was measured to assess the overall change in size distribution among various *Cassiopea*-algal associations.

Data Analysis

The crown size measurements as well as mortality were analyzed using a nested analysis of variance (ANOVA). JMP version 4.0.0 (Academic) was used to generate all statistics.

PART 4: RESULTS

The objective of this experiment was to examine variation among symbionts in their effect on host growth and mortality. Partners were chosen from as many different sites as possible, increasing the chance of observing natural variation. All flasks inoculated with algal isolates showed visible signs of infection within one week. During the experiment, two polyp lineages exhibited fungal contamination, and it is likely that this secondary infection had an effect on the overall health of polyps. As a result, these lineages were removed from the analysis. However, the absence of these two lineages has little effect on the outcome of the analyses. A one-way analysis of variance (ANOVA) revealed significant differences between all jellyfish-algal combinations for both change in size (μm) ($p < 0.0001$; $F = 26.97$; D.F. = 2, 65) and mortality ($p < 0.0001$; $F =$ D.F. = 2, 65). Some combinations suffered high mortality while others experienced little or no mortality (Figure 3.4). Additionally, various jellyfish-algal combinations either grew, stayed the same size, or shrunk (Figure 3.5). Controls (polyps devoid of symbiotic algae) for each polyp lineage suffered little or no mortality during the experiment. Additionally, the controls changed little in size (μm) during the experiment. It is not unusual for invertebrate hosts to shrink when

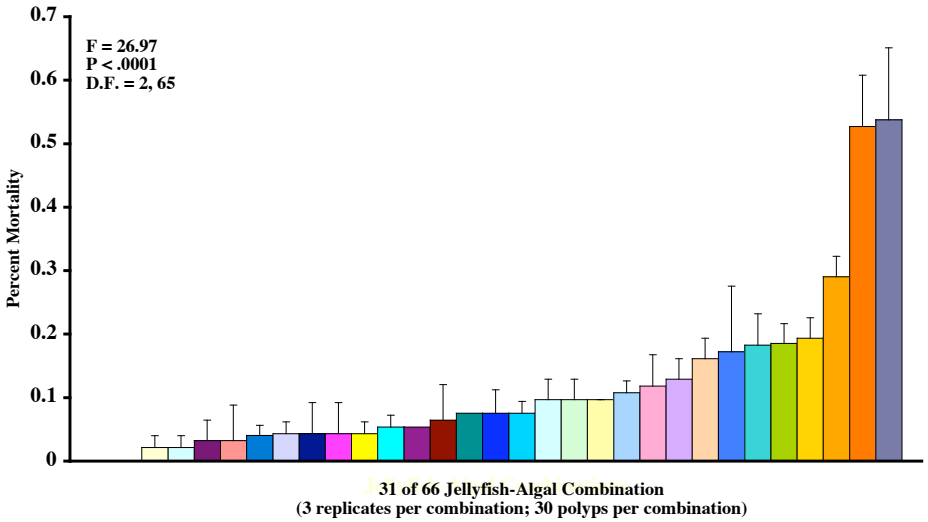


Figure 3.4: Mortality among *Cassiopea*-algal combinations.

Thirty-one of the 66 combinations represented by each bar on the x-axis. Lines above each bar are standard error bars (3 replicates per combination). Y-axis is the proportion of individuals that died for each combination. ANOVA revealed significant variation among the 66 combinations for mortality ($P<0.0001$).

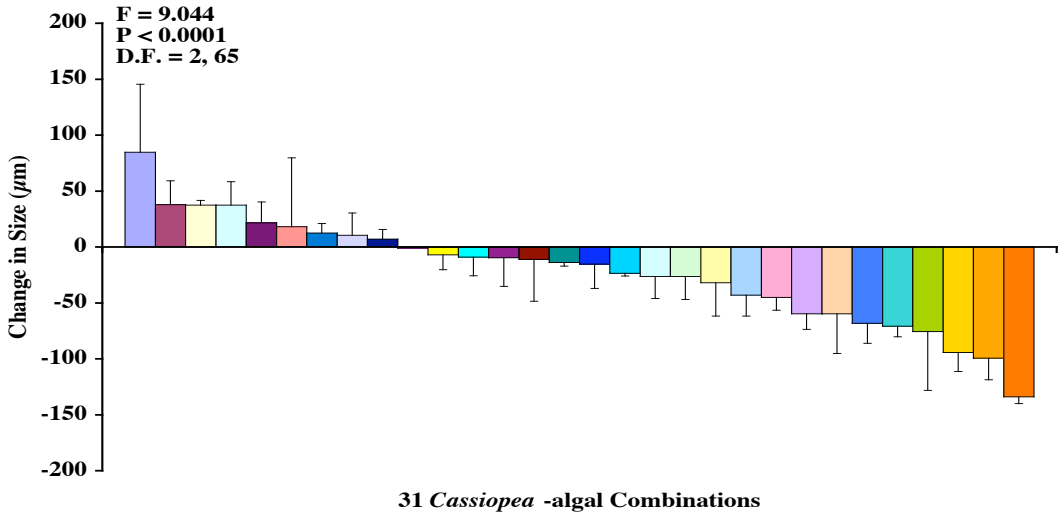


Figure 3.5: Change in size (μm) among *Cassiopea*-algal combinations. Thirty-one of the 66 combinations represented by each bar on the x-axis. Lines above each bar represent standard error bars (3 replicates per combination). Y-axis is the change in size in microns for each experimental combination. ANOVA revealed significant variation among the 66 combinations ($P<0.0001$).

they are stressed, and because not all combinations shrunk, I am confident that shrinkage was not a result of laboratory artifact protocol but rather due to the algal isolate within the host. Additionally, there is a significant correlation ($p < 0.0001$) between change in size (μm) and mortality (Figure 3.6); that is, when polyps shrink, they generally die.

No single host lineage did equally well with all algal isolates (Figure 3.7-A, B); for instance, there was significant variation across all combinations involving larvae collected from medusa 4 for both mortality ($p < 0.001$) and growth ($p = 0.006$). For example, polyps from medusa 4 containing algae isolated from medusa 1 and 3 had similar mortality but experienced differential growth patterns. Additionally, polyps containing algae isolated from medusa 5 had the highest mortality and changed little in size. Overall, for different combinations within and across polyp lineages, there is a range of symbiotic outcomes.

No single algal isolate had the same effect on mortality and growth across *Cassiopea* hosts (Figure 3.7-C, D). For instance, the effect of algae from medusa 4 on both mortality ($P<0.0001$) and growth ($P=0.0007$) depended upon the origin of the polyps. Polyps collected from medusa 7 had the highest mortality when infected with algae isolated from medusa 3 while polyps collected from medusa 4 suffered little mortality when infected with algae isolated from medusa 3. Further, polyps from medusa 4 grew when infected with algae isolated from medusa 3 while polyps from medusa 7 shrunk. Unmistakably, intraspecific symbiont variation is present and it can no longer be assumed that algal symbionts are equally compatible with individuals from the same host species. Further, results indicate that, on average, novel combinations (partners that originate from

different medusae) suffer more mortality and slower growth (Figure 3.8) than maternal combinations (partners that originate from the same medusa).

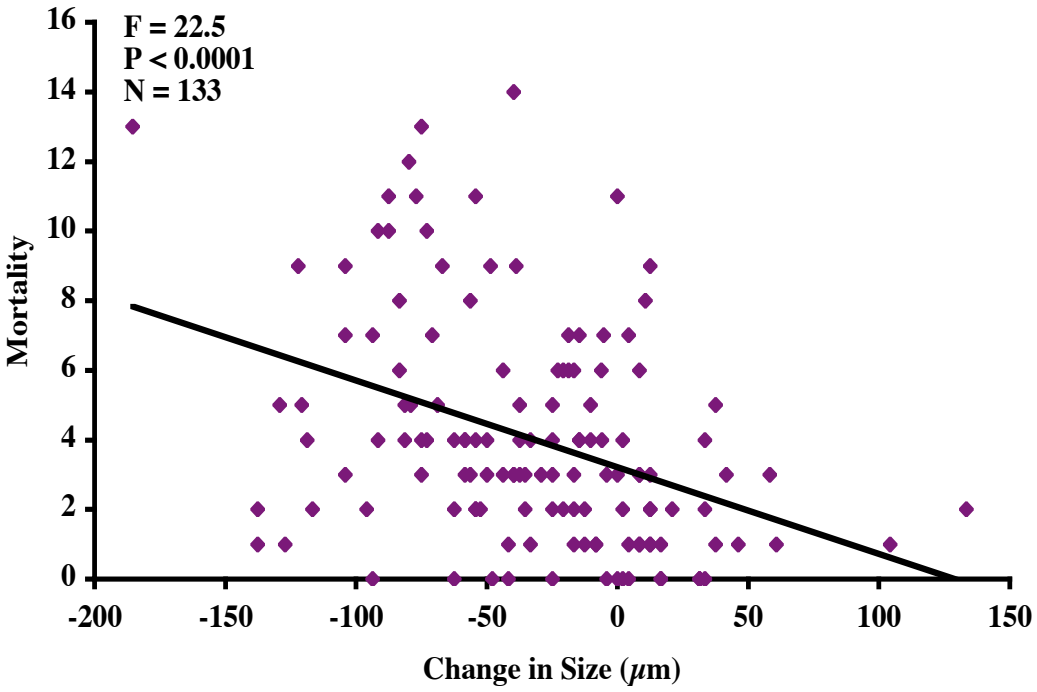


Figure 3.6: Correlation between size (μm) and mortality.

Correlation between overall change in size (μm) on the x-axis and mortality on the y-axis. When polyps shrink, mortality increases.

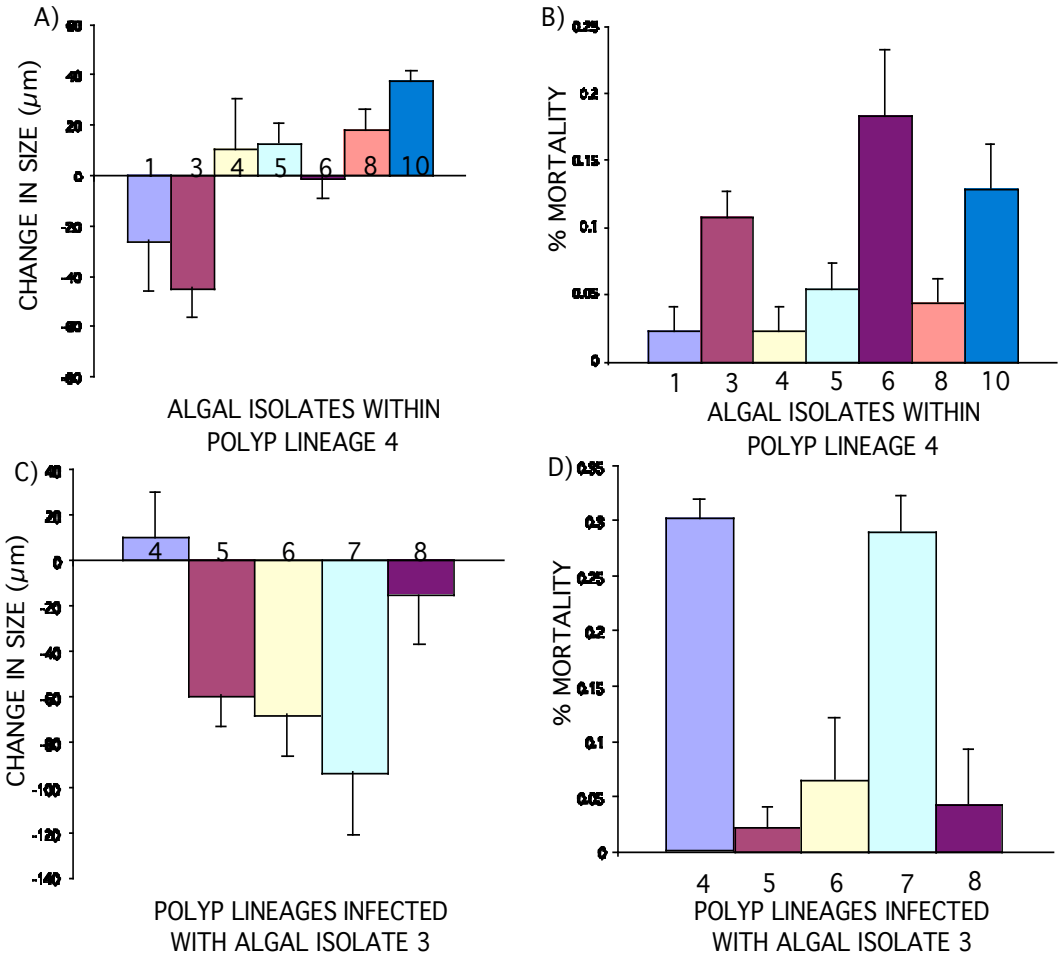


Figure 3.7: No single polyp lineage or algal isolate performed equally well.

A) Change in size (μm) for host lineage 4 when infected with 7 different algal isolates. Each bar represents the average change in size (μm) of the 3 replicates per combination. The bars are standard error bars. B) Mortality (%) across host lineage 4 infected with each of 7 algal isolates. Each bar represents the average mortality of the 3 replicates per combination. The bars are standard error bars. C)

Change in size (μm) among 5 polyp lineages infected with algal isolate 3. Each bar represents the average change in size (μm) of the 3 replicates per combination. The bars are standard error bars. D) Mortality (%) among 5 polyp lineages infected with algal isolate 3. Each bar represents the average mortality of the 3 replicates per combination. The bars are standard error bars.

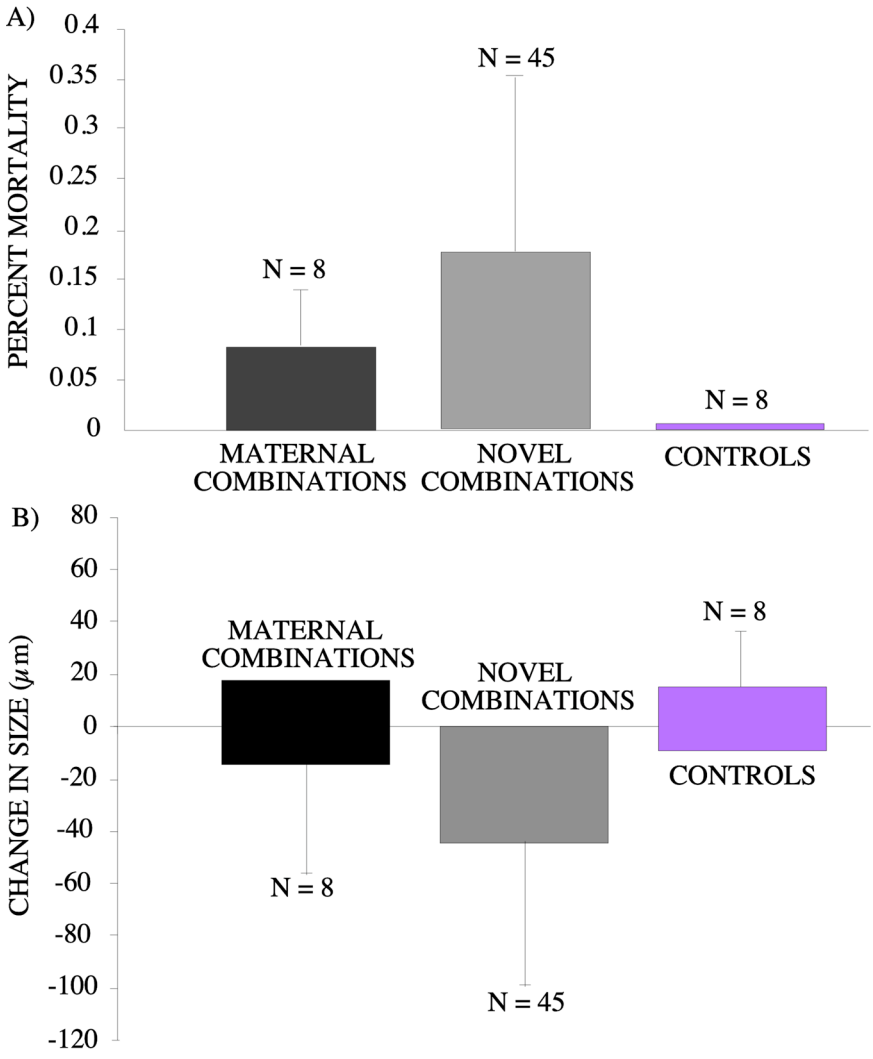


Figure 3.8: Mortality among combination types.

A) Mortality (%) among different combinations grouped as maternal and novel combinations. The number above the standard error bar represents the number of combinations included in each group. B) Change in size (μm) among different combinations grouped as maternal and novel combinations. The number above the standard error bar represents the number of combinations included in each group.

PART 5: DISCUSSION

Many researchers have suggested that marine invertebrates remain flexible in their specificity for algal symbionts as a means of coping with environmental variability. This seems reasonable since hosts with broad compatibility will rarely suffer from lack of access to partners, while specialization clearly entails the cost of a reduced chance of finding suitable mutualists (Parker, 1999). However, this study suggests that there is not only strong host specificity for a single specific symbiont species but also for specific isolates (genotypes) within the algal species. Several studies investigating legumes and their nitrogen fixing bacteria have found extremely specialized symbiotic associations (Young and Matthews, 1982; Kneen and LaRue, 1984; Devine, 1987). For example, Parker and Wilkinson (1997) found that 60% of populations of *Amphicarpaea bracteata,* sampled over a 1,000-km region, were dominated by plants with specialized *rhizobium* genotypes. Thus, symbiotic specialization, at least in these terrestrial symbioses, does not appear to be a rare anomaly.

Investigating symbiotic specialization in other systems has been limited by difficulties of maintaining partners outside the association, manipulating interactions with different partners, and the necessity of large sample sizes from many collection sites. Some researchers have obviated these difficulties using systems such as the *Acromyrmex*-fungus association (Bot, Rehner, and Boomsma, 2001). Additionally, using the *Cassiopea*-algal system, many of these difficulties are overcome. It is evident that intraspecific symbiont variation exists among symbionts isolated from various *Cassiopea* hosts. One can no longer assume that all symbionts are equally benevolent across the same host species. However, because larvae and algal isolates were collected from a single medusa at each site, it is difficult to quantify the observed variation. Nonetheless, this design is the simplest and most direct way to test for algal isolate effects on host fitness.

If the presence of intraspecific symbiont variation is a between-site phenomenon rather than a within site phenomenon, then there are several important ecological and evolutionary implications. That is, processes like migration, colonization, proliferation, growth, and potentially the response to stress (i.e. bleaching events) are determined by the symbiont *in hospite,* even if the symbiont is from the same taxon. If there is spatial variation in symbionts, certain host lineages may be excluded from a habitat not because of inferior adaptation to abiotic factors but simply because they are incompatible with local algal symbionts. Scientists have long contemplated the source of symbiont variation and whether it is limited due to the lack of evidence of sexual reproduction in zooxanthellae. The zooxanthellae gene pool as a whole may be quite diverse but perhaps within local populations, genetic diversity has decreased

as hosts and symbionts become locally adapted to one another and to prevailing environmental conditions.

To my knowledge, this is the first time empirical intraspecific symbiont variation and differential compatibility among marine algal-invertebrate symbioses has been investigated, a necessary first step towards determining the influence that host-symbiont interactions might have in dictating symbiotic outcomes. In future studies it will be important to extend this approach to a larger sample of algal isolates, to analyze the relative magnitude of variation within as well as between populations.

VARIATION IN HOST SYMBIONT COMPATIBILITY AMONG *CASSIOPEA*-ALGAL SYMBIOSES

Chapter 4: Intra-specific Symbiont Variation among *Cassiopea xamachana* hosts – Is it Driven By Local Adaptation?

PART 1: ABSTRACT

Mutualistic symbioses have long been of interest to evolutionary biologists. It is therefore surprising how few empirical studies have addressed the evolutionary ecology of these associations. In particular, there are a paucity of data available that address the following crucial questions: 1) what factors align the interests of symbiotic partners so that their relationship remains mutually beneficial and evolutionarily stable? 2) what is the degree of ecological and genetic variation in symbionts across multiple populations of a single host species? and, 3) what evolutionary mechanisms drive intraspecific symbiont variation if it exists? Although there is no general theory of mutualism, conventional wisdom suggests that mutualisms are best defined as reciprocal exploitations that nonetheless provide net benefits to the partners involved. Contemporary theory regarding the evolution of virulence has identified several factors that may help align host and symbiont interests. However, the extent to which natural systems conform to these theoretical expectations and what factors are most responsible for maintaining cooperative symbioses remains unclear. I have used the *Cassiopea*-algal complex to examine symbiont variation at the level of different individual hosts, and across multiple host populations, to address the

following general question: what evolutionary and ecological factors influence endosymbiotic mutualisms?

PART 2: INTRODUCTION

Broadly defined, coevolution refers to the joint evolution of two or more taxa that have close ecological relationships and reciprocal selective pressures that make the evolution of either taxon partially dependent on the evolution of the other (Ehrlich and Raven, 1964). The geographic theory of coevolution is based on the idea that structured populations of interacting species experience local differences in the intensity of selection they impose on each other (Thompson, 1994; 1999). This can lead to a geographic patchwork for traits involved in the interaction in space. Mosaics of this kind may be particularly common for structured populations of hosts interacting with a variable community of obligate symbionts (Thompson,1994; 1999), particularly if selection is fluctuating and populations are out of phase with each other (Lively, 1999). Theoretical models have revealed that, depending on the rates of migration by hosts and parasites, host-parasite coevolution can generate local adaptation by parasites. Local adaptation by parasites is generally expected if host migration is low, and parasites disperse at the same rate (or slightly more) than their hosts (Ladle *et al.*, 1993; Judson, 1995; Gandon *et al.*, 1996). However, given the dynamic nature of host-parasite coevolution, the degree of local adaptation may vary in time and occasionally disappear. The first rigorous study of local adaptation by natural populations of parasites (Parker,1985) showed that fungal isolates from geographically distant populations were more infective to host plants drawn from

the same geographic region. Conversely, Ebert (1994) has shown that microsporidian gut parasites behave more virulently towards foreign *Daphnia* hosts. He describes this phenomena as local maladaptation. In an effort to disentangle the inconsistent results between Parker and Ebert's studies, Lively (1999) simulated models of host-parasite coevolution. He showed that parasite migration and virulence interact to affect the degree of local adaptation by parasites. As migration decreases and virulence increases, the strength of local adaptation by parasites increases. Lively predicts that local adaptation will be most striking when highly virulent parasites are involved. This is because the strength of selection on the host is positively related to parasite virulence, and strong selection for host resistance along with selection for parasites to overcome host defenses, is more likely to lead to population differentiation in the face of gene flow (Wright, 1931).

How, and whether, the geographic mosaic of coevolution differs between mutualistic and antagonistic interactions still requires careful modeling, experimentation, and analyses of natural populations. Studies conducted using the legume-*Rhizobia* symbiosis suggests that mutualisms evolve toward less population-level and species-level specialization (Wilkinson *et al.*, 1996). Natural selection on parasitic associations may act to cause populations to diverge from one another more often than in mutualistic associations. Nonetheless, selection on mutualisms may at least sometimes produce geographic mosaics (Thompson, 1999). In fact, theoretical models by Parker (1999) have revealed that symbiotic mutualisms can favor geographic structuring through positive frequency-

dependent selection for the most common local host genotypes resulting in a mosaic pattern of differentiation. It would seem that, mutualistic associates are no different from participants in other types of ecological interactions (i.e. host-parasite systems and predator-prey systems) in that populations are often differentiated for traits affecting interactions. However, for mutualistic associations, few strong generalizations have emerged, either empirical or theoretical, regarding the coevolutionary significance of geographic variation. This is in part because most investigators of marine algal-invertebrate symbioses assume that all symbionts are equally benevolent not only within individual hosts, but across the same host species. As a result, the natural variation among algal symbionts in terms of their effect on host fitness has been ignored. Thus, empirical studies addressing variation in cooperation within the same host species across multiple populations are virtually nonexistent. The first experiment described below examines variation among experimentally manipulated *Cassiopea*-algal combinations using multiple hosts and symbionts from several populations across the Florida Keys. The second experiment described below evaluates and quantifies variation among *Cassiopea*-algal combinations in terms of host-symbiont interaction effects and local adaptation.

PART 3: METHODS

Experiment 1 – geographic variation re-examined

Larvae and algal symbionts were collected from three medusae at two northern sites and two southern sites in the Florida Keys as described in chapter 3 (figure1). Polyps collected from each medusa were split into six groups. These

polyp groups were infected with algal isolates from a nearest-neighbor site and a distant-site (Figure 4.2). Nearest-neighbor combinations consist of partners that originate in close geographic proximity. For example, northern site polyps are infected with algae from the second northern site or vice versa. Distant-site combinations consist of partners that originate from geographically distant-sites. For instance, polyps from one southern site are infected with algae from one of the northern sites. There were 69 different *Cassiopea*-algal combinations, 3 replicates per combination, and 25 experimental polyps per replicate (Figure 4.2). Collection of larvae and the isolation of algal symbionts from each medusae at each site as well as infection and maintenance of experimental polyps followed the protocol described in chapter 3.

Experiment 2 – Host-symbiont interaction effects explored

If the observed variation is geographically structured, one might expect to find local adaptation in this system. For example, little or no migration and regional variation (salinity, pH, temperature, depth) might influence the evolutionary dynamics of the system. Thus, an additional experiment was designed to investigate the structure of variation among *Cassiopea*-algal symbioses in more detail and, to determine whether host-symbiont interactions drive the geographic variation. Larvae and algal symbionts were collected from three medusae at the same four sites in the Florida Keys (Figure 4.1). This experiment was conducted to examine four types of *Cassiopea*-algal combinations (Figure 4.3): maternal combinations (partners originate from the same medusa), same-site combinations (partners originate from within the same-

site but different medusae), nearest-neighbor combinations (partners originate from nearby sites), and distant-site combinations (partners originate from geographically distant-sites).

Fitness measures

Host fitness was assessed in two ways during the course of both experiments: host survival and host growth. Mortality was assessed twice a week by recording the number of polyps surviving in each flask. I could not track individual polyps during the course of the experiment, so I assessed polyp growth by determining the change in size distribution of polyps in each flask when all polyps became noticeably infected to the end of the experiment. The size distribution of polyps in each flask was determined by measuring the diameter of the polyp crown (as described in chapter 3) for all polyps in a flask. Polyp crown measures were made using a compound microscope and a calibrated ocular micrometer. The experiment was terminated after 30 days. Additionally, the algal mitotic index (MI), a measure of algal reproduction, was determined for the second experiment described above. This was accomplished by squashing 8 polyps per replicate per combination and counting the number of mitotically dividing algal cells per 200 algal cells. Two replicate counts per polyp were made using a hemocytometer and a compound light microscope.

Analysis

Host size distribution, host survival, and algal MI were analyzed using nested and factorial ANOVA's with JMP statistical package version 4.0.0 (Academic).

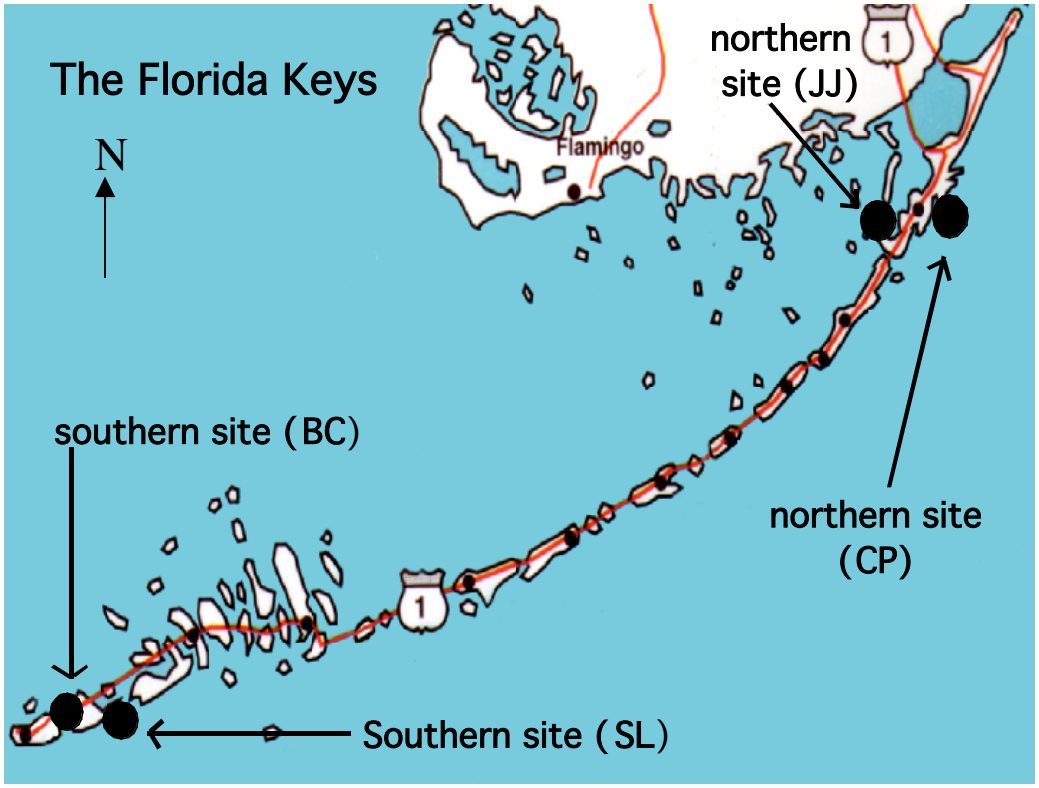


Figure 4.1: The four collection sites in the Florida Keys.

Two northern sites and two southern sites separated by ~160km. Larvae and algae were collected from three medusa at each of the four sites.

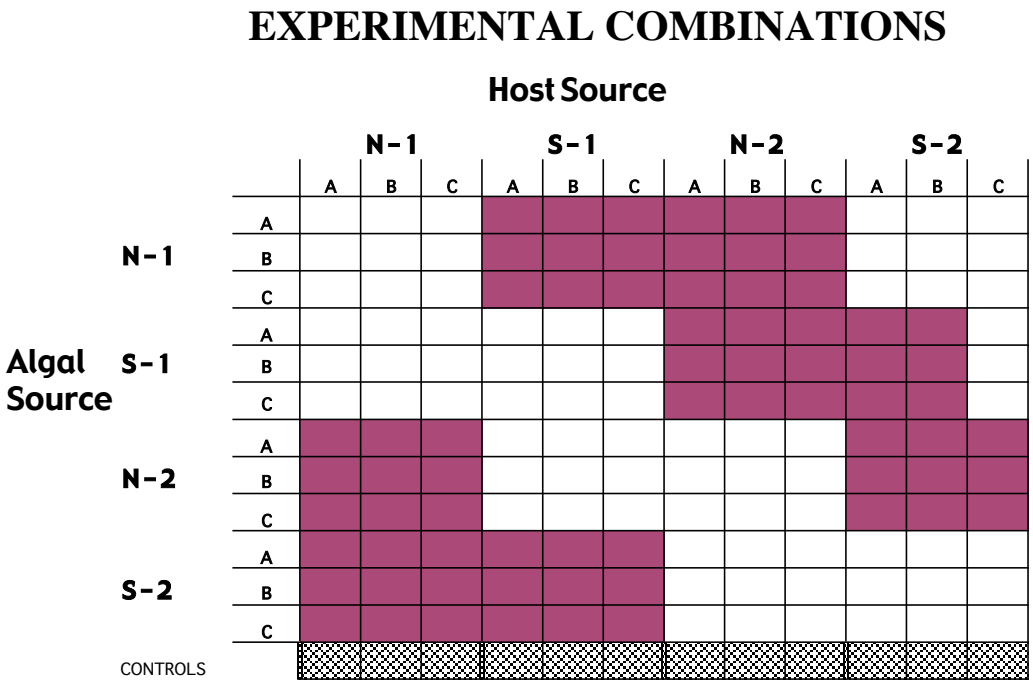


Figure 4.2: Experimental design for the geographic structure experiment.

There were 69 different combinations, 3 replicates per combination, and 25 experimental polyps per replicate. There were 2 control replicates per polyp lineage (speckled squares). Columns represent polyp origin and rows algal origin. Colored squares represent jellyfish-algal combinations used in the experiment.
N-1 = Northern site 1 on Figure 4.9, S-1 = southern site 2 on Figure 4.9, etc. A, B, and C = one of three medusa collected at each site.

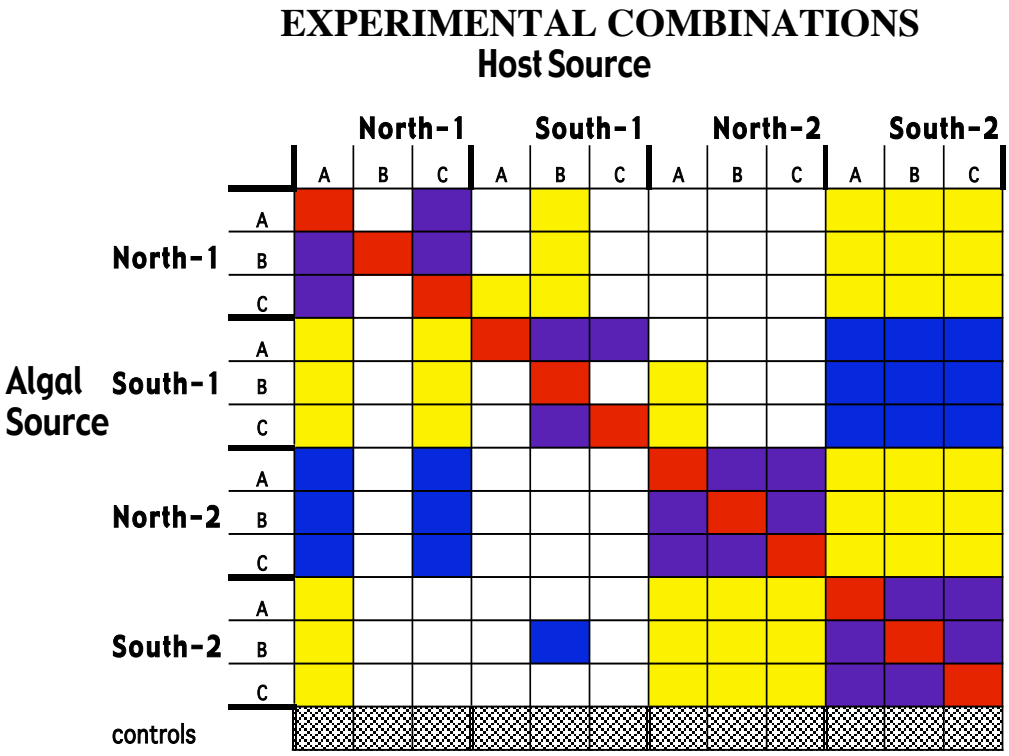


Figure 4.3: Experimental design including 89 combination types.

Maternal combinations (red diagonal), same-site combinations (purple squares), nearest neighbor combinations (blue squares), and distant site combinations (yellow squares). Three replicates per combination, 25 experimental polyps per replicate, and 2 control replicates per polyp lineage. When possible, polyps from a medusa were infected with its maternal algal isolate, 2 same-site algal isolate, 3 nearest-neighbor algal isolate, and 3 distant-site algal isolates.

PART 4: RESULTS

Experiment 1 – Variation in host-symbiont compatibility examined

There was little or no mortality across the controls; they shrank or changed very little in size as already observed in the previous experiment. All polyps inoculated with algal isolates showed visible signs of infection within one week. A one-way ANOVA revealed significant variation among the 69 combinations for both change in size (μm) ($p < 0.0001$) and mortality ($p < 0.0001$) as expected (Chapter 3). However, when the 69 combinations were grouped according to nearest neighbor and distant-site combinations, ANOVA revealed significant variation between the groups (Figure 4.4) for both change in size (μm) ($p < 0.001$) and mortality ($p < 0.001$). Distant-site combinations had higher mortality than nearest-neighbor combinations. Additionally, distant-site combinations shrank while nearest-neighbor combinations grew (Figure 4.4).

Experiment 2 –Geographic variation and host-symbiont interaction effects

Controls suffered little mortality and shrank as observed in the two previous experiments. All flasks inoculated with algal isolates showed visible signs of infection within one week. A one-way ANOVA revealed significant variation among the 89 combinations for both change in size (μm) ($p < 0.001$) and mortality ($p < 0.0001$). When the 89 combinations were grouped according to combination type (Figure 4.5), there were significant differences between the groups for change in size (μm) ($p <.001$) and mortality ($p <.001$). Nearest-neighbor combinations have lower mortality than distant combinations, but higher mortality than same-site or maternal combinations. Nearest-neighbor combina-

tions grew more and suffered less mortality than distant-site combinations, as observed in the previous experiment. However, nearest-neighbor combinations had lower survival and growth when compared with maternal and same-site combinations. There was no significant difference between same-site and maternal combinations for growth and mortality (Figure 4.5). Clearly, there is a geographic component to the variation observed among these *Cassiopea*-algal combinations.

In order to investigate host-symbiont interactions, a subset of data, two sites where both sites had all possible combinations, was analyzed using a 2-way factorial ANOVA (Figure 4.6). Analysis revealed significant host-symbiont interaction effects for both change in size (μm) ($p < 0.001$) and mortality ($p < 0.001$). Southern site polyps suffered more mortality when infected with northern site algae while northern site polyps suffered more mortality when infected with southern site algae (Figure 4.7). Additionally, northern site polyps grew more when infected with northern site algae; however, southern site polyps grew less when infected with northern site algae (Figure 4.8). This result suggests that the observed variation in compatibility among *Cassiopea*-algal combinations depends in part upon the geographic origin of symbiotic partners.

Mitotic Index (MI) for algae from a given site was the same across all polyp types ($p > 0.05$; D.F. = 59; Figure 4.9). For example, BC algae had similar MI in BC and SL polyps. Likewise, SL algae had similar MI in SL and JJ polyps. There was, however, a significant algal effect ($p < 0.001$; $F = 68.0091$; D.F. = 60; Figure 4.10a). That is, there were significant differences among algal strains from

the four sites for MI. JJ algae (northern site 2 Figure 4.1) had the highest MI while BC algae (from southern site 1 Figure 4.1) had the lowest MI. Overall, southern site algae (BC and SL algal isolates) had lower MI within hosts and northern site algae (JJ and CP algal isolates) had high MI within the hosts. Conversely, there were no significant differences among the cultured algal isolates for MI (Figure 4.10b; $p > 0.05$; $F = 1.1810$; D.F. = 12). There was no significant correlation between host mortality and algal MI, nor was there a significant correlation between host growth and algal MI.

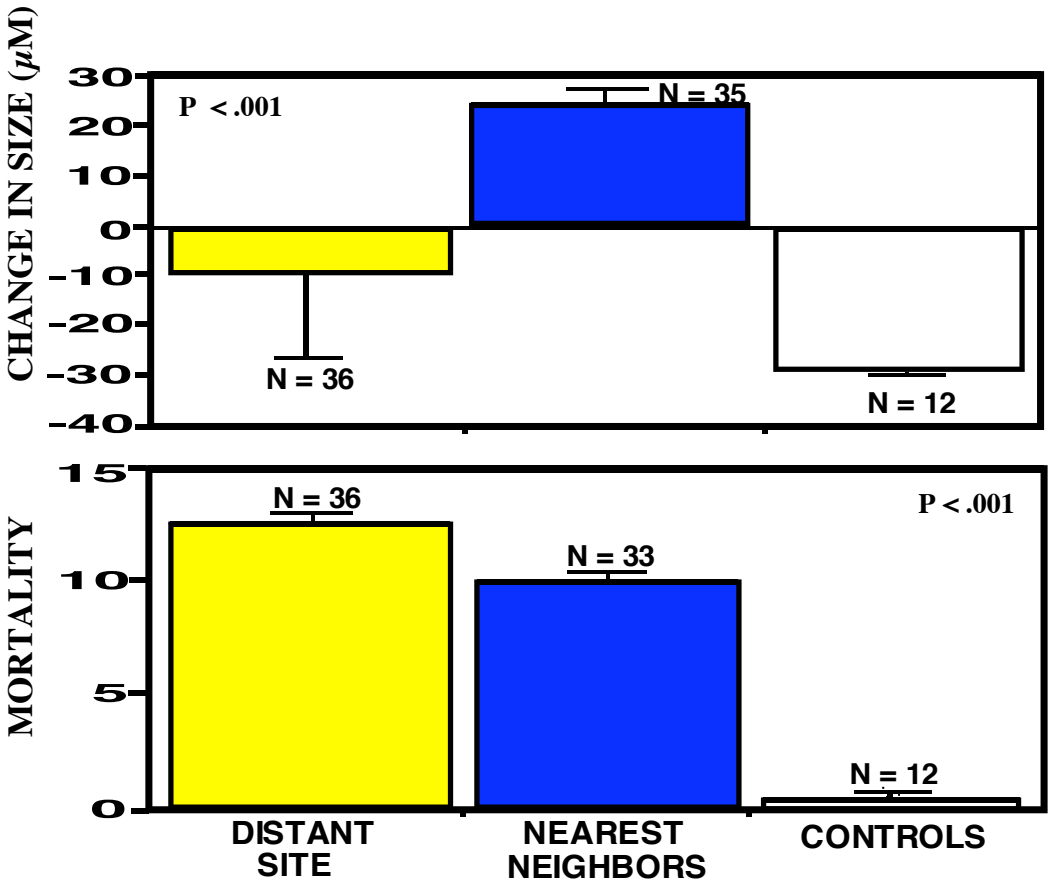


Figure 4.4: Experiment 1 – ANOVA.

Results from ANOVA for change in size (μm) ($p < .001$) and mortality ($p < .001$) among combinations grouped as nearest-neighbor or distant-site combinations and controls. Top: Change in size (μm). Bottom: Number of individuals that died out of 25. The number above the standard error bar is the number of combinations in each group.

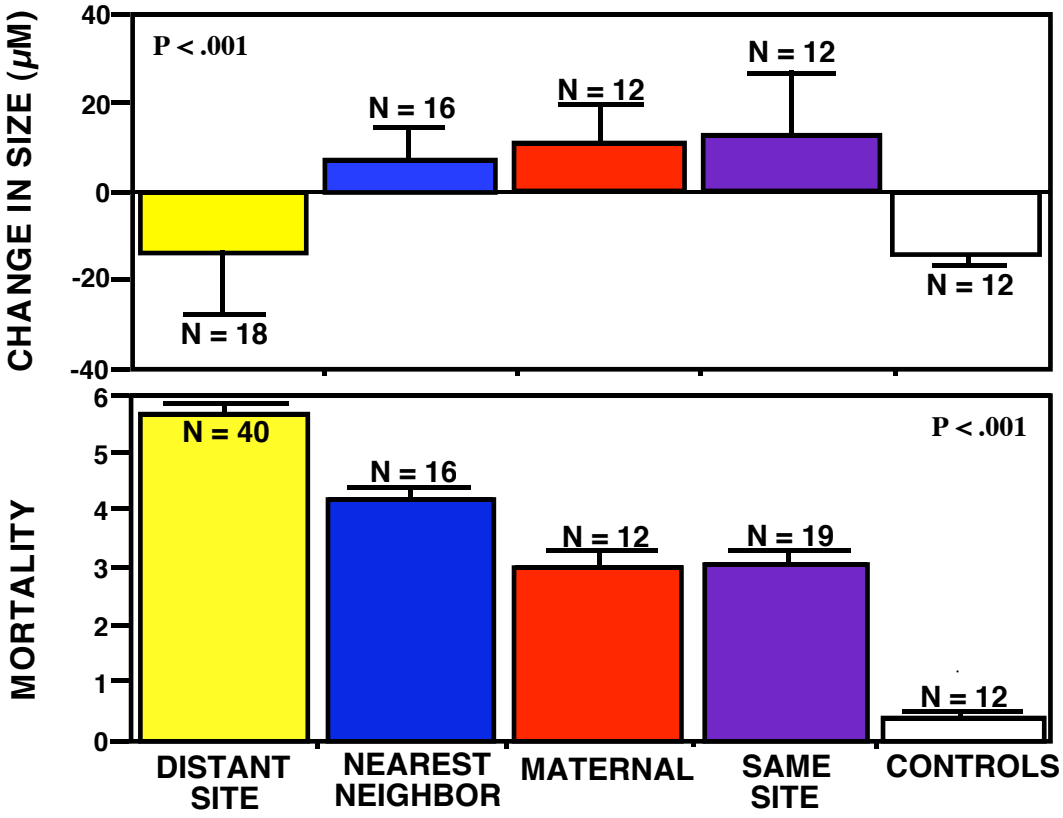


Figure 4.5: Experiment 2 – ANOVA.

Results from ANOVA for change in size (μm) ($p < .0001$) and mortality ($p < .0001$) among combinations grouped on the x-axis as distant-site, nearest-neighbor, same-site, maternal combinations or aposymbiotic controls. The numbers above the standard error bars are the number of combinations in each group. Controls suffer little or no mortality and shrink, as seen in the previous experiments. See text for a description of the results and Figure 4.3 for the experimental design matrix.

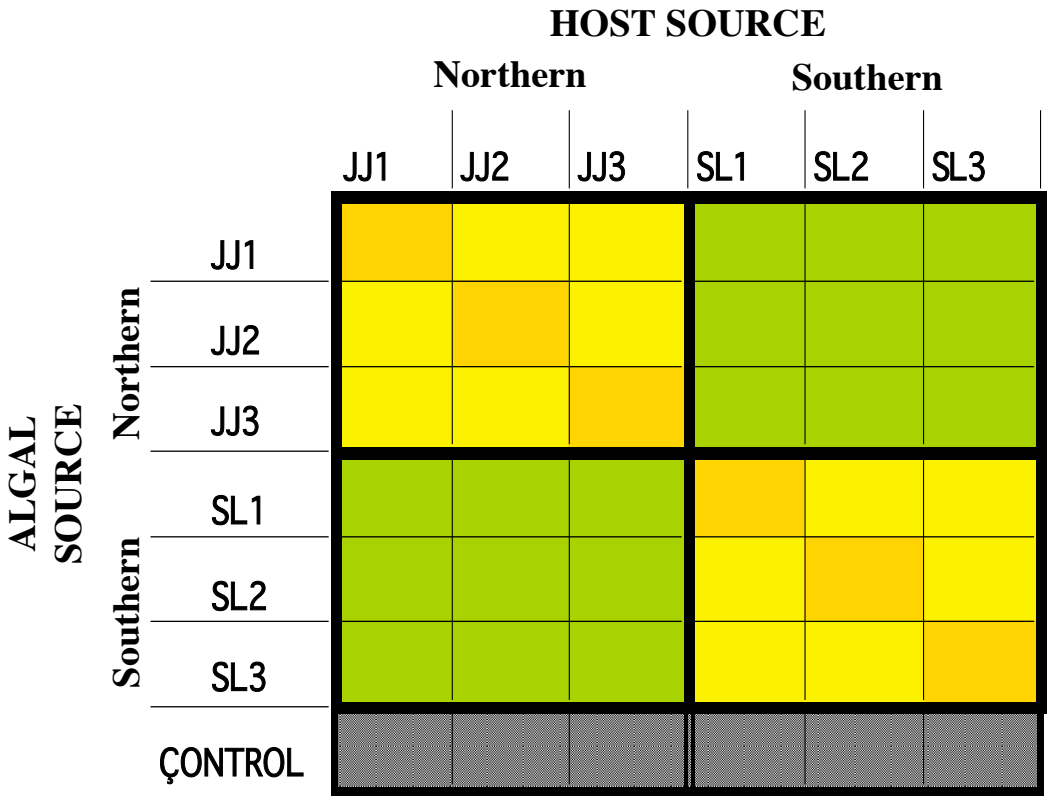


Figure 4.6: Experiment 2 – a subset of data analyzed.

This is the bottom right hand corner of experimental design depicted in Figure 4.3. The light green squares are distant-site combinations, the diagonal tan squares are maternal combinations, and the light yellow squares represent same-site combinations.

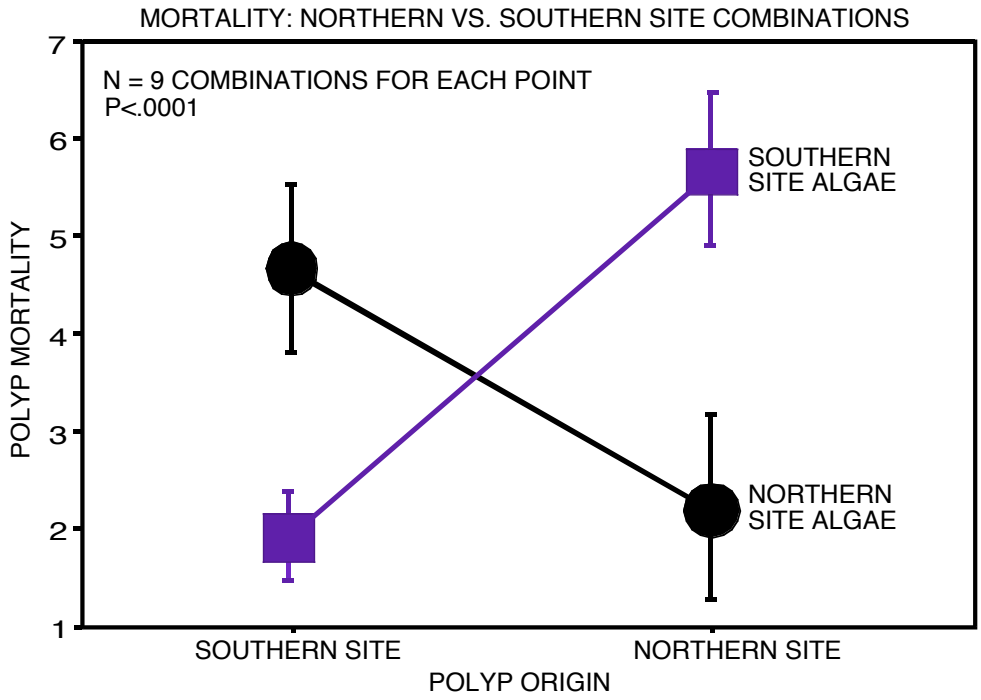


Figure 4.7: Host-symbiont interaction effects for mortality.

Significant host-symbiont interaction ($p < 0.0001$) for mortality when a subset of the data is analyzed for all pairwise combinations between a southern site and a northern site. A full factorial ANVOA was performed using all pairwise combinations between the second northern site (N2=JJ) and the second southern site (S2=SL). See Figure 4.6 for subset of data analyzed.

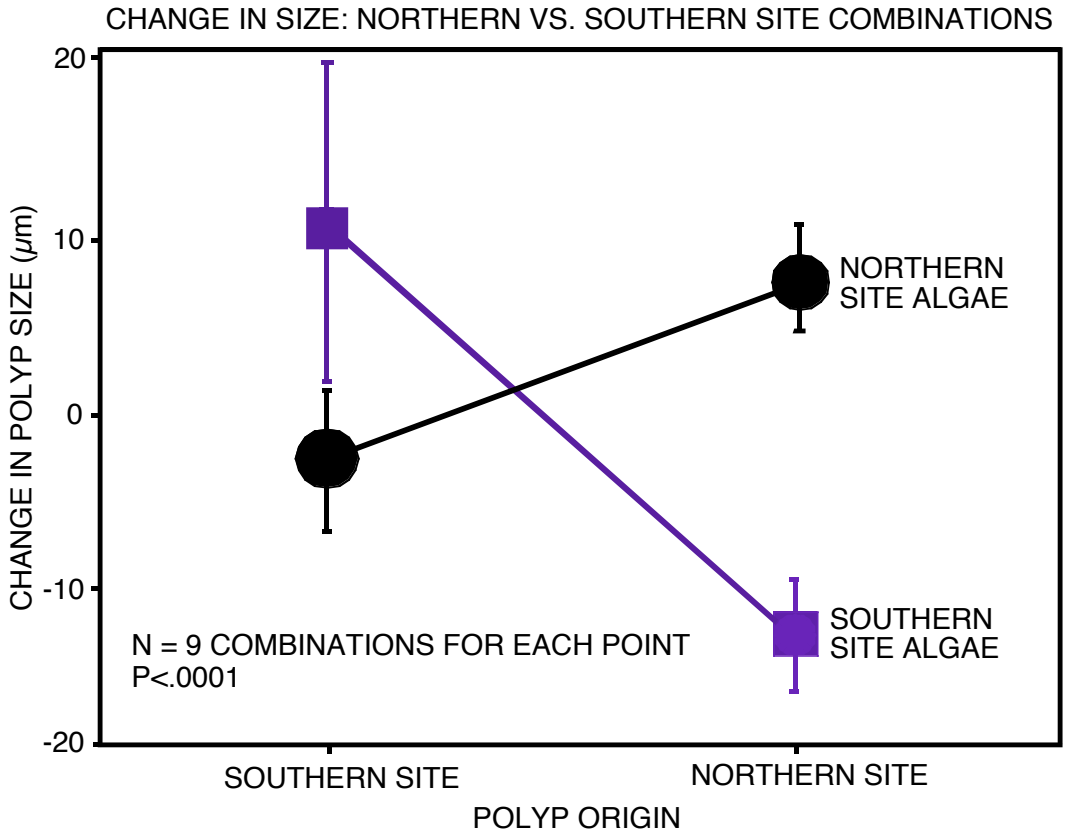


Figure 4.8: Host-symbiont interaction effects for change in size (μm).

Significant host-symbiont interaction ($p < 0.0001$) for change in size (μm) when a subset of the data is analyzed for all pairwise combinations between a southern site and a northern site. See also Figure 4.6 for subset of data analyzed.

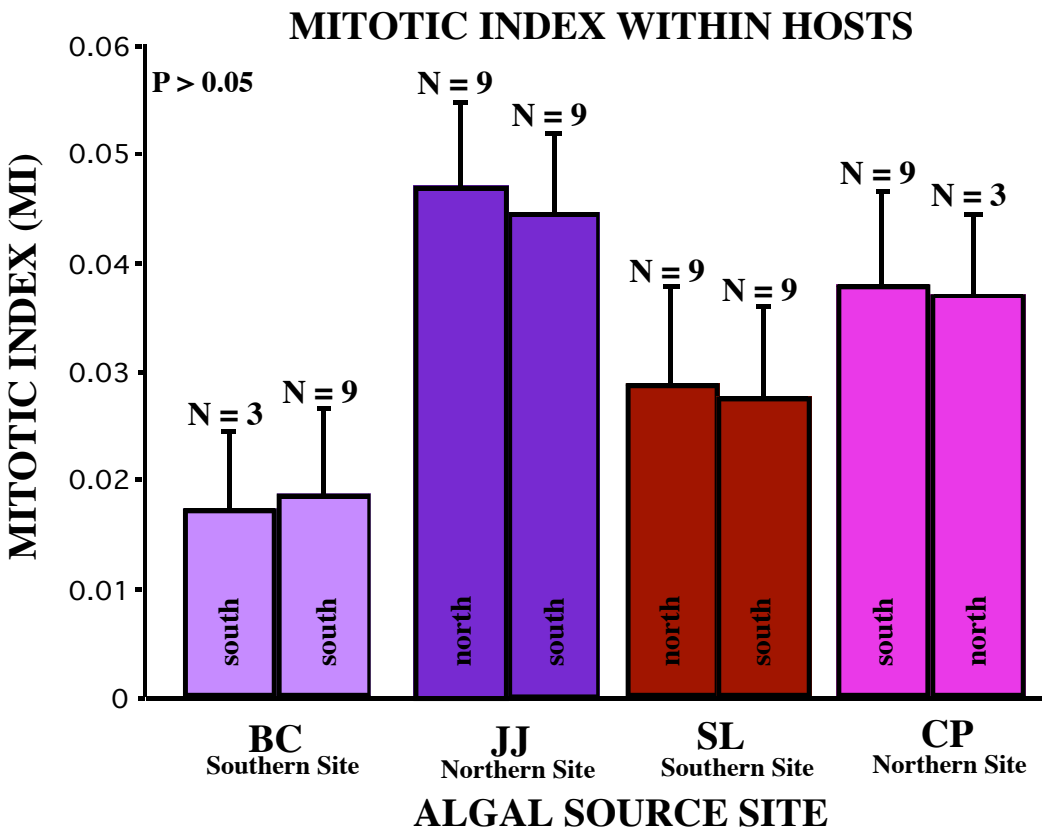


Figure 4.9: Mitotic Index (MI).

Individual bars represent polyps infected with each algal isolate. The number above the standard error bar represents the number of combinations included in the analysis. For example, the first bar represents southern polyps infected with algae from BC. There is no significant difference in BC algae MI within different polyp lineages.

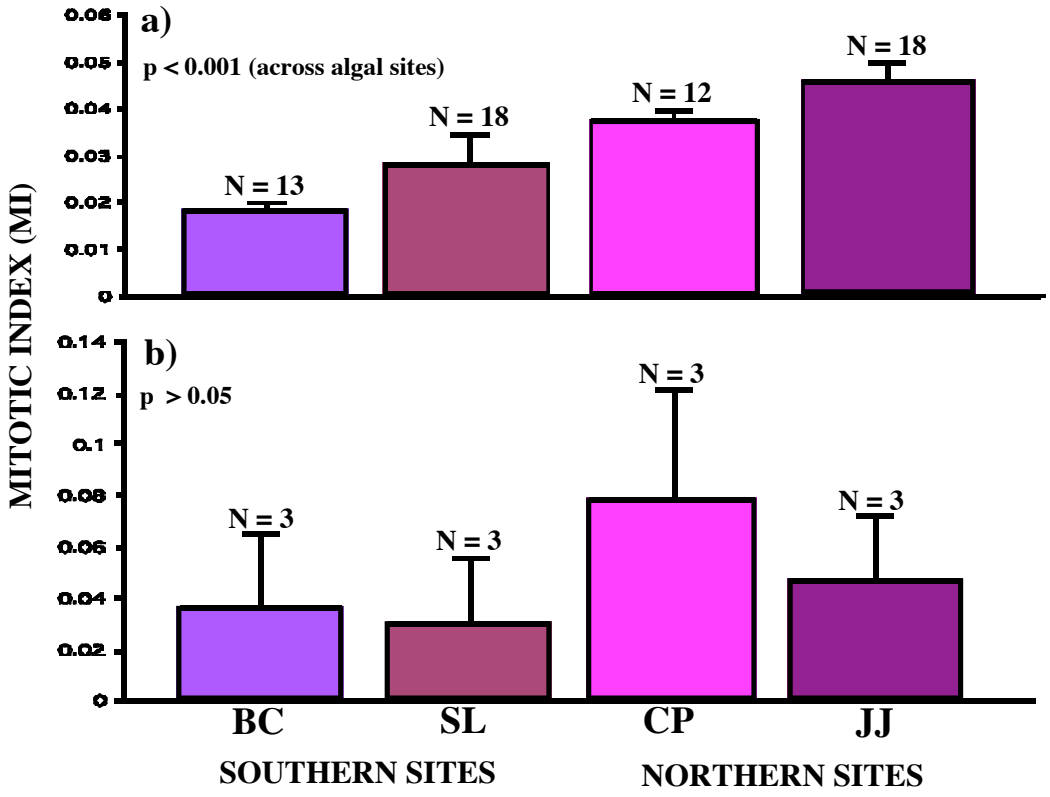


Figure 4.10: Comparison of MI in host and MI in culture.

a) The y-axis represents Mitotic Index (MI) within the host. The x-axis in both graphs represents the algal isolate origin and the algal origin is categorized as northern or southern site origin. Southern site algae have lower MI than northern site algae. b) The y-axis represents MI in culture. No significant difference in MI across algae in culture. Each bar is the average of three algal isolates collected at each of the four sites.

PART 5: DISCUSSION

Overall, there is intraspecific symbiont variation, a fundamental variable for evolutionary adaptation, among the algal symbionts inhabiting *Cassiopea* hosts. In these experiments, the growth and survival of host lineages was significantly altered when interacting with various algal isolates. The observed variation is depends on the geographic origin of symbiotic partners. Specifically, combinations in which partners originate in close geographic proximity (same-site or maternal combinations) suffer less mortality and grow more than combinations whose partners originate from geographically distant-sites. Finally, the structure of the variation appears to be driven by significant host-symbiont interaction effects at least at the two sites where all pairwise combinations were performed. Therefore, the two sites are very different selective environments for the algal isolates. These results are consistent with the interpretation that *Cassiopea xamachana* are generally better adapted to sympatric algal mutualists than to algal isolates from distant-sites.

Results from these studies have important evolutionary and ecological implications regarding migration and colonization processes in invertebrate hosts that are obligately dependent upon symbiotic algae for growth and survival. The results imply that compatibility with the local symbiont population could be a key factor influencing the potential for the establishment and proliferation of any given host immigrant. Certain host genotypes might be excluded from a habitat not because of inferior adaptation to macroscopic features of the non-symbiotic environment (i.e. predators, climate, water quality), but simply because they are

incompatible with indigenous algal mutualists necessary for proliferation in that particular habitat. Migrant lineages are likely to have reduced longevity and fecundity and will likely be displaced by resident associates. Over the long term, the local host-symbiont population would become genetically homogeneous as incompatible symbioses are intensely selected against. Positive frequency-dependent selection might characterize local *Cassiopea*-algal symbioses, where partners adapted to the most common phenotypes accrue larger fitness advantages (Law, 1985; Parker, 1995; 1999). Meanwhile, mutualistic associates adapted to rare phenotypes will be eliminated because the chance of encountering a compatible partner is low. The above argument (1985) was for a single population of mutualists in isolation, but invokes interesting questions when extended to larger geographic scales. It is likely that some degree of spatial differentiation exists among one or both partner species due to drift and adaptation to various environmental factors among sites. For example, the host and symbiont genepool in any given location might consist of genotypes best suited to prevailing environmental conditions (salinity, turbidity, temperature, etc.). These initial differences might be magnified by positive frequency-dependent selection on the holibiont (*Cassiopea*-algal complex). This would generate a geographic mosaic with each locally dominant set of mutualists displaying resistance to invasion by rare immigrant phenotypes who are adapted to different partner phenotypes (Parker, 1999). The role of spatially varying environmental factors among the study sites used in this experiment deserves attention. For example, future experiments implementing environmental stress might provide an explanation for

some of the factors that control the distribution and abundance of different algal symbionts in nature.

Given the differential growth and survival among local and allopatric combinations, it is clear that the origin of algal symbionts plays an important role in host growth and survival. However, the extent to which *Cassiopea* hosts can adaptively control the local composition of algal symbionts remains an unknown. Because there was no 'polyp' main effect on algal reproduction (MI), it is difficult to interpret how hosts might alter algal fitness and thus modify the symbiont gene pool. All *Cassiopea* polyps became infected when subjected to various algal isolates even if the algal isolate provided inferior symbiotic benefits (lower growth and survival). This may imply that *Cassiopea* hosts lack the ability to discriminate between 'good' and 'bad' algae - unless hosts can detect differences between 'good' and 'bad' algae when they are present at the same time (Bull and Rice, 1991). Unfortunately, the design of these experiments did not allow for partner choice since polyps were forced to interact with one algal isolate at a time. It is likely that, in nature, aposymbiotic polyps encounter a range of symbiont genotypes. Nonetheless, this design is the simplest and most direct way to test for algal genotype effects on host fitness.

It is also possible that the algal symbionts used in these studies represent symbiotic partners that have survived the host's 'sieve' and have successfully established an association. That is, the algae used to infect polyps across sites are already the "crème de la crème" since they were isolated directly from sexually mature female medusa. However, finding spatially correlated variation suggests

that hosts might have at least some selective impact on algal populations. In chapters 5 and 6 these ideas are investigated further.

It is curious that there were no significant differences among algal isolates in MI when grown in culture. Moreover, *in hospite*, MI was lower for southern site algae and higher for northern site algae regardless of which polyp lineage the symbiont occupied. Freshly isolated algae were used in the infection experiments, and algal MI in each host lineage might be an artifact of this protocol, particularly if the algae cells were not completely free of host tissue. Conversely, in culture and free from host control, algal MI converges across algal isolates. Does this imply that free-living algal symbionts behave as generalists, facilitating migration, but specialize on a particular host genotype when they establish symbioses? Because the algal symbionts are acquired directly from the environment, there is an element of chance as to which algal symbiont a jellyfish juvenile will encounter. Perhaps having a relatively plastic, free-living stage facilitates the establishment of a successful symbiosis, allowing algal symbionts a variety of possible responses to varying "jellyfish environments". Plasticity would serve to maintain genetic variability within the symbiont population and slow subpopulation divergence. Given the significant host-symbiont interactions detected during this study, plasticity is likely limited.

Alternatively, perhaps sexually reproduced larvae as well as asexually reproduced planulae rarely disperse, but settle and proliferate in the 'home' habitat where the local symbiont gene pool is relatively homogeneous. If migration between subpopulations is minimal for both hosts and symbionts, then

local adaptation could proceed rapidly and eventually lead to population divergence. This is one possible explanation for the significant difference in MI between Northern and Southern algae *in hospite*. Future studies should include an investigation of *Cassiopea* and algal symbiont population genetic structure both within and between collection sites. Additionally, comparisons of MI *in hospite* between polyps infected with freshly isolated algae and algae collected from the water column might help disentangle the MI results reported above.

Frequent abiotic disturbances such as hurricanes undoubtedly cause extinctions and provide a potential mixing of nearby *Cassiopea*-algal subpopulations. Thus, it is likely that the metapopulation of *Cassiopea*-algal symbioses consists of subpopulations in a continuum of evolutionary states (e.g. a stable geographic mosaic). Patches such as JJ (a northern site in this study) might be in a relatively stable homogeneous state characterized by *Cassiopea*-algal specialists. JJ is a well-protected bayside site with relatively stagnant water. Many researchers have suggested that marine invertebrates remain flexible in their specificity for algal symbionts as a means of coping with environmental variability. This seems reasonable since hosts with broad compatibility will rarely suffer from lack of access to partners, while specialization clearly entails the cost of a reduced chance of finding suitable mutualists (Parker, 1999). However, this study suggests that there is not only strong host specificity for a single specific symbiont species but also for specific isolates (genotypes) within the algal species. Several studies investigating legumes and their nitrogen-fixing bacteria have found extremely specialized symbiotic associations (Young and Matthews,

1982; Kneen and LaRue, 1984; Devine, 1987). For example, Parker and Wilkinson (1997) found that 60% of populations of *Amphicarpaea bracteata,* sampled over a 1,000-km region, were dominated by plants with specialized *rhizobium* genotypes.

Conversely, a site such as SL (a southern site in this study) may be characterized by a more variable population of hosts and symbionts. Perhaps this site is oceanside and more susceptible to storm surges/hurricanes. Thus, this population might have an evolutionary history molded by frequent extinction-recolonization events. Alternatively, given the sites location, perhaps oceanic mixing maintains a more variable population of hosts and symbionts. Either of these scenarios might explain the greater within site variation observed among same-site and maternal combinations from SL. In fact, a maternal combination from this site suffered high mortality and little growth. This site is located on the oceanside of the Florida Keys with heavy boat traffic and increased water flow due to canal dredging. These are potentially two ends of the spectrum with many subpopulations likely falling between these extremes. Future studies should focus on within-site and nearest-neighbor site variation in order to determine the extent of local variation. Further, future investigations of host-symbiont interaction effects should examine interactions across a variety of collections sites.

To my knowledge, this is the first investigation of intraspecific host-symbiont compatibility among marine algal-invertebrate symbioses; however, researchers have found similar results in terrestrial symbioses and host-parasite associations. The first rigorous investigation of local adaptation by natural

populations of parasites was conducted by Parker (1985). He showed that fungal pathogens were more infective to host plants (*Amphicarpaea bracteata*) originating from the same geographic region. Using a reciprocal cross-infection design, Lively (1989) found similar results among trematode-snail associations across several lakes in New Zealand (also Lively *et al*., 1996). Trematodes were most infective to sympatric hosts providing strong evidence for local adaptation of trematode parasites to local snail hosts (reviewed in Lively, 1999). Over the last decade, evidence of local adaptation of parasites to their respective hosts has been observed in several systems: anther-smut fungus to *S. albina* (Alexander, 1989), microsporidian gut parasites of *Daphnia* (Ebert, 1994), trematode worms of fish (Balabeni and Ward, 1993), and pulmonata snails (Manning *et al*., 1995). In fact, Ebert showed (1994) that geographic distance negatively affected infectivity by parasites. Theoretical work (Lively, 1999) corroborates these empirical results showing that sympatric parasites are better at infecting locally common host genotypes than remote or foreign parasites, which are likely to be tracking different host genotypes. The results from my study clearly mimic this trend. That is, symbionts are most benevolent towards local or sympatric host genotypes than to foreign host genotypes. In the case of my system, both host and symbiont gain larger fitness advantages by associating with local or sympatric partners. Like many parasites, algal symbionts have short generation times. Furthermore, *Cassiopea xamachana* are reproductive year around. Perhaps asexually generated host clones settle locally keeping local conditions relatively homogenous in terms of host genetics. Meanwhile, symbionts become adapted to

the most common host genotype. This scenario would facilitate rapid adaptation of algal symbionts to *Cassiopea* hosts.

It would be interesting to discover the extent to which other algal-invertebrate symbioses conform to these results. I would expect as much, if not more, local adaptation in sedentary invertebrates obligately associated with marine algal symbionts i.e. sponges or reef-building corals. Over the last several decades, it has become clear that most invertebrate hosts are restricted to associating with one or a few algal symbiont species. However, no studies have examined variation among algal symbionts beyond species level differences. This is curious since migration, colonization, survival, growth, and reproduction appear to be dependent upon the algal symbiont *in hospite* – even if the symbionts are from the same taxa! Most investigators of algal-invertebrate symbioses have assumed that all symbionts are equally benevolent across multiple populations of the same host species, and, generally, their investigations are restricted to single sites/populations. Given the results from my study, it might be important for researchers to extend their investigations to include a range of sites, before making broad generalizations about specific associations based on single site data.

VARIATION IN HOST SYMBIONT COMPATIBILITY AMONG *CASSIOPEA*-ALGAL SYMBIOSES

Chapter 5: Intra-specific Symbiont Variation among *Cassiopea xamachana* hosts – Are Environmentally Available Symbionts Equally Compatible Across *Cassiopea* Hosts?

PART 1: ABSTRACT

It has become clear that not all *Cassiopea xamachana* algal symbionts are equally compatible across *C. xamachana* hosts. Further, variation among different host-symbiont combinations appears to be geographically structured, and there is some evidence that suggests the presence of local adaptation of algal symbionts to *Cassiopea* hosts. However, a concern with previous studies (chapter 4) has been that the algae isolated from each female medusa may not accurately represent the naturally available symbiont pool at each of the collection sites. And, it is likely that aposymbiotic polyps (polyps devoid of algae) encounter a range of compatible symbionts locally. In order to address this concern, an experiment was conducted to examine variation in *Cassiopea*-algal compatibility between polyps and environmentally available algae collected from a variety of locations in the Florida Keys. In this experiment, seawater from each collection site, presumably containing algal symbionts released from local hosts, was used to initiate infection instead of algae isolated directly from female medusae. Overall, results corroborate previous studies to a remarkable degree. Same-site combinations (jellyfish-algal combinations in which *Cassiopea* polyps and seawater come from

the same site) and nearest-neighbor combinations (jellyfish-algal combinations in which partners originate from sites in close geographic proximity) suffered less mortality than distant site combinations (jellyfish-algal combinations in which partners originate from geographically distant sites). In fact, there was no significant difference in survivorship between same-site combinations and nearest-neighbor combinations. Additionally, analysis indicates that there are highly significant interactions between site of host origin and site of algal origin.

PART 2: INTRODUCTION

For mutualistic endosymbiotic associations, few strong generalizations have emerged, either empirical or theoretical, regarding the evolutionary significance of geographic variation. This is, in part, because most investigators of marine algal-invertebrate symbioses have assumed that all symbionts are equally benevolent not only within individual hosts, but across the same host species. As a result, the natural variation among algal symbionts, in terms of their effect on host fitness, has largely been ignored. Thus far, this dissertation has shown that variation in host-symbiont compatibility among manipulated *Cassiopea*-algal symbioses is significant. This variation appears to be geographically structured, an expectation associated with the geographic mosaic theory of coevolution (Thompson 1994, 1999). Researchers have suggested that the dynamic nature of host-parasite associations make them ideal candidates for natural systems that conform to the geographic mosaic theory of coevolution. However, if the zooxanthellae inhabiting many marine invertebrates span a genetic continuum ranging from highly selfish/virulent symbionts to benevolent/cooperative

symbionts, then the same dynamic nature should exists between host-symbiont associations. In this case, a mosaic can occur if selection is fluctuating, rather than directional, and the fluctuations among populations are out of phase with each other (Lively, 1999). Mosaics such as these might be common for structured populations of hosts interacting with a variable population of obligate symbionts. Overall, it is important to determine the extent to which mutualisms conform to geographic mosaics and what factors are most responsible for determining conformity among symbiotic partners where mosaics exist.

On the other hand, there are several potential reasons for the variation I have observed in host-symbiont compatibility (Chapter 3 and 4). First, it is possible that the observed variation in host fitness simply reflects one-sided adaptation by a species (either the host or symbiont) to environmental differences with causes that are completely external to the mutualism (Parker, 1999). Secondly, because algal associates were isolated directly from female medusae, they may not accurately reflect the available pool of symbionts at any given location. Perhaps variation among symbionts within a site is high yet the design of previous experiments may have overestimated intraspecific symbiont variation, especially if *Cassiopea* medusae house only a single specific symbiont genotype in any given site. Thirdly, it is possible that within a given site there is a diversity of symbiont genotypes maintained by migration between sites. As a result, all sites have roughly equal representation of symbiont genotypes, but local hosts are compatible with only a subset of these symbionts, and the most compatible symbiont genotypes vary across sites (possibly due to host population structure).

If this is this case, then previous experiments may have overestimate population structure in the free-living symbionts since I used 'locally compatible' symbionts isolated directly from the hosts. In other words, symbionts isolated directly from the medusa already survived the 'selective sieve' that occurs when the association is established. Fourth, it is possible that both the algal symbionts and *Cassiopea* hosts have population structure; as a result, not all symbiont genotypes are equally represented across all sites. In this case, the results from previous experiments should roughly mimic the results that would be obtained if seawater from each site, presumably containing the environmentally available symbionts, were used to establish the symbiosis. In this manner, one can examine the effect of a local symbiont gene pool on jellyfish from that site, nearest neighbor sites, and distant sites. All things being equal, if each combination type performs equally well, then variation within a site will mask variation between sites. If true, then this system does not conform to a geographic mosaic but rather a panmictic population of compatible symbionts present in the water column.

This study re-examines variation among experimentally manipulated *Cassiopea*-algal combinations using multiple hosts and symbionts from several populations across the Florida Keys. In this experiment, algal symbionts inhabiting the seawater at each site, as opposed to isolated directly from female medusa, were used to infect *Cassiopea* hosts. Additionally, this study encompasses all pairwise combinations of origination sites for both symbiont and hosts. The data from this study allow me to determine if the observed variation

among *Cassiopea*-algal combinations is structured or random with respect to naturally available symbiont genotypes.

PART 3: METHODS

Collection of larvae from each medusa at each site as well as the maintenance of experimental polyps followed the protocol described in chapter 3 and 4. Planula larvae were collected from three similarly sized medusa as well as seawater at two northern sites and two southern sites in the Florida Keys (figure1). Larvae were allowed to settle to the polyp stage as described in chapters 3 and 4. Polyps collected from each medusa were split into four groups. These polyp groups were placed in 100μm-filtered seawater collected from their site of origin, from a nearest-neighbor site and from two distant-sites (Figure 5.2). Same site combinations consisted of partners that originated from within the same site. Nearest-neighbor combinations consisted of partners that originate in close geographic proximity. For example, northern site polyps were infected with algae from the second northern site or vice versa. Distant-site combinations consisted of partners that originate from geographically distant-sites. For instance, polyps from one southern site were infected with algae from one of the northern sites. Overall, there were 44 different *Cassiopea*-algal combinations, 3 replicates per combination, and 30 experimental polyps per replicate. Additionally, polyps devoid of algae for each host lineage were maintained as controls (3 replicates per host lineage, 30 uninfected polyps per replicate). Once polyps appeared an orange-brown, color indicative of algal infection, they were maintained in ASW (artificial salt water) and maintained as described in previous experiments

(chapter 3 and 4). Size and mortality were negatively correlated in previous experiments (chapter 3 and 4); that is, when polyps shrink, they tend to die. Thus, in this study, host fitness was assessed in terms of survival only. Mortality was assessed twice a week by recording the number of polyps remaining in each flask. The experiment was terminated after 30 days.

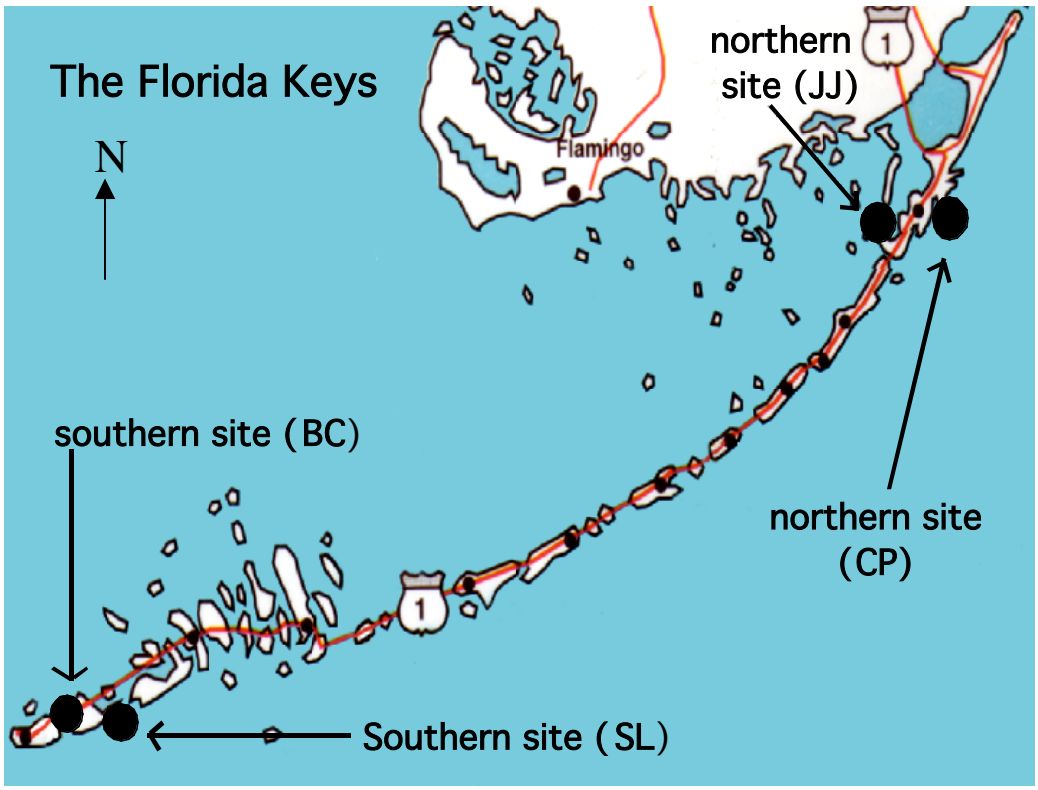


Figure 5.1: The four collection sites in the Florida Keys.

Two northern sites and two southern sites separated by ~160km. Water and larvae were collected from three medusa at each of the four sites.

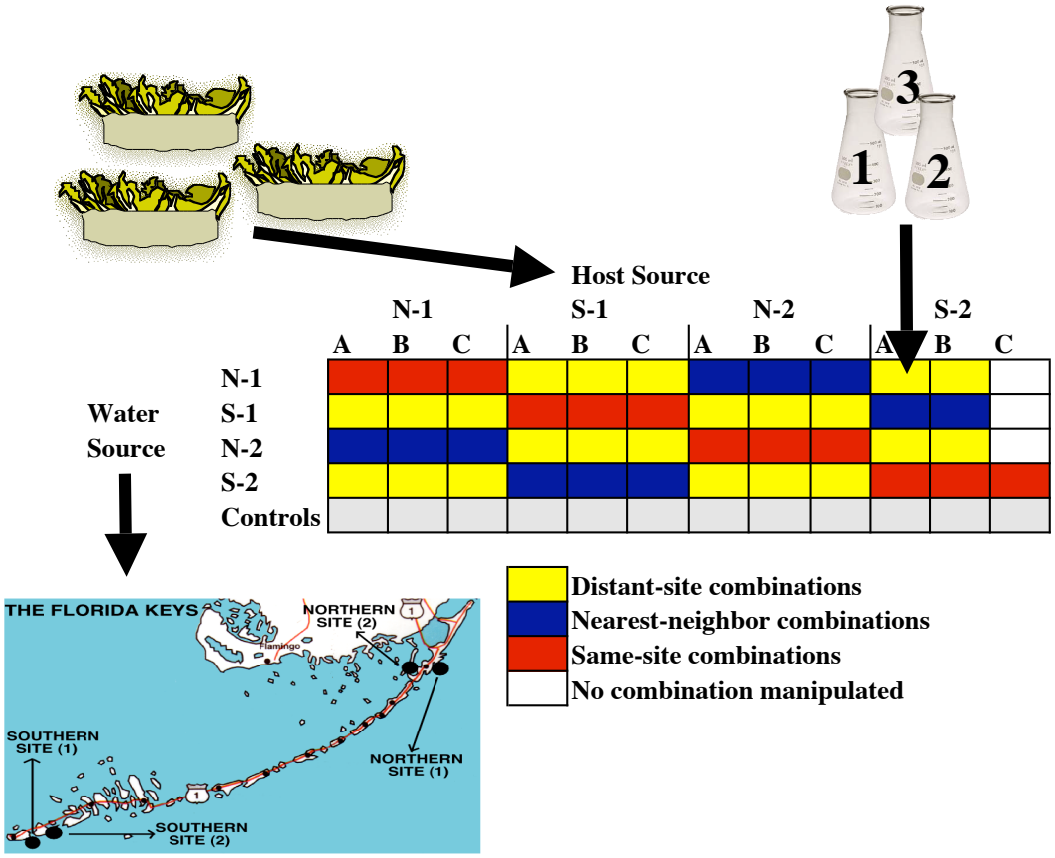


Figure 5.2: Experimental design.

Columns represent polyp source while rows represent water source. White squares = no combination; diagonal (red squares) = same-site combinations; yellow squares = distant-site combinations; blue squares = nearest-neighbor combinations. There were 46 different combinations, 3 replicates per combination. Each replicate contained 30 experimental polyps.

PART 4: RESULTS

All experimental flasks showed visible signs of infection within two weeks. Prior to subjecting polyp lineages to various water sources, one lineage from a southern site suffered almost complete mortality. As a result this lineage was necessarily omitted from the investigation. As in previous studies (Chapter 3 and 4), a one-way ANOVA revealed significant differences ($p < 0.0001$; D.F. = 44, 130; $F = 3.5481$) between all jellyfish-algal combinations for mortality (Figure 5.3). Some combinations suffered nearly 100% mortality while others experienced little or no mortality (Figure 5.3). Controls (polyps devoid of symbiotic algae) for each polyp lineage suffered little or no mortality during the experiment (there were 2 control replicates per polyp lineage). The controls for one polyp lineage (last column on Figure 5.2) suffered more mortality than would be expected based on previous experiments. One replicate had 4 polyps die and the other 3 during the coarse of the experiment. This polyp lineage exhibited high larval mortality just after collection and, as a result, I was unable to perform all pairwise combinations with this lineage. Those polyps that survived in this lineage took an exceptionally long time to settle to the polyp stage. It is likely that these polyps were either underdeveloped or sick to begin with.

When the 47 combinations were grouped according to combination type (Figure 5.4), there were significant differences between the groups for mortality ($p < 0.001$; $F = 19.2232$; D.F. = 2, 130). As before, distant-site combinations have the highest mortality. However, in this experiment, there was no significant difference in mortality between nearest-neighbor and same-site combinations.

Recall that, because I used water to infect newly settled polyps, there were no maternal combinations in this study.

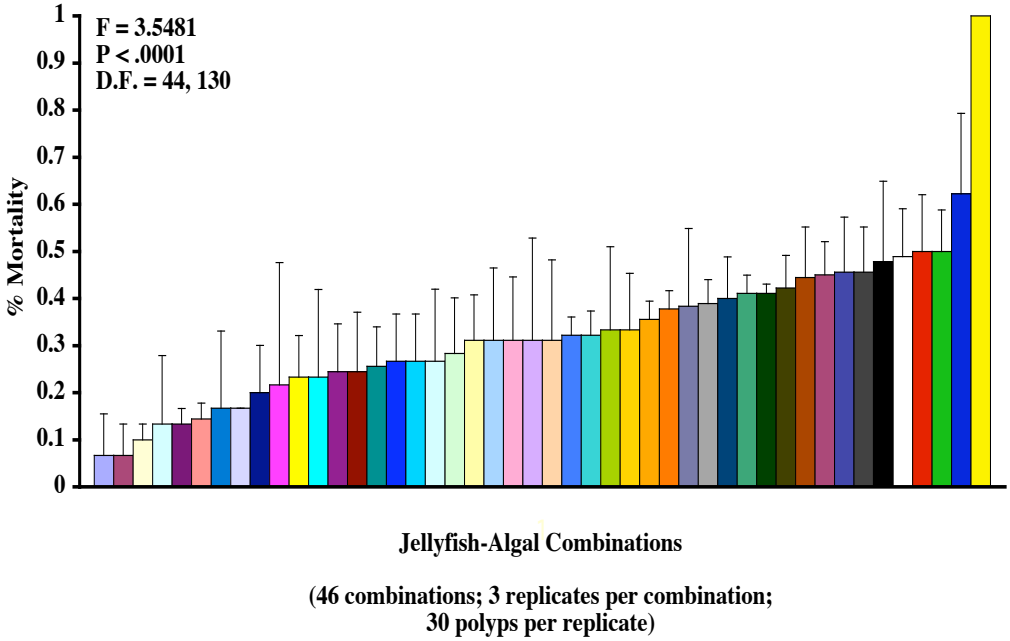


Figure 5.3: Mortality across 46 different combinations.

Forty-seven combinations represented by each bar on the x-axis. Lines above each bar are standard deviation bars (3 replicates per combination). Y-axis is the proportion of individuals that died for each combination. ANOVA revealed significant variation among the 47 combinations for mortality (P<.0001).

In order to investigate host-symbiont interactions, all pairwise combinations between water source and host source (Figure 5.1) from each site were analyzed using a Full Factorial 2-way ANOVA. Analysis revealed significant ($p < 0.001$; $F = 4.9071$; D.F. = 9) host-symbiont interaction effects for

mortality (Figure 5.5). Southern site polyps (BC and SL) suffered more mortality when infected with northern site algae (JJ and CP), while northern site polyps suffered more mortality when infected with southern site algae (Figure 5.5). This result suggests that the observed variation among *Cassiopea*-algal combinations depends upon the geographic origin of symbiotic partners. Interestingly, polyp lineages originating in the northern keys did almost as well with algae from the south (distant site algae) as they did with algae from the north (same-site or nearest neighbor algae) (Figure 5.6). For example, survival decreased on average by 10-12% when they were infected with algae from distant sites (Figure 5.5). However, polyp lineages originating in the southern keys suffered a 15-25% increase in mortality when infected with algae from distant northern sites. Additionally, all polyp lineages infected with algae originating from JJ water (a northern site) experienced similar mortality (Figure 5.5). In general, however, polyps infected with algae from sites in close geographic proximity survived more often then polyps infected with algae from geographically distant sites ($p < 0.0001$; $F = 20$; D.F. = 3); although, it would appear that most of this result is due to reduced mortality in southern site polyps when infected with southern site algae (Figure 5.6).

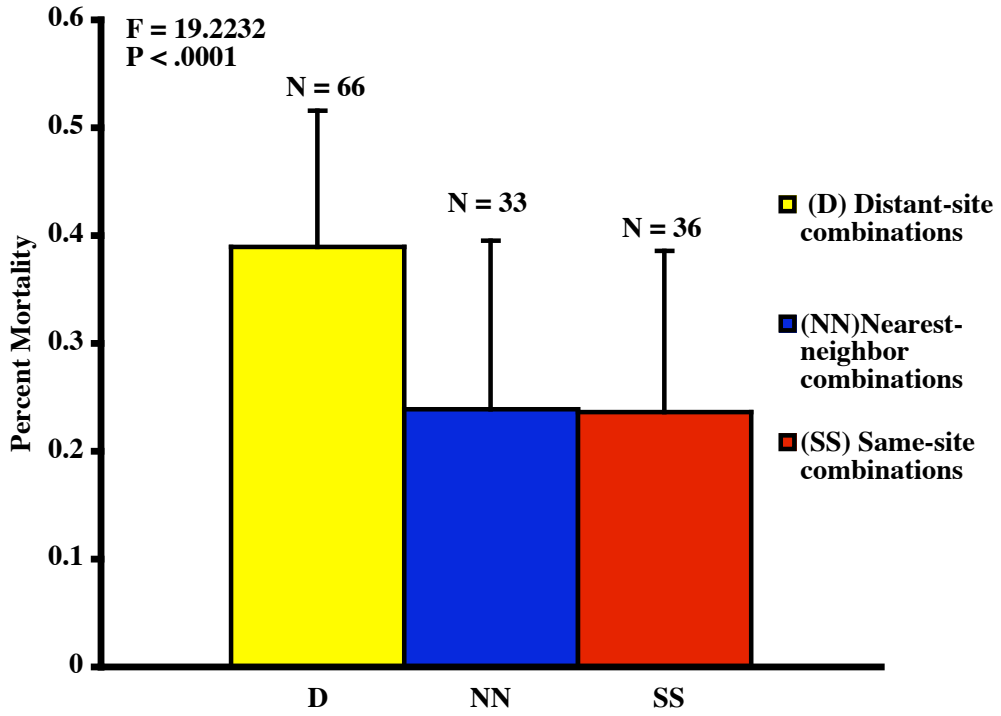


Figure 5.4: ANOVA for combination types.

Results from ANOVA for change in size (μm) ($p < .0001$) and mortality ($p < .0001$) among combinations grouped as distant-site, nearest neighbor, same-site, or maternal combinations. The numbers above the standard error bars are the number of combinations in each group. Controls suffer little or no mortality and shrink as seen in the previous experiments. See text for a description of the results. See Figure 5.2 for experimental design matrix.

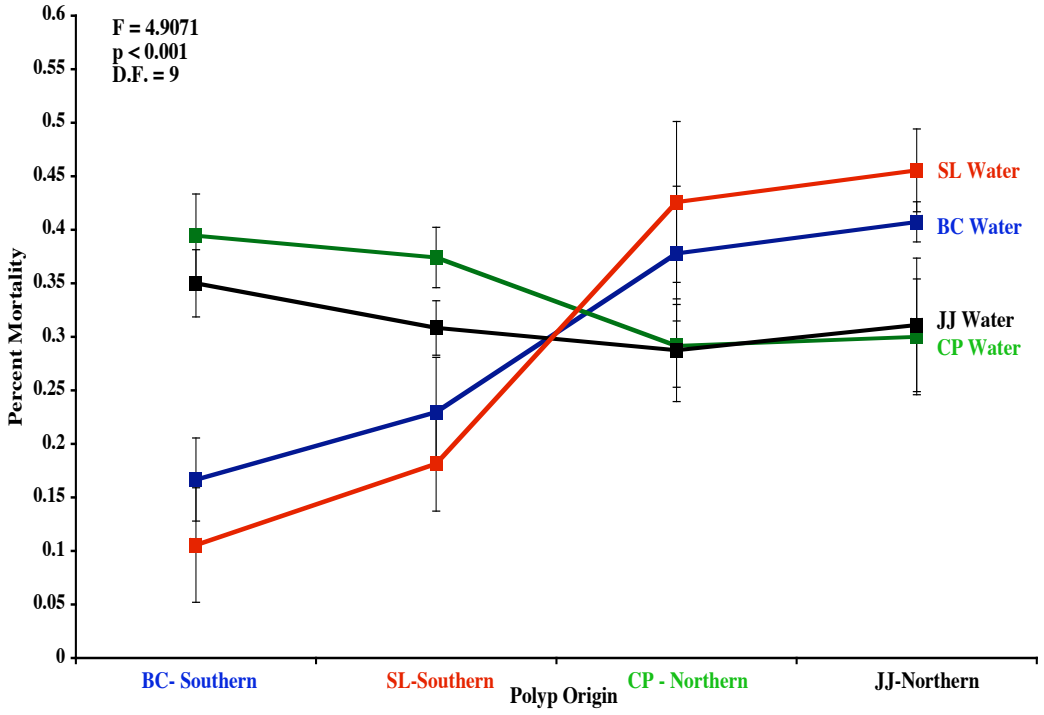


Figure 5.5: Full factorial ANOVA reveals significant host-symbiont interactions.

There are significant host-symbiont interactions ($p < 0.0001$) for mortality when the data is analyzed using a full factorial 2-way ANOVA for all pairwise combinations between the two southern sites and the two northern sites.

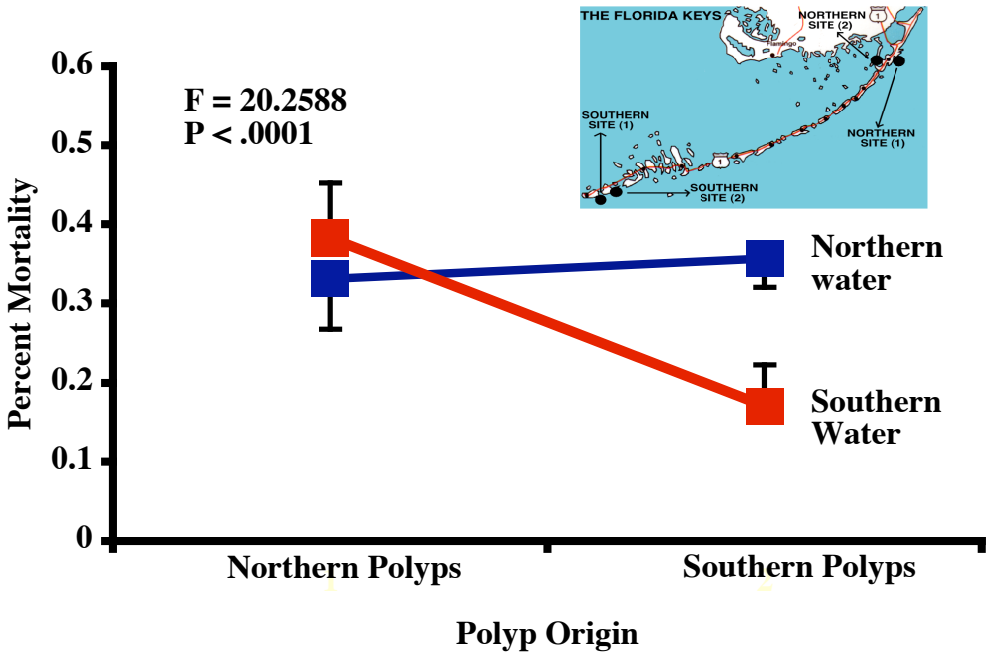


Figure 5.6: Significant host-symbiont interaction – south vs. north.

Significant host-symbiont interaction ($p < 0.0001$) for mortality when the data is grouped into southern and northern combinations and analyzed for all pairwise combinations. Top right corner: A map of the Florida Keys showing the two northern and the two southern collection sites.

PART 5: DISCUSSION

Overall, the results of study mimic the results from my previous studies (Chapter 3 and 4). There is intraspecific symbiont variation among the algal symbionts inhabiting the water across these collection sites. Moreover, the survival of host lineages was significantly altered when interacting with algae inhabiting the water collected from each site. The observed variation is dependent on the geographic origin of symbiotic partners. In other words, combinations in which partners originate in close geographic proximity (same-site or nearest-neighbor combinations) suffer less mortality than combinations whose partners originate from geographically distant sites. Finally, the structure of the variation appears to be driven by significant host-symbiont interaction effects; therefore, the four collection sites would appear to be very different selective environments for the symbiotic algae.

These results are consistent with the interpretation that *Cassiopea xamachana* are generally better adapted to local co-occurring algal mutualist than to algal isolates from distant-sites (chapter 4). The differential survivorship among distant-site combinations versus same-site and nearest-neighbor combinations indicates that the identity of the algal symbiont can strongly affect the intensity of selection for or against a particular host type. In other words, the results provide evidence of pronounced local adaptation of algal symbionts to *Cassiopea* hosts or vice versa. Additionally, it is possible that both *Cassiopea* hosts and their respective algal symbionts are coadapted to one another. However, since nearest neighbor and same-site combinations performed equally well, it is

likely that the algal symbionts and/or hosts migrate to some extent. This idea is explored further in chapter 6.

Further, the results presented here show that the algae isolated directly from female medusa appear to be representative of the algae normally acquired from the environment. The locally available algal symbionts at each site were not equally compatible with all polyp lineages used in this study. In fact, the observed variation in host-symbiont compatibility remained geographically structured. Thus, it seems plausible to suggest that both the algal symbionts and *Cassiopea* hosts have population structure and, as a result, not all symbiont genotypes are equally distributed across all sites. This makes intuitive sense in light of the pronounced host-symbiont interactions observed in this study as well as in the previous study.

The results obtained in this study have some implications regarding the adaptive bleaching hypothesis (Buddemeier and Fautin, 1993). This hypothesis suggests that bleaching is an adaptive response to changes in local environmental conditions, which allows invertebrate hosts to acquire novel symbionts best suited to prevailing environmental conditions. In other words, the host has the ability to switch symbionts when current symbionts have less than optimal or negative fitness effects. If this hypothesis were true, I would have expected *Cassiopea* polyps to bleach algal symbionts that were disadvantageous in an effort to acquire a new symbiotic partner. Instead, polyps that contained algal symbionts from foreign water sources died. Adaptive bleaching does not appear to be an option for theses polyp hosts. That is, *Cassiopea* polyps do not appear to be able to

prevent infection by symbionts that might kill them. Perhaps, in the future, a more rigorous test of this hypothesis would be informative.

Overall, the next step towards bridging the gaps in our understanding of the evolutionary dynamics of *Cassiopea*-algal symbioses should be examining the population genetic structure of both host and symbiont in order to quantify the observed variation in terms of genetics. In the following chapter I explore the use of ISSR molecular markers to quantify intraspecific genetic variation among the algal symbionts inhabiting *Cassiopea* hosts.

VARIATION IN HOST SYMBIONT COMPATIBILITY AMONG *CASSIOPEA*-ALGAL SYMBIOSES

Chapter 6: Population Genetic Structure of the Algal Symbionts inhabiting *Cassiopea xamachana*

PART 1: ABSTRACT

The studies described in this dissertation have revealed marked variation in host-symbiont compatibility among *Cassiopea*-algal symbioses in the Florida Keys. The observed variation depends on the geographic origin of symbiotic partners. It is possible that Cassiopea hosts are capable of housing one of several species of symbiotic algae. If so, this would explain the geographic variation. If, however, *Cassiopea* hosts a single specific symbiotic species, then localized population genetic structure in either hosts, symbionts, or both might explain variation in host-symbiont compatibility. I harvested algal isolates from *Cassiopea* medusa across four sites in the Florida Keys in order to investigate both species level differences and intraspecific symbiont variation. Species differences were assayed using RFLP analysis of the 5' end of the large subunit r-DNA gene. Intraspecific genetic variation and population genetic structure among algal symbionts were assessed using ISSR molecular markers. Results indicate that the algal symbionts inhabiting *Cassiopea* are members of a single species, *Symbiodinium microadriaticum*. ISSRs revealed significant genetic variation among isolated algae. Furthermore, significant population genetic structure was detected among the algal symbionts. These results indicate that local populations

of symbionts are at least partially genetically isolated from one another and this may in part explain the observed geographic variation in host-symbiont compatibility (Chapter 3 – 5).

PART 2: INTRODUCTION

The algal symbionts inhabiting marine invertebrates are not obviously different from each other. For this reason, symbionts from hard and soft corals, anemones, giant clams, and jellyfish were historically classified as a single pandemic species, *Symbiodinium microadriaticum* Freudenthal (Freudenthal, 1962; Trench and Blank, 1987). Since then, different *Symbiodinium* species have been described from morphological, biochemical, physiological, behavioral, and genetic evidence. It is now clear that symbiotic dinoflagellates are a large, heterogeneous complex of cryptic taxa (Blank and Trench, 1986; Rowan and Powers, 1991; Rowan *et al*., 1997; Wilcox, 1998). Because of their morphological uniformity and difficulties in culturing, traditional taxonomic approaches have only provided limited information on the biological diversity within the symbiotic dinoflagellates. Recently, molecular genetic methods have been applied to *Symbiodinium*-like zooxanthellae, obviating many of the difficulties previously associated with zooxanthellae identification and classification (Rowan *et al.*, 1997). For example, Wilcox (1997) examined generic and species relationships among 11 symbiotic dinoflagellate isolates using large-subunit ribosomal RNA (lsrRNA) gene sequences. His results indicated that morphological similarities among the taxa examined did not correspond with molecular phylogeny. Additionally, Rowan *et al*. (1997), using RFLPs of small-subunit ribosomal RNA

(ssrRNA) genes, identified three distantly related taxa of *Symbiodinium* within a single species of coral. Their results revealed that corals can harbor dynamic, multispecies communities of *Symbiodinium*. Because of these and other studies, the "one host-one symbiont" mentality, and the idea that *Symbiodinium microadriaticum* is the only symbiotic dinoflagellate found among different Cnidarian hosts, has been re-evaluated.

Today we know that symbiont taxonomic diversity associated with cnidarians is high with symbiotic algae comprising at least 5 or 6 clades. *Cassiopea xamachana* is believed to harbor only one symbiont, *Symbiodinium microadriaticum* (Fitt and Trench, 1981); however, no empirical studies have examined symbiont diversity extensively within this host species. Recent field investigations have revealed significant variation between same-site, nearest neighbor, and distant site jellyfish-algal combinations in terms of host mortality and growth (Chapter 3, 4, and 5). These investigations focused on host-symbiont interaction effects as a potential explanation for the observed variation. If, however, *Cassiopea xamachana* can harbor one of several possible *Symbiodinium*-like algal species, then an alternative explanation for the variation among jellyfish-algal combinations is the presence of different species of algae within *Cassiopea* hosts. In order to discuss with any certainty the evolutionary dynamics of the *Cassiopea*-algal associations, it is critical to determine the genetic structure of the algal symbionts within individual *Cassiopea* hosts, as well as within and between *Cassiopea* populations. Additionally, if *Cassiopea* houses a single symbiont species, *Symbiodinium microadriaticum*, then it is important to

evaluate intraspecific symbiont variation as a means of explaining the geographic structure associated with host-symbiont compatibility.

Intrapopulation genetic studies using DNA fingerprinting techniques such as random amplified polymorphic DNA (RAPD) (Alberto *et al.*, 1997; Coyer *et al.,* 1997, Lanham and Brennan, 1999), amplified fragment length polymorphisms (AFLP) (Lanham and Brennan, 1999), allozymes (Benzie *et al.*, 1997), and isozymes (Skov *et al.*, 1997) have been relatively successful in elucidating genotypic variation within a species, particularly in marine algae. For instance, Skov *et al.* (1997), found genetic variation among clones of the diatom *Psuedo-nitzschia pseudodelicatissima*; however, no correlation was found with geographic location. On the contrary, Coyer *et al.* (1997), revealed that geographic proximity in individuals of *Postelsia* (Phaeophyceae) did reflect genetic relatedness. Additionally, strong spatial differentiation was shown in populations of *Caulerpa* (Chlorophyta) species (Benzie *et al.*, 1997).

A relatively new molecular technique, called intersimple sequence repeats (ISSRs), has been developed to explore intrapopulational variation (Zietkiewicz *et al.*, 1994). Simple sequence repeats (SSRs or microsatellites) are short, hypervariable elements distributed throughout the genomes of eukaryotes (Abbot, 2001). This molecular marker technique involves using primers (derived from di- and trinucleotide repeats) complimentary to microsatellites to PCR-amplify regions between microsatellite loci, rather than providing information about variation in a particular microsatellite locus (Zietkiewicz *et al.*, 1994) (Figure 6.1). This results in anonymous, typically dominant, di-allelic Mendelian markers

when divergence in SSR sites or chromosomal structural rearrangements occur (Wolfe and Liston, 1998; Wolfe *et al.*, 1998). The resulting bands are electrophoresed in an agarose gel and viewed under UV light. The pattern of PCR products obtained can be considered a signature or fingerprint of the analyzed DNA template (Zietkiewic *et al.*, 1994). ISSR markers are attractive tools for disentangling genetic relatedness for several reasons: small amounts of DNA may be used, small reaction volumes and amounts of enzymes are needed for PCR, the hypervariability of banding patterns (2-100 bands per PCR sample), no specialized apparatus or kits required other than those needed for standard PCR techniques, and banding patterns are easily scorable (Wolfe *et al.*, 1998). A brief review of the literature reveals that ISSR markers exhibit higher levels of polymorphism than RAPD markers (bands/marker) while, at the same time, having greater reproducibility and being about as expensive (Nagaoka and Ogihara, 1997; Vis, 1999; Lanham and Brennan, 1999; McGregor *et al.*, 2000).

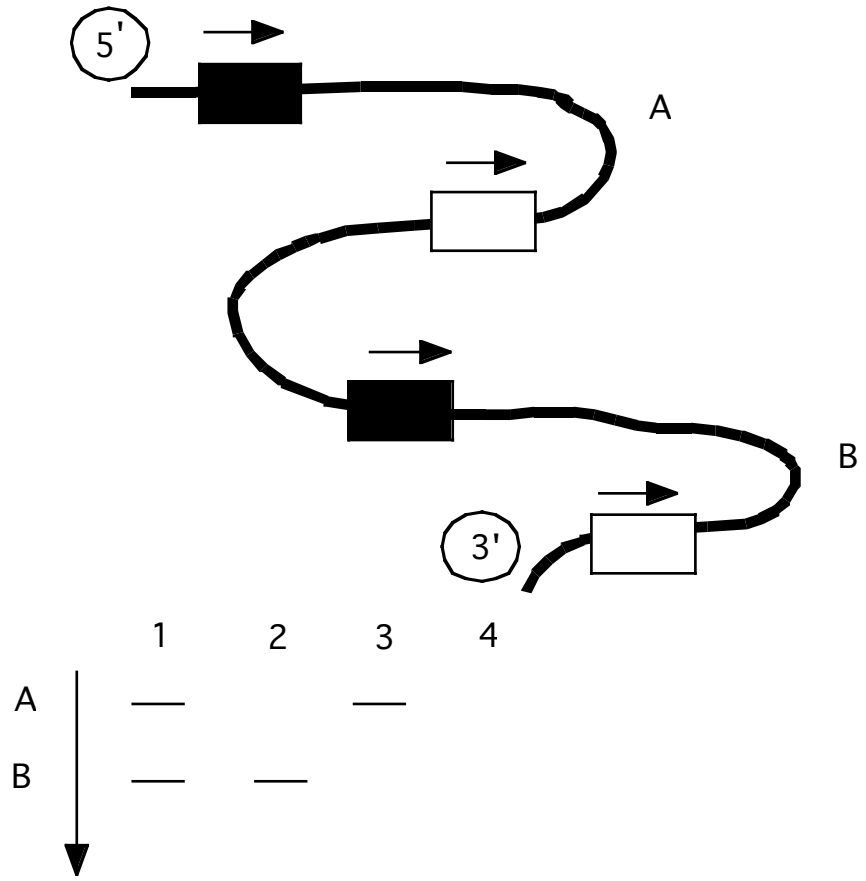


Figure 6.1: Schematic of ISSR primer annealing and the possible banding patterns generated.

A and B refer to intersimple sequence repeat regions that are amplified if primer sequences anchored on the 5^1 end of the microsatellite regions are intact. Solid boxes represent primer sequences oriented in the 5^1 direction and clear boxes represent primer sequences on the complimentary strand. If all primer sites are present, two bands will result (lane 1), if one or more primer sites are absent, one or both bands may be absent (lanes 2-4).

To date, many researchers have successfully quantified variation within a single species using ISSR markers, For example, Vis (1999), investigated genetic variability among gametophytes of *Batrachospermum boryanum,* and found the technique useful in distinguishing among individual gametophytes within a population. ISSR's have also been used with cultivated plants to elucidate genetic relationships among peas (Lu et al., 1996), maize (Taramino and Tingey, 1996), wheat (Devis *et al*., 1995; Nagaoka and Ogihara, 1997), blueberry cultivars (Levi and Rowland, 1997), *Penstemon* (Wolfe *et al*., 1998), mangroves (Ge and Sun, 1999), and potatoes (McGregor *et al*., 2000). Although ISSRs have been used by plant biologists for a variety of applications, only recently have they been used in animal population studies (Reddy *et al.,* 1999; Kostia *et al*., 2000; Abbot, 2001). Overall, ISSR markers are becoming a powerful technique for DNA fingerprinting and evaluating genetic polymorphisms within species.

In this study I used two molecular techniques (RFLP, ISSR) to explore species-level genetic differences as well as intraspecific genetic diversity among the algal symbionts inhabiting *Cassiopea xamachana* in the Florida Keys. More specifically, I use the data to address the following question: Are the algal symbionts found within *C. xamachana* members of the same species and, if so, are populations genetically structured along a geographic cline?

Restriction fragment length analysis of the 5' end of the lsRNA gene was used to determine if *C. xamachana* harbored a single or multiple species of symbionts. This technique was chosen because it has been shown to readily discriminate among closely related symbiotic dinoflagellates (Rowan and Powers,

1991). Initially, I attempted to use AFLP analysis to examine intraspecific genetic variation, as this technique can provide more information for a given effort than ISSRs or other PCR based fingerprinting techniques. However, this molecular technique did not work with the algal symbionts found within *Cassiopea* because *Eco*R1 did not cut the DNA (Figure 6.2), probably because of methylation and the presence of non-standard nucleotides in dinoflagellate genomes (Spector, 1984). Therefore, ISSR analyses were used to examine levels of intraspecific genetic variation. One typical drawback of ISSRs is that they are dominant markers, and thus may underestimate genetic variability (Lynch and Milligan 1992). However, *Symbiodinium* are normally haploid (Santos and Coffroth, 2003), thus obviating this difficulty.

PART 3: METHODS

Algae harvesting and maintenance

Symbiotic algae from Cassiopea xamachana were collected from twenty medusae at each of four sites in the Florida Keys (Figure 6.3). Algal isolates were collected by clipping a small portion of each hosts' tentacle (Chapter 2). Each tentacle was placed in ASW (artificial salt water) in a 50ml plastic centrifuge tube and transported to Keys Marine Laboratory, Florida Keys, USA. Each tentacle portion was then placed in a changing bowl and the host tissue containing the highest concentration of algal cells was removed. The infected host tissue was rinsed with a squirt bottle containing ASW (artificial seawater), placed in 25ml of ASW in a 50ml centrifuge tube, and ground using a tissue homogenizer. Homogenized samples were then centrifuged for 3 minutes at 3500rpm. The

supernatant was discarded and any remaining visible host tissue was removed from the pellets using a glass pasture pipette or water bottle containing ASW. The remaining pellet was resuspended in 20ml FSW (filtered seawater) and centrifuged again. This was repeated at least four times in order to reduce the amount of host tissue present in the pellet and to decrease the possibility of fungal contamination. The final algal pellet was resuspended in 10ml of FSW. Algae were then maintained in ASW for five days to allow any remaining host tissue to degrade, minimizing the potential for host DNA contamination in subsequent analyses (T.P. Wilcox, pers. com.). Each day samples were centrifuged and rinsed twice with 10ml of ASW. On the fifth day, 100μl of the algal suspension was plated onto 1% agar plates made with F/2 algal growth media. The remainder of the suspension was then centrifuged, and the resulting pellet frozen at –20°C for later analysis.

Plated algal cells were then allowed to grow by asexual division at room temperature (25ºC) under fluorescent lights (40" F40DX full spectrum bulbs) on a 14hr light: 10hr dark cycle at 25^{O}C. After two weeks, individual algal colonies, each consisting of a single, clonally replicated cell line, were selected and cultured in 5ml F/2 liquid algal media. This allowed for the acquisition of uniclonal algal strains for genetic assays. After 2 months, a portion of the liquid algal cultures were centrifuged and the resulting pellet frozen (-20^{0}C) for later analysis.

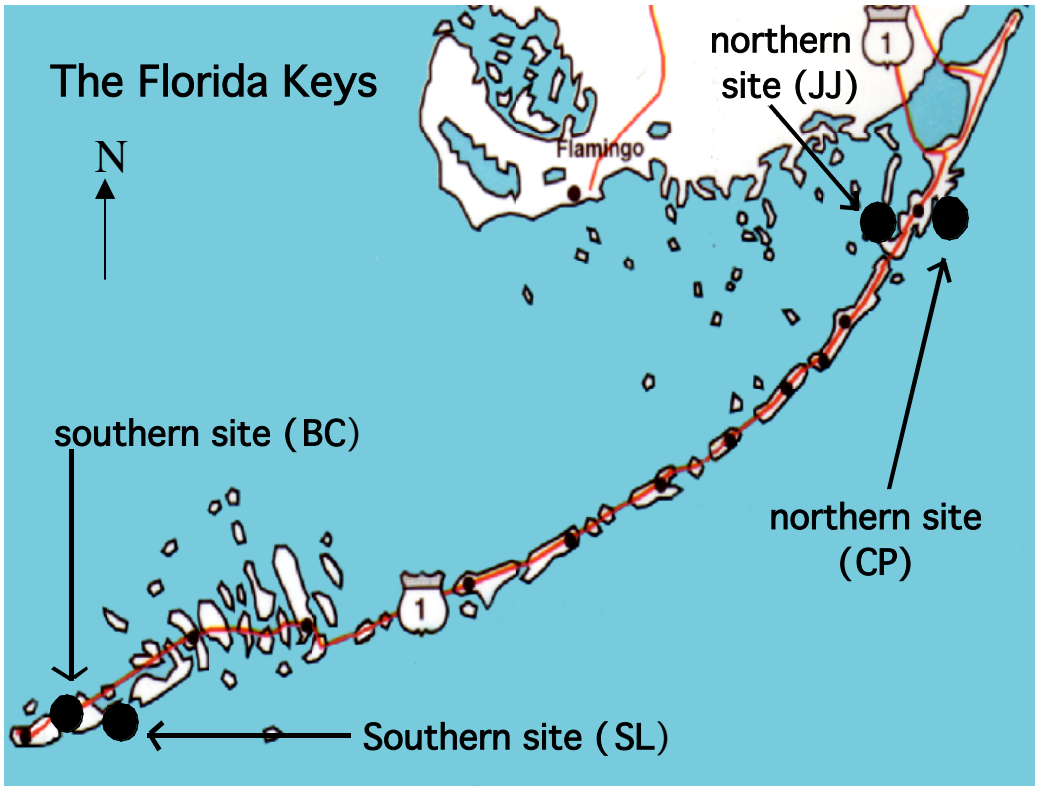


Figure 6.3: The four collection sites in the Florida Keys.

Two northern sites and two southern sites separated by ~160km. Algal isolates were collected from twenty similar-sized male and female *Cassiopea* medusae at each of four sites – two northern sites (JJ and CP) and two southern sites (SL and BC).

DNA Extraction

The algal isolates were thawed and ground in 1.5ml microcentrifuge tubes in 200μl of 2XCTAB + B-ME and 10mg/ml of Proteinase K. The samples were then incubated at 55^{0}C for 2 hours or until the algal cells were lysed. DNA was then extracted using the DNeasy kit (Qiagen, Inc.) following the manufacturers protocol. DNA extractions were then quantified by running 10μl of each sample on a 1% agarose gel. Gels were stained with EtBr and visualized on a UV transiluminator. DNA concentration was estimated by comparison with DNA standards of known concentration run concurrently with the samples.

RFLP Analysis of the lsRNA gene

The 5' end of the lsRNA gene was amplified using the primers ls1.5 (5' – CGCTGAATTTAAGCATATAAGTAAG - 3') and 1.3 (5' – AACGATTTGCACGTCAGTATC- 3') (Wilcox 1997, 1998). Reaction volume was 25μl consisting of 1-7μl of DNA depending on the quantity of DNA extracted, 1mM each of dNTP (20mM each of dATP, dTTP, dGTP, and dCTP), 10μM primer (Ls 1.5 and Ls 1.3), 25mM $MaCl_2$, and 1X reaction buffer with 1 unit Taq. PCR amplification was performed in a thermocycler as follows: initial denaturation 94^{0}C for 2 minutes; 35 cycles of 94^{0}C for 30 seconds, 52^{0}C for 30 seconds, 72^{0}C for 1 minute; and a final extension at 72^{0}C for 7 minutes followed by a 6^{0}C soak. Amplification products were then checked by running 5μl of each reaction on ethidium bromide-stained, .8% agarose gels. The gels were electrophoresed for 15-30 minutes at 105V. Gels were then visualized on a UV transiluminator and product size estimated using a Hind III ladder size standard.

In addition to the algal isolates, ls1.5-1.3 was amplified from genomic DNA isolated from cultures of three known *Symbiodinium* species, *S. microadriaticum* (the putative symbiont of *C. xamachana*), *S. kawagutii* (from *M. verrucosa*), and *S. bermudense* (from *A. pallida*) 5μl of each PCR reaction were used in restriction digests with TaqI and Hha. For each digest, 10 units of enzyme were added directly to 10μl of PCR product. Samples used in the TaqI restriction digest were incubated in the thermocycler at 72^0C for two hours while Hha restriction digests were incubated at 37^0C for 4-6 hours. The samples were characterized on ethidium bromide-stained 3% gels (1% low-melt agarose: 2% standard agarose) in 1 X TBE. The gels were electrophoresed for 2.5 hours at 80V. Gels were viewed under UV light, and the images were recorded using a digital camera. Fragment sizes were estimated using a Hind III ladder size standard.

ISSR Analysis

Ten primers were tested with three to four algal isolates to determine the suitability of primers for the entire population (Table 6.1). ISSR amplification was performed in 25μl reactions consisting of 1-7μl of DNA, depending on the quantity of DNA extracted, 1mM each of dNTP (250μM each of dATP, dTTP, dGTP, and dCTP), 10μM of a single primer, 25mM $MaCl_2$, and 1X reaction buffer with 1 unit *Taq* DNA polymerase. Amplifications were performed in a thermocycler as follows: initial denaturation 94^0C for 1.5 minutes; 35 cycles of 94^0C for 45 seconds, 52^0C for 45 seconds, 72^0C for 1.5 minutes; and a final extension at 72^0C for 7 minutes followed by a 6^0C soak. Replicate reactions for each isolate (fresh and cultured) were done with each primer to evaluate the

reproducibility of the results among PCR reactions. PCR reactions were electrophoresed on ethidium bromide-stained 1.5% agarose gels in 1 X TBE buffer. The gels were electrophoresed for 3 hours at 80V. Fragment sizes were estimated against a 1-kb ladder size standard. Gels were viewed under UV light, and the images were recorded using a digital camera. From the gel image the size of each band was determined, and its presence in a sample recorded, using Gel Reader (v1.1, Wilcox, unpubl. software). Bands were scored as present (1) or absent (0).

Data analysis

The index proposed by Nei and Li (1979) was used to calculate genetic similarities among algal isolates ($S_{ij} = 2N_{ij}/(N_i + N_j)$ where N_{ij} = the number of bands in common between algal isolates i and j, N_i and N_j are the number of bands for algal isolates i and j respectively). The similarity index was calculated using FragerX (v1.0, Wilcox, unpubl. Software).

The population genetic structure among algal isolates from each of the four collection sites was examined using genetic distances in an analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992). The genetic distances were calculated using a Euclidean distance metric: $\partial^2_{jk} = (p_j - p_k)'W(p_j - p_k)$, where W is a matrix of differential weights for the various sites, p_i is a vector of allele presence/absence scores for individual i, and p_j is the equivalent vector for individual j. In this study, W is equal to I, the identity matrix, because all sites are assumed to be independent and equally informative. This metric is commonly employed to estimate population differences (Nei and Tajima, 1981). The degree

of genetic separation among each population was examined by calculating pairwise Φ_{st} values. If Φ_{st} values are significantly different from zero, then the populations are considered genetically isolated. To determine if pairwise Φ_{st} values were significantly different from zero, the expected null distribution of Φ_{st}, assuming no population structure, was estimated by permutation analysis. One thousand permutations of the observed distance matrix were performed to generate the null distribution. These calculations were performed in Arlequin (v2.001, Schneider *et al*., 2000).

Additionally, to visualize any apparent population structure, a minimum spanning tree (MST) was generated from the genetic distance matrix (Excoffier *et al*., 1992; Rohlf, 1973; Prim 1957). An MST is an open network, where no hypothetical nodes are assumed, that minimizes the total number of steps necessary to connect all individual genotypes. Thus, it is equivalent to a Wagner parsimony tree with known internal nodes. The minimum spanning tree was calculated using a modification of a clustering algorithm created by Rohlf (1973) and implemented in Arlequin (v2.001, Schneider *et al*., 2000). Rohlf's algorithm is guaranteed to find the MST for a given set of distances (Rohlf 1973).

Primer number:	Primer Sequence:
3	$(CA)_7GG$
5*	$(CA)_6RG$
8	$(CT)_8TG$
9*	$(CT)_8RG$
13	$(GA)_6CC$
17*	$(GT)_6GG$
19	$(GT)_7TC$
20	$(GT)_7CGA$
21	$(GT)_6YR$
23	$(GT)_6RG$

Table 6.1: ISSR primers and primer sequences tested for amplification of bands in each algal isolate.

*denotes a primer used to obtain data for analysis.

PART 4: RESULTS

Results from the RFLP analysis of the lsrRNA gene indicate that there are no species level differences among the algal isolates used in this study. The algal symbionts inhabiting *Cassiopea xamachana* hosts are members of a single dinoflagellate species, *Symbiodinium microadriaticum* (Figure 6.4). All of the primers used for the ISSRs produced amplification products from the test individuals with the exception of four primers (Table 6.2). Because these PCR reactions did not produce bands in the tested individuals, it is most likely that these particular microsatellite loci were not present in the genome or were not in close enough proximity to each other to be amplified by this technique (Vis, 1999). The number of distinct bands for each primer ranged from 2-25 and the bands amplified per individual ranged from 0-12 (Table 6.2).

From the ten tested primers, I chose primers 5, 9, and 17 to study the genetic variation among the algal isolates. These primers were chosen because they produced clear, consistent banding patterns within individual isolates and were polymorphic among isolates (Figure 6.5). Additionally, replicate PCR amplifications of each individual produced the same banding patterns, demonstrating reproducibility of results. These three primers produced a total of 66 distinct fragments among the 28 algal isolates (Table 6.2). Fragments ranged in size from 181bp (primer 17) to 2376bp (primer 5). The percentage of polymorphic bands ranged from 74 to 96 (Table 6.2). There were 8 bands found in all algal isolates and three additional bands were nearly monomorphic across all taxa. In many cases the cultured algal isolates and the freshly isolated algae

had similar and often identical banding patterns (Figure 6.6a and b). Percent similarity ranged from 24% to 100% with most pairwise comparisons being between 60% to 80% (Figure 6.6a and b). Overall, algae collected from medusa within a site were more closely related (Table 6.3; $p < 0.001$; $F = 3.49$). Results from AMOVA revealed significant differences among the algae collected from each of the four sites/populations with the exception of CP isolates (northern site) and SL isolates (Table 6.4). This result suggests that there are at least three distinct genetic populations (Figure 6.7 and 8). The 27 algal isolates generally cluster by site (Figure 6.7 and 8); however, they do not cluster by geographic location (i.e. north or south).

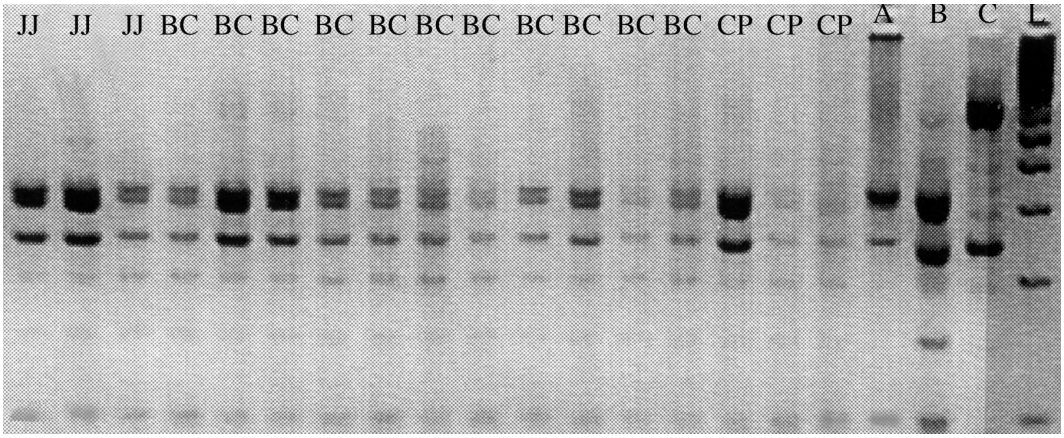


Figure 6.4: RFLP example gel.

RFLP analysis of the lsRNA gene using *Taq*I for the restriction digest. The algae collected from *Cassiopea* medusa are members of the same species, *Symbiodinium microadriaticum*. JJ and CP – northern sites; SL (not shown here) and BC– southern sites; Positive controls: A – *S. microadriaticum*; B – *S. bermudense*; C - *S. kawagutii*; L - Hind III ladder.

Primer number:	Primer Sequence 51→ 31:	Amplifica-tion:	Total no. of bands:	No. of bands Amplified/ isolate	Percent polymorph-ism
3	(CA)7GG	Yes	21	0-1	NA
5*	(CA)6RG	Yes	242	2-14	96%
8	(CT)8TG	No	-	-	NA
9*	(CT)8RG	Yes	192	6-12	95%
13	(GA)6CC	Yes	241	5-12	NA
17*	(GT)6GG	Yes	232	7-18	74%
19	(GT)7TC	No	-	-	NA
20	(GT)7CGA	Yes	251	6-10	NA
21	(GT)6YR	No	-	-	NA
23	(GT)6RG	No	-	-	NA

Table 6.2: Primer table.

Summary of ISSR primers, success at amplification, total number of bands scored, and number of bands scored per algal isolate.

*denotes a primer used to obtain data for analysis

[1] Number of bands based on three isolates tested in preliminary primer screening.

[2] Number of bands based on 15 algal isolates (two replicates/isolate)

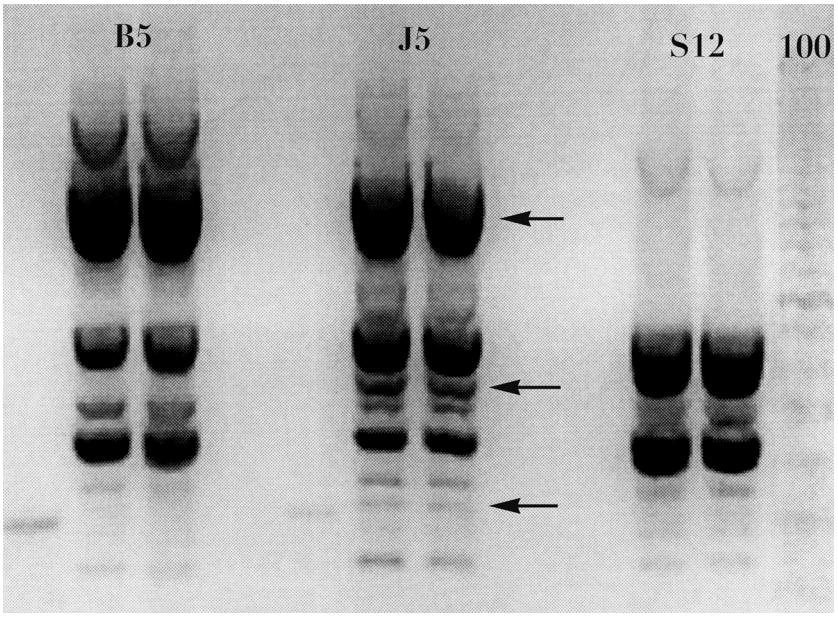


Figure 6.5: Example ISSR gel.

A portion of an ethidium bromide-stained 1.5% agarose gel and the ISSR banding pattern produced from PCR amplification with primer 5. Arrows illustrate intraspecific variation between three algal isolates, B5, J5, and S12 (two replicates per isolate). 100 – is 100kb ladder.

	B3	B3C	B5	BJC	B11	B11C	B15	B15C	C2C	C11	C11C	C14	C14C	C16
B3	1.00	0.74	0.67	0.70	0.58	0.61	0.49	0.58	0.54	0.62	0.57	0.57	0.51	0.54
B3C		1.00	0.76	0.79	0.60	0.67	0.51	0.64	0.53	0.70	0.65	0.52	0.56	0.56
B5			1.00	0.79	0.65	0.76	0.65	0.82	0.55	0.63	0.64	0.54	0.51	0.61
BJC				1.00	0.55	0.65	0.53	0.67	0.47	0.63	0.58	0.50	0.51	0.51
B11					1.00	0.70	0.63	0.64	0.67	0.63	0.62	0.67	0.59	0.69
B11C						1.00	0.71	0.72	0.67	0.70	0.71	0.59	0.67	0.69
B15							1.00	0.77	0.63	0.56	0.64	0.76	0.63	0.69
B15C								1.00	0.64	0.64	0.66	0.68	0.60	0.67
C2C									1.00	0.76	0.71	0.78	0.67	0.76
C11										1.00	0.92	0.73	0.70	0.78
C11C											1.00	0.74	0.74	0.81
C14												1.00	0.71	0.84
C14C													1.00	0.72
C16														1.00

Figure 6.6a: Similarity matrix for the 28 algal isolates used in this study.

Specimen abbreviations = site (S and B i.e. northern sites or J and C i.e. southern sites); number = individual isolates; C at the end of the abbreviation = cultured isolates. BJC = cultured isolates B5 and J17 were identical so they were combined in the matrix. Matrix is continued on the next page. Percent similarity ranged from 24% to 100% with most pairwise comparisons being between 60% to 80%.

	C16C	J5	J5C	J10	J10C	J16	J16C	J17	S12	S12C	S17	S17C	S20	S20C
B3	0.63	0.64	0.75	0.75	0.73	0.82	0.71	0.55	0.56	0.60	0.61	0.24	0.67	0.66
B3C	0.68	0.60	0.58	0.71	0.60	0.82	0.67	0.49	0.58	0.51	0.69	0.30	0.72	0.68
B5	0.63	0.65	0.56	0.67	0.55	0.66	0.58	0.47	0.63	0.53	0.65	0.42	0.68	0.63
B5CJ17C	0.63	0.65	0.60	0.67	0.51	0.78	0.62	0.47	0.53	0.57	0.62	0.26	0.65	0.60
B11	0.64	0.48	0.54	0.58	0.52	0.51	0.51	0.57	0.73	0.58	0.75	0.38	0.72	0.68
B11C	0.71	0.56	0.50	0.68	0.52	0.57	0.59	0.43	0.70	0.50	0.72	0.43	0.69	0.75
B15	0.64	0.67	0.60	0.57	0.64	0.46	0.58	0.44	0.56	0.57	0.66	0.58	0.58	0.60
B15C	0.73	0.64	0.50	0.62	0.57	0.58	0.60	0.53	0.64	0.50	0.60	0.55	0.70	0.65
C2C	0.81	0.59	0.61	0.58	0.64	0.51	0.63	0.52	0.67	0.63	0.63	0.38	0.75	0.71
C11	0.79	0.60	0.55	0.65	0.54	0.67	0.60	0.55	0.69	0.53	0.74	0.35	0.86	0.85
C11C	0.80	0.65	0.57	0.69	0.52	0.65	0.58	0.53	0.76	0.54	0.77	0.38	0.81	0.86
C14	0.79	0.67	0.68	0.61	0.60	0.53	0.63	0.67	0.70	0.58	0.66	0.47	0.76	0.68
C14C	0.75	0.56	0.54	0.64	0.52	0.54	0.59	0.52	0.67	0.54	0.66	0.32	0.69	0.75
C16	0.86	0.66	0.60	0.67	0.56	0.54	0.58	0.61	0.81	0.62	0.79	0.39	0.86	0.79
C16C	1.00	0.68	0.62	0.69	0.58	0.65	0.68	0.60	0.79	0.64	0.75	0.38	0.85	0.81
J5		1.00	0.82	0.71	0.68	0.70	0.78	0.57	0.54	0.71	0.63	0.32	0.59	0.61
J5C			1.00	0.75	0.77	0.74	0.72	0.55	0.55	0.76	0.61	0.31	0.60	0.62
J10				1.00	0.76	0.79	0.79	0.55	0.71	0.72	0.70	0.33	0.70	0.75
J10C					1.00	0.68	0.77	0.53	0.47	0.77	0.57	0.30	0.56	0.58
J16						1.00	0.70	0.51	0.58	0.60	0.63	0.26	0.66	0.68
J16C							1.00	0.51	0.50	0.71	0.59	0.35	0.62	0.64
J17								1.00	0.55	0.56	0.58	0.08	0.57	0.55
S12									1.00	0.56	0.74	0.39	0.77	0.79
S12C										1.00	0.62	0.26	0.58	0.64
S17											1.00	0.34	0.79	0.75
S17C												1.00	0.36	0.38
S20													1.00	0.85
S20C														1.00

Figure 6.6b: Similarity matrix continued.

See figure legend on previous page for a description of the sample abbreviations.

Source of variation	d.f.	Sum of squares	Variance component	% of variation
Among population	3	70.359	2.19113	22.3
Within population	25	190.917	7.63667	77.70
Total	28	261.276	9.82779	100

Table 6.3: ISSR AMOVA results

Site	CP	JJ	BC	SL
CP	0.00	**	**	NS
JJ	.38	0.00	**	**
BC	.28	.16	0.00	*
SL	.06	.23	.13	0.00

Table 6.4: Φst results - Comparison of pairs of sites:

CP and JJ are northern sites and SL and BC are southern sites.

* significant Φst values. Significance level = 0.01

** significant Φst values. Significance level = 0.05

NS = not significant

Figure 6.7: MST.

Minimum spanning tree (below) was generated using the genetic distances calculated for each algal isolate. C=CP and J=JJ, the two northern sites. S=SL and B=BC, the two northern sites. The numbers denote the different algal isolates and the C at the end of the abbreviation denotes a cultured isolate. Algal isolates generally cluster by site as indicated by circles (purple circle = CP; green circle = JJ; yellow circle = SL; and red circle = BC). There was no evidence of clustering by geographic location (i.e. north or south). Arrows indicate algal isolates that do not cluster with their respective populations and numbers on branches are the number of mutational steps between each genotype/node.

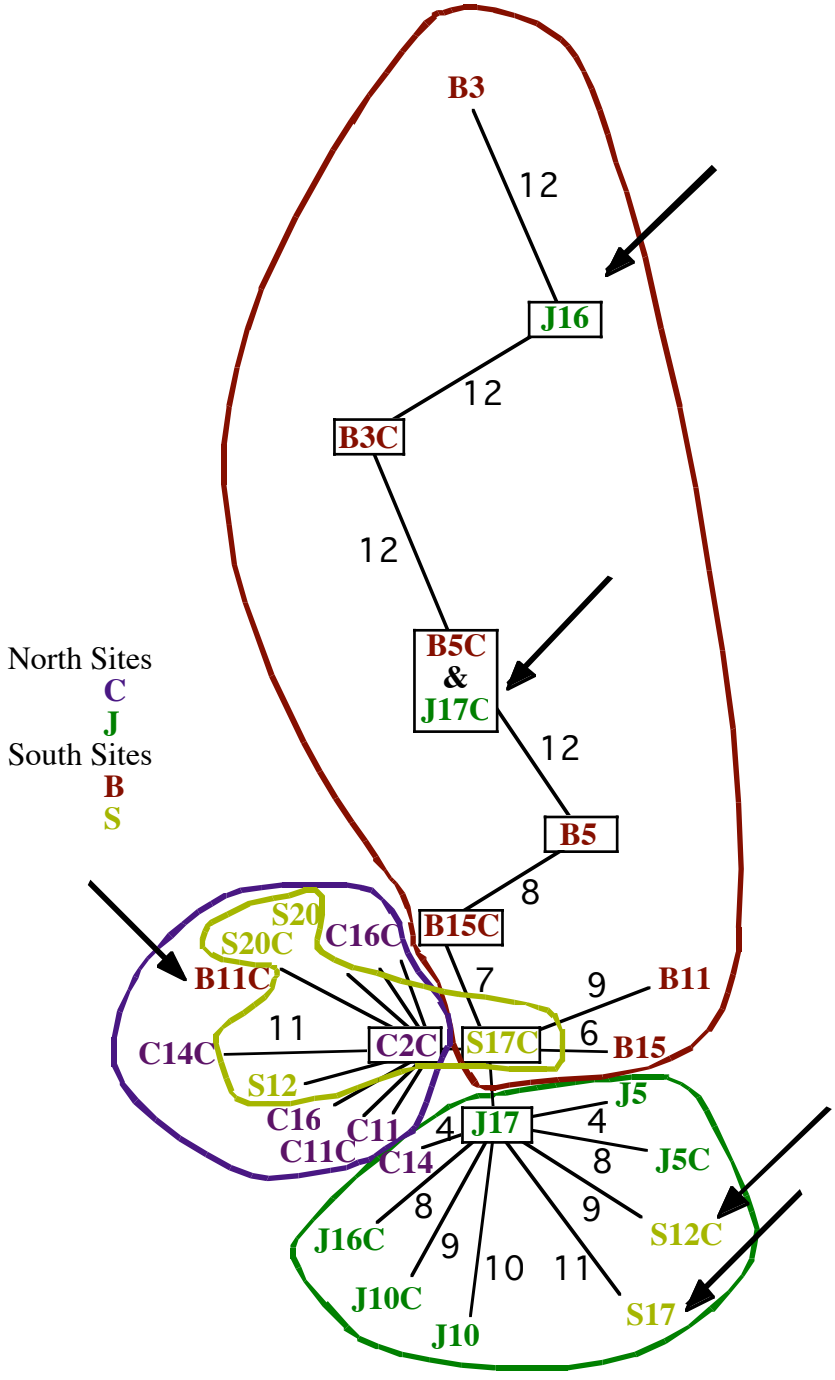
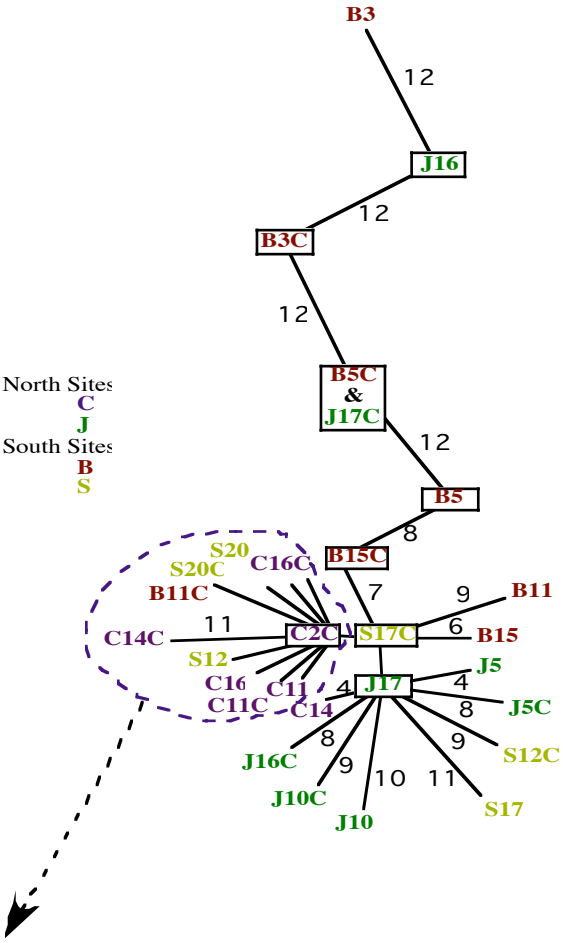

North Sites
C
J
South Sites
B
S
B3
12
J16
12
B3C
12
B5C
&
J17C
12
B5
8
B15C
7
9
B11
6
B15
S20
S20C
C16C
B11C
11
C14C
C2C
S17C
S12
C16
C11
C11C
C14
4
J17
J5
4
8
J5C
9
S12C
8
J16C
9
10
11
J10C
J10
S17

Figure 6.8: Enlarged portion of MST from Figure 6.7.

Minimum spanning tree (below) was generated using the genetic distances calculated for each algal isolate. C=CP and J=JJ, the two northern sites. S=SL and B=BC, the two northern sites. The colored numbers denote the different algal isolates and the C at the end of the abbreviation denotes a cultured isolate. The numbers on branches are the number of mutational steps between each genotype/node.


B3
12
J16
12
B3C
12
B5C
&
J17C
12
B5
8
B15C
7
9
B11
6
B15
J5
4
J5C
8
S12C
9
S17
11
10
J10
9
J10C
8
J16C
4
J17
4
C14
8
C11
C11C
C16
S12
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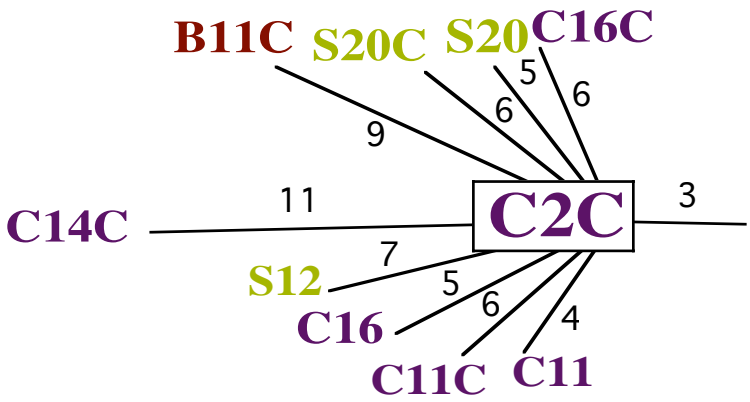
C14C
C2C
S17C
S20
S20C
C16C
B11C
North Sites
C
J
South Sites
B
S


B11C
S20C
S20
C16C
5
6
6
9
11
C14C
C2C
3
7
S12
5
6
C16
4
C11C
C11

PART 5: DISCUSSION

Summary of results

Many questions about variation in *Cassiopea*-algal compatibility have remained unanswered in part because of the unresolved nature of zooxanthellae diversity. In this study I used two molecular techniques (RFLPs, ISSRs) to explore species-level genetic differences as well as intraspecies polymorphisms among the algal symbionts inhabiting *Cassiopea xamachana* in the Florida Keys. Data generated by the RFLP markers indicate that there are no species-level differences among the algal isolates used in this study. The algal symbionts inhabiting *C. xamachana* are members of a single dinoflagellate species, *Symbiodinium microadriaticum*.

The population genetic structure revealed by ISSR markers suggests that there is a great deal of diversity among the algal symbionts within *Cassiopea* hosts. The banding patterns generated by ISSR markers were clear and readily scored and replicate PCR amplifications of each individual produced the same banding patterns. In many cases the cultured algal isolates and the freshly isolated algae had similar if not identical banding patterns, though this was not always the case (Santos *et al*., 2001). Further, the analysis of molecular variance (AMOVA) revealed that there was significant population genetic structure in the in the algal symbionts. Examination of Φ_{st} indicates the presence of at least three distinguishable algal populations. That is, these algal isolates are not part of a single, panmictic population; however, they share some degree of gene flow, as evidenced by the five isolates that do not cluster with their respective populations.

Overall, the current study has demonstrated that ISSR molecular markers are useful in distinguishing genetic relatedness among algal isolates collected from these four sites.

Significance of symbiont genetic diversity

There appears to be considerable genetic variation among the algal isolates inhabiting *Cassiopea* medusae. The level of polymorphism (74-96%) detected in this study is high; though within the range seen in other studies. For example, Wolfe *et al.*, (1998) found 72-95% polymorphic bands among *Penstemon*, and McGregor et al., (2000) detected 78.8% polymorphic bands among the potato, *Solanum tuberosum*. Finally, Davis *et al.*, (1999) and Schlueter *et al.*, (1998) found high levels of diversity among the seagrass *Thalassia testudium* in the Florida Keys.

The amount of genetic diversity observed among the algal isolates indicates possible recombination through sexual reproduction. Despite inconsistent reports of sexual reproduction in *Symbiodinium* (Freudenthal, 1962; Taylor, 1974), and lack of sexual reproduction in this genus (Schoenberg and Trench, 1980; Rowan and Powers, 1991a, Trench, 1997), sexual processes have been reported for other dinoflagellates (Pfiester and Anderson, 1987). The possibility that sexual reproduction occurs in *Symbiodinium microadriaticum* symbionts seems likely when the ISSR data are considered. Sexual reproduction might afford symbionts the opportunity to adapt to local hosts at a faster rate then would be expected if they were restricted to clonal reproduction (Bell, 1982; Bell and Maynard-Smith, 1987; West *et al.*, 1999). However, unequivocal evidence for

sexual reproduction will require direct observation of recombinants, which is as yet an elusive goal.

Significance of population genetic structure

The data collected during this study did reveal significant population genetic structure across the four collection sites. One reasonable explanation for these results is that migration rates of symbionts are low among the sites studied (Hartl and Clark, 1999). Low migration rates can result from limited dispersal capabilities and/or significant barriers to migration (Larson *et al.*, 1984). It seems reasonable that symbiotic dinoflagellates would be unlikely to actively migrate the 160km that separate the northern and southern study sites. Furthermore, prevailing currents around the Florida Keys move water from Florida Bay out through the keys to the Atlantic, with locally entrained flows primarily moving south and west along the islands. Thus, passive migration between northern and southern sites via currents, though possible, is probably rare. However, migration of the hosts would also mean migration of their *in hospite* symbionts. If symbiont migration is low, then host migration must also be low. If low migration is largely responsible for the population genetic structure seen among these symbiont populations, then future studies on host population genetic structure should parallel the results for the symbionts.

However, the genetic data do not exhibit the same marked division between northern and southern sites as found in the analysis of host-symbiont compatibility (Chapter 4 and 5). This may be due to low sample sizes or that the use of only three primers might be insufficient to detect a conspicuous division

between northern and southern sites. It is also possible that low migration rates do not completely explain the population genetic structure observed for the symbionts.

A second possibility for the observed population genetic structure is related to the fact that the Florida Keys represents the northern extent of the *Cassiopea xamachana* range. The populations used in these studies could have an evolutionary history molded by unusual edge effects and frequent bottlenecks as *Cassiopea* attempted to establish themselves at the edge of their range in populations that are regularly subjected to extinction and re-colonization. If only one or a few jellyfish colonized a location, then established populations will likely have high among-population genetic variation (Hartl and Clark 1999). If populations undergo frequent extinctions and re-colonization, interpopulation genetic equilibrium will rarely be reached.

A third possibility is that the population genetic structure is being driven by selection for host-symbiont compatibility if the success of a symbiont genotype is dictated by its compatibility with the most common local host genotype. It is possible that this is a simple process of independent local adaptation of symbionts to *Cassiopea* hosts at each site. This is intuitively plausible since the algal symbionts have greater evolutionary potential than their *Cassiopea* hosts. The symbiotic algae have shorter generation times than their hosts, and this, together with high reproductive rates, will increase rates of evolution (Hafner *et al.*, 1994) and thereby the turnover of novel adapted symbiont variants. Thus, the symbionts might have evolved to specialize on the

most common host genotype(s) within its own population; thus, reducing its mean performance on hosts from foreign sites. Alternatively, intraspecific symbiont variation may arise as an unexpected outcome of geographic divergence caused by drift or selective agents unrelated to the mutualism. Organisms may track changes among mutualist partners in their own local habitat, but there is no selection to adapt to changes that take place in among partners in distant areas. Given an extended period of geographical isolation, symbionts in one population may lose traits necessary for successful symbioses with partners from other Caribbean sites.

It is fairly clear that these algal isolates are capable of migrating to some extent as evidenced by the five isolates that did not cluster with their respective populations. However, in larger populations very small rates of natural selection may overcome the effect of immigration (Wehrhahn and Powell, 1987), and local adaptation is possible if habitats have remained stable long enough (where long enough is relative to generation time). Given that generation times of the symbionts are on the order of weeks and days, and the study populations have been stable over the entire study period (6 years), it seems likely that adaptation to their local hosts could play an important role in maintaining population structure in the symbionts.

Allopatric invaders

It is also worth noting that some algal genotypes (5 of 28) do not cluster with their respective populations. For instance, algal isolates collected from CP and SL were not genetically distinct from one another, perhaps suggesting gene

flow or dispersal between these two sites. Although CP is a northern site and SL is a southern site, both are oceanside collection sites. This is perhaps evidence for oceanic mixing whereby gene flow dilutes local adaptation. On the other hand, the lack of population structure observed between the isolates from these two sites could be indicative of a recent colonization of one or both of these sites. Additionally, of all the sites I have collected from over the last six years, SL is the most variable in terms of population density. That is, the jellyfish are often completely absent from this site, especially after strong storms (pers. obs.). Since hurricanes and strong storms often sweep the Florida Keys, extinction and re-colonization events are likely to occur frequently at locations more susceptible to storm surges, maintaining low levels of genetic diversity between some sites.

Conversely, JJ and BC are fairly isolated, well-protected bayside sites; thus, the placement of several JJ and BC isolates outside their respective population clusters is interesting yet, more difficult to interpret. Migration between these sites might occur in a stepping stone fashion across intermediate sites characterized by heterogeneous symbiont populations. Most biological systems are characterized by the uneven distribution of individuals into a series of populations that show varying degrees of connectedness i.e. the metapopulation (Levins, 1969, 1970; Hanski and Gilpin, 1991, 1997). Thus, it would be premature to assume that all *Cassiopea*-algal populations exhibit the same degree of population genetic structure. Processes such as gene flow, genetic drift, and various forms of natural selection within and among individual populations is likely transitory and ephemeral. Long-term monitoring of *Cassiopea*-algal

compatibility among these four sites will enhance our understanding of the spatial and temporal patterns associated with this system. Future studies should also focus on including more intermediate sites in the Florida Keys.

Conclusions

It would appear that *Cassiopea* tends to co-occur with specific algal genotypes that confer particularly high mutualistic benefits. Partners in a mutualism can impose strong selection pressures on each other (Thompson, 1982, 1994; Schemske, 1983; Wilkinson *et al.*, 1996). Therefore, geographic variation in one species may lead to parallel population differentiation in its mutualist partner, because selection should favor compatibility between co-occurring genotypes rather than between partners from allopatric or foreign sites. Two predictions follow from this argument: 1) mutualistic benefits should be higher in symbioses between genotypes that co-occur at the same site (same-site combinations) and, 2) genetically similar hosts of a species ought to show higher compatibility with each other's symbiont (maternal combinations). Previous studies (Chapter 3) have revealed marked variation in host-symbiont compatibility among *Cassiopea*-algal symbioses as well as significant host-symbiont interactions among four sites (Chapter 4 and 5). Maternal combinations and same-site combinations survive more often and grow more than distant site combinations. Results from the current study help to explain, in part, the significant *Cassiopea*-algal interactions described in the previous chapters. It is likely that there is a relationship between geographic differentiation in *Cassiopea* and geographic differentiation in the symbiotic algae. Coevolutionary host-

symbiont interactions may be responsible for the spatial genetic differentiation of the algal populations; however, the population genetic structure of *Cassiopea* remains unresolved.

Evolutionary research to date has only begun to consider the extent of intraspecific specialization within mutualistic associations in nature. It has been argued that narrow specialization should be rare in mutualisms due to ecological and selective processes unique to this type of association (Schemske, 1983; Howe, 1984; Law, 1985; Thompson, 1994). Studies of legumes and *Rhizobiaceae* have been cited as evidence supporting this conclusion (Law, 1985). The results presented here suggest that intraspecific specialization in *Cassiopea*-algal mutualisms may be as common as in other, antagonistic interactions (Lively, 1999; Ebert, 1994; Alexander, 1989, 1990). However, it is difficult to predict whether the results obtained in this study will be representative of outcomes in other mutualistic associations.

VARIATION IN HOST SYMBIONT COMPATIBILITY AMONG *CASSIOPEA*-ALGAL SYMBIOSES

Chapter 7: Conclusions

PART 1: RESEARCH SYSTEM AND OBJECTIVES

The *Cassiopea*-algal symbiosis is an excellent model system for examining a variety of evolutionary and ecological questions. First, it consists of relatively long-lived hosts with stable symbiont populations that are acquired by horizontal transmission every generation. This creates the potential for spatially heterogeneous selection if the genetic composition of partner populations differs. Second, it is likely that the symbiont spends most if not all of its lifetime within the tissues of the host and symbiont reproduction within the host has a direct effect on host fitness. Third, thousands of larvae can be collected readily from *Cassiopea* medusa and maintained as algal-free polyps indefinitely. The algae are equally easy to acquire from *Cassiopea* medusa and can be used immediately in infection experiments or cultured for later use. Fourth, *Cassiopea*-algal symbioses are ubiquitous along 160km of coastline in the Florida Keys and multiple populations are readily accessible. Finally, varying levels of interaction among *Cassiopea*-algal combinations can be assessed in terms of symbiont reproduction (mitotic index within the host), host growth, and host longevity.

This dissertation broadly addresses variation in host-symbiont compatibility (chapter 3) but, more specifically, how variation is structured and

maintained (chapters 4-6) and, most importantly, the evolutionary and ecological implications of the observed variation. I have developed and used the *Cassiopea*-algal complex to examine the following general questions: 1) Are enodsymbionts equally benevolent across a single host species? 2) does geography play a role in structuring variation in host-symbiont compatibility? and, 3) does intraspecific symbiont variation drive host-symbiont interaction effects, thus dictating the symbiotic outcome?

PART 2: OVERVIEW OF EXPERIMENTAL RESULTS

Overall, there is intraspecific symbiont variation, a fundamental variable for evolutionary adaptation, among the algal symbionts inhabiting *Cassiopea xamachana* hosts. It can no longer be assumed that all symbionts are equally benevolent across the same host species. In the studies presented in this dissertation, the growth and survival of host lineages was significantly altered when interacting with different algal isolates. The observed variation in host-symbiont compatibility dependent on the geographic origin of symbiotic partners. In other words, combinations in which partners originate in close geographic proximity (same-site or maternal combinations) suffer less mortality and grow more than combinations whose partners originate from geographically distant-sites. Additionally, the structure of the variation appears to be driven by significant host-symbiont interaction effects; therefore, the collection sites used in these studies are very different selective environments for the algal isolates. These results are consistent with the interpretation that *Cassiopea xamachana* are generally better adapted to co-occurring algal mutualists than to algal isolates

from distant-sites. Additionally, the differential growth and mortality between local and non-local combinations provide evidence of pronounced local adaptation of algal symbionts to *Cassiopea xamachana* hosts.

Further, the results presented in chapter 5 showed that the algae isolated directly from female medusa and environmentally available symbionts have similar effects on host fitness. The locally available algal symbionts at each site were not equally compatible with all polyp lineages used in this study. In fact, the observed variation in host-symbiont compatibility remained geographically structured. Thus, it seems plausible to suggest that both the algal symbionts and *Cassiopea* hosts have population structure and, as a result, not all symbiont genotypes are equally distributed across all sites. This makes intuitive sense in light of the pronounced host-symbiont interactions observed in this study as well as in the previous study.

The molecular investigations reported here are among the first steps towards better understanding the population genetic structure of the algal symbionts found within *Cassiopea xamachana* hosts. Based on the restriction fragment length polymorphisms (RFLPs), it appears that *Cassiopea xamachana* harbors only a single algal symbiont, *Symbiodinium microadriaticum*. Moreover, the ISSR data suggests that there is a great deal of intraspecific genetic variation within this species. Additionally, the AMOVA revealed that there was significant population genetic structure. Examination of Φ_{st} indicates the presence of at least three distinguishable algal populations. That is, these algal isolates are not part of a single, panmictic population and gene flow is constrained.

PART 3: DISCUSSION OF THE RESULTS

Results from these studies have important evolutionary and ecological implications regarding migration and colonization processes in invertebrate hosts that are obligately dependent upon symbiotic algae for growth and survival. The results imply that compatibility with the local symbiont population could be a key factor influencing the potential for the establishment and proliferation of any given host immigrant. Certain host genotypes might be excluded from a habitat not because of inferior adaptation to macroscopic features of the environment (i.e. predators, climate, water quality), but simply because they are incompatible with indigenous algal mutualists necessary for proliferation in that particular habitat. Migrant lineages are likely to have reduced longevity and fecundity and will be rapidly displaced by resident associates. Over the long term, the local host-symbiont population would become increasingly homogeneous as incompatible symbioses are intensely selected against.

Positive frequency-dependent selection might characterize local *Cassiopea*-algal symbioses, where partners adapted to the most common phenotypes accrue larger fitness advantages (Law, 1985). Meanwhile, mutualistic associates adapted to rare phenotypes will be eliminated because the chance of encountering a compatible partner is low. The above argument (1985) was for a single population of mutualists in isolation, but invokes interesting questions when extended to larger geographic scales. Results from this dissertation suggest that there is a significant degree of spatial differentiation among algal symbionts. And, it is likely that this differentiation exists among *Cassiopea* hosts as well due

to drift and adaptation to various environmental factors among sites. For example, the host and symbiont gene pool in any given location might consist of genotypes best suited to prevailing environmental conditions (salinity, turbidity, temperature, etc.). These initial differences might be magnified by positive frequency-dependent selection on the holibiont. This would generate a geographic mosaic with each locally dominant set of mutualists displaying resistance to invasion by rare immigrant phenotypes which are adapted to different partner phenotypes (Parker, 1999). However, the role of spatially varying environmental factors deserves attention and future studies should include stress experiments (particularly increased ultraviolet light and temperature), which might provide an explanation for some of the factors that control the distribution and abundance of different algal symbionts in nature.

Frequent abiotic disturbances such as hurricanes undoubtedly cause extinctions and provide a potential mixing of nearby *Cassiopea*-algal populations. Thus, it is likely that the metapopulation of *Cassiopea*-algal symbioses consists of subpopulations in a continuum of evolutionary states (e.g. a stable geographic mosaic). Patches such as JJ (a northern site in this study) might be in a relatively stable homogeneous state characterized by *Cassiopea*-algal specialists. This is a well-protected bayside site with relatively stagnant water. Conversely, a site like SL (a southern site in this study) may be characterized by a more variable population of hosts and symbionts. This might explain the greater within-site variation observed (Chapters 4 and 5) among same-site and maternal combinations from SL as well as the fact that SL algal isolates were genetically

similar to CP isolates. In fact, a maternal combination from SL suffered high mortality and little or no growth (Chapter 4). SL is located on the oceanside of the Florida Keys with heavy boat traffic and increased water flow due to canal dredging. These are potentially two ends of the spectrum with many subpopulations likely falling between these extremes. The ISSR data lends support to these ideas.

The AMOVA revealed significant population genetic structure among sites used in these studies and analysis of Φ_{st} revealed three distinct algal population (BC, JJ, and SL/CP). The results indicate that SL medusa (southern site) contain algae that do not appear to members of a unique population. Indeed, the majority of the SL isolates co-cluster with CP isolates (northern site). Additionally, 5 of 28 algal isolates cluster outside of their respective populations perhaps suggesting limited migration between sites. For instance, CP is a northern site and SL is a southern site but, both are oceanside collection sites. This is perhaps evidence for oceanic mixing whereby gene flow dilutes local adaptation. On the other hand, the lack of population structure observed between the isolates from these two sites could be indicative of a recent colonization of one or both of these sites. Since hurricanes and strong storms often sweep the Florida Keys, extinction and re-colonization events are likely to occur frequently at locations more susceptible to storm surges maintaining low levels of genetic diversity between some sites.

Conversely, JJ and BC are fairly isolated, well-protected bayside sites; thus, the placement of several JJ and BC isolates outside their respective

population clusters is interesting yet, more difficult to interpret. Migration between these sites might occur in a stepping stone fashion across intermediate sites characterized by heterogeneous symbiont populations. Most biological systems are characterized by the uneven distribution of individuals into a series of populations that show varying degrees of connectedness i.e. the metapopulation (Levins, 1969, 1970; Hanski and Gilpin, 1991, 1997). Thus, it would be premature to assume that all *Cassiopea*-algal populations exhibit the same degree of population genetic structure. Processes such as gene flow, genetic drift, and various forms of natural selection within and among individual populations is likely transitory and ephemeral. Long term monitoring of *Cassiopea*-algal compatibility among these four sites will enhance our understanding of the spatial and temporal patterns associated with this system. Future studies should also focus on including more intermediate sites in the Florida Keys.

PART 4: FUTURE DIRECTIONS

Evolutionary studies

Given the extent of geographic variation in *Cassiopea*-algal compatibility within the Florida Keys, it would be intriguing to investigate if the structured variation in host-symbiont compatibility is a Caribbean-wide phenomenon. Additionally, it would be interesting to discover the extent to which other algal-invertebrate symbioses conform to these results. I would expect as much, if not more, local adaptation in sedentary invertebrates obligately associated with marine algal symbionts (i.e. sponges or reef-building corals).

Ecological studies

The actual mechanism of algal loss from marine invertebrate hosts under stressful conditions (bleaching) remains an enigma, and much more is still to be learned about the dynamics of algae within invertebrate hosts. However, if host-symbiont interactions play a role in determining symbiotic outcomes, these interactions might also determine the ability of the association to withstand environmental perturbation. Future investigations should focus on *Cassiopea* recovery from bleaching events and acclimation to changed environmental parameters. Further, it would be interesting to determine whether hosts are capable of acquiring new symbiont genotypes (adaptive bleaching hypothesis) using ISSRs to identify specific genotypes.

Genetic studies

Future investigations should increase sample size by sampling from intermediate and distant Caribbean sites and increase the number of ISSR primers used. This would allow one to investigate the stability of the population genetic structure across a larger region of the metapopulation. Additionally, it will be intriguing to investigate the population genetic structure of *Cassiopea xamachana* medusae within and between the sites as a means of explaining a portion of the variation in host-symbiont compatibility. To date, no one has pursued this research avenue. Additionally, it would be interesting to investigate the affects that bridge and highway construction have had on subdividing the *Cassiopea*-algal metapopulation in the Florida Keys due to restricted water flow between Keys. Finally, with the ability to fingerprint various algal symbionts, it would be

interesting to investigate host-symbiont compatibility using known algal genotypes. Of particular interest would be a series of cross-infection experiments in which polyps are infected with same-site algae as well as allopatric algal invaders from that site. I would expect little variation in growth and survival among polyps infected with closely related algal isolates regardless of their geographic origin.

PART 5: GLOBAL IMPLICATIONS AND CONCLUDING REMARKS

If there is a single overriding message from the studies documented in this dissertation, it is that host-symbiont compatibility plays a vital role in determining symbiotic outcomes. To my knowledge, this is the first investigation of intraspecific host-symbiont compatibility among marine algal-invertebrate symbioses and it will be interesting to discover the extent to which other algal-invertebrate symbioses conform to these results. Furthermore, this is the first time ISSR molecular markers have been used to investigate intraspecific variation among symbiotic dinoflagellates. Over the last several decades, it has become clear that most invertebrate hosts are restricted to associating with one or a few algal symbiont species. However, no other studies have examined variation among algal symbionts beyond species level differences. This is curious since migration, colonization, survival, growth, and reproduction appear to be dependent upon the algal symbiont *in hospite* – even if the symbionts are from the same taxa!

For marine invertebrates, particularly coral, the question of how host performance is affected by algal genotypes has become a serious concern. Over

the last several decades, the frequency and intensity of coral bleaching (loss of algal symbionts) has increased. More recently, I have observed increased bleaching among *Cassiopea* medusa in the Florida Keys. Some researchers have suggested that bleaching is an adaptive response to changes in local environmental conditions, particularly increased UV light and temperature, which allows an invertebrate host to acquire novel symbionts best suited to prevailing environmental conditions (Buddemeier and Fautin, 1993). In other words, the host has the ability to switch symbionts when current symbionts reduce fitness. Adaptive bleaching does not appear to be an option for this invertebrate host since *Cassiopea* polyps do not appear to be able to prevent infection by symbionts that might kill them. If hosts cannot switch symbiont genotypes, and most algal-invertebrate symbioses are obligate, then what is the fate of coral reefs in lieu of increased global warming? Perhaps not all symbionts are lost during bleaching events and remaining *in hospite* populations gradually recover within the host as the symbionts become acclimated to prevailing environmental conditions (Wilcox, *in prep*.). With the ability to fingerprint algal genotypes, answers to these types of questions are within reach.

It would seem that the results obtained in this dissertation broadly mimic the results acquired during investigations of antagonistic interactions (Lively, 1999; Bot *et al*., 2001). For instance, Lively (1999) has shown that parasite migration and virulence interact to affect the degree of local adaptation by parasites. As migration decreases and virulence increases, the degree of local adaptation by parasites increases. Lively predicts that local adaptation will be

most striking when highly virulent parasites are involved. This is because the strength of selection on the host is positively related to parasite virulence, and strong selection for host resistance along side selection for parasites to overcome host defenses, is more likely to lead to population differentiation in the face of gene flow (Wright, 1931). Perhaps mutualisms behave similarly to parasitic associations. Instead, however, migration and compatibility interact to affect the degree of local adaptation by algal symbionts. For example, as migration decreases and compatibility increases, the strength of local adaptation by algal symbionts increases. Thus, in mutualistic associations, local adaptation will be most striking when highly compatible, most likely obligate, symbionts are involved. As Lively (1999) would suggest, this is because the strength of selection on the host is positively related to symbiont incompatibility, and strong selection for host resistance to incompatible or "selfish" algal symbionts along side selection for compatible symbionts, is more likely to lead to population differentiation, even in the face of gene flow (Wright, 1931).

Today there is still no widely tested theory of mutualism; however, studies such as those presented in this dissertation, bring investigators of endosymbiotic mutualisms one step closer to understanding the nature of host-symbiont dynamics. Over the last few decades, the dynamics of host-parasite interactions have become increasingly well understood. It is interesting and exciting that the results presented here parallel many of the results found in the host-parasite literature. As a result, I would suggest that intimate interactions among species, whether cooperative or antagonistic, span a continuum of potential outcomes,

particularly when investigators manipulate interactions. The continuum ranges from highly advantageous to extremely disadvantageous and in many cases will be driven by local adaptation as partners evolve to align their interests (mutualism) or disentangle them (parasitism). The outcome is a geographic mosaic across the metapopulation of each associate.

The studies detailed in this dissertation were a necessary first step in beginning to describe and interpret the evolutionary and ecological dynamics of the *Cassiopea*-algal symbiosis. In future studies, it will be important to extend this approach to a larger sample of *Cassiopea* and algal isolates, to analyze the relative magnitude of variation within and between populations across a larger portion of the metapopulation. Nevertheless, the enormity of differential survival and growth among *Cassiopea* hosts observed in these studies is noteworthy and has opened numerous doors for future investigations.

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Vita

Adrienne Joy Sloan was born in Dodge City, Kansas on December 27, 1971, the daughter of Joyce Neal Sloan and Francis Gilbert Sloan. After graduating from St. Agnes Academy, Houston, Texas, in 1990, she entered Loyola University, New Orleans, Louisiana. She received the degree of Bachelor of Science from Loyola in December 1994. During the next year, she was employed as a Mass Spectrometer Technician at Rice University, Houston, Texas under the auspice of The University of Houston, Houston, Texas. In January 1996 she entered graduate school at The University of Houston. During her first few years of graduate studies, Adrienne spent several semesters training in molecular biology techniques at The University of Texas, Austin, Texas. In January 2002 she transferred to The University of Texas to complete her dissertation work. During her graduate studies she was awarded six teaching assistantships, four research assistantships, and has cumulatively been awarded $46,900.00 from six organizations for the pursuit of her scientific inquires.

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