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Variation in symbiont uptake in the early ontogeny of the upside-down jellyfish, *Cassiopea* spp.

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ABSTRACT

The upside-down jellyfish *Cassiopea*, like many cnidarians, form obligate symbioses with dinoflagellates belonging to the genus *Symbiodinium* (commonly known as zooxanthellae). In adult *Cassiopea*, the symbiosis is specific, with a given *Cassiopea* species hosting a particular symbiont phylotype throughout broad distributions. However multiple phylotypes of *Symbiodinium* can infect the scyphistoma (polyp) stage of development, making *Cassiopea* spp. an ideal model to study the effects of symbiont phylotype on host development and proliferation. To assess the flexibility of symbiont acquisition and to understand how symbiont identity affects the early stages of *Cassiopea* development, symbiont uptake and host developmental traits were monitored in two species of *Cassiopea* that were exposed to multiple symbiont phylotype in laboratory and field experiments. Scyphistomae of *Cassiopea ornata* and *Cassiopea xamachana* both demonstrated flexibility in their symbiosis at the scyphistoma stage during which they acquired a range of laboratory cultured *Symbiodinium*. The presence of symbionts in *C. ornata* increased planuloid production relative to uninfected controls, and the rate at which symbionts accumulated in the polyp tissues varied with symbiont phylotype. Laboratory infected *C. xamachana* polyps continued to take up novel/additional symbiont types when transferred to the field; however, novel uptake occurred significantly less frequently in polyps that harbored homologous (ITS type-A1) symbionts prior to field placement. Similarly, ephyrae of *C. ornata* were able to acquire additional symbiont types even when already infected with *Symbiodinium* ITS type C1. Our findings demonstrate that the symbiosis is flexible within the early ontogeny of *Cassiopea*, but that associating with the "right" symbiont may provide a developmental advantage for that host.
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1. Introduction

Dinoflagellates in the genus *Symbiodinium* form symbioses with many cnidarians, including the upside-down jellyfish species that comprise the genus *Cassiopea*. In this mutually beneficial symbiosis, the symbionts provide the host with photosynthetically produced carbohydrates in exchange for inorganic and organic nutrients and a light-rich environment free from predators (Muscatine and Porter, 1977).

These symbiotic algae exhibit deep genetic divergence and based on nuclear small subunit ribosomal DNA (18S rDNA), *Symbiodinium* have been divided into nine clades (A–I) (Rowan and Powers, 1991; Carlos et al., 1999; LaJeunesse and Trench, 2000; LaJeunesse, 2001; Pochon and Gates, 2010; reviewed by Coffroth and Santos, 2005). The genetic differences among *Symbiodinium* clades are comparable to those separating genera, families, and even orders among other dinoflagellates (Rowan and Powers, 1992). The clades are further subdivided, according to variation within the chloroplast (cp) 23S rDNA (Coffroth and Santos, 2005; Santos et al., 2002; Santos et al., 2003), internal transcribed spacer region of nuclear rDNA (LaJeunesse, 2001) and other molecular markers (LaJeunesse and Thornhill, 2011; Pochon et al., 2012). Diverse symbiotic partnerships result from the specificity and flexibility seen in both host and algae.

Cassiopea harbor *Symbiodinium* intracellularly within the subtentacular region, and the proboscis (Hofmann et al., 1978), as well as the oral arms (tentacles) and tissues (Colley and Trench, 1983).Unlike typical jellyfish, *Cassiopea* spp. medusa rest inverted on the bottom of tropical inshore marine waters facilitating the symbionts' access to sunlight. During the life cycle, sexually mature adult medusae produce aposymbiotic (symbiont-free) larvae that settle on an appropriate substrate and develop into a scyphistoma (polyp) (Fleck and Fitt, 1999). In this stage, scyphistomae greater than 1 mm in polyp diameter may produce asexual buds (planuloids) near the base of the polyp that settle and develop into additional scyphistomae (Hofmann et al., 1996). Upon infection with the symbiont, the scyphistomae are capable of undergoing metamorphosis (strobilation) and producing free-swimming ephyra (young medusa).

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Many adult coral–algal symbioses show consistency in their harbored symbiont types (Baker et al., 2013; Goulet, 2006; Goulet and Coffroth, 2003), however further work has shown that in their early ontogeny, some aposymbiotic cnidarians are found with symbiotic diversity surpassing that of the adult form (Abrego et al., 2009; Coffroth et al., 2001; Poland et al., 2013; Thornhill et al., 2006). Given this apparent initial lack of specificity for a particular *Symbiodinium* phylotype, it is unclear what governs the selection for the final symbiont type that is found in the adult host.

This study investigated flexibility in symbiont uptake for two *Cassiopea* spp. when offered multiple phylotypes of *Symbiodinium* at different stages of development. We subsequently examined host growth and development for *Cassiopea* harboring specific symbiont phylotypes by measuring the rate of planuloid production by scyhistomae, symbiont accumulation rate within the host, and host survivorship. Although evidence of coevolution between host and symbiont is lacking (van Oppen et al., 2001), data such as these are important in accessing the potential benefits to harboring specific phylotypes by establishing if there is a developmental advantage to harboring one phylotype over another. Furthermore, to understand how this and other reef symbioses will respond to threats posed by global climate change, it is important to understand how the symbiosis is established and maintained, and the consequences of particular associations on host fitness.

2. Materials and methods

2.1. Study organism

Cassiopea xamachana is a widely distributed Caribbean species, which, at the medusa (adult) stage, typically harbors one specific type of symbiont *Symbiodinium microadriaticum* (cp-23S rDNA type A194 based on variation in the chloroplast 23S rDNA (cp-23S rDNA), ITS type A1 (Thornhill et al., 2006)). *C. xamachana* organisms for this research were reared from larvae collected in the Florida Keys. Ephyra and polyps of *Cassiopea ornata* were generously donated by the Monterey Bay Aquarium (Monterey, CA) as approximately 2 month old polyps that harbored *Symbiodinium* Clade C (cp-23S rDNA type C180, ITS-type C1). Although symbiont specificity is less studied in *C. ornata*, observations have suggested that Indo-Pacific *Cassiopea* might naturally harbor Clade C *Symbiodinium* in the field. There is debate over the taxonomy of *Cassiopea* spp., and these latter polyps from Monterey Bay Aquarium were identified as *C. ornata* based on sequence homology with those described by Holland et al. (2004) (see results).

2.2. Larval collection and scyphistoma rearing

C. xamachana larvae were collected from various-sized female medusae (diameter = 10–23 cm) from Key Largo and Long Key sites (Florida Keys, FL) by pipetting the developing embryos from the base of the tentacles of female colonies. The planulae that developed from these embryos were rinsed with artificial seawater (ASW, Instant Ocean™, Blacksburg, VA) at a salinity of ~33 ppt, and then filtered through a 64 μm mesh. Planulae were kept overnight in fingerbowls with 200 ml of an antibiotic seawater mixture (0.1 g Neomycin and 0.13 g Streptomycin dissolved in 1 L of ASW) and then transferred into ASW with degrading mangrove leaves which serve as settlement substrate. Mangrove leaves were collected from the shore adjacent to the Keys Marine Lab (Long Key, FL) and were either rinsed with the photosynthetic inhibitor herbicide, Diuron ((3, 4-dichlorophenyl)-1, 1-dimethylurea (DCMU)) or incubated in DCMU (concentration 3.4×10^{-6} mol l^{-1}) for 3–5 days to reduce the number of algal symbionts potentially found on the mangrove leaves as they are not proliferating due to poor (non-photosynthesizing) conditions (Hofmann et al., 1996). After the larvae metamorphosed to scyphistomae, and prior to inoculations with the desired culture phylotype, polyps were confirmed to be aposymbiotic by microscopic examination (Zeiss compound microscope at 40×) by their lack of any visible symbiont cells within the tissues or tentacles. Symbiont cells in other treatments were readily identified by their size, shape, organelles and/or golden color which was easily visible due to the transparent nature of host tissues. Once metamorphosed, the polyps used for the field experiment were fed *Artemia* nauplii daily and raised in the lab for one month with daily water changes. The polyps used for lab experimentation were fed and cleaned three times a week. All animals were maintained under a 14:10 h light/dark regime at 26 °C and in ASW at 32–34 ppt.

2.3. Symbiodinium identification

To identify the symbiont phylotype commonly found in *C. xamachana* at our study sites, DNA was extracted from tentacle clippings and the ITS gene was amplified following the protocols of Santos et al. (2003). The PCR products were sequenced at High Throughput Genomics Center (htseq.org Seattle, WA). We compared the ITS sequence from our cultured *S. microadriaticum* to the *in hospite* symbionts of adult, field *C. xamachana* to confirm a 100% match for this gene of both samples to *S. microadriaticum* sequences published on GenBank using Accession Number **AF333505** as a type sample (Santos et al., 2003).

Samples of *Symbiodinium* Clade C collected from *C. ornata* were also subjected to molecular analysis and identified as *Symbiodinium* ITS-type C1 or cp23S-type C180 based on the hypervariable region of the chloroplast 23S rDNA. A BLAST (Altschul et al., 1990) search found that the symbiont type ITS matched 99.7% with *Symbiodinium* sp. type C1 (Accession Number **EU074964**).

Following the initial sequencing, symbiont types were distinguished via length heteroplasmy in domain V of chloroplast large subunit (cp23S) ribosomal DNA (Santos et al., 2003). Samples were preserved in 95% ethanol (EtOH) for later DNA extraction following a 2× Cetyl trimethyl ammonium bromide (CTAB) protocol for *Symbiodinium* DNA isolation (modified from Coffroth et al., 1992 by the addition of "bead-beating" with glass beads to rupture the symbiont cell wall). The cp-23S gene was amplified with polymerase chain reaction (PCR) according to Santos et al. (2003). The PCR product was then run on a 6.5% Long Ranger acrylamide gel using LI-COR's Long ReadIR 4200 DNA Sequencer along with size standards for fragment size analysis.

2.4. Cassiopea identification

A molecular phylogeny presented by Holland et al. (2004) demonstrated cryptic species diversity within the traditional morphologically based *Cassiopea* groupings particularly among Pacific phylotypes. To identify the *Cassiopea* used for this study, we employed morphological, geographical, and for the Pacific samples, molecular diagnostics (Holland et al. 2004).

Caribbean *Cassiopea* larvae that were collected from adult individuals in the Florida Keys were identified as *xamachana* based on genetically supported tentacle morphology differences between this species and the co-occurring Caribbean *C. frondosa* (Holland et al. 2004). *C. xamachana* is a widely distributed Caribbean species, which, at the medusa (adult) stage, typically harbors one specific type of symbiont, *S. microadriaticum* (cp-23S rDNA type A194, ITS type A1, Thornhill et al., 2006).

Holland et al. (2004) tentatively identified several genetically distinct, but morphologically cryptic, species of Pacific *Cassiopea* based on sequence comparisons of the COI genes from widely distributed samples. Following this protocol, we determined the COI sequence of our Pacific aquarium samples (High Throughput Genomics Center (htseq.org, Seattle, WA)), which was identical among our samples confirming a single species (accession number **KF683387**). While our samples were not an exact match to any *Cassiopea* COI sequences published in Genbank as of May 2013, a phylogenetic reconstruction following Holland et al. (2004), grouped these Indo-Pacific aquarium species

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