Laboratory results: Genotypes of symbionts detected in Orbicella faveolata recruits of different ages and different history of symbiont exposure (McIlroy/Coffroth Coral Reefs, 2017) (SymBioSys project)

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Project
» Ontogenic change in Cnidarian-algal symbioses: A genomic and ecologic perspective (SymBioSys)

Contributors	Affiliation	Role
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Abstract
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Dataset Description

Orbicella faveolata recruits inoculated with either *Symbiodinium microadriaticum*, *Symbiodinium minutum,* or maintained aposymbiotic (Control). These recruits were then exposed to different symbiont types at different ages and sampled 1-2 months later for symbiont genotyping.. These data were used in McIlroy and Coffroth (2017).

- Methods, data processing, and results reported in McIlroy, S.E. and Coffroth, M.A., 2017
- Collection and rearing methodology in McIlroy S.E. *et al,* 2016
- Electrophoretic fragment length analysis methods in Santos, S.R. *et al.,* 2003

Methods & Sampling

Methodology from McIlroy & Coffreoth, Coral Reefs (2017):

Coral collection and rearing is described in detail in McIlroy et al. (2016). Briefly, we collected gametes from multiple spawning *Orbicella faveolata* colonies at two reefs in the Florida Keys, FL at Alligator Reef (24°48.7710 N, 80°40.1670 W) and Looe Key (24°32.6930 N, 81°24.5620 W) in 2011. Eggs and sperm from each site were mixed immediately for fertilization (Miller and Szmant 2006). At the laboratory, gametes and later embryos from all sites were combined and maintained in closed, 18-20-L tanks with 1.0-lm filtered seawater (FSW). Tanks were outdoors under a metal structure for shade with water temperatures ~28-30 degrees C, similar to

ambient temperatures at the spawning sites. Ceramic tiles (5 cm x 5 cm, preconditioned for 10 d in 1.0-um FSW) served as a settlement substrate. Full FSW changes were done initially 1-3 times d-1; then every 1-3 d following settlement.

To assess ontogenetic effects on initial symbiont uptake, a set of recruits was maintained aposymbiotic. To measure the effect of initial infection on symbiont uptake, inoculations with 500 cells mL-1 of one of two cultured *Symbiodinium* strains, A194 or B184 (Table 1), were done repeatedly from the late larval stage through settlement and were terminated when visible infection was observed (~2 weeks). The cultures were *Symbiodinium* species present in other hosts throughout the Caribbean (LaJeunesse et al. 2012; Mellas et al. 2014) and are readily acquired by *O. faveolata* in the laboratory (Voolstra et al. 2009; McIlroy et al. 2016), but they are not the *Symbiodinium* species that are associated with adult *O. faveolata* colonies in the field (Table 1). Corals may respond differently to symbiont species dominating the symbiosis in the field, but these species have yet to be brought into culture, so this hypothesis could not be tested.

Two weeks post settlement, recruits were transferred to the University of Miami Experimental Hatchery (Virginia Key, Miami, FL) where they were maintained in indoor tanks under 5-20 umol photons m-2 s-1 in FSW (i.e., no exposure to exogenous symbionts), and fed weekly with Zeigler (Gardners, PA) larval diet (\100 lm in size). The light level was similar to that measured in reef crevices at the spawning site (McIlroy et al. 2016), the preferred settlement environment of this species (Miller 2014). Each month we took tiles from each treatment (A194, B184, aposymbiotic), removed 10 recruits per treatment for genotype analysis, and transferred the tiles with the remaining~20 recruits to an indoor seawater system of coarsely filtered (~20 lm) natural seawater with environmental symbionts at the same temperature and under the same low light levels. Other sources of symbionts included 1-2-yr-old, laboratory-reared *O. faveolata* corals with *in hospite* symbiont communities dominated by D206 and infected recruits (A194 and B184 treatments) from this study that were no longer physically isolated from each other (Fig. 1). One month after transfer we sampled the recruits (~20 per initial treatment) into 95% ethanol for symbiont genotyping.

To genotype *Symbiodinium* within recruits, we extracted the total genomic DNA (Coffroth et al. 1992), amplified the hypervariable region of the chloroplast 23S rDNA gene, and ran an electrophoretic fragment length analysis (Santos et al. 2003). All PCRs included positive (cultured *Symbiodinium*) and negative controls. Where no amplification occurred after three attempts, *Symbiodinium* was assumed to be absent or below the level of detection of this technique (10-1000 Symbiodinium cells; Santos et al. 2003) and was reported as "none detected". Within the *Symbiodinium* B184 treatment, we randomly selected four post-exposure recruit DNA extractions, and amplified and sequenced a species-level marker, B7Sym15, which can distinguish our culture species from other potential environmental types including the *Symbiodinium* B184 that occurs in *O. faveolata* adults (Finney et al. 2010; LaJeunesse et al. 2012).

For symbiont uptake in the aposymbiotic treatment, we compared the frequency of infected and uninfected polyps after exposure to environmental symbionts with Fisher's exact test (FET; R package RVAideMemoire v 0.9-52). Where significant, we used post hoc multiple comparison with Bonferroni adjustment. We limited the comparisons a priori to pairs of consecutive months. For the A194 and B184 infected recruits, we used FET to compare the frequency with which the two pre-inoculated recruits harbored additional symbionts.

Data Processing Description

BCO-DMO Processing:
- original file: DATASET_McIlroyetal2016_Orbicella Symbiont Genotypes.xlsx
- modified conventional header with dataset name, PI name, version date, reference information
- changed parameter names to be BCO-DMO compatible

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Data Files

File	
McIlroy_2017_sym_genotypes.csv	(Comma Separated Values (.csv), 417 bytes) MD5:ff496b0670b1635fb2f08f27af64181e
Primary data file for dataset ID 714453	

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Related Publications

McIlroy, S. E., & Coffroth, M. A. (2017). Coral ontogeny affects early symbiont acquisition in laboratory-reared recruits. Coral Reefs, 36(3), 927–932. doi:10.1007/s00338-017-1584-7
Results

McIlroy, S. E., Gillette, P., Cunning, R., Klueter, A., Capo, T., Baker, A. C., & Coffroth, M. A. (2016). The effects ofSymbiodinium(Pyrrhophyta) identity on growth, survivorship, and thermal tolerance of newly settled coral recruits. Journal of Phycology, 52(6), 1114–1124. doi:10.1111/jpy.12471
Methods

Santos, S. R., Gutierrez-Rodriguez, C., & Coffroth, M. A. (2003). Phylogenetic identification of symbiotic dinoflagellates via length heteroplasmy in Domain V of chloroplast Large Subunit (cp23S)-Ribosomal DNA Sequences. Marine Biotechnology, 5(2), 130–140. doi:10.1007/s10126-002-0076-z
Methods

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Parameters

Parameter	Description	Units
Symbiodinium_treatment	Species of Symbiodinium used for larval inoculation	unitless
age_transfer_mon	The age (in number of months following spawning) that recruits were transferred from treatments to exposure to environmental symbionts	months
exposure_mon	The number of months between transfer and sampling for symbiont genotype analysis	months
Sym_genotype_184	The number of recruits sampled in which symbiont genotype 184 was detected in the recruits. Nomenclature follows cp23s nomenclature established in Santos et al. 2003.	unitless
Sym_genotype_A194	The number of recruits sampled in which symbiont genotype A194 was detected in the recruits. Nomenclature follows cp23s nomenclature established in Santos et al. 2003.	unitless
Sym_genotype_B184_D206	The number of recruits sampled in which symbiont genotypes B184 and D206 were detected in the recruits. Nomenclature follows cp23s nomenclature established in Santos et al. 2003.	unitless
Sym_genotype_A194_D206	The number of recruits sampled in which symbiont genotypes A194 and D206 were detected in the recruits. Nomenclature follows cp23s nomenclature established in Santos et al. 2003.	unitless
Sym_genotype_D206	The number of recruits sampled in which symbiont genotype D206 was detected in the recruits. Nomenclature follows cp23s nomenclature established in Santos et al. 2003.	unitless
Sym_genotype_none	The number of recruits sampled in which no symbiont genotypes were detected in the recruits. Nomenclature follows cp23s nomenclature established in Santos et al. 2003.	unitless

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Instruments

Dataset-specific Instrument Name	LI-COR Long ReadIR 4200 DNA Sequencer
Generic Instrument Name	Automated DNA Sequencer
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	ceramic tiles
Generic Instrument Name	Ceramic tile settlement plate
Generic Instrument Description	An artificial colonization substrate made of ceramic tiles. It is used to determine the extent of colonization and/or the diversity of settled organisms in a marine or artificial environment.

Dataset-specific Instrument Name	
Generic Instrument Name	Thermal Cycler
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

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Project Information

Ontogenic change in Cnidarian-algal symbioses: A genomic and ecologic perspective (SymBioSys)

Coverage: Florida Keys, Bahamas, Panama, Mexico

PROJECT SUMMARY:
The symbiosis between corals (Cnidaria:Hexacorallia:Scleractinia) and photosynthetic dinoflagellate symbionts (Alveolata: Dinophycea: Symbiodinium) provides the foundation and structure of the coral reef ecosystem, as well as significant contributions to global carbon and biogeochemical cycles. Given the importance of this symbiosis to the coral-algal holobiont and the reef ecosystem, understanding the mechanisms governing the establishment and long term maintenance of this symbiosis is essential. The overall aim of this project is to

identify the mechanisms and selective processes that lead to the final assemblage of symbionts harbored by adult hosts. This question will be approached from two perspectives, ecologic and genomic, with the specific aims of determining (1) if different Symbiodinium strains differentially affect fitness of corals as the adult settles into a mature symbiosis (2) if competition among symbionts or environmental conditions contribute to the final host-symbiont pairing and (3) how host/symbiont transcriptomes varying as the symbiont community within a host is winnowed to the final assemblage found in the adult host. Traits that directly affect coral fitness (i.e. growth, survivorship, energy production) will be measured under different environmental conditions over the ontogeny of coral recruits that are experimentally infected with different types of Symbiodinium. Concurrently, high throughput gene expression profiling will be used to follow changes in gene expression between host and symbiont. Together, these data will be used to validate or falsify the hypotheses that the final symbiont assemblage found in the adult host is determined by (a) host selection (b) competition among symbionts and/or (c) environmental condition.

This study pools the expertise of two labs that have focused on these aspects of the symbiosis. The Coffroth lab pioneered the studies on early ontogeny of the symbiosis and symbiont diversity and will continue to take the lead in the ecological studies. The Medina lab is at the forefront in the development and utilization of genomic technology to study transcriptomic changes during the establishment and breakdown of the symbiosis. Furthermore, the Medina lab has the coral microarrays to be used in this study and in 2009 will also have oligo arrays for two Symbiodinium species based on 454 EST data. Although several groups have initial studies of the host transcriptome, none have combined an approach that examines the host and the symbiont in a single experiment. This will be a powerful approach as it will allow the investigators to track complementary changes in gene expression between host and symbiont and relate those to turnover in the symbiont community as the final symbiont complement is established.

The data resulting form the study will bridge an important gap in our understanding of the establishment and maintenance of coral-Symbiodinium symbiosis. Understanding the mechanism(s) regulating the establishment of the symbiosis will broaden our knowledge and help to predict the response of this symbiosis to future climate conditions. As in the past, the genomic tools (arrays, ESTs) will be made readily available to researchers via array distribution at cost, microarray analysis training, or sequence data, providing valuable resources to continue exploring these systems.

In conjunction the Aquarium of Niagara, Coffroth will develop educational and outreach programs to train and disseminate information on coral reefs to local area teachers and the general public. The Medina lab will continue to produce science and environment podcasts in multiple languages (English, Spanish and Hmong) with undergraduate students at UC Merced and will continue to collaborate with the California Academy of Sciences (CAS) in their coral reef outreach efforts. Additionally, this work will result in the training and mentoring of a postdoctoral fellow, at least one graduate student and at least 2 undergraduates. Through this project these students will have the opportunity to participate in research in both a lab and field setting, learning a range of ecological, molecular and algal culturing techniques. The extensive culture collection housed at the University at Buffalo is an important resource that is available to researchers worldwide which the proposed funding will help to maintain. Our EST annotations are publicly available through our EST database (http://montastraea.psu.edu/SymBioSys/).

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0926822

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