Sequencing reads_(2bRAD) for Montastrea cavernosa, Siderastrea siderea, Agaricia agaricites and Porites astreoides from Florida Reefs

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Project
» Barriers to cross-shelf coral connectivity in the Florida Keys (KeysCoralPopgen)

Contributors	Affiliation	Role
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Abstract
Sequencing reads_(2bRAD) for broadcasters Montastrea cavernosa and Siderastrea siderea sampled in the Lower Florida Keys and brooders Agaricia agaricites and Porites astreoides sampled in Florida coral reefs, from Dry Tortugas to Fowley Rocks.

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Coverage

Spatial Extent: **N**:25.58826 **E**:-80.10108 **S**:24.45597 **W**:-83.0424
Temporal Extent: 2015 - 2019

Dataset Description

Data is published in Rippe et al., 2021.

Methods & Sampling

Broadcasters Montastrea cavernosa and Siderastrea siderea sampled in the Lower Florida Keys and brooders Agaricia agaricites and Porites astreoides sampled in Florida coral reefs, from Dry Tortugas to Fowley Rocks. The first broadcasted transect was sampled in 2015, the second broadcaster transect sampled in 2017. Brooders were sampled in 2019

Tissue samples were collected using hammer and chisel and kept on 100% ethanol and the minimum logistically feasible temperature (-20C or -80C) until processed.

Genomic DNA was extracted from tissue samples using a modified phenol-chloroform procedure. First, samples were transferred to an extraction solution comprising 800 µL CTAB extraction buffer (2% CTAB, 100

mM Tris pH 8, 20 mM EDTA, 1.4 M NaCl), 1.6µL beta merceptoethanol, 1µL proteinase K, and 1µL RNAse A. Tissue was separated from the skeletal matrix and macerated by bead beating in a BioSpec MiniBeadbeater-96 for 15 seconds using 150-212 µm glass beads and then incubated at 42°C for at least one hour. Skeletal fragments were pelleted by spinning in a tabletop centrifuge at maximum speed for 15 minutes. The aqueous phase was then transferred to a clean tube, and 800 µL phenol-chloroform/isoamyl alcohol was added and vortexed into solution for 2-3 seconds. Samples were centrifuged at maximum speed at 4°C for 20 minutes to separate the DNA from organic contaminants. The aqueous supernatant was transferred to a clean tube to which 550 µL ice cold isopropanol was added and gently mixed by inverting. After incubating at -20°C for 20 minutes, samples were again centrifuged at 4°C and maximum speed for 20 minutes to pellet DNA. The supernatant was discarded, 1 mL 80% ethanol added, and the sample was centrifuged at 4°C and maximum speed for 5 minutes. The supernatant was discarded and sample tubes were allowed to air dry for 15-20 minutes. Lastly, pelleted DNA was dissolved in 30 µL warm (65°C) nuclease-free water. Isolated DNA was then further purified using the Zymo Clean and Concentrator-10 kit and normalized to a concentration of 12.5 ng/µL.

2b-RAD libraries were prepared according to the protocol maintained at https://github.com/z0on/2bRAD_denovo (see related software publications). Briefly, 50 ng DNA was then digested in a reaction comprising 1 U BcgI restriction enzyme, 1X NEB Buffer #3, 20 µM SAM in a total reaction volume of 6 µL. Digests were incubated at 37°C for one hour followed by 20 minutes at 65°C to inactivate the enzyme. Barcodes were then attached in two stages, first by ligation to the digested DNA fragments and secondly via PCR amplification. Ligation reactions consisted of 1X T4 ligase buffer, 400 U T4 ligase, and 0.25 µM of two double-stranded ligation adaptors, one of which contained an internal barcode, combined with 6 µL digested DNA in a total reaction volume of 20 µL. Ligation reactions were held at 4°C for 12 hours followed by 20 minutes at 65°C to inactivate the enzyme. Samples with unique ligation barcodes were then pooled and a second barcode was attached via PCR with thermocycler conditions set to 30 seconds at 70°C, followed by 15 cycles of 20 seconds at 95°C, 3 minutes at 65°C and 30 seconds at 72°C. PCR was carried out using 10 µL of pooled ligations with 1X Titanium Taq polymerase, 1X Titanium Taq buffer, 200 µM dNTPs, 0.12 µM unique ILL-BC barcode, 0.12 µM TruSeq-UN barcode, and 0.2 µM each of P5 and P7 adaptors in a total reaction volume of 50 µL.

RNA for TagSeq was isolated using RNAqueous kit (Ambion). DNA contamination was removed by treatign with RNA-seq free DNAse (Ambion). The TagSeq libraries were prepared following the “simplified” version of the protocol, which is maintaned on GitHub (see related software publications).

Data Processing Description

Sequencing reads were trimmed of adaptors and filtered to remove reads containing low-quality bases.

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Data Files

File
all_concatenate.csv (Comma Separated Values (.csv), 104.73 KB) MD5:245391156323b50fd36a974e62476724 Primary data file for dataset ID 874274

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Related Publications

Matz, M. V. (2022). *z0on/2bRAD_denovo: best RAD-seq method ever* (Version v1) [Computer software]. Zenodo. https://doi.org/10.5281/ZENODO.7392161 https://doi.org/10.5281/zenodo.7392161 *Software*

Matz, M. V. (2022). *z0on/tag-based_RNAseq: Tag-Seq: low-cost alternative to RNAseq* (Version v1) [Computer software]. Zenodo. https://doi.org/10.5281/ZENODO.7392165 https://doi.org/10.5281/zenodo.7392165 *Software*

Rippe, J. P., Dixon, G., Fuller, Z. L., Liao, Y., & Matz, M. (2021). Environmental specialization and cryptic genetic divergence in two massive coral species from the Florida Keys Reef Tract. Molecular Ecology, 30(14), 3468–3484. Portico. https://doi.org/10.1111/mec.15931
Results

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Parameters

Parameter	Description	Units
Accession	Accession number	unitless
SPUID	Sample identification	unitless
Organism	Organism name: Montastraea cavernosa, Sidereastrea siderea, Porites astreoides, Agaricia agaricites	unitless
Isolate	Isolate type	unitless
BioProject	Bioproject number	unitless
Depth	Sampling depth	meters (m)
Lat	Latitude of sampling, south is negative	decimal degrees
Long	Longitude of sampling, west is negatice	decimal degrees
coral_type	Coral Type: brooder or broadcaster	unitless
transect	Transect number 1 or 2	unitless

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Instruments

Dataset-specific Instrument Name	Illumina HiSeq 2500
Generic Instrument Name	Automated DNA Sequencer
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

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Project Information

Barriers to cross-shelf coral connectivity in the Florida Keys (KeysCoralPopgen)

NSF Award Abstract:

Coral reefs in the Florida Keys are in severe decline, which is the most prominent along the offshore reef tract while many nearshore reefs retain high coral cover. Why then coral larvae produced from surviving nearshore patches do not recolonize offshore reefs? The investigators hypothesize that such cross-shelf migrants do not survive in the new habitat due to genetic specialization for different environmental conditions, specific to their reef of origin. This project will analyze genetic diversity of coral populations to quantify the severity of this barrier in three common coral species. This will be the first study to assess the strength of ecological barriers to coral dispersal across the seascape, which will fill an important knowledge gap that currently precludes informed assessment of threats to Florida reefs and development of accurate models to forecast their future. The project includes a variety of broader impact activities. Public outreach: This project is very well poised to raise public awareness of ongoing biodiversity loss. The investigators regularly give public lectures at diverse Austin venues, such as Science Under the Stars, Science in the Pub, and Nerd Nite. The progress of the project will be followed by press releases, materials on the University of Texas public outreach web page Know and in The Daily Texan. K-12 outreach: Two interns from Crockett High School and will be involved in the research. Graduate education: The project will be the main PhD theme for one full-time graduate student. Undergraduate education: The primary investigator regularly employs undergraduates. In this project they will participate in field experiments and sample processing, and later assigned individual sub-projects. Promotion of rapid data sharing: All sequencing data will be made available for unconditional use prior to publication. Specifically, the investigators will rapidly share new coral genome data, as well as data on genome-wide variation in coral populations.

The project consists of four parts, each of which is designed to demonstrate the action of divergent selection among nearshore and offshore reefs and to obtain quantitative estimate of its demographic impact. (1) To look for genomic signatures of ongoing selection between inshore and offshore habitats. The research team will perform genome-wide genotyping in three coral species representing alternative life histories and replicate the nearshore-offshore population comparison along the Florida Reef Tract. (2) To confirm continuous action of selection by comparing the extent of inshore-offshore divergence among juveniles and adults. Juveniles are presumed to have experienced local selection for shorter time and hence should show less cross-shelf divergence at the candidate loci. (3) To demonstrate association of genotypes at the candidate loci with local fitness by quantifying in situ growth and survival of genotyped juveniles. This part as well as part 2 is expected to provide quantitative estimates of the strength of selection against cross-shelf immigrants. (4) To verify the obtained estimates by simulating genome evolution under divergent selection and confirming that the proposed selection scenario is compatible with the observed genomic signatures.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1737312

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