DNA microsatellite alleles for hatchery-produced oyster cohorts

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Project

» CAREER: Linking genetic diversity, population density, and disease prevalence in seagrass and oyster ecosystems (Seagrass and Oyster Ecosystems)

Contributors	Affiliation	Role
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Abstract

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Coverage

Spatial Extent: **N**:34.6951 **E**:-76.6183 **S**:30.0224 **W**:-81.4199

Temporal Extent: 2012-04 - 2012-06

Acquisition Description

In April 2012, we collected 100 adult oysters (80-100 mm shell length) from 3-5 separate reefs at each of 6 sites: St. Augustine, FL (FL-1; 30.0224, -81.3287), Jacksonville, FL (FL-2; 30.4446, -81.4199), Sapelo Island, GA (GA/SC-1; 31.4777, -81.2726), Ace Basin, SC (GA/SC-2; 32.4846, -80.6001), Masonboro, NC (NC-1; 34.1510, -77.8551), and Middle Marsh, NC (NC-2; 34.6951, -76.6183). They were held in flowing seawater tanks or suspended in cages from docks in their home region for 2-3 weeks until 30 oysters from each site could be tested and certified as disease free. The remaining 70 oysters were then shipped on ice to a single hatchery facility in Florida (Research Aquaculture Inc., Tequesta, FL; 26.9607, -80.0931) at the end of April.

The adult oysters from each site were used as the broodstock to produce 6 separate site-specific "cohorts" (one cohort per site). From their arrival at the hatchery, the adult oysters were held for 2 weeks until they were ready to spawn under the same conditions in separate flow-through seawater systems to prevent cross-contamination. All families were manually spawned (i.e., strip spawned) on May 7 (see details below). Because the original FL-1 family did not produce many offspring, the remaining broodstock oysters from this site were spawned on June 1 using the same process. Due to variation in ripeness and sex, the number of oysters spawned and the ratio of males to females varied across broodstock (Table 1 of Hughes et al., 2019), though our broodstock numbers for each cohort are comparable to those commonly used in hatchery settings (30-60 individuals; Morvezen et al. 2016).

The broodstock oysters from each source site were strip spawned, sexed, and fertilized on the same day by a team of 7 people, who each had a specific job to perform: shucking the animals, sampling and preparing tissue for microscopic analysis of sex, identifying the sex, stripping the male sperm, stripping the female eggs, mixing the sperm and eggs after all of the animals from a particular source were stripped, overseeing the process and keeping track of broodstock source. We sanitized equipment between individuals and again between broodstock sources. Stripping was done by broodstock source independently and quickly so that the sperm and eggs would remain viable, and all viable sperm and eggs were used. During the gamete mixing process, the eggs from all females and the sperm from all males were first pre-mixed and then combined to ensure equal access of gametes to one another. We allowed 30-60 minutes for fertilization; once 75-90% of the eggs were fertilized, they were moved to larval tanks. All larvae were retained except for minimal numbers of

individuals in each cohort that did not grow or had improper development. Larval culture occurred in 60-gallon conical tanks utilizing a flow-through seawater system with Banjo screens that is commercially used in multiple bivalve hatcheries (e.g., Taylor Shellfish in WA; Cherrystones in VA).

Over a period of 3 days the week of May 28, oysters were sieved on a 250-micron sieve and settled on crushed oyster cultch in a recirculating flow-through system. The week of June 11, once they reached 800 microns in size, they were moved into a nursery facility compliant with state regulations, again under flow-through seawater conditions (salinity = 32 ppt, temperature = 30ºC). In the hatchery and nursery stages, the oysters were fed a mixed diet of T. isochrysis, Chaetocerous gracilis, and Tetraselmis via a constantly running peristaltic pump. Although growth was similar during the larval culture phase, some cohorts produced more juvenile oysters ("spat") than others during settlement, despite following the same procedures for all. To maintain consistency in their growing conditions, we selected a random sample of each cohort to yield similar total abundances across cohorts on June 18. At the end of June (June 27) at approximately 4mm in size, the 6 cohorts were transferred to a common flow-through facility at the Whitney Marine Biological Laboratory in St. Augustine, FL. To assess genetic diversity within and between oyster cohorts produced in the hatchery, 50 individuals were haphazardly collected from each juvenile cohort prior to the start of the field experiments and preserved at -80°C for genetic analysis. This sample size is sufficient to estimate allele frequencies accurately (Hale et al. 2012).

To extract DNA, we ground each tissue sample with a pestle, and used the tissue centrifugation protocol from the Omega Bio-Tek E-Z 96 Tissue DNA Kit. We determined genetic diversity and population structure using 12 highly variable microsatellite loci developed for C. virginica: Cvi9, Cvi11, and Cvi13 from Brown et al. (2000); Cvi1i24b, Cvi2i23, Cvi2j24, and Cvi2k14 from Reece et al. (2004); Cvi4313E-VIMS from Carlsson and Reece (2007); and RUCV1, RUCV66, RUCV73, and RUCV74 from Wang and Guo (2007). We amplified four loci in each multiplexed polymerase chain reaction (PCR) using the Qiagen Type-It Microsatellite PCR Kit. Each 10 l reaction consisted of 1 l DNA template, 5 l 2X type-it multiplex master mix (Qiagen), 2.4 l water, and 0.2 l each 10 M primer. Using a T100 thermal cycler (Bio-Rad), PCR cycling conditions included initial activation/denaturation at 95°C for 5 min, followed by 28 cycles of 95°C for 30 sec, 60°C for 90 sec, and 72°C for 30 sec, and final extension at 60°C for 30 min. PCR products were separated on a 3730xl Genetic Analyzer (Applied Biosystems) with the internal size standard GeneScan 500 LIZ (Applied Biosystems), and fragment analysis was performed using GeneMarker version 2.6 (SoftGenetics).

We created panels for each multiplexed reaction in GeneMarker, which included bins that were assigned manually for all alleles; the same panels were used to score all samples, and the alignment of the panels was checked prior to each analysis to account for any run-to-run

variation and to identify any new alleles. We used these panels to do a preliminary first assignment of alleles based on peak position and bin position, but every sample was then scored manually for all loci to examine signal intensity, to confirm the presence/absence of alleles, and to identify any reruns. A subset of samples was then rerun (at least 15% per multiplex PCR reaction) and manually scored again to confirm any uncertain allele calls and account for any genotyping error.

Processing Description

We used rarefaction and extrapolation in EstimateS to confirm that our sample size was sufficient to characterize genetic diversity accurately (see Appendix S2 of Hughes et al., 2019 for details). We then examined measures of genetic diversity per locus and per source site and assessed cohort structure and differentiation for both the juvenile oyster cohorts produced in 2012 and the adult oysters sampled in 2014 using GenoDive version 2.0b27 (Meirmans and Van Tienderen 2004). We calculated allele number (a), observed (H_o) and expected (H_e) heterozygosity, and inbreeding coefficient (G_i , which is analogous to F_i (Nei 1987)) (Meirmans and Van Tienderen 2004). We also calculated genetic relatedness using STORM (R; Frasier 2008), a metric that accounts for the frequency of alleles to determine the relatedness, or genetic (dis)similarity, of a population. In addition, we calculated pairwise F (Weir and Cockerham 1984) and analysis of molecular variance (AMOVA) (Michalakis and Excoffier 1996) using GenoDive to assess cohort structure and differentiation. Finally, we used the M-ratio test (Garza and Williamson 2001) to determine whether any of the 2012 or 2016 (see below) juvenile cohorts showed evidence of a population bottleneck.

Our juvenile oyster cohorts differed from each other in several measures of genetic diversity, so we tested whether genetic variation predicted ecological trait variation across cohorts in our experiments. We focused on effective allelic diversity, a metric similar to allelic richness (i.e., number of alleles) that weights the number of alleles by their frequencies to determine the effective number of alleles in the population (Meirmans and Van Tienderen 2004). Thus, effective allelic diversity integrates information about the number of alleles and their distribution (Meirmans and Van Tienderen 2004). This metric is sensitive to variation in sample size (Meirmans 2013), so we held sample sizes consistent across groups of juvenile and adult oysters (Table 1 of Hughest et al., 2019). Effective allelic diversity was highly correlated with both allelic richness (positive; $R^2 = 0.96$; $y = 1.71x + 1.49$) and genetic relatedness (negative; $R^2 = 0.98$; $y = -0.08x + 0.52$), so we focused only on effective allelic diversity here. Results of analyses including allelic richness rather than effective allelic diversity were similar (Appendix S3 of Hughes et al., 2019). Our response variables included: initial size (average shell height per tile before being deployed in the field);

survival in the absence of predation (number of live oysters in cages at the end of the experiment, modeled with a binomial generalized linear model (GLM) with logit link); survival in the presence of predation (number of live oysters on open tiles at the end of the experiment, modeled with a binomial GLM with logit link); final size (average shell height per tile at the end of the experiment); and oyster growth (standardized as (final oyster shell height - initial oyster shell height) / initial oyster shell height for each individual per tile). Because we hypothesized that differences in initial oyster size may affect oyster performance, we included initial size as a covariate in our analyses. In all analyses, we tested linear models including a fixed effect of effective allelic diversity with experimental site as a random factor to account for differences in experimental duration or other unmeasured variables. Analyses were run in R software (version 3.0.2) using the packages lme4 and lmerTest (which calculates F and P-values using the Sattterthwaite approximation for degrees of freedom).

Several characteristics of the broodstock may have contributed to the observed variation in juvenile oyster genetic diversity, including variation in broodstock genetic diversity, effective population size (Ne, calculated as (4 * Number of females * Number of males / (Number of females + Number of males)), and/or sex ratio. We did not sample genetic diversity of the 2012 broodstock used to produce our original 6 cohorts at the time of spawning, precluding a direct analysis of the relationship between broodstock genetic diversity and cohort genetic diversity. To address this gap, we first examined the relationship between effective allelic diversity of the adult oysters from each site (sampled in 2014) and the juvenile oyster cohorts (spawned and sampled in 2012). We conducted our analyses on a subset of the adult oysters sampled in 2014 to maintain consistent sample sizes with juveniles (Table 1 of Hughes et al., 2019). In addition, we produced three additional oyster cohorts in 2016 in the same hatchery using broodstock from three field sites in FL (FL-3: 30.0224, -81.3287; FL-4: 30.4446, -81.4199; FL-5: 29.7181, -84.9739) and the same methods as described above. We assessed effective allelic diversity of the 2016 juvenile cohorts using the same methods as described above and also measured the following variables on the broodstock: genetic diversity (effective allelic diversity), effective population size (Ne), and sex ratio (number of males to females). We then tested whether there were significant correlations between these predictors and effective allelic diversity of the 2016 juvenile cohorts. Thus, the analyses of broodstock effective population size and sex ratio include 9 cohorts (the original 6 plus the additional 3), whereas the analysis of broodstock genetic diversity only includes the later 3 cohorts. Analyses were conducted with R statistical software (version 3.0.2) using the lm and glm functions in the lme4 package.

To evaluate the potential for reproductive skew, we ran a parentage analysis of the 2016 broodstock and juveniles using Cervus (version 3.0.7; Kalinowski et al. 2007), assigning each juvenile to the most likely male and female parents from the known pool of broodstock.

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Related Publications

Brown, B. L., Franklin, D. E., Gaffney, P. M., Hong, M., Dendanto, D., & Kornfield, I. (2000). Characterization of microsatellite loci in the eastern oyster, Crassostrea virginica. Molecular Ecology, 9(12), 2216–2218. doi:10.1046/j.1365-294X.2000.105333.x

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Reece, K. S. (2004). Microsatellite Marker Development and Analysis in the Eastern Oyster (Crassostrea virginica): Confirmation of Null Alleles and Non-Mendelian Segregation Ratios. Journal of Heredity, 95(4), 346–352. doi:10.1093/jhered/esh058

Wang, Y., & Guo, X. (2007). Development and Characterization of EST-SSR Markers in the Eastern Oyster Crassostrea virginica. Marine Biotechnology, 9(4), 500–511. doi:10.1007/s10126-007-9011-7

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-Statistics for the Analysis of Population Structure. Evolution, 38(6), 1358. doi:10.2307/2408641

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Parameters

Parameter	Description	Units
STUDY	unique identifier for the 3 components of this dataset	unitless
POPULATION	unique identifier for the site/populations sampled	unitless
SAMPLE_ID	unique identifier for each individual in this dataset	unitless
Cvi4313E_a	allele 1 for locus Cvi4313E	bp (base pairs)
Cvi4313E_b	allele 2 for locus Cvi4313E	bp (base pairs)
RUCV73_a	allele 1 for locus RUCV73	bp (base pairs)
RUCV73_b	allele 2 for locus RUCV73	bp (base pairs)
RUCV74_a	allele 1 for locus RUCV74	bp (base pairs)
RUCV74_b	allele 2 for locus RUCV74	bp (base pairs)
Cvi1i24b_a	allele 1 for locus Cvi1i24b	bp (base pairs)
Cvi1i24b_b	allele 2 for locus Cvi1i24b	bp (base pairs)
Cvi2i23_a	allele 1 for locus Cvi2i23	bp (base pairs)
Cvi2i23_b	allele 2 for locus Cvi2i23	bp (base pairs)
RUCV1_a	allele 1 for locus RUCV1	bp (base pairs)
RUCV1_b	allele 2 for locus RUCV1	bp (base pairs)
Cvi11_a	allele 1 for locus Cvi11	bp (base pairs)
Cvi11_b	allele 2 for locus Cvi11	bp (base pairs)
RUCV66_a	allele 1 for locus RUCV66	bp (base pairs)
RUCV66_b	allele 2 for locus RUCV66	bp (base pairs)
Cvi9_a	allele 1 for locus Cvi9	bp (base pairs)
Cvi9_b	allele 2 for locus Cvi9	bp (base pairs)
Cvi13_a	allele 1 for locus Cvi13	bp (base pairs)
Cvi13_b	allele 2 for locus Cvi13	bp (base pairs)
Cvi2k14_a	allele 1 for locus Cvi2k14	bp (base pairs)
Cvi2k14_b	allele 2 for locus Cvi2k14	bp (base pairs)
Cvi2j24_a	allele 1 for locus Cvi2j24	bp (base pairs)
Cvi2j24_b	allele 2 for locus Cvi2j24	bp (base pairs)

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Instruments

Dataset-specific Instrument Name	3730xl Genetic Analyzer (Applied Biosystems)
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	T100 thermal cycler (Bio-Rad)
Generic Instrument Name	PCR Thermal Cycler
Generic Instrument Description	General term for a laboratory apparatus commonly used for performing polymerase chain reaction (PCR). 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. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

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Project Information

CAREER: Linking genetic diversity, population density, and disease prevalence in seagrass and oyster ecosystems (Seagrass and Oyster Ecosystems)

Coverage: Coastal New England

NSF Award Abstract: Disease outbreaks in the ocean are increasing, causing losses of ecologically important marine species, but the factors contributing to these outbreaks are not well understood. This 5-year CAREER project will study disease prevalence and intensity in two marine foundation species - the seagrass Zostera marina and the Eastern oyster Crassostrea virginica. More specifically, host-disease relationships will be explored to understand how genetic diversity and population density of the host species impacts disease transmission and risk. This work will pair large-scale experimental restorations and smaller-scale field experiments to examine disease-host relationships across multiple spatial scales. Comparisons of patterns and mechanisms across the two coastal systems will provide an important first step towards identifying generalities in the diversity-density-disease relationship. To enhance the broader impacts and utility of this work, the experiments will be conducted in collaboration with restoration practitioners and guided by knowledge ascertained from key stakeholder groups. The project will support the development of an early career female researcher and multiple graduate and undergraduate students. Students will be trained in state-of-the-art molecular techniques to quantify oyster and seagrass parasites. Key findings from the surveys and experimental work will be incorporated into undergraduate courses focused on Conservation Biology, Marine Biology, and Disease Ecology. Finally, students in these courses will help develop social-ecological surveys and mutual learning games to stimulate knowledge transfer with stakeholders through a series of workshops. The relationship between host genetic diversity and disease dynamics is complex. In some cases, known as a dilution effect, diversity reduces disease transmission and risk. However, the opposite relationship, known as the amplification effect, can also occur when diversity increases the risk of infection. Even if diversity directly reduces disease risk, simultaneous positive effects of diversity on host density could lead to amplification by increasing disease transmission between infected and uninfected individuals. Large-scale field restorations of seagrasses (Zostera marina) and oysters (Crassostrea virginica) will be utilized to test the effects of host genetic diversity on host population density and disease prevalence/intensity. Additional field experiments independently manipulating host genetic diversity and density will examine the mechanisms leading to dilution or amplification. Conducting similar manipulations in two marine foundation species - one a clonal plant and the other a non-clonal animal - will help identify commonalities in the diversity-density-disease relationship. Further, collaborations among project scientists, students, and stakeholders will enhance interdisciplinary training and help facilitate the exchange of information to improve management and restoration efforts. As part of these efforts, targeted surveys will be used to document the perceptions and attitudes of managers and restoration practitioners regarding

genetic diversity and its role in ecological resilience and restoration.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1652320

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