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Examination of Polymorphism in the Serine Proteinase Inhibitor Gene in the Eastern Oyster, Crassostrea virginica.

Aine Lehane
aine_lehane@my.uri.edu

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Aine Lehane, University of Rhode Island Undergraduate

Introduction

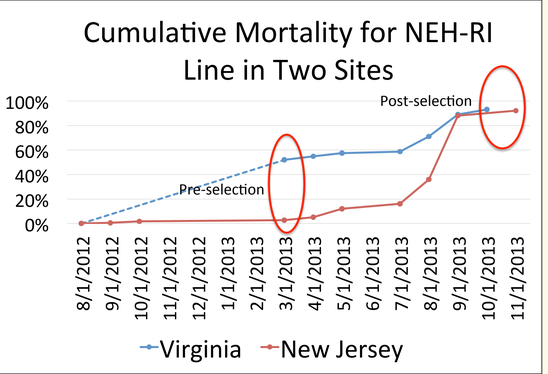
Oysters are a keystone species. They are filter-feeders, reef-builders, and are extremely important within the shellfish aquaculture industry (Zhang and Guo, 2010). Eastern oysters, *Crassostrea virginica*, are produced in aquaculture and are susceptible to Dermo disease. Dermo is an infection causing widespread tissue damage and blockage of blood vessels in oysters and is often fatal. The emergence and spread of Dermo over the last few decades has severely impacted wild populations and the production potential for oyster culture. Millions of dollars are lost each year in aquaculture and millions more are being spent to restore wild populations (Lutz et al., 2011). One of the best options for prevention of Dermo involves identifying oysters with genetic resistance to the disease and breeding them for aquaculture use (Ewart and Ford, 1993). To date, only one gene has been associated repeatedly with Dermo resistance in the eastern oyster: the serine proteinase inhibitor 1 gene (CvSI-1) (Yu et al., 2010; see Table 1). CvSI-1 has been linked to Dermo resistance in eastern oysters and is thought to provide host defense against Dermo by possibly inhibiting proliferation of the parasite (La Peyre et al., 2010). The purpose of this study was to develop an assay for genotyping a single nucleotide polymorphism (SNP) within the CvSI-1 gene and confirm its association with disease resistance in two selected oyster populations. Positive controls (known genotypes at the CvSI-1 SNP198 locus) were identified via amplification and sequence analysis and additional unknown samples were genotyped using a high resolution melting (HRM) assay and compared to the control. Genotypes were used to identify allele frequency shifts following selection by Dermo. A significant difference in allele frequencies before and after Dermo induced mortality suggests the CvSI-1 SNP198 locus is associated with resistance.

Table 1: Evidence of CvSI-1 SNP198 association with Dermo resistance

	AA	AC	CC
Family			
Before Selection	0	51.1	48.9
After Selection	0	36.2	63.8
Selected Line			
Susceptible	20.8	58.3	20.8
Resistant	2.1	29.2	68.8
Wild Type	14.9	31.9	53.2

Genotype frequencies at CVSI-1 SNP198 in different oyster populations. The CC genotype is associated with Dermo resistance. An increase in CC genotype is observed after selection in a family, in a resistant line and in the wild type. (Adapted from Yu and Guo, 2011).

Figure 1:Mortality of NEH-RI at NJ and VA



Dermo-related selection events at Virginia and New Jersey sites resulted in 40% and 90% mortality, respectively.

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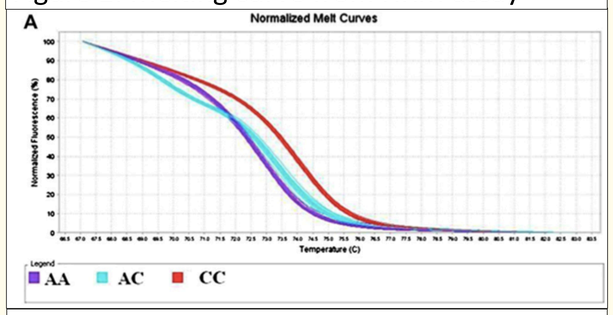
Study Design

An earlier study to evaluate the performance of six selected oyster lines at 5 geographic locations along the east coast of the U.S. with documented disease pressure was completed. Measures of performance included growth, survival and a combined metric for yield. Survival was measured at monthly intervals to identify spikes in mortality, and disease prevalence and intensity were measured concurrently. Oysters were sampled before and after significant mortality events with the expectation that post-mortality samples represent selected, disease-resistant populations.

For this study, before and after mortality samples from a line with undocumented levels of Dermo resistance (NEH-RI) that had been deployed at NJ and VA sites will be genotyped at the CvSI-1 SNP198 locus to further confirm this gene's association with disease resistance. At both sites the NEH-RI line experienced peak disease-induced mortality between March and November of 2013. These individuals were used as samples for this project (Figure 1).

Figure 2: Melting curve from HRM assay



Raw data from an HRM assay, where each colored line represents a different genotype. To determine curve genotype identity, control samples, whose genotypes are determined by direct sequencing must be included in the assay to serve as a point of comparison. (Taken from Yu and Guo, 2011)

Methods

All oyster samples will be genotyped using the qPCR-based High Resolution Melting (HRM) assay. This assay relies on subtle differences in melting temperature among PCR products to discriminate genotype (Figure 2). In order to assign the proper genotype to a melting curve, samples with known genotype must be run alongside unknown samples to serve as controls.

To identify control samples, a portion of the CvSI-1 sequence containing SNP198 was amplified in 28 oyster DNA extracts using the primers listed in Table 2. PCR products were run on a 1.5% agarose gel to confirm successful amplification. Amplification products were purified according to the AMPure XP protocol and submitted to the URI GSC for sequencing.

Sequence data were examined in the software Finch TV, and each sample sequenced was assigned a genotype at CvSI-1 SNP198.

Table 2: Primers used to amplify and sequence CvSI-1 to identify control samples

Primer	Sequence (5'->3')	Product Size
SPI-125 fwd	GTGCGCAAGTGGCTATGTAA	432 bp
SPI-2 rev	GATTTATTTCAAAACAAGAAAACC	

Primer SPI-125 fwd is a novel primer designed specifically for this study. Primer SPI-2 rev was taken from Yu and Guo 2011.

Results

All DNA samples successfully amplified with primer pair SPI-125 fwd/SPI-2 rev and generated PCR products of the expected size. However, several samples generated two DNA bands rather than one (Figure 3) suggesting an insertion/deletion polymorphism within the amplicon, a phenomenon common in oysters. Two-banded amplicons cannot be sequenced directly, therefore only samples with one distinct band were submitted to the GSC for sequencing.

Sequence data was obtained for a total of 12 individuals and genotypes at the CvSI-1 SNP198 locus were distributed as 60% AA and 40% CC (Table 3). One individual might be a heterozygote (AC) at this locus, but sequence data suggests some additional polymorphisms that interfere with clear interpretation of the genotype (Figure 4).

Figure 3: Agarose gels showing successful amplification of the CvSI-1 locus. Red arrows indicate samples that could not be sequenced directly due to multiple bands.

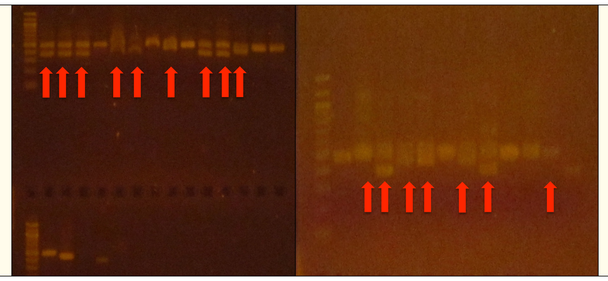
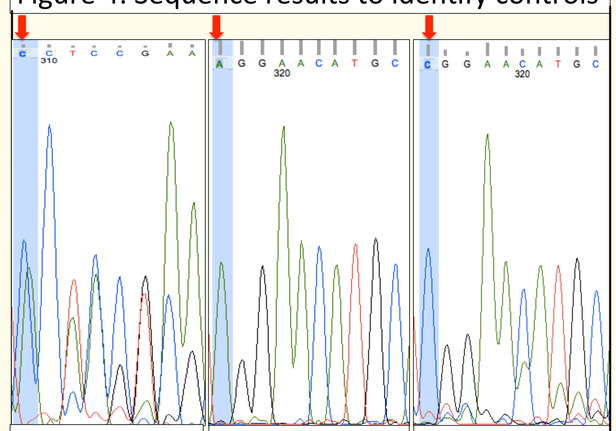


Figure 4: Sequence results to identify controls



NRV1013- 47 Possible AC heterozygote	PGS-22 Sequencing results AA homozygote	PGS-209 Sequencing results CC homozygote

Table 3: Genotypes at the SNP198 Locus

Sample ID	SNP198 Locus
PGS- 62	AA
PGS- 116	AA
PGS- 209	CC
PGS- 215	AA
PGS- 220	AA
PGS- 244	AA
PGS- 250	AA
PGS- 253	AA
NRJ11-41	CC
NRV1013- 18	CC
NRV1013- 39	CC
NRV1013- 47	AC?

Conclusions

Seven individuals were identified as good candidates for the AA genotype control while 4 individuals can be used as the CC genotype control in the CvSI-1 SNP198 HRM genotyping assay. Additional sequencing needs to be done to isolate an individual with the AC genotype at this locus. Once the three genotype controls have been identified, DNA extracted from NEH-RI oysters collected in NJ and VA in the spring and subsequent fall of 2013 will be genotyped at CvSI-1 SNP198 to validate this marker's association with Dermo resistance.

References

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