Epigenetics in offspring of oysters infected with *Perkinsus marinus*

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Abstract

Oysters are important organisms in maintaining the health of their aquatic ecosystems as well as supporting the aquaculture industry. Through filter feeding, they remove bacteria and other particles out of the water, keeping the water clean. Because of this, their innate immune systems are constantly exposed to different microbes and pathogens. The Eastern oyster (*Crassostrea virginica),* is a widespread keystone species susceptible to such pathogens. One parasite of particular concern is *Perkinsus marinus,* which degrades the tissues of its host overtime. However, through epigenetic processes that allow preconditioning and phenotypic plasticity, it is possible that the offspring of these oysters will develop resistance to this parasite. In order to test epigenetic processes in the Eastern oyster, we can compare the offspring of two groups: one group that is exposed to *P. marinus,* and another group that is not. Through preconditioning, we expect to find the offspring of the exposed group more resistant to parasitic infection. This research aims to uncover a mechanism which allows aquatic organisms to acclimate to a rapidly changing environment, helping them thrive in the face of stress.

Introduction

Epigenetics is the study of potentially heritable changes in gene expression that do not involve changes to the underlying DNA sequence — a change in phenotype without a change in genotype. Perhaps the best-known epigenetic process, in part because it has been easiest to study with existing technology, is DNA methylation. This is the addition or removal of a methyl group (CH3), predominantly where cytosine bases occur consecutively (Weinhold, 2006). Epigenetic change is a regular and natural occurrence but becomes more likely given certain factors, including age, environment, lifestyle, and disease state (Brouwer, 2012). For example, if an organism is living in the presence of a stressor, this can affect their offspring's phenotype, changing how they react to this same stressor without re-shaping their genome. Although DNA methylation is considered to be an ancient evolutionary mechanism, the process is not fully understood (Roberts, 2013). This process also involves preconditioning, which is when an organism is living in certain environmental conditions for an extended period of time and begins to acclimate to those conditions (Munguia 2013).

Epigenetic markers are commonly used to measure immunity and resistance in organisms. Invertebrates such as shellfish do not have an adaptive immune system, and instead rely on innate immunity to combat pathogens. Despite this protection, these organisms remain susceptible to numerous diseases and infections. This is problematic for both the organisms and their consumers. Americans consume an average of 15.8 pounds of fish and shellfish per year, making oysters an economically important food source (National Oceanic Atmospheric Administration, 2017).

In addition to playing a substantial role in feeding the human population, they are also beneficial to their native environments. Not only do they provide food for fish, crustaceans, and sea otters, but oysters and mussels are also filter feeders, consuming phytoplankton and thereby improving water quality, preventing eutrophication and hypoxic events. As generations of oysters settle on top of each other and grow, they also form reefs that provide structured habitats for many fish and crabs (National Oceanic Atmospheric Administration, 2017). However, the efficiency of their filtration poses a threat; it exposes these organisms to many harmful substances, including contaminants, pathogens, and parasites.

One parasite of a particular concern, *Perkinsus marinus,* is a species of protist belonging to the phylum Perkinsozoa. It is a protozoan parasite of the Eastern Oyster *Crassostrea virginica*, and is prevalent along the Atlantic and Gulf coasts of the United States. This parasite is the cause of Dermo disease, which degrades oyster tissue over time, devastating natural and farmed oyster populations (Joseph, 2010). *P. marinus* proliferates *in vivo* most rapidly at water temperatures above 18°C and salinities greater than 15°C ; however, experimental infections have been achieved at 10°C and 3°C (Kern, 2011). *P. marinus* is transmitted from infected to uninfected oysters through filter feeding of infective particles (Powell, 2017). Transmission is commonly attributed to the release of parasites through the feces of live infected oysters and decaying tissues of deceased oysters. This is often followed by increases of free *P. marinus* in the water column (Powell, 2017). Because these modes of contracting *P. marinus* are nearly impossible to manage on such a large scale, we must measure the oysters' ability to withstand them. Thus, observing the role of epigenetic processes in shellfish could significantly improve our understanding of the shellfish immune system (Roberts, 2013). One way of doing this is by looking at the way oysters respond to environmental stressors across generations. Usually, phenotypic plasticity refers to the ability of an organism to change its phenotype in response to changes in the environment (Price, 2003). This process provides them with a buffer against these changes. But by measuring the degree to which phenotypic plasticity influences immunity in oysters across generations, we can gain more information on transgenerational plasticity and how DNA methyl groups change overtime. This is essential for understanding all organisms' responses to change. We can look for certain influences that create differences in DNA methyl group patterns and use that to learn how an organism deals with stressful conditions on an epigenetic level. And as the ocean continues to face stressors such as climate change and ocean acidification, this would provide us the tools necessary for predicting future responses from an array of organisms.

Observational or Theoretical Motivation for Research

Oysters are a model organism to do epigenetic research on. Like other invertebrates, they do not have an adaptive immune system, and instead rely on their innate immune systems. (Zhang, 2014). Because of this, we can observe if, and how, innate immune systems respond to the presence of parasites through observation of DNA methylation and survivorship. Oysters are also constantly in the presence of microbes, many of which are potential pathogens. The fact that they can thrive in these microbe-rich environment as filter-feeders is a testament of their immune system's resilience (Zhang, 2014). As many other mollusks feed this exact same way, this makes *C. virginica* an exemplary subject to research. We can then expand the results from this study across all other species of invertebrates who face the detriments of parasitic infections.

Taking it a step further than parasitic immunity, understanding the role that epigenetics plays in preconditioning could change the way scientists look at innate immunity or adaptation in response to major environmental changes or threats. What if an organism's phenotype is changed by the environment and then inherited? What does this mean for adaptation? Instead of changes in the DNA sequence that occur throughout long periods of evolution, organisms have the potential to quickly change their gene expression without altering the actual DNA sequence. This research has the potential to uncover a mechanism that helps invertebrates thrive in the face of rapid environmental change. This is critical now more than ever, as climate change and ocean acidification continue to wreak havoc on the ocean. Understanding oyster's amazing resilience against biotic and abiotic stressors may provide insights to the evolution of the immune and stress response systems in protostome invertebrates (Zhang, 2014).

Oysters are also of ecological and economic importance. They are keystone species in estuarine ecology and provide important ecological services as filter-feeders and reef-builders (Zhang, 2014). Oysters support major aquaculture and fishery industries worldwide. Aquaculture production of oysters amounted to 4.7 million metric tons (FAO, 2014). Because of their economic and ecological importance, oysters are popular models to study molluscan biology, development, innate immunity and stress adaptation (Zhang, 2014). Studies on oysters' immune and stress responses may contribute not only to our understanding of host-defense and adaptation, but also to the development of superior stocks for aquaculture (Kocher, 2008). We could implement these epigenetic mechanisms in testing all shellfish resilience to parasites, and see which species survives. This information could help manage aquaculture by dictating which shellfish to grow on farms, contributing to cost-effective practices and sustainability.

Epigenetics are a part of fundamental physiological processes that apply to everything with DNA- including humans. Research has been done on DNA methylation over an organism's lifetime, but very few studies have observed these epigenetic processes across generations. For example, a study executed in 2005 found that water fleas (*Daphnia magna*), exposed to microsporidian parasites produce more offspring in the early stages of exposure to compensate for future loss of reproductive success (Chadwick, 2005). The study of DNA methylation is providing remarkable insight into gene regulation and the complex mechanisms associated with phenotypic variation and the response to stressors. DNA methylation is present in species from prokaryotes to humans, however there is dramatic diversity in characteristics - from species where this phenomenon is absent, to cases where the genome is globally methylated (Roberts, 2013).

Research Question

Do oysters that are infected with *Perkinsus marinus* produce offspring that are more immune to this disease because of preconditioning?

Hypothesis

One hypothesis is that the offspring of oysters infected with *P. marinus* will be more resistant to this parasite than the ones whose parents were not infected, because of phenotypic variation and epigenetic processes that respond to environmental stressors. Through changes of gene expression, the next generation of oysters will be able to withstand the parasite because of preconditioning. This happens through DNA methylation, which is the addition of a methyl group to a cytosine base. This could contribute to phenotypic plasticity by increasing the number of transcriptional opportunities (Roberts, 2011).

An alternative hypothesis is that epigenetic processes will have no effect on immunity, and offspring will be just as susceptible regardless of exposure in prior generations.

Experimental Design and Methods

This experiment will be conducted using 24 *Crassostrea virginica* oysters collected from the Gulf of Mexico off the coast of Florida. I will separate them into 2 groups of 12 oysters. One group will be the “naive” group and the other will be the “exposed” group. Each group will be placed in separate tanks with a continuous flow of seawater adjusted to a temperature of 19 °C, which is the average temperature of the Gulf (National Centers for Environmental Information, 2017). After an acclimation period of 8 to 12 days, the experiment will proceed (Thompson, 2012). I will inject the exposed group of 12 oysters with the parasite *Perkinsus marinus*, and inject the naive group of oysters with a placebo to account for any error. I will look at the epigenomes of both groups, and take note of varying DNA methylation patterns.

Once acclimated, environmental conditions will be manipulated to trigger spawning in the oysters. Temperatures in both tanks will be increased to 25°C, and salinity will be above 10 ppt in order to induce spawning (Wallace 2001), (University of Maryland).

After the production of offspring from each tank, I will separate them again into new tanks based on whether or not their parents were infected with *P. marinus.* When the offspring reach their juvenile stage, I will begin to observe their survival and mortality rates, noting any difference in these rates between the two groups.

The final part of the experiment will involve infecting both groups of offspring with *P. marinus* to test any potential beneficial or detrimental epigenetic processes on immunity. Different dosages ranging from 2 to 12 µl of the parasite will be injected into all of the offspring in both tanks. I will then measure the performance, feeding rate, growth rate, and mortality rate of the offspring. Any signs of infection among offspring, such as lesions on the tissue will be noted. The main effects of *P. marinus* infection in *C. virginica* range from pale appearance of the digestive gland, reductions in condition index, impaired gametogenic development, reduced hemolymph protein concentrations and lysozyme activity, to severe emaciation, gaping, shrinkage of the mantle away from the outer edge of the shell, slowed growth and occasionally the presence of puss-like pockets, which will all be measured. (Ford and Tripp, 1996). Survival and mortality will also be recorded. In order to measure the full effects of preconditioning over multiple generations, I will also observe the offspring's oocyte size, fertilization rate, and overall fitness. In terms of epigenetics, the epigenomes of the parents will be compared to those of their offspring in search of any changes in DNA methyl group patterns.

Anticipated Results

Due to preconditioning and phenotypic plasticity, I anticipate that the offspring of oysters infected with *P. marinus* will have some sort of immunity, or will have more tolerance to this parasite (fig 1). This would signify that an epigenetic process has occurred which allows the exposed oysters to alter their phenotype, and then pass this down to their offspring. Meanwhile, the offspring whose parents were not exposed to the parasite will have higher mortality rates, as no phenotypic change in gene expression occurred. Thus, the offspring were not able to inherent any traits that allowed them to adjust to this same stressor.

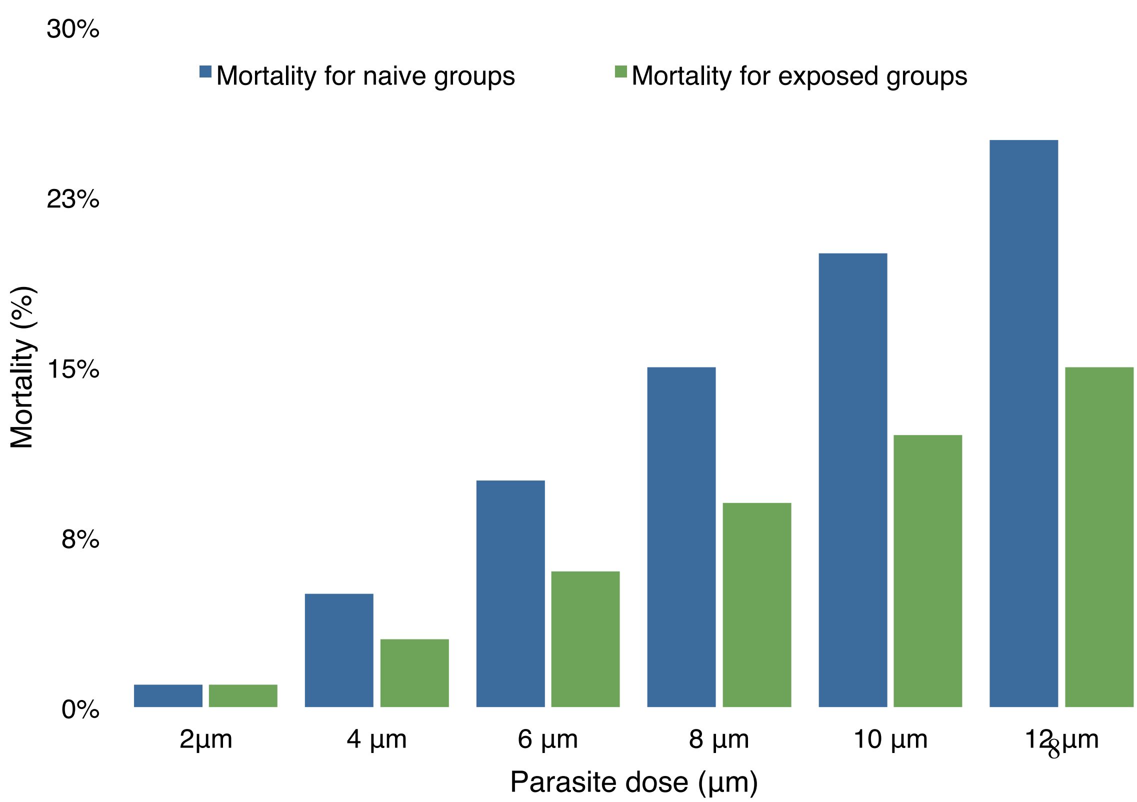

Figure 1. Mortality rates in offspring of naive and exposed groups of oysters after being injected with *P. marinus.* Offspring of the exposed parental groups had significantly higher survivorship than than the offspring of the naive groups.

Discussion

The offspring of exposed groups having more resistance to parasitic infection could contribute to our understanding of defense and immunity mechanisms in invertebrates. If there are any changes in DNA methyl group patterns on the genome of the offspring, we can assume that an epigenetic process has occurred in which gene expression has been altered. In species that experience a diverse range of environmental conditions, these processes have evolved in order to increase the potential phenotypes in a population to improve the chances of survival (Gavery, 2010). From these results, it is possible that oysters are able to alter their phenotype based on their parents' exposure, making them less susceptible to *P. marinus.* This could redefine modes of adaptation, immunity, and how the aquaculture industry chooses what shellfish to farm.

Alternatively, if both groups of offspring show no resistance to the parasite, there is a likelihood that the offspring were not preconditioned to withstand *P. marinus.* Through further assessment, we could quantify whether or not this had to do with the lack of a transgenerational epigenetic process in the offspring. This would mean that methyl group patterns on the epigenome were not inherited, revealing that these processes only occur over the span of an organism's lifetime, rather than across generations. However, it is also possible that even though the exposed group of parent oysters were infected, their methyl group patterns did not change at all. Thus, no epigenetic response in the presence of this parasite occurred. From here, we could conduct further research investigating what conditions trigger changes in methyl groups and in phenotype.

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