Exploring the Genetic Variability of Zebra Mussels *Dreisenna polymoprha*

Maxwell Carr
Aquatic Biology Program
Biology Department
Bemidji State University

Zebra Mussels *Dreisenna polymorpha* are one of the most well-known invasive species here in the United States. This species can take over watersheds very quicky, causing damage to both the ecosystem and man-made structures. The basic physical behavior of this species has been well documented, but the invasive North American Zebra Mussel is much more aggressive and therefore causes more damage in the Great Lakes region compared to its native waters. Genetic variation may be playing a very large role in how and why *D. polymorpha* is causing such large problems for stakeholders in the United States. This review cites both past and current literature in order to discover possible reasons why *D. polymorpha* is "mutating".

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Background of *Dreisenna polymorpha*

Native to the waters of Europe, such as the Caspian Sea, Zebra Mussels *D. polymorpha* are relatively small, about the size of a human fingernail. This species is considered invasive in many parts of the United States, specifically around the Great Lakes region. *D. polymorpha* was inadvertently brought to the U.S via the trading ship ballast water that was then expelled into the freshwaters of the Great Lakes. *D. polymorpha* are very effective filter feeders, possessing both inhalant and exhalant siphons. An adult is capable of filtering up to 1 L of water per day (Snyder et al. 1997). with their primary diet consisting of zooplankton and bacteria. They are also very prolific reproducers; an adult female can release up to one million eggs (Snyder et al. 1997) into the water over the course of one single year.

Why are they bad?

Unlike native freshwater bivalves, *D. polymorpha* share similar characteristics to marine bivalves, meaning they can become so densely populated that "reefs" of Zebra Mussels may occur. Since *D. polymorpha* become vastly dense so quick, they outcompete native mollusks and invertebrates for food and this could lead to a decline in population or possible extinction for those native organisms. Since the introduction *of D. polymorpha* in the Hudson River estuary in 1991, there was a very sharp decline in all species of native bivalves between 1992 and 1999. The estimated decrease in native bivalve population size was between 65% and 100%. The population and recruitment of unionid species were also negatively impacted (Strayer and Malcom 2007).

D. polymorpha are also the only freshwater bivalve to attach to other hard surfaces such as dock posts (Leppäkoski et al. 2002). Zebra Mussels' likelihood to attach to hard surfaces in large numbers has led to many problems including clogging of water intake pipes and in some cases even ruining boat motors. This is called mussel biofouling and generally only takes place in marine ecosystems. Although they have only been in North America for about 35 years, biofouling is probably the number one impact on stakeholders. Biofouling by *D. polymorpha* have caused major problems such as clogging of the water-cooling intake pipes of water treatments centers and power stations across the Great Lakes region. It is estimated that this biofouling costs the U.S. upwards of one billion dollars each year (Aldridge et al. 2006).

Another large impact these mussels have on infested waters is water clarity. A study done on Oneida Lake located in New York in 1991 concluded that the increase of water clarity was due directly to the presence of *D. polymorpha* and their intense filter feeding, rather than a lack of nutrients. After the invasion of *D. polymorpha* in Lake Oneida, water clarity increased from 6.7 to 7.8 m (Greeson 1971). While some may argue this is a good thing for murky lakes, a sudden increase in water clarity due to high rates of phytoplankton filtration is not good. This will cause more light to be available throughout the water column and possibly change growth rates of certain plant species and maybe even feeding times of fish (Macisaac and Hugh 1996). Plants that receive more light due to water clarity improvement caused by *D. Polymorpha* are able to grow much more rapidly and for longer periods of time. This may frustrate stakeholders who enjoy the lake.

Genetic Variation in Nature

Genetic variation is the difference in DNA among individuals of the same species. There are multiple causes of genetic variation, including mutation and genetic recombination. In some cases, genetic variation may become so extreme that it causes speciation. Speciation occurs when a group of organisms within the same species separates from the rest, reproduces, and ultimately creates a new species with its own unique genetic differences.

Natural selection and mutation are the ultimate sources of genetic variation, but other mechanisms such as type of sexual reproduction and genetic drift contribute as well. In mammalian species, smaller populations often lose genetic variability relatively fast within the gene pool due to inbreeding. This decrease in genetic variation can lead to negative impacts on the population, such as lesser ability to resist disease, stunted growth rates, and inferior ability to react properly to environmental change (Lacy 1997).

A study done on the genetic population structure and spatial distribution regarding the genetic diversity of *Radix balthica*, a species of air breathing freshwater snail native to the waters of Europe and Asia found that a relatively recent change in climate has given rise to a large change in range expansion for this species (Pfenninger et al. 2011). This sudden change in habitat expansion was found to actually have a considerable negative impact on genetic variation due to recurrent population bottlenecks and founder effects. Population bottlenecking occurs when the population size is reduced for more than one generation, whereas a founder effect occurs when an entire new colony is formed in a new location by a few members of the original population. This drastic decrease in genetic variation may also be enhanced by *R. balthica*'s ability to reproduce asexually (Pfenninger et al. 2011). Asexual reproduction often causes little to no genetic variability within a population due to the fact that there is no fusion of gametes and therefore no change in chromosomes, making the offspring basically a clone of the parent organism.

Compared to *R. balthica, D. polymorpha* seem to be much more ready to adapt to environmental stimulus because the population gene pool stays relatively large, mostly due to their prolific sexual reproduction. In warmer waters and systems with plenty of rocky substrate and phytoplankton, *D. polymorpha* are able to have multiple successful spawning windows. Whereas, when *R. balthica* spread to more temperate waters, the method of reproduction changed from sexual to asexual, resulting in the gene pool slowly getting smaller and ultimately resulting in a "genetic dead-end" (Pfenninger et al. 2011). The term "genetic dead-end" refers to organisms that only reproduce asexually, over a long time this causes each offspring to be an exact replica of the parent organism, basically meaning that each organism within that population is going to have the same allelic characteristics. Although *D. polymorpha* may seem like an organism that would fit this category because on the outside there are rarely any phenotypical differences from population to population, this is not the case because studies have indicated that there are many allelic differences from population to population.

The Genetics of *D. polymorpha*

Due to stakeholder interest caused by all the problems *D. polymorpha* have caused, the complete genetic code was sequenced for *D. polymorpha*. Now that the full genetic code has been sequenced, scientists can use comparative analysis as well as RNA sequencing experiments to provide important insight on allelic characteristics such as thermal tolerance, shell formation, and formation of the byssal thread fibers.

Since their introduction to the Great Lakes in 1988 many comparative studies have been made between the invasive populations found here in North America and the original populations found in Europe. It has been noted that although the two populations are extremely similar in basic biological function, the North American Zebra Mussel is different in regard to population dynamics such as life span, growth rates, potential population range limits, calcium and pH tolerances, and differences in ratio between veligers and adults (Mackie and Schloesser 1996). This difference in genetic variation between the native species and the invasive species is very important as it can help scientists make well educated hypotheses as to why *D. polymorpha* behaves much differently in the U.S compared to its native waters.

A research study performed along a latitudinal gradient of the Mississippi River that sampled seven different populations of *D. polymorpha,* using gel electrophoresis and estimated heterozygosity showed large genetic differences among these populations. These genetic differences were observed by analyzing the leucine aminopeptidease locus (Lap) as well as the manose phosphate isomerase (Mpi). A Mantel's test was then performed and indicated a strong correlation between genetic distance and geographic distance. These results showed that nonrandom factors such as natural selection may be having a significant impact on the Lap gene locus and possibly on linked genes as well (Elderkin et al. 2001). Within the Mississippi River, gene flow would generally balance out allelic differences between populations. These results suggest that selection would have to be quite strong in order to overcome recruitment from populations found upstream.

Why is this an important topic to explore?

By examining the allelic differences of *D. polymorpha* populations throughout the U.S and then comparing those findings to the data of *D. polymorpha* in their native waters it can provide biologists and other researchers the capability to make very well-educated hypotheses as to which populations in the U.S came from which areas across seas. This comparison of data could also possibly provide helpful insight regarding how to better control *D. polymorpha* as an invasive species.

Within the field of ecology, scientists have often combined genetic variation data with various risk assessment tools to evaluate the future relative success of invasive species. This allows for variables such as number of founding individuals, population sources and genetic variants to be analyzed. This amount and type of data can provide great insight as to how and why invasive species, specifically, *D. polymorpha* can successfully establish and spread very quickly. Genetic evidence strongly suggests that in the case of *D. polymorpha* and other similar invasive species such as the Quagga Mussel *D. bugensis,* three main factors contribute to these two species high risk level of invasion effectiveness. These three factors are multiple founding sources, relatively high genetic variability in comparison to the founding population and large amounts of founding organisms being introduced at the same time (Stepien et al. 2005).

Now that biologists have sequenced the entire genome of *D. polymorpha*, studies have been performed regarding gene expression stress indicators such as Catalase, Cytochrome, Glutathione and heat-shock proteins HSP70 and HSP90. One study that used real-time polymerase chain reaction (PCR) while analyzing many gene expression stress indicators, showed a defined pattern of coordinated responses after adult Zebra Mussels had been exposed to mercury, copper and cadmium. Oxidative stress was also observed in the digestive gland and gills of the adults, which was most likely caused by the metal exposure. Furthermore, a very similar, yet partial genetic response was observed in *D. polymorpha* larvae (veligers) after being exposed to the same metals (Navarro et al. 2011). These results strongly indicate that *D. polymorpha* develop stress related gene responses very early in life. This could potentially be very helpful for scientists trying to find a way to control this invasive species as well as other similar invasive species such as *D. bugensis*. But, if Zebra Mussels are here to stay, they are at least a very good bio-indicator for detecting pollutants within a watershed.

Why is *D. polymorpha* "mutating"

Most scientists and other peer reviewed sources conclude that the main reason Zebra Mussels behave differently here in North America compared to their native waters of Russia and the Ukraine is due to the difference of water temperature. Data collected from native northern European populations of *D. polymorpha* showed an upper thermal tolerance of only 28 °C (Hernandez et al. 1995). Considering water temperatures exceed 28 °C quite often in the here in the United States, biologists have speculated that natural selection for thermal tolerance among specific populations of *D. polymorpha* is occurring.

A research study performed in 1994 collected samples of *D. polymorpha* from the lower Mississippi River as well as the Niagara River. The sample specimens were then exposed to a lethal water temperature of 33 °C and the time until death was recorded. Although, a multiple factor ANOVA test indicated there was no difference in thermal tolerance temperature between the two groups, a least squares regression analysis indicated that the sample mean thermal tolerance temperature of the specimen group from the lower Mississippi River had increased. These results suggest a possible seasonal pattern of thermal tolerance regulation (Hernandez et al. 1995).

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