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Interactions of Heterozygosity and Fitness in Unionid Bivalves (Mollusca)

Introduction

Numerous studies have documented relationships between heterozygosity of enzyme loci and measures of fitness of marine bivalves (Mitton and Grant 1984). These studies have assumed that higher viability (survivorship) and growth rate reflect greater fitness, while higher rates of metabolism and protein turnover are associated with lower fitness. These effects have been examined at single enzyme loci and across loci (multiple locus heterozygosity). In the oyster (*Crassostrea virginica*), multiple locus heterozygosity is positively correlated with survival of post–settlement juveniles (Zouros et al. 1983). When individual loci are examined, growth rates of oysters, mussels (*Mytilus edulis*), and clams (*Mulinia lateralis*) are greater in heterozygotes than in homozygotes (Singh and Zouros 1978, Zouros et al. 1980, Koehn and Gaffney 1984, Gaffney 1990), although a few studies have documented greater fitness of a homozygous genotype under certain conditions in *Mytilus* (Hilbish and Koehn 1985a, b). In addition, individuals with higher multiple locus heterozygosity have higher growth rates in *Mulinia* and *Mytilus* (Garton et al. 1984, Gaffney et al. 1990). Most of these studies have concluded that increased growth rate associated with heterozygosity is due to greater energetic efficiency. Metabolic rates (measured as oxygen consumption) are negatively correlated with multiple locus heterozygosity (Koehn and Shumway 1982, Diehl et al. 1985, 1986), implying lower maintenance costs. In *Mytilus*, protein turnover rates are also lower for heterozygotes than homozygotes, resulting in more protein available for growth and reproduction of heterozygotes(Hawkins et al. 1989). Thus, greater heterozygosity leads to greater energetic efficiency, which is then translated into higher growth rates and greater survival.

In contrast to marine bivalves, little is known of the physiological ecology and genetics of freshwater bivalves. Shell length (and hence growth rate) of zebra mussels (*Dreissena polymorpha*) is positively correlated with multiple locus heterozygosity, and single locus effects (average shell length greater for heterozygotes than homozygotes) are present at two loci (Garton and Haag 1991). In terms of life history, zebra mussels (Family Dreissenidae) are similar to marine bivalves in that they exhibit external fertilization, produce planktonic veliger larvae, and attach to hard substrates by means of a byssus. The common freshwater bivalves of the Family Unionidae, however, have internal fertilization, produce parasitic glochidial larvae, and burrow into soft substrates (Mackie 1984). No studies of genetic interactions with physiological energetics have been published for these organisms, although developmental stability (decreased morphological variability) is positively correlated with multiple locus heterozygosity in *Lampsilis radiata* and *Elliptio complanata* (Kat 1982).

The recent invasion of the North American Great Lakes by the zebra mussel provides an opportunity to examine heterozygosity–fitness interactions in populations of unionid bivalves. Because of their preference for hard substrates, zebra mussels attach to native unionid clams at levels up to 10,000 zebra mussels per unionid (Hebert et al. 1989, 1991, Schloesser and Kovalak in press). Fouling by zebra mussels has a severe cost to unionids. In a series of field experiments, female *Lampsilis* suffered extremely high mortality when encrusted with zebra mussels (Figure 1). Mortality was probably due to some sort of energetic constraint (i.e., starvation) since parallel experiments with another unionid

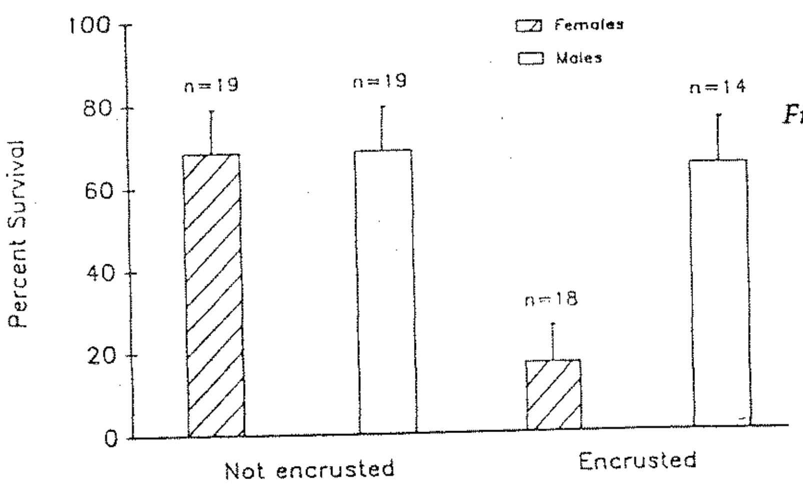

Figure 1. Percent survival of Lampsilis radiata *in three-month field experiments. Error bars represent one standard error. Females suffered greater mortality when encrusted by zebra mussels (ANOVA, p<0.05). Results are from Haag et al. (in prep.).*

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species (*Amblema plicata*) resulted in no mortality effects but did show reduced glycogen levels and decreased cellulase enzyme activity (an indicator of stress in bivalves (Farris et al. 1988)) in individuals encrusted by zebra mussels (Haag et al. in prep.). Glycogen reserves and cellulase activity were much lower in females than in males, further indicating the vulnerability of females to fouling by zebra mussels. In Lake St. Clair, lipid reserves of unionids were negatively correlated with biomass of attached zebra mussels (Hebert et al. 1991), additional evidence of the energetic cost of zebra mussel fouling of unionids. If unionid bivalves exhibit heterozygosity–related interactions with metabolic efficiency similar to those noted for marine bivalves, the energetic costs associated with encrustation by zebra mussels provide a mechanism for examining these relationships in unionids.

In order to determine whether heterozygosity is associated with fitness of unionids and if encrustation by zebra mussels results in changes in genetic structure of unionid populations, I propose to answer the following questions.

- Is unionid fitness and viability positively correlated with single and/or multiple locus heterozygosity?

- Are the responses of unionids to fouling by zebra mussels determined by single locus genotypes and are these responses sex specific? Are these responses due to increased physiological fitness (measured as energy reserve, growth rate, and fecundity) of heterozygotes?

- Does increased multiple locus heterozygosity of unionids decrease the magnitude of zebra mussel fouling effects? Is this decrease in response the result of positive correlations of multiple locus heterozygosity and physiological fitness?

These questions will be addressed by testing the following hypotheses.

1) In manipulative field experiments, heterozygous unionids will have lower mortality and stress (higher cellulase activity) and greater energy reserves (glycogen, lipids) and fecundity than homozygotes. The differences between heterozygotes and homozygotes will be greater when zebra mussels are attached than when zebra mussels are absent. Effects of zebra mussels will be greater on female unionids than on males.

2) In manipulative field experiments, multiple locus heterozygosity of unionids will be negatively correlated with mortality and stress and positively correlated with energy reserves and fecundity. Multiple locus heterozygosity effects will be greater for females than for males.

3) In surveys of unionid populations, those fouled by zebra mussels will show greater frequencies of heterozygotes and higher multiple locus heterozygosity than similar populations that are not fouled.

4) In surveys of unionid populations, magnitude of fouling by zebra mussels (number or biomass of attached zebra mussels) will be positively correlated with stress and negatively correlated with energy reserves and fecundity of unionids. The slopes of these relationships will be steeper for homozygotes than heterozygotes (greater impact on homozygotes).

Confirmation or rejection of these hypotheses will allow me to determine whether heterozygosity is associated with fitness of unionids. If such a relationship exists, this study will elucidate the mechanisms by which zebra mussels may alter abundance and genetic structure of unionid populations. If these hypotheses are rejected, the design of the project will also allow me to examine correlations between specific genotypes and fitness.

Materials and Methods

This study is designed to determine whether single locus or multiple locus heterozygosity are major factors determining fitness of freshwater bivalves. It uses the energetic stress provided by zebra mussel fouling as a manipulation that tests hypotheses of greater metabolic efficiency with increasing heterozygosity. The methods described below will allow me to determine whether, in fact, there is a direct relationship between genotype and ability to respond to energetic challenges. By combining field experiments (which are limited in geographic scope) with field surveys, the project will enable me to ascertain whether experimental conclusions are generalizable across populations. At the completion of the study, I will be able to conclude whether heterozygosity-related interactions are a major force structuring unionid populations and if the presence of zebra mussels will alter genetic structure of these populations.

Because little is known of the physiological ecology and genetics of unionid populations, the initial portion of the study must be spent examining the energetic status and genetic structure of natural populations. Earlier work indicates *Lampsilis radiata* is a good candidate for use in this project. This species is relatively common in Lake Erie and throughout lakes and streams in Ohio (Haag et al. in prep., D. W. Garton pers. comm.). Procedures for analyzing biochemical fitness have been developed by myself and colleagues (Haag et al. in prep.), growth rate studies have been published or are in progress (Bailey and Green 1988, Janke unpub. data), and the species appears to contain reasonable levels of intrapopulation genetic variation at a number of enzyme loci (Kat 1982, D. W. Garton unpub. data). In addition, *Lampsilis* is sexually dimorphic based on external shell morphology, allowing sex-related responses to be examined.

<u>Year 1</u>

The first year of the project will be spent surveying *Lampsilis* populations throughout Lake Erie and inland lakes and streams in Ohio, Indiana, and Illinois. Lake Erie sites will include South Bass Island and Kelley's Island, which contain large *Lampsilis* populations (personal observation), and other sites determined in consultation with Drs. D. Stansbery and T. Watters of the Ohio State University Museum of Zoology. Inland sites will include eutrophic lakes or reservoirs (lentic habitat similar to Lake Erie) and streams. These sites will also be determined after consultation with Drs. Stansbery and Watters. Most Lake Erie individuals will be fouled with zebra mussels (Haag et al. in prep.), while other populations should be free of zebra mussel encrustation. Divers will collect unionids, assessing population size and relative abundance of unionid species. *Lampsilis* will be retained, frozen immediately and returned to the laboratory for morphological, biochemical, and electrophoretic analyses. The extent of zebra mussel fouling will also be assessed. Morphological analysis will allow determination of age structure, sex ratio and growth rates of populations. Energetic status will be determined by examining weight-specific glycogen and lipid content and cellulase activity. These procedures have already been developed for use with unionids by myself and colleagues (Farris et al. 1988, Haag et al. in prep.). Starch gel and/or cellulose acetate gel electrophoresis techniques will be used to determine genetic variation of populations. These techniques, modified for use with unionids, have been developed in the laboratory of D. W. Garton, my Ph.D. advisor. Completion of these population surveys will provide information for identifying populations to be used in field experiments.

In addition to conducting surveys, the first year of the project will be used to intensively monitor one *Lampsilis* population from Lake Erie, a mainland lake, and a stream in order to determine the reproductive cycle of this species. Earlier work indicates that *Lampsilis* females brood young for long periods of time, releasing glochidia larvae from April through August (Clarke 1981). Monitoring the reproductive cycle of populations from Lake Erie and inland lakes and streams will allow me to know when to begin field experiments in order to examine effects of zebra mussel fouling on fecundity of unionids.

Year 1 will also be used to refine techniques for handling mussels, removing small pieces of mantle tissue from living unionids, and running electrophoretic analyses on the mantle tissue. Survivorship of unionids following removal of mantle tissue will also be monitored. Methods for individually identifying unionids (such as plastic tags or shell marks) will be developed.

At the end of Year 1, I will have identified populations of *Lampsilis* for use in field experiments in Years 2 and 3, and have perfected the techniques needed for conducting those experiments. In addition, I will have collected data that reveal patterns of energetic status and genetic variation in populations of *Lampsilis* over a large portion of its range and have gathered details on the reproductive cycle of this species.

<u>Years 2 and 3</u>

The second and third years of the project will be used to conduct manipulative field experiments and more extensive surveys of *Lampsilis* populations. The results of these activities will be used to test the hypotheses stated earlier.

Field experiments will be conducted at the South Bass Island site on Lake Erie and at an inland lake site to be determined during the surveys conducted in Year 1. *Lampsilis* will be collected from a single population and all zebra mussels will be removed. Individuals will be from a single size and age class, removing size and age as factors in the experiments. Genotypes of each individual will be determined by electrophoresing a small piece of mantle tissue. Individuals will be marked and assigned to treatment groups based on sex and genotype. Zebra mussels will be allowed to attach to half of the individuals for the Lake Erie experiments. The experiments will be a 2x2x2 factorial design, with sex, genotype, and zebra mussel encrustation as factors for the Lake Erie experiments and a 2x2 factorial design with sex and genotype as factors for the inland lake experiments. Mortality, growth rate, stress (measured as cellulase activity), energy reserves, and fecundity will be used as response variables. Genotype will be classified as homozygous or heterozygous at each polymorphic locus. Thus, for each of these loci, an experiment will be performed. For example, an individual may be classified as a homozygote for analyses examining one locus while being classified as a heterozygote when a second locus is examined. Because of this locus-by-locus method, sample size of homozygotes versus heterozygotes will vary with each experiment but such problems should be tractable using ANOVA procedures for unbalanced designs. Effects of multiple locus heterozygosity will be examined by determining mean heterozygosity for the population and then classifying individuals as having average heterozygosities above or below the mean. This will also result in a 2x2x2 factorial design with sex, level of heterozygosity, and zebra mussel encrustation as factors for Lake Erie experiments and a 2x2 design for the inland lake experiments. In addition, multiple regression techniques will be used to examine relationships between multiple locus heterozygosity and the response variables.

Populations of *Lampsilis* will be surveyed in Lake Erie and at mainland sites. These surveys will compare growth rate, stress, energy reserves, and fecundity versus single locus genotype at all polymorphic loci using ANOVA techniques. Multiple regression techniques will be used to examine these factors as functions of multiple locus heterozygosity. For populations suffering from zebra mussel fouling, multiple regression techniques will be used to account for the effects of both genotype and zebra mussel encrustation.

Significance of Project

This study is designed to test whether basic theories in ecological genetics may be extended to account for differences in fitness of unionid populations. Previous work on fitness-heterozygosity interactions has been confined to marine bivalves, which possess life history patterns very different from those of most freshwater bivalves. Thus, this work will examine the generality of theories developed from research on marine bivalves. One of the unique aspects of the proposed project is its use of an invasion event as the selective force determining fitness of native populations. By relying on this "natural" event and using field experiments and surveys, this project will assess fitness of unionids under conditions much more realistic than normally exist in laboratory studies, utilizing an agent of selection that may have severe effects on native bivalve populations. This proposed research takes advantage of a large-scale perturbation of the Lake Erie ecosystem to measure fitness of native populations.

In addition to its contribution to understanding of ecological genetics theory, this project will also provide valuable information regarding the potential consequences of the invasion of North American aquatic ecosystems by the zebra mussel. The North American Unionidae contain many endemic species, such that vulnerability to zebra mussels could represent a threat to a large number of species, many of which are considered endangered or threatened. Government agencies charged with monitoring endangered and threatened species will be particularly concerned with any detrimental effects zebra mussels may have on native unionids. Therefore, this project can further elucidate basic interactions between genetics and ecology of populations while also providing data that may be applied by management agencies seeking to protect endangered species.

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