1993

Genetic Population Structure of a Species Complex of Blue Mussels (*Mytilus* Spp.).

Shane Kevin Sarver

Louisiana State University and Agricultural & Mechanical College

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_disstheses

Recommended Citation

Sarver, Shane Kevin, "Genetic Population Structure of a Species Complex of Blue Mussels (*Mytilus* Spp.)." (1993). *LSU Historical Dissertations and Theses*. 5545.
https://digitalcommons.lsu.edu/gradschool_disstheses/5545

This Dissertation is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Historical Dissertations and Theses by an authorized administrator of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

U·M·I

University Microfilms International
A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
313/761-4700 800/521-0600

Order Number 9401567

**Genetic population structure of a species complex of blue
mussels (*Mytilus* spp.)**

Sarver, Shane Kevin, Ph.D.

The Louisiana State University and Agricultural and Mechanical Col., 1993

U·M·I

300 N. Zeeb Rd.
Ann Arbor, MI 48106

GENETIC POPULATION STRUCTURE OF A
SPECIES COMPLEX OF BLUE MUSSELS (MYTILUS SPP.)

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Zoology and Physiology

by

Shane K. Sarver

B.S., California Polytechnic State University, 1983

M.S., Humboldt State University, 1989

May 1993

ACKNOWLEDGEMENTS

I would like to thank my major professor, Dr. David W. Foltz for his support during my time at Louisiana State University. I would also like to thank the members of my graduate committee, Drs. Joseph Siebenaller, Kenneth Brown, William Stickle, Jay Geaghan, Alan Biel, and former committee member William Wolters. I would also like to thank Ron Bouchard for his help with photography, Elizabeth Li and Dr. Patricia Wozniak for help with statistical matters, Jeffrey Tamplin for help with preparing figures, and my lab partner Dr. Masaya Katoh. And finally, I would like to thank my wonderful wife Amy Jennings for her unwavering support.

TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGEMENTS	ii
ABSTRACT	iv
CHAPTER	
1 INTRODUCTION	
Species Complexes in Marine Bivalves	1
Heterozygosity-Related Phenomena	9
2 GENETIC AND ENVIRONMENTAL INTERACTION IN A SIBLING SPECIES COMPLEX OF <u>MYTILUS</u>	
Introduction	14
Materials and Methods	19
Results	29
Discussion	57
3 THE POSSIBLE EXISTENCE OF NULL ALLELES IN THE MUSSEL <u>MYTILUS TROSSULUS</u>	
Introduction	72
Materials and Methods	74
Results	76
Discussion	80
4 SUMMARY AND CONCLUSIONS	92
REFERENCES	96
VITA	107

ABSTRACT

Mussels representing two species (Mytilus trossulus and M. galloprovincialis) were collected from 28 littoral locations on the Pacific coast of the USA, and examined for genetic variation at 15 allozyme loci. Eleven samples from a region of suspected hybridization between the two species were also analyzed for variation in seven shell characters. Analysis of allozyme data by principal components analysis revealed three groups based on first principal component scores, and were identified as M. trossulus, M. galloprovincialis and hybrids. Canonical discriminate analysis of seven shell characters was less successful in separating mussels based on morphological criteria alone. To explore the possible effect of environmental variation on the distribution of M. trossulus and M. galloprovincialis, each sample location was characterized for four habitat characteristics: (1) temperature, (2) salinity, (3) tidal range and (4) degree of exposure to wave action which were used as independent variables in a series of multiple regression analyses with proportion of M. trossulus and M. galloprovincialis as dependent variables. Low temperature, temperature range, low salinity and salinity range had statistically significant effects on the proportion of M. trossulus at each location, while temperature range, low salinity and salinity range had statistically significant

effects on the proportion of M. galloprovincialis at each location. Mytilus trossulus was more likely to occur at locations with lower temperatures and greater salinity variation than M. galloprovincialis. An ANOVA using a randomized complete block design was used to study the effect of tidal height on allele frequencies for a single allozyme locus, octopine dehydrogenase (Odh, E.C. 1.5.1.11.). Tidal height was determined not to have a significant effect on Odh allele frequencies for Mytilus in California, in contrast to a previous study of Mytilus populations in Europe where Odh allele frequencies varied significantly among samples from different tidal heights. In a separate study, an electrophoretic survey of a natural Mytilus trossulus population found ten active alleles for the octopine dehydrogenase (Odh) locus. A deficiency of heterozygous genotypes resulted in a statistically significant ($P < 0.005$) departure from Hardy-Weinberg expectations. In vitro specific activity was determined for 207 mussels representing 17 different Odh genotypes. The Odh heterozygotes had an average specific activity 19% greater than that of homozygous genotypes ($P < 0.05$).

Chapter 1

INTRODUCTION

Marine bivalves have been the subject of a number of genetic surveys in recent years and several noteworthy trends have emerged from these studies. First, genetic studies have revealed that several nominal species are actually morphologically cryptic complexes of sibling species or semispecies that can be sympatric over broad geographic distances (Ó Foighil and Eernisse, 1988; Väinölä and Varvio, 1989; Koehn, 1991). Second, studies with natural populations of marine bivalves have consistently shown a positive correlation between fitness related characters (such as growth) and heterozygosity for enzyme loci (see Mitton and Grant, 1984 and Zouros and Foltz, 1987 for reviews). Third, studies of natural populations of marine bivalves have consistently revealed deficiencies of heterozygous individuals, compared to expected population-wide frequencies (reviewed by Zouros and Foltz, 1984).

Species Complexes in Marine Bivalves

Morphologically similar or identical natural populations that are reproductively isolated are termed "sibling species" (Mayr, 1936). Allozyme studies have demonstrated the occurrence of sibling species complexes for a number of marine invertebrates (Manwell and Baker, 1963;

Grassle and Grassle, 1976; Steiner et al., 1977; Murphy, 1978; Thorpe et al., 1978; McDonald and Koehn, 1988, Ó Foighil and Eernisse, 1988; Kwast et al., 1990; Palmer et al., 1990; Liu et al., 1991; Sarver et al., 1992). These sibling species complexes are of interest because they often show little intraspecific differentiation over large geographic scales, are often sympatric over broad geographic areas, and sometimes maintain their genetic integrity in spite of the occurrence of hybridization. The morphological variation observed in these sibling species complexes is not always concordant with the genetic variation (Liu et al., 1991).

One of the most extensively studied marine organisms, the blue mussel Mytilus edulis Linnaeus, 1758, was until recently thought to be the common mussel in cold and temperate coastal waters worldwide (e.g., Soot-Ryen, 1955). Studies using allozyme electrophoresis have shown Mytilus edulis, in fact, to be a complex of morphologically similar but electrophoretically distinct forms (McDonald et al., 1991). In Europe, two forms of Mytilus (M. edulis and the Mediterranean mussel M. galloprovincialis Lamarck, 1819) have traditionally been recognized on morphological grounds (Seed, 1972). Allozyme electrophoresis has shown that M. galloprovincialis also occurs sympatrically with M. edulis in parts of Britain, Ireland, and France (Skibinski et al. 1983; Gosling, 1984). Furthermore, where the two types of

mussels occur sympatrically, some populations have been found to hybridize extensively, while in other locations the two forms are distinct, with little hybridization (Skibinski et al. 1983).

In North America, a similar situation is now thought to exist. In eastern North America, Koehn et al. (1984) found that Mytilus populations were differentiated into three groups: (Group I) populations south of Cape Cod, (Group II) populations throughout the Gulf of Maine, Gulf of St. Lawrence, areas of both southern and northern Newfoundland, and southern Hudson Bay, and (Group III) populations in southeastern Nova Scotia, northern Newfoundland and Hudson Strait, Quebec. Group I was distinguished on the basis a single locus leucine aminopeptidase (Lap), and this is thought to be the result of intraspecific natural selection. Groups II and III were differentiated on the basis of diagnostic multilocus genotypes. Based on this information, Koehn et al. (1984) suggest that Group III mussels might be a different species than Group II mussels (Mytilus edulis). In one location Groups II and III mussels were found to occur sympatrically, with no indication of hybridization.

On the Pacific coast of North America, allozyme studies indicate that putative Mytilus edulis populations are differentiated into two genetically distinct groups (McDonald and Koehn, 1988; Sarver and Loudenslager, 1991). Based on a comparison of Pacific coast blue mussels with

Mediterranean and Atlantic Mytilus, McDonald and Koehn (1988) concluded that mussels from southern California were electrophoretically similar to M. galloprovincialis from the Mediterranean, and mussels from northern California were similar to mussels from eastern Canada and the Baltic Sea (Group III mussels). McDonald and Koehn (1988) proposed the recognition of these as two separate species, Mytilus trossulus Gould, 1850 in northern California and M. galloprovincialis in southern California. Based on the genetic similarity of mussels from southern California to Mytilus from the Mediterranean, McDonald and Koehn (1988) suggested the accidental introduction of M. galloprovincialis into the relatively warm waters of southern California. M. galloprovincialis has apparently been introduced into several geographically widespread areas in recent times, presumably as a result of man's shipping activities. Mytilus galloprovincialis was apparently introduced to Japan (Wilkins et al., 1983), Hong Kong (Lee and Morton, 1985), and South Africa (Grant and Cherry, 1985). The lack of M. galloprovincialis from aboriginal shell middens in South Africa, and from prior museum collections in South Africa and Japan support the position that M. galloprovincialis was introduced. Analyses of middens from archeological sites of aboriginal southern Californians have revealed the presence of "Mytilus edulis" in large numbers (Sarver and Loudenslager, 1991). The dates

of these sites range from 4000 to 5000 b.p., and pre-date the arrival of Europeans to California. Therefore M. galloprovincialis may not be a recent immigrant to southern California, although M. galloprovincialis may have been introduced to southern California and subsequently displaced a native blue mussel.

Although the need for a thorough taxonomic revision of the genus Mytilus is generally recognized, there is still some controversy as to the nature of geographic differentiation observed. For example, while some (e.g., Varvio et al., 1988) regard North Sea Mytilus to be taxonomically distinct from Mytilus in the Baltic, others (e.g., Johannesson et al., 1990) regard the genetic differentiation to be a result of natural selection. In eastern Canada, Gartner-Kepkay et al. (1983) found that M. edulis inhabiting more estuarine sites were genetically differentiated from populations inhabiting more oceanic sites, and that genetic similarities correlated with environment type rather than geographic distance between sites. Gartner-Kepkay et al. (1983) suggest that these genetic differences were due to the action of natural selection. McDonald and Koehn (1988) suggest that the differences observed by Gartner-Kepkay et al. (1983) may in fact be due to the overlap of two species of Mytilus (M. edulis and M. trossulus) in the area of their study. Theisen (1978) observed clines for three enzyme loci in the

Baltic and North Sea and argued that natural selection was acting to maintain this cline. On the other hand, Varvio et al. (1988) suggested that the clines observed by Theisen (1978) resulted from secondary contact between M. trossulus and M. edulis.

Patterns of microgeographic differentiation are also subject to different interpretations. For example, Gosling and McGrath (1990) observed significant differences in allelic and genotypic frequencies for the octopine dehydrogenase (Odh) locus (octopine: NAD oxidoreductase; E.C. 1.5.1.11) between populations of Mytilus from different tidal levels on the west coast of Ireland, a region where M. edulis and M. galloprovincialis co-occur. Mussels higher up the shore exhibited a higher frequency of alleles characteristic of M. galloprovincialis. Similarly, Sarver (1989) observed similar differences in Odh genotypes between two samples of mussels differing in tidal height, and in contrast to the study of Gosling and McGrath (1990) this was a location where only a single species (M. trossulus) was found. The physiological role of octopine dehydrogenase in molluscan tissues is thought to be in maintaining redox balance in an analogous manner to lactate dehydrogenase in vertebrate tissues (Gäde and Grieshaber, 1986). Mussels living at a higher location in the intertidal zone will experience a greater period of emersion and therefore must sustain longer periods of anaerobic metabolism than mussels

living lower in the intertidal zone. Natural selection could act on the Odh locus if different allozymes had dissimilar influences on anaerobic metabolism. It is not known whether the differentiation in Odh genotype frequencies in mussels from different tidal heights that was reported by Sarver (1989) and McGrath and Gosling (1990) was due to taxonomic differences between mussels at differing tidal heights or adaptive differences among ODH allozymes.

The region between Cape Mendocino and Point Conception along the California coast appears to be a region of overlap and possible hybridization between Mytilus trossulus and M. galloprovincialis. This area may be a transition zone between the two species where, depending on the local hydrographic conditions, some locations favor M. trossulus and some locations favor M. galloprovincialis (Sarver and Loudenslager, 1991). Studies in Europe have found evidence that in areas where both M. edulis and M. galloprovincialis occur, M. galloprovincialis favors the more exposed locations while M. edulis favors sheltered habitats (Gosling, 1984). It is not known whether there is any correlation between habitat type and the distributions of M. trossulus and M. galloprovincialis along the Pacific coast of the United States.

Although there is some evidence of hybridization between Mytilus trossulus and M. galloprovincialis on the central coast of California, little is known about the

nature of this zone of hybridization (Koehn, 1991). Regions of hybridization are of general interest to evolutionary biologists because they provide natural laboratories for the study of processes of evolution and speciation (Barton and Hewitt, 1985; Harrison, 1990). For example, the pattern of genotype frequencies across a hybrid zone can give good estimates of parameters such as gene flow and selection pressure (Mallet et al., 1990).

The first part of this dissertation focuses on intergradation and hybridization between Mytilus trossulus and M. galloprovincialis in California. In the transition zone between M. trossulus and M. galloprovincialis, fine scale sampling was conducted in a variety of habitats. Because it is not known why M. trossulus and M. galloprovincialis remain genetically distinct in the face of hybridization the potential ecological differences between the habitats of M. trossulus and M. galloprovincialis were examined. Several habitat types are contrasted in the transition zone: exposed vs. sheltered sites, estuarine vs. non-estuarine sites, and high intertidal vs. low intertidal sites. This part of the study provides a detailed description of the region of overlap and hybridization between M. galloprovincialis and M. trossulus, and furnishes information regarding the maintenance of the "genetic integrity" of M. trossulus and M. galloprovincialis.

The sampling design allows comparison with previous studies of Mytilus. For example, Gosling and McGrath (1990) have indicated that differences in Odh genotype exist between mussels from different tidal levels in areas of Ireland. Genotypic variation in mussel populations of differing tidal heights could be a result of natural selection acting on the Odh locus, or taxonomic differences between mussels at different tidal heights. This was investigated by collecting Mytilus spp. at different tidal heights from locations that contain "pure" M. trossulus, "pure" M. galloprovincialis, and mixed populations from California. If there are differences in Odh allele frequencies in the intertidal, the pattern of Odh differentiation among samples should be informative in discerning the nature of differentiation at the Odh locus.

Heterozygosity-Related Phenomena

Numerous studies of natural populations of marine bivalves have reported significant deficiencies of heterozygotes compared to Hardy-Weinberg expectations for a number of allozyme loci (Berger, 1983; Singh and Green, 1984; Zouros and Foltz, 1984). The origin of such deficiencies is uncertain, but most studies have sought to explain deficiencies of heterozygotes in marine bivalve populations by invoking non-random mating, selection or population structure. Partial inbreeding, through the

mating of related individuals, can generate excess homozygosity. However, inbreeding is thought to be negligible for organisms such as marine bivalves which have life history patterns with external fertilization, planktonic larvae, and extended dispersal. Natural selection can also generate deficiencies of heterozygotes. Selection against heterozygotes (underdominance) during larval life can theoretically generate deficiencies of heterozygotes in post-metamorphic juvenile and adult marine bivalves. However, the fitness differences among allozyme genotypes necessary to generate the heterozygote deficiencies commonly observed in marine bivalves are much larger than those that have been reported (Gaffney et al., 1990). The pooling of genetically differentiated subpopulations (Wahlund effect) can yield an apparent deficiency of heterozygotes. Large variances in allele frequencies are necessary in order to generate commonly observed heterozygote deficiencies, yet for most marine bivalves large-scale geographic variation in allozyme allele frequencies is relatively small (Zouros and Foltz, 1984). The lack of geographic variation in allozyme allele frequencies would seem to indicate a limited role of the Wahlund effect in generating heterozygote deficiencies in marine bivalves. With regard to allozyme loci, much less attention has been given to the possibility that heterozygote deficiencies result from the existence of nonreactive alleles (null alleles).

The occurrence of heterozygote deficiencies in marine bivalves is even more curious when coupled with the observation (often in the same studies) that the degree of multilocus heterozygosity is positively correlated with fitness related traits such as growth and fitness (Zouros, 1987; Gaffney et al., 1990). The genetic mechanism for heterozygosity-fitness correlations is currently not known, but possible explanations for heterozygosity-fitness correlations in marine bivalves include: fitness advantages associated with allozyme heterozygosity (overdominance), linked gene effects, and null activity alleles. The occurrence of heterozygote deficiencies and (allozyme) heterozygosity-fitness correlations in marine bivalves constitutes one of the more puzzling problems in population genetics.

Previous work with marine bivalves has suggested that some allozyme loci might be harboring recessive null activity alleles at moderate frequencies (Skibinski et al., 1983; Mallet et al., 1985; Foltz, 1986a). Null activity alleles, if present, will usually go undetected in routine electrophoretic surveys because an active/null heterozygote is often indistinguishable (on an electrophoretic gel) from an active/active homozygote. As a result of the mis-scoring of active/null heterozygotes as active/active homozygotes, estimates of apparent heterozygosity are reduced. If active/null heterozygotes have reduced fitness relative to

active/active genotypes, then active/active heterozygotes will have an apparent fitness advantage over apparently homozygous genotypes because some active/null heterozygotes are mis-scored as active/active homozygotes. Thus, null activity alleles could theoretically account for both heterozygote deficiencies and heterozygosity-fitness correlations that have been observed in marine bivalves.

Part of this dissertation will examine the occurrence of heterozygote deficiencies and heterozygosity-growth correlations in marine bivalves by focusing on the possible existence of null activity alleles. This study examines a highly polymorphic enzyme locus in Mytilus trossulus (Odh) that has been shown to exhibit deficiencies of heterozygotes and correlations between heterozygosity and growth (Koehn and Gaffney, 1984; Zouros et al., 1988; Gosling, 1989). The approach for detecting null activity alleles for the Odh locus in M. trossulus is to use an assay specific for the enzyme ODH in conjunction with allozyme electrophoresis in a manner similar to that described by Katoh and Foltz (1989). In their study, Katoh and Foltz (1989) measured leucine aminopeptidase (LAP) enzyme activity in oysters derived from pair matings from Foltz (1986b). Foltz (1986a) found evidence for the occurrence of a null allele for the Lap-2 locus in the oyster Crassostrea virginica due to anomalous segregation patterns in progeny of pair matings. Katoh and Foltz (1989) found that presumptive active/null

heterozygotes had approximately half the enzyme specific activity of active/active homozygotes. For the Mytilus ODH data, I will calculate a presumptive null activity allele frequency, and determine if a null allele can account for the observed heterozygote deficiency.

Chapter 2

GENETIC AND ENVIRONMENTAL INTERACTION IN A SIBLING SPECIES COMPLEX OF MYTILUS

Introduction

The common mussel Mytilus edulis has been the subject of many studies by population geneticists. Most of the early studies focused on genetic variation over relatively small geographic distances (e.g., Milkman and Beaty, 1970; Theisen, 1978; Gartner-Kepkay et al., 1980; 1983, Bulnheim and Gosling, 1988) and found that mussel populations were highly structured (genetically), with some sharp genetic discontinuities observed over short distances, sometimes forming allele frequency clines (Koehn et al., 1976; Theisen, 1978). The apparent highly structured nature of mussel populations seemed to be at odds with the ability of mussel larvae to be dispersed great distances, which would tend to have a homogenizing effect on the genetic structure of mussel populations (Koehn et al., 1976). The genetic population structure and the life history characteristics of mussels led several researchers to implicate natural selection as the cause of the genetic structure of mussel populations (Theisen, 1978; Gartner-Kepkay et al., 1980; 1983; Johannesson et al., 1990).

More recent surveys of genetic variation in Mytilus have examined genetic variation over much greater geographic

distances, in some cases the entire geographic range (e.g., Koehn et al., 1984; Varvio et al., 1988; Koehn, 1991; McDonald et al., 1991). These broad geographic surveys of genetic variation indicated that the common mussel "Mytilus edulis" was actually a morphologically cryptic sibling species complex. It is now known that some of the early reports of clines and genetic discontinuities over short distances (e.g., Theisen, 1978; Bulnheim and Gosling, 1988) were actually due to the overlap of two previously unrecognized species. One notable exception is the cline in Lap allele frequencies that occurs at the entrance to Long Island Sound and Cape Cod, which is regarded as the result of natural selection (Koehn et al., 1980; Hilbish et al., 1982), and not due to secondary contact between different lineages of Mytilus (Hilbish and Koehn, 1985).

Patterns of microgeographic differentiation have been subject to different interpretations. For example, Gosling and McGrath (1990) observed differentiation for the octopine dehydrogenase locus (Odh) in samples of Mytilus from different tidal heights and interpreted this differentiation to indicate a higher frequency of M. galloprovincialis in upper shore locations than the co-occurring M. edulis. Furthermore, the differentiation observed by Gosling and McGrath (1990) was repeated in replicate samples from different locations and in samples from different years, which make it unlikely that stochastic processes (such as

drift) were responsible their results. Sarver (1989) also observed differentiation for Odh between two samples differing in tidal height, but in contrast to the results of Gosling and McGrath (1990) this was a location where only a single species (M. trossulus) was found. It is possible that the differentiation of Odh allele frequencies observed by Sarver (1989) and Gosling and McGrath (1990) was due to natural selection rather than taxonomic differentiation.

The common blue mussel is now thought by some (e.g., McDonald et al., 1991) to consist of at least three species: Mytilus edulis (Linnaeus), M. galloprovincialis (Lamarck), and M. trossulus (Gould). Mytilus edulis occurs in the eastern USA, northern Europe, Argentina, the Falkland Islands, and the Kerguelen Islands. Mytilus galloprovincialis is common to the Mediterranean, western Australia, Tasmania, New Zealand, and has been found to occur sympatrically with M. edulis in parts of Great Britain, Ireland and France (Koehn, 1991; McDonald et al., 1991). Mytilus galloprovincialis has been introduced into Japan, Hong Kong, South Africa, and possibly southern California (Wilkins et al., 1983; Grant and Cherry, 1985; Lee and Morton, 1985; McDonald and Koehn, 1988). Mytilus trossulus has been identified in the northern Pacific from Siberia to central California, as well as in the Canadian Maritimes, and the Baltic Sea (Koehn, 1991; McDonald et al., 1991).

In areas where two species of Mytilus (excluding the eastern Pacific Mytilus californianus) come into contact, there is always hybridization between them. Hybridization occurs between M. edulis and M. galloprovincialis in a widespread region from northern Spain to northwest France (Gosling, 1984). Hybridization occurs between M. edulis and M. trossulus in several locations in the Gulf of Lawrence and hybridization between M. trossulus and M. galloprovincialis occurs along the central California coast (McDonald and Koehn, 1988; Koehn, 1991).

An unusual feature of the regions of hybridization in Mytilus is that they do not always exist as smooth clines connecting pure populations of each species, but instead can occur as a patchwork or mosaic pattern (Skibinski and Beardmore, 1979; McDonald and Koehn, 1988). For example, in Europe M. edulis and M. galloprovincialis come into contact in a geographically widespread region from the British Isles to northern Spain, with pure, mixed and hybrid populations occurring in patchwork pattern throughout the region. This is in contrast to most studies of hybrid zones which have dealt with terrestrial vertebrates and invertebrates, and have usually interpreted hybrid zones as smooth clines along linear transects (Harrison and Rand, 1989). Mosaic hybrid zones have been described for several plant (Briggs, 1962; Alston and Turner, 1963) and animal (Gartside, 1980; Howard, 1982) species and are thought to result from a patchy

environment where the interacting taxa differ in their response to physical or environmental factors (Harrison and Rand, 1989). One exception to the usual mosaic pattern that occurs in Mytilus populations in Europe, was reported for a region of transition between M. edulis in the North Sea to M. trossulus in the Baltic Sea where a gradual cline in allele frequencies, typical of most vertebrate or terrestrial invertebrate hybrid zones occurs (Väinölä and Hvilson, 1991). Mosaic hybrid zones typically observed in Mytilus contact zones differ from steep clines that are commonly described in the literature on hybrid zones in that the complex nature of the environment appears to be the major factor determining the fate of these species boundaries.

The present study has several objectives. The first objective is to examine the region of contact between Mytilus trossulus and M. galloprovincialis to provide a detailed description of this region with special reference to the distribution of M. trossulus, M. galloprovincialis, and possible hybrids. Secondly, by analyzing samples from different habitats in the region of contact between M. trossulus and M. galloprovincialis this study will explore the possible ecological factors that keep these two species distinct despite the potential for hybridization. And finally, this study will compare Odh frequencies in samples from high and low tidal locations from pure M. trossulus locations, pure

M. galloprovincialis locations and mixed locations in an effort to discern the nature of differentiation at this locus that has been reported in previous studies.

Materials and Methods

Mussels (Mytilus spp.) were collected (at random with respect to size) from 28 intertidal locations along the Pacific coast of the USA in 1990-1991 (Table 2.1), air shipped live to Louisiana State University in Baton Rouge, and then frozen whole at -70°C. For animals larger than 15 mm, the posterior adductor muscle and part of the digestive gland were removed and homogenized in an equal volume of homogenization buffer. Mussels smaller than 15 mm were homogenized whole. Homogenized tissue preparations were centrifuged and the supernatants were collected for electrophoretic analysis.

Starch gel electrophoresis was performed using standard procedures (e.g., Richardson et al. 1986; Murphy et al. 1990). Enzyme names and locus abbreviations for the 15 enzyme loci used in this study were taken from Shaklee et al. (1990) (Table 2.2), except that Aap (peptidase II, demonstrated with DL-alanyl-beta-naphthylamide) and Lap (leucine aminopeptidase, demonstrated with L-leucine-beta-naphthylamide) were taken from Young et al. (1979), Fdh (formaldehyde dehydrogenase) was taken from Balakirev and Zaykin (1990) and Odh (octopine dehydrogenase) was taken

Table 2.1. Collection locations of Mytilus spp.

Collection locality	Symbol	Sample size	Species*	Collection date
Juneau, AK	AK	40	T	05/15/91
Crescent City, CA	CC	48	T,G,H	06/19/90
Eureka Slough 1, CA (high intertidal)	ES1H	48	T,H	06/20/90
Eureka Slough 1, CA (low intertidal)	ES1L	48	T	06/20/90
Eureka Slough 2, CA (high intertidal)	ES2H	48	T	06/20/90
Eureka Slough 2, CA (low intertidal)	ES2L	48	T	06/20/90
Woodley Island, CA	WI	48	T	06/20/90
Bodega Bay, CA (high intertidal)	BBH	40	T,G,H	06/27/91
Bodega Bay, CA (low intertidal)	BBL	40	T,G,H	06/27/91
Tomales Bay, CA (high intertidal)	TBH	60	G,H	06/22/90
Tomales Bay, CA (low intertidal)	TBL	30	G,H	06/22/90
Berkeley, CA	BK	35	T,G,H	06/24/90
San Rafael, CA	SR	48	T,G,H	06/24/90
Benicia, CA	BN	41	T,H	06/24/90
Sausalito, CA	SS	48	T,G,H	06/24/90
Salinas River 1, CA	SL1	44	T,G,H	06/25/90
Salinas River 2, CA	SL2	47	T,G,H	07/05/91
Moss Landing, CA (high intertidal)	MLH	40	G,H	07/05/91
Moss Landing, CA (low intertidal)	MLL	40	G,H	07/05/91
Morro Bay, CA	MB	41	G	06/26/90
Point San Luis, CA (high intertidal)	SLH	48	G	06/27/90
Point San Luis, CA (low intertidal)	SLL	47	G	06/27/90
Hazard Beach, CA	HZ	47	G	06/26/90
Gaviota, CA	GV	48	G	06/28/90
Santa Barbara, CA	SB	48	G	06/28/90
Ventura, CA	VN	48	G	06/28/90
Newport Bay, CA	NP	48	G	06/29/90
Marina del Rey	LA	40	G,H	06/29/90
Total	28	1256		

* T=Mytilus trossulus, G=Mytilus galloprovincialis, H=M. trossulus x M. galloprovincialis hybrid

Table 2.2. Enzymes analyzed by electrophoresis in Mytilus spp. Substrate is shown in parenthesis.

Enzyme	Abbreviation	Number of loci	E.C. Number
Aconitase	AC	2	4.2.1.3
Aspartate aminotransferase	AAT	2	2.6.1.1
Esterase (4-methylumbelliferyl acetate)	EST	1	2.7.3.3
Formaldehyde dehydrogenase	FDH	1	1.2.1.1
Fructose-bisphosphatase	FBP	1	3.1.3.11
Glucose-6-phosphate isomerase	GPI	1	5.3.1.9
Glutathione reductase	GR	1	1.6.4.2
Mannose-6-phosphate isomerase	MPI	1	5.3.1.8
Octopine dehydrogenase	ODH	1	1.5.1.11
Peptidase (leucyl-alanine)	AP	1	3.4.-.-
Peptidase (DL-alanyl-beta-naphthylamide)	AAP	1	3.4.-.-
Peptidase (L-leucine-beta-naphthylamide)	LAP	1	3.4.-.-
Phosphoglucomutase	PGM	1	2.7.5.1
Total		15	

from Livingstone et al. (1990). Multiple loci coding the same enzymes but of differing electrophoretic mobilities (isozymes) were numbered with "1" denoting the faster migrating system and "2" denoting the slower migrating system. Alleles were assigned numbers representing electrophoretic mobility with the most common allele in Mytilus trossulus scored as "100", all other alleles were assigned numbers according to their mobility relative to the common allele.

Conventional statistics for allozyme data (allele frequencies, observed and expected heterozygosities, and genetic distances) were calculated using formulas from Hedrick (1985). The nested ANOVA method of Weir and Cockerham (1984) was used to analyze the genetic structure within and among populations. Hierarchical F-statistics were calculated for Mytilus trossulus and M. galloprovincialis separately with hybrids excluded from the analysis. Three hierarchical levels were recognized for both M. trossulus and M. galloprovincialis: F_{ID} (individual within collection) as a measure of departure of genotypic frequencies from Hardy-Weinberg expected values; F_{DS} (microgeographic genetic differentiation) and F_{ST} (macrogeographic genetic differentiation) (Table 2.3), For M. trossulus, F_{ST} represents genetic differentiation of subregions within the total sample (populations north of Cape Mendocino vs. populations south of Cape Mendocino); F_{DS}

Table 2.3. Hierarchical F-statistics adapted from Weir and Cockerham (1984). F_{ST} is the genetic differentiation of subregions within the total sample, F_{DS} is the genetic differentiation of demes within subregions, F_{ID} is the genetic differentiation of individuals within demes.

Source of variation	Variance component	F-Statistics
Subregion within Total (macrogeographic differentiation)	σ^2_{ST}	$F_{ST} = \sigma^2_{ST} / \sigma^2_{GT}$
Deme within subregion (microgeographic differentiation)	σ^2_{DS}	$F_{DS} = \sigma^2_{DS} / (\sigma^2_{GT} - \sigma^2_{ST})$
Individual within deme (individual within collection)	σ^2_{ID}	$F_{ID} = \sigma^2_{ID} / (\sigma^2_{ID} + \sigma^2_{GI})$
Gene within individual	σ^2_{GI}	
Total	σ^2_{GT}	

represents genetic differentiation of populations within subregions (locations within regions north of Cape Mendocino and regions south of Cape Mendocino). The hierarchy was similar for M. galloprovincialis, but the subregions for F_{ST} were locations north of Point Conception and locations south of Point Conception. The multiple-locus allozyme genotypes of individual mussels were analyzed using principal components analysis in the PRINCOMP procedure in Statistical Analysis System (SAS Institute INC., 1985). Each allele was treated as a separate variable, with a value equal to the number of copies (0, 1, or 2) of the allele for each individual mussel. First principal component scores were used to classify individual mussels as M. galloprovincialis, M. trossulus, or as a presumptive hybrid (Campton, 1987).

Morphological measurements were taken on shells from populations located within the suspected region of hybridization. Seven shell measurements were measured with calipers to the nearest 0.05 mm, following Beaumont et al. (1989): (1) shell length (sl), (2) shell height (sh), (3) shell width (sw), (4) length of the anterior adductor muscle scar (aams), (5) length of the posterior adductor muscle scar (pam), (6) length of posterior byssal retractor scar (lbrs), and (7) width of posterior retractor scar (Fig 2.1).

Morphological data were subjected to canonical discriminate analysis using the CANDISC procedure in Statistical Analysis

Figure 2.1. Shell morphology measurements used in this study. All measurements were taken as described in Beaumont et al. (1989). sl:shell length; sh:shell height; sw:shell width; aams:length of the anterior adductor muscle scar; pam:length of the posterior muscle scar; lbrs:length of the byssal retractor muscle scar; wbrs: width of the byssal retractor muscle scar.

system (SAS, Inc. 1985), using the principal component scores of allozyme data for classification.

Each sampling location was characterized for four habitat characteristics: (1) temperature, (2) salinity, (3) tidal regime, and (4) whether the sample was located in a sheltered habitat or subject to wave action. The environmental information was gathered from published sources and is summarized in Table 2.4. A multiple regression analysis was performed for both Mytilus trossulus and M. galloprovincialis, where the dependent variable in the analysis was percent species at each sampling location, and the independent variables were low temperature, temperature range, low salinity, salinity range, intertidal or subtidal, and open coast or sheltered. Statistical analyses were done using the regression (REG) procedure of the Statistical Analysis System (SAS Institute Inc., 1985).

To test for an effect of tidal height on single locus allele frequencies (Odh), an ANOVA was performed using a randomized complete block design. For this analysis, individual mussels were first categorized as Mytilus trossulus, M. galloprovincialis, or presumptive hybrid based on first principal component scores of multilocus genotypes (see Results section for details). Weighted Odh allele frequencies for M. trossulus, M. galloprovincialis (hybrids were excluded from the analysis) were then determined for the two diagnostic alleles Odh¹⁰⁰ (M. trossulus) and Odh¹¹⁰ (M.

Table 2.4. Habitat characteristics for Mytilus samples.

Location	Temperature Range (°C)	Salinity (‰)	Tidal ¹ Regime	Habitat ²
Crescent City ^j	8-18	33-35	S	B
Tomales Bay High ^a	6.7-23.8	21.5-36.5	I-H	B
Tomales Bay Low ^a	6.7-23.8	21.5-36.5	I-L	B
Woodley Island ^{b,c}	9.0-15	30.0-33.5	S	B
Bodega Bay high ^d	10-25	33-35	I-H	B
Bodega Bay Low ^d	10-25	33-35	S-L	B
Eureka Slough1 High ^{b,f}	9.3-16	5-28	I-H	B
Eureka Slough1 Low ^{b,f}	9.3-16	5-28	I-L	B
Eureka Slough2 High ^{b,f}	9.3-16	5-28	I-H	B
Eureka Slough2 Low ^{b,f}	9.3-16	5-28	I-L	B
Berkeley Marina ^{g,h}	10-20	3-35	S	B
San Rafael ^{g,h}	9.9-22	15-26	S	B
Benicia ^{g,h}	9.1-20	0-17	I	B
Sausalito ^{g,h}	11.5-16	7.5-32	S	B
Salinas River1 ⁱ	13-22	13-33	I-L	B
Salinas River2 ⁱ	13-22	13-33	I-H	B
Moss Landing High ⁱ	11.5-22	23-33	I-H	B

Table 2.4 (cont.)

Location	Temperature Range (°C)	Salinity (‰)	Tidal ¹ Regime	Habitat ²
Moss Landing Low ⁱ	11.5	23-33	S-L	B
Morro Bay ^c	10-23	33-35	S	B
Point San Luis High ^j	9.4-21	33-35	I-H	O
Point San Luis Low ^j	9.4-21	33-35	S-L	O
Hazard Beach ^j	9.4-21	33-35	I	O
Gaviota ^j	10.7-23	33-35	I	O
Santa Barbara ^j	10.7-23	33-35	S	B
Ventura ^j	10.7-23	33-35	S	B
Newport Bay ^{j,k}	12-25	13-33	I	B
Marina del Rey ^{j,k}	12-25	33-35	S	B

¹Tidal regime is defined as subtidal (S) (never exposed at low tide) or intertidal (I) (can be exposed at low tide) high (H) or low (L) in the intertidal zone.

²Habitat is defined as bay (B) or open coast (O).

^a(Haydock, 1964)

^b(Malachowski, 1988)

^c(Machell & DeMartini, 1971)

^d(Shaw, Allen, & Stone, 1974)

^e(Barrett, 1963)

^f(Sarver, 1989)

^g(Tison, 1967)

^h(Dedini, Schemel & Turnbreull, 1981)

ⁱ(Nybakken, Cailliet & Broenkow, 1977)

^j(Churgin, 1974)

^k(Garthwaite, 1986)

galloprovincialis) for each sample. At each sampling location a high and a low collection was made. The sampling locations and collections used in this analysis were Bodega Bay (BBH, BBL), Eureka Slough 1 (ES1H, ES1L), Eureka Slough 2 (ES2H, ES2L), Salinas River (SL1 [high], SL2 [low]), Moss Landing (MLH, MLL), Tomales Bay (TBH, TBL) and Port San Luis (SLH, SLL). The sample allele frequencies were considered the dependent variable, the locations were treated as blocks and species and tidal height were considered as treatments. The analysis was conducted twice, once for Odh¹⁰⁰ and once for Odh¹¹⁰. The ANOVA's were done using the general linear models (GLM) procedure of the Statistical Analysis System (SAS Institute, Inc., 1985). The effects of species and tidal height on Odh¹⁰⁰ and Odh¹¹⁰ allele frequencies were tested using linear contrasts in the GLM procedure.

Results

The electrophoretic survey of 15 allozyme loci found 14 polymorphic loci (Table 2.5). The mean number of alleles per locus was similar in all collections for both Mytilus galloprovincialis and M. trossulus (2.07-4.15) with the exception of BBL for M. galloprovincialis (1.23) which consisted of only a single mussel. Mean individual heterozygosity was also similar in all collections for both M. galloprovincialis and M. trossulus (0.23-0.45) (Table 2.6). Analysis of individual multilocus genotypes by

Table 2.5. Mytilus spp.: allele frequencies at 15 allozyme loci in 28 collections. Locality abbreviations are given in Table 2.1, locus abbreviations are given in Table 2.2.

Locus/	Collection Locality													
	AK	CC	ES1H	ES1L	ES2H	ES2L	WI	BBH	BBL	TBH	TBL	BN	SR	SS
(AAP)														
114	0.00	0.02	0.00	0.03	0.00	0.00	0.00	0.08	0.00	0.87	0.86	0.05	0.59	0.19
104	0.05	0.07	0.06	0.07	0.05	0.06	0.06	0.06	0.08	0.07	0.04	0.00	0.02	0.03
100	0.71	0.66	0.59	0.41	0.46	0.64	0.54	0.59	0.69	0.05	0.04	0.50	0.17	0.44
98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
92	0.20	0.24	0.34	0.47	0.45	0.28	0.31	0.23	0.23	0.00	0.04	0.44	0.22	0.33
86	0.04	0.01	0.01	0.02	0.04	0.02	0.09	0.04	0.00	0.00	0.00	0.01	0.00	0.01
(AAT-1)														
133	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
133	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
100	1.00	0.98	1.00	0.97	1.00	0.95	0.95	0.99	0.99	1.00	1.00	0.96	1.00	1.00
74	0.00	0.01	0.00	0.03	0.00	0.04	0.05	0.01	0.01	0.00	0.00	0.02	0.00	0.00
(AAT-2)														
290	0.05	0.01	0.03	0.02	0.02	0.00	0.01	0.01	0.00	0.04	0.07	0.02	0.05	0.03
190	0.18	0.10	0.05	0.08	0.05	0.13	0.07	0.11	0.10	0.04	0.05	0.05	0.02	0.09
134	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00	0.00	0.00	0.00
100	0.77	0.89	0.92	0.90	0.93	0.87	0.92	0.86	0.87	0.92	0.88	0.93	0.93	0.88
(AC-1)														
112	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
110	0.01	0.02	0.00	0.01	0.00	0.02	0.01	0.03	0.05	0.03	0.04	0.05	0.02	0.02
100	0.54	0.47	0.43	0.28	0.45	0.39	0.38	0.36	0.54	0.74	0.72	0.48	0.50	0.49
90	0.45	0.50	0.52	0.70	0.53	0.58	0.58	0.58	0.41	0.23	0.24	0.46	0.43	0.47

Table 2.5. (cont.)

Locus/	Collection Locality													
	AK	CC	ES1H	ES1L	ES2H	ES2L	WI	BBH	BBL	TBH	TBL	BN	SR	SS
(AC-1) (cont.)														
85	0.00	0.00	0.05	0.01	0.02	0.01	0.03	0.03	0.00	0.00	0.00	0.01	0.03	0.02
80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
(AC-2)														
150	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.06	0.06	0.00	0.04	0.00
125	0.08	0.06	0.08	0.13	0.07	0.16	0.05	0.16	0.22	0.61	0.62	0.06	0.49	0.19
100	0.89	0.90	0.89	0.83	0.88	0.78	0.91	0.74	0.73	0.31	0.28	0.91	0.46	0.79
67	0.03	0.02	0.02	0.04	0.05	0.06	0.03	0.09	0.05	0.02	0.04	0.03	0.01	0.02
60	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(AP)														
153	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
131	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.05	0.03	0.18	0.14	0.00	0.04	0.04
119	0.05	0.01	0.02	0.02	0.06	0.07	0.04	0.06	0.04	0.25	0.24	0.12	0.21	0.09
114	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.01	0.00
112	0.19	0.13	0.16	0.18	0.11	0.08	0.17	0.17	0.17	0.25	0.28	0.23	0.27	0.15
100	0.63	0.80	0.75	0.75	0.74	0.76	0.68	0.67	0.72	0.32	0.34	0.54	0.41	0.63
97	0.13	0.06	0.07	0.05	0.07	0.09	0.09	0.05	0.04	0.00	0.00	0.11	0.03	0.09
92	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
(EST-D)														
150	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
144	0.00	0.02	0.00	0.00	0.00	0.00	0.04	0.03	0.01	0.00	0.00	0.03	0.02	0.00
119	0.04	0.03	0.03	0.01	0.03	0.05	0.05	0.01	0.00	0.00	0.00	0.04	0.02	0.01
100	0.95	0.93	0.93	0.92	0.94	0.90	0.84	0.96	0.96	1.00	0.93	0.91	0.89	0.95
74	0.00	0.02	0.04	0.05	0.03	0.03	0.07	0.00	0.00	0.00	0.04	0.00	0.01	0.01

Table 2.5. (cont.)

Locus/	Collection Locality													
	AK	CC	ES1H	ES1L	ES2H	ES2L	WI	BBH	BBL	TBH	TBL	BN	SR	SS
(EST-D) (cont.)														
64	0.01	0.00	0.00	0.01	0.00	0.02	0.00	0.00	0.03	0.00	0.03	0.02	0.05	0.03
60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
(FDH)														
145	0.00	0.00	0.00	0.00	.	0.03	0.00	0.00	0.00	0.02	0.00	0.00	0.01	0.00
140	0.29	0.13	0.16	0.18	.	0.21	0.26	0.29	0.20	0.00	0.08	0.12	0.16	0.09
119	0.00	0.04	0.00	0.00	.	0.00	0.04	0.01	0.05	0.08	0.00	0.04	0.03	0.09
100	0.71	0.77	0.79	0.74	.	0.73	0.64	0.68	0.70	0.89	0.92	0.79	0.79	0.77
63	0.00	0.06	0.05	0.08	.	0.03	0.04	0.03	0.05	0.01	0.00	0.05	0.01	0.05
60	0.00	0.00	0.00	0.00	.	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(FBP)														
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(GPI)														
140	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
136	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.18	0.07	0.02	0.05	0.03
134	0.00	0.07	0.07	0.04	0.04	0.01	0.03	0.11	0.08	0.72	0.84	0.06	0.57	0.20
112	0.08	0.24	0.20	0.16	0.27	0.23	0.13	0.05	0.09	0.02	0.00	0.32	0.02	0.14
110	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.01	0.00	0.03	0.00	0.01	0.00	0.00
100	0.79	0.59	0.67	0.68	0.63	0.68	0.70	0.58	0.57	0.04	0.09	0.56	0.31	0.53
87	0.10	0.04	0.04	0.09	0.03	0.06	0.00	0.15	0.18	0.00	0.00	0.02	0.03	0.07
80	0.03	0.06	0.02	0.03	0.01	0.02	0.07	0.04	0.08	0.00	0.00	0.01	0.02	0.03
75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00

Table 2.5. (cont.)

Locus/	Collection Locality													
	AK	CC	ES1H	ES1L	ES2H	ES2L	WI	BBH	BBL	TBH	TBL	BN	SR	SS
(GR)														
116	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.01	0.00
114	0.01	0.02	0.02	0.02	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
111	0.09	0.05	0.03	0.04	0.03	0.07	0.04	0.00	0.04	0.01	0.02	0.00	0.00	0.03
103	0.20	0.40	0.20	0.30	0.46	0.43	0.38	0.30	0.21	0.07	0.07	0.24	0.20	0.23
100	0.70	0.49	0.71	0.59	0.44	0.46	0.49	0.66	0.66	0.87	0.82	0.69	0.76	0.71
95	0.00	0.04	0.04	0.05	0.07	0.02	0.07	0.04	0.09	0.03	0.07	0.06	0.02	0.03
93	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.01	0.00	0.00	0.01	0.00
(LAP)														
107	0.00	0.02	0.03	0.00	0.00	0.00	0.00	0.01	0.00	0.02	0.03	0.00	0.01	0.01
103	0.01	0.11	0.05	0.10	0.08	0.13	0.09	0.18	0.13	0.34	0.26	0.11	0.22	0.17
100	0.55	0.28	0.47	0.29	0.32	0.37	0.35	0.44	0.41	0.58	0.67	0.33	0.59	0.45
90	0.41	0.51	0.35	0.54	0.54	0.49	0.47	0.35	0.41	0.03	0.02	0.51	0.16	0.34
82	0.03	0.05	0.10	0.07	0.06	0.01	0.09	0.01	0.05	0.03	0.02	0.05	0.02	0.03
78	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
(MPI)														
125	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
121	0.00	0.01	0.01	0.03	0.00	0.01	0.02	0.05	0.03	0.03	0.03	0.00	0.02	0.00
100	1.00	0.94	0.94	0.95	0.98	0.91	0.90	0.80	0.88	0.03	0.09	0.89	0.29	0.63
91	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.11	0.05	0.94	0.88	0.07	0.67	0.31
64	0.00	0.03	0.05	0.02	0.02	0.08	0.08	0.04	0.04	0.00	0.00	0.04	0.02	0.05
60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01

Table 2.5. (cont.)

Locus/	Collection Locality													
	AK	CC	ES1H	ES1L	ES2H	ES2L	WI	BBH	BBL	TBH	TBL	BN	SR	SS
(ODH)														
126	0.04	0.02	0.04	0.01	0.07	0.02	0.01	0.01	0.03	0.00	0.00	0.00	0.01	0.03
110	0.06	0.06	0.12	0.09	0.05	0.08	0.13	0.12	0.08	0.69	0.67	0.17	0.47	0.22
107	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100	0.46	0.48	0.39	0.54	0.50	0.46	0.49	0.49	0.59	0.03	0.05	0.45	0.30	0.35
94	0.35	0.30	0.36	0.25	0.29	0.28	0.23	0.19	0.24	0.19	0.19	0.24	0.15	0.28
68	0.08	0.14	0.07	0.08	0.07	0.15	0.12	0.15	0.06	0.09	0.09	0.13	0.07	0.10
65	0.01	0.00	0.01	0.03	0.02	0.01	0.01	0.03	0.00	0.00	0.00	0.01	0.00	0.00
48	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.02
(PGM)														
112	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
110	0.01	0.02	0.00	0.04	0.02	0.06	0.06	0.01	0.02	0.01	0.03	0.01	0.00	0.00
106	0.11	0.11	0.12	0.16	0.12	0.17	0.11	0.20	0.06	0.01	0.02	0.11	0.00	0.06
100	0.45	0.41	0.47	0.43	0.44	0.38	0.43	0.41	0.49	0.06	0.09	0.43	0.23	0.36
91	0.39	0.34	0.30	0.32	0.34	0.34	0.29	0.24	0.29	0.31	0.19	0.28	0.26	0.32
73	0.04	0.07	0.06	0.05	0.05	0.01	0.06	0.11	0.10	0.41	0.51	0.13	0.35	0.17
70	0.00	0.05	0.05	0.00	0.03	0.02	0.02	0.03	0.04	0.16	0.14	0.04	0.13	0.07
65	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.03	0.02	0.00	0.01	0.02
60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00

Table 2.5. (cont.)

Locus/	Collection Locality													
	BK	SL1	SL2	MLH	MLL	MB	SB	SLH	SLL	GV	HZ	VN	LA	NP
(AAP)														
114	0.69	0.36	0.29	0.24	0.34	0.90	0.85	0.94	0.93	0.91	0.82	0.88	0.41	0.82
104	0.01	0.01	0.46	0.65	0.55	0.03	0.07	0.02	0.00	0.05	0.08	0.08	0.54	0.08
100	0.13	0.38	0.17	0.10	0.10	0.06	0.07	0.01	0.02	0.04	0.06	0.03	0.05	0.09
98	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
92	0.16	0.25	0.07	0.01	0.01	0.01	0.00	0.02	0.04	0.00	0.04	0.01	0.00	0.01
86	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(AAT-1)														
133	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
133	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
74	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(AAT-2)														
290	0.03	0.05	0.05	0.04	0.04	0.06	0.02	0.09	0.07	0.06	0.01	0.10	0.03	0.04
190	0.10	0.18	0.06	0.10	0.15	0.20	0.07	0.05	0.14	0.06	0.05	0.08	0.09	0.13
134	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100	0.87	0.77	0.89	0.86	0.81	0.74	0.91	0.86	0.79	0.88	0.94	0.82	0.88	0.83
(AC-1)														
112	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
110	0.06	0.03	0.01	0.03	0.09	0.06	0.08	0.04	0.06	0.07	0.07	0.08	0.00	0.06
100	0.57	0.56	0.85	0.71	0.66	0.74	0.65	0.70	0.77	0.74	0.71	0.65	0.95	0.73
90	0.37	0.41	0.14	0.25	0.25	0.20	0.27	0.26	0.17	0.19	0.22	0.26	0.05	0.19
85	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.02
80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 2.5. (cont.)

Locus/	Collection Locality													
	BK	SL1	SL2	MLH	MLL	MB	SB	SLH	SLL	GV	HZ	VN	LA	NP
(AC-2) (cont.)														
150	0.02	0.07	0.07	0.07	0.08	0.04	0.10	0.12	0.15	0.09	0.14	0.07	0.09	0.08
125	0.50	0.43	0.65	0.68	0.65	0.61	0.67	0.53	0.63	0.58	0.58	0.77	0.56	0.66
100	0.47	0.45	0.28	0.24	0.24	0.32	0.21	0.35	0.20	0.32	0.28	0.15	0.35	0.24
67	0.01	0.05	0.00	0.01	0.03	0.03	0.02	0.00	0.02	0.01	0.00	0.01	0.00	0.02
60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(AP)														
153	0.00	0.03	0.02	0.00	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
131	0.23	0.10	0.11	0.11	0.13	0.12	0.15	0.07	0.20	0.12	0.15	0.11	0.12	0.08
119	0.20	0.20	0.16	0.27	0.30	0.27	0.31	0.24	0.32	0.39	0.18	0.26	0.37	0.32
114	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.01	0.00	0.02	0.00	0.00
112	0.30	0.18	0.32	0.33	0.42	0.35	0.43	0.40	0.29	0.28	0.39	0.35	0.28	0.35
100	0.24	0.45	0.38	0.29	0.11	0.26	0.10	0.28	0.17	0.20	0.28	0.26	0.23	0.25
97	0.03	0.04	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
92	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(EST-D)														
150	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
144	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
119	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100	0.99	0.95	0.97	1.00	0.99	0.95	0.97	0.96	0.99	1.00	0.99	0.98	1.00	0.96
74	0.01	0.02	0.01	0.00	0.00	0.03	0.03	0.02	0.01	0.00	0.00	0.02	0.00	0.04
64	0.00	0.01	0.00	0.00	0.01	0.02	0.00	0.02	0.00	0.00	0.01	0.00	0.00	0.00
60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 2.5. (cont.)

Locus/	Collection Locality													
	BK	SL1	SL2	MLH	MLL	MB	SB	SLH	SLL	GV	HZ	VN	LA	NP
(FDH)														
145	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
140	0.01	0.18	0.07	0.04	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.01	0.00	0.00
119	0.09	0.00	0.06	0.08	0.08	0.17	0.08	0.13	0.07	0.02	0.09	0.08	0.06	0.04
100	0.87	0.82	0.87	0.88	0.92	0.82	0.92	0.86	0.89	0.98	0.91	0.91	0.93	0.96
63	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00
(FBP)														
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(GPI)														
140	0.00	0.00	0.04	0.02	0.01	0.00	0.03	0.02	0.02	0.01	0.00	0.00	0.00	0.02
136	0.00	0.07	0.05	0.20	0.09	0.07	0.10	0.07	0.10	0.16	0.16	0.10	0.08	0.04
134	0.79	0.42	0.64	0.71	0.89	0.91	0.85	0.91	0.86	0.81	0.84	0.88	0.90	0.93
112	0.10	0.05	0.03	0.00	0.01	0.02	0.02	0.00	0.01	0.01	0.00	0.01	0.01	0.00
110	0.00	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.01
100	0.11	0.40	0.20	0.05	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00
87	0.00	0.03	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
80	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(GR)														
116	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
114	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
111	0.02	0.02	0.07	0.03	0.01	0.04	0.05	0.06	0.04	0.00	0.03	0.02	0.00	0.00
103	0.25	0.26	0.24	0.10	0.13	0.14	0.28	0.17	0.10	0.17	0.07	0.12	0.13	0.24

Table 2.5. (cont.)

Locus/	Collection Locality													
	BK	SL1	SL2	MLH	MLL	MB	SB	SLH	SLL	GV	HZ	VN	LA	NP
(GR) (cont.)														
100	0.68	0.64	0.62	0.81	0.85	0.79	0.59	0.72	0.78	0.80	0.90	0.81	0.83	0.73
95	0.05	0.04	0.07	0.06	0.01	0.03	0.08	0.05	0.08	0.03	0.00	0.05	0.04	0.03
93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(LAP)														
107	0.00	0.01	0.07	0.04	0.01	0.07	0.04	0.01	0.04	0.01	0.02	0.02	0.02	0.00
103	0.23	0.23	0.26	0.31	0.49	0.29	0.29	0.36	0.32	0.36	0.19	0.32	0.35	0.32
100	0.64	0.50	0.54	0.58	0.47	0.64	0.64	0.61	0.63	0.62	0.78	0.63	0.61	0.68
90	0.09	0.21	0.12	0.06	0.03	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.00
82	0.04	0.05	0.01	0.01	0.00	0.00	0.02	0.02	0.01	0.01	0.00	0.02	0.01	0.00
78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(MPI)														
125	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
121	0.00	0.01	0.01	0.05	0.01	0.04	0.01	0.02	0.02	0.03	0.00	0.01	0.03	0.02
100	0.19	0.41	0.17	0.04	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.02	0.00	0.01
91	0.81	0.57	0.80	0.91	0.96	0.96	0.98	0.97	0.96	0.96	1.00	0.97	0.97	0.97
64	0.00	0.00	0.02	0.00	0.03	0.00	0.00	0.01	0.00	0.01	1.00	0.00	0.00	0.00
60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(ODH)														
126	0.01	0.06	0.02	0.01	0.02	0.02	0.03	0.01	0.01	0.00	0.00	0.04	0.00	0.00
110	0.57	0.44	0.41	0.64	0.64	0.70	0.60	0.72	0.71	0.75	0.69	0.60	0.70	0.69
107	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.02	0.00	0.00	0.00	0.00	0.03
100	0.13	0.21	0.27	0.09	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00
94	0.20	0.20	0.20	0.13	0.24	0.12	0.19	0.12	0.14	0.18	0.16	0.27	0.19	0.20

Table 2.5. (cont.)

Locus/	Collection Locality													
	BK	SL1	SL2	MLH	MLL	MB	SB	SLH	SLL	GV	HZ	VN	LA	NP
(ODH) (cont.)														
68	0.09	0.09	0.10	0.13	0.09	0.13	0.17	0.15	0.12	0.07	0.15	0.09	0.04	0.08
65	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(PGM)														
112	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
110	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.00
106	0.03	0.07	0.04	0.00	0.01	0.01	0.02	0.00	0.03	0.00	0.04	0.02	0.00	0.01
100	0.11	0.22	0.23	0.08	0.03	0.04	0.03	0.04	0.03	0.03	0.00	0.05	0.04	0.03
91	0.30	0.33	0.29	0.26	0.35	0.29	0.35	0.27	0.16	0.19	0.39	0.26	0.26	0.25
73	0.40	0.28	0.40	0.53	0.43	0.39	0.43	0.44	0.59	0.59	0.38	0.47	0.50	0.48
70	0.09	0.09	0.04	0.10	0.14	0.18	0.16	0.20	0.16	0.18	0.18	0.18	0.14	0.20
65	0.03	0.00	0.00	0.03	0.04	0.07	0.01	0.03	0.03	0.00	0.01	0.01	0.06	0.03
60	0.04	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00

Table 2.6. Estimation of genetic variability for 13 loci (Fdh and Gr were excluded because of missing data) for populations of M. trossulus and M. galloprovincialis (hybrids have been excluded). N=mean number of alleles per locus $\pm$ standard error; P=frequency of polymorphism determined by the 95% criterion. Observed (H_{obs}) and expected (H_{exp}) heterozygosity are means summed across all loci $\pm$ standard error. F=mean fixation index summed across loci.

Collection	N	P	H_{obs}	H_{exp}	F
<u>Mytilus trossulus</u>					
AK	3.31 $\pm$ 0.47	0.69	0.28 $\pm$ 0.02	0.34 $\pm$ 0.03	0.12
CC	4.00 $\pm$ 0.39	0.76	0.30 $\pm$ 0.02	0.35 $\pm$ 0.03	0.08
ES1H	3.76 $\pm$ 0.51	0.85	0.32 $\pm$ 0.02	0.36 $\pm$ 0.03	0.07
ES1L	3.92 $\pm$ 0.43	0.85	0.31 $\pm$ 0.02	0.36 $\pm$ 0.03	0.14
ES3H	3.62 $\pm$ 0.49	0.77	0.32 $\pm$ 0.02	0.35 $\pm$ 0.03	0.08
ES3L	3.85 $\pm$ 0.44	0.92	0.35 $\pm$ 0.02	0.38 $\pm$ 0.03	0.08
WI	4.07 $\pm$ 0.47	0.85	0.34 $\pm$ 0.02	0.38 $\pm$ 0.03	0.09
BBH	4.15 $\pm$ 0.55	0.77	0.35 $\pm$ 0.02	0.36 $\pm$ 0.04	0.02
BBL	3.53 $\pm$ 0.39	0.77	0.31 $\pm$ 0.02	0.36 $\pm$ 0.03	0.12
BK	2.46 $\pm$ 0.31	0.85	0.27 $\pm$ 0.05	0.38 $\pm$ 0.08	0.25
SR	3.00 $\pm$ 0.39	0.85	0.38 $\pm$ 0.04	0.39 $\pm$ 0.07	0.02
BN	3.77 $\pm$ 0.38	0.85	0.32 $\pm$ 0.02	0.38 $\pm$ 0.03	0.13
SS	3.77 $\pm$ 0.41	0.85	0.30 $\pm$ 0.02	0.37 $\pm$ 0.03	0.20
SL1	2.85 $\pm$ 0.32	0.85	0.25 $\pm$ 0.04	0.40 $\pm$ 0.06	0.31
SL2	2.77 $\pm$ 0.39	0.77	0.42 $\pm$ 0.06	0.43 $\pm$ 0.05	0.05
<u>Mytilus galloprovincialis</u>					
BBH	2.07 $\pm$ 0.26	0.69	0.38 $\pm$ 0.08	0.41 $\pm$ 0.09	0.08
BBL	1.23 $\pm$ 0.12	0.24	0.23 $\pm$ 0.12	0.23 $\pm$ 0.09	0.00
TBH	3.31 $\pm$ 0.47	0.69	0.27 $\pm$ 0.02	0.31 $\pm$ 0.03	0.10
TBL	3.15 $\pm$ 0.36	0.85	0.26 $\pm$ 0.02	0.31 $\pm$ 0.04	0.12
BK	3.00 $\pm$ 0.37	0.77	0.27 $\pm$ 0.02	0.32 $\pm$ 0.04	0.12
SR	3.23 $\pm$ 0.39	0.77	0.27 $\pm$ 0.02	0.33 $\pm$ 0.04	0.23
SS	2.77 $\pm$ 0.38	0.77	0.32 $\pm$ 0.04	0.37 $\pm$ 0.07	0.19
SL1	2.92 $\pm$ 0.38	0.77	0.31 $\pm$ 0.03	0.37 $\pm$ 0.05	0.14
SL2	2.84 $\pm$ 0.38	0.61	0.25 $\pm$ 0.02	0.32 $\pm$ 0.03	0.14
MLH	3.15 $\pm$ 0.41	0.77	0.31 $\pm$ 0.02	0.34 $\pm$ 0.03	0.08
MLL	3.46 $\pm$ 0.42	0.69	0.32 $\pm$ 0.02	0.35 $\pm$ 0.03	0.06
MB	3.39 $\pm$ 0.47	0.69	0.31 $\pm$ 0.02	0.33 $\pm$ 0.03	0.06
HZ	2.77 $\pm$ 0.36	0.69	0.26 $\pm$ 0.02	0.31 $\pm$ 0.03	0.11
SLH	3.31 $\pm$ 0.38	0.69	0.27 $\pm$ 0.02	0.31 $\pm$ 0.03	0.08
SLL	3.53 $\pm$ 0.43	0.69	0.28 $\pm$ 0.02	0.31 $\pm$ 0.03	0.11
GV	3.15 $\pm$ 0.41	0.69	0.25 $\pm$ 0.02	0.31 $\pm$ 0.03	0.12
SB	3.46 $\pm$ 0.43	0.69	0.29 $\pm$ 0.02	0.33 $\pm$ 0.03	0.06
VN	3.61 $\pm$ 0.46	0.69	0.30 $\pm$ 0.02	0.33 $\pm$ 0.03	0.03
LA	3.00 $\pm$ 0.42	0.69	0.25 $\pm$ 0.02	0.31 $\pm$ 0.03	0.11
NP	3.23 $\pm$ 0.39	0.69	0.27 $\pm$ 0.02	0.32 $\pm$ 0.03	0.12

principal components analysis (Gr and Fdh were excluded because of missing data) separated three groups based on the first principal component scores. The first principal component explained 13% of the total variance, and reflected variation at the Mpi, Gpi, Odh, Ap, Pgm, Ac-1, Ac-2, and Aap loci. The remaining principal components each explained less than 3% of the variance, and represented variation not associated with differences between M. galloprovincialis and M. trossulus. The first principal component scores for Mytilus galloprovincialis ranged from -0.55 to -4.32. Mytilus trossulus had first principal scores ranging from 1.45 to 6.07, while hybrid mussels were interpreted as having first principal scores between -0.55 and 1.45 (Fig.2.2). Mussels that were genetically intermediate between M. galloprovincialis and M. trossulus were rare in the 28 collections, and only 57 out of 1149 (5%) mussels were classified as possible hybrids (Fig.2.3; Fig.2.4).

The genetic structure within and among populations of Mytilus galloprovincialis and M. trossulus was analyzed using hierarchical F statistics (F_{ID} , F_{DS} , and F_{ST}). There was little evidence for genetic heterogeneity in either M. galloprovincialis or M. trossulus over micro- or macrogeographic scales: F_{DS} and F_{ST} values were not statistically different from zero (Table 2.7). Mean F_{ID} values, representing average departure from Hardy-Weinberg genotypic proportions were significantly different from

Figure 2.2. Map of central California coastline showing twelve sampling locations (abbreviations as in Table 2.1) with histograms of first principal component scores from allozyme data (above the abscissas) and distribution of first canonical variable scores from morphological data (below abscissas). Open, filled and hatched bars represent M. trossulus, M. galloprovincialis, and presumptive hybrid mussels respectively based on principal-component scores.

Figure 2.3. Map of California showing sixteen sampling locations with pie diagrams representing the percentages of M. trossulus (open), M. galloprovincialis (filled), and presumptive hybrid mussels (hatched) at each location.

— - AK

Figure 2.4. Map of central California coastline showing twelve sampling locations with pie diagrams representing the percentages of M. trossulus (open), M. galloprovincialis (filled), and presumptive hybrid mussels (hatched) at each location.

Table 2.7. Mytilus spp.: Hierarchical F-statistics, with means and standard errors (S.E.).

Locus	<u>Mytilus trossulus</u>			<u>Mytilus galloprovincialis</u>		
	F _{ID}	F _{DS}	F _{ST}	F _{ID}	F _{DS}	F _{ST}
<u>Aap</u>	0.224	0.014	0.027	0.120	0.146	0.167
<u>Aat-1</u>	-0.021	0.009	-0.004	-0.003	0.004	0.000
<u>Aat-2</u>	0.048	-0.007	0.018	-0.008	0.010	-0.002
<u>Ac-1</u>	0.039	0.017	-0.006	0.112	0.032	-0.006
<u>Ac-2</u>	0.090	0.025	0.006	0.114	-0.004	0.007
<u>Ap</u>	0.097	0.000	0.005	0.249	0.003	-0.001
<u>Est-D</u>	0.146	0.012	-0.003	0.056	0.014	-0.010
<u>Gpi</u>	0.165	0.006	0.011	0.104	0.023	-0.005
<u>Lap</u>	0.202	0.005	0.002	0.249	0.001	0.000
<u>Mpi</u>	0.060	0.032	0.025	0.166	-0.007	0.014
<u>Odh</u>	0.152	-0.003	0.002	0.045	0.002	0.004
<u>Pgm</u>	0.033	0.001	0.001	0.071	0.005	0.002
Mean	0.122	0.007	0.007	0.131	0.018	0.015
S.E.	0.025	0.003	0.003	0.033	0.012	0.016

zero, based on standard errors, for both M. galloprovincialis and M. trossulus. The mean F_{ID} values, and 21 of 24 single locus F_{ID} values were positive, indicating an overall deficiency of heterozygotes for both species.

Canonical discriminant analysis of seven morphological variables using the allozyme-derived classification of each mussel as M. trossulus, M. galloprovincialis, or presumptive hybrid was somewhat successful in separating mussels based on morphological criteria. The first canonical variable separated M. trossulus (mean=-1.401) from M. galloprovincialis (mean=1.132), but presumptive hybrids (mean=0.465) could not always be separated from M. trossulus or M. galloprovincialis based on morphological criteria alone. Examination of canonical coefficients showed that all morphological variables had fairly high positive correlations. High positive correlations of the first canonical coefficient for three shell measurements: length, width, and height are probably not a result of actual differences between M. galloprovincialis and M. trossulus, but instead reflect size differences among certain collections due to differences in growth rate or age structure (Table 2.8). Byssal retractor muscle scar width, posterior adductor muscle scar length, and length of the byssal retractor muscle scar had high positive correlations for the first canonical coefficient (Table 2.9).

Table 2.8. Mytilus spp.: mean values (± 1 standard error) for seven shell measurements in twelve collections (not separated by species). See text for explanation of shell measurement abbreviations.

Sample	sl	sh	sw	aams	pam	lbrs	wbrs
AK	44.6 $\pm$ 1.4	20.9 $\pm$ 0.5	8.4 $\pm$ 0.3	3.9 $\pm$ 0.1	7.8 $\pm$ 0.3	10.6 $\pm$ 0.4	2.7 $\pm$ 0.1
BBH	23.8 $\pm$ 0.9	13.5 $\pm$ 0.5	5.3 $\pm$ 0.2	1.9 $\pm$ 0.1	4.1 $\pm$ 0.2	6.5 $\pm$ 0.3	1.7 $\pm$ 0.1
BBL	28.3 $\pm$ 1.5	17.0 $\pm$ 0.8	5.5 $\pm$ 0.3	2.0 $\pm$ 0.1	4.7 $\pm$ 0.3	7.5 $\pm$ 0.4	2.0 $\pm$ 0.1
BK	49.4 $\pm$ 2.4	27.0 $\pm$ 1.3	11.3 $\pm$ 0.8	3.3 $\pm$ 0.2	9.7 $\pm$ 0.6	13.5 $\pm$ 0.7	3.5 $\pm$ 0.2
BN	25.1 $\pm$ 0.9	13.2 $\pm$ 0.5	5.5 $\pm$ 0.2	1.9 $\pm$ 0.1	4.0 $\pm$ 0.2	7.2 $\pm$ 0.3	1.7 $\pm$ 0.1
LA	44.2 $\pm$ 1.3	24.0 $\pm$ 0.8	9.4 $\pm$ 0.3	2.6 $\pm$ 0.3	8.8 $\pm$ 0.3	12.2 $\pm$ 0.4	3.8 $\pm$ 0.1
ML	39.9 $\pm$ 1.5	23.0 $\pm$ 0.8	8.3 $\pm$ 0.3	3.0 $\pm$ 0.1	6.8 $\pm$ 0.3	10.8 $\pm$ 0.5	3.4 $\pm$ 0.1
MLH	47.8 $\pm$ 1.6	24.8 $\pm$ 0.8	10.8 $\pm$ 0.4	3.8 $\pm$ 0.1	9.6 $\pm$ 0.3	13.4 $\pm$ 0.5	3.3 $\pm$ 0.1
MLL	51.5 $\pm$ 1.8	27.4 $\pm$ 0.9	12.0 $\pm$ 0.5	3.7 $\pm$ 0.1	10.7 $\pm$ 0.4	13.5 $\pm$ 0.7	4.1 $\pm$ 0.2
ML2	44.8 $\pm$ 1.8	25.1 $\pm$ 1.0	9.5 $\pm$ 0.4	3.7 $\pm$ 0.2	8.5 $\pm$ 0.4	12.8 $\pm$ 0.6	4.1 $\pm$ 0.2
SR	53.6 $\pm$ 2.1	28.2 $\pm$ 1.1	11.3 $\pm$ 0.4	3.8 $\pm$ 0.1	10.3 $\pm$ 0.5	15.2 $\pm$ 0.7	3.6 $\pm$ 0.2
SS	34.5 $\pm$ 1.9	18.7 $\pm$ 1.0	6.2 $\pm$ 0.4	2.4 $\pm$ 0.1	5.1 $\pm$ 0.3	9.3 $\pm$ 0.5	2.2 $\pm$ 0.1

Table 2.9. Mytilus spp.: Total canonical structure of seven morphological variables.

Variable	First	Second
	Canonical	Canonical
	Variable	Variable
Shell length	0.697	0.134
Shell height	0.812	0.274
Shell width	0.748	-0.004
Anterior adductor muscle scar length	0.494	0.379
Posterior adductor muscle scar length	0.784	0.100
Byssal retractor muscle scar length	0.627	0.301
Byssal retractor muscle scar width	0.921	-0.070

Table 2.10. ANOVA table from multiple regression analysis of the effect of environmental parameters on the geographic distribution of M. trossulus.

Source of variation	Degrees of Freedom	Mean Square	F Value	Prob > F
Model	6	7570.71	26.38	0.0001
Low Temperature	1	2821.88	9.83	0.0052
Temperature Range	1	21874.28	76.22	0.0001
Low Salinity	1	4011.30	13.98	0.0013
Salinity Range	1	3022.54	10.53	0.0041
Habitat Type	1	345.25	1.20	0.2858
Tidal Height	1	113.59	0.40	0.5364
Error	20	287.00		
Total	26			

Table 2.11. ANOVA table from multiple regression analysis of the effect of environmental parameters on the geographic distribution of M. galloprovincialis.

Source of variation	Degrees of Freedom	Mean Square	F Value	Prob > F
Model	6	7643.57	27.11	0.0001
Low Temperature	1	964.55	3.42	0.0792
Temperature Range	1	22357.82	79.29	0.0001
Low Salinity	1	4845.42	17.18	0.0005
Salinity Range	1	3703.62	13.13	0.0017
Habitat Type	1	737.04	2.61	0.1216
Tidal Height	1	4.68	0.02	0.8988
Error	20	281.97		
Total	26			

The results of the multiple regression analysis of the environmental data are presented in Table 2.10 and Table 2.11. The proportion of variation in the distribution of Mytilus trossulus and M. galloprovincialis explained by the model is 88.8% ($R^2=0.888$) and 89.1% ($R^2=0.891$) respectively. Temperature range, low salinity, and salinity range had statistically significant influences on the distribution of M. galloprovincialis. The low temperature of the temperature range, temperature range, the low salinity of the salinity range, and salinity range had statistically significant influences on the distribution of M. trossulus. Habitat type (open vs. sheltered sites) and tidal type (high vs. low) did not have statistically significant effects on the distributions of either M. galloprovincialis or M. trossulus.

The results of the analysis of variance for the effect of tidal height, collection, and species on Odh¹⁰⁰ and Odh¹¹⁰ allele frequencies are presented in Table 2.12 and Table 2.13. For the frequency of each allele, the sources of variation were: collection (block), species (treatment), tidal height (treatment), and residual (error) variation. For both Odh¹⁰⁰ and Odh¹¹⁰ allele frequencies, tidal height did not have any significant effect. The only significant variation in Odh allele frequencies was due to species (Mytilus trossulus vs. M. galloprovincialis). The least squares mean allele frequencies of Odh¹¹⁰ for Mytilus

Table 2.12. ANOVA table for the effect of tidal height on the frequency of the octopine dehydrogenase 110 allele (Odh¹¹⁰) with hybrids removed from the analysis.

Source of variation	Degrees of Freedom	Mean Square	F Value	Prob > F
Model	8	6.56	98.60	0.0001
Block (location)	6	0.20		
Species	1	51.26	771.03	0.0001
Tidal Height	1	0.0006	0.01	0.9245
Error	10	0.66		
Total	18			

Table 2.13. ANOVA table for the effect of tidal height on the frequency of the octopine dehydrogenase 100 allele (Odh¹⁰⁰) with hybrids removed from the analysis.

Source of variation	Degrees of Freedom	Mean Square	F Value	Prob > F
Model	8	4.02	25.30	0.0001
Block (location)	6	0.18		
Species	1	31.05	195.63	0.0001
Tidal Height	1	0.001	0.01	0.9254
Error	10	0.16		
Total	18			

galloprovincialis were 0.614 and 0.617 for high and low samples respectively, and 0.130 and 0.129 in M. trossulus for high and low samples respectively. For Odh¹⁰⁰, the least squares mean allele frequencies for M. galloprovincialis were 0.092 and 0.044 for high and low samples respectively, and 0.436 and 0.494 in M. trossulus for high and low samples.

Discussion

Previous genetic studies of Mytilus spp. on the Pacific coast of the U.S. have focused on broad geographic surveys of allozyme variation that have primarily addressed the taxonomic status of Mytilus spp. (McDonald and Koehn, 1988; Sarver and Loudenslager, 1991). The present study included more extensive sampling from a variety of habitats in the region of overlap between M. trossulus and M. galloprovincialis, included additional allozyme loci, and focused on the possible mechanisms that keep M. trossulus and M. galloprovincialis genetically distinct in spite of hybridization. Also, because different electrophoretic conditions were used from previous studies, allele homologies among studies were not determined. Regardless of the differences between this study and previous studies of Mytilus spp. on the Pacific coast of the U.S., several common points have emerged. First, the overall geographic distribution of M. trossulus and M. galloprovincialis

reported in this study is largely in agreement with previous studies (McDonald and Koehn, 1988; Koehn, 1991; Sarver and Loudenslager, 1991). Mostly pure populations of M. trossulus occur north of Cape Mendocino and mostly pure M. galloprovincialis populations occur south of Point Conception. Also, genetically intermediate mussels (presumptive M. trossulus x M. galloprovincialis hybrids) are relatively rare, comprising only 5% of the mussels analyzed in this study compared with approximately 12% from McDonald and Koehn (1988). McDonald et al. (1991) observed the occurrence of M. trossulus x M. galloprovincialis hybrids at Crescent City, Westport, Tomales Bay, San Rafael, and Port San Luis. The present study also observed M. trossulus x M. galloprovincialis hybrids at Crescent City, Tomales Bay and San Rafael, as well as at Bodega Bay, several locations in San Francisco Bay, in Monterey Bay (Moss Landing and Salinas River sites) and Los Angeles (Marina del Rey). Hybrid mussels were not observed at Port San Luis in this study, nor did McDonald et al. (1991) note the occurrence of hybrids as far south as Los Angeles. The occurrence of hybrid mussels as far north as Crescent City and as far south as Los Angeles (a range of approximately 1200 km) indicates that hybrids may occur, although in low frequencies, over a larger area than previously suspected (Sarver and Loudenslager, 1991).

As with most previous studies of Mytilus and other marine bivalves, this study noted the occurrence of deviations from Hardy-Weinberg expectations for genotypic proportions in the form of heterozygote deficiencies (positive F_{ID} values in Table 2.4). In this study the strongest deficiencies were observed for the Aap, Lap, Gpi and Odh loci in M. trossulus and the Ap, Lap, Mpi and Aap loci in M. galloprovincialis. The origin of heterozygote deficiencies in marine bivalves has been the subject of a great deal of speculation (for reviews see Zouros and Foltz, 1984; Zouros, 1987), and several explanations have been suggested to account for heterozygote deficiencies, including self-fertilization, null alleles, mis-scoring of gels, population mixing and natural selection. The possible role of null alleles in generating heterozygote deficiencies in Mytilus is explored in Chapter 3 of this dissertation.

The taxonomic status of electrophoretically and/or morphologically distinguishable forms of Mytilus has been the subject of considerable debate for a number of years. For example, some authors have suggested members of the Mytilus edulis complex are ecotypes or varieties (Gosling, 1984; Johannesson et al., 1990), while others advocate the recognition of the members of the Mytilus edulis complex as separate species (McDonald and Koehn, 1988; McDonald et al., 1991). Any debate about taxonomy at the species level is largely dependent on the species definition which is used.

A number of authors (e.g., Woodruff et al. 1988; Kwast et al., 1990; Liu et al. 1991; Sarver et al., 1992) have suggested that the evolutionary species concept (Wiley, 1981), which places greater emphasis on genetic integrity of separate evolutionary lineages regardless of the potential for hybridization, should be used rather than the biological species concept. Using the evolutionary species concept, the combination of a large genetic distance ($D=0.39$), the genetic homogeneity of populations over broad geographic ranges ($F_{ST}=0.007$ and 0.015 for M. trossulus and M. galloprovincialis, respectively), differences in shell morphology, the overall rarity of genetically intermediate mussels (hybrids), and the maintenance of genetic integrity despite the potential for hybridization, supports the recommendation of McDonald et al. (1991) that M. trossulus, M. galloprovincialis and M. edulis be regarded as separate species.

There have been a number of studies using gross shell morphology to distinguish between Mytilus edulis and M. galloprovincialis (Lewis and Seed, 1969; Seed, 1972; 1974; Beaumont et al., 1989;). Most of these studies have shown that morphological traits alone can not always distinguish between M. galloprovincialis and M. edulis (Koehn, 1991). This is due in part to plasticity Mytilus shell morphology in relation to local habitat conditions (Seed, 1968), and because many of the early studies that examined

morphological differences between M. edulis and M. galloprovincialis were done in areas now known to be regions where the two species overlap and hybridize (Seed, 1972; 1974). In general, the morphological characters that have proven most useful in distinguishing M. edulis and M. galloprovincialis are length of the adductor muscle scar and length of the hinge plate (Beaumont et al., 1989). Fewer studies have examined the morphological characteristics of M. trossulus in relation to either M. edulis and/or M. galloprovincialis. In a study of mussels from 36 locations in the Northern Hemisphere and nine locations in the Southern Hemisphere, McDonald et al. (1991) characterized mussels using both allozyme electrophoresis and morphological analysis using 18 shell characters. Restricting their analysis to areas containing only pure populations of M. edulis, M. galloprovincialis or M. trossulus, McDonald et al. (1991) were able to distinguish the 3 species of Mytilus based on multilocus genotypes derived from allozyme electrophoresis and morphological characteristics. Using canonical variates analysis of 18 shell characters, McDonald et al. (1991) could separate M. galloprovincialis and M. trossulus into separate groups based on first canonical variable scores. The shell characters that provided the greatest differences between M. galloprovincialis and M. trossulus were number of hinge teeth and width of the byssal retractor scar, but when these characters were analyzed singly there

was considerable overlap between species. The present study analyzed variation in seven shell characteristics, so it is not directly comparable to McDonald et al. (1991), but the results from this study also showed that in pure populations, M. trossulus and M. galloprovincialis could be grouped into separate clusters with some overlap using canonical discriminant analysis (Fig 2.2). The most informative morphological characters in this study were byssal retractor muscle scar width, posterior adductor muscle scar length and byssal retractor muscle scar length. In general, M. galloprovincialis had a wider byssal retractor muscle scar, a greater posterior adductor muscle scar length, and a greater byssal retractor muscle scar length than M. trossulus.

In populations that contained mixtures of Mytilus galloprovincialis, M. trossulus and hybrid mussels it was not possible to separate the three groups into three non-overlapping clusters using canonical discriminate analysis of morphological data. This is consistent with other studies of morphological variation in hybridizing marine invertebrates. For example, Sarver et al. (1992) describe a similar finding in a study of the ribbed mussels Geukensia demissa and G. granosissima, with morphological differences clear in pure populations, but in mixed and hybridizing populations morphological discrimination was less distinct between species. As in the study of Sarver et al. (1992),

it is not possible to determine if the breakdown in morphological discrimination observed in this study was the result of environmental effects or introgressive hybridization.

A number of studies of Mytilus have demonstrated significant genetic differentiation between populations that are well within the dispersal capabilities of the planktonic dispersal stage of mussel larvae. This finding has prompted many researchers to implicate strong natural selection as a factor shaping the genetic structure of natural mussel populations. For example, Gartner-Kepkay et al. (1980; 1983) found microgeographic genetic difference among geographically proximal populations, and the populations clustered according to their environment rather than geographic proximity. Theisen (1978) observed strong clines for several enzyme loci in populations of Mytilus in the Danish Belt Sea which connects the North Sea with the Baltic Sea. Both Gartner-Kepkay et al. (1983) and Theisen (1978) argued that natural selection was responsible for the observed patterns of genetic differentiation. It is now known that the areas studied by Gartner-Kepkay et al. (1980; 1983) and Theisen (1978) are regions where different species of Mytilus come into contact and hybridize (M. edulis and M. trossulus in the Canadian Maritimes and M. edulis and M. trossulus in the North Sea and Baltic Sea). In light of

these recent findings the studies of Gartner-Kepkay et al. (1980; 1983) and Theisen (1978) need to be reevaluated.

Microgeographic genetic differentiation has also been reported among mussel populations in the United Kingdom. The situation in the British Isles differs from that of Gartner-Kepkay et al. (1980; 1983) and Theisen (1978) because it was known that Great Britain and Ireland are areas where two species of Mytilus (M. edulis and M. galloprovincialis) come together to form mixtures of the two species (Ahmad and Beardmore, 1976; Skibinski and Beardmore, 1979; Gosling and Wilkins, 1981). The geographic differences in allele frequencies that are observed in the United Kingdom are thought to represent differences in the distribution of the two species of mussel. For example, Gosling and Wilkins (1981) compared allele frequencies for three enzymes (Phi, Lap and Pgm) in Mytilus populations from two types of habitat in Ireland, exposed sites, and bay sites with known pure samples of M. galloprovincialis from Venice and M. edulis from France. Gosling and Wilkins (1981) observed differences in allele frequencies between exposed sites and sheltered sites, and interpreted these differences to indicate that M. galloprovincialis was more prominent at exposed sites and M. edulis predominates at sheltered locations. A similar finding was observed by Skibinski et al. (1983) in areas of southwest England, northeast England and Scotland where both M. edulis and M.

galloprovincialis are also known to occur. Skibinski et al. (1983) observed higher frequencies of M. galloprovincialis alleles for the Est-D locus at exposed locations than at less exposed locations. Moreover, M. galloprovincialis has recently been shown to be able to withstand greater wave forces than M. edulis, due to greater strength of attachment to the substrate, which is presumed to make it less susceptible to dislodgement from wave action than M. edulis (Willis and Skibinski, 1992).

Data presented in the present study indicate that microgeographic genetic differentiation of mussel populations along the California coastline results from heterogeneity in the proportions of Mytilus trossulus and M. galloprovincialis among sites. There is no evidence for microgeographic genetic differentiation within either M. trossulus or M. galloprovincialis as illustrated by the low F_{DS} values for both M. trossulus and M. galloprovincialis.

The results of the multiple-regression analysis suggest that several environmental parameters significantly influence the geographic distribution of Mytilus trossulus and M. galloprovincialis along the California coastline. The variables in the multiple-regression that significantly influenced the proportion of M. trossulus were low annual temperature, temperature range (the difference between high annual temperature and low annual temperature), low annual salinity and salinity range (the difference between high

annual salinity and low annual salinity). The most important variables in the multiple-regression influencing the proportion of M. galloprovincialis were temperature range, low salinity, and salinity range. Mytilus trossulus was associated with lower temperatures, and habitats that experienced greater salinity variation. On the other hand, M. galloprovincialis was associated with higher temperatures and locations with little salinity variation.

It is possible that the significant influences of temperature and salinity on the distributions of Mytilus trossulus and M. galloprovincialis demonstrated by the multiple regression analyses are just spurious correlations, and the distributions of the two species of bay mussels are influenced by other factors such as current patterns, patterns of larval dispersal or historical distributional patterns of the two species. The effect of such factors as current patterns or patterns of larval dispersal are more likely to be important over broad geographic scales. A second set of multiple regression analyses were performed, this time assessing the effects of temperature and salinity on the distributions of M. trossulus and M. galloprovincialis only over microgeographic scales (samples in fig. 2.2 and 2.4). Wave exposure (open coast or bay) and tidal height (intertidal or subtidal) were shown in the previous analyses not to be significant, and were not included in the second set of analyses. To further remove

the effect of macrogeographic differences, three bay systems were used as blocks in the multiple regression analyses: Monterey Bay, San Francisco Bay and Tomales/Bodega Bay. The results of the second set of analyses indicate that salinity is more important than temperature in influencing the distributions of M. galloprovincialis and M. trossulus. For example, low annual temperature and temperature range were not statistically significant for either M. trossulus or M. galloprovincialis ($P > 0.05$). Low salinity and salinity range were statistically significant for both M. trossulus and M. galloprovincialis. It appears that salinity (low salinity and salinity range) is more important than temperature (low temperature and temperature range) in determining the distributions of M. galloprovincialis and M. trossulus on the California coast.

The ecological relationship between Mytilus trossulus and M. galloprovincialis in California appears to be different than the ecological relationship between M. edulis and M. galloprovincialis in the contact zone in Europe. In contrast to the situation in the United Kingdom, degree of exposure (sheltered vs. open coast) does not appear to be a significant factor influencing the distributions of M. trossulus and M. galloprovincialis (Table 2.10; 2.11). This may be related to the occurrence of M. californianus which is confined to the Pacific coast of North America from the Aleutian Islands to Mexico, where

it occurs principally on exposed shorelines (Soot-Ryen, 1955). Salinity appears to be the most important environmental factor affecting the distribution of M. trossulus and M. galloprovincialis over microgeographic scales in the contact zone in central California. In San Francisco Bay, the distribution of M. trossulus and M. galloprovincialis varied considerably among sites that were separated by less than 10 kilometers. For example, the Benicia and Sausalito samples had a higher proportion of M. trossulus (90% M. trossulus/ 10% hybrids and 75% M. trossulus/ 7% hybrids/ 18% M. galloprovincialis, respectively) than Berkeley or San Rafael (77% M. galloprovincialis/ 6% hybrids/ 17% M. trossulus and 54% M. galloprovincialis/ 24% hybrids/ 22% M. trossulus, respectively).

San Francisco Bay is a large, geographically and hydrographically complex estuary consisting of two distinct subestuaries (Conomos et al., 1985). The North Bay lying between the connection to the Pacific Ocean and the confluence of the Sacramento-San Joaquin River System, is a partially mixed estuary dominated by seasonal varying river flow. The South Bay is a tidally oscillating lagoon estuary that typically has higher salinities than the North Bay (Conomos et al., 1985). Benicia is located in the Carquinez Strait which separates San Pablo Bay from Suisun Bay in the North Bay, and is characterized by low average salinities

(mean=10-15‰), and is reported to be the eastern most location to find Mytilus in San Francisco Bay (Mark Stephenson, Associate Biologist, Mussel Watch Program, Moss Landing California, CA, pers. comm.). Sausalito, on the other hand, is located near the mouth of the bay near the Golden Gate Bridge, and typically has cooler (more oceanic) temperatures than other locations in San Francisco Bay. Salinities at Sausalito are also typical of the Pacific Ocean but can vary considerably during periods of heavy rainfall, when the water in the bay can become stratified, with a lens of low salinity water that can reach as far as the mouth of the Bay (Dedini et al., 1981). San Rafael is located in San Pablo Bay and has somewhat higher temperatures and higher salinities than Benicia because of the complex circulation patterns in San Francisco Bay. Data for Berkeley Marina are somewhat contradictory with a greater salinity range (3-35‰) than San Rafael, but higher temperatures than Sausalito and Benicia. Mytilus trossulus predominates at Benicia and Sausalito and M. galloprovincialis is dominant at San Rafael and Berkeley.

The effect of salinity on the distribution of Mytilus trossulus and M. galloprovincialis can also be seen for samples collected in Monterey Bay. Moss Landing High (MLH) and Moss Landing Low (MLL) had a greater proportion of M. galloprovincialis (90% M. galloprovincialis/ 10% hybrids and 97% M. galloprovincialis/ 3% hybrids, respectively) than two

other sites in Monterey Bay, Salinas River 1 (SL1) and Salinas River 2 (SL2) (37% M. galloprovincialis/ 24% M. trossulus/ 39% hybrids and 61% M. galloprovincialis/ 12% M. trossulus/ 27% hybrids, respectively). The Salinas River samples experience greater salinity variation than the Moss Landing samples as a result of their proximity to the mouth of the Salinas river (Nybakken et al., 1977).

The macrogeographic distribution of Mytilus trossulus and M. galloprovincialis is also consistent with the suggestion that M. trossulus is a euryhaline, cool water mussel and M. galloprovincialis is a warm water, oceanic mussel (e.g., Johannesson et al., 1990). For example, a rather sharp thermal gradient of 4°C occurs near Point Conception, which is the focal point of a faunal change between the Californian and Oregonian biogeographic provinces. The region near Point Conception appears to be northern limit to the distribution of pure M. galloprovincialis populations. Also, because of the arid nature of southern California, there are very few estuarine habitats south of Monterey.

Gosling and McGrath (1990) demonstrated genetic differences between mussel populations at different tidal heights using two diagnostic enzyme loci Est-D and Odh. Gosling and McGrath (1990) found statistically different frequencies in Odh allele frequencies between mid and lower-shore mussels. Furthermore, mussels higher up in the

intertidal had higher frequencies of Odh alleles associated with Mytilus galloprovincialis and mussels lower in the intertidal had alleles characteristic of M. edulis. There was no significant heterogeneity among samples for Est-D. Gosling and McGrath (1990) concluded that the differences between upper and lower shore mussels were due either to taxonomic differences or the action of natural selection. The results of the multiple-regression analysis indicates that there was no tendency for either M. trossulus or M. galloprovincialis to be associated with high shore or low shore environments in the present study. A test for the action of natural selection acting on the Odh locus was done using an ANOVA for the effect of position in the intertidal zone (high vs. low) on Odh allele frequencies. The only significant variable in the ANOVA was species; no significant effect of either position in the intertidal or collection locality on Odh allele frequencies could be demonstrated. Thus in contrast to Gosling and McGrath, no microgeographic differentiation of Odh allele frequencies attributable to taxonomic differences between upper and lower shore mussels, or natural selection was observed in the present study.

Chapter 3

THE POSSIBLE EXISTENCE OF NULL ALLELES IN THE MUSSEL

MYTILUS TROSSULUS

Introduction

There have been many studies investigating the genetic structure of marine bivalve populations. From these studies several noteworthy trends have emerged. Studies of marine bivalves consistently report apparent overdominance in fitness components (such as growth) for allozyme genotypes (Singh and Zouros, 1978; Zouros et al., 1980; Zouros and Foltz, 1987). The genetic interpretation of these findings has been complicated by several other characteristics of marine bivalve genetics. For example, allozyme loci that have been found to show a strong correlation between heterozygosity and fitness components usually exhibit a deficiency of heterozygotes compared to Hardy-Weinberg predictions. Furthermore these two phenomena appear to be related, in that a correlation between heterozygosity and growth rate in bivalves is usually associated with heterozygote deficiency at the same set of loci (Zouros, 1987).

A number of models to explain heterozygosity-fitness correlations have been suggested. The main distinction among these models is in the role allozyme loci play in the heterozygosity-fitness correlations. One hypothesis

suggests that the allozyme loci themselves are responsible for the correlation between heterozygosity and fitness (Koehn et al., 1988). In a study of a single age cohort of Mulinia lateralis, Koehn et al. (1988) found that growth was correlated with heterozygosity for loci coding for enzymes in glycolytic and protein catabolic pathways. Intermediacy in enzyme phenotype conferred by heterozygosity (Kacser and Burns, 1981) was suggested to be important in maintaining flux in these important metabolic pathways. Apparent overdominance can also result from the action of closely linked modifier genes or through misclassification of genotypes from normal Mendelian patterns of inheritance and expression (Zouros and Foltz, 1987). For example, the scored allozyme loci may not be responsible for the heterozygosity-fitness correlations, but instead they may serve as markers for linked loci (Ohta, 1971; Zouros, 1987). The linked gene model assumes that homozygotes for allozyme loci are more likely to be homozygous for linked detrimental genes than heterozygotes for these same loci (associative-overdominance). The higher the frequency of deleterious genes in the population, the stronger the effect under the associative-overdominance model. Null activity alleles could also be responsible for heterozygosity-fitness correlations (Foltz, 1986). For example, if a null activity allele is segregating at an allozyme locus, null heterozygotes will inadvertently be scored as homozygotes.

If null activity alleles have negative affects on fitness, then apparent homozygotes might show reduced fitness or growth relative to heterozygotes. Other possible explanations for heterozygosity-fitness correlations and deficiencies of heterozygotes include molecular imprinting or a high rate of aneuploidy (Zouros et al., 1988; Chakraborty, 1989; Gaffney et al., 1990).

Several studies have reported dominance or overdominance in specific activities of allozyme genotypes in marine bivalves for loci that have shown apparent overdominance in growth rate and exhibited deficiencies of heterozygotes (Hilbish and Koehn, 1985; Pogson, 1991). The present study examines a highly polymorphic enzyme locus in another bivalve species Mytilus trossulus that has been demonstrated in this and related Mytilus species to show correlations between heterozygosity and growth rate and to exhibit significant heterozygote deficiencies: the octopine dehydrogenase locus (octopine: NAD oxidoreductase; E.C. 1.5.1.11) (Koehn and Gaffney, 1984; Zouros et al., 1988; Gosling, 1989).

Materials and Methods

Mussels (Mytilus trossulus) were collected from Humboldt Bay, California and shipped live to Baton Rouge, Louisiana. Posterior adductor muscle was dissected from each mussel and used to make enzyme extracts. Buffer (10 mM

Tris-HCl pH 7.0 at 20°C) was added to each tissue sample to give a 10-fold (v:w) dilution. Samples were homogenized and centrifuged at 49,500 x g for 20 min at 0-4°C.

Horizontal starch-gel electrophoresis was performed using standard methods (Selander et al., 1971). Several gel buffer systems (nos. 2, 3, 4, 9 from Selander et al. 1971) were utilized in an effort to detect all mobility variants (electromorphs). Electromorphs were designated by numbers indicating approximate mobility relative to the most common electromorph which was designated "100". Allelic homologies to electromorphs from previous studies were not determined (McDonald and Koehn, 1988; Bulnheim and Gosling, 1988; Sarver and Loudenslager, 1991). Also, "0" was used to designate a presumed null or enzymatically non-reactive allele.

The enzyme activity assay was modified from Gäde and Grieshaber (1975). The standard assay medium contained 100 μ mol tris-HCl pH 9.0 at 20°C, 30 μ mol NAD⁺, 10 μ mol octopine, and 25 μ l of enzyme preparation in a final volume of 1.025 ml. Enzyme activities were determined by monitoring the increase in absorbance at 340 nm. One unit of enzyme activity was defined as the quantity of enzyme required to convert 1 μ mol of substrate to product/min at 20°C. Total protein was determined by the method of Bradford (1976) as modified by Read and Northcote (1981), with bovine serum albumin as a standard. Standard curves

for estimation of total protein were generated by the method of McPherson (1985). Enzyme specific activity was calculated as units of activity per mg total protein. All activity measurements and total protein determinations were done in duplicate.

Standard formulas were used to calculate allele frequencies, fixation indices and G-tests for fit to expected genotypic proportions (Hedrick, 1985). The procedure for the estimation of allele frequencies under a null-allele model was done using the computer program of Reed and Schull (1968), which uses numerical methods to find maximum likelihood estimates of the allele frequencies and the G-statistic for goodness-of-fit. The statistical model is a modification of the Hardy-Weinberg model to include a null allele (e.g., model 2.1 of Gart and Nam, 1988). Least-squares mean specific activity and standard error for each enzyme genotype were calculated using the SAS General Linear Models (GLM) procedure (SAS, 1985). The effect of heterozygosity on specific activity was tested using linear contrasts in the GLM procedure (Sokal and Rohlf, 1981, p. 236).

Results

In all, 10 active alleles were observed for the Odh locus in Mytilus trossulus; their respective frequencies are presented Table 3.1. Table 3.2 shows the observed and

expected frequencies of the 17 most common genotypes, which jointly made up 95% (316/331) of the sample; the other nine genotypes were present only once or twice in the sample. One individual with no ODH activity was observed, but was not included in the sample of 331 mussels. The observed (H_o) and expected heterozygosity (H_e) were 0.565 and 0.667 respectively. Comparison of observed and expected genotypic proportions showed a significant ($G=79.4$, degrees of freedom=45, $P < 0.005$) deficiency of heterozygotes, as revealed by the fixation index F ($F=0.153$). Analysis of the data after pooling rare alleles did not significantly reduce the large G , showing that the G value was inflated not by low expected numbers for some genotypes. The observation of one non-reactive individual and a significant heterozygote deficiency suggested the possibility of a null allele segregating at the Od*h* locus. A second analysis of the genotype frequencies was performed allowing for the possibility of a null allele (Od*h*⁰). The revised allele frequencies (after omitting the non-reactive individual to give a conservative estimate of the null allele frequency) are shown at the right in Table 1, and the expected genotype frequencies under the null-allele model are shown at the right in Table 3.2. The null allele model with an Od*h*⁰ allele frequency of 0.053 fit the data better than the basic Hardy-Weinberg model, but there was still a poor fit of observed to expected genotype frequencies ($G=27.3$, degrees

Table 3.1. Odh allele frequency estimates ($\pm$ 1 standard error) for Mytilus trossulus.

Allele	Observed Frequency	Frequency with Null (<u>Odh</u> ⁰)
<u>Odh</u> ⁰	-	0.053 $\pm$ 0.015
<u>Odh</u> ⁵⁵	0.003 $\pm$ 0.002	0.003 $\pm$ 0.002
<u>Odh</u> ⁶⁸	0.006 $\pm$ 0.003	0.006 $\pm$ 0.003
<u>Odh</u> ⁷³	0.010 $\pm$ 0.004	0.011 $\pm$ 0.004
<u>Odh</u> ⁷⁵	0.085 $\pm$ 0.011	0.075 $\pm$ 0.010
<u>Odh</u> ⁹²	0.008 $\pm$ 0.003	0.008 $\pm$ 0.003
<u>Odh</u> ⁹⁵	0.237 $\pm$ 0.017	0.226 $\pm$ 0.017
<u>Odh</u> ¹⁰⁰	0.509 $\pm$ 0.019	0.486 $\pm$ 0.021
<u>Odh</u> ¹¹³	0.103 $\pm$ 0.012	0.096 $\pm$ 0.012
<u>Odh</u> ¹²²	0.030 $\pm$ 0.007	0.027 $\pm$ 0.006
<u>Odh</u> ¹³⁰	0.009 $\pm$ 0.004	0.009 $\pm$ 0.004

Table 3.2. Octopine dehydrogenase specific activity (SA, in units/mg protein, $\pm$ 1 standard error), coefficients for linear contrast (C), number of mussels assayed for activity (N_1) and for allozyme genotype (N_2), and expected genotype frequencies without (E_1) and with (E_2) a null allele for the *Odh* locus in *Mytilus trossulus*.

Genotype	N_1	SA	C	N_2	E_1	E_2
73/100	4	.1008 $\pm$.0328	0	5	3.57	3.35
75/75	4	.1387 $\pm$.0328	4	4	2.33	5.48
75/95	5	.2118 $\pm$.0303	-2	12	13.30	11.90
75/100	13	.1715 $\pm$.0177	-2	30	28.55	25.63
75/113	2	.2345 $\pm$.0479	-2	2	5.76	5.04
75/122	4	.1311 $\pm$.0328	-2	4	1.69	1.42
95/95	21	.1235 $\pm$.0151	3	28	18.53	25.40
95/100	32	.1765 $\pm$.0126	-2	69	80.04	70.32
95/113	6	.2042 $\pm$.0303	-2	15	16.15	13.84
100/100	87	.1513 $\pm$.0076	4	99	85.65	95.31
100/113	13	.1740 $\pm$.0177	-2	24	34.67	29.81
100/122	3	.1664 $\pm$.0378	-2	4	10.20	8.39
100/130	2	.0857 $\pm$.0479	0	3	3.06	2.87
113/113	5	.1664 $\pm$.0303	4	10	3.45	6.78
113/122	2	.1639 $\pm$.0479	-2	3	2.06	1.65
113/130	2	.2017 $\pm$.0479	0	2	0.62	0.56
122/122	3	.1387 $\pm$.0378	3	3	0.29	1.31
others	-	-	0	15	21.08	21.94

of freedom=10, $P < 0.05$), indicating that occurrence of a null allele could not by itself explain all of the heterozygote deficiencies. Putting the single presumed null homozygote back into the analysis increased the null frequency only slightly (to 0.063), and did not improve the goodness of fit.

Specific activity was determined for 207 individual mussels which represented the 17 most common Odh genotypes (Table 3.2). A linear contrast between heterozygous and homozygous genotypes revealed a significant difference in average specific activity between heterozygous genotypes ($N=87$, mean specific activity= 0.1758 ± 0.0081) and homozygous genotypes ($N=120$, Mean= $0.1480, \pm 0.0068$). Other than the difference between homozygous and heterozygous genotypes, there was no detectable difference in average specific activity among Odh genotypes (Table 3.3).

Discussion

Ten active alleles were found for the Odh locus in Mytilus trossulus (Table 3.2). Fewer alleles were reported for Odh in M. trossulus in previous studies (Bulnheim and Gosling, 1988; McDonald and Koehn, 1988; Sarver and Loudenslager, 1991). The use of varied electrophoretic conditions has uncovered additional mobility variants in other studies (Beaumont and Beveridge, 1983). The heterozygote

Table 3.3. Analysis of variance table for enzyme specific activity. Mean square values should be multiplied by 10^4 .

Source of Variation	Degrees of Freedom	Mean Square	F
Among genotypes	16	2.87	1.61
Homozygotes vs. Heterozygotes	1	8.81	4.95*
Error	190	1.78	

*P < 0.05

deficiency, as measured by the fixation index (F), was less than an earlier study of the same population (Sarver, 1989).

Analysis of Odh activity in this study showed that heterozygotes had higher average specific activities than homozygous genotypes. When two homozygous genotypes have different specific activities, the heterozygote should be exactly intermediate unless specific activity is controlled in a non-additive fashion (Kacser and Burns, 1981). One test for overdominance for an allozyme locus is to compare the number of cases in which the specific activity of the heterozygote exceeds that of the homozygote. By chance alone, heterozygotes would be expected to exceed the specific activities of the homozygotes 33% of the time. For Odh, in 7 of 9 comparisons, the heterozygotes exceeded the specific activities of the corresponding homozygotes, a significant departure from expectation ($G=7.46$, degrees of freedom=1, $P < 0.01$).

Overdominance or dominance of enzyme specific activity has occasionally been reported (Hilbish and Koehn, 1985; Patarnello et al., 1989; Pogson, 1991). For example, Patarnello et al. (1989) observed significantly higher specific activity of heterozygous genotypes for the Pgi locus in the amphipod Gammarus insensibilis. Moreover, in mortality experiments, Patarnello et al. (1989) observed significantly higher mortality of Pgi homozygotes over Pgi heterozygotes at 27°C. Patarnello et al. (1989) suggest

that Pgi in G. insensibilis may be the subject of natural selection. Activity variation among genotypes of leucine aminopeptidase (Lap) in Mytilus edulis has also been shown to be related to fitness. Differential activity among Lap genotypes has been related to genotype-dependent mortality in relation to environmental salinity and temperature (Hilbish et al., 1982). As in this study, some of the enzymes showing correlations between heterozygosity and growth or overdominance for biochemical attributes are monomers, which eliminates intragenic complementation as an explanation for the apparent overdominance (Pogson, 1991).

The greater activity of the ODH heterozygotes may be a result of greater protein production of the heterozygous genotypes. Pogson (1991) found highly significant differences in phosphoglucumutase (PGM) activity among Pgm-2 genotypes for the Pacific oyster Crassostrea gigas. The three most common heterozygotes expressed higher specific activities than the corresponding homozygotes. For all other kinetic properties that Pogson studied, including $V_{\max}/K_m$ ratios, pH-dependent activities and temperature stabilities (Pogson, 1989), the heterozygotes were intermediate to the corresponding homozygotes. Pogson (1991) attributed the apparent overdominance in specific activity to genotype-dependent differences in steady-state enzyme concentrations. Earlier studies showed the Pgm-2 locus to be one of five loci involved in a significant

correlation between heterozygosity and growth in C. gigas. Pogson (1991) argues that as a result of greater flux capacities of heterozygotes, oysters heterozygous for the common Pgm-2 allele may reach a higher adult body weight than homozygotes. In the fish Fundulus heteroclitus, Crawford and Powers (1990) observed that the specific activity of LDH-B₄ differed in fish depending on LdhB genotype. Moreover, the LDH-B₄ allelic isozymes were shown to have the same k_{cat} values, so the differences in enzyme activity were suggested to be a function of different enzyme concentrations. Using antibody preparations, Crawford and Powers (1990) showed that enzyme activity differences were correlated with differences in LDH-B₄ concentrations. In a study of Mytilus edulis, Koehn et al. (1980) showed significant differences in specific activity among alleles for the Lap locus. Furthermore, Koehn et al. (1980) demonstrated that in estuarine samples the differences in specific activity among Lap genotypes were due to lower concentrations of one homozygous genotype.

In the present study, the presence of a null-activity allele could affect ODH concentration and thus ODH activity. As a result, specific activities of active/null heterozygotes would be reduced. Previous studies of marine bivalves have suggested the occurrence of null alleles at moderate frequencies for some allozyme loci (Skibinski et al., 1983; Mallet et al., 1985; Foltz, 1986). Null alleles

usually go undetected in electrophoretic surveys, because active/null heterozygotes are scored as active/active homozygotes, resulting in apparent heterozygote deficiencies in population surveys. If a null allele is segregating at an enzyme locus, the proportion of presumed homozygotes that are actually null/active heterozygotes will vary inversely with the frequency of the active allele. Because of this, the rare occurrence of homozygous genotypes has been suggested as evidence for the occurrence of a null allele (Skibinski et al., 1983). Rare homozygous genotypes frequently go unreported in the literature because researchers usually pool such homozygous genotypes into synthetic allele categories for statistical purposes. Rare homozygous genotypes, and departures from Hardy-Weinberg equilibrium have been observed for the Odh locus in Mytilus trossulus (Sarver, 1989). Departures from expected Mendelian patterns of inheritance in breeding experiments can sometimes be attributed to null alleles. For example, Foltz (1986) detected abnormal segregation patterns in pair matings of oysters which were attributed to the existence of null alleles for two enzyme loci (Lap-2 and Mpi). Furthermore, Katoh and Foltz (1989) found that leucine aminopeptidase (LAP) specific activity was significantly reduced in oysters presumed to be active/null heterozygotes from pair matings.

If a null activity allele is segregating at the Odh locus in Mytilus trossulus, the mean activity of the homozygotes would be reduced because some of the homozygotes would actually be undetected active/null heterozygotes. On the other hand, the mean activity of heterozygotes would not be affected by the presence of a null activity allele. If active/null heterozygotes are present in significant numbers, then specific activities of single banded or apparently homozygous individuals should have a greater variance than heterozygous genotypes. However, a Bartlett's test for heterogeneity of variance between heterozygotes and homozygotes revealed no detectable heterogeneity of variance for Odh. Furthermore, inspection of specific activity residual values obtained from analysis of variance, revealed no departure from normality ($P > 0.05$). And finally, a further check for homogeneity of variances between heterozygous and apparently homozygous genotypes was obtained by taking the ratio of mean square errors (MSE) for the two classes. The MSE for Odh heterozygotes was 8.97×10^{-6} and the corresponding value for Odh homozygotes was 5.81×10^{-6} . Thus, Odh heterozygotes had significantly greater variance ($F=1.54$, degrees of freedom =75, 115. $P < 0.05$ from one tailed test) in specific activity than homozygotes, opposite of the prediction of a null allele model. Undetected null alleles do not appear to be an adequate explanation of apparent overdominance in specific activity for Odh.

As discussed previously, loci that demonstrate apparent overdominance for fitness related traits usually also exhibit deficiencies of heterozygotes (Zouros, 1987). Several theories have been suggested to account for deficiencies of heterozygotes and apparent overdominance simultaneously; they include: inbreeding, aneuploidy, or molecular imprinting (Gaffney et al., 1990). If significant inbreeding were to occur, then a deficiency of heterozygotes would be evident. Homozygosity as a result of inbreeding could result in reduced fitness due the increased expression of deleterious recessive genes (linked to allozyme genes). The reproductive biology of marine bivalves make it seem unlikely that closely related individuals would be able to mate in natural populations where population sizes are very large (in the many millions) and larval dispersal distances are presumed to be great. Differential expression of genes depending on whether they are maternally or paternally derived (molecular imprinting) has been suggested as a possible mechanism for the co-occurrence of heterozygote deficiencies and apparent overdominance (Chakraborty, 1989). A molecular imprinting model has yet to be supported by any direct experimental evidence for marine bivalves. Moreover, Moore and Haig (1991) suggest that imprinting will only evolve in those species where offspring are nourished directly from maternal tissues because there is a potential conflict between the interests of the paternal and maternal

genes within an embryo. For example, the more nourishment an embryo derives from its mother, the larger it will be at birth, but also the greater the cost to the mother and her future reproductive success. Significant imprinting effects are not expected under this model in oviparous taxa because the amount of yolk is determined before fertilization or immediately afterwards.

Several studies have shown high rates of aneuploidy in marine bivalves (Thiriot-Quievreux, 1984; 1986). Furthermore, in two oyster species, individuals showing a high frequency of aneuploid cells had reduced growth rates compared to normal individuals (Thiriot-Quievreux, 1986; Thiriot-Quievreux et al., 1988). It is not known if the occurrence of aneuploidy would result in mis-scoring of allozyme heterozygotes as homozygotes, so the significance of aneuploidy for heterozygote deficiencies is not known.

Data from studies that have examined heterozygote deficiencies and heterozygosity-growth correlations in progeny from restricted pair matings, have indicated that heterozygote deficiencies and heterozygosity-growth correlations may have separate causes. Analyses of data from cohorts derived from single families or restrictive matings have found in virtually every case no significant correlation between heterozygosity and growth. On the other hand, heterozygote deficiencies are usually present in progeny from pair matings. The studies of laboratory

crosses of marine bivalves have provided support for the "associative-overdominance" explanation for heterozygosity-fitness correlations. The associative-overdominance model regards allozyme loci merely as markers for recessive deleterious genes that are responsible for heterozygosity-fitness correlations. This model assumes that the allozyme loci are in gametic disequilibrium with linked detrimental genes. Under this model, homozygotes for allozyme loci are more likely (on average) to be homozygous for linked detrimental genes than heterozygous genotypes. Thus, heterozygosity-growth correlations are only observed in large samples from random mating populations and not observed in progeny from pair matings. On the other hand, if allozyme genes are tightly linked to detrimental genes, then "allele specific" differences in growth or fitness should be observed in progeny of pair matings in marine bivalves. Single locus growth rate differences have been reported in several studies of pair matings in marine bivalves (Beaumont et al., 1983; Adamkewicz et al., 1984; Foltz and Chatry, 1986) but it should be emphasized that these results do not allow discrimination between the possibility that the growth effect is due to a linked gene or the allozyme locus.

When biochemical properties are measured for different allozyme genotypes, the usual pattern is for the biochemical properties of the heterozygote to be intermediate to the

corresponding homozygotes (Gillespie and Langley, 1974; Paigen, 1979). Overdominance exists when the phenotypic attribute (in this case specific activity) of a heterozygous genotype exceeds the values of the two corresponding homozygous genotypes. The findings of this study are significant because of the unusual occurrence of "overdominant" specific activity for an enzyme locus. The data presented here indicate that a null allele model does not adequately explain heterozygote deficiencies or apparent overdominance in activity for Odh. Other studies have reached similar conclusions about the limited role of null alleles in generating heterozygote deficiencies and apparent overdominance (Gaffney et al., 1990; Pogson, 1991). Null alleles are probably not the cause of heterozygote deficiencies and apparent overdominance in marine bivalves. While this study has demonstrated apparent overdominance for specific activity for Odh in Mytilus, the fitness consequences of Odh genotype are still not known. The physiological role of ODH in molluscan tissues is thought to be in maintaining redox balance in a manner analogous to lactate dehydrogenase (LDH) in vertebrate tissues (Gäde and Grieshaber, 1986). The determination of the cause of apparent overdominance of specific activity for Odh was not part of this study, but intragenic complementation can probably be excluded because of the monomeric subunit structure of ODH. More studies are needed to determine if

apparent overdominance of specific activity for Odh is a result of genotype-dependent differences in enzyme concentration. And finally, further studies are needed to examine the possible roles of linked genetic elements, regulatory genes, molecular imprinting, and aneuploidy in generating deficiencies of heterozygotes and overdominance for fitness in marine bivalves.

CHAPTER 4

SUMMARY AND CONCLUSIONS

Two sibling species of a complex of marine mussels (Mytilus spp.) was studied for genetic and morphological variation in a region where they come together on the Pacific coast of the USA. Using allozyme electrophoresis and multivariate statistical methods, 28 samples of mussels could be separated into three distinct groups, and identified as M. trossulus, M. galloprovincialis and their hybrids. Morphologically, M. trossulus and M. galloprovincialis from allopatric populations could be separated into two overlapping groups, but in regions of sympatry and hybridization, morphological discrimination was obscure. The central California coast was found to contain populations that consisted of a mixture of M. trossulus, M. galloprovincialis and their hybrids in varying degrees. The structure of the region of intergradation and hybridization between M. trossulus M. galloprovincialis was that of a mosaic or patchwork pattern that is likely to occur as a result of a spatially complex, patchy environment.

The geographic distribution of M. trossulus and M. galloprovincialis was found to be related to the salinity and temperature regimes of the sampling locations and not related to such factors as degree of exposure to wave action or location in the intertidal zone. This is different from

the contact zone between M. edulis and M. galloprovincialis in Europe, where the distribution of the two species is largely related to degree of wave exposure, with M. galloprovincialis found in open coast locations and M. edulis found in bays. No relationship was found between tidal height and the distribution of M. galloprovincialis and M. trossulus.

In contrast to a study of European populations of Mytilus, which found differences in allele frequencies for Odh (an allozyme locus that is diagnostic between M. edulis and M. galloprovincialis) between samples of mussels collected from different tidal heights. In the present study, there was also no significant differences in Odh frequencies between samples from different tidal heights.

It appears that the different species of Mytilus are ecologically distinct and genetic integrity of the different species of Mytilus is maintained as a result of heterogeneity of the environment. Also, data presented in the present study indicate that the ecological factors acting to maintain genetic integrity between M. edulis and M. galloprovincialis are different than the ecological factors maintaining genetic integrity between M. trossulus and M. galloprovincialis. Further research is needed to determine the nature of the biological mechanisms that interact with the environment to keep the different species of Mytilus distinct in spite of the possibility of

hybridization. And finally, future studies of the genetic architecture of the species differences in Mytilus may provide valuable insight into the nature of species boundaries and the process of speciation.

Another study was undertaken to investigate common observations in Mytilus and other marine bivalves of the co-occurrence of heterozygote deficiencies and heterozygosity-fitness correlations. The octopine dehydrogenase locus (Odh) in Mytilus trossulus was chosen because it had previously been reported to exhibit both deficiencies of heterozygotes and show positive correlations between heterozygosity and fitness related traits (e.g., Koehn and Gaffney, 1984). In an electrophoretic examination of a natural population of Mytilus trossulus ten active alleles were revealed for the Odh locus, and a statistically significant deficiency of heterozygotes was observed. The possible existence of a null activity allele at the Odh locus was investigated because research had indicated that recessive null alleles at moderate frequencies could theoretically account for both heterozygote deficiencies and heterozygosity-fitness correlations (Skibinski et al., 1983). The specific activity variation of octopine dehydrogenase in Mytilus trossulus revealed that heterozygotes had on average higher specific activity than homozygotes. The results indicated that null activity alleles may be partly responsible for the observed

heterozygote deficiencies at the Odh locus in Mytilus trossulus, but nulls cannot explain all the deficiencies observed for Odh, and cannot account for the apparent overdominance in enzyme activity observed in this study. Thus, it seems unlikely that the occurrence of null activity alleles are solely responsible for the common observations of heterozygote deficiencies and heterozygosity-fitness correlations. It is possible that some other form of aberrant gene expression could be involved in heterozygote deficiencies and heterozygosity-fitness correlations in marine bivalves. Other possible explanations include molecular imprinting and aneuploidy, but these models are difficult to test and will require additional research.

REFERENCES

- Adamkewicz, L., S.R. Taub, & J.R. Wall, 1984. Genetics of the clam Mercenaria mercenaria. II. Size and genotype. *Malacologia* 25:525-533.
- Ahmad, M. & J.A. Beardmore, (1976). Genetic evidence that the "Padstow Mussel" is Mytilus galloprovincialis. *Mar. Biol.* 9:139-147.
- Alston, R.E. & B.L. Turner, 1963. Natural hybridization among four species of Baptisia (Leguminosae). *Am J. Bot.* 50:159-173.
- Balakirev, E.S. & D.V. Zaykin, 1990. Allozyme variability of formaldehyde dehydrogenase - a new gene marker in marine invertebrates. *Isozyme Bull.* 23:91.
- Barrett, E.M., 1963. The California oyster industry. *Calif. Fish & Game Fish. Bull.* 123:1-103.
- Barton, N.H. & G.M. Hewitt, 1985. Analysis of hybrid zones. *Ann. Rev. Ecol. Syst.* 16:113-148.
- Beaumont, A.R. & C.M. Beveridge, 1983. Resolution of phosphoglucumutase isozymes in Mytilus edulis. *Mar. Biol. Lett.* 4:97-103.
- Beaumont, A.R., C.M. Beveridge, & M.D. Budd, 1983. Selection and heterozygosity within single families of the mussel Mytilus edulis (L.). *Mar. Biol. Lett.* 4:151-161.
- Beaumont, A.R., R. Seed, & P. Garcia-Martinez, 1989. Electrophoretic and morphometric criteria for the identification of the mussels Mytilus edulis and M. galloprovincialis. *Proc. Eur. Mar. Biol. Symp.* 23:251-258.
- Berger, E.M., 1983. Population genetics of marine gastropods and bivalves. In: Russell-Hunter, W.D. (ed.) *The Mollusca, Volume 6, Ecology*. Academic Press, New York, p. 563-596.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248-254.
- Briggs, B.G., 1962. Interspecific hybridization in the Rununculus lappaceus group. *Evolution* 16:372-390.

- Briggs, J.C., 1974. Marine zoogeography. McGraw-Hill, New York.
- Bulnheim, H.P. & E. Gosling, 1988. Population genetic structure of mussels from the Baltic sea. *Helgolander Meeresunters.* 42:113-129.
- Campton, D.E., 1987. Natural hybridization and introgression in fishes: methods of detection and genetic interpretations pp. 168-191. In N. Ryman and F. Utter (eds.), *Populations Genetics and Fishery Management*. University of Washington Press, Seattle.
- Chakraborty, R., 1989. Can molecular imprinting explain heterozygote deficiency and hybrid vigor? *Genetics* 122: 713-717.
- Churgin, J., 1974. Temperature, salinity, oxygen and phosphorus in waters off the United States. NOAA Environmental Data Center.
- Conomos, T.J., R.E. Smith, & J.W. Gartner, 1985. Environmental setting of San Francisco Bay. *Hydrobiologia* 129:1-12.
- Crawford, D.L., & D.A. Powers, 1990. Molecular basis of evolutionary adaptation in two latitudinally extreme populations of Fundulus heteroclitus. *Proc. Natl. Acad. Sci.* 86:9365-9370.
- Dedini, L.A., L.E. Schemel & M.A. Turnbull, 1981. Salinity and temperature measurements in San Francisco waters, 1980. U.S. Geol. Survey Open-File Rep. 82-125, Menlo Park Calif., 130 pp.
- Foltz, D.W., 1986a. Null alleles as a possible cause of heterozygote deficiencies in the oyster Crassostrea virginica and other bivalves. *Evolution* 40:869-870.
- Foltz, D.W., 1986b. Segregation and linkage studies of allozyme loci in pair crosses of the oyster Crassostrea virginica. *Biochem. Genet.* 24:941-956.
- Foltz, D.W. & M. Chatry, 1986. Genetic heterozygosity and growth rate in Louisiana oysters (Crassostrea virginica). *Aquaculture* 57:261-269.
- Gäde, G. & M. Grieshaber, 1975. Partial purification and properties of octopine dehydrogenase and the formation of octopine in Anodonta cygnea L. *J. comp. Physiol.* 102:149-158.

- Gäde, G. & M.K. Grieshaber, 1986. Pyruvate reductases catalyze the formation of lactate and opines in anaerobic invertebrates. *Comp. Biochem. Physiol.* 83B:255-272.
- Gaffney, P.M., T.M. Scott, R.K. Koehn & W.J. Diehl, 1990. Interrelationships of heterozygosity, growth rate and heterozygote deficiencies in the coot clam, Mulinia lateralis. *Genetics* 124:687-699.
- Gart, J.J. & J. Nam, 1988. The equivalence of two tests and models for HLA data with no observed double blanks. *Biometrics* 44:869-873.
- Garthwaite, R., 1986. The genetics of California populations of Geukensia demissa (Dillwyn) (Mollusca): further evidence on the selective importance of leucine aminopeptidase variation in salinity acclimation. *Biol. J. Linn. Soc.* 28:343-358.
- Gartner-Kepkay, K.E., L.M. Dickie, K.R. Freeman & E. Zouros, 1980. Genetic differences and environments of mussel populations in the Maritime Provinces. *Can. J. Fish. Aquat. Sci.*, 37:775-782.
- Gartner-Kepkay, K.E., E. Zouros, L.M. Dickie, & K.R. Freeman, 1983. Genetic differentiation in the face of gene flow: a study of mussel populations from a single Nova Scotian embayment. *Can. J. Fish. aquat. Sci.* 40:443-451.
- Gartside, D.F., 1980. Analysis of a hybrid zone between chorus frogs of the Pseudoacris nigrita complex in the southern United States. *Copeia* 1980:56-66.
- Gillespie, J.H. & C.H. Langley, 1974. A general model to account for enzyme variation in natural populations. *Genetics* 76:837-848.
- Gosling, E.M., 1984. The systematic status of Mytilus galloprovincialis in western Europe: a review. *Malacologia* 25:551-568.
- Gosling, E.M., 1989. Genetic heterozygosity and growth rate in a cohort of Mytilus edulis from the Irish coast. *Mar. Biol.* 100:211-215.
- Gosling, E.M. & D. McGrath, 1990. Genetic variability in exposed-shore mussels, Mytilus spp., along an environmental gradient. *Mar. Biol.* 104:413-418.

- Gosling, E.M. & N.P. Wilkins, 1981. Ecological genetics of the mussels Mytilus edulis and M. galloprovincialis on Irish coasts. Mar. Ecol. Prog. Ser. 4:221-227.
- Grant, W.S. & M.I. Cherry, 1985. Mytilus galloprovincialis Lmk. in southern Africa. J. Exp. Mar. Biol. Ecol. 90:179-191.
- Grassle, J.P. & J.F. Grassle, 1976. Sibling species in the marine pollution indicator, Capitella capitella (Polychaeta). Science 192:567-569.
- Harrison, R.G., 1990. Hybrid zones: windows on the evolutionary process. Ox. Surv. Evol. Biol. 7:69-128.
- Harrison, R.G. & D.M. Rand, 1989. Mosaic hybrid zones and the nature of species boundaries. pp. 111-133 In: Speciation and its consequences (Otte, D. & J.A. Endler eds.) Sinauer, Sunderland.
- Haydock, C.I., 1964. An experimental study to control oyster drills in Tomales Bay California. Calif. Fish & Game 50(1):11-28.
- Hedrick, P., 1985. Genetics of populations. Jones & Bartlett, Boston.
- Hilbish, T.J. & R.K. Koehn, 1985. The exclusion of a role of secondary contact in an allele frequency cline in the mussel Mytilus edulis. Evolution 39:432-443.
- Hilbish, T.J., L.E. Deaton, & R.K. Koehn, 1982. Effect of an allozyme polymorphism on regulation of cell volume. Nature 298:223-228.
- Hillis, D.M., M.T. Dixon, & A.L. Jones, 1991. Minimal genetic variation in a morphologically diverse species (Florida tree snail, Liguus fasciatus). J. Hered. 82:282-286.
- Howard, D.J., 1986. A zone of overlap and hybridization between two ground cricket species. Evolution 40:34-43.
- Johannesson, K., N. Kautsky & M. Tedengren, 1990. Genotypic and phenotypic differences between Baltic and North sea populations Mytilus edulis evaluated through reciprocal transplantations. II. Genetic variation. Mar. Ecol. Prog Ser. 59:211-219.
- Kacser, H. & J.A. Burns, 1981. The molecular basis of dominance. Genetics 97:639-666.

- Katoh, M. & D.W. Foltz, 1989. Biochemical evidence for the existence of a null allele at the leucine aminopeptidase-2 (Lap-2) locus in the oyster Crassostrea virginica (Gmelin). *Genome* 32:687-690.
- Koehn, R.K., 1991. The genetics and taxonomy of species in the genus Mytilus. *Aquaculture* 94:125-145
- Koehn, R.K. & P.M. Gaffney, 1984. Genetic heterozygosity and growth rate in Mytilus edulis. *Mar. Biol.* 82:1-7.
- Koehn, R.K., B.L. Bayne, M.N. Moore & J.F. Siebenaller, 1980. Salinity related physiological and genetic differences between populations of Mytilus edulis. *Biol. J. Linn. Soc.* 14:319-334.
- Koehn, R.K., R.I.E. Newell & F. Immermann, 1980. Maintenance of an aminopeptidase allele frequency cline by natural selection. *Proc. Natl. Acad. Sci., USA* 77:5385-5389.
- Koehn, R.K., J.G. Hall, D.J. Innes, & A.J. Zera, 1984. Genetic differentiation of Mytilus edulis in eastern North America. *Mar. Biol.* 79:117-125.
- Koehn, R.K., W.J. Diehl & T.M. Scott, 1988. The differential contribution by individual enzymes of glycolysis and protein catabolism to the relationship between heterozygosity and growth rate in the coot clam, Mulinia lateralis. *Genetics* 118:121-130.
- Koehn, R.K., F.J. Turano, & J.B. Mitton, 1973. Population genetics of marine pelecypods. II. Genetic differences in microhabitats of Modiolus demissus. *Evolution* 27:100-105.
- Koehn, R.K., R. Milkman & J.B. Mitton, 1976. Population genetics of marine pelecypods. IV Selection, migration and genetic differentiation in the blue mussel Mytilus edulis. *Evolution* 30:2-32.
- Kwast, K.E., D.W. Foltz, & W.B. Stickle, 1990. Population genetics and systematics of the Leptasterias hexactis (Echinodermata:Asteroidea) species complex. *Mar. Biol.* 105:477-489.
- Lewis, J.R. & R. Seed, 1969. Morphological variations Mytilus from southwest England in relation to the occurrence of M. galloprovincialis Lamarck. *Cah. Biol. mar.* 10:231-253.

- Lee, S.Y. & B. Morton, 1985. The introduction of Mytilus galloprovincialis into Hong Kong. *Malac. Rev.* 18:107-109.
- Liu, L.L., D.W. Foltz, & W.B. Stickle, 1991. Genetic population structure of the southern oyster drill Stramonita (=Thais) haemastoma. *Mar. Biol.* 111:71-79.
- Livingstone, D.R., W.B. Stickle, M.A. Kapper, S. Wang, & W. Zurburg, 1990. Further studies on the phylogenetic distribution of pyruvate oxidoreductase activities. *Comp. Biochem. Physiol.* 97B:661-666.
- Machell, J.R. & J.D. DeMartini, 1971. An annual reproductive cycle of the gaper clam Tresus capax (Gould) in South Humboldt Bay, California. *Calif. Fish & Game* 57(4):274-282.
- Malachowski, M., 1988. The reproductive cycle of the rock scallop Hinnites giganteus in Humboldt Bay, California. *J. Shellf. Res.* 7(3):341-348.
- Mallet, A.L., E. Zouros, K.E. Gartner-Kepkay, K.R. Freeman & L.M. Dickie, 1985. Larval viability and heterozygote deficiency in populations of marine bivalves: evidence from pair matings of mussels. *Mar. Biol.* 87: 165-172.
- Mallet, J., N. Barton, G.M. Lamas, J.C. Santisteban, M.M. Muedas & H. Eeley, 1990. Estimates of selection and gene flow from measures of cline width and linkage disequilibrium in Heliconius hybrid zones. *Genetics* 124:921-936.
- Manwell, C., C.M.A. Baker, 1963. A sibling species of sea-cucumber discovered by starch-gel electrophoresis. *Comp. Biochem. Physiol.* 10:39-53.
- Mayr, E., 1936. *Animal species and evolution*. Harvard University Press, Cambridge, Massachusetts, 797 pp.
- McDonald, J.H. & R.K. Koehn, 1988. The mussels Mytilus galloprovincialis and M. trossulus on the Pacific coast of North America. *Mar. Biol.* 99: 111-118.
- McDonald, J.H., R. Seed, & R.K. Koehn, 1991. Allozymes and morphometric characters of three species of Mytilus in the northern and southern hemispheres. *Mar. Biol.* 111:323-333.
- McPherson, G.A., 1985. Analysis of radioligand binding experiments: a collection of computer programs for the IBM PC. *J. Pharmacol. Meth.* 14:213-228.

- Milkman, R. & L.D. Beaty, 1970. Large-scale electrophoretic studies of allelic variation in Mytilus edulis. Biol. Bull. 139:430.
- Mitton J.B. & M.C. Grant, 1984. Associations among protein heterozygosity, growth rate, and developmental homeostasis. Ann. Rev. Ecol. Sys. 15:479-499.
- Moore, T. & D. Haig, 1991. Genomic imprinting in mammalian development: a parental tug of war. Trends Genet. 7:45-49.
- Munksgaard, C., 1990. Electrophoretic separation of morphologically similar species of the genus Rissoa (Gastropoda: Prosobranchia). Ophelia 31:97-104.
- Murphy, P.G., 1978. Collisella austrodigitalis sp. nov.: a sibling species of limpet (Acmaeidae) discovered by electrophoresis. Biol. Bull mar. biol. Lab. Woods Hole 155:193-206.
- Murphy, R.W., J.W. Sites, D.G. Buth, & C.H. Haufler, 1990. Proteins I: Isozyme electrophoresis. In: Hillis, D.M., Moritz, C. (eds.) Molecular Systematics. Sinauer, Sunderland, Massachusetts. pp. 45-126.
- Nybakken, J., G. Caillet & W. Broenkow, 1977. Ecological and hydrographic studies of Elkhorn Slough, Moss Landing Harbor and nearshore waters. Moss Landing Marine Laboratories, Moss Landing, California, 465 pp.
- Ó Foighil, D., & D.J. Eernisse, 1988. Geographically widespread, non-hybridizing, sympatric strains of the hermaphroditic, brooding clam Lasaea in the northeastern Pacific Ocean. Biol. Bull. 175:218-229.
- Ohta, T., 1971. Associative overdominance caused by linked detrimental mutations. Genet. Res. 18:277-286.
- Palmer, A.R., S.D. Gayron, & D.S. Woodruff, 1990. Reproductive, morphological, and genetic evidence for two cryptic species of northeastern Pacific Nucella. Veliger 33:325-338.
- Paigen, K., 1979. Acid hydrolases as models of genetic control. Ann. Rev. Genet. 13:417-466.
- Patarnello, T., P.M. Bisol & B. Battaglia, 1989. Studies on differential fitness of PGI genotypes with regard to temperature in Gammarus insensibilis (Crustacea: Amphipoda). Mar. Biol. 102:355-359.

- Pogson, G.H., 1989. Biochemical characterization of genotypes at the phosphoglucomutase-2 locus in the Pacific oyster, Crassostrea gigas. Biochem. Genet. 27:571-589.
- Pogson, G.H., 1991. Expression of overdominance for specific activity at the phosphoglucomutase-2 locus in the Pacific oyster, Crassostrea gigas. Genetics 128:133-141.
- Read, S.M. & D.H. Northcote, 1981. Minimization of variation in the response to different proteins of the Coomassie Blue G dye-binding assay for protein. Anal. Biochem. 116:53-64.
- Reed, T.E. & W.J. Schull, 1968. A general maximum likelihood estimation program. Amer. J. Hum. Genet. 20: 579-580.
- Richardson, B.J., P.R. Baverstock, & M. Adams, 1986. Allozyme electrophoresis: A handbook for animal systematics and population studies. Academic Press, Orlando, Florida.
- Sarver, S.K., 1989. Genetic differentiation of the mussel Mytilus edulis along the coast of California and in Humboldt Bay. M.S. Thesis, Humboldt State University, Arcata, California, 52 pp.
- Sarver, S.K. & E.J. Loudenslager, 1991. The genetics of California populations of the blue mussel: further evidence for the existence of electrophoretically distinguishable species or subspecies. Biochem. Syst. Ecol. 19: 183-188.
- Sarver, S.K., M.C. Landrum & D.W. Foltz, 1992. Genetics and taxonomy of ribbed mussels (Geukensia spp.). Mar. Biol., in press.
- SAS, 1985. SAS User's Guide: Statistics. Version 5 Edition. SAS Institute, Inc., Cary, North Carolina.
- Seed, R., 1968. Factors influencing shell shape in the mussel Mytilus edulis. J. Mar. Biol. Assoc. U.K. 48:561-584.
- Seed, R., 1972. Morphological variations in Mytilus from the French coasts in relation to the occurrence and distribution of M. galloprovincialis. Cah. Biol. mar. 13:357-384.

- Seed, R., 1974. Morphological variations in Mytilus from the Irish coasts in relation to the occurrence and distribution of M. galloprovincialis Lamarck. *Cah. Biol. mar.* 15:1-25.
- Selander, R.K., M.H. Smith, S.Y. Yang, W.E. Johnson & J.B. Gentry, 1971. Biochemical polymorphism and systematics in the genus Peromyscus. I. Variation in the old field mouse (Peromyscus polionotus). *Stud. Genet.* 6:49-90.
- Shaklee, J.B., F.W. Allendorf, D.C. Morizot, & G.S. Whitt, 1990. Gene nomenclature for protein-coding loci in fish. *Trans. Amer. Fish. Soc.* 119:2-15
- Shaw, E, J. Allen, & R. Stone, 1974. Notes on the collection of Shiner Perch Cyanogaster aggregata in Bodega Bay, California. *Calif. Fish & Game* 60(1):15-22.
- Singh, S.M. & R.H. Green, 1984. Excess allozyme homozygosity in marine molluscs and its possible biological significance. *Malacologia* 25:569-581.
- Singh, S.M. & E. Zouros, 1978. Genetic variation associated with growth rate in the American oyster (Crassostrea virginica). *Evolution* 32: 342-353.
- Skibinski, D.O.F. & J.A. Beardmore, 1979. A genetic study of intergradation between Mytilus edulis and M. galloprovincialis. *Experientia* 35:1442-1444.
- Skibinski, D.O.F., J.A. Beardmore & T.F. Cross, 1983. Aspects of the population genetics of Mytilus (Mytilidae; Mollusca) in the British Isles. *Biol. J. Linn. Soc.* 19: 137-183.
- Sokal, R.R. & F. J. Rohlf, 1981. *Biometry*, second edition. W.H. Freeman & Co., San Francisco.
- Soot-Ryen, T., 1955. A report on the family Mytilidae (Pelecypoda). *Allan Hancock Pacific Exped.* 20:1-175.
- Steiner, W.W.M., E.A. Lisowski & D. Osterbur, 1977. Biochemical differences in sympatric color morphs of an aquatic isopod (Asellus brevicauda). *Comp. Biochem. Physiol.* 56B:371-374.
- Theisen, B.F., 1978. Allozyme clines and evidence for strong selection in three loci in Mytilus edulis L. (Bivalvia) from Danish waters. *Ophelia* 17:135-142.
- Thiriou-Quievreux, C., 1984. Karyotypes of some Ostreidae and Mytilidae (Bivalvia). *Malacologia* 25:465-476.

- Thiriot-Quievreux, C., 1986. Study of aneuploidy in spats of Ostreidae (Bivalvia). *Genetica* 70:225-231.
- Thiriot-Quievreux, C., T. Noel, S. Bougrier & S. Dallot, 1988. Relationships between aneuploidy and growth rate in pair matings of the oyster Crassostrea gigas. *Aquaculture* 75:89-96.
- Thorpe, J.P., J.A. Beardmore & J.S. Ryland, 1978. Genetic evidence for cryptic speciation in the marine bryzoan Alcyonidium gelatinosum. *Mar. Biol.* 49:27-32.
- Tison, J.C., 1967. Surface water temperature and density: Pacific Coast North and South America and Pacific ocean Islands. Coast and Geodetic Survey Pub. 31-3 U.S. Department of Commerce.
- Väinölä, R., Hvilson, M.M. 1991. Genetic divergence and a hybrid zone between Baltic and North Sea Mytilus populations (Mytilidae: Mollusca). *Biol. J. Linn. Soc.* 43:127-148.
- Väinölä, R., Varvio, S.-L. 1989. Biosystematics of Macoma balthica in northwestern Europe. *Proc. Eur. Mar. Biol. Symp.* 23:309-316.
- Varvio, S.-L., R.K. Koehn & R. Väinölä, 1988. Evolutionary genetics of the Mytilus edulis complex in the North Atlantic region. *Mar. Biol.* 98:51-60.
- Weir, B.S., & C.C. Cockerham, 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358-1370.
- Wilkins, N.P., K. Fujino, & E.M. Gosling, 1983. The Mediterranean mussel Mytilus galloprovincialis Lmk. in Japan. *Biol. J. Linn. Soc.* 20:365-374.
- Willis, G.L. & D.O.F. Skibinski, 1992. Variation in strength of attachment to the substrate explains differential mortality in hybrid mussel populations (Mytilus galloprovincialis and M. edulis) populations. *Mar. Biol.* 112:403-408.
- Wiley, E.O., 1981. Phylogenetics: the theory and practice of phylogenetic systematics. John Wiley & Sons, New York.
- Woodruff, D.S., K.C. Staub, E.S. Upatham, V. Viyanant, & H.-C. Yuan, 1988. Genetic variation in Oncomelania hupensis: Schistosoma japonicum transmitting snails in China and the Philippines are distinct species. *Malacologia* 29:347-361.

- Wright, S., 1978. Evolution and the genetics of populations. Volume 4. Variability within and among natural populations. University of Chicago Press.
- Zouros, E. & D.W. Foltz, 1984. Possible explanations of heterozygote deficiency in bivalve molluscs. *Malacologia* 25:583-591.
- Zouros, E., 1987. On the relation between heterozygosity and heterosis: an evaluation of the evidence from marine mollusks. *Isozymes* 15: 255-270.
- Zouros, E. & D.W. Foltz, 1987. The use of allelic isozyme variation for the study of heterosis. *Isozymes* 13:1-59.
- Zouros, E., M. Romero-Dorey & A.L. Mallet, 1988. Heterozygosity and growth in marine bivalves: further data and possible explanations. *Evolution* 42: 1332-1341.
- Zouros, E., S.M. Singh & H.E. Miles, 1980. Growth rate in oysters: an overdominant phenotype and its possible explanations. *Evolution* 34: 856-867.

VITA

Shane K. Sarver was born in Wisconsin on February 14th, 1958. He graduated from California Polytechnic State University, San Luis Obispo in 1983 with a B.S. degree in Biology. He also received a M.S. degree in Fisheries from Humboldt State University in 1989. He entered the Ph.D. program in the Department of Zoology and Physiology at Louisiana State University in 1987. His research publications include a study on the population genetics of Mytilus (Biochemical Systematics and Ecology 19(3):183-188) based on his master's research. Another research paper investigates the genetics and taxonomy of ribbed mussels (Geukensia spp.) (Marine Biology, 113:385-390). A third paper has also recently been published (Genetica, 85:231-239). Currently, he is a candidate for the Degree of Doctor of Philosophy in Zoology.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Shane K. Sarver

Major Field: Zoology

Title of Dissertation: GENETIC POPULATION STRUCTURE OF A SPECIES COMPLEX
OF BLUE MUSSELS (MYTILUS SPP.)

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

August 19, 1992