EVOLUTIONARY GENETICS AND BIOGEOGRAPHY OF THE MARINE BRYOZOAN MEMBRANIPORA MEMBRANACEA (CHEILOSTOMATA): IMPLICATIONS FOR POPULATION HISTORY, DISPERSAL ROUTES, AND TAXONOMY.

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

Heidi Regula Schwaninger

May 1999

BIOGRAPHICAL SKETCH

Heidi Schwaninger was born in Liestahl, Switzerland and grew up in nearby Schaffhausen. After completing high school she trained in horticulture and forestry. After graduation, Heidi left Switzerland with fond memories of the mountains and the forests. She worked as a horticulturist in England and the United States, emigrating to Oregon and moving to Alaska. Unexpectedly, the Last Frontier offered new educational possibilities. At Anchorage Community College she discovered the beauty of algebra and calculus and considered majoring in mathematics. A transfer to the University of Washington in Seattle brought intellectual expansion to new horizons. She became acquainted and fascinated with scientific research and the marine world. She vigorously pursued undergraduate research both in neurobiology and oceanography, and participated in salmon research in the remote Wood River Lakes system in Alaska. After receiving an award for excellence in undergraduate research, Heidi graduated summa cum laude in zoology and biological oceanography. Exploration of ground new to her continued during her graduate studies at Cornell where Heidi combined biogeography, population genetics and molecular techniques during her dissertation research. Upon graduation from Cornell, Heidi intends to continue along the trajectory of learning and scientific research.

For my mother Olga Schwaninger-Walter

and for John

ACKNOWLEDGMENTS

This project would not have been possible without the help of many people and funding agencies. First, I would like to thank my graduate committee. Drew Harvell, the chair, provided advise, encouragement, connections, samples, high quality editing, and financial support especially during the initial stages of this project when funding was scarce. Richard Harrison and Chip Aquadro have been supportive mentors by sharing their time and expertise in bringing this project to fruition. Rick was especially helpful letting me use his lab during the later stages of this project. Further, I am grateful to Judith Winston now at the Virginia Museum of Natural History, who was a reliable source of information about Membranipora, be it taxonomy, distributions, literature, potential suppliers of samples, and supplying key samples. She was a virtual fourth committee member.

My research was funded by a National Science Foundation Dissertation Improvement Grant, grants from the Mellon Foundation, the Roosevelt Fund of the American Museum of Natural History, Cornell's Section of Ecology and Systematics, Cornell's and national chapters of Sigma Xi, and NSF grants to C.D. Harvell. Stipend and summer support were supplied by a Clare Booth Luce Fellowship. The Cornell Graduate School and the Moore Fund provided travel funding. Finally, the Office of Naval Research sponsored my participation in a course on molecular techniques at the Wriggly Marine Laboratory on Catalina Island.

My project could not have taken on such a broad scope without the help of many people who sent samples. I thank the following individuals

for their efforts in obtaining tissue samples: Grethe Buntse, Juan Cancino, Dennis Gordon, Steve Hageman, Drew Harvell, Ewan Hunter, Mats Lindegarth, Carol McFadden, Claus Nielsen, Arne Noeverang, Dr. Pulpeiro, Michelle Ridgeway, Laura Roberson, Jen Schaeffer and Judith Winston. I also thank the South Australian Department of Fisheries for issuing the permit for collection of Membranipora membranacea in the Marine Reserve at Port Noarlunga, and the South Australian Museum for supporting these collections.

I thank members of the Harvell and Harrison labs, especially Steve Bogdanowicz, Ben Normark, Kiho Kim, Richard Broughton, Scott Stanley, Chris Willett, and Laura Katz for their help over the years, be it in the lab, on computers, or giving me feedback on presentations and writing. Bernie May and Merrill Peterson helped to get this project going by introducing me to Allozyme gels and Bernie by offering me a place to work in his laboratories. Bryan Danforth's very positive influence in one of these labs was also much appreciated.

It has been a pleasure to interact with the Staff of Ecology and Systematics and Corson Hall during my time here. I thank especially LuAnne Kenjerska, Alberta Jackson and Linda Harrington for their efficient and creative solutions to all sorts of problems. I also thank Shirley for her many cheerful "good morning, sweetie".

Finally, I thank my companion John Hale for his unflagging friendship, support and encouragement throughout my undergraduate and graduate years.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

POPULATION STRUCTURE OF THE WIDELY-DISPERSING MARINE BRYOZOAN MEMBRANIPORA MEMBRANACEA (CHEILOSTOMATA): IMPLICATIONS FOR POPULATION HISTORY, BIOGEOGRAPHY, AND TAXONOMY*

Abstract

Morphologically plastic, cryptic, or geographically widespread species pose similar challenges to the evolutionary biologist: their taxonomic status is often unclear yet must be known to study almost any aspect of their biology, ecology, evolution, or biogeography. The marine bryozoan Membranipora membranacea (Linnaeus) is morphologically plastic and geographically widespread in temperate oceans of the northern and southern hemisphere, and its taxonomy is unclear. This study examined genetic relationships among allopatric populations and sympatric morphs of this species, or species complex. Allozymes were used to elucidate the relationships among four widely-separated populations, two in the North Atlantic and two in the North Pacific Ocean. Allozymes and mtDNA sequencing were used to clarify the genetic relationships among three sympatric morphs that might correspond to the species M. villosa (Hincks) and M. membranacea in the Northeastern Pacific (Washington State). Populations in the North Atlantic and North Pacific had no fixed allelic differences at the loci tested but were separated by an average Nei's genetic distance of 0.581, suggesting their near-sibling species status. Populations

* Schwaninger HR (accepted) Population structure of the widely-dispersing marine bryozoan Membranipora membranacea (Cheilostomata): implications for population history, biogeography, and taxonomy. Marine Biology.

from Friday Harbor (Washington) and Catalina Island (California) were not significantly differentiated, which was attributed to high gene flow. Populations on either side of the North Atlantic were genetically indistinguishable, which is most likely due to the recent establishment of the West Atlantic populations from European founders. At Friday Harbor, sympatric morphs varying in their spination and spine inducibility were genetically indistinguishable, supporting the hypothesis that M. villosa is an induced phenotype of M. membranacea and not a distinct species in the Northeastern Pacific. Since such phenotypic plasticity is common in cheilostome bryozoans, the morphospecies concept must be used with caution.

Introduction

Species that are morphologically plastic or are geographically widespread pose interesting challenges because their taxonomic status might influence how we interpret their ecological interactions, their evolutionary history, and how we assess biodiversity in general. For example, alternative morphologies in interbreeding cichlid fishes endemic to Cuatro Cinegas basin of Mexico suggests that their morphological radiation has been achieved through polymorphism within a species rather than through speciation (Sage and Selander 1975). At least three species were recognized based on their food source (snail, fish, detritus/algae) and corresponding tooth type (Kornfield and Koehn 1975 and refs. therein). This situation parallels that of the cichlid species flock of eastern African lakes (Fryer and Iles 1972). However, in African cichlids

single lakes contain monophyletic groups of genetically distinct species (Meyer et al. 1990) representing a case of rapid speciation from a common ancestor. Allozyme analyses of the Mexican cichlids have found no genetic differentiation either at a morphological, temporal or geographic scale (Kornfield and Koehn 1975, Sage and Selander 1975, Kornfield et al. 1982). When it was observed that offspring from the same (wild caught) broods produced offspring with two different (out of the three) tooth morphologies, a single polymorphic species was suggested, one that diversified by polymorphism rather than speciation (Sage and Selander 1975). Similarly, the genetic relationships of geographic populations of the threespine stickleback needed to be clarified before its biogeographic history could be reconstructed. This superspecies is polymorphic with respect to life history (marine, fresh water, and anadromy), and fresh water populations have derived morphologies that probably evolved multiple times under appropriate conditions (eg., Bell and Foster 1994). This has caused much taxonomic confusion because subspecies status had been assigned based on morphology (Penczak 1966; Miller and Hubbs 1969). Genetic analyses have helped to clarify relationships and to test clear biogeographic hypotheses in this case (Haglund et al. 1992; Orti et al. 1994).

Sibling species present the opposite problem. These are species that are difficult or impossible to distinguish based on morphological characters (Mayr and Ashlock 1991). This definition might apply to many widely distributed or cosmopolitan species as well as morphologically cryptic sympatric species. The genetic relatedness of distant populations of cosmopolitan species will determine what potential mechanisms will be invoked to explain the observed pattern of variation. The underlying

causes of these distributions could range from vicariance, differentiation of peripheral isolates, and/or dispersal events in the distant past driven by climatic and geologic changes (eg., Vermeij 1978, 1989) to recent dispersal aided by humans (Elton 1958; Carleton and Geller 1993).

Similarly, the discovery of cryptic sympatric species challenges the current ecological understanding of those species and the ecosystem they are a part of and may suggest research directions that explore the newly discovered complexity. For example, species of zooxanthellae, shrimp and corals that appeared to be generalists with respect to environmental gradients, food sources, behavior, or life history have been shown to be more specialized and finely tuned to their niche (Knowlton and Jackson 1994). This generalization is cogently illustrated by Montastraea annularis which, like all reef-building corals, is obligately associated with photosynthetic endosymbiotic zooxanthellae (Rowan and Knowlton 1995). Molecular genetic analysis of the symbionts revealed that, contrary to traditional beliefs, a single individual of a coral species acted as host to dynamic, multi-species communities of zooxanthellae. The composition of the symbiont community followed gradients of solar irradiance both within a coral colony and with depth (Rowan and Knowlton 1995; Rowan et al. 1997). The dynamic nature of the symbiont community was suggested to protect some corals from bleaching (if they host a spectrum of irradiance-tolerant genotypes), help corals cope with seasonal environmental change and possibly with long-term global warming (Rowan et al. 1997). These few examples show that molecular methods often offer an independent alternative to meet the taxonomic challenges of

intraspecific morphological plasticity and the recognition of sibling species where morphology may not be sufficient.

The marine bryozoan Membranipora membranacea is both morphologically plastic and widely distributed, giving rise to taxonomic confusion about the relationships of populations and/or species within and between oceans. M. membranacea is a colonial invertebrate growing on kelps and has a simple morphology offering few taxonomic characters. Osburn (1950) recognized three species of Membranipora along the North American west coast: M. membranacea (Linnaeus), M. villosa (Hincks), and M. serrilamella (Osburn). M. serrilamella is distinguished by the presence of a serrated cryptocyst (inner extension of the zooid wall, Figure 1.1), M. villosa by the presence of a cryptocyst and numerous spines on the frontal membrane and lateral zooid walls, and M. membranacea by the lack of both characters (Figure 1.1). However, several authors have found variation in these taxonomic characters within single colonies (O'Donoghue 1926; Pinter 1969; Seed 1976). Yoshioka (1982a) found that the M. villosa form was temporally and spatially correlated with the presence of nudibranchs in the field and suggested that these predators induced this form. In laboratory experiments, Harvell (1984, 1986, 1991) confirmed that M. membranacea exposed to nudibranchs grew M. villosa-like spines. In a laboratory common-garden experiment 178 colonies were exposed to the nudibranch cue. 80.3% of the colonies produced spines, 13.5% remained unspined, and 6.2% were constitutively spined (spined before exposure to the cue) (Harvell 1998). Thus, at least two of the three species described along the North American west coast might simply be an

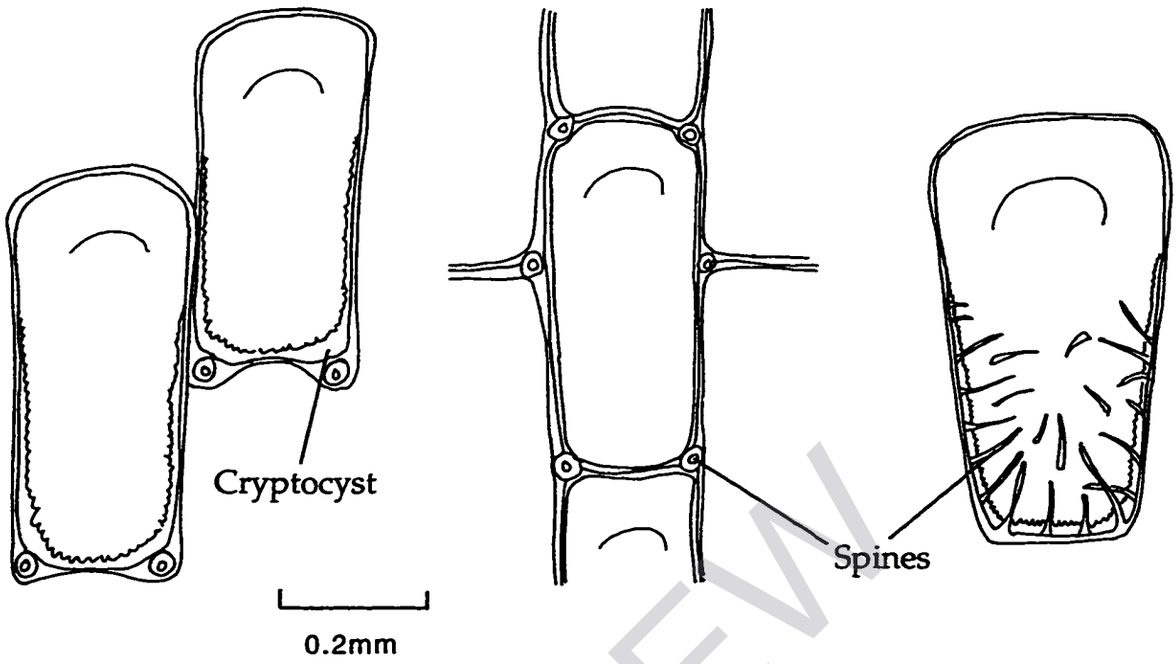

Figure 1.1. Membranipora spp. Zooids of three species. M. serrilamella is characterized by the presence of a crypotcyst, M. membranacea by the absence of a cryptocyst and spines on zooid walls, and M. villosa by the presence of both characters. Redrawn from Osburn (1950).

expression of the polymorphism of one species (M. membranacea) induced by exposure to the nudibranch predator and other environmental conditions some time before the type specimens were collected. But since there are three distinct morphotypes there is also the possibility that these are different species. Still other variation in colony form may be associated with substrate type, because M. membranacea grows on many species of

brown algae. In fact, aspects of colony morphology do seem to vary with substrate. In southern California, few stolons (elongated specialized zooids produced in response to intraspecific competition; Harvell and Padilla 1990) are made on Macrocystis integrifolia but many on Laminaria spp. (Roberson and Harvell, unpublished data). These observations raise the question of whether colonies on different kelps are genetically distinctive or phenotypically plastic in response to different substrates. There is currently no information about plasticity associated with the M. serrilamella phenotype.

Similarly, on a global scale the relationship of Membranipora membranacea described from various geographic locales is unclear (Osburn 1950; Yoshioka 1982b). Morphological differences between geographic populations of this species have been reported in temperate waters of the northern and southern hemisphere (Osburn 1950) raising the question of whether these allopatric populations belong to the same species. For example, the planktotrophic larvae of M. membranacea of the US West Coast are smaller and differ in ornamentation from those in European waters (Robertson 1908; Atkins 1955). The populations of M. membranacea in the West Atlantic are reported to be recent invaders (Berman et al. 1992; Lambert et al. 1992), but the source population is unknown. In the absence of a solid taxonomic or evolutionary framework we cannot interpret such variation nor can we explain how the global distribution of the species or species complex has come about.

The present study determined the relationships among populations of Membranipora membranacea at varying spatial scales and assessed whether different inducible spine morphologies correspond to different

species. This study uses allozyme allele frequencies and mitochondrial DNA sequences to address the following questions: 1) How closely related are populations in the North Atlantic and North Pacific? Did the Northwest Atlantic invaders come from Europe? 2) Are local populations in Friday Harbor, Washington genetically structured? 3) Are populations on different host algae from the same site genetically differentiated? 4) Are the different morphs of M. membranacea at Friday Harbor genetically distinct species?

Methods

Sampling and preservation

Genetic structure

Samples of Membranipora membranacea (L.) and M. serrilamella Hincks were collected from 1993 to 1995 at locations shown on Table 1.1 and Figure 1.2. Relationships among populations in different oceans (Table 1.1A), in regions within oceans (Table 1.1A,B), and among local groups growing on different hosts (Table 1.1C) were determined in different collections. At Bamfield Marine Station (Vancouver Island, British Columbia, Canada), colonies were collected from kelps, Macrocystis integrifolia and Nereocystis luetkeana, while at Friday Harbor (Washington, USA) colonies were collected from N. luetkeana and Laminaria groenlandica. This comparison of colonies from different host algae was necessary because M. membranacea grows on different hosts worldwide so any geographic analysis could be confounded by host species, i.e. Macrocystis spp. at Catalina and Bamfield, Laminaria spp. at Friday Harbor, the Isle of Man (UK) and Appledore Island (Maine, USA).

Table 1.1. *Membranipora membranacea* and *M. serrilamella*. Collection sites, sample sizes, loci analyzed and year collected, organized by question

Variation in question sample location (code)	n	Allozymes Loci[a]	mtDNA (n)	Year collected
A) Inter vs intra Ocean (North Atlantic, North Pacific)				
Isle of Man, England (IOM)	29	Pep-La, Tpi, Gpi	---	1992
Appledore Island, Maine (AI)	29	Ada, Pp, Pgd, Idh,	---	1992
Friday Harbor, WA (FH)	30	Np	---	1992
Catalina Island, CA (CAT)	31	(Monomorphic: Sod Mdh, EstFa-1, Dia n=10 per pop)	---	1992
B) Local populations (San Juan Island)				
Dock (DO)	27	Pep-La, Tpi, Gpi,	---	1992
Shady Cove (SC)	28	Ada	---	1992
West Side (WS)	27	Pp, Pgd, Idh, Np	---	1992
Turn Island (TI)	28		---	1992

Table 1.1 (Continued)

Variation in question sample location (code)	n	Allozymes Loci[a]	mtDNA (n)	Year collected
C) Substrates (Bamfield, BC and Friday Harbor)				
FH *N. lutkeana* (FHN)	27	Ada, Gpi, Pgd	---	1993
FH *L. groenlandica* (FHL)	80		---	1992
Bamfield *N. lutkeana* (BFN)	26		---	1993
Bamfield *M. integrifolia* (BFM)	27		---	1993
D) Morphs (Friday Harbor)				
Constitutively spined (CS)	4	Pgd, Gpi, Np, Tpi	6	1995
Constitutively unspined (CU)	11	Ada, Idh	7	1995
Inducibly spined (IS)	18	(Monomorphic: Mdh, Me)	6	1995
M. serrilamella, Japan (M.ser)	2	----	2	1995

[a] Loci abbreviations: Ada= adenosine deaminase, Dia= diaphorase, EstFa-1= esterase, Gpi= glucose phosphate isomerase, Idh= isocitrate dehydrogenase, Me= malic enzyme, Mdh= malate dehydrogenase, Np= nucleoside phosphorylase, Pep-la = peptidase, leucyl-alanine, Pgd= phosphogluconate dehydrogenase, Pp= inorganic pyrophosphatase, Sod= superoxide dismutase, Tpi= triosephosphate isomerase