BIOGEOGRPAHY AND DIVERSITY OF FRESHWATER MUSSELS OF THE SOUTHEAST UNITED STATES

by

SCOTT THOMAS SMALL

(Under the Direction of John P. Wares)

ABSTRACT

Biodiversity is the variation of life at all levels of biological organization (Gaston and Spicer 2004). However, it is rare that biodiversity is actually studied at all levels of organization. To form a more cohesive picture of biodiversity, this means that we must consider data ranging from genes to species (or even higher taxonomic units), and from various spatial scales. It is only with data from multiple spatial scales that we begin to understand how species are distributed in a region, how species diversity is generated and maintained, and how evolution within species shapes patterns in communities.

Here we investigate patterns of species and genetic diversity in communities of freshwater mussels (*Unionidae*) in the southeast United States. We approach this problem by combining numerical models with empirical data from regional and community scales. My methods combine the fields of biogeography, community ecology, and population genetics to elucidate the driving forces that maintain biodiversity in freshwater communities.

Our results provide evidence for a regional vicariant event that is responsible for structuring diversity in freshwater mussels and possibly other freshwater taxa. Numerical simulations provide evidence for the non-neutral structuring of mussel communities influenced

by both positive and negative density dependence. Patterns of genetic diversity within populations support the contemporary formation of species within the Altamaha River in Georgia with moderate spatial structuring of populations.

In conclusion we find that by collecting data on 2 aspects of biodiversity, species diversity and genetic diversity, we are able to form a complete picture of forces shaping diversity in the southeast United States. Future goals of our study are to test hypotheses of mechanisms structuring freshwater communities, providing valuable data for comparison across drainages and taxa.

INDEX WORDS: Biodiversity, Genetic diversity, Species abundance, Population genetics, Freshwater Mussels, Unionids, Biogeography, Community ecology,

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To Melissa, the constant in my life

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There are many people and experiences throughout my life that have inspired my love of science. First and foremost are my parents whose encouragement to explore the world through inquiry and imagination has led to many mistakes. Thank you, Mom and Dad, for letting me make those mistakes and learn the valuable lessons that follow. Thank you to my family whose tolerance and acceptance has always given me strength. Thank you to all of my friends and adopted families who have always seen fit to care for me with infinite patience, nary a difference between myself and their own. To everyone else who has given me a chance and trusted me to find my own solutions, your faith in me has surely been tested but hopefully never lost. Finally to Melissa, who continues to teach me lessons of life far beyond science, reminding me what makes it all worthwhile.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Biodiversity is the variation of life at all levels of biological organization (Gaston and Spicer 2004). However, it is rare that biodiversity is actually studied at all levels of organization. To form a more cohesive picture of biodiversity, this means that we must consider data ranging from genes to species (or even higher taxonomic units), and from various spatial scales. It is only with data from multiple spatial scales that we begin to understand how species are distributed in a region, how species diversity is generated and maintained, and how evolution within species shapes patterns in communities.

In my research I strive to combine data from disciplines of ecology and evolution. I don't think anyone would argue that the two disciplines are not entwined, yet few studies have honesty attempted to integrate both fields – often one or the other is treated as a nuisance parameter, or ignored entirely. My research offers a rare opportunity to integrate datasets from both population genetics and ecology over various spatial scales in an effort to understand how biodiversity is generated and maintained.

My research focused on the rivers of southeastern United States. The complex river systems of the southeastern U.S. are traditionally delineated into two provincial regions: the Atlantic Slope and Gulf of Mexico drainages. In my research I have included rivers east from

the Mississippi River to south of the Pee Dee River, creating a semicircle of rivers that bridge the Appalachian Divide, encompassing the Coastal Plains drainages of Mississippi, Alabama, Georgia, Florida, and South Carolina. The focal taxon were freshwater mussels in the family Unionidae (Table 1). Freshwater mussels were a natural choice as they are ubiquitous throughout the world but reach some of the highest diversity in the southeastern United States (Williams, Warren et al. 1993). Within the last 20 years, freshwater mussels have seen resurgence in research (Kat 1983, Turner, Trexler et al. 2000; Curole, Foltz et al. 2004; Mock, Brim-Box et al. 2004; Bogan and Roe 2008; Elderkin, Christian et al. 2008). However, a majority of this research has been taxonomic or ecological, focusing on contemporary processes among freshwater mussels while overlooking freshwater mussel evolution and the role of historical processes on their current patterns of diversity and biogeography.

Over the next three chapters I will present data from 3 separate spatial scales using freshwater mussels and numerical models to determine the connections between species and genetic diversity and to understand what mechanisms are generating and maintaining species diversity in freshwater ecosystems of the southeast U.S.

Comparative Phylogeography

I begin my dissertation by examining patterns of species diversity and distribution among the rivers of the southeast U.S. Freshwater mussels are not vagile and are usually restricted to rivers where they were born; however patterns of species distribution and genetic relatedness seem to suggest that mussels have moved among rivers as recently as the Pleistocene epoch (Bermingham and Avise 1986; Swift 1986; Mayden 1988). Baring anthropogenic interference, mussels can only move among rivers when the rivers exchange water. Rivers may exchange

fauna through the process of remodeling or headwater capture by another drainage (Sepkoski and Rex 1974). There appears to be two recent time periods in history that would have allowed for such faunal exchange between rivers. First is the retreat of the sea after the coastal inundation of the Pliocene epoch. As the sea retreated the coastal rivers flowed freely until channels were carved into the substrate (Bermingham and Avise 1986). Due to the sandy substrate of the coastal plains and fluctuating sea levels is has not been possible to reconstruct the historical river channels (Mayden 1988). A second opportunity for mussel movement is post-glacial flooding, such as the proposed Appalachian River following the last glacial maximum (Swift 1986). The massive influx of freshwater from the melting glaciers heavily remodeled coastal rivers by increasing flood plains, creating connections that did not previously exist. Some of these remodeling events, such as headwater captures, are still evident today in the Piedmont highlands where glacial deposits allow the reconstruction of historic rivers (Mayden 1988).

Community Ecology

The second chapter of my dissertation begins by investigating mechanism that generate and maintain species diversity at the community scale. I consider a local community as a component of the larger regional metacommunity (Hubbell 2001) and through computer simulation test how different interaction among species influence species richness and genetic diversity. Factors that shape species diversity and distributions in communities have been at the forefront of ecology since 1959 when Hutchinson asked in his homage to Santa Rosalia, "Why are there so many kinds of animals?" (Hutchinson 1959). The answer is not simple and many different models have been proposed through the years attempting to explain species diversity (Fisher, Corbet et al. 1943; Preston 1948; Hutchinson 1959; Levins 1970; May 1975; MacArthur

and Wilson 1967; Hubbell 1979; Pacala and Tilman 1993; Hubbell 2001). Two main classes of models that have received heavy debate are equilibrial and nonequilibrial models, referring to the steady state distribution of species in the community (Chave, Mueller-Landau et al. 2002). Equilibrial models are based on intrinsic differences between species such as life history, competition, or habitat preference. Intrinsic differences between species create deterministic species distributions where the community returns to the steady state following any type of perturbation (Chave, Mueller-Landau et al 2002). In contrast nonequilibrial models are stochastic in nature relying on the balance of antagonistic forces (i.e. immigration and extinction) (MacArthur and Wilson 1967; Caswell 1976; Hubbell 1979). The stochastic nature of the nonequilibrial models creates a steady state distribution where species identities are interchangeable; the nature of the steady state distribution is such that following a perturbation the community may change in species composition (Chave, Mueller-Landau et al. 2002). The mechanisms underlying both equilibrial and nonequilibrial models seems easy to distinguish, but current techniques comparing species abundance distributions from communities are unable to differentiate between these two classes of models (Bell 2000).

Population Genetics

The third chapter of my dissertation investigates patterns of neutral genetic diversity in 17 species of freshwater mussel in the Altamaha River in Georgia. Here I consider intraspecific variation between the Altamaha River and 3 tributaries: Oconee, Ocmulgee and Ohoopee Rivers. Intraspecific genetic variation can be used to infer demography, genetic diversity, selection, and life history parameters for populations that make up a species (Tajima 1989). There have been few genetic studies of freshwater mussel populations, and no studies using multiple species

within a single drainage. Previous studies on freshwater mussels populations using allozymes and microsatellite markers have found low levels of genetic diversity and low levels of gene flow (Mulvey, Lydeard et al. 1997; King, Eackles et al. 2002; Curole, Foltz et al. 2004; Mock, Brim-Box et al. 2004;). Mussel populations within drainages are spatial segregated with most genetic diversity among populations (Turner, Trexler et al. 2000). An interesting pattern is that there is more segregation (given by higher values of F_{ST} (Wright 1951)) in small tributaries and less in larger lowland rivers (Turner, Trexler et al 2000; Wares and Turner 2003). Correlation of river size and spatial patterns of genetic diversity have been attributed to differences in fish host vagility, as larger fish are typically more vagile and tend to inhabit larger rivers (Wares and Turner 2003).

Life History

Freshwater mussels are confined to freshwater environments with little tolerance to saltwater and no overland vagility (Graf 1997; Sepkoski and Rex 1974; Atrill and Rundle1996). Similar to other bivalves, they are sedentary filter feeders subsisting on algae and bacteria that drift in the water column. Freshwater mussels are generally gonochoristic, although there are exceptions (van der Schalie 1970; Hoeh and Frazer 1996). Fertilization of unionid ova occurs within the mantle cavity of the female; the male's sperm is ejected into the water column and must be entrained in the female's respiratory current to reach the ova (Brusca and Brusca 1990; Graf and Ó Foighil 2000). Sperm are packaged in spermatozeugmata (Edgar 1965; Lynn 1994; Waller and Lasee 1997), similar to the way sperm are packaged in brooding oysters (Ó Foighil 1989).

In striking contrast to other bivalves, freshwater mussels have a parasitic lifecycle.

Freshwater mussel larvae are parasitic, generally upon fishes though exceptions have been reported where metamorphosis can take place without a host (Howard and Anson 1923; Allen 1924; Parodiz and Bonetto 1963; Kondo 1990), and one species, *Simpsonaias ambigua* (Say 1825), uses an amphibian (*Necturus*) (Howard 1915; Clarke 1985). Larvae generally obtain hosts passively (Lefevre 1910), although some genera, such as *Lampsilis*, have evolved morphological and behavioral modifications (Kat 1984; O'Brien and Brim-Box 1999) for attracting potential hosts.

The unique life cycle and strict habitat preference of freshwater mussels have made then vulnerable to extinction. The past 40 years have seen a dramatic decline in both individual abundance and species diversity of native mussels in both the U.S. and Canada. Though it is unclear what effect recent species invasions have had on freshwater mussel populations, direct effects of water pollution and habitat degradation have been blamed for recent extinctions (Williams, Warren et al. 1993). It is estimated that over 70% of North American mussel species are listed as imperiled at the federal or state level and are in need of some type of protection, in comparison to 7% of bird and mammal species (Master 1990).

Summary

The imperiled status and unique life history of freshwater mussels (i.e., reliance upon freshwater fishes for not only survival but dispersal) make them an ideal system to study evolutionary processes. It is with this thought in mind that I utilized freshwater mussels to explore biodiversity in the rivers of the southeast U.S. In three chapters I explore the relationship of diversity at the regional, community, and population scale. My goal is to quantify levels of species and genetic diversity as well as develop tools to quantify biodiversity in an

effort to improve conservation of freshwater mussels and the ecosystems they inhabit.

Table 1.1: Classification and distribution of freshwater mussels.

Classification	Genera	Species	Distribution
C: *Bivalvia*	---	---	---
Sc: *Paleioeterodonta*	---	---	---
O: *Unionoida*	159	829	---
F: *Margaririferidae*	2	5	Na,Or,Pa
F: *Anodontinae*	14	61	Na,Or,Pa
F: *Unioninae*	106	615	Na,Au,Et,Or,Pa
F: *Hyriinae*	10	55	Nt
F: *Hyridellinae*	8	27	Au
F: *Iridinidae*	6	22	Et
F: *Mycetopodidae*	10	40	Na
F: *Etheriidae*	3	4	Na,Et,Or

Geographic abbreviations: Au=Australasian; Et=Ethiopian; Na=Nearctic; Nt=Neotropical; Or=Oriental; Pa=Palearctic.

CHAPTER 2

COMPARATIVE PHYLOGEOGRAPHY OF FRESHWATER MUSSELS OF THE SOUTHEASTERN UNITED STATES

Introduction

The goal of comparative phylogeography is to evaluate the topological and chronological congruence of molecular phylogenies across a common biogeographic barrier in multiple species pairs (Avise 1998; Edwards and Beerli 2000). Comparisons among species allow inference into common mechanisms that have influenced the evolutionary, demographic, and distributional histories of taxa in an ecological region (Bermingham and Moritz 1998). Knowledge concerning the different ages of species and their areas of extent serve to enhance our understanding of processes that create and maintain species diversity at both local and regional levels. Improving our understanding of processes that promote and maintain diversity will allow us to focus conservation efforts on biologically diverse regions containing endemic species.

The southeastern United States (U.S.) provides the perfect example of endemic regional biodiversity as it contains high levels of endemic freshwater species such as gastropods, fish, crayfish, and mussels (Master 1990). There have been two proposed hypotheses to explain contemporary species distribution and diversity in the southeastern U.S, *i)* source-dispersal and *ii)* multiple vicariance. Under the hypothesis of source-dispersal, it is believed that a single

ancestral drainage was a cradle for most of the present species diversity; contemporary patterns of species distribution are then due to coastal flooding, headwater captures, and anadromous fish migration which allow dispersal of species among rivers (Sepkoski and Rex 1974). Under the hypothesis of vicariance biogeography, a once widespread ancestor species was fragmented into smaller isolated populations by the formation of a barrier to gene flow; these species then diverged becoming reproductively isolated (Pflieger 1971; Mayden 1985; Mayden 1987; Wiley and Mayden 1985). These two hypotheses are by no means mutually exclusive and attempts to treat them as such may lead to data outliers that cannot be explained by either mechanism. A better hypothesis would incorporate mechanisms of both vicariance and source-dispersal to explain contemporary pattern of species diversity and distribution.

Despite the presence of mussels throughout the southeastern U.S., it has not been tested whether the distribution of freshwater mussels can be explained by either of these hypotheses. In this paper we use freshwater mussel taxa that are distributed throughout the drainages of the southeastern U.S. to test for both topological and chronological congruent patterns of biogeographic vicariance and dispersal. Freshwater mussels are ideal for this task because of an extrinsic link to fish dispersal; mussels do not migrate except during their larval life stage where they rely on fish in a parasitic life cycle. Hence any movement made by fish during times of sea level change or head-water capture would be preserved in the genome of freshwater mussels. By sequencing multiple loci from mussel species distributed throughout the drainages of the southeastern U.S., we can uncover historical patterns of divergence and determine the role of vicariance events on the species formation in mussels and freshwater animals in general.

If we find that multiple mussel taxa support a single divergence time it would follow that a single dispersal/vicariant event was most likely responsible for species formation, linking species

in evolutionary history. Lieberman (2000) advocated the importance of identifying community-wide events as they serve to link species within an evolutionary framework and create a stable community (Lieberman 2000). Species in a stable community share an evolutionary history important to both the biotic stability of the community and inference of environmental factors affecting both past and future diversity (Lieberman 2000). Incorporating history of the entire community and its implied stability allow us to draw inferences on the long-term sustainability of species relationships within that community, an important factor for conservation management.

Here we use a comparative phylogeographic approach to determine the relative importance of glacial and interglacial cycles on speciation of freshwater mussels. We test the congruence of divergence times among species to determine the stability of contemporary mussel communities. Five widespread genera (*Alasmidonta, Elliptio, Lampsilis, Pleuorbema*, and *Villosa*) are sampled from the Altamaha (GA), Satilla (GA), Apalachicola-Flint-Chattahoochee (GA, AL, FL), Alabama-Coosa (GA, AL), Savannah (GA), Tennessee (TN), Ocklockonee (FL), Ogeechee (GA), St. Mary's (FL) St. John's (FL), and Choctowhatchee (FL) for both mitochondrial and nuclear intron sequence data. We utilize Bayesian phylogenetic construction and incorporate genealogical variance to estimate possible divergence times as well as the likelihood of multiple vicariance events. We use data from 5 mussel genera to answer the following questions: how old is the MRCA for each species group? Are molecular phylogenies similar in topology and chronology? Is there evidence of a phylogeographic break across the Appalachian Divide?

Materials and Methods

We targeted 12 drainage basins in our study covering an area from the Mississippi River east to south of the Pee Dee River, encompassing a majority of the rivers in the southeastern U.S. (Figure 2.1). We grouped drainages into either the Atlantic Slope or the eastern Gulf of Mexico Coastal Plains (Gulf Coast) based on the terminus of the rivers. The rivers of the Atlantic Slope and eastern Gulf Coast and their freshwater faunas constitute discrete biogeographic systems (Sepkoski and Rex 1974). The Appalachian Divide separates the Atlantic Slope region and the eastern Gulf Coast, effectively isolating the Unionid fauna of the coastal rivers from direct contact with the species of the interior basin (Johnson 1970). Freshwater taxa of mussels are distributed on both sides of the divide in most of the contemporary river systems. The Atlantic Slope contains the Altamaha, Savannah, Satilla, Ogeechee, and Santee rivers while the Gulf of Mexico contains the Apalachicola-Chattahoochee-Flint (ACF), Coosa, Mobile, Choctawhatchee, Suwannee, Ochlockonee, and Tennessee.

Mussel specimens were collected between 2007-2009 from taxonomic collections at Auburn University, University of Alabama, and North Carolina State (Appendix A). We focused our specimen collection on 5 genera, *Alasmidonta, Elliptio, Lampsilis, Pleurobema, Villosa*, that co-occur in most of the drainages (Table 2.1). From each genus we avoided species under taxonomic revision (Williams and Bogan 2008), species with purported anthropogenic introductions, and widespread congeners consisting of subspecies. Our final list comprised 18 and 37 species from the Atlantic Slope and Gulf of Mexico drainages, respectively.

Tissue was collected from the foot, abductor mussel, or mantle of mussel specimens and preserved in 95% EtOH for later DNA isolation. DNA from the collected tissue was then isolated using a modified CTAB (cetyl trimethylammonium bromide) isolation protocol based on Campbell, Serb et al.

(2005). Samples were first homogenized using liquid nitrogen and micro-pestles. We next added CTAB to a final volume of 300ul with an addition of 25mg/ml Proteinase K solution (Gentra). Samples were allowed to digest at 55 ° Celsius for 1-2 hours or until no solid tissue remained in the tube. DNA was precipitated using a chloroform wash step followed by the addition of 100% isopropanol. DNA was then eluted in 40ul of H_2O and stored at -80° Celsius until time of use.

We used PCR to amplify 2 loci (*16S,CO1*) from the mitochondrial genome and 2 loci (*ANT, NELTRS*) from the nuclear genome; NADH sequences were obtained from Genbank (Appendix B). For mitochondrial loci (*16S*, *CO1*) we obtained primer sequences from Campbell, Serb et al. (2005) and Folmer, Black et al. (1994), respectively. For nuclear loci we designed primers from the successful amplification alignment, purification, and cloning of degenerate primers provided in Jarman, Robert et al. (2002). PCR amplifications were performed in 20μl volumes consisting of 0.5µM each primer, 0.8mM total dNTPs, 3mM $MgCl_2$, and 1U Taq polymerase (Promega). Annealing temperatures for each locus were as follows: *16S*, 50°; COI, 40°; *NELTRS*, 50°; *ANT*, 54°. PCR products were prepared for sequencing using Exonuclease I and Antarctic Phosphatase (New England Biolabs, Ipswich, MA, USA). Sequencing reactions were carried out in 10μl volumes with 80ng of prepared template, 0.6µM primer, 0.6µl BigDye Terminator (Applied Biosystems, Foster City, CA, USA) and 3.4 µl Better Buffer (The Gel Company). Sequence reactions were cleaned and precipitated with 4 volumes 75% isopropanol, suspended in Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) and run on an ABI 3730 at the University of Georgia. For each locus, sequence data were edited using CODONCODE ALIGNER v.2.06 (CodonCode Corporation, Dedham, MA, USA). Sequences were aligned using ALIGNER'S built-in 'end-to-end' algorithm, examined and edited for likely artifacts caused by poly-N repeats and other apparent insertions, and disassembled/realigned. PHRED (Ewing et al.

1998) quality scores < 30 were investigated visually, and recoded as ambiguities (N) if not readily classified.

Phylogenetic Analysis

The program BEAST version 1.4.8 (Drummond and Rambaut 2007) was used to make inferences about demographic history based on mtDNA and nDNA sequence phylogenies within the study populations. BEAST is a Bayesian phylogenetic program that uses MCMC to sample the posterior distribution of gene trees, coalescence events and demographic parameters through time given observed DNA sequence data in addition to priors on substitution model and demographic models (Drummond and Rambaut 2007).

We used the program JMODELTEST (Posada 2008) to estimate which nucleotide substitution models best fitted the observed data. Priors on parameters associated with the substitution model in BEAST were based on the values chosen by the Akaike information criterion (AIC) (Akaike 1974) in JMODELTEST. The best-fit model was HKY85+ Γ for mitochondrial loci (*16S, CO1)* and GTR + Γ for the nuclear loci (*NELTRS, ANT*). For the molecular clock, we used a normal distributed substitution rate prior for *16S* with 95% of the probability density between 5.4×10–9 and 1.36×10–8, *CO1* with a 95% of the probability density between 2.4×10–8 and 4.8×10–8 (Rawson and Hillbish 1995), and nDNA with 95% of the probability density between 8.30×10–9 and 2.1×10–8 (Wares, Pankey et al. 2009).

We used a relaxed molecular clock model as implemented in BEAST, as preliminary analyses showed evidence of rate heterogeneity (Drummond, Ho et al. 2006). Species trees in BEAST were estimated from aligned sequence data with enforcement of intraspecific monophyly and starting trees were randomly configured from a Yule prior (Aldous 2001), which assumes a constant speciation rate per lineage. All other parameters within BEAST were given uniform

distributions, where parameters were adjusted using the suggestions from the BEAST log file (Drummond and Rambaut 2007). MCMC chains were run with 10^7 iterations with trees sampled every1000 iterations. The first 10% of the iterations were discarded as burn-in throughout. Log-files were analyzed in TRACER version 1.4 (Rambaut & Drummond 2003), and effective sample sizes (ESS) were used to evaluate MCMC convergence within chains. LOGCOMBINER version 1.4.8 (Drummond and Rambaut 2007) was used to combine data from independent chains with identical settings into a composite chain to assess the robustness of parameter estimates.

Results

We analyzed 838 unique sequences from 5 genera encompassing 49 species. Species were sampled across 12 distinct drainages all containing the Atlantic slope-Gulf of Mexico drainage split except *Pleurobema* (Table 2.2). We found that in most cases the mitochondrial loci (*CO1, NADH*) provided more variation than the nuclear loci.

All BEAST runs were found to have good convergence across 3 independent chains with an ESS of greater than 200. Mean substitution rates were found to be within reasonable bounds for both mitochondrial and nuclear loci in invertebrates (Drake, Charlesworth et al. 1998). Drainage split times were inferred from the mean posterior distribution averaged over all loci within the sample. The mean substitution rate at each locus was used to estimate a divergence time between drainages and across the Appalachian Divide.

Mitochondrial DNA typically gave older ages for species, except in the genus *Villosa* where the nuclear DNA gave slightly older times (Table 2.3). The ages for species of *Lampsilis*, *Alasmidonta*, and *Pleurobema* were similar with a mean value of 6-7 mya. In contrast species of *Villosa* and *Elliptio* had a mean value of 2-3 mya. Nuclear data produced slightly different

results with similar mean ages for species of *Lampsilis*, *Alasmidonta*, *Villosa*, and *Pleurobema* of 4-6 mya, while ages for species of *Elliptio* were younger with a mean value of 2 mya (Table 2.3). All trees were rooted with the outgroup *Margaritifera margaritifera,* a freshwater mussel outside of the family Unionidae.

Gene phylogenies for each genus gave different topologies but "similar" chronologies. Overall the topologies of the different genera returned phylogenetic trees that were congruent with geographically contiguous drainages. The most common pattern was the grouping between the Atlantic slope and Gulf Coast drainages, however the Tennessee drainage was often the exception to this pattern grouping with the Atlantic Slope (Figure 2.2).

In the genus *Alasmidonta,* species from different drainages were reciprocally monophyletic with the Savannah and ACF having the fewest substitutions separating the terminal nodes. However sampling issues restricted us to only the Savannah, Tennessee, and Altamaha for 2 mitochondrial loci restricting our ability to reconstruct drainages patterns using *Alasmidonta*. The genus *Elliptio* showed patterns of polyphyly in the Altamaha drainage with *Elliptio shepardiana* grouping with species from the Ogeechee drainage. Most other drainages for the genus *Elliptio* were reciprocally monophyletic, except Florida where limited sample size (n=2) does not allow us to make any concrete conclusions as to its placement. The genus *Lampsilis* also produced a polyphyletic Altamaha drainage with some species being more closely related to species from the Tennessee drainage than other Altamaha species. Other drainages within the *Lampsilis* genus were reciprocally monophyletic in respect to the Gulf Coast and Atlantic slope groupings with the exception of the Tennessee. Sparse sampling in the genus *Pleurobema* returned a polyphyletic Coosa drainage with a monophyletic Tennessee. The genus

Villosa showed patterns of polyphyly in the Tennessee drainage with some species grouping with the Gulf drainages and some with the Coosa drainage, all other drainages were monophyletic.

Even though we found different topologies among the genera the chronologies were similar. Timing of speciation events were similar in *Alasmidonta*, *Lampsilis*, and *Pleurobema* at ~5-7 mya with a more recent event affecting these genera as well as *Villosa* at ~3 mya (Figure 2.2). The genera of *Elliptio* and *Villosa* share a speciation event between ~2-3 mya with *Elliptio* having the most recent event at ~1-2 mya (Figure 2.2).

Appalachian Divide: Atlantic Slope-Gulf Coast split

We found evidence for 2 different vicariance times corresponding to the Pliocene (*Alasmidonta,* and *Lampsilis*) and the Pleistocene (*Elliptio, Villosa*) across the Appalachian divide (Figure 2.3; Table 2.4). *Elliptio* species showed slightly different patterns across loci with an Atlantic-Gulf Coast split of 0.93 (0.5-1.4) million years ago (mya) using 3 mitochondrial loci and 2 nuclear intron loci. Later dates were found for *16S* and *NADH* (2.617-4.12 (0.6-7)) owing mainly to the lack of resolution at *16S* and poor sampling for *NADH*. *Villosa* species had a median divergence time of 1.84 (0.57-17) mya with both mitochondrial and nuclear loci providing similar divergence dates. However *16S* was an exception with an earlier divergence time and large variance. *Lampsilis* species showed evidence of an earlier split time, when compared to *Elliptio,* of 5.6 (1-20) mya with a slightly later times for the nuclear locus *NELTRS* of 3.75 (2.28-5.47). *Alasmidonta* species were similar to species of *Lampsilis* but were by far the earliest split time with an Atlantic-Gulf split of 7.9 (2.12-17) million years. However only 2 loci were available for *Alasmidonta* and *CO1* was only half as divergent as values found in other species.

Bayesian posterior estimates of divergence times per locus present a pattern of 2 separate vicariance events, one corresponding to *Elliptio* and *Villosa* at approximately 0.8-2.2 mya and a second corresponding to *Lampsilis* and *Alasmidonta* at approximately 4.8-6.5 mya. The 95% credible intervals for the posterior distribution between the *Elliptio-Villosa* group and the *Alasmidonta-Lampsilis* group do not overlap (Table 2.4), further supporting 2 separate events.

Discussion

In the southeast United States two main hypotheses have been proposed to explain the distribution of freshwater taxa among drainages. One proposed hypothesis is the source-pool where a central river high in species diversity seeded the surrounding rivers (Sepkoski and Rex 1974). This hypothesis proposed that various headwater captures, and flooding in the Pleistocene allowed the movement of species among adjunct rivers creating the present pattern of species distribution. A competing hypothesis proposed that fragmentation of a widespread ancestral species, existing before the Pleistocene, created the present pattern of species distribution. Mayden (1988) as well as others (Pflieger 1971; Mayden 1985; Mayden 1987; Wiley and Mayden 1985; Bermingham and Avise 1986; Avise 1992) provide support for a Pliocene vicariant event in freshwater fish taxa, refuting the source-dispersal hypothesis as the main mechanism responsible for contemporary freshwater fish species distributions. The current distribution of freshwater mussel species in the southeastern U.S. supports both the source-dispersal and vicariant hypotheses.

The hypotheses of source-dispersal and vicariance may not be mutually exclusive. This is in fact the case for freshwater mussels where we find evidence supporting both vicariance events and dispersal events. Vicariance events can be most clearly seen as the congruent

bifurcation of phylogenetic trees among different genera. This pattern is evident in Figure 2.2A where the genera of *Lampsilis*, *Pleurobema*, and *Alasmidonta* share a diversification event and the genera of *Elliptio* and *Villosa* also share a diversification event. Later diversification events are also shared among genera such as the ~3-4 mya diversification of *Lampsilis*, *Pleurobema*, *Alasmidonta*, and *Villosa* and the much later diversification event of *Villosa* and *Elliptio*. Concluding congruence among diversification events is difficult due to confounding factors such as species life history, substitution rates, and ancestral population sizes (Edwards and Beerli 2000), but the support of multiple genera all with a similar pattern point to a general vicariant event that influenced the whole southeastern mussel fauna.

Dispersal events are more rare in the dataset possibly because of the exclusion of widespread species from the analysis. Dispersal events are evident in the genera of *Lampsilis*, *Villosa*, and *Pleurobema* with dispersal events typically involving the Coosa or the Altamaha and Tennessee. Dispersal events are identified by a species from a specific area, i.e. Coosa drainage, finding a most recent common ancestor with species from another drainage. Timing of the dispersal events corresponds with diversification events and is concordant between the genus *Lampsilis* and *Pleurobema* for species exchange involving the Altamaha, Tennessee, and Coosa drainages.

We find evidence for multiple divergence times in our 5 genera of mussels with *Elliptio* and *Villosa* species corresponding to a later Pleistocene divergence time, while other genera had an earlier divergence time. Multiple divergence times were also found by Bermingham and Avise (1986) in the study of 4 fish species of the southeastern U.S> that spanned the Appalachian divide. Bermingham and Avise (1986) found two separate divergence times, one for *Lepomis* dating to the Pliocene and a second later time for *Amia* dating to the Pleistocene.

Bermingham and Avise (1986) propose that if a second vicariance event occurred in the Pleistocene it might have erased the earlier event in the genus *Amia*, while at the same time not affecting *Lepomis*. Pleistocene stream remodeling, notably the headwater capture of the Chattahoochee by the Savannah (Swift, Gilbert et al. 1986), may also have led to species exchange, but it may not be expected due to the rarity of many fish and mussels species in headwater reaches. In the species of *Elliptio* and *Villosa* we did not find any evidence supporting the movement of species via the headwater capture, but did find evidence in *Villosa* species that supports the Pleistocene connection of the Tennessee with the Mobile drainage through the presence of the hypothesized Appalachian River (Swift, Gilbert et al. 1986; Mayden 1988).

The recent divergence time in both species of *Elliptio* and *Villosa* are fitting given the recent taxonomic difficulties in delineating species groups in both these genera. The overlooked consequence of a more recently diverged *Elliptio* and *Villosa* is that they are relatively newcomers to freshwater communities of mussels. If communities of freshwater mussels that have existed for million of years recently acquired 2 new genera it is likely that the community is not yet at equilibrium (Lieberman 2000). A community not yet at equilibrium may still be experiencing species extinctions or natural selection through either direct or indirect competition for resources or fish hosts. If the recent introduction of mussels into the rivers can be seen to only happen with the introduction of a new fish host, then the fish community may also be relatively young and consequently not at equilibrium.

Appalachian Divide: Atlantic-Gulf split

Our results provide evidence for a widespread pre-Pleistocene ancestor in species of *Alasmidonta* and *Lampsilis* with a deep phylogenetic divergence between Atlantic slope and Gulf Coast drainages of approximately 5-7 mya. However we also found support for a more recent divergence in *Elliptio* and *Villosa* species dating to 0.7-1.8 mya also between Atlantic slope and Gulf Coast drainages.

Our estimated divergence times for the Atlantic-Gulf split are concordant with 2 other studies estimating vicariance in fish species of the Atlantic and Gulf Coast. Bermingham and Avise (1986) found a divergence time in *Lepomis* separating species of the Atlantic Slope drainages from the Gulf Coast drainages dating back to the Pliocene, and Mayden (1988) found evidence of a pre-Pleistocene vicariance event in 7 fish taxa of the Eastern and Central highlands. Bermingham and Avise (1986) offer the explanation that saltwater inundation of the lowlands during the Pliocene interglacial (50-80m above present-day sea levels), isolated and fragmented a once widespread species. Then as the seas receded from the Pliocene high sea-level stand, it would have been possible for fish to disperse along the lowlands, enabling taxa within major lineages to colonize adjacent coastal rivers.

The major genetic effects of the Pliocene 1 million year-long, high sea-level stand on both mussels and fish species would have been *i)* extinction of locally differentiated species in the smaller Coastal Plain rivers, *ii)* attendant reduction of overall levels of genetic diversity within each species, *iii)* significant sequence divergence between lineages that had survived in refugia of either piedmont headwaters or Floridian highlands (Bermingham and Avise 1986; Baer 1998). Isolation of mussel taxa with fish taxa for long periods of time would also have allowed for the specification of host-parasite relationships and may account for some of the

contemporary patterns of species-specific parasitism see among different genera of mussels, where some mussel species are specialists and other are generalists.

Reproductive isolation in mussels

Species formation and reproductive isolation are poorly understood in freshwater mussel taxa. Broadening our understanding of vicariance events and their frequency between drainages serve to inform future hypotheses of speciation and reproductive isolation in freshwater mussel species. If it is believed that vicariance events are responsible for most species formation then the presence of multiple endemic species within drainage may be due to sympatric speciation rather than reticulate allopatry (van Veller, Kornet et al. 2000). Determining the role of speciation will serve to inform taxonomic classifications and provide testable hypotheses for reproductive isolating mechanisms that have received much debate in recent times.

Very little is understood about possible mechanism responsible for speciation in freshwater mussels. It has been suggested that mechanisms such as *Lysin* recognition proteins similar to the genus *Mytilus* may be acting to prevent species hybridization (Riginos and McDonald 2003), but there is also the possibility of reproductive isolation via a break down of fish-host relationships with hybrid individuals (Kat 1985; Kat 1986). If our results on mussel vicariance, as well as the corroboration of data from fish taxa, are any indication of speciation processes in the Unionids it might be possible that while the first burst of species formation arose via allopatric speciation, while subsequent, within drainage endemism is due entirely to sympatric of parapatric speciation. This hypothesis arises from the strong reciprocal monophyly found in most mussel and fish species for a particular drainage. As most fish and mussel species find a most recent common ancestor within a drainage before finding an ancestor between drainages, it could be assumed that after the initial allopatric separation, a secondary burst of

species formation occurred within drainages. Although our data suggests that within drainage speciation may have occurred as recently as 1 mya in species of *Lampsilis* in the Coosa drainage. Species within drainages are typically reciprocally monophyletic but harbor vast amounts of genetic diversity leading to shallow branches between species. Speciation within a drainage either via sympatric or parapatric speciation would most likely require the formation of prezygotic barriers to reproductive isolation more so than in the case of allopatric speciation (Coyne and Orr 2004). However it is possible that within drainage speciation events are linked to introductions of new fish hosts by dispersal from other drainages, which would allow specialization and possible diversification into different species.

Conclusions

Although our study is the largest study of freshwater mussel phylogeography performed to date and the only study to include a single copy nuclear locus, there still remain many unanswered questions as to historical relationships among drainages. It would be of value to extend our study to include rivers north of the Savannah River especially northern rivers that have experienced recent glaciations and recolonizations. It would also be useful to include rivers in the Mississippi and Ohio systems to form a more conclusive picture of freshwater mussel speciation as it relates to more extensively studied fish distributions (Mayden 1988). We think it would be worthwhile, however difficult, to examine genetic divergence in fish species in the same rivers we examined divergence in mussel species. As a comparison of this nature may uncover parallel patterns of divergence as is sometimes witnessed in host-parasite relationships (Page 1994), and may shed light on possible fish-host relationships in freshwater mussel species.

When 2 or more groups display patterns congruent in time and space, the patterns are probably the result of common history. Our paper tests the concordance of species divergence

with glacial and interglacial vicariance to determine whether divergence times are temporally congruent among mussel genera. Our ability to differentiate between hypotheses that have significantly influenced the ecological and evolutionary history of the biota in the southeast United States will assist in taxonomic reconstruction of species relationships within the region and allow inference into long-term community stability in freshwater ecosystems.

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Table 2.1: Mussel specimens collected from drainages on the (A. Gulf Coast and (B. Atlantic Slope.

Gulf of Mexico Drainage			
Apalachicola-Chattahoochee-Flint	**Coosa/Mobile**	**Tennessee**	**Yellow/Chocotawhatchee**
Alasmidonta triagulata	---	*Alasmidonta marginata*	---
---	---	*Alasmidonta virdis*	---
Elliptio fumata	*Elliptio arca*	*Elliptio dilatata*	*Elliptio mcmichaeli*
Elliptio purpurella	*Elliptio arctata*	---	---
Hamiota subangulata	*Hamiota altilis*	*Lampsilis abrupta*	*Hamiota australis*
---	*Hamiota perovalis*	*Lampsilis fasciola*	---
---	*Lampsilis ornata*	*Lampsilis ovata*	---
---	---	*Lampsilis virescens*	---
Pleurobema pyriforme	*Pleurobema georgianum*	*Pleurobema clava*	---
---	*Pleurobema decisum*	*Pleurobema oviforme*	---
---	*Pleurobema hanleyianum*	*Pleurobema gibberum*	---
Villosa villosa	*Villosa nebulosa*	*Villosa iris*	*Villosa choctawensis*
---	*Villosa umbrans*	*Villosa taeniata*	*Villosa constricta*
---	---	*Villosa trabalis*	*Villosa vaughaniana*
---	---	*Villosa vanuxemensis*	---

Atlantic Slope Drainage				
Savannah	**Ogeechee**	**Altamaha**	**Satilla**	**St. Mary's/St. John's**
Alasmidonta varicosa	*Alasmidonta arcula*	*Alasmidonta arcula*	---	---
Elliptio fraterna	*Elliptio folliculata*	*Elliptio dariensis*	*Elliptio downiei*	*Elliptio buckleyi*
Elliptio waccamawensis	*Elliptio hopetonensis*	*Elliptio shepardiana*	---	---
---	---	*Elliptio spinosa*	---	---
Lampsilis cariosa	*Lampsilis dolabraeformis*	*Lampsilis dolabraeformis*	---	---
Lampsilis radiata	---	*Lampsilis splendida*	---	---
---	---	*Villosa delumbis*	---	*Villosa amygdala*

Table 2.2: Summary of genetic data collected for freshwater mussels used in comparison among drainages

Genus	Marker	Inds.	Species	Base Pairs	# inform characters	Drainages
Alasmidonta	16S	18	4	530	117	ACF,ALT,TEN
	CO1	26	4	701	182	ALT,SAV,TEN
Elliptio	16S	55	13	533	140	ACF,ALT,SAV,TEN,COO,GUL,FLD,SAT,OGE
	CO1	86	13	663	315	ACF,ALT,SAV,TEN,COO,GUL,FLD,SAT,OGE
	NADH	17	12	909	193	ACF,ALT,SAV,TEN,COO,GUL,FLDOGE
	NELTRS	42	11	660	135	ACF,ALT,SAV,TEN,COO,GUL,FLD,SAT,
	ANT	39	10	372	120	ACF,ALT,SAV,TEN,COO,GUL,FLD,SAT,OGE
	LTRS	55	12	578	251	ACF,ALT,SAV,TEN,COO,GULSAT,OGE
Pleurobema	16S	15	7	537	89	ACF,TEN,COO
	CO1	32	11	681	327	ACF,TEN,COO
	NELTRS	11	3	605	47	ACF,TEN,COO
Lampsilis	16S	102	10	534	175	ACF,ALT,SAV,TEN,COO,GUL
	CO1	118	11	645	270	ACF,ALT,SAV,TEN,COO,GUL
	NADH	12	7	950	236	ACFSAV,TEN,COO
	NELTRS	38	10	617	129	ACF,ALT,SAV,TEN,COO,GUL
Villosa	16S	38	8	538	149	ACF,ALT,SAV,TEN,COO
	CO1	56	11	665	289	ACF,ALT,SAV,TEN,COO,GUL
	NADH	41	9	898	254	ACF,ALTTEN,COO,GUL,FLD
	NELTRS	22	6	606	138	ACF,ALT,COO,GUL,FLD

ACF=Apalachicola-Chattahoochee-Flint; ALT=Altamaha; SAV=Savannah; TEN=Tennessee; COO=Coosa; GUL=Choctawhatchee, Suwannee; FLD=St.John's, St. Mary's; SAT=Satilla; OGE=Ogeechee.

Table 2.3: Summary of species ages.
Summary of species ages for the 5 genera examined in this study. We report the mean, median and 95% Bayesian credible intervals from the program BEAST (Drummond and Rambaut 2007). Mean μ is the given as the average rate of substitutions per million years per gene.

	Locus	Mean (MYA)	Median (MYA)	95 % CI		mean *u/million years*
Alasmidonta	16S	12.48	11.04	3.00	24.00	0.010
	CO1	6.34	5.79	2.00	11.00	0.033
Elliptio	CO1	0.71	0.67	0.39	1.10	0.036
	NADH	2.62	2.29	0.40	5.60	0.050
	ANT	1.15	1.09	0.58	1.86	0.014
	NELTRS	0.79	0.76	0.48	1.15	0.005
Lampsilis	16S	5.59	5.33	3.06	8.68	0.013
	CO1	5.23	5.79	3.00	29.00	0.036
	NADH	5.62	5.38	2.25	9.87	0.042
	NELTRS	3.75	3.64	2.28	5.47	0.005
Villosa	16S	1.89	1.60	0.49	4.17	0.012
	CO1	1.57	1.86	0.68	2.56	0.037
	NADH	2.11	2.01	0.92	3.40	0.052
	NELTRS	1.76	1.71	1.07	2.55	0.005

Table 2.4: Summary of divergence times.
Summary of divergence times for the Atlantic Slope-Gulf Coast estimated from mtDNA (*16S, CO1, NADH*) and nDNA (*NELTRS, ANT*) for each genus of mussel. We report the mean, median, and 95% credible intervals from the Bayesian analysis by Beast (Drummond and Rambaut 2007).

Genus	Locus	Mean (MYA)	Median (MYA)	95 % CI		mean *u/million years*
Alasmidonta	16S	12.83	11.35	4.23	25.25	0.010
	CO1	6.36	5.82	2.40	11.50	0.033
Elliptio	CO1	2.67	2.59	1.74	3.70	0.036
	NADH	5.24	4.90	2.30	9.00	0.042
	ANT	1.16	1.10	0.58	1.87	0.014
	NELTRS	2.90	2.89	1.84	4.36	0.005
Lampsilis	16S	5.81	5.50	3.00	9.24	0.013
	CO1	3.92	1.45	0.25	14.68	0.036
	NADH	5.62	5.38	2.25	9.87	0.042
	NELTRS	3.79	3.67	2.30	5.52	0.005
Pleurobema	16S	12.58	10.42	2.90	27.50	0.012
	CO1	6.93	6.52	3.37	11.27	0.037
	NELTRS	6.10	5.54	2.75	10.65	0.005
Villosa	16S	2.16	18.20	1.20	4.19	0.012
	CO1	1.98	1.90	1.12	2.99	0.037
	NADH	3.38	3.30	2.25	4.61	0.052
	NELTRS	6.21	6.02	3.78	8.98	0.005

Figure 2.1
A map of the southeast United States showing in bold the rivers sampled for this project. The Mississippi is labeled for reference only and was not included in this study.

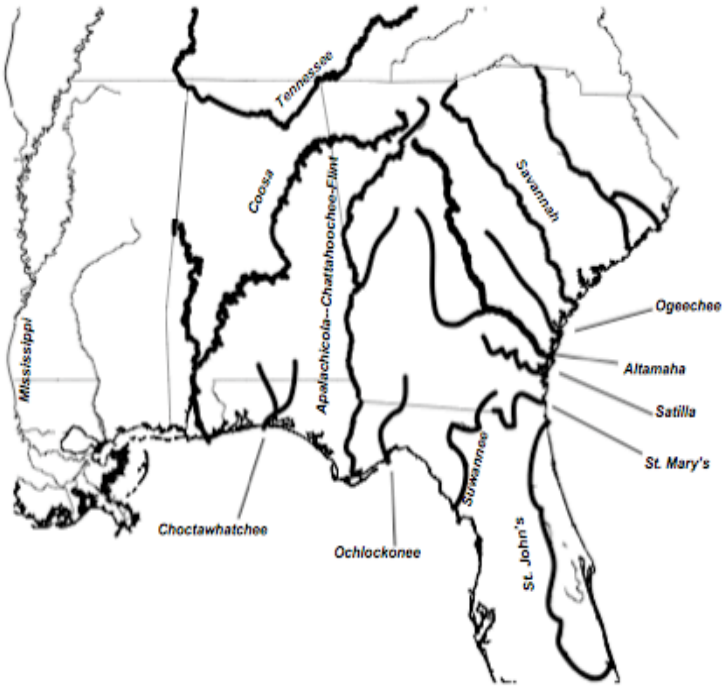
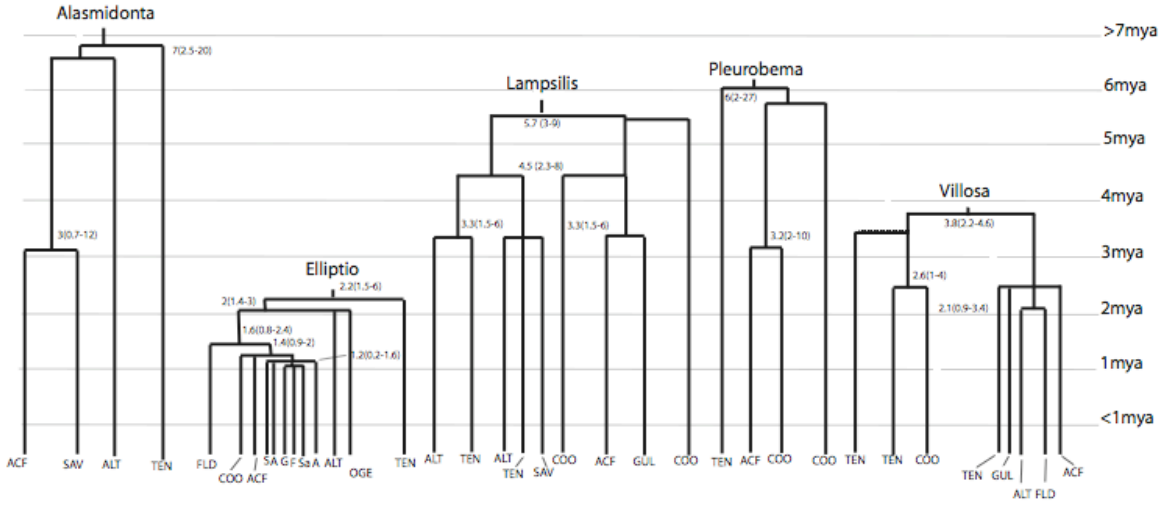

Tennessee
Coosa
Apalachicola--Chattahoochee-Flint
Savannah
Mississippi
Ogeechee
Altamaha
Satilla
St. Mary's
Suwannee
St. John's
Choctawhatchee
Ochlockonee

Figure 2.2
Comparison of species trees from 5 genera with tips labeled as river drainages. Mean age with 95% credible intervals on branching events are given using the mean substitution rate as given by BEAST (Drummond & Rambaut 2007). a) Concatenated tree for 3 mitochondrial markers (*16S, CO1, NADH*). b) Species phylogeny for the nuclear marker (*NELTRS, ANT*).

A.


Alasmidonta
Lampsilis
Pleurobema
Villosa
Elliptio
>7mya
6mya
5mya
4mya
3mya
2mya
1mya
<1mya
7(2.5-20)
3(0.7-12)
5.7 (3-9)
4.5 (2.3-8)
3.3(1.5-6)
3.3(1.5-6)
6(2-27)
3.2(2-10)
3.8(2.2-4.6)
2.6(1-4)
2.1(0.9-3.4)
2.2(1.5-6)
2(1.4-3)
1.6(0.8-2.4)
1.4(0.9-2)
1.2(0.2-1.6)
ACF SAV ALT TEN FLD COO ACF SA G F Sa A ALT OGE TEN ALT TEN ALT TEN SAV COO ACF GUL COO TEN ACF COO COO TEN TEN COO TEN GUL ALT FLD ACF

B.

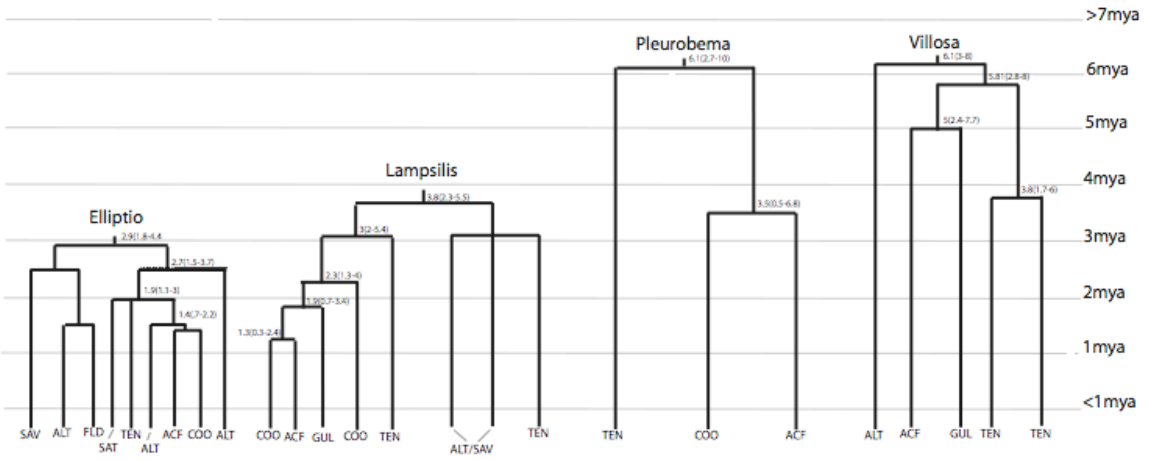
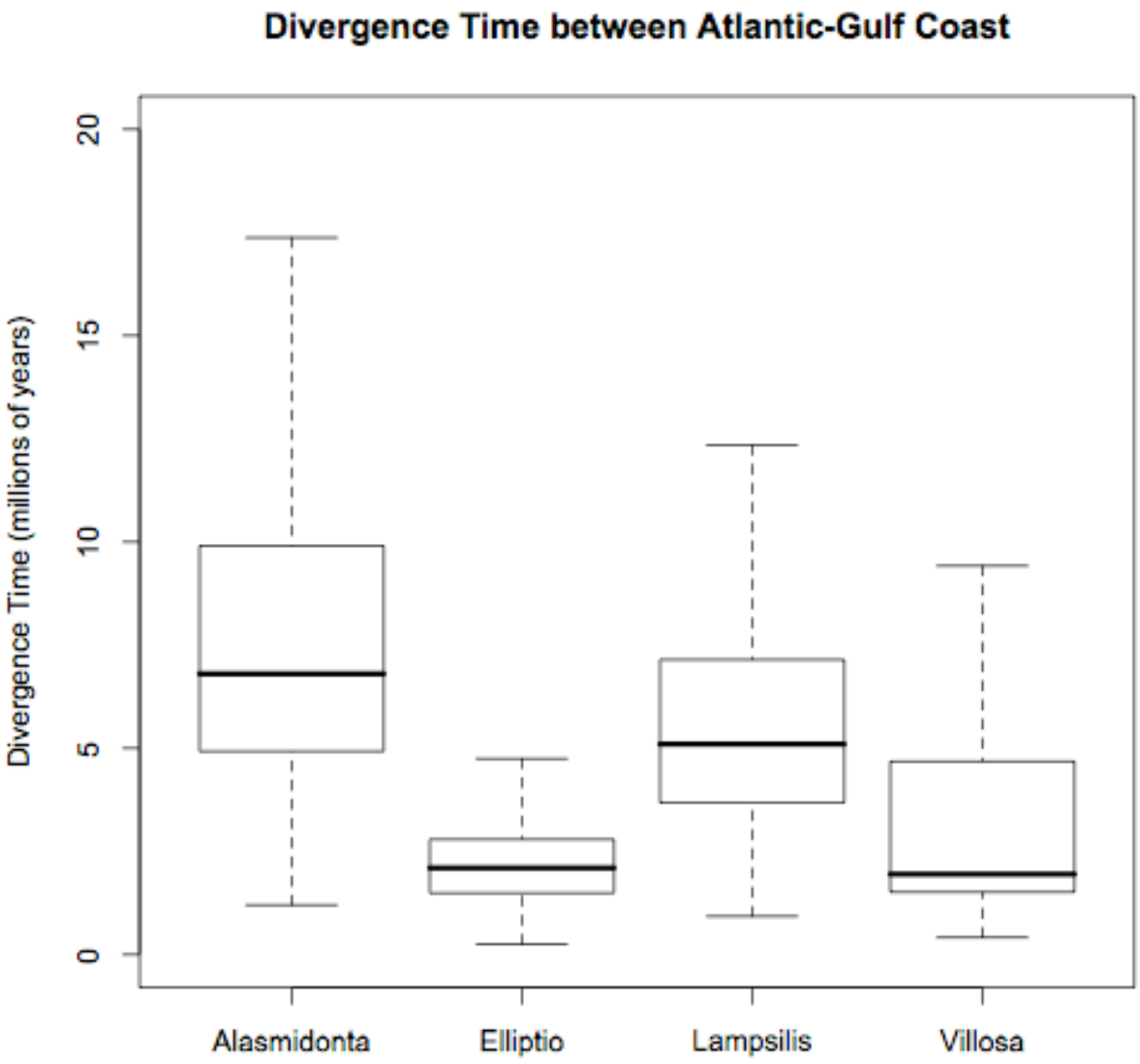

Elliptio
Lampsilis
Pleurobema
Villosa
>7mya
6mya
5mya
4mya
3mya
2mya
1mya
<1mya
2.9(1.8-4.4
2.7(1.5-3.7)
1.9(1.1-3)
1.4(0.7-2.2)
3(2-5.4)
2.3(1.3-4)
1.8(0.7-3.4)
1.3(0.3-2.4)
6.1(2.7-10)
3.5(0.5-6.8)
6.1(3-8)
5.81(2.8-8)
5(2.4-7.7)
3.8(1.7-6)
SAV ALT FLD / SAT TEN / ALT ACF COO ALT COO ACF GUL COO TEN ALT/SAV TEN TEN COO ACF ALT ACF GUL TEN TEN

Figure 2.3
Boxplot showing the divergence time between the Atlantic Slope and Gulf Coast drainages for 4 genera of freshwater mussels. Boxes delineate upper and lower quartiles, dark lines show medians, and dashed lines extend to the last observation within 1.5X interquartile range of the boxes


Divergence Time between Atlantic-Gulf Coast
Divergence Time (millions of years)
0
5
10
15
20
Alasmidonta
Elliptio
Lampsilis
Villosa

CHAPTER 3

ABUNDANCE AND GENETIC DIVERSITY OF SESSILE ORGANISMS IN A NEUTRAL COMMUNITY

Introduction

What maintains species diversity in communities? Many mechanisms have been proposed to explain observed patterns in species-area curves and relative abundance distributions (Hutchinson 1959; Levins 1970; May 1975; Hubbell 1979; Pacala and Tilman 1993). Few studies have examined the ability of species diversity and genetic diversity to differentiate between these mechanisms (Chave, Mueller-Landau et al. 2002; Vellend 2005; Hu and He 2006), however historically, ecologists have categorized mechanisms of species diversity into two categories: species interactions and environmental influences. Recently Clark, Dietze et al. (2007) reviewed how mutli-dimensional environmental mechanisms help to structure diversity, here we will focus on species interactions only with the intention of later adding environmental stochasticity to our models.

Species interactions can be broadly partitioned into equilibrial or nonequilibrial mechanisms (Chave, Mueller-Landau et al. 2002). Equilibrial mechanisms are based on functional differences between species, such as life history differences (Grubb 1977), habitat preference (Ashton 1969; Ashton 1998), or competition (Pacala and Tilman 1993). In contrast,

nonequilibrial mechanisms are best explained as a balance between birth and death, or in broader terms, speciation and extinction (MacArthur and Wilson 1967; Caswell 1976; Hubbell 1979).

To determine which forces are at work in a community researchers have relied on the distribution of species abundance and relationships between species and area. Chave, Mueller-Landau et al. (2002) and others (McGill 2003; Volkov, Banavar et al. 2005) have shown that sole use of the species abundance distribution cannot differentiate between equilibrial and nonequilibrial mechanisms. A particular case presented by Volkov, Banavar et al. (2005) has shown that both dispersal limitation and negative conspecific density dependence explain species abundance distributions of numerous tropical tree datasets equally well. In the case of Volkov, Banavar et al. (2005), dispersal limitation is a nonequilibrial mechanism slowing the exclusion of rare species in the community, while negative density dependence is an equilibrial mechanism increasing the mortality rate of common species in proportion to abundance (Chave 2004). Both Volkov, Banavar et al. (2005) and Chave, Mueller-Landau et al. (2002) endorse the use of dynamic (i.e. multiple sample periods) rather than static (i.e. single sample period) diversity data to differentiate between hypotheses of different mechanisms, but the widespread availability of static species diversity data creates an intriguing problem.

Here, we propose combining data both from species abundance distributions and genetic diversity to increase the power to differentiate between hypotheses of equilibrial and nonequilibrial mechanisms. Vellend (2005) examined the correlation between species diversity and genetic diversity and found parallel processes often can influence both. Vellend (2005) concludes that species diversity and genetic diversity should be positively correlated in communities, but did not explore the usefulness of parallel processes for differentiation between mechanisms that maintain diversity. Here we use a continuous landscape model of a neutral

community (Bell 2000; Hubbell 2001; Chave, Mueller-Landau et al. 2002; Hu, He et al. 2007) paired with a genetic model (Kimura 1983) to test the ability of a combined species/genetic dataset to differentiate between dispersal limitation and conspecific density dependence mechanisms in a community of sessile organisms. We test the ability to differentiate between processes maintaining species diversity in communities with 50 to 400 species, using 1 kilobase (kb) of selectively neutral DNA sequence data. We compare the distribution of species-abundance and species-area relationships among the models, as well as the distribution of genetic diversity and its relationship with area. Our results provide the expected distribution of genetic diversity in a community and prove that the inclusion of DNA sequence data does increase our ability to differentiate mechanism maintaining species diversity.

Model Overview

The added complexity of both species interaction and mutation makes analytical solutions intractable; here all of our results are from simulation. Our model is an individual based, spatially explicit model of a community of sessile organisms where neutral evolution occurs through substitution of base pairs at a single non-recombining locus. The nature of the model makes it easy to change species-level life history parameters as well as change the model of mutation. Here we present a simple case of two extremes of dispersal and density dependence in a neutral model framework easily extended to include metapopulation dynamics as well as niche and competitive mechanisms.

Neutral Model

We used the unified neutral model of biodiversity (UNTB) to describe community dynamics for our simulations (Hubbell 2001). The UNTB model represents an extension of the

Theory of Island Biogeography (MacArthur and Wilson 1967) where the number of species in a community (or island) depends upon the rate of immigration from outside the community and the rate of extinction. Hubbell's original derivation of the UNTB included two parameters: J_M the number of individuals in the metacommunity, and ν the speciation rate. The product of J_M and ν give Hubbell's fundamental biodiversity parameter $\theta=J_M\nu$ (here referred to as θ_H to avoid confusion with measures of genetic diversity). Hubbell's fundamental biodiversity parameter is a measure of the effective speciation rate in the community $\nu=\theta/J_M$.

We deviate from Hubbell's (2001) original model by representing species on a spatially explicit landscape (Chave, Mueller-Landau et al. 2002). The spatially explicit representation of individuals in the UNTB does not change the underlying dynamics of the model as equilibrium abundances and transition probabilities are still defined by Hubbell's original equations (Hubbell 2001, Chave, Mueller-Landau et al. 2002, Hu, He et al. 2007). We also take the approach of simulating the entire metacommunity, represented by Hubbell as J_M and here and elsewhere as J (Bell 2000; Chave, Mueller-Landau et al. 2002; Hu, He et al. 2007). The benefit to modeling the metacommunity is that we avoid the artificial distinction of dividing a continuous community into partitions of a local community and metacommunity. Also the resulting data on spatial distribution of species can be used to infer the variance in migration rates, treated as a constant in other Neutral models.

In the Neutral Model, the total simulated metacommunity at any time, t, is composed of J individuals of S total species, where each individual belongs to species $i \in \{1, \ldots, S\}$. The J individuals can be seen as competing for the same resource, in this case space, but easily extended to represent other resources. The simulated community is represented by a square lattice of J cells, where $\sqrt{J}$ is the length of a single side of the lattice. At most one individual

can occupy a single cell, and there are no empty cells. The absence of empty cells is termed the zero-sum model (Hubbell 2001) and has been found to be equivalent to models with empty space (Etienne 2007). We chose the zero-sum assumption because it decreases computation time to reach equilibrium (Chave, Mueller-Landau et al. 2002). The lattice represents a finite community with boundary conditions handled by the conserved reflection of propagules that disperse outside of the lattice. Simulations of finite communities or communities with definable boundaries best represent islands of habitat. In our case, all simulations were performed on the largest allowable lattice, by memory allocation, of $\sqrt{J}$=256 cells.

In the context of an empirical community of sessile organisms, each cell can be considered to represent any spatial scale. The spatial lattice could represent a forest of trees species where a cell is a 5 m x 5 m area or an intertidal community where a cell is a 5 cm x 5 cm area (Botkin, Janik et al. 1972; Shugart 1984; Chave, Mueller-Landau et al. 2002).

In our simulated community, the abundance of species *i* at time *t* is given as $n_i(t)$, where $J = \sum_{i=1}^{S} n_i$. The transition probabilities for a single species, *i*, with initial abundance N_i is then given by,

$$\Pr\langle N_i - 1 | N_i \rangle = \mu\left(\frac{N_i}{J}\right)\left(\frac{J-N_i}{J-1}\right)$$

$$\Pr\langle N_i | N_i \rangle = 1 - \mu + \mu\left(\frac{N_i}{J}\right)\left(\frac{N_i-1}{J-1}\right) + \mu\left(\frac{J-N_i}{J}\right)\left(\frac{J-N_i-1}{J-1}\right)$$

$$\Pr\langle N_i + 1 | N_i \rangle = \mu\left(\frac{J-N_i}{J}\right)\left(\frac{N_i}{J-1}\right)$$

where μ is the probability of one death per time step. The transition matrix contains $J+1$ states corresponding to the *k* different abundances of species *i* with absorbing states corresponding to abundances *0* and *J,* representing extinction and dominance (Hubbell 2001).

To extrapolate the single species case to the entire community involves representing the abundance states for each species. The transition matrix for the entire community is complex even for small communities (*J>10*) (Etienne, Apol et al. 2007) however an approximation derived by Volkov (2003) can be used to determine the stationary state of a community at equilibrium. For example a community of *J=4* will have 5 possible states, where state is defined as the number of individuals of that species at any given point in time. A simple example with *J=4* would be 4 species with abundance (1,1,1,1), 3 species with abundance (1,1,2), 2 species with abundance (2,2) or (1,3), or 1 species with abundance and (4). The transition matrix T is then given by:

$$T = \begin{bmatrix} \nu + \frac{1}{4}(1-\nu) & \frac{3}{4}(1-\nu) & 0 & 0 & 0 \\ \frac{2}{4}\left(\frac{2}{4}\nu + \frac{2}{4}\nu\right) & \frac{2}{4}\left(\frac{2}{4}\nu + \frac{2}{4}\nu + \frac{1}{4}(1-\nu)\right) + \frac{2}{4}\left(\frac{2}{4}(1-\nu) + \frac{2}{4}(1-\nu)\right) & \frac{2}{4}\left(\left(\frac{1}{4}\right)(1-\nu)\right) & \frac{2}{4}\left(\left(\frac{2}{4}\right)(1-\nu)\right) & 0 \\ 0 & \left(\frac{2}{4}\nu + \frac{2}{4}\nu\right) & \frac{2}{4}(1-\nu) & \frac{2}{4}(1-\nu) & 0 \\ 0 & \frac{3}{4}\left(\frac{1}{4}\nu + \frac{3}{4}\nu\right) & \frac{3}{4}\left(\left(\frac{1}{4}\right)(1-\nu)\right) & \frac{3}{4}\left(\left(\frac{3}{4}\right)(1-\nu)\right) + \frac{1}{4}\left(\frac{1}{4} + \frac{3}{4}\nu\right) & \frac{1}{4}\frac{3}{4}(1-\nu) \\ 0 & 0 & 0 & \nu & 1-\nu \end{bmatrix}$$

where ν represents the probability of speciation. For a species with abundance n the total probability of speciation then equals νn (Etienne, Apol et al. 2007).

Speciation under the UNTB is modeled as a constant probability ν, which leads to a speciation probability per species of νn_i. The effective speciation rate, represented as the variable θ_H, is equivalent to νJ, which is the number of new species added to the community every time step (Chave, Mueller-Landau et al. 2002). This type of speciation was deemed point-mutation speciation by Hubbell (2001) and represents the formation of a new species that is instantaneously reproductively isolated. Hubbell's use of speciation has been widely criticized

(Ricklefs 2006), although Hubbell (2001) points out that some plant species exhibit point-mutation speciation with polyploidy formation.

Dispersal

We examined the effects of two different kinds of propagule dispersal of our simulated species: global dispersal where all propagules are able to disperse throughout the entire community, and nearest-neighbor dispersal where propagules are only able to disperse to neighboring cells. Dispersal distance can greatly alter the spatial patterns of species abundance and distribution of genetic diversity. Dispersal distances can vary among different species in a community (Thorson 1950; Caley, Carr et al. 1996), such as long distance dispersal in intertidal communities and relatively local dispersal in plant communities (Harper 1977; Wilson 1993; Ouborg, Piquot et al. 1999). Though we recognize the importance of many different kinds of dispersal, i.e. from Gaussian kernels to rare long-distance events (Clark, Silman et al. 1999) our goal was to understand the utility of our approach for extreme cases before allowing more complex dispersal scenarios.

Conspecific Density Dependence

In real communities, both intra and interspecific density dependence are important factors for facilitating species coexistence (Molofsky, Durrett et al. 1999; Bulleri, Bruno et al. 2008). Here, we examine the case of negative density dependence where an individual is disadvantaged when the spatial density of conspecifics is high. Mechanisms driving negative density dependence are diverse, but there has been much support for a specific mechanism of negative density dependence named the Janzen-Connell effect (Janzen 1970; Connell 1971; Condit, Hubbell et al. 1992; Packer and Clay 2000). The Janzen-Connell effect is defined as the reduced fitness of an individual due to a high concentration of species-specific pests or pathogens

stemming from high local abundance of shared hosts (Janzen 1970; Connell 1971). Janzen-Connell effects create a "rare species" advantage that can alter species abundance distributions. Chave, Mueller-Landau et al. (2002) have shown that the inclusion of negative density dependence increases species richness when compared to the standard neutral model of Hubbell (2001).

We modeled the effects of negative density dependence (hereafter density dependence) in a globally dispersed community. Density dependence was modeled as the linear-decreasing probability of a propagule establishing as the proportion of conspecific individuals occupying neighboring sites increased (Chave, Mueller-Landau et al. 2002). We calculated the proportion, q, of the eight nearest neighboring cells of the propagule-occupied cell that were occupied by individuals of the same species. The probability that the propagule survives and establishes at a site was defined by $w=1-aq$, where a determines the strength of the density-dependent interaction (Chave, Mueller-Landau et al. 2002).

Mutational Model

After each birth event a propagule mutated with a probability of $\mu=2.4 \times 10^{-8}$ per site, equivalent to the substitution rate of mitochondrial COI in a large variety of organisms (Brown, George et al. 1979). We used a Jukes-Cantor mutation model to simulate neutral evolution at a single non-recombining locus of length 1kb (Jukes and Cantor 1969). The Jukes-Cantor model of mutation assumes equal rates of substitutions between base pairs and equal frequencies of each nucleotide base. Jukes-Cantor (JC69) corrects for successive substitutions at a single site, which violate the assumptions of the infinite sites model of mutation (Kimura 1969). The transition matrix for the Jukes-Cantor model is

$$\begin{bmatrix} 1-3\alpha & \alpha & \alpha & \alpha \\ \alpha & 1-3\alpha & \alpha & \alpha \\ \alpha & \alpha & 1-3\alpha & \alpha \\ \alpha & \alpha & \alpha & 1-3\alpha \end{bmatrix}$$

where α represents the transition probability from one base (A,T,C,G) to another. JC69 assumes equal probabilities for the transition and transversion substitution rates. We note that our model can incorporate any model of mutation; we used the JC69 model for simplicity.

We simulated genomes for each species in the community by creating a tree of 1000 individuals in MS (Hudson 2002) from a single population. Sequences were then evolved along the genealogy using SEQ-GEN (Rambaut and Grassly 1997) with a θ_W value per 1000bp sequence equal to 1 and a Jukes-Cantor model of mutation (Jukes and Cantor 1969). A separate genealogy was created for each species at the start of model (as we are mainly interested in within species variation), where genomes of individuals randomly placed on the grid were sampled with replacement from its 1000 simulated sequences.

Algorithm for community evolution

The simulation algorithm consists of $T=10^5$ time steps where a single time step is analogous to a single generation. One time step contains J iterations so that every individual has an equal chance to both dies and reproduce. In each of J iterations an individual is randomly selected from the community to die with another individual randomly selected to give birth and fill the empty space. When an individual gives birth, propagules have an equal probability of dispersing to any site in the community (under the global dispersal model) representing J number of offspring or a community-wide seed shadow. In our model only a single offspring survives to disperse to a new site during a single generation; the propagule is dispersed to a new site in accordance with the dispersal model, either global dispersal or nearest neighbor dispersal. Thus

for each time step there are J deaths and J successive births representing a continuous-time Moran process (Moran 1958), where all individuals have an equal probability of both dying and giving birth. There is a small but non-zero probability that an individual can both die and give birth in the same time step, however the probability ($=1/J^2$) approaches zero as the size of the community increases.

Global dispersal is modeled as a random process where the propagule is dispersed with equal probability to any site. Dispersal limitation is modeled as nearest neighbor dispersal and consists of the propagule moving in 1 of 8 cardinal directions a distance of 1 cell. The direction of dispersal is given by a Uniform [0,7] distribution where individuals dispersing off of the lattice reflect back onto the lattice in a direction equal to $7-x$, where x is the original cardinal direction. Individuals occupying corner cells were treated as a special case where propagules dispersing off the lattice were redrawn for a new direction. Dispersal under the density dependence model is similar to global dispersal but the probability of a propagule establishing at the randomly chosen site, given that it has arrived at the site, is given by $w=1-aq$, where q is the proportion of the 8 surrounding cells occupied by similar species, and a is a weighted parameter dictating the strength of density dependence. Hence density dependence creates a probability landscape where the propagule has varying success of establishment. If establishment is unsuccessful, another individual is randomly chosen to give birth. This process continues until a propagule successfully establishes in the cell.

New species join the community through the process of speciation. Speciation occurs with probability ν and an individual of an extant species become a new species with a probability of νm_i. New species inherit the genome of the former species, conserving phylogenetic history between species in the community.

Mutations occur after successful dispersal with probability μ. If a mutation occurs, a site is selected using a uniform distribution from all possible sites; the transition matrix under the Jukes-Cantor model of mutation gives the direction of change given that a mutation has occurred.

The summary algorithm for T time steps is then:

Repeat J times…

1. Draw a random cell from J possible cells to die.
2. Draw a cell to replace the individual that just died.
    a. (global dispersal) randomly from all cells .
    b. (nearest neighbor dispersal) randomly from the 8 nearest cells at a distance of 1 cell in all directions.
3. The propagule disperses to the chosen cell
    a. (No Density dependence) the propagule establishes with a probability of 1.
    b. (Density dependence) the propagule establishes with a probability of $w=1-aq$. If unsuccessful establishment repeat step 2-3.
4. The successfully dispersed propagule mutates with a probability of μ, where the Jukes-Cantor transition matrix gives the site-specific direction of the change.
5. The propagule becomes a new species with probability ν.

Simulations

To investigate the distribution of genetic diversity under different models of community assembly we ran simulations for combinations of the following parameters: *i)* UNTB with global dispersal (i.e. classic neutral model) and no density dependence *ii)* UNTB with nearest-neighbor

dispersal and no density dependence *iii)* UNTB with global dispersal and density dependence. Five simulations were run for each value of θ_H, which was set to 5, 10, 20, 40 or 60 and a lattice size of J=65,536. Mutation was set to 2.4×10^{-8} per site for the 1kb locus. We ran each model for $T=10^5$ time steps, which allowed simulations to reach equilibrium. Community equilibrium was determined when the rates of extinction and speciation were equal, or when no further directional changes in the forms of species-area and relative-abundance curves were observed (steady state) (Chave, Mueller-Landau et al. 2002; Hu, He et al. 2007) (Table 1).

We tested the influence of starting conditions on our model to determine the robustness of the equilibrium state. We compared data from simulations using different starting genealogies, amounts of genetic diversity, number of species, and the influence of random v. exponentially seeded communities. We found that these initial starting conditions values had no effect on the equilibrium condition of the model. This is due to the effect of lineage sorting at both the species and genetic level, whereas all species in a community present at the equilibrium are derived from a single common ancestor in the past. Based on these results we used an initial species richness value of 100 for species and a nucleotide diversity value of θ_W of 1 per locus (Watterson 1975). Individuals of species were randomly seeded on the lattice until all cells were occupied.

During the simulation runs, we recorded data every 5,000 time-steps starting at time = *J/2*, which was shown by Chave, Mueller-Landau et al. (2002) to be the time to reach equilibrium for the UNTB model with a θ_H value of 5. We recorded data on species-area curves, relative-abundance distributions, and a subsample of the community (*J/16*) for genetic data. We subsample the genetic data from the community to avoid violating the assumption of the coalescent model where the effective population size (N_e) must be much less than the census size

(N). Failure to subsample the genetic data would lead to an excess of singleton polymorphisms in the data set, inflating measures of θ_W based on the number of segregating sites (Wakley and Takahashi 2003).

Species abundance data were analyzed in R (R Development Core Team 2005) for both species-area curves and relative-abundance distributions. Species-area curves were calculated using a sliding window of 16x16 cells dividing the community into 256 non-overlapping quadrats that completely covered the simulation grid. This curve shows how species richness changes with sample size in a given community (Condit, Hubbell et al. 1996). Relative-abundance distributions were also calculated in R by averaging across all 5 runs for each combination of parameters and calculating the mean and 95% confidence intervals from the 5 runs. We display these distributions as rank-abundance curves, where species are ranked from highest to lowest abundance.

Genetic diversity data were analyzed using R for both rank-diversity curves and diversity-area curves. For diversity-area curves we divided the community into 256 non-overlapping quadrats each containing 256 cells, and used COMPUTE (Thornton 2003) to calculate the number of segregating sites per species (Watterson 1975) for each increasing sample area. To avoid bias due to the paucity of segregating variation in species with small census sizes (rare species) we used only the 20 most abundant species to compile a diversity-area curve (Vellend 2005). We then performed a simple linear regression in R for the genetic diversity-area curve. For rank-genetic diversity curves we displayed θ_W (Watterson 1975) as calculated in COMPUTE (Thornton 2003) for each species ranked from highest to lowest abundance.

We used a Mann-Whitney U test (Mann and Whitney 1947), as implemented in R, to test for statistically significant difference between underlying mechanisms of dispersal and density

dependence for species diversity data. To account for multiple comparisons we use Bonferroni corrected value of alpha = 0.0169 (n=3) to determine statistical significance for each θ_H value. We used a two sample Kolmogorov-Smirnov test, as implemented in R, to compared the rank-genetic diversity distribution for each of the model combinations with statistical significance given by a Bonferroni corrected value of alpha = 0.0169 (n=3).

Results

As a result of our simulations we found that communities with dispersal limitation and density dependence have higher species richness than the UNTB model with global dispersal. Density dependence produced communities with higher species richness for all values of θ_H examined, while dispersal limited communities had species richness values closer to the UNTB model (Figure 3.1a-3.5a). The trend for dispersal limitation was similar to the UNTB model but with more rare species, creating an 'S' shaped curve. Trends were consistent across all values of θ_H examined, except $\theta_H=60$ where we found the dispersal limitation model to be more similar to the UNTB model in number of rare species (Figures 3.1a-3.5a).

Spatial distribution of species in the dispersal limitation model tended toward more clumped species distributions while models with density dependence tended towards under-dispersed species distributions in comparison to the UNTB model. Species-area curves illustrate this difference wherein global dispersal is a saturating curve; dispersal limitation is a linear relationship with gains in species even at the maximum area sampled. Models with density dependence had greater numbers of species than the UNTB models but gave a similar shaped curve (Figures 3.1b-3.5b).

Statistical comparison between models across different values of θ_H produced mixed results. We were able to statistically differentiate the density dependence model from all others models for every value of θ_H, except for $\theta_H=5$ where the density dependence and UNTB model were only marginally different. We were not able to differentiate the UNTB model from the dispersal limitation model for any values of θ_H examined (Table 3.2).

The dispersal limitation model had the greatest amount of genetic diversity across all values of θ_H. The UNTB model with global dispersal had similar shaped curves to the dispersal limitation model but did not contain species with the higher amounts of genetic diversity. Models with density dependence had the greatest amount of species with intermediate genetic diversity, but few species with high values (Figures 3.1c-3.5c).

The spatial distribution of genetic diversity was similar to the species-area curves. The UNTB model and the density dependence model both produce similar relationships with area when plotted on a log-log plot. The slopes for the UNTB model and the density dependence models were similar but not the same (0.15 v. 0.18), however the intercepts were quite different (1.7 v. 0.38) owing to greater amount of diversity in the UNTB model. The dispersal limitation model had a much steeper slope than either the UNTB model or the density dependence models (0.53), however the intercept was lower reflecting that genetic diversity is more spatially segregated. Graphs represent only the 20 most abundant species as the correlation between genetic diversity and area becomes convoluted with the inclusion of rare species (Vellend 2005) (Figures 3.1d-3.5d).

Comparisons between models using the distribution of genetic diversity were able to statistically differentiate the density dependence model from the other models for cases where $\theta_H<40$, values above $\theta_H=40$ were less significant. The density dependence and dispersal

limitation model were similar for values of $\theta_H > 40$, but significantly different at values $\theta_H < 40$. The UNTB and dispersal limited models were not statistically different for any values of θ_H (Table 2).

Discussion

Our approach to modeling biodiversity is to include data from the distribution of species and genetic diversity. The expected distribution of species in a dispersal-limited community has been examined by previous studies (Bell 2000; Chave, Mueller-Landau et al. 2002; Hu, He et al. 2007) and our results are similar. The distribution of genetic diversity in a community has not been described for dispersal-limited and density dependent communities, and have only been hinted at for the UNTB model (Etienne and Olff 2004). The truly novel insight of our work is combining these data sets, with the eventual goal of describing the expected distribution of genetic diversity under different models of community assembly.

Species Diversity

The three models examined here—dispersal limitation, density dependence, and UNTB—have all been shown to generate species diversity in communities (Bell 2000; Hubbell 2001; Chave, Mueller-Landau et al. 2002; Hu, He et al. 2007). Each mechanism is similar in that it limits the strength of interspecific competition and increases intraspecific interactions. The increase in intraspecific interactions creates a dynamic where the abundance of a species is self-limiting, thus decreasing competitive exclusion of new species. The coexistence of species imposed by limiting dispersal distances reduces the rate at which species drift to extinction in the Neutral framework, producing communities similar to the UNTB model (Durrett and Levin 1994). Communities with limited dispersal have higher numbers of species than the UNTB

model but also have no stable equilibrium value to which the abundance will return if perturbed. This is largely due to the spatial scaling of the species where most species are clumped together resulting in patchy distributions. Reproduction in clumped species creates a situation where propagules land on sites occupied by neighbors of the same species increasing intraspecific competition and retarding rates of increase and decrease in species abundance. At larger scales the species-area curves will have a slope of 1, as increasing area will always continue to find locally unique species (Bramson, Cox et al. 1996). This is in contrast to species-area relationships under the UNTB model where global dispersal ensures that species are ubiquitous in the community. Local species richness is higher under the UNTB and the number of species that can be packed into a community will rise as community becomes larger, producing a saturated curve.

Coexistence under density dependence creates a dynamic where species fitness declines with increasing abundance. Under density dependence species tend towards equal abundance with greater number of species with intermediate abundances. The equilibrium created by density dependence has been shown to be more robust to disturbance returning to equilibrium values after being perturbed (Chave, Mueller-Landau et al. 2002). Due to the local disadvantage of common species, clumping patterns are broken up and species richness is higher than in the UNTB and dispersal models. In space, the community becomes a mosaic of different habitats where for each species suitability is dependent on the presence/absence of conspecifics. The species-area curves are similar in shape to those produced under the UNTB model but species richness values are greater at the asymptote.

Genetic Diversity

Genetic diversity in our models is governed entirely by the forces of drift and dispersal and therefore has no effect on the distribution of species abundances (Kimura 1983; Hubbell 2001). However, species abundances, influencing genetic drift and mutation, do influence the pattern of genetic diversity in our model. Under the equivalence assumption of the UNTB it has been shown that species can be represented as an infinite alleles model (Etienne, Alonso et al. 2007) where the expected distribution of genetic diversity in a community is given by Ewens' sampling distribution (Ewens 1972). Ewens' sampling distribution represents the probability that there are a_1 alleles represented once in the sample, and a_2 alleles represented twice, and so on. Typically Ewens' distribution is used to describe alleles at a single locus but the distribution also seems to describe the rank genetic diversity for an entire community (Etienne and Olff 2004). Under our three models different mechanisms of community assembly create different distributions of genetic diversity. The dispersal limited model had on average more diversity than both the UNTB model and the density dependence model owing to the spatial segregation of species in the community. Spatially segregated species maintain higher diversity because individuals can only disperse to neighboring sites; this creates high amounts of spatially- unique genetic diversity within each species. Diversity-area relationships are represented by an increasing curve, similar to species accumulation, which does not saturate with area. Models with density dependence do not have clumped species distributions subsequently producing more species with intermediate abundances, which increases the frequency of common alleles in species populations. The shape of the diversity-area curve for density dependence was also similar to the species-area curve but with the opposite association when compared to the UNTB

model. Hence, the curves were similar in shape but the UNTB curve had more diversity than the density dependence curve.

If it could be conceived that the distribution of genetic diversity in a community is analogous to the distribution of alleles at a locus we can contribute patterns of genetic diversity—due to different mechanisms—to processes of ecology. It has been theorized that the distribution of neutral genetic diversity in the UNTB should be described by Ewens' distribution (Etienne and Olff 2004). A similar pattern can be seen in the density dependence model where the increase in intermediate species creates a truncated curve where most species have similar amounts of neutral diversity. Thus a community under density dependence can be likened to a locus under negative selection where the fate of diversity at a locus is constrained. Under negative selection there are more rare alleles than expected under neutrality and very few alleles are at high frequency, such is the distribution of genetic diversity in the density dependent community. In contrast a community under limited dispersal could be likened to a locus under positive selection or balancing selection. Under balancing selection there are more common alleles than expected under neutrality and very few rare alleles. The dispersal-limited communities had greater values of genetic diversity than either the UNTB or density dependence models as shown by the fat tail on the curve of genetic diversity in the community.

Combined datasets

Species diversity and genetic diversity are correlated through parallel processes in communities (Antonovics 1976; Chave 2004; Vellend 2005). However no one has tested whether the inclusion of genetic diversity can help differentiate mechanisms maintaining species diversity in communities. We found that the combined datasets provided support for differentiating between processes of dispersal and density dependence when both the distribution

of species abundance and genetic diversity were used. Comparisons between models using the distribution of genetic diversity were able to statistically differentiate the density dependence model from the other models for cases where $\theta_H<40$, values above $\theta_H=40$ had lower significance due to the decreased population sizes of species in more species rich communities. Smaller population sizes increase the strength of genetic drift and create more stochastic noise in the model, making it more difficult to use genetic diversity (often 0 or <<1) to differentiate between the models.

Our original hypothesis was based on the research of Volkov, Banavar et al. (2005) where a test of two models—symmetrical density dependence and dispersal limitation—provided equally good fit to species abundance data from tropical forests. Our simulations did not find a strong similarity between density and dispersal limited species abundance distributions, possibly owing to the high variance in our models. Other causes of the discrepancy might be the strength of density dependence and severity of dispersal limitation used in our simulation. Volkov, Banavar et al. (2005) used an analytical solution (Volkov, Banavar et al. 2003), rather than a numeric simulation, to calculate species abundance curves. Values of dispersal and density dependence were then estimated from maximum likelihood methods to fit the species data. What would be useful would be to use the values of dispersal and density dependence estimated by Volkov, Banavar et al. (2005) in our models to then determine if genetic data can be used to differentiate between mechanisms in a real data set. Another difference between our results and the Volkov, Banavar et al. (2005) results is our use of a continuous landscape model (CLM) versus the island-mainland model (IMM). Volkov, Banavar et al. (2005) used the analytical solution to the IMM because the CLM did not exist at the time of publication. Hu, He et al.

(2007) has now provided an analytical solution to the CLM providing a more realistic model for estimating diversity in local communities.

Application to real communities

There are many limitations to the models that we have tested in this paper. First of all, the size of our simulated communities is unrealistically small. However, work by Chave, Mueller-Landau et al. (2002) and others (Bramson, Cox et al. 1998) have shown that species diversity in communities can be described by a scaling function. Under the scaling function the number of species in the total community of arbitrarily large size can be deduced from information on smaller systems (Chave, Mueller-Landau et al. 2002). The relationship between smaller communities and projection to larger communities is linear in all cases except nearest neighbor dispersal, which is logarithmic (Chave, Mueller-Landau et al. 2002). A second realism that was difficult to represent was the process of speciation in a community. Speciation is still poorly understood in many systems, especially long-lived species (Coyne and Orr 2004). Ricklefs (2003) criticized Hubbell's neutral model for its unrealistic assumption about species formation, which typically over predicts the presence of rare species in communities. Some of these concerns have been addressed (Etienne, Alonso et al. 2007), but Hubbell states that the presence of cryptic species in real communities and our difficulty in actually defining species is the cause of the discrepancy (Ricklefs 2003).

Despite the false assumptions in the neutral model of biodiversity it has been shown time and time again to provide a good fit to species distributions in varying types of communities; this may not be because species are truly equivalent but because at larger scales species differences do not matter (Chave 2004). We have yet to test whether our description of genetic diversity in a community provides a good fit to empirical data, mainly because datasets of this complexity are

not readily available. However, with the dropping price of genetic sequencing and the recognized utility of genetic markers, datasets may soon become available.

Future models and tests

The integration of genetic models with newer methods of generating species distributions has not yet been explored but since we have shown that density dependence and dispersal limitation both affect the site frequency distribution of genetic diversity in predictable ways, it may be possible to use coalescent algorithms to simulate genetic diversity in future endeavors. The incorporation of the analytical solution to the neutral model of biodiversity (Volkov, Banavar et al. 2003) as well as similar algorithms (Etienne and Olff 2004; Hu, He et al. 2007; Etienne, Alonso et al. 2007) will allow us to reduce the variation in our results providing better fits to empirical species data in future models. Analytical models will make it easier to extend to more complex mechanisms of community assembly such as niche partitioning, life history trade-offs, and disturbance. By limiting genetic diversity to have no fitness effects in our current models we have decoupled the effect of genetic diversity on species abundances. Thus, a final endeavor would be to modify our model to include more complex genetic models that incorporate recombination and selection.

Conclusions

The goal of our project was to understand how mechanisms of dispersal and density structure species abundances and genetic diversity. Whether information from both can be used to discriminate between competing hypotheses of community assembly remains uncertain as some parameter combinations allow us to statistically differentiate between models while other do not. The utility of our approach can be applied immediately by predicting the distribution of genetic diversity in communities where neutral mechanisms have already been described. By

understanding the distribution of genetic diversity in communities we will have a better understanding of how biodiversity is structured and maintained, which will aid in conservation of imperiled species and ecosystems.

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Table 3.1: List of the parameters used in the model as well as the range of values for each parameter.

Parameter	Value
Area (J)	65536 cells
N (Σn_i)	65536 individuals
Time (t)	10^5
Species (S)	100
θ_H	5,10,20,40,60
ν	5/N; 10/N; 20/N; 40/N; 60/N
θ_W (per sequence)	1
μ	2.4×10^{-8}
a (density model only)	1.0

Table 3.2: Table of p-values for the comparison between the UNTB, density dependence, and dispersal limitation models of community assembly.
The upper triangle contains the p-values comparing the distribution of genetic variation among the models using a two-sample Kolmogorov-Smirnov test, while the lower triangle contains the p-values comparing the distribution of species abundance using a Mann-Whitney U test.

	Neutral	***Density Dependence***	***Dispersal Limited***
θ_H***=5***			
Neutral	--	<0.0001	0.736
Density Dependence	0.027	--	0.001
Dispersal Limited	0.300	<0.0001	--
θ_H***=10***			
Neutral	--	<0.0001	0.091
Density Dependence	0.001	--	<0.0001
Dispersal Limited	0.483	<0.0001	--
θ_H***=20***			
Neutral	--	0.005	0.993
Density Dependence	0.001	--	0.001
Dispersal Limited	0.279	<0.0001	--
θ_H***=40***			
Neutral	--	0.022	0.339
Density Dependence	0.005	--	0.187
Dispersal Limited	0.210	<0.0001	--
θ_H***=60***			
Neutral	--	0.025	0.990
Density Dependence	0.002	--	0.193
Dispersal Limited	0.067	0.016	--

Figure 3.1.
Each plot compares the three models of community assembly: UNTB (solid line), density dependence (long-dash line), dispersal limited (dotted line) for $\theta_H=5$. A) log plot of the distribution of species abundances with mean and 95% confidence intervals; B) plot of species-area curve from 0 to 65,536 square units; C) plot of the distribution of genetic diversity as given by θ_W (Watterson 1975): D) log-log plot of genetic diversity (segregating sites) with increasing area sampled from 0 to 65,536 square units.

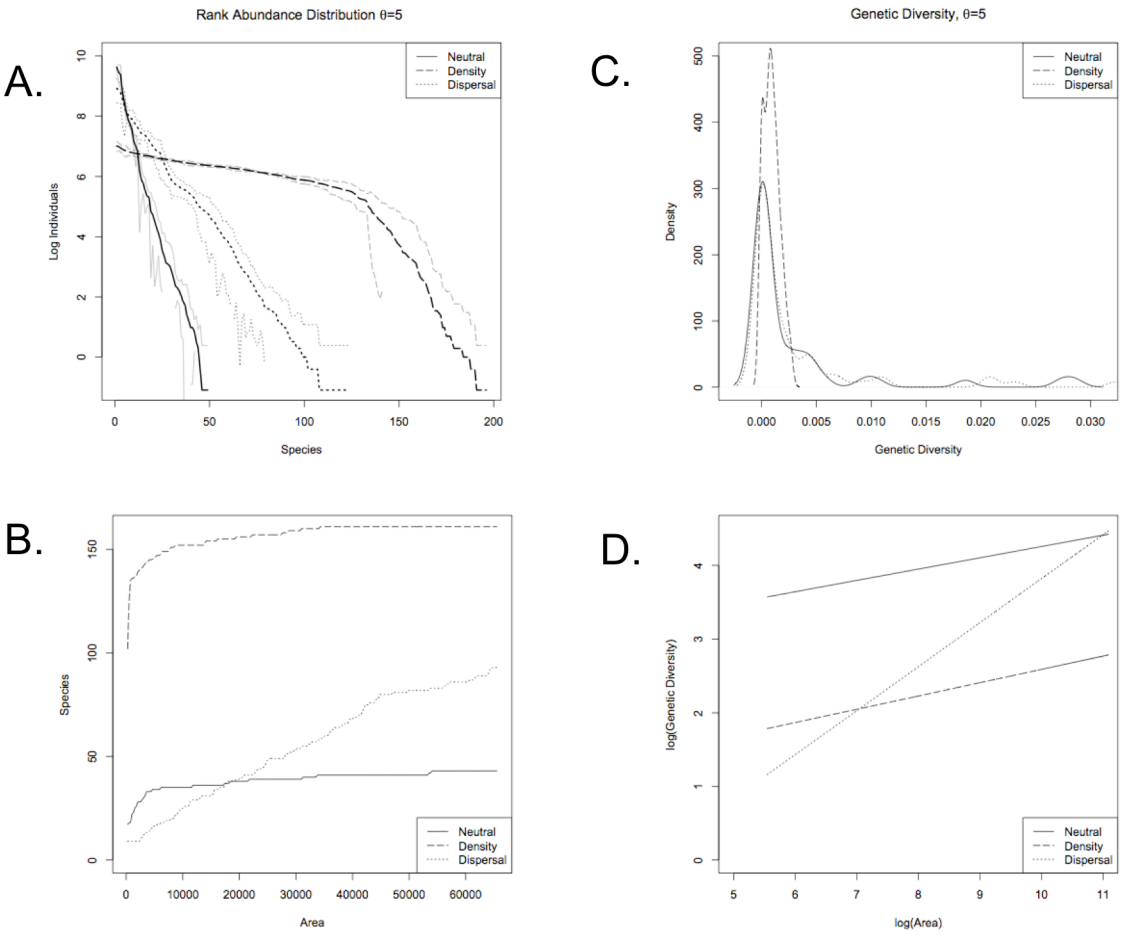
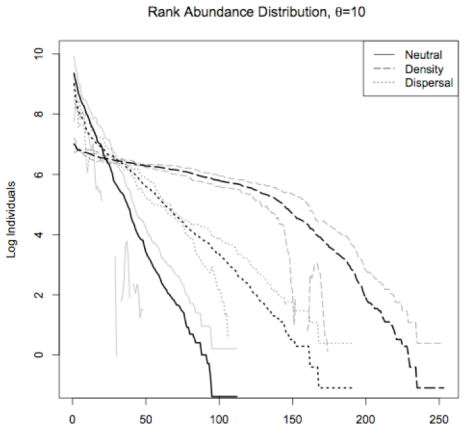
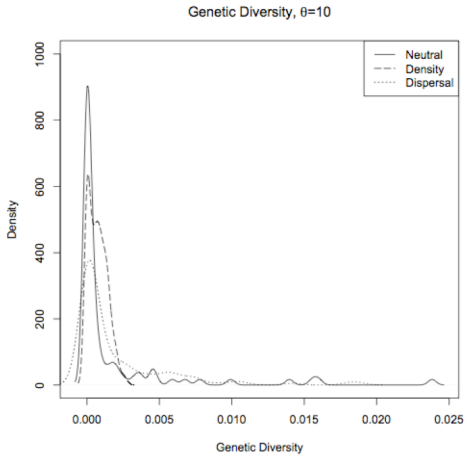

A.
Rank Abundance Distribution θ=5
Log Individuals
Species
Neutral
Density
Dispersal
C.
Genetic Diversity, θ=5
Density
Genetic Diversity
Neutral
Density
Dispersal
B.
Species
Area
Neutral
Density
Dispersal
D.
log(Genetic Diversity)
log(Area)
Neutral
Density
Dispersal

Figure 3.2.
Each plot compares the three models of community assembly: UNTB (solid line), density dependence (long-dash line), dispersal limited (dotted line) for $\theta_H=10$. A) log plot of the distribution of species abundances with mean and 95% confidence intervals; B) plot of species-area curve from 0 to 65,536 square units; C) plot of the distribution of genetic diversity as given by θ_W (Watterson 1975): D) log-log plot of genetic diversity (segregating sites) with increasing area sampled from 0 to 65,536 square units.

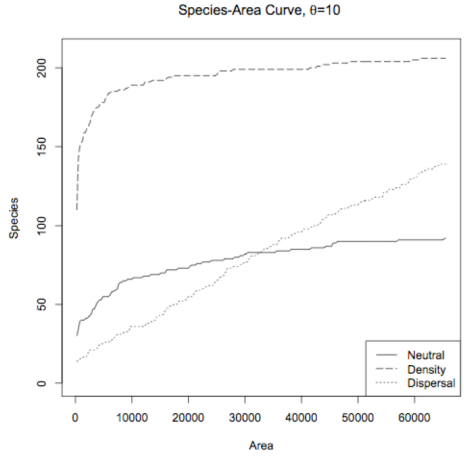
A.


Rank Abundance Distribution, θ=10
Neutral
Density
Dispersal
Log Individuals
0
2
4
6
8
10
0
50
100
150
200
250

C.


Genetic Diversity, θ=10
Neutral
Density
Dispersal
Density
0
200
400
600
800
1000
0.000
0.005
0.010
0.015
0.020
0.025
Genetic Diversity

B.


Species-Area Curve, θ=10
Species
0
50
100
150
200
0
10000
20000
30000
40000
50000
60000
Area
Neutral
Density
Dispersal

D.

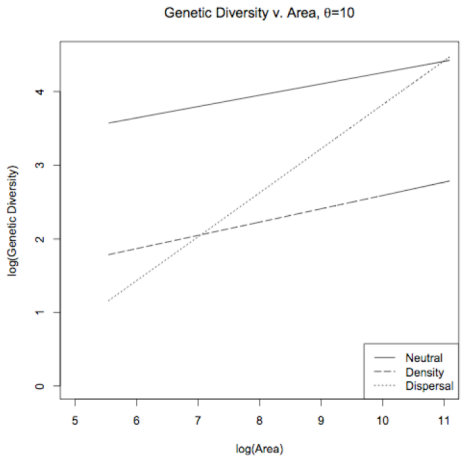
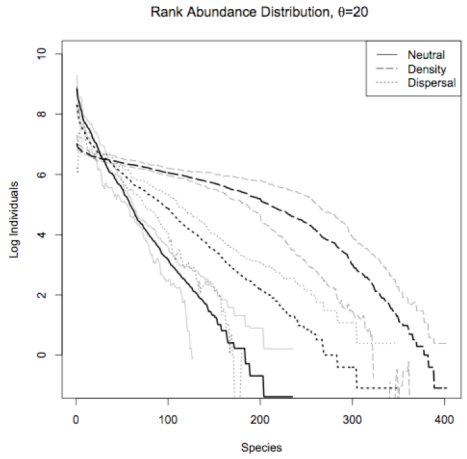
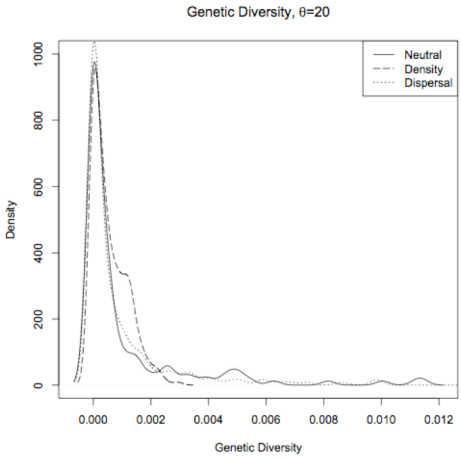

Genetic Diversity v. Area, θ=10
log(Genetic Diversity)
0
1
2
3
4
5
6
7
8
9
10
11
log(Area)
Neutral
Density
Dispersal

Figure 3.3.
Each plot compares the three models of community assembly: UNTB (solid line), density dependence (long-dash line), dispersal limited (dotted line) for $\theta_H=20$. A) log plot of the distribution of species abundances with mean and 95% confidence intervals; B) plot of species-area curve from 0 to 65,536 square units; C) plot of the distribution of genetic diversity as given by θ_W (Watterson 1975): D) log-log plot of genetic diversity (segregating sites) with increasing area sampled from 0 to 65,536 square units.

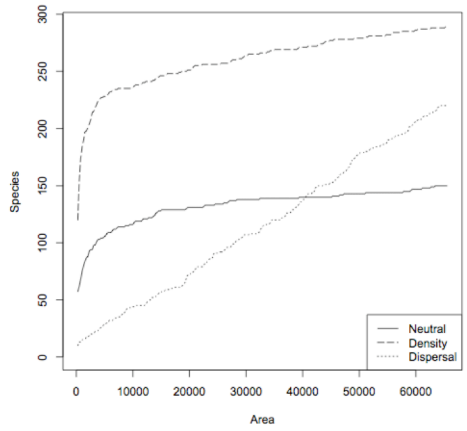
A.


Rank Abundance Distribution, θ=20
Neutral
Density
Dispersal
Log Individuals
Species
0
2
4
6
8
10
0
100
200
300
400

C.


Genetic Diversity, θ=20
Neutral
Density
Dispersal
Density
Genetic Diversity
0
200
400
600
800
1000
0.000
0.002
0.004
0.006
0.008
0.010
0.012

B.


Species-Area Curve, θ=20
Neutral
Density
Dispersal
Species
Area
0
50
100
150
200
250
300
0
10000
20000
30000
40000
50000
60000

D.

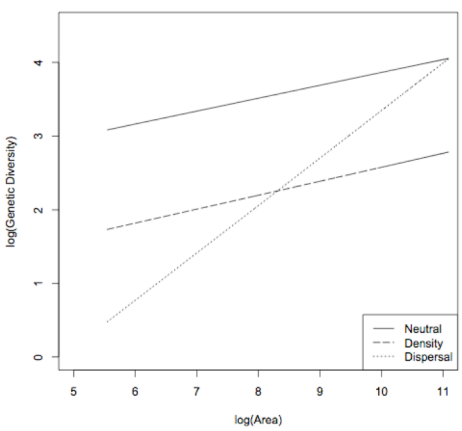
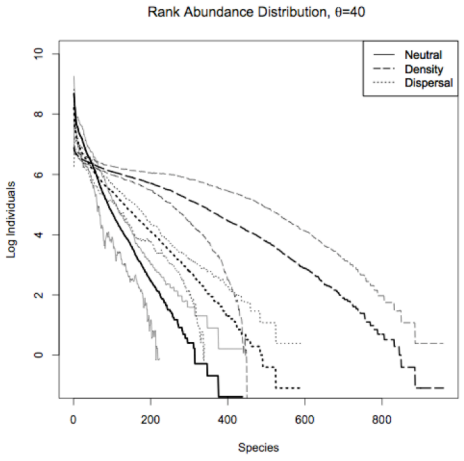
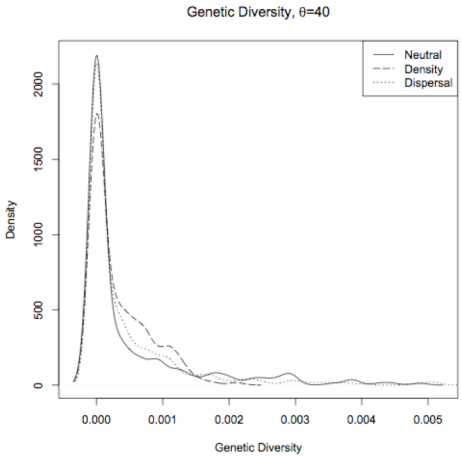

Genetic Diversity v. Area, θ=20
Neutral
Density
Dispersal
log(Genetic Diversity)
log(Area)
0
1
2
3
4
5
6
7
8
9
10
11

Figure 3.4.
Each plot compares the three models of community assembly: UNTB (solid line), density dependence (long-dash line), dispersal limited (dotted line) for $\theta_H=40$. A) log plot of the distribution of species abundances with mean and 95% confidence intervals; B) plot of species-area curve from 0 to 65,536 square units; C) plot of the distribution of genetic diversity as given by θ_W (Watterson 1975): D) log-log plot of genetic diversity (segregating sites) with increasing area sampled from 0 to 65,536 square units.

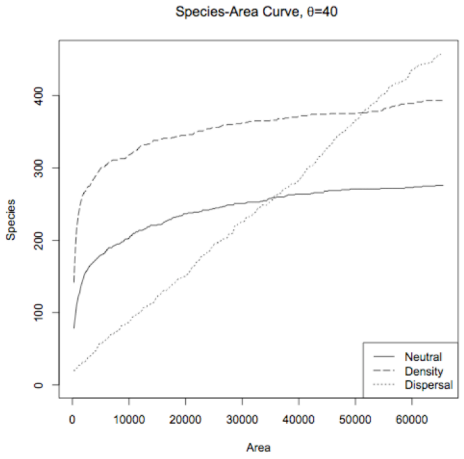
A.


Rank Abundance Distribution, θ=40
Neutral
Density
Dispersal
Log Individuals
Species
0
2
4
6
8
10
0
200
400
600
800

C.


Genetic Diversity, θ=40
Neutral
Density
Dispersal
Density
Genetic Diversity
0
500
1000
1500
2000
0.000
0.001
0.002
0.003
0.004
0.005

B.


Species-Area Curve, θ=40
Neutral
Density
Dispersal
Species
Area
0
100
200
300
400
0
10000
20000
30000
40000
50000
60000

D.

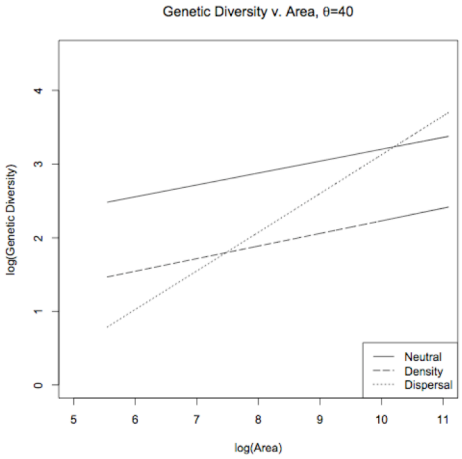
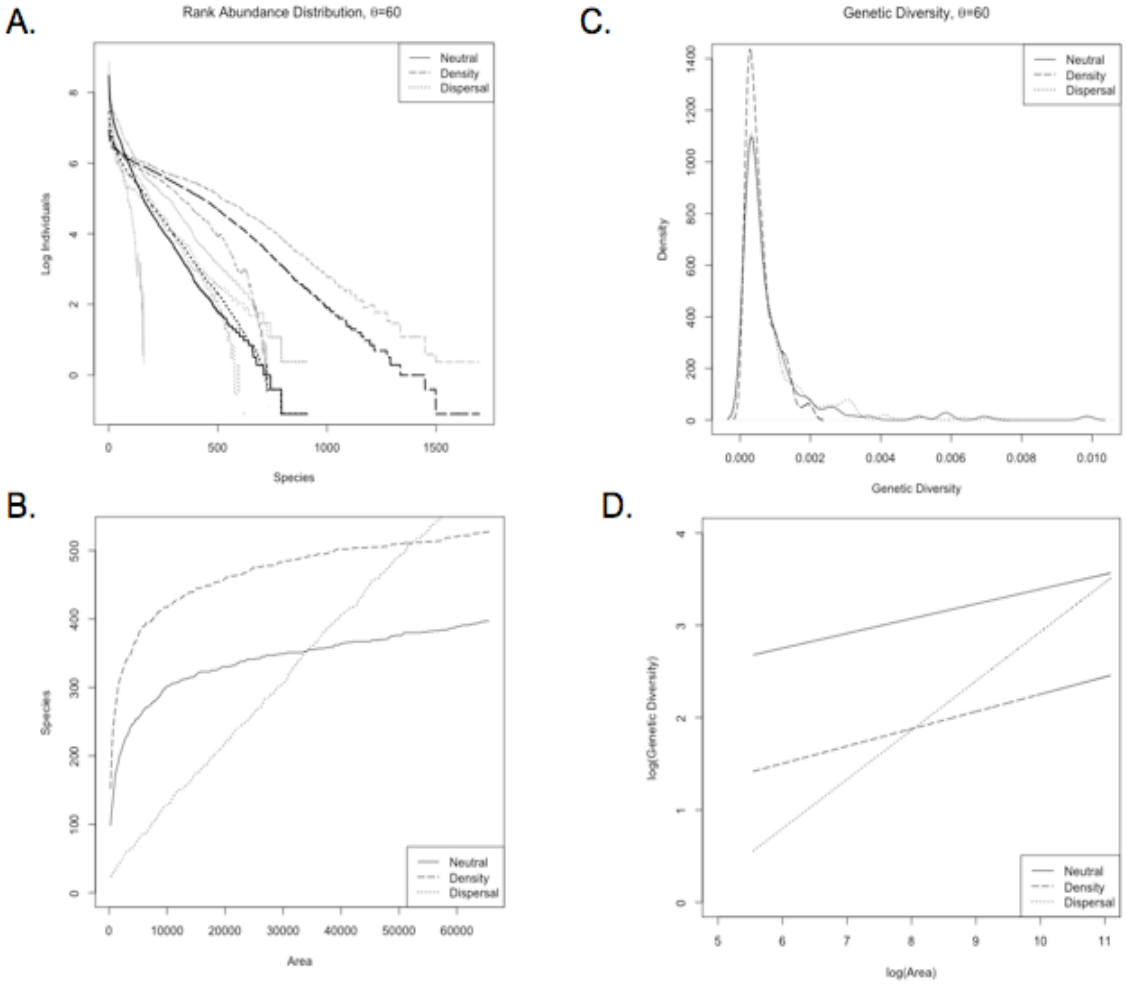

Genetic Diversity v. Area, θ=40
Neutral
Density
Dispersal
log(Genetic Diversity)
log(Area)
0
1
2
3
4
5
6
7
8
9
10
11

Figure 3.5.
Each plot compares the three models of community assembly: UNTB (solid line), density dependence (long-dash line), dispersal limited (dotted line) for $\theta_H=60$. A) log plot of the distribution of species abundances with mean and 95% confidence intervals; B) plot of species-area curve from 0 to 65,536 square units; C) plot of the distribution of genetic diversity as given by θ_W (Watterson 1975): D) log-log plot of genetic diversity (segregating sites) with increasing area sampled from 0 to 65,536 square units.


A.
Rank Abundance Distribution, θ=60
Neutral
Density
Dispersal
Log Individuals
Species
B.
Species
Area
Neutral
Density
Dispersal
C.
Genetic Diversity, θ=60
Neutral
Density
Dispersal
Density
Genetic Diversity
D.
log(Genetic Diversity)
log(Area)
Neutral
Density
Dispersal

CHAPTER 4

POPULATION GENETICS OF MUSSELS (UNIONIDAE) IN THE ALTAMAHA RIVER DRAINAGE, GEORGIA

Introduction

North America's native freshwater mussel species are disappearing. It is estimated that 70% of the nearly 300 species of freshwater mussels native to North America are extinct, endangered, or in decline (Williams, Warren et al. 1993). The causes of decline are not wholly understood but believed to be a combination of habitat loss and pollution; however, there has been little evaluation of changes due to interspecific competition, hybridization, and other historical contingencies. The cryptic nature of these taxa – both that they lie mostly hidden in benthic sediments, and that many species are morphologically difficult to distinguish – is one reason it has been difficult to evaluate the problem of decline across the entire community using traditional methods.

The Altamaha River in southeastern Georgia is home to 16 native species of freshwater mussel with 7 endemic to this basin. While declining numbers, available habitat, and drought potentially threaten all 16 species of mussels, there are three species of particular concern that show a trend of decline in the Altamaha Basin (Wisniewski, Krakow et al. 2005). In 2002, *Elliptio spinosa* was recognized for listing under the Endangered Species Act due to a sharp decline in abundance from previous surveys (Wisniewski, Krakow et al. 2005). Other mussel

species, *Alasmidonta arcula* and *Pyganodon gibbosa,* show a similar trend and are classified as Imperiled or Vulnerable to imperilment (Neves, Bogan et al. 1997; O'Brien 2002).

Management of these species raises important questions: if we do not know what factors are contributing to mussel species decline, then how can we prevent further threats to native species and how do we manage species that are currently imperiled? To identify factors that are causing species decline we need basic natural history data including habitat use and life history parameters (population size, sex ratio, and population structure). Generally, life history parameters are estimated through a series of ecological assays, such as surveys, lab experiments, or mark-recapture studies. However, in extreme cases – where the species has declined to threatened or endangered status – there may be too few individuals to retrieve information through these classical methods.

Conservation genetic studies have typically proceeded in a species-by-species fashion, but it is clear that more direct evaluation of expected diversity and life history patterns in species of concern can be made when a larger community of species is examined simultaneously and placed in a comparative framework. Inference based on the estimated phylogenetic relationships of a group of species can be used to improve our understanding of how rare species interact with their environment, as well as predict life history characteristics of severely threatened populations in a timely manner (Lockwood, Russel et al. 2002; Fisher and Owens 2004; Jones, Hallerman et al. 2006; Whiteley, Spruell et al. 2006). Obtaining comparative data from a large number of species, across as many sites in the Altamaha River basin as possible, allows us to understand long-term gene flow (propagule movement) of mussel species, the relationship between genetic diversity, species density, and reproductive life history among related species (e.g. variance in reproductive success).

In this study, we used DNA sequence and codominant marker data collected for common *Elliptio* species (*E. shepardiana, E. hopetonensis, E. dariensis, E. icterina*) and other relatively common Unionid mussels (*Lampsilis splendida, L. dolabraeformis*, and *Villosa delumbis*) to establish a range of genetic diversity data that can be used to predict life history parameters – such as the ratio of the inbreeding effective population to population census size – for the rare (and thus minimally sampled) species *Elliptio spinosa* and *Alasmidonta arcula* in the Altamaha River basin. Geographic patterns of genetic diversity provide estimates of variance in reproductive success, as well as indirect estimates of dispersal among sites. The goal is to apply these data toward testing general hypotheses of mussel decline, establishing conservation-related baselines for Georgia mussel species, and inferring the taxonomic status and relationships of mussel species endemic to the Altamaha basin.

Specifically, here we focus on the inference that can be made by combining population genetic and phylogenetic approaches to infer the likely range of demographic, ecological, and other natural history traits in *E. spinosa* based on information gleaned from its congeners. This "comparative" approach is becoming a common and important tool in conservation biology and natural history studies (Garland and Ives 2000; Purvis, Gittleman et al. 2000; Belshaw, Grafen et al. 2003). While limited sample sizes continue to constrain our ability to determine some life history parameters, these indirect estimates can improve our knowledge of rare species at a time when it is being strongly considered for federal listing and management; such data may be useful for listing objectives with the other imperiled taxa in the Altamaha as well (Moritz 2002; Wares, Alo et al. 2004; Palsbøll, Berube et al. 2007).

Materials and Methods

Specimen Collection

We collected samples for genetic analysis from the Altamaha River Basin in Georgia, USA over a two-year period (2006-2007). The Altamaha River Basin, located in the southeast region of the United States, is the largest drainage system in Georgia and one of the largest along the east coast, covering nearly 37,000 km^2. The river is formed by the confluence of the Ocmulgee and Oconee Rivers and flows east 215 river-km (rkm) until it enters the Altamaha Sound and the Atlantic Ocean. The Altamaha River averages 50-70 m in width and 2-3 m in depth with some areas in excess of 5 m (Heidt and Gilbert 1978). It has an average gradient of 0.13 m per km (EPD 2003) and average discharge of 381 m^3/s near Doctortown, Georgia (Rogers and Weber 1994). The streambed is comprised predominantly of sand with large woody debris distributed throughout the river via erosion and deposition.

Sampling habitats within the Altamaha River were delineated according to methods specified in Meador (2008). Within each habitat, Nine 10m x 1m transects were randomly placed perpendicular to flow, sampling was then conducted along each transect using tactile searches along the sediment surface. SCUBA equipment was used in areas with a depth greater than 1.5 m, or where conditions were too hazardous to sample using a mask and snorkel. Habitats within the Ocmulgee, Oconee, and Ohoopee rivers were specifically targeted to locate individuals of *Elliptio spinosa* and *Alasmidonta arcula*. We carried out timed searches for 15-30 minutes per person using tactile and visual searches along the sediment surface. Captured mussels were placed in a mesh bag for the duration of the search.

At each sample site we collected mantle tissue from 18-20 individuals per species, when available. Individuals were first identified to species by morphology and then a 1-mm2 piece of

tissue was excised from the mantle (Berg, Haag et al. 1995). The protocol of Berg, Haag et al. (1995) has been shown to limit mortality and stress on collected individuals. One hundred individuals from non-listed species were collected as morphological vouchers and deposited at the University of Georgia Museum of Natural History. All tissue and specimens were preserved in 95% ethanol for later DNA extraction.

Abundance Estimation

We returned to the Altamaha River in the 2008 to obtain abundance data for species of mussels not sampled in Meador, Peterson et al. (unpublished man). We selected three sites previously visited by Meador (2008) for which data were collected and performed mark-recapture sampling using the Robust Design (Pollock 1982). The Robust Design consists of data collected during primary and secondary sampling periods (Pollock 1982). Secondary sampling periods are conducted during primary periods under the assumption that sample habitats were closed to mortality and emigration; however, mortality and emigration are possible between primary periods. Our primary occasions occurred at approximately six-week intervals, spanning 5 months (June-October). Within each primary period, we conducted two secondary sampling periods no more than 24h apart. Within each site, we sampled mussels from five 10m x 1m transects that were randomly placed perpendicular to flow in a delineated 150m^2 of habitat. Captured mussels were identified to species and affixed with a shellfish tag to the ventral valve for later identification.

We analyzed data collected from the Robust Design sampling period using Program MARK (White and Burnham 1999). We used estimates of capture and emigration provided by MARK using the best fit model of Meador, Peterson et al. (unpublished man.) to make

corrections on the data for sites visited in the Altamaha River (Kendall 1999; Meador 2008). The abundance corrections for each site were calculated using the equation

$$\hat{N}^{\circ} = \frac{\hat{N}}{1-\hat{\gamma}}c$$

where $\hat{N}^{\circ}$ is the superpopulation, $\hat{N}$, is the estimated surface abundance from Meador (2008), *c,* is the capture probability, and $\hat{\gamma}$, is the estimated temporary emigration during the last primary period using the best fit model of Meador, Peterson et al. (unpublished man.). We then used the corrected abundance data for all sites to calculate the average density of each species of mussel and performed a crude projection on the total abundance of mussels in the Altamaha River given estimates of available habitat—215 rkm with an average width of 50-70 meters (Heidt and Gilbert 1978).

DNA extraction

We isolated DNA from the collected tissue snips using a modified CTAB (cetyl trimethylammonium bromide) isolation protocol (based on Doyle and Doyle 1987; Campbell, Serb et al. 2005). First we subsampled a smaller piece of tissue ~1mm x .25mm, when applicable, from our collected sample preserved in 95% ethanol, then homogenized the sample using liquid nitrogen and micro-pestles. We next added CTAB to a final volume of 300ul with an addition of 25mg/ml Proteinase K solution (Gentra). Samples were allowed to digest at 55 ° Celsius for 1-2 hours or until no solid tissue remained in the tube. DNA was precipitated using a chloroform wash step followed by the addition of 100% isopropanol. DNA was then eluted in 40ul of H_2O and stored at -80° Celsius until time of use.

Primer Design and Sequence Amplification

For mitochondrial loci (*16S*, *CO1*) we obtained primer sequences from Campbell, Serb et al. (2005) and Folmer, Black et al. (1994), respectively. PCR amplifications were performed in 20µl volumes consisting of 0.5µM each primer, 0.8mM total dNTPs, 3mM $MgCl_2$, and 1U Taq polymerase (Promega). Annealing temperatures for each locus were as follows: *16S*, 50°; COI, 40°. Successful reactions were prepared for sequencing using Exonuclease I and Antarctic Phosphatase (New England Biolabs, Ipswich, MA, USA). Sequencing reactions were carried out in 10µl volumes with 80ng of prepared template, 0.6µM primer, 0.6µl BigDye Terminator (Applied Biosystems, Foster City, CA, USA) and 3.4 µl Better Buffer (The Gel Company). Sequence reactions were cleaned and precipitated with 4 volumes 75% isopropanol, suspended in Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) and run on an ABI 3730 at the University of Georgia.

For each locus, sequence data were edited using CODONCODE ALIGNER v.2.06 (CodonCode Corporation, Dedham, MA, USA). Sequences were aligned using ALIGNER'S built-in 'end-to-end' algorithm, examined and edited for likely artifacts caused by poly-N repeats and other apparent insertions, and disassembled/realigned. PHRED (Ewing, Hillier et al. 1998) quality scores < 30 were investigated visually, and recoded as ambiguities (*N*) if not readily classified.

Microsatellite Design and Amplification

Microsatellite markers, designed by Ward, Shaw et al. (in press) and amplified using the protocols found therein, were shown by to have reliable cross-genus amplification, in *Elliptio hopetonensis, E. dariensis, E. icterina, E. shepardiana, E. complanata* and *E. spinosa* (Ward, Shaw et al. *in press*). Data from cross-genus screening tests (using samples n>6 for each species) were evaluated using MICROCHECKER (Oosterhout, Hutchinson, et al. 2004) for heterozygosity, null alleles, and deviation from Hardy-Weinberg equilibrium. We found that 8

loci (*ECO1*,*ECO2*, *ECO8*, *ECO14*, *ECO16*, *ECO21*, *ECO23*, *ECO29*) amplified reliably (>50%) with a group of 4 loci (*ECO1*,*ECO2*,*ECO23*,and *ECO29*) being in Hardy-Weinberg equilibrium. Only microsatellites in Hardy-Weinberg equilibrium were analyzed for the above species (Table 4.1).

Sequence Analysis

We used the program MRBAYES (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) to construct a multi-locus phylogeny for mussels collected within the Altamaha River Basin. The concatenated data set, including all sequenced loci, was generated with the fused matrix export option in MESQUITE (Maddison and Maddison 2009) and used only individuals for which at least 2 loci were fully sequenced. We assessed the best-fit model of molecular evolution using JMODELTEST (Posada 2008); AIC (Akaike 1973) was used to choose the simplest model that provided the best fit to the data. MRBAYES was run using the optimized model parameters output from JMODELTEST (Posada 2008) where each locus in the concatenated data set was unlinked. Bayesian analysis was run with 4 heated MCMC chains and 2 independent runs for each analysis involving a minimum of 2×10^6 MCMC generations with sample frequency f=1000; if the standard error between independent runs was not less than 0.05 at this point, additional sets of 1×10^6 generations were run until this standard was reached. The first 25% of generations were discarded as burn-in, an approach verified through graphical analysis of the stabilization of likelihood values.

We used COMPUTE (Thornton 2003) to calculate diversity statistics for sequenced loci such as the standardized number of segregating sites, θ (Watterson 1975), and nucleotide diversity (π), the average number of pair-wise differences between sequences in a sample (Nei and Li 1979). We also used COMPUTE to calculate the value and significance of Tajima's *D*

statistic (Tajima 1989), a test to determine if sequence variation is consistent with the neutral theory of molecular evolution (Kimura 1968). Neutrality tests like Tajima's *D* are commonly used to detect the influence of selection on a gene, but can also be potentially informative about the demographic forces that have affected a population in the past (Tajima 1989). The significance of Tajima's *D* was assessed by 10,000 coalescent simulations in COMPUTE (α=0.05).

To test for genetic differentiation between populations, here defined by the site-specific sampling locality, we used DNASP (Rozas, Sanchez-DelBarrio et al. 2003) to calculate pairwise population divergence (F_{ST}) (Wright 1951) for each species x site combination and *SNN*, the nearest-neighbor statistic (Hudson 2000). *SNN* is a measure of how often the 'nearest neighbors' of a sequence are from the same locality in geographical space and is particularly suitable when haplotype diversity is large and sample sizes are small (Hudson 2000). A Mann-Whitney U test was used to compare the distributions of F_{ST} among each species. To quantify the partitioning of genetic variation within the Altamaha River Basin we performed an analysis of molecular variance (AMOVA; Excoffier, Smouse et al. 1992) in ARLEQUIN (Schneider, Roessli et al. 2000). For AMOVA, Φ-statistics were used to estimate the relative contribution of molecular variance at three levels: (I) among the 3 primary tributaries (Ocmulgee River, Ohoopee River, Oconee River) and the Altamaha River (Φ_{CT}); (II) among populations within the Rivers (Φ_{SC}), and; (III) within populations (Φ_{ST}). Significance of AMOVA values was assessed by 1,000 permutations under the Jukes-Cantor model of molecular evolution (Jukes and Cantor 1969). Lastly, we performed Mantel tests (Mantel 1967) to test for correlations between geographic distance and genetic distance using the program IBDWS (Jensen, Bohonak et al. 2005) with significance determined by 1000 permutations.

We calculated effective population size (*Ne*) to the corrected abundance (*N*) ratios to determine reproductive variance in mussels of the Altamaha River Basin. Recent work has led to the development of demographic models for predicting *Ne* without explicit genetic data (Nunny and Elam 1994), allowing us to test the most likely demographic models that would produce a similar value of *Ne* for freshwater mussel species. *Ne* values were calculated using an estimate of sequence diversity, θ, and the equation $N_e=\theta/x\mu$ (Kimura 1968), where *x* is twice the copy number of the locus. Mutation rates were assumed to be similar as those estimated for the bivalve species *Mytilus* (Rawson and Hilbish 1995; Stillman and Reeb 2001) for mitochondrial DNA. For *N* we used estimates from the projected abundance of mussels in the Altamaha (see above).

We performed Ancestral State Reconstruction using the program MESQUITE (Maddison and Maddison 2009). Ancestral State Reconstruction (ASR) is the inference of a character state or trait by use of a phylogenetic tree relating taxonomic units. Cunningham (1999) showed that the corroboration of more than one phylogenetic reconstruction method avoids the shortcomings of each alone, so we used both parsimony and likelihood methods to reconstruct ancestral states. Parsimony reconstruction methods find the ancestral states that minimize the number of steps of character change given the tree and observed character distribution, while likelihood reconstruction methods find the ancestral states that maximize the probability the observed states would evolve under a stochastic model of evolution (Schluter, Price et al.1997; Pagel, 1999). The likelihood reconstruction finds, for each node, the state assignment that maximizes the probability of arriving at the observed states in the terminal taxa, given the model of evolution, and allowing the states at all other nodes to vary (Maddison and Maddison 2009). We used ASR to infer the value of the effective population size to abundance ratio in the endangered species *E.*

spinosa. The MESQUITE analysis used both the Bayesian mitochondrial tree of mussel species from the Altamaha River (see above) and a character matrix of the ratio of genetic effective population size (*Ne*) and total corrected abundance (*N*).

Microsatellite Analysis

We scored size fragments from microsatellite loci using GENEMARKER V1.6 (SoftGenetics). Size fragments were binned using the program MSATALLELE V1.0 (Alberto 2009); suspect alleles were checked visually in GENEMARKER, with 10% of individuals redundantly amplified to check consistency of scoring. Microsatellites were then tested for fit to Hardy-Weinberg equilibrium, a test of non-random association of alleles within diploid individuals (Hardy 1908; Weinberg 1908), and fit of the stepwise mutation model (SMM) using the program MICROCHECKER (Oosterhout, Hutchinson et al. 2004).

We analyzed microsatellite data using the programs FSTAT (Goudet 2002) and ARLEQUIN (Schneider, Roessli et al 2000). We calculated Nei's diversity statistics (Nei 1987) and Wright's (1951) F_{IS} values in FSTAT (Goudet 2002). Nei's diversity statistics provide information on the distribution of genetic variation both among and within populations. Wright's F_{IS} value is a measure of the deviation from Hardy-Weinberg equilibrium within subpopulations where positive values indicate a deficiency of heterozygotes and negative values indicate excess (Wright 1951). We used ARLEQUIN (Schneider, Roessli et al 2000) to calculate pair-wise R_{ST} values (Slatkin 1995), Hardy-Weinberg equilibrium, and the G-W statistic (Garza and Williamson 2001). Slatkin (1995) designed R_{ST}, analogous to Wright's F_{ST} (Wright 1951), to take into account the Stepwise mutation model of microsatellite loci. The G-W statistic compares the ratio of the number of alleles to range in allele size, where values deviating from 1 indicate past bottlenecks (Garza and Williamson 2001).

Results

Specimen Collection

Over the course of two years (2006-2007) we collected over 2000 tissue samples from 13 species of Unionids in the Altamaha River Basin. We sampled 51 sites in the Altamaha River, 9 sites in the Ohoopee River, 7 sites in the Oconee River, and 19 sites in the Ocmulgee River; sites were counted as long as they contained 1 individual of any mussel species (Figure 4.1). The numbers of tissue samples collected were in proportion to the number of species sampled, except in the case of *E. spinosa* where only 6 individuals were sampled in total (Table 4.1). For *E. spinosa* we bolstered our sample size using DNA from 8 additional individuals collected by P. Johnson (Research Institute Tennessee Aquarium) from Coon Island located in the Altamaha River in 2005.

Abundance Corrections

We calculated abundance estimations using a mark-recapture study designed to estimate the capture probability and temporary emigration of species that were not evaluated in Meador, Peterson et al. (unpublished man). Our results for *L. dolabraeformis* and *P. gibbosa* were similar to those found by Meador, Peterson et al. (unpublished man); *A. arcula* and *L. splendida* were not accurately estimated from our study as small sample size created too much variance. Therefore for *A. arcula* and *L. splendida* we used estimates of capture probability and temporary emigration from Meador (unpublished man). Data for the remaining species, mainly *Elliptio*, were estimated with relatively small [95%] credible intervals.

Applying the corrections for both capture probability and temporary emigration allowed us to estimate the size of the superpopulation from the raw count data collected by Meador

(2008) for each species. We found that *E. hopetonensis* had the highest average predicted abundance (N=43,926) followed by *E. dariensis* (N=10,252), E. shepardiana (N=8,342), *L. dolabraeformis* (N=5,928), *E. icterina* (N=5,570), *L. splendida* (3,596), and *A. arcula* (N=319). We also estimated abundance corrections for P. gibbosa (N=454), *U. imbecillis* (N=2,270), and *V. delumbis* (N=1,072), but due to small sample size in our mark-recapture study the variance around our estimates of the capture probability and temporary emigration of these species was high (Table 4.2).

The above estimates were only useful for estimating the abundance at focal sites sampled by Meador (2008), so a coarse projection of the total number of individuals per species in the Altamaha River was calculated based on the maximum amount of available habitat. For the coarse projection we used our abundance corrections to calculate the density of each species (inds/m^2), we then projected to the total amount of available habitat in the Altamaha River assuming a river length of 215km and average width of 50m. Density was calculated by correcting the raw count data at every site mussels were found at in Meador (2008). We then used the total area sampled by Meador (2008), including all sites that were visited, along with the mean abundance corrections to estimate a density of individuals for each species. These proportional results suggested maximal population sizes for *E. hopetonensis* of N=7.7 x 10^6 followed by *E. dariensis* (N=1.8 x 10^6), *E. shepardiana* (N=1.5 x 10^6), *L. dolabraeformis* (N=1.0 x 10^6), *E. icterina* (N=9.8 x 10^5), *L. splendida* (6.3 x 10^5), and *A. arcula* (N=5.6 x 10^4) (Table 4.2).

Sequence Analysis

We used a Bayesian phylogenetic method to reconstruct the relationship between mussel species within the Altamaha River Basin. Phylogenies using mtDNA (*16S* and *CO1*) show

strong posterior densities (>60%) in support of all genera proposed on morphological classification and former studies (Campbell, Serb et al. 2005) (Figure 4.2). Species classifications based on morphology were also strongly supported except in the genus *Elliptio* where the species of *E. hopetonensis*, *E. dariensis*, and *E. icterina* remain polyphyletic. However, within the genus *Elliptio* we did find strong posterior densities supporting the species status of *E. shepardiana and E. spinosa* (Figure 4.2). Further analysis shows that with the exclusion of *E. icterina,* relationships among *E. hopetonensis* and *E. dariensis* were resolved (Figure 4.3).

We used estimates of θ (Watterson 1975) to determine the amount of sequence diversity at each locus for each species. For gene diversity as quantified by θ, *E. hopetonensis* showed the highest value for *16S* (θ=0.0103) where *E. angustata* had the highest value of *CO1* (θ=0.0212). The trend for all species was for *CO1* to harbor higher diversity than *16S*.

In our analysis mtDNA was typically negative for Tajima's D, however only 5 species were significantly negative (*A. arcula, E. dariensis, E. icterina, E. shepardiana, L. dolabraeformis)*. Of the species that showed significantly negative Tajima's D values for mtDNA, only *E. dariensis* was not significant at both loci (*16S; D*= -1.98; *CO1;D*=-1.0) (Figure 4.4).

To understand how migration and dispersal affect mussel species in the Altamaha River Basin, we utilized various techniques to measure genetic differentiation between and within populations of mussels. We calculated genetic differentiation among sites using S_{NN}, rivers (AMOVA), and species (F_{ST}) to test for patterns of restricted gene flow. For both the S_{NN} (Hudson 2000) and F_{ST} statistics (Wright 1951) we found the greatest amount of genetic

structure in the mitochondrial loci (*CO1>16S*). The distributions of F_{ST} for each locus show a similar pattern to the SNN statistic.

Comparisons of pairwise F_{ST} among sites revealed common patterns among species. All *Elliptio* species share a high F_{ST} value ~82-86km from the confluence which is also an area of high genetic diversity for *E. hopetonensis, E. dariensis*, and *E. icterina*. Other common patterns are seen ~71km from the confluence in *A. arcula* and *E. dariensis* as well as ~96km from the confluence between *A. arcula, E. dariensis, E. hopetonensis*, and *E. shepardiana*. A final correlation of high diversity and high F_{ST} is found in *E. shepardiana* and *E. icterina* ~119km from the confluence. A Mann-Whitney U test comparing the distributions of F_{ST} for each species was significantly different between *A. arcula* versus all *Elliptio* species, and *E. dariensis* versus all other *Elliptio* species (Bonferroni corrected α =0.0102). A Mantel test of isolation by distance was non-significant on all loci and species tested.

We used AMOVA (Excoiffer, Smouse et al. 1992) to test the hierarchical partitioning of molecular variance among regions, among populations within regions, and within populations in the Altamaha River Basin. We divided mussel populations into 4 regions: Ocmulgee River, Oconee River, Ohoopee River, and Altamaha River based on the collection site and tested the significance both among and within regions. Among region structure was only found in one species, *A. arcula* (*CO1*) (Table 4.3). Structure 'among population within regions' was significant at locus *16S* for all species except *A. arcula* (which harbors no detectable variation), while locus *CO1* only showed significant structure in species *A. arcula* and *E. icterina* (Table 4.3).

Genetic effective population size (*Ne*) is an important parameter for inferring the levels of neutral genetic variation and the ability of a population to respond to natural selection.

Populations with low *Ne* may suffer from inbreeding depression or exhibit a reduced response to natural selection (Frankham 1995). We estimated *Ne* for 13 species in the Altamaha River Basin from mitochondrial (Ne_f, Figure 4.5) loci. Effective size calculated from mitochondrial data, Ne_f, based on maternally inherited DNA is a good estimate of the effective size of females in each species (Figure 4.5).

We calculated the ratio of the effective population size (*Ne*) to abundance (*N*) derived from estimates of *Ne* and *N* from an average of all populations in the Altamaha River. The ratio of N_e/N for all species was between 0.03-0.15, with species within the genus *Elliptio* exhibiting lower N_e/N ratios than species from other genera (Table 4.4). Estimates from *A. arcula* seem to be extremely high, either owing to the poor sampling of *A. arcula* during abundance sampling or recent and rapid demographic change in abundance. Estimates for *U.imbecillis*, *P. gibbosa*, and *V. delumbis* suffer from wide credible intervals due to small sample sizes in the mark-recapture study, and are not reliable. We plotted the relationship of N_e/N ratio vs. abundance (*N*) for mtDNA (Figure 4.6) to demonstrate the interdependent relationship between projected abundance and the ratio of N_e/N.

Microsatellite Analysis

We used estimates of θ_H (Watterson 1975), as calculated from homozygosity, and the number of alleles per locus to denote the diversity at each of 4 microsatellite loci in six species of *Elliptio*. Elliptio species had the highest diversity at *ECO1* and *ECO23* with lower diversity at locus *ECO2* and *ECO29*. The trend was slightly different for allelic diversity with *ECO1* and *ECO29* having the most alleles (alleles=19.5, alleles=18.3) and *ECO2* and *ECO23* having fewer alleles (alleles=11.6, alleles=15.3). For 6 species within the Altamaha River Basin we then estimated θ_H for among sites. In the microsatellite loci there was a trend of higher diversity ~ 32-

35km from the confluence and ~80km from the confluence. These trends are general as for each species/locus combination there was some variation as to where the highest diversity was located.

We calculated multilocus pair-wise R_{ST} (Slatkin 1995) values for each site to examine concordant patterns of genetic structure along the length of the Altamaha River in 6 species from the genus *Elliptio*. *E. dariensis* and *E. icterina* shared a high R_{ST} value ~36km (site 66) from the confluence while *E. icterina* and *E. hopetonensis* shared a high R_{ST} value ~88km (site 33) from the confluence. *E. shepardiana* had higher values of R_{ST} at both ~96km (site 4) and ~115km (site 38) from the confluence. A Mann-Whitney U test comparing the distributions of R_{ST} for each species was significantly different between *E. shepardiana* versus all other *Elliptio* species (Bonferroni corrected α =0.0127).

Discussion

The data collected for Altamaha mussel species allow us to make inference into long-term management strategies of not just the listed species but non-listed species as well. Non-listed species are not as critical for current management, but the data will provide baseline information on size of reproductive and standing population sizes, local diversity, gene flow, and help to establish the range of natural variation for these traits across the whole community. Based on the baseline data established in our study for both common and listed species, it is now possible to determine a change in species status that warrants reclassification (Hoffmann and Dabborn 2007). It is our goal that data collected for the mussel community will serve to inform future studies on life history and species interactions in the Altamaha watershed, including the

identification of fish hosts for particular species through genetic identification of glochidia (Gerke and Tiedemann 2001).

Classification of freshwater mussels species is hampered by phenotypic plasticity in intra-specific shell morphology and wide spread polytomies (Davis 1983; Campbell, Serb et al. 2005). Campbell, Serb et al (2005) used 3 loci from the mitochondria, where previous studies have used mitochondrial loci and ITS sequence from the nuclear genome (Serb, Buhay et al. 2003). The mitochondrial dataset showed high levels of polyphyly in the genus *Elliptio* between the species of *E. complanata, E. icterina, E. dariensis*, and *E. hopetonensis*. The removal of *E. icterina* and *E. complanata* resolved the polytomies and created two monophyletic groupings of *E. dariensis* and *E. hopetonensis* (Figure 4.3). The remaining *Elliptio* species, *E. spinosa* and *E. shepardiana*, were both strongly supported as monophyletic species independent of *E. icterina*. We did find evidence for the misidentification of *E. angustata* as our comparison of *E. shepardiana* with 6 type specimens from museum collections (A. Bogan, North Carolina Musuem of Natural Sciences) split our *E. shepardiana* specimens into two intermediately (~50%) supported clades.

We utilized the evolutionary relationship among mussel species to infer the value of N_e/N in *E. spinosa* for which there was not enough data available to calculate by tradition methods. Both likelihood and parsimony-based methods of reconstruction provided estimates of N_e/N that were less than 0.10, the trend for the genus *Elliptio* (Table 4.4). This value is typical for natural populations (Frankham 1995) and suggests that over the long-term history of these species, similar characteristics pertaining to gender ratios, variance in reproductive success, and overall demographic history define life history for the genus *Elliptio*. It is possible that *E. spinosa* has a dramatically different N_e/N than what was estimated from ancestral reconstruction as states can

only be reconstructed for values extant within contemporary lineages. Oakley and Cunningham (2000) showed that it is difficult to assume the evolutionary trajectory of a character without some knowledge of the range the character could assume. The inclusion of ancestors with known state values or a root species with known trait values could reduce variance making the inference of states more accurate (Oakley and Cunningham 2000). When more data become available on unknown life history parameters, e.g. fish hosts and specific habitat preference, it will be possible for us to more accurately reconstruct N_e/N values in *E. spinosa*, determining the critical differences that make *E. spinosa* vulnerable to extinction while other *Elliptio* species seem unaffected (Wisniewski, Krakow et al. 2005).

Genetic diversity among studied species exhibit substantial levels of nucleotide variation, given the long lifespan, overlapping generations, and high juvenile mortality associated with fish host transformation (Haag and Warren 2003; Berg, Christian et al. 2007). This is also surprising noting that recent surveys of species abundance turned up few individuals for some species (Wisniewski, Krakow et al. 2005). Nei (1975) showed that extended periods of low abundance increases the rate of genetic drift in populations reducing genetic diversity; in contrast Lande (1993) notes that in cases of species with long generation times, overlapping generations (Waples 1998), or rapid and recent decline, patterns of genetic diversity may not be affected. This may very well be the case for *E. spinosa* in the Altamaha River, where censuses have turned up few individuals while genetic diversity estimates remain substantial, although lower than all other species of *Elliptio* within the Altamaha River.

The power of multi-species, multi-locus datasets is the ability to compare data for all species in a community, providing corroboration as to whether community-wide events or species-specific factors have affected populations. We calculated Tajima's D value for all

species from the Altamaha River Basin and found patterns of demographic expansion, as given by negative Tajima's D values at more than 1 locus, in multiple species (Figure 4.4). The concordance of multiple genes within a genome having similar values of Tajima's D provides support for a demographic event rather than locus specific selection. *A. arcula, E. dariensis, E. icterina, E. shepardiana,* and *L. dolabraeformis* had significantly negative Tajima's D values at more than 1 locus, suggesting that these species are expanding from a historic demographic bottleneck. The lack of significant values at other species does not rule out a single demographic event in the Altamaha River, as there was a negative, though nonsignificant, trend for other species. Various factors like generation time, length of the bottleneck, and severity of the bottleneck could have reduced the signal of a past event on the species genome. It is also plausible that other species *E. hopetonensis*, *L. splendida, U. imbecillis*, *P. gibbosa*, and *V. delumbis* either did not experience a bottleneck or have had supplemental genetic variation reintroduced into their geographic range from an outside source.

We obtained estimates of genetic structure from F_{ST}, which is analogous to migration under a stepping stone model of migration, for a subset of mussel species in the Altamaha River Basin (Wright 1951; Whitlock and Barton 1999). Estimates of F_{ST} indicate moderate population structure across sites in the Altamaha River Basin; no specific strong barriers to gene flow are recovered from our analysis. Multi-locus F_{ST} values allowed us to compare the genetic structure among species, where we would assume that species with similar host fish (i.e. similar dispersal) would show similar patterns of genetic structure. We compared the distribution of F_{ST} values across congeners of the genus *Elliptio,* with the expectation that the distribution of F_{ST} should be similar for species using similar hosts. We found that *E. dariensis* was significantly different (Bonferroni corrected α=0.0102) from all other *Elliptio* species. For comparison we also tested

the similarity between *A. arcula* and *Elliptio* species and found that all comparisons were also significantly different. This does not mean that *A. arcula* and *E. dariensis* are using a similar fish host as there may be several guilds of appropriate host species in this basin. However, it does suggest that not all species interact with the same set of hosts, or that some other element of reproductive life history influences the pattern of gene flow in species like *E. dariensis* (Hamrick and Godt 1996).

The location of sites with high F_{ST} share few commonalities except that they are all downstream of the Ohoopee River confluence and with the exception of site 1, are all upstream of Rayonier Paper Products. Biological causes may stem from variant behavior of fish hosts either congregating or absent at these sites. Environmental causes may stem from increased siltation and nutrient loading due to the presence of agriculture or pollution. An examination of site 1 in particular would be interesting, as Shoults-Wilson, Peterson et al. (in press) have shown Rayonier to be an emitter of industrial pollutants with possible consequences for the health of freshwater mussel species.

As a final test of the distribution of genetic variation, we hierarchically partitioned genetic diversity into 4 regions: Altamaha, Ocmulgee, Ohoopee, and Oconee Rivers. Regional structure was only statistically significant in one species, *A. arcula*, suggesting either a reduced rate of migration between the rivers or a difference in selection between rivers exists for this species. Significant values were mainly found among populations but within regions (Φ_{SC}), where the locus *16S* showed the same pattern of significance for all species. Puzzling is that *CO1* only showed significant Φ_{SC} value for *A. arcula* and *E. icterina.* Due to the high mutation rate of *CO1* it reflects a more contemporary pattern of gene flow in comparison to *16S*, whereas

a barrier may have existed in the past impeding gene flow between rivers where today it may no longer exist.

The observed differences in N_e across species are a reflection of different life history characteristics that cause reproductive success to vary. To quantify the differences in N_e across species it is important to compare it with the adult abundance estimate (N). N_e/N in an idealized population, i.e. random mating, a 1:1 sex ratio, nonoverlapping generations, Poisson variance in reproductive success, and temporally stable population number, should have N_e/N values that approach 1. Deviations from idealized assumptions can skew N_e/N ratios in either direction. In Altamaha mussel species we found N_e/N to be 0.03-0.15 for species of *Elliptio*, 0.09 to 0.10 for species of *Lampsilis*, and >1 in *A. arcula*. Frankham (1995) analyzed N_e/N ratios for wildlife species and found a mean of 0.11, whereas Nunney and Elam (1994) testing a variety of demographic models found a mean of <0.25. Both *Elliptio* and *Lampsilis* species fall into these supported ranges, however the deviation of *A. arcula* cannot be explained by any demographic model and is probably due to error in estimation of adult abundance or a recent and rapid reduction in abundance that has not affected genetic diversity. If *A. arcula* prefers a rare habitat or one that was not readily sampled it would not have been encountered as often leading to an error in estimation of adult abundance. Alternatively, a recent and rapid reduction in the abundance of *A. arcula* individuals irrespective of genotype could skew the N_e/N value to be greater than 1. The similar values of N_e/N within each genus seem to suggest similar life history characteristics for each species, however alternative explanations like inter-site environmental variability have yet to be fully explored.

Following the correction on the raw count data we made a coarse projection based on density of mussels captured during occupancy sampling and the proportion of available mussel

habitat in the Altamaha River to get a better estimate of the total abundance of mussels in the Altamaha River. We assumed that all habitats in the Altamaha River have a uniform distribution of mussels, and estimated the available habitat as 215rkm x 50m (length x average width) (Heidt and Gilbert 1978). Our projection is gross at best as we estimate total abundance assuming a uniform distribution of mussels in slack-water habitat. and do not take into account habitat heterogeneity between pool, swift-water, and slack-water sites that are shown to affect capture probability and emigration in Meador (2008). It is important to note that this gross projection does not take into account habitat-specific differences in abundance which were found to be substantial in Meador (2008). Since habitats are not in equal proportion the extrapolation of the slack-water habitats were used as they account for a large proportion of sites visited usedThey are very large and the habitats are not in equal proportion.Based on our personal observations we know both of these assumptions to be untrue, however the trend of our assumption will skew the estimate of mussels to be greater than the actual number. Typically mussels are not uniformly distributed within a habitat, preferring a clumped or over-dispersed distribution (Downing 1993). It is also not typical for mussels to inhabit all areas of the river with some species preferring slow moving or fast moving currents. A better projection should use estimates from other rivers that are characteristically similar to the Altamaha until data on density and habitat preference are collected for the Altamaha River. As more data become available, our coarse projection can easily be corrected to reflect the new information on habitat preference and habitat use by freshwater mussel species.

Our microsatellite analysis had some contradictory results when compared with our sequence data. Microsatellite markers have been proven to be the informative for studies on gene flow, in that reduced gene flow and subtle population structure have been demonstrated with microsatellites when other genetic markers filed to detect genetic heterogeneity among

samples (Bentzen, Taggart et al. 1996). The primary reason for the use of microsatellites in detecting population structure is their generally high allelic diversity, which adds statistical power to tests of allele-distribution homogeneity (Estoup, Rousett et al. 1998; Ross, Shoemaker et al. 1999). Our microsatellite data showed a lower amount of genetic structure when compared to the mitochondrial sequence data. There were significant R_{ST} values across sites for microsatellite loci (see results) but these did not correspond to areas of high values of F_{ST} found in sequence data. Contradictions in our microsatellite data may represent homoplasy at these markers leading to a lack of differentiation when compared with sequence data. Differences between the mitochondrial versus nuclear genome can indicate dispersal differences among gamete stages due to the male gamete being haploid and transmitting only half the DNA. This model fits well with freshwater mussels because males broadcast sperm into the water column, but females brood young, which are later dispersed by fish. In *E. complanata,* successful fertilization was strongly correlated with the density of mussels within a 0.5 m radius (Downing 1993), which suggests that male gametes disperse only over very short distances. Similarly, male gametes of other mussel species may not travel as far as the fully formed offspring via the fish host. A second explanation for the discrepancy in genetic structure is scoring error based on the presence of null alleles, large allele drop, or stutter. Though we did not find any sign of large allele drop when we checked our data in MICROCHECKER, we did find some evidence for the presence of null alleles. Individuals with null alleles were rescored, repeated, or excluded from future analysis. We did find some loci with stutter and attempted to mediate scoring errors by using MSATALLELE (Alberto 2009), where data is binned according to the distribution of size fragments. Future analyses will test the use of larger bin categories to reduce scoring errors due to the presence of stutter (Turner, Wares et al. 2002; Gold and Turner 2002).

Extensions

When we first began this project our intention was to integrate ecology with a large-scale population genetic study. Though we have touched on many aspects of comparative methods for inferring factors that shape diversity, we have as yet not connected them back to the physiology of the Altamaha River. Landscape genetics is a discipline defined by the integration of genetic information, e.g. diversity and gene flow, with physiological aspects of the environment to explain patterns in genetic data that may be due to demography or natural selection. At each site we collected genetic samples and environmental data that is considered important for classifying aquatic invertebrate habitat (Ward and Tockner 2001; Thompson and Townsend 2006) such as: salinity, temperature, dissolved oxygen, conductivity, turbidity, nitrate, and flow rate. Future analyses will use canonical correspondence analysis (CAA) to quantify the amount of variation in genetic data explained by environmental variables, allowing the inference of management decision with regard to environmental variation.

Future Analyses

We have collected a considerable data set describing genetic diversity and genetic structure in 13 species within the Altamaha River Basin. During our initial analyses we encountered some incongruencies (microsatellites) that warrant further testing as well as interesting developments that have informed new hypotheses. It is our goal to continue working with this dataset to understand how life history and the environment have shaped diversity in the Altamaha River Basin.

We intend to use the methods of Turner, Wares et al. (2002) to fit a demographic model to each species in an attempt to infer the values of life history parameters that create a similar value of N_e/N. We will be building a demographic model similar to Turner, Wares et al. (2002)

however we will fit our model using a pseudo-likelihood approach. Pseudo–likelihood approaches are useful when estimating multiple parameters with unknown distributions that would make the likelihood function intractable if not impossible to maximize. The current difficulty with this approach is the paucity of life history data available from freshwater mussel species as a prior. It is likely that there will be multiple combinations of parameters that produce similar values of N_e/N for each mussel species; however by using prior data from *E. complanta* for generation time, maturation time, average life span, and variance in both male and female reproductive success, we hope to provide a range of possible life history parameters in Altamaha mussels species (Downing 1993).

Turner, Wares et al (2002) also advocate comparing N_e from temporal estimate and coalescent estimates to determine the stability of both contemporary and historical population size. Temporal estimates calculate N_e strictly based on allelic variance between two time periods, providing an estimate of N_e between these sampled periods (Waples 1998; Turner and Salter 2001). On the other hand, the coalescent N_e provides an estimate on the long-term inbreeding effective size of the population. These two estimates are expected to be the same in large populations of constant size (Whitlock and Barton 1997), but differ under conditions such as population fluctuation. Though we did not collect temporally variant samples, we did keep track of the size of each individual that we sampled. When a regression of age v. size becomes available we can use size data as a proxy for age, allowing us to calculate N_e for multiple time periods. We can then compare the estimates of variance N_e to the coalescent estimate of N_e to investigate contemporary versus historic factors that have influenced effective size in Altamaha species.

Conclusion

In conclusion, our project the lays the necessary foundation for future projects that deal with mussel conservation in both the Altamaha River and other drainages in Georgia. In our project we have: proven the utility of 8 microsatellite markers for use in the genus *Elliptio* (Ward, Shaw et al. unpub man), quantified genetic variation across the Altamaha River Basin, estimated sites with high gene diversity, provided locations of sites with impeded gene flow, compared life history characteristics in freshwater mussels species with the goal of eventually defining idiosyncratic traits that make species more or less vulnerable to extinction, and finally we have established a baseline of genetic data that can be used for comparison against future studies on freshwater mussels in the Altamaha River Basin. To our knowledge our project is the largest genetic study of freshwater mussels and one of only a handful of datasets capable of comparing ecological data with genetic data for a whole community of organisms. We are hopeful that the resultant publications from this dataset will provide valuable insight for the basis of conservation management in the Altamaha River as well as set a standard for future population genetic studies in freshwater invertebrates.

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Table 4.1: Summary of genetic data collected from the Altamaha River Basin 2006-2008

Summary of sequence and microsatellite data collected for each species in number of individual sequences. *Aa=Alasmidonta arcula, Eang=Elliptio angustata, Ec=E. complanta, Ed=E. dariensis, Eh=E. hopetonensis, Ei=E. icterina, Esh=E. shepardiana, Esp=E. spinosa, Ld=Lampsilis dolabraeformis, Ls=L. splendida, Pg=Pyganodon gibbosa, Tp=Toxolasma parvus, Ui=Utterbackia imbecillis, Vd=Villosa delumbis*.

Species	**Collected**	**Mitochondrial**		**Microsatellites**			
	Tissue	*CO1*	*16S*	*ECO1*	*ECO2*	*ECO23*	*ECO29*
Aa	76	69	59	*NA*	*NA*	*NA*	*NA*
Eang	18	4	6	2	2	2	2
Ec	47	42	47	35	35	35	35
Ed	326	271	301	160	160	160	160
Eh	529	320	396	210	210	210	210
Ei	203	176	183	105	105	105	105
Esh	353	153	158	114	114	114	114
Esp	8	8	8	6	6	6	6
Ld	270	124	189	*NA*	*NA*	*NA*	*NA*
Ls	178	25	120	*NA*	*NA*	*NA*	*NA*
Pg	46	12	43	*NA*	*NA*	*NA*	*NA*
Tp	2	2	2	*NA*	*NA*	*NA*	*NA*
Ui	24	21	22	*NA*	*NA*	*NA*	*NA*
Vd	83	60	67	*NA*	*NA*	*NA*	*NA*
TOTAL	**2163**	**1287**	**1601**	**595**	**595**	**595**	**595**

Table 4.2: Abundance corrections on raw count data for species from the Altamaha River Basin 2006-2008.

Corrected abundance for raw count data from the Altamaha River, where N MRC is the corrected abundance as given in MARK, N MRC –JM is the corrected abundances as given in Meador, Peterson et al. (unpubman), projected abundances calculated from density estimates of mussels per m^2 then extrapolated to a total area of 10,850 km^2 (215 km x 50 m).

Species	Raw Count (JM)	N (hat) MRC STS	N (hat) MRC JM	Total Abundance (corrected)	Total Abundance (projected)
Alasmidonta arcula	51	18	33 (15-107)	319	56298
Elliptio dariensis	2716	1450	NA	10252	1805240
Elliptio hopetonensis	7081	1856	NA	43926	7734224
Elliptio icterina	420	193	NA	5570	980765
Elliptio shepardiana	1752	308	NA	8342	1468933
Lampsilis dolabraeformis	811	491	535 (387-681)	5928	1043811
Lampsilis splendida	364	277	925 (730-1227)	3596	633297
Pyganodon gibbosa	27	16	18 (5-160)	454	80032
Villosa delumbis	127	21	NA	1072	188859
Utterbackia imbecillus	49	7	NA	2270	399847

Table 4.3: AMOVA results for species collected from Altamaha River Basin 2006-2008
AMOVA results for comparison of genetic structure as divided among regions (Phi CT), among populations within regions (Phi SC), and within populations (Phi ST). The variance associated with each statistic as well as the contribution to the total variance is given by Variance and % Total. P-values are significant at alpha=0.05.

Species	Locus	Stats	Variance	% Total	Phi stat (SC,ST,CT)	P value
Alasmidonta arcula	*16s*	Among Pops/region (Phi SC)	0	0	-0.04448	0.39883+-0.01521
		Within Pops (Phi ST)	0	0	-0.0673	0.42424+-0.01621
		Among Regions (Phi CT)	0.05331	100	-0.02185	0.64809+-0.01308
	CO1	Among Pops/region (Phi SC)	0.30301	30.77	0.20972	0.00391+-0.00233
		Within Pops (Phi ST)	0.14301	14.52	0.45286	<0.00001
		Among Regions (Phi CT)	0.53888	54.71	0.30766	0.01369+-0.00309
Elliptio Dariensis	*16s*	Among Pops/region (Phi SC)	0.63927	3.15	0.59372	<0.00001
		Within Pops (Phi ST)	11.66073	57.5	0.60652	<0.00001
		Among Regions (Phi CT)	7.9795	39.35	0.03152	0.35973+-0.01215
	CO1	Among Pops/region (Phi SC)	0	0	-0.00576	0.56403+-0.01367
		Within Pops (Phi ST)	0	0	-0.00787	0.63050+-0.01574
		Among Regions (Phi CT)	3.20359	100	-0.0021	0.53763+-0.01526
Elliptio hopetonensis	*16s*	Among Pops/region (Phi SC)	1.05	5.71	0.53092	<0.00001
		Within Pops (Phi ST)	9.20575	50.06	0.55773	<0.00001
		Among Regions (Phi CT)	8.13335	44.23	0.05715	0.20919+-0.01204
	CO1	Among Pops/region (Phi SC)	0.05322	1.19	0.03046	0.06843+-0.00831
		Within Pops (Phi ST)	0.1342	3.01	0.04203	0.03519+-0.00598
		Among Regions (Phi CT)	4.27125	95.8	0.01194	0.05963+-0.00535
Elliptio icterina	*16s*	Among Pops/region (Phi SC)	0.03432	0.22	0.52563	<0.00001
		Within Pops (Phi ST)	8.3486	52.45	0.52665	<0.00001
		Among Regions (Phi CT)	7.53453	47.34	0.00216	0.30108+-0.01473
	CO1	Among Pops/region (Phi SC)	-0.17	-6.13	0.36342	<0.00001
		Within Pops (Phi ST)	1.12	38.57	0.32437	<0.00001
		Among Regions (Phi CT)	1.97872	67.56	-0.06134	0.28348+-0.01359
Elliptio shepardiana	*16s*	Among Pops/region (Phi SC)	1.8	6.87	0.82862	<0.00001
		Within Pops (Phi ST)	20.28645	77.17	0.84039	<0.00001
		Among Regions (Phi CT)	4.19572	15.96	0.06869	0.33920+-0.01046
	CO1	Among Pops/region (Phi SC)	-0.15386	-17.14	0.0036	0.36657+-0.01802
		Within Pops (Phi ST)	0.00378	0.42	-0.16719	0.43011+-0.01456
		Among Regions (Phi CT)	1.0477	116.72	-0.1714	0.94526+-0.00650

Table 4.4: The estimated ratio of effective population size to abundance for species from the Altamaha River Basin 2006-2008.
The ratio of effective size to abundance with 95% credible intervals for mussels species where both genetic and abundance data are available. Values for *E. spinosa* are inferred from ancestral state reconstruction using MESQUITE.

Species	Marker	Ne/N
Alasmidonta arcula	*16s*	0.66
	CO1	0.34
Elliptio dariensis	*16s*	0.14
	CO1	0.06
Elliptio hopetonensis	*16s*	0.04
	CO1	0.02
Elliptio icterina	*16s*	0.23
	CO1	0.08
Elliptio shepardiana	*16s*	0.05
	CO1	0.03
Elliptio spinosa***	*16s*	0.05
	CO1	0.03
Lampsilis dolabraeformis	*16s*	0.23
	CO1	0.09
Lampsilis splendida	*16s*	0.11
	CO1	0.15
Pyganodon gibbosa	*16s*	0.83
	CO1	0.15
Utterbackia imbecillus	*16s*	NA
	CO1	0.03
Villosa delumbis	*16s*	0.55
	CO1	0.22

*** Inferred from Ancestral State Reconstruction

Figure 4.1
Map of the Georgia showing the Altamaha River Basin and three main tributaries sampled in this project. Sampling locations are marked as filled circles; location is approximate.

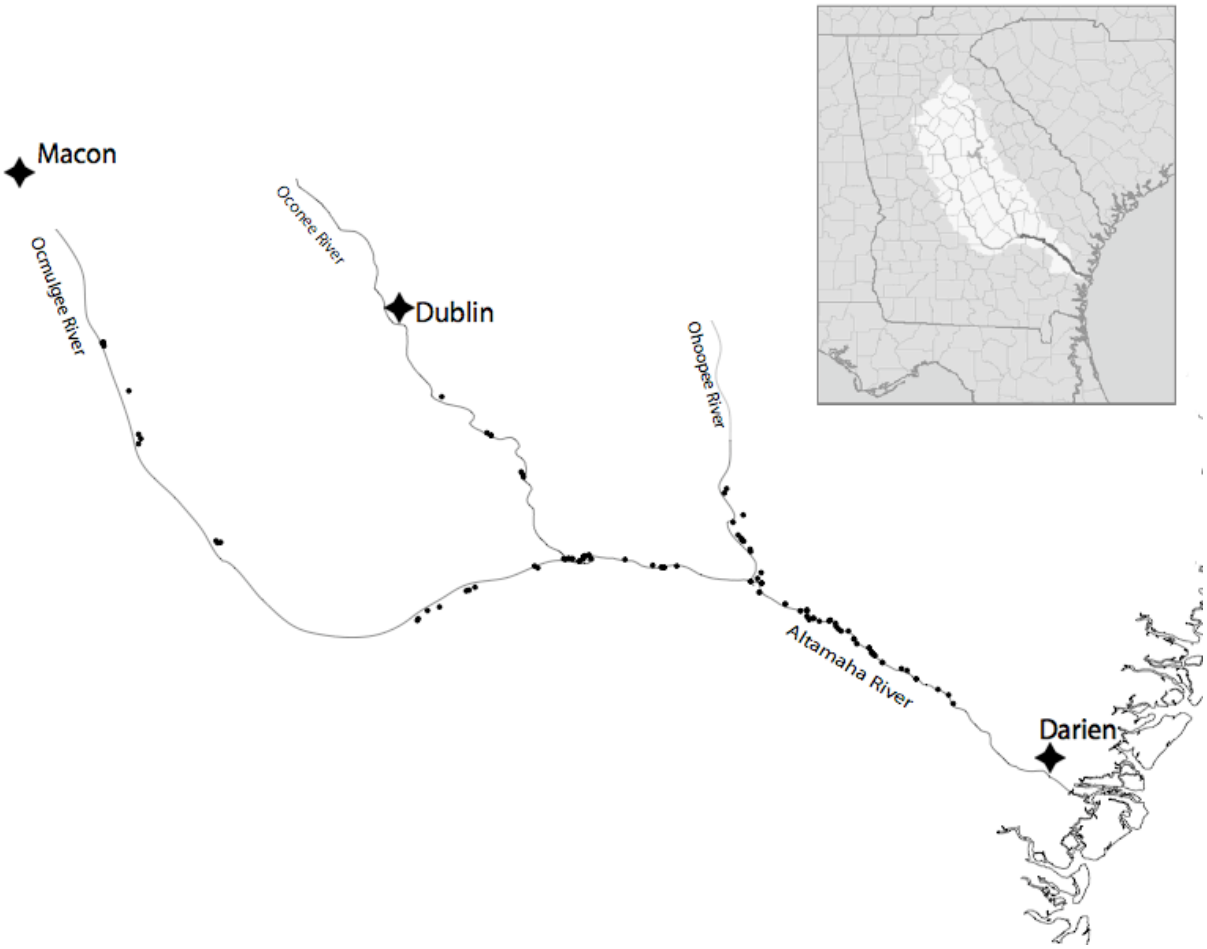

Macon
Ocmulgee River
Oconee River
Dublin
Ohoopee River
Altamaha River
Darien

Figure 4.2
Bayesian phylogeny of 2 genes from the mitochondria, 16s and CO1. Monophyletic groupings are collapsed for simplification.

Bayesian Phylogeny mtDNA (CO1, 16s)

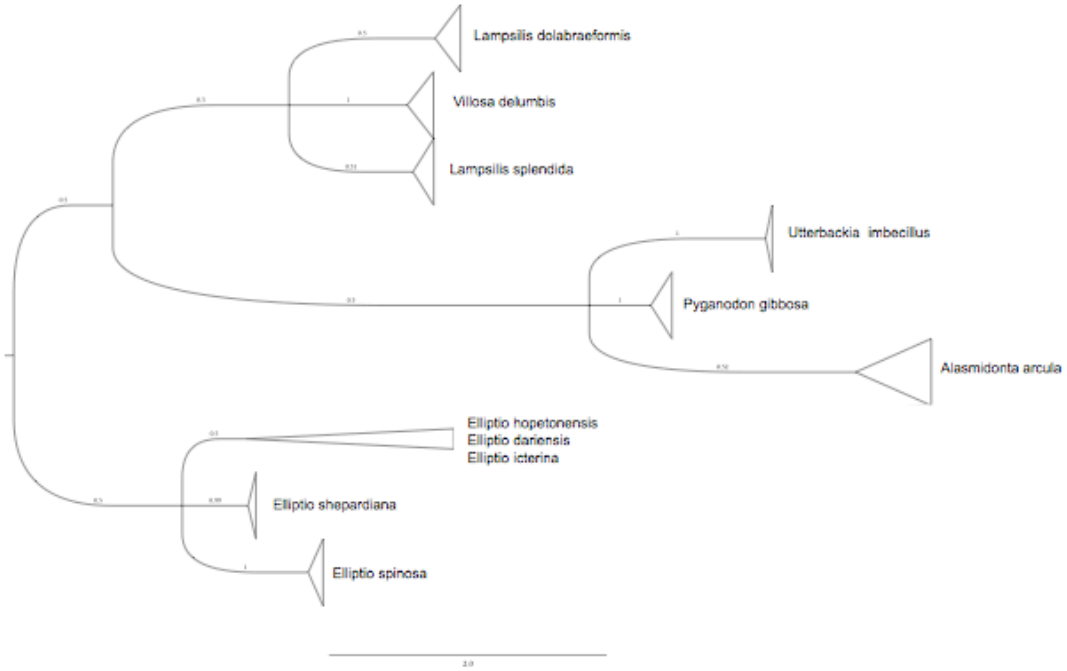


Figure 4.3
Bayesian phylogeny of 2 genes from the mitochondria, 16s and CO1, with the removal of *Elliptio icterina*. Monophyletic groupings are collapsed for simplification.

Bayesian Phylogeny mtDNA (CO1,16s)

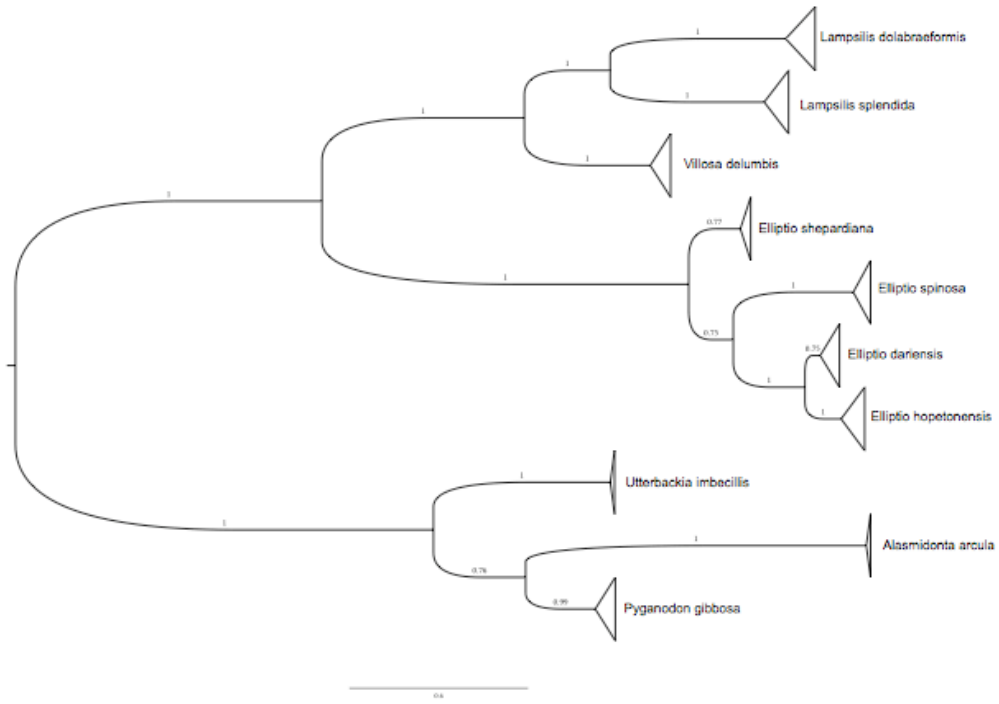


Figure 4.4
Summary of Tajima's D statistic (Tajima 1989) for 2 mitochondrial and 3 nuclear loci for all species sampled in the Altamaha River Basin.

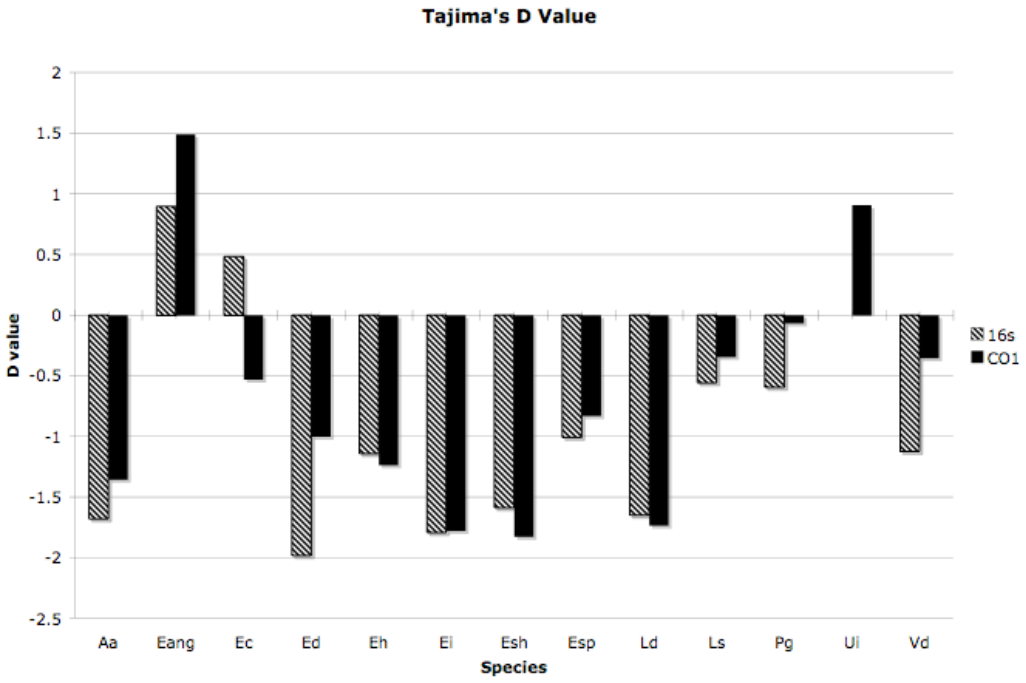

Tajima's D Value
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
D value
Aa
Eang
Ec
Ed
Eh
Ei
Esh
Esp
Ld
Ls
Pg
Ul
Vd
Species
16s
CO1

Figure 4.5
Boxplot showing the effective population size of 2 mitochondrial loci for all species sampled in the Altamaha River Basin. Boxes delineate upper and lower quartiles, dark lines show medians, and dashed lines extend to the last observation within 1.5X interquartile range of the boxes.

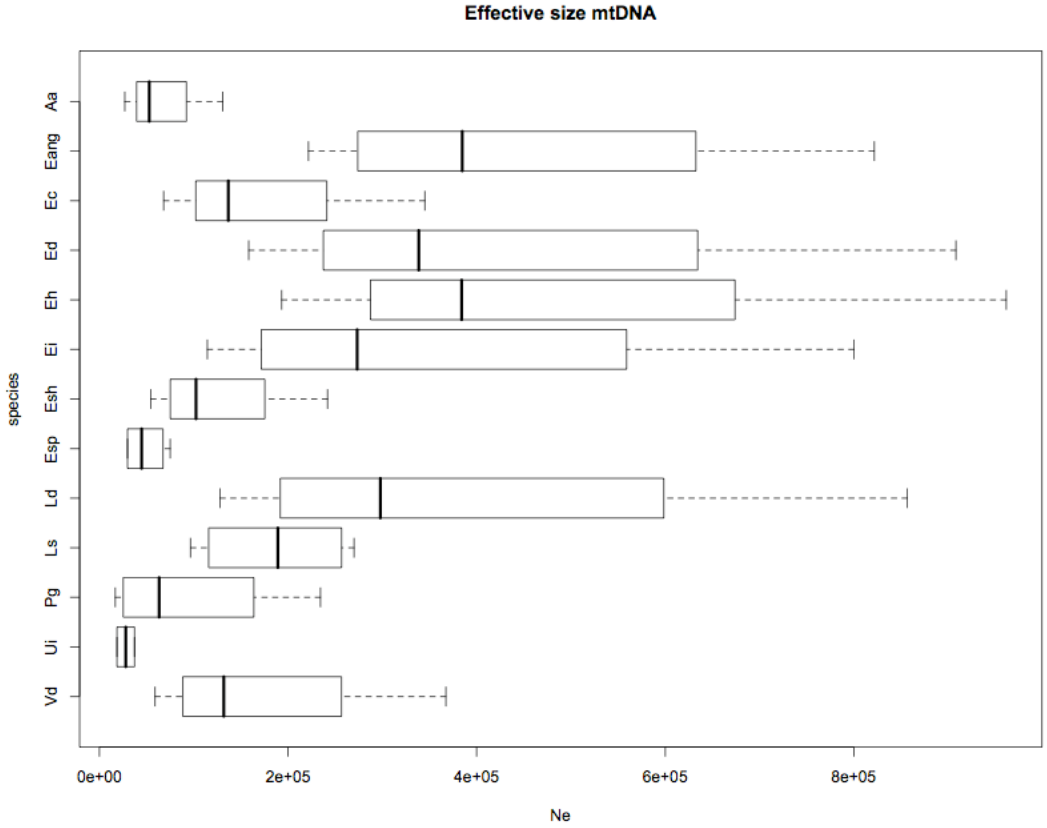
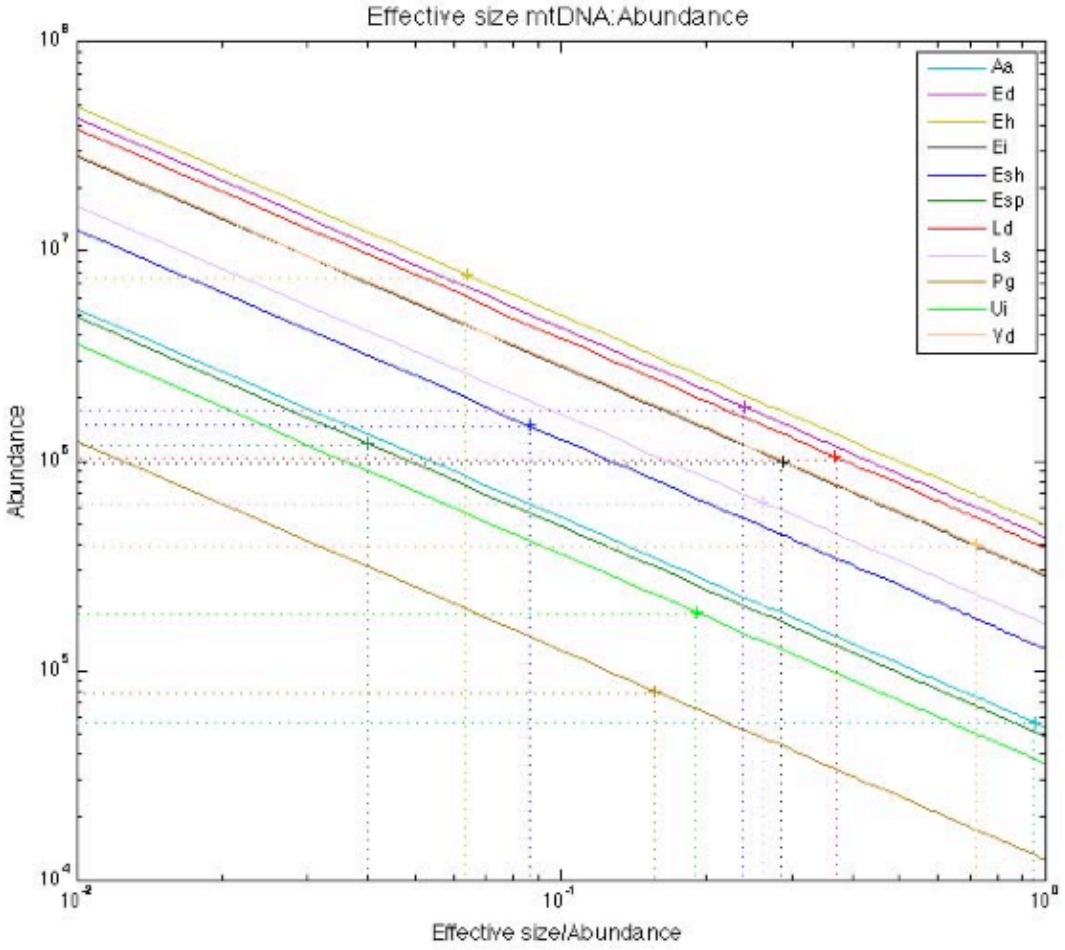

Effective size mtDNA
species
Aa
Eang
Ec
Ed
Eh
Ei
Esh
Esp
Ld
Ls
Pg
Ui
Vd
0e+00
2e+05
4e+05
6e+05
8e+05
Ne

Figure 4.6
Ratio of the effective population size to the abundance of mussels in the Altamaha River from 2 mitochondrial loci. The cross marks represent the projected abundance of each mussels species as given by the assumption that all available habitat is colonized (215km long with an average width of 50 m).


Effective size mtDNA:Abundance
Abundance
Effective size/Abundance
Aa
Ed
Eh
Ei
Esh
Esp
Ld
Ls
Pg
Ui
Yd

CHAPTER 5

CONCLUDING REMARKS

Summary

In the past three chapters I have attempted to elucidate mechanisms that generate and maintain biodiversity in freshwater mussels. The unique design of my dissertation allows the examination of biodiversity at both coarse and fine spatial scales. It is my hope that the data collected here for freshwater mussels can be extended to include other species allowing us to make inferences into forces that shape diversity in freshwater ecosystems.

In Chapter 1 I examined the regional distribution of freshwater mussel species in an attempt to explain the macroevolutionary processes that generate species diversity. I determined the effects of geological and paleo-climatic events using genetic data from species distributed among drainages throughout the Southeast United States. My results indicate that there were two major bursts of speciation in the history of the Southeast United States mussel taxa; one dating to the Pliocene and a second event dating to the Pleistocene.

The Pleistocene speciation was more likely the result of faunal exchange between rivers as patterns of species dispersal (e.g. paraphyletic species groupings) are prevalent in both the Coosa and Tennessee drainages from this time. During the Pliocene, high sea levels may have isolated ancestral populations of mussels, allowing genetic differences to accumulate. When the sea levels receded the rivers of the coastal plains were ill defined and probably free to exchange

fauna through interconnections. The importance of the Pliocene and Pleistocene speciation events is not only the casual mechanisms, but also the ubiquity of the event. The simultaneous speciation of freshwater mussels serves to link species in an evolutionary framework allowing sufficient time for competitive exclusion and co-adaptation. Inferences into processes of species formation in freshwater mussels highlight the importance of allopatric speciation in generating species diversity. However, there is evidence for either sympatric (or parapatric) speciation in some drainages, as sister species have a MRCA within the same drainage.

In Chapter 2 I examined the utility of genetic diversity for differentiating processes of community assembly in sessile organisms. I used MATLAB to simulate 3 different neutral community mechanisms: global dispersal, nearest neighbor dispersal, and conspecific density dependence. For each model community, I constructed a genetic model that kept track of selectively neutral mutations at a single locus. My results indicate that the combined use of genetic and species distributions aid the interpretation of mechanisms that structure communities. The distribution of species diversity in communities does in fact present different patterns that are easy to differentiate. However, in real data sets the forces of density dependence and dispersal are not as extreme leading to intermediate values of species distributions that are indistinguishable. It is also unclear to what degree real communities' history, e.g. disturbance, may affect the underlying equilibrium distribution of species. Where the species distributions failed to differentiate assembly mechanisms, we found that the distribution of genetic diversity as well as its spatial distribution did add statistical power. The novel insight was the description of the expected distribution of genetic diversity for a community of organisms under the UNTB. Previous studies had only hinted at the expected distribution of genetic diversity under the UNTB model but it had not been shown empirically.

In Chapter 3 I examined the pattern of genetic diversity for 17 species of freshwater mussel from the Altamaha River drainage here in Georgia. I found substantial genetic structure within populations of mussels, patterns of recent demographic expansion, and effective population sizes that differ among species. Genetic structure did not follow a pattern indicative of dispersal limitation, especially as tests of isolation by distance were not significant. More often there were a few populations that had atypical amounts of genetic diversity relative to the surrounding river. These populations were most prominent ~80 rkm from the confluence of the Ocmulgee and Oconee Rivers; populations close to the confluence were relatively well mixed. Previous work has shown that estimating the distribution of mussels was more successful when habitat preferences of fish were used. So, it is possible that a peculiarity in the fish host is creating these patterns of genetic structure.

A large majority of the mussel species displayed a negative Tajima's D value, which is indicative of populations under selection or undergoing demographic expansion. As of yet I have not dated the demographic expansion but if it is similar across species then it provides evidence for a general event that affected all mussel species in the Altamaha River. If the bottleneck is disjointed temporally among species, an important question is what life history characteristic made some species vulnerable while others were not.

Finally I looked at the reproductive variance of freshwater mussel species. By comparing the effective population size (N_e) and the abundance (N), I was able to determine how reproductive variance was correlated with phylogeny. Species within a genus tend to have similar values of N_e/N and hence similar life history strategies. A future goal is to fit life history models to explain the ratio of N_e/N in mussel species.

The final synthesis of data from all three chapters of my dissertation will require the utilization of methods from ecology, phylogenetics, and population genetics. The analysis of data will require new methods to understand individual variance in genes scale to the level of whole communities of species.

Conclusion

My dissertation encompasses a new way to study biodiversity. Although I only investigate a single taxonomic group, freshwater mussels are representative of larger processes in freshwater ecosystems. Freshwater mussels influence their ecosystem by cycling nutrients and creating livable substrate for many other freshwater organisms. Given more resources and more time it would be beneficial to choose representative organisms from each trophic level of the ecosystem, providing key insights into how diversity is generated and maintained within and among species.

Future directions for my project need to include data on how habitat heterogeneity and disturbance affect the species composition in communities. This is especially important in lotic systems as flood pulses and droughts can alter habitat availability and change species composition. I can also improve my project by incorporating data on how functional processes respond to spatial-temporal heterogeneity. Species within an ecosystem have functional roles that affect the stability of the ecosystem. It is important to understand what functional role each species plays and how interaction and functional redundancy affect overall ecosystem processes.

As humans enter a new era of energy consumption, water resources will become more valuable. Even here in the Southeast United States where water seems plentiful, recent climate changes have created disputes over water rites. As water becomes more valuable, our lotic

ecosystems will fall victim to increased impoundment and diverting, which in times of drought may mean disaster for freshwater species. It is important that we garner an understanding of freshwater ecosystems now so we can predict what affect an increase in anthropogenic water use may have. Once we are able to predict response of lotic ecosystems to changing use, we can adapt management policies to protect the diversity and functionality of freshwater ecosystems.

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APPENDIX A

Table A.1: Museum accession numbers

Museum accession numbers for mussel samples used in to determine the divergence of drainages in Chapter 2.

Genus	Species	Accession #	CAT #	Museum	Drainage
Alasmidonta	triangulata	29995	1	NC	ACF
Alasmidonta	triangulata	29995	3	NC	ACF
Alasmidonta	triangulata	46874	4	NC	ACF
Alasmidonta	marginata	29158	7	NC	TENN
Alasmidonta	marginata	29158	9	NC	TENN
Alasmidonta	virdis	30864	12	NC	TENN
Alasmidonta	undulata	45406	15	NC	SAV
Alasmidonta	undulata	28550	16	NC	SAV
Alasmidonta	varicosa	29140	18	NC	SAV
Alasmidonta	varicosa	29140	19	NC	SAV
Alasmidonta	varicosa	29140	20	NC	SAV
Elliptio	fumata	45601	23	NC	ACF
Elliptio	fumata	45601	24	NC	ACF
Elliptio	fumata	45601	25	NC	ACF
Elliptio	fumata	45601	26	NC	ACF
Elliptio	purpurella	45613	27	NC	ACF
Elliptio	purpurella	45613	28	NC	ACF
Elliptio	mcmichaeli	No Cat	37	NC	Gulf
Elliptio	mcmichaeli	No Cat	38	NC	Gulf
Elliptio	arca	30081	42	NC	Coosa
Elliptio	arca	No Cat	51	NC	Coosa
Elliptio	arca	No Cat	52	NC	Coosa
Elliptio	arca	No Cat	53	NC	Coosa
Elliptio	folliculata	30206	56	NC	Ogeechee
Elliptio	downeii	30029	57	NC	Satilla
Elliptio	downeii	30029	58	NC	Satilla
Elliptio	downeii	30029	59	NC	Satilla
Elliptio	downeii	30029	60	NC	Satilla
Elliptio	downeii	30029	61	NC	Satilla
Elliptio	downeii	30029	62	NC	Satilla
Elliptio	waccamawensis	29742	63	NC	SAV
Elliptio	waccamawensis	29742	65	NC	SAV
Elliptio	buckleyi	29818	77	NC	FLPENN
Elliptio	buckleyi	29818	78	NC	FLPENN
Elliptio	buckleyi	29818	79	NC	FLPENN
Elliptio	dilatata	MMNS 6518	84	NC	TENN
Elliptio	waccamawensis	45967	112	NC	SAV
Elliptio	waccamawensis	45967	113	NC	SAV
Elliptio	dariensis	29106	125	NC	Altamaha
Elliptio	dariensis	29106	126	NC	Altamaha
Elliptio	dariensis	30011	127	NC	Altamaha
Elliptio	dariensis	29115	128	NC	Altamaha

Elliptio	dariensis	29115	129	NC	Altamaha
Elliptio	hopetonensis	29103	130	NC	Altamaha
Elliptio	hopetonensis	29103	131	NC	Altamaha
Elliptio	hopetonensis	29103	132	NC	Altamaha
Elliptio	hopetonensis	30032	133	NC	Altamaha
Elliptio	hopetonensis	30032	134	NC	Altamaha
Elliptio	hopetonensis	45627	135	NC	Altamaha
Elliptio	angustata	45632	136	NC	Altamaha
Elliptio	angustata	45632	137	NC	Altamaha
Elliptio	angustata	45632	138	NC	Altamaha
Elliptio	angustata	45636	139	NC	Altamaha
Elliptio	angustata	45636	140	NC	Altamaha
Elliptio	angustata	45636	141	NC	Altamaha
Elliptio	dilatata	173	173	UA	TENN
Elliptio	arca	503	503	UA	Etowah
Elliptio	mcmichaeli	3410	3410	UA	Choctawhatchee
Elliptio	arctata	6438	6438	AU	Coosa
Elliptio	arctata	9400	9400	AU	Mobile
Elliptio	arctata	9662	9662	AU	ACF
Elliptio	purpurella	9679	9679	AU	ACF
Elliptio	folliculata	9749	9749	AU	Pee Dee
Elliptio	arctata	15322	15322	AU	ACF
Elliptio	purpurella	15323	15323	AU	ACF
Elliptio	fraterna	15325	15325	AU	ACF
Elliptio	purpurella	15339	15339	AU	ACF
Elliptio	fraterna	15340	15340	AU	ACF
Elliptio	arctata	15343	15343	AU	ACF
Elliptio	fumata	15865	15865	AU	ACF
Elliptio	mcmichaeli	3200	3200	AU	Gulf
Lampsilis	subangulata	No Cat	29	NC	ACF
Lampsilis	subangulata	No Cat	30	NC	ACF
Lampsilis	australis	46409	39	NC	Gulf
Lampsilis	australis	46409	40	NC	Gulf
Lampsilis	australis	46409	41	NC	Gulf
Lampsilis	ornata	No Cat	48	NC	Coosa
Lampsilis	ornata	No Cat	49	NC	Coosa
Lampsilis	ornata	No Cat	50	NC	Coosa
Lampsilis	cariosa	30208	66	NC	SAV
Lampsilis	radiata	45429	68	NC	SAV
Lampsilis	radiata	30003	69	NC	SAV
Lampsilis	radiata	27928	70	NC	SAV
Lampsilis	radiata	28949	71	NC	SAV
Lampsilis	fasciola	29745	86	NC	TENN
Lampsilis	fasciola	29745	87	NC	TENN
Lampsilis	fasciola	29745	88	NC	TENN
Lampsilis	virescens	30642	90	NC	TENN
Lampsilis	altilis	45043	110	NC	Coosa
Lampsilis	cariosa	41138	122	NC	SAV
Lampsilis	cariosa	41138	123	NC	SAV

Lampsilis	cariosa	41138	124	NC	SAV
Lampsilis	ornata	182	182	UA	Mobile
Lampsilis	altilis	538	538	UA	Etowah
Lampsilis	subangulata	604	604	UA	ACF
Lampsilis	subangulata	645	645	UA	ACF
Lampsilis	perovalis	646	646	UA	Mobile
Lampsilis	ornata	734	734	UA	Coosa
Lampsilis	ornata	735	735	UA	Coosa
Lampsilis	ornata	736	736	UA	Coosa
Lampsilis	ornata	739	739	UA	Coosa
Lampsilis	ornata	1151	1151	UA	Mobile
Lampsilis	ornata	1152	1152	UA	Mobile
Lampsilis	ornata	1179	1179	UA	Mobile
Pleurobema	pyriforme	29454	31	NC	ACF
Pleurobema	pyriforme	29454	32	NC	ACF
Pleurobema	pyriforme	29454	33	NC	ACF
Pleurobema	pyriforme	29454	34	NC	ACF
Pleurobema	oviforme	27782	91	NC	TENN
Pleurobema	oviforme	27779	92	NC	TENN
Pleurobema	oviforme	27804	93	NC	TENN
Pleurobema	decisum	29405	109	NC	Coosa
Pleurobema	oviforme	27787	117	NC	TENN
Pleurobema	oviforme	27779	118	NC	TENN
Villosa	villosa	47076	36	NC	ACF
Villosa	villosa	27443	81	NC	ACF
Villosa	trabalis	28978	94	NC	TENN
Villosa	taenita	29180	95	NC	TENN
Villosa	taenita	No Cat	96	NC	TENN
Villosa	taenita	No Cat	97	NC	TENN
Villosa	taenita	No Cat	98	NC	TENN
Villosa	taenita	No Cat	99	NC	TENN
Villosa	vanuxemensis	No Cat	100	NC	TENN
Villosa	vanuxemensis	No Cat	101	NC	TENN
Villosa	vanuxemensis	No Cat	102	NC	TENN
Villosa	vanuxemensis	No Cat	103	NC	TENN
Villosa	iris	29744	104	NC	TENN
Villosa	iris	35326	105	NC	TENN
Villosa	iris	29744	106	NC	TENN
Villosa	iris	35325	108	NC	TENN
Villosa	vanuxemensis	46339	119	NC	TENN
Villosa	vanuxemensis	45645	120	NC	TENN
Villosa	vanuxemensis	45645	121	NC	TENN
Villosa	umbrans	416	416	UA	Etowah
Villosa	delumbis	610	610	UA	SAV
Villosa	villosa	2745	2745	UA	ACF
Villosa	nebulosa	2804	2804	UA	Etowah
Villosa	delumbis	3067	3067	UA	Altamaha
Villosa	delumbis	3068	3068	UA	Altamaha
Villosa	delumbis	3070	3070	UA	Altamaha

Villosa	choctawensis	35324	45	NC	Gulf
Villosa	choctawensis	35324	46	NC	Gulf
Villosa	choctawensis	35324	47	NC	Gulf
Villosa	delumbis	611	611	UA	SAV

Table A.2: Genebank accession numbers

Genebank accession numbers for sequences used to determine divergence of drainages in Chapter 2.

1: AF093844 Alasmidonta varic...[gi:22001084]
2: AF156502 Alasmidonta margi...[gi:5107865]
3: U72563 Alasmidonta trian...[gi:1698812]
4: AY654995 Elliptio arca cyt...[gi:56800566]
5: DQ383427 Elliptio arctata ...[gi:91992191]
6: AF156507 Elliptio dilatata...[gi:5107870]
7: AF156506 Elliptio dilatata...[gi:5107869]
8: AF231751 Elliptio dilatata...[gi:16755104]
9: U72557 Elliptio dilatata...[gi:1698814]
10: AF385132 Lampsilis altilis...[gi:18157625]
11: AF385131 Lampsilis altilis...[gi:18157624]
12: AF385130 Lampsilis altilis...[gi:18157623]
13: AF385129 Lampsilis altilis...[gi:18157622]
14: AF385116 Lampsilis altilis...[gi:18157609]
15: AF385108 Lampsilis altilis...[gi:18157594]
16: AF385107 Lampsilis altilis...[gi:18157592]
17: AF385106 Lampsilis altilis...[gi:18157590]
18: AF385105 Lampsilis altilis...[gi:18157588]
19: AF385092 Lampsilis altilis...[gi:18157562]
20: EF033305 Hamiota subangula...[gi:124298350]
21: EF033266 Hamiota subangula...[gi:124298272]
22: AF385128 Lampsilis subangu...[gi:18157621]
23: AF385127 Lampsilis subangu...[gi:18157620]
24: AF385126 Lampsilis subangu...[gi:18157619]
25: AF385104 Lampsilis subangu...[gi:18157586]
26: AF385102 Lampsilis subangu...[gi:18157582]
27: AF385103 Lampsilis subangu...[gi:18157584]
28: AF385120 Lampsilis peroval...[gi:18157613]
29: AF385119 Lampsilis peroval...[gi:18157612]
30: AF385118 Lampsilis peroval...[gi:18157611]
31: AF385117 Lampsilis peroval...[gi:18157610]
32: AF385115 Lampsilis peroval...[gi:18157608]
33: AF385096 Lampsilis peroval...[gi:18157570]
34: AF385095 Lampsilis peroval...[gi:18157568]
35: AF385094 Lampsilis peroval...[gi:18157566]
36: AF385093 Lampsilis peroval...[gi:18157564]
37: AF385091 Lampsilis peroval...[gi:18157560]
38: AF385125 Lampsilis austral...[gi:18157618]
39: AF385124 Lampsilis austral...[gi:18157617]
40: AF385123 Lampsilis austral...[gi:18157616]
41: AF385122 Lampsilis austral...[gi:18157615]
42: AF385121 Lampsilis austral...[gi:18157614]
43: AF385101 Lampsilis austral...[gi:18157580]
44: AF385100 Lampsilis austral...[gi:18157578]
45: AF385099 Lampsilis austral...[gi:18157576]
104: AY655017 Pleurobema ovifor...[gi:56800609]
105: AY655068 Pleurobema ovifor...[gi:56068158]
106: AY655067 Pleurobema ovifor...[gi:56068157]
107: EF619919 Pleurobema plenum...[gi:156567919]
108: EF619920 Pleurobema plenum...[gi:156567921]
109: U72558 Pleurobema pyrifo...[gi:1698830]
110: AY613839 Pleurobema pyrifo...[gi:54610951]
111: AY613841 Pleurobema rubrum...[gi:54610955]
112: AY655018 Pleurobema rubrum...[gi:56800611]
113: EF033291 Pleurobema sintox...[gi:124298322]
114: EF033253 Pleurobema sintox...[gi:124298246]
115: AY655019 Pleurobema sintox...[gi:56800613]
116: AF156509 Pleurobema coccin...[gi:5107872]
117: AF156508 Pleurobema coccin...[gi:5107871]
118: DQ191418 Pleurobema sintox...[gi:77632480]
119: AY655084 Villosa vanuxemen...[gi:56068174]
120: AF156526 Villosa vanuxemen...[gi:5107889]
121: AF156525 Villosa vanuxemen...[gi:5107888]
122: AF385133 Villosa villosa U...[gi:18157626]
123: AF385109 Villosa villosa U...[gi:18157596]
124: DQ191422 Villosa iris larg...[gi:77632484]
125: AY655083 Villosa iris 16S ...[gi:56068173]
126: AF156524 Villosa iris UMMZ...[gi:5107887]
127: AF156523 Villosa iris UMMZ...[gi:5107886]
128: DQ220726 Villosa fabalis c...[gi:78172546]
129: U72574 Villosa delumbis ...[gi:1698839]
130: AY655054 Medionidus acutis...[gi:56068144]
131: AY655005 Medionidus acutis...[gi:56800585]
132: AY654991 Amblema elliottii...[gi:56800558]
133: AY655029 Amblema elliottii...[gi:56068119]
134: AY655077 Strophitus subvex...[gi:56068167]
135: AY655021 Strophitus subvex...[gi:56800617]
136: AY655001 Lasmigona holston...[gi:56800578]
137: AY654996 Epioblasma capsae...[gi:56800568]
138: DQ208520 Epioblasma capsae...[gi:77456050]
139: DQ208521 Epioblasma capsae...[gi:77456051]
140: DQ208522 Epioblasma capsae...[gi:77456052]
141: DQ208519 Epioblasma capsae...[gi:77456049]
142: DQ208518 Epioblasma capsae...[gi:77456048]
143: DQ208517 Epioblasma capsae...[gi:77456047]
144: DQ208516 Epioblasma capsae...[gi:77456046]
145: DQ208515 Epioblasma capsae...[gi:77456045]
146: DQ208514 Epioblasma capsae...[gi:77456044]
147: DQ208513 Epioblasma capsae...[gi:77456043]
148: DQ208512 Epioblasma capsae...[gi:77456042]

46: AF385098 Lampsilis austral...[gi:18157574]
47: AF385097 Lampsilis austral...[gi:18157572]
48: EF033303 Lampsilis ovata i...[gi:124298346]
49: EF033262 Lampsilis ovata i...[gi:124298264]
50: AY613826 Lampsilis ovata c...[gi:54610925]
51: AY655048 Lampsilis ovata 1...[gi:56068138]
52: AF385135 Lampsilis ovata U...[gi:18157628]
53: AF385111 Lampsilis ovata U...[gi:18157600]
54: AY365193 Lampsilis ornata ...[gi:39726228]
55: NC_005335 Lampsilis ornata ...[gi:41057409]
56: AF385136 Lampsilis ornata ...[gi:18157629]
57: AF385112 Lampsilis ornata ...[gi:18157602]
58: AF049520 Lampsilis ornata ...[gi:3894376]
59: AF156520 Lampsilis fasciol...[gi:5107883]
60: AY238480 Lampsilis radiata...[gi:29838616]
61: AY498703 Lampsilis radiata...[gi:40748064]
62: DQ060171 Margaritifera mar...[gi:70728070]
63: DQ060167 Margaritifera mar...[gi:70728063]
64: AY579088 Margaritifera mar...[gi:50897882]
65: AY579087 Margaritifera mar...[gi:50897881]
66: AY579130 Margaritifera mar...[gi:50897932]
67: AY579129 Margaritifera mar...[gi:50897930]
68: DQ272382 Margaritifera fal...[gi:83031776]
69: DQ272383 Margaritifera fal...[gi:83031778]
70: DQ272381 Margaritifera fal...[gi:83031774]
71: DQ272380 Margaritifera fal...[gi:83031772]
72: DQ272379 Margaritifera fal...[gi:83031770]
73: DQ272378 Margaritifera fal...[gi:83031768]
74: DQ272377 Margaritifera fal...[gi:83031766]
75: DQ272376 Margaritifera fal...[gi:83031764]
76: DQ272375 Margaritifera fal...[gi:83031762]
77: DQ272374 Margaritifera fal...[gi:83031760]
78: AY579084 Margaritifera fal...[gi:50897878]
79: AY579128 Margaritifera fal...[gi:50897928]
80: AY579127 Margaritifera fal...[gi:50897926]
81: AY579126 Margaritifera fal...[gi:50897924]
82: AY655013 Pleurobema clava ...[gi:56800601]
83: AY655060 Pleurobema clava ...[gi:56068150]
84: AF231754 Pleurobema clava ...[gi:16755110]
85: EF619918 Pleurobema cordat...[gi:156567917]
86: EF619917 Pleurobema cordat...[gi:156567915]
87: AY613831 Pleurobema cordat...[gi:54610935]
88: DQ383431 Pleurobema decisu...[gi:91992199]
89: AY613832 Pleurobema decisu...[gi:54610937]
90: AY655014 Pleurobema decisu...[gi:56800603]
91: AF232801 Pleurobema decisu...[gi:11526863]
92: AF232776 Pleurobema decisu...[gi:11526838]
93: AY655015 Pleurobema georgi...[gi:56800605]
94: AY613834 Pleurobema georgi...[gi:54610941]
149: DQ208511 Epioblasma capsae...[gi:77456041]
150: DQ208510 Epioblasma capsae...[gi:77456040]
151: DQ208509 Epioblasma capsae...[gi:77456039]
152: DQ208508 Epioblasma capsae...[gi:77456038]
153: DQ208507 Epioblasma capsae...[gi:77456037]
154: DQ208506 Epioblasma capsae...[gi:77456036]
155: DQ208505 Epioblasma capsae...[gi:77456035]
156: DQ208504 Epioblasma capsae...[gi:77456034]
157: DQ208503 Epioblasma capsae...[gi:77456033]
158: AY655037 Epioblasma capsae...[gi:56068127]
159: AY094372 Epioblasma capsae...[gi:32482510]
160: DQ208538 Epioblasma floren...[gi:77456068]
161: DQ208537 Epioblasma floren...[gi:77456067]
162: DQ208536 Epioblasma floren...[gi:77456066]
163: DQ208535 Epioblasma floren...[gi:77456065]
164: DQ208534 Epioblasma floren...[gi:77456064]
165: DQ208533 Epioblasma floren...[gi:77456063]
166: DQ208532 Epioblasma floren...[gi:77456062]
167: DQ208531 Epioblasma floren...[gi:77456061]
168: DQ208530 Epioblasma floren...[gi:77456060]
169: DQ208529 Epioblasma floren...[gi:77456059]
170: DQ208528 Epioblasma floren...[gi:77456058]
171: DQ208527 Epioblasma floren...[gi:77456057]
172: DQ208526 Epioblasma floren...[gi:77456056]
173: DQ208525 Epioblasma floren...[gi:77456055]
174: DQ208524 Epioblasma floren...[gi:77456054]
175: DQ208523 Epioblasma floren...[gi:77456053]
176: AY094373 Epioblasma floren...[gi:32482512]
177: AY094374 Epioblasma floren...[gi:32482514]
178: DQ479949 Epioblasma torulo...[gi:94450809]
179: DQ479948 Epioblasma torulo...[gi:94450807]
180: DQ479947 Epioblasma torulo...[gi:94450805]
181: DQ479946 Epioblasma torulo...[gi:94450803]
182: DQ479945 Epioblasma torulo...[gi:94450801]
183: DQ479944 Epioblasma torulo...[gi:94450799]
184: DQ220724 Epioblasma torulo...[gi:78172542]
185: DQ208544 Epioblasma torulo...[gi:77456074]
186: DQ208543 Epioblasma torulo...[gi:77456073]
187: DQ208542 Epioblasma torulo...[gi:77456072]
188: DQ208541 Epioblasma torulo...[gi:77456071]
189: DQ208540 Epioblasma torulo...[gi:77456070]
190: DQ208539 Epioblasma torulo...[gi:77456069]
191: AY655038 Fusconaia barnesi...[gi:56068128]
192: AY613822 Fusconaia barnesi...[gi:54610917]
193: DQ206791 Toxolasma parvus ...[gi:77997705]
194: DQ206790 Toxolasma parvus ...[gi:77997703]
195: DQ206789 Toxolasma parvus ...[gi:77997701]
196: DQ206788 Toxolasma parvus ...[gi:77997699]
197: DQ206787 Toxolasma parvus ...[gi:77997697]

95: AY655063 Pleurobema georgi...[gi:56068153]
96: AY655062 Pleurobema georgi...[gi:56068152]
97: DQ383432 Pleurobema gibber...[gi:91992201]
98: AY655064 Pleurobema gibber...[gi:56068154]
99: AY613835 Pleurobema gibber...[gi:54610943]
100: AY655016 Pleurobema hanley...[gi:56800607]
101: AY613836 Pleurobema hanley...[gi:54610945]
102: AY655066 Pleurobema hanley...[gi:56068156]
103: AY655065 Pleurobema hanley...[gi:56068155]
198: DQ206786 Toxolasma parvus ...[gi:77997695]
199: DQ206785 Toxolasma parvus ...[gi:77997693]
200: DQ206784 Toxolasma parvus ...[gi:77997691]
201: AY655022 Toxolasma parvus ...[gi:56800619]
202: AY238482 Toxolasma parvus ...[gi:29838618]
203: AF231756 Toxolasma lividus...[gi:16755114]
204: AY655023 Toxolasma texasie...[gi:56800621]
205: AY655078 Toxolasma texasie...[gi:56068168]