CONVERGENT EVOLUTION IN LIVEBEARING FISHES

A Thesis

by

NICHOLAS JOSEPH TROENDLE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2012

Major Subject: Wildlife and Fisheries Sciences

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	Mariana Mateos
Committee Member,	Adam Jones
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ABSTRACT

Convergent Evolution in Livebearing Fishes. (May 2012)

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Co-Chairs of Advisory Committee: Thomas J. DeWitt, Mariana Mateos

The directionality and consistency of evolution has long been a subject of contention among evolutionary biologists since the days of Darwin. However, it is unknown how much can be quantified and how much results from more complex variables. It is also unknown whether evolution is consistent or whether it occurs differently in each system.

My study focuses on predation and habitat as ecological gradients that may create convergent evolution in livebearing fishes. In Chapter I, I focus on predation as a mechanism for driving convergent evolution in *Gambusia affinis*. A suite of 7 microsatellite markers was used in order to determine independence of morphological evolution. Mantel tests were used to compare genetic, phenotypic, geographical and environmental distances among the six focal populations. These tests showed that there was a significant correlation between genetic and geographic distance but no significant correlation between genetic and phenotypic distances, which may indicate that phenotypic divergence has arisen independently in multiple instances.

The second chapter focuses on a unique form of convergence that arose during speciation of three livebearing fishes, which we termed "convergent speciation." I focus on habitat type as a selective pressure in the lake system of Lake Catemaco, Mexico and the surrounding rivers. Lake Catemaco has been isolated from the surrounding rivers for approximately 1.2 million years and during that time several endemic species have evolved in the lake. This provides an excellent study system for studying convergent divergence. To test the theory of convergent speciation in this system, a MANOVA was conducted. The effect of habitat was an important source of variance in the system, indicating that habitat is a likely driving force responsible for convergent speciation in the system. Using discriminant functions I was able to correctly predict the habitat of fish of six different species between 68% and 71% of the time. This may indicate that evolutionary response to habitat is consistent across taxa (i.e., convergent divergence is taking place).

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I would also like to thank Carlos Santamaria of the Mateos lab for all of his help with the microsatellite analysis and Mark Renshaw and Dr. John Gold's lab for the aid in analyzing the microsatellites.

Thanks also to Dr. Thomas DeWitt for his countless hours sitting down with me going through data analysis, hashing out the details of this project and working out the kinks.

NOMENCLATURE

DFA	discriminant function analysis
LDA	linear discriminant function analysis
QDA	quadratic discriminant function analysis
AMOVA	analysis of molecular variance
MANOVA	multivariate analysis of variance
PCA	principal components analysis
PCR	polymerase chain reaction
MYA	million years ago
k	kilometers
m	meters
mm	millimeters

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CHAPTER I

INTRODUCTION

Convergent evolution is the idea that alternative lineages exposed to similar evolutionary conditions evolve similar traits (Futuyma, 2009). Examples of this process are evident in widely phylogenetically distinct organisms (e.g., independent evolution of wings in birds, bats and insects), and intraspecific variation among populations (e.g., replicated beak form in finches, or trophic ecotypes in fishes) (Grant *et al.* 2004). Convergence has historically been considered to arise from two phenomena; convergent evolution and parallel evolution. These two processes can be thought of as ends of a continuum, rather than distinctly different concepts (Arendt & Reznick 2007). The traditional idea is that distantly related organisms evolve similar phenotypes via different developmental and genetic pathways. The term convergent evolution is generally reserved for this type of convergence in phenotype, via alternative genetic and developmental processes. Conversely, closely related organisms are believed to evolve similar phenotypes through genetically and developmentally similar evolutionary pathways; which are historically termed parallel evolution. Drawing distinctions between these two terms can be problematic because often convergence is partly the same at the mechanistic level, and partly different. Moreover, often convergence is observed, but there is incomplete (or a total lack of) information about genetic and

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This thesis follows the style of Evolution.

developmental basis of the traits. Arendt and Reznick (2007) describe several cases of distantly related organisms converging through the same pathways. They describe evolution of the Mc1r gene in taxa spanning the classes Reptilia, Mammalia and Actinopterygii. All of these taxa evolve the Mc1r gene through similar pathways even though they are highly divergent taxonomically. They cite cavefish pigment loss as an example of closely related species evolving similar phenotypes through different genetic mechanisms. There are two species of Mexican cavefish which have deletions in the ocular albinism 2 gene. However, even though the deletion is in the same gene, it is not the same deletion. Arendt and Reznick (2007) cite this as evidence that the evolutionary loss of pigment has occurred independently, though the distinction between parallel and convergent evolution blurs at the specific gene (as opposed to sequence within the gene) level. Because there are clear problems with the historical definitions of convergent and parallel evolution, Arendt and Reznick (2007) proposed that the distinction should be abandoned and a single term, convergent evolution, should be used. For the purpose of this thesis, I will use the single term convergent evolution to describe evolution of similar genetic or phenotypic characteristics regardless of phylogenetic relatedness, as recommended by Arendt and Reznick (2007).

Convergent evolution is apparent in many natural systems spanning diverse taxa. Examples exist from plants, fish, invertebrates, birds, mammals and bacteria. For example, three species of lizard, (*Aspidoscelis inornata, Sceloporus undulates* and *Holbrookia maculate*) evolved convergently due to the shared habitat provided in White Sands, New Mexico (Rosenblum & Harmon 2011). The three species of White Sand

lizards evolved body coloration to match their surroundings, and all three species also evolved larger heads and longer toes than their dark soil counterparts. Givnish *et al.* (2005) conducted a study in which they determined that fleshy fruit in monocots have arisen at least 21 times in their evolutionary history. They also discovered that monocots tend to evolve leaves with venation arranged in nets rather than in parallel when they are in shaded habitats. This concerted evolution due to shaded habitats has occurred across many species of monocots, providing an excellent example of related species independently evolving the same phenotypic qualities to adapt to a similar environment.

Fish provide an excellent study group for studying convergence because isolated bodies of water (e.g., drainage basins, ponds, streams, lakes, rivers or oceans, or even regions within these habitats are sufficiently different, either ecologically or spatially), providing separate, self-contained evolutionary crucibles. Numerous classical examples of convergence exist in fishes, in a wide range of systems. Some examples are trophic convergence (Keast & Winemiller 1991), benthic and limnetic forms (Rundle & Schluter 2004), flow gradients (Langerhans *et al.* 2003) and predator associated behavior (Arendt & Reznick 2005), life history (Reznick *et al.* 2007), and morphology (Langerhans & DeWitt 2004). Predator associated morphology is seen in *Gasterosteus aculeatus* (the three-spine stickleback), *Poecilia reticulatea* (guppies) and *Gambusia* (mosquitofish) (Colismo *et al.* 2005; Cresko *et al.* 2004; Endler & Reznick 1982; Langerhans *et al*, 2004).

Livebearing fish of the family Poeciliidae (hereafter "livebearers") have become a particularly widely studied group, shedding light on evolution of life-history traits, morphological evolution, and evolutionary responses to predation. They are good model organisms because they live in a broad range of physical habitats and often adapt well to laboratory use, including breeding. Many species are highly fecund and/or extreme-environment hardy. Testament to their adaptability, *Gambusia* is now considered to be the most invasive fish worldwide and the most widespread fish in the world, being represented on every continent except Antarctica (Alcaraz & García-Berthou 2007; Keller & Lodge 2009). Work on morphological diversification of livebearers across ecological gradients serves as a basis for the present work. In Chapter II, I explore further the shared pattern of phenotypic difference in body shapes of livebearers across predation gradients. DeWitt and colleagues (Langerhans & DeWitt 2004), demonstrated that for three species of livebearers, diversity across predator gradients is so repeatable that they could actually predict the predator regime of fish from multiple populations and species based only on knowledge of morphology. They showed through linear discriminant function analysis of body morphology that 78% of fish could be classified to the correct predator regime. This was constant across all three species of livebearers indicating that the evolutionary results of predation are highly conserved in Poeciliidae (Langerhans. & DeWitt 2004). It has been established that predation has a significant effect on the morphology of *Gambusia affinis* (Langerhans *et al.* 2004). Common predators for *Gambusia* are *Micropterus salmoides (*Largemouth Bass), *Lepomis cyanellus (*Green Sunfish) and *Lepomis macrochirus* (Bluegill) (Langerhans *et al.* 2004).

Langerhans *et al.* (2004) thoroughly studied six particular populations within Brazos County, Texas, USA: three containing these predators and three without them. They found that significant morphological shifts for males, females and juvenile mosquitofish as a result of predation regime. Langerhans *et al.* (2004) also found that morphological differences among populations had a heritable (i.e., quantitative genetic basis), and subsequent work as yet unpublished indicated a lack of phenotypic plasticity in mosquitofish body shape in response to predators. What remained undetermined was whether the replicated pattern of divergence observed among the six focal populations represented separate instances of divergent evolution. To address this, I performed a population genetic analysis of the six focal populations to determine whether I could detect evidence for either extreme: one evolutionary event, followed by differential colonization of habitats, or multiple separate evolutionary events. The population genetic analysis composes Chapter II of this Thesis.

A second focus of this Thesis, which is covered in Chapter III, expands from the intraspecific focus of Chapter II, to a broadly comparative analysis of six species of livebearers across a flow gradient. I compare three livebearing species from Lake Catemaco, Mexico, to their three ancestral species from the surrounding rivers. Lake Catemaco was isolated from the surrounding rivers 1-2 MYA and since then, several of the livebearers have taken new evolutionary paths (Mateos *et al.* 2002; McEachran & DeWitt 2008). Chapter III focuses on the idea that the fish in the lake were be subjected to different evolutionary pressures than those left in the streams, which could result in convergent divergence of the lake endemics toward a morphology more suited to lotic

environments. If convergent divergence has occurred, I expect to see: (a) very similar patterns of morphology among all three species of the lake ecotype and among all three species of the river ecotype; but (b) divergence between the two ecotypes, consistent with traditional patterns of convergence.

CHAPTER II

GENETIC ANALYSIS OF EVOLUTIONARY INDEPENCENCE OF REPLICATED BODY SHAPE VARIANTS IN LIVEBEARING FISH

Introduction

The course of evolutionary diversification is affected by both deterministic factors such as selection and assortative mating and stochastic factors such as genetic drift. However, only selection produces substantial systematic change in organismal form over time and space (Futuyma 2009). The systematic component of diversification should in principle lead to replicated patterns of divergence whenever the pattern of selection is replicated (Arendt & Reznick 2007; Hudson *et al.* 2011). However, even when separate lineages experience identical selection, random factors and any unique history leading up to a given period of diversification may reduce the fidelity of replicated divergence (Langerhans & DeWitt 2004; Langerhans 2010; Ruehl *et al.* 2011). It is interesting to ask then, to what degree can we expect convergent evolution (shared evolutionary response to a shared selection gradient) and to what degree have history and random factors impacted diversification?

Repeated or convergent evolution is the tendency for organisms of different lineages (e.g., species or populations within species) to adapt in similar ways to similar selective pressures. Gould (1989) suggested that if we were to be able to restart evolution from previous states, it would progress differently in each instance because of

the endless variables involved. However, it is clear that, in many instances evolution has played out in similar fashion, producing replicated adaptations in multiple instances of a given environmental context. To quote Gompel and Prud'homme (2009), "Similar solutions evolve in response to similar problems." Haldane (1932) said, "Related species will vary in similar directions and be subject to similar selective influences. They may therefore be expected to evolve in parallel." However, a repeated pattern of phenotype X in habitat 1 and phenotype Y in habitat 2 is not sufficient to infer replicated evolution. It could be that one instance of divergence occurred, and habitats were differentially colonized multiple times by the lineages with the best phenotype-environment matching (Ruehl *et al.* 2011). I would like to be able to determine when scenario 1, (one divergence followed by differential colonization) occurs, versus the alternative of separate instances of evolutionary divergence. Because convergent evolution is driven by similar selective pressures, it is essential to study selective pressures that we can be certain are held in common.

Common selective pressures that can lead to convergent evolution include habitat structure and limitation, availability of food, and predation. In fishes, predation has been found to be a particularly common cause of convergent evolution in several systems including guppies, sticklebacks, and *Gambusia.* For example, predation plays a significant evolutionary role in the retention or reduction of pelvic armor plates in the three-spine stickleback (Bell *et al.* 1993; Marchinko 2009). Armor plating is reduced in populations that do not have fish predators, whereas increased armor was found in populations where predators were present. In *Gambusia* and other livebearers, it has

been repeatedly documented that predation is a powerful evolutionary force driving phenotypic divergence (Langerhans & DeWitt 2004; Langerhans & Reznick 2009). The strong selection typically imposed by predation combined with the ease of determining whether a site contains predators or not, makes predation an excellent selective pressure to study.

This study focuses on a small livebearing species, *Gambusia affinis,* the Western Mosquitofish. I examined six populations of *Gambusia* in Brazos County, Texas, USA, representing two types of habitats. One habitat type contains larger predatory fish such as bass, green sunfish and blue gill, whereas the other habitat is free of predatory fish. Sampling included three populations of each habitat type. These six populations have been thoroughly studied with an emphasis on morphological differences associated with predation (Langerhans & DeWitt 2004). It has been shown repeatedly that populations of fish with piscivorous predators evolve a morphology associated with fast burst swimming speed. This morphology involves overall streamlining, reduction of the head and body cavity and an increase in the area of the caudal peduncle region. Their gonads increase in size, whereas all other organs in the body decrease in size (DeWitt unpublished results). Increased gonad size is a very common evolutionary response to predation. It is a simple tradeoff where organisms sacrifice other biological processes in favor of early sexual maturation and high fecundity (Reznick 1983). On the other hand, fish in non-predation environments, have much deeper bodies, larger heads, smaller caudal peduncle regions and smaller gonads. These fish are not adapted for burst swimming speed, but may be more suited for efficient navigation of complex habitats

such as reeds and grasses (Langerhans & Reznick 2009). This pattern is seen across a wide variety of livebearers including *Brachyrphaphis, Poecilia* and *Gambusia* (Langerhans & DeWitt 2004). These authors were able to use discriminate function analysis to correctly predict predator regime of individual fish across all three genera.

My study focuses on these six populations in order to determine whether we are truly seeing repeated evolution in these populations or whether the phenomenon stems from a common ancestor or standing variation. To address this question, I used a suite of seven microsatellite markers. I examined genetic, phenotypic (morphology), geographic and environmental distances between the six populations in an attempt to determine whether these three distances were correlated. If evolution is independent I expect to see stronger correlation between genetic and geographic distances than between genetic and phenotypic distances. If genetic distances are more closely tied to the phenotypic distances, then I would assume that evolution is not independent but stems from pre-existing variation that is brought about by predation.

Methods

Collection and Sites

Fish were collected using dip nets and seines from three sites lacking predatory fish and three sites with predatory fish. Predatory fish included native sunfishes, predominantly *Lepomis cyanellus, Micropterus salmoides* and *Lepomis macrochirus*. These sites were selected based on previous findings demonstrating predator driven divergent morphology that aids escape swimming for mosquitofish, (Langerhans *et al.*

2004, 2005; Langerhans & DeWitt 2004). Site locations and sample sizes are given in Table 2-1. The fish were euthanized and preserved in 75% ethanol. A small section of the caudal peduncle was removed in order to perform DNA extraction.

Table 2-1 Population characteristics

Shows locations, sample sizes and predation regimes of each of the six sample populations

Population	Location(Coordinates)	Sample Size	Predation Regime
Riverside Pond	30º38.1′N, 96º27.9′W	19	Predator
Riverside Ditch	30º38.0′N, 96º28.4′W	34	Non-Predator
Central Park	30º36.6′N, 96º17.6′W	20	Predator
Autumn Circle	30º38.4′N, 96º19.7′W	20	Non-Predator
Hensel Park	30º37.5′N, 96º20.8′W	17	Non-Predator
University Oaks	30º37.2′N, 96º18.8′W	21	Predator

Molecular Methods

DNA extraction was performed with the PUREGENE® DNA Purification Kit (Gentra Systems, Minneapolis, MN). Sample sizes consisted of 18 to 34 individuals per population. I used PCR to amplify seven previously described microsatellite loci (Spencer *et al.* 1999; Purcell *et al.* 2010), found to be variable in my samples. The protocol initiated with a 5 minute denaturation period at 95°C followed by 40 cycles of 30 seconds at 95°C, 30 seconds at the annealing temperature for each primer, and 1

minute at 72°C. The final extension period was 5 minutes at 72°C. The annealing temperatures for each primer, primer names and sequences are listed in table 2-2.

Table 2-2 Microsatellite loci

Shows sequences of the microsatellite loci used, repeat motif and the annealing temperature used

Locus	Primer Sequence	Repeat Motif	AT (°C)
Gafu2	F: CTC CAA ACA CAC GTC CAA TAA TC R: AGT TTC CCC AGC CGT TCA T	$(CA)_{17}$	53
Gafu3	F: CTCAGCCGTCATTTAGTCTCAT R: GCA CAT AAC ATG GAA ACA GTA AAC	$(GT)_{33}$	53
Gafu5	F: TGGGCCTTGTCTTGCTTT R: AAG CCG CGG ATA TTC ATG	$(GA)_7A_2(GA)_{11}$	54
Mf-6	F: ACGCCTATTGGTCGCCTGAT R:TTTGATTTCCTGGATTCTGACTGA	GT	54
Gafu7	F:CACAGAACAACACAGAAACTGGAGG R: TGC CGA TGG ATG TTC CTG TTA G	$(AG)_{22}$	55
Gaaf9	F: GGTGCAAATCCGCAGCTTG R: *GGGAAATACTCCTGGACTCG	$(ACAG)_{14}$	55
Gaaf11	F: ACTCAAGGCTGCCATACTGC R: *GGACTTAAGAGTGCCATCTGTC	$(ACAG)_{16}$	55

PCR products were run on agarose gels to confirm amplification. If amplification was confirmed, PCR products were visualized in an ABI 377 automated sequencer with the Genescan ®-400 HD Rox Size Standard (Applied Biosystems) for sizing. I carried out allele sizing and scoring using Applied Biosystem's Genescan ® 3.1.2 and Genotyper ® version 2.5 software and 95% coverage was attained (i.e., Genescan was able to score 95% of the samples automatically). The remaining 5% were interpolated manually (for the ANOVA only) in order to avoid errors that GenAlEx makes by failing to average over entire loci. The resulting data were imported into GenAlEx (Peakall & Smouse 2006) which was then used to generate pairwise Nei's

genetic distances. I obtained summary statistics for data (Table 2-3), including allelic richness, inbreeding coefficient F_{IS} and pairwise F_{ST} (Table 2-4) with FSTAT v. 2.9.3.2 (Goudet 1995). Number of alleles, expected heterozygosity and observed heterozygosity were obtained with GenAlEx (Peakall & Smouse 2006). I also used GenAlEx and Genepop (Raymond & Rousset 1995; Rousset 2008) to test loci for Hardy-Weinberg equilibrium and linkage disequilibrium and to determine the number of distinct populations. Any loci found to be out of Hardy-Weinberg equilibrium were then examined with the program Bottleneck (Maruyama & Fuerst 1985; Cornuet & Luikart 1999) under the infinite allele model, to determine if founder events or recent reduction in effective population size was a likely cause of the disequilibrium. In addition, loci were examined utilizing Microchecker and FreeNA (Chapuis & Estoup 2007; Oosterhout *et al.* 2005), in order to test for null alleles and potential scoring errors due to stuttering.

Analysis

For comparison with the genetic distance matrix, I estimated phenotypic, environmental, and geographic distance matrices between populations. The phenotypic distance matrix was inferred with the original morphometric data from these populations reported in (Langerhans & DeWitt 2004). Landmark data (10 landmarks per fish) were superimposed and subjected to principal components analysis to remove null vectors. These principal components were then used to calculate a general Euclidian distance matrix among populations. This provides a distance matrix showing the most closely

related individuals based upon their morphology. The environmental distance matrix among populations was calculated with dummy variables (1 and 0) to indicate whether two populations shared or did not share a predator regime respectively. Two geographic distance matrices were calculated: (a) linear distance between latitude and longitude coordinates ("crow-flies" distance); and (b) drainage connection lengths ("fish-swims" distance). The drainage path distances follow the connections between sites that would occur during floods, based on an interactive version of FEMA's Preliminary Digital Flood Map for Brazos County, Texas (http://ims.bryantx.gov/fema/viewer.htm). This was the final geographic distance matrix used because it was found to show better correlations and because of the nature of the system. Since this study examined aquatic organisms it is more logical to use the drainage distance than "crow-flies" distance.

In GenAlEx (Peakall & Smouse 2006), I also conducted an analysis of molecular variance (AMOVA), focused on the predator regimes. This analysis was conducted to provide additional support for our hypothesis. If a significant portion of the variance lies among the two predator regimes then it would suggest that there was a single evolutionary event followed by adaptive radiation. However, if the variance lies within the predator regimes then this would lend further support to the independence hypothesis.

In order to assess whether the phenotypic differentiation observed was consistent with a single genetic diversifying event, or multiple independent events, I provide a graphical interpretation to visualize genetic distance. I performed UPGMA cluster

analysis (Figure 2-2) in JMP (version 5.0, SAS, Cary, N.C.) utilizing the microsatellite loci in order to determine which populations were most closely related.

To test for associations between population matrices for genetics (G), phenotype (P), environmental (E) and "fish-swims" geographic (L) distances. Significance of the matrix correlations were assessed by bootstrapping to a total of 9,999 randomizations. If a single differentiation occurred followed by differential colonization, I would expect the three predator sites to be more genetically similar to each other than to the three no-predator sites. This would result in a strong pattern of association between the G and E matrices. In contrast, if G were more closely correlated with L it would be consistent with independent origins.

Results

Six distinct genetic populations were detected using GenAlEx. None of the loci were in linkage disequilibrium and therefore apparently are unlinked. These populations exhibited considerable deviation from Hardy-Weinberg equilibrium. Of 42 tests for Hardy-Weinberg equilibrium, 14 were found to be in equilibrium while the remaining 28 were out of equilibrium (Table 2-3). Due to the high number of loci in Hardy-Weinberg disequilibrium, I conducted a bottleneck analysis to determine whether evidence for a reduction in effective population size exists.

The bottleneck analysis showed that five of the six populations display excess heterozygosity under the infinite allele model (IAM), however, only two of these had p values below .05, which could indicate that those two populations have under-gone a

recent bottleneck event (Table 2-5). When effective population size is reduced due to a bottleneck or founder event, both the allele number and the heterozygosity are reduced at polymorphic loci evolving under the infinite allele model. However, the allele number is reduced more rapidly than the heterozygosity resulting in an observed excess heterozygosity (Maruyama & Fuerst 1985). However, this was only found under the IAM. However, the stepwise mutation model showed that the populations overall displayed heterozygote deficiency.

Using Micro-Checker (Oosterhout *et al.* 2005) and FreeNA (Chapuis & Estoup 2007), I found that two loci had potential miss-scoring due to stuttering, (Gafu-5 and Gafu-7). I also found that there were possible null alleles present in all loci other than Gafu 3 and Gafu 9. In order to determine what effect these factors had on the data I used FreeNA to calculate Cavalli-Sforza and Edwards genetic distances, both corrected and uncorrected for null alleles. These genetic distances were then compared and it was found that they only differed at the third decimal place.

Table 2-3 Summary statistics

*N: Number of samples genotyped; N_A/N_P: Number of alleles and private alleles detected; AR: Allelic richness as implemented by FSTAT; H_O: Observed heterozygosity as implemented by GenAlEx; H_E: Expected heterozygosity as implemented by GenAlEx. Asterisks significant departures from HWE calculated with GenAlEx. (*: p < .05, **: p < .01,***:p<.001;); F_{IS}: Inbreeding coefficient as implemented by FSTAT; Suggested null alleles by program MICRO-CHECKER indicated in italics.*

Hensel Park							Autumn Circle					
Locus	N	N_A/N_P	A.R.	H_O	H_E	F_{IS}	N	N_A/N_P	A.R.	H_O	H_E	F_{IS}
Gafu2	*17*	*6/0*	*3.812*	*0.471*	*0.702*	*0.357*	*20*	*6/0*	*3.916*	*0.500*	*0.738***	*0.345*
Gafu3	17	10/3	5.000	0.706	0.848***	0.197	20	18/4	3.193	0.550	0.911***	0.418
Gafu5	*17*	*3/0*	*2.000*	*0.176*	*0.611****	*0.726*	*20*	*4/1*	*1.000*	*0.250*	*0.666****	*0.64*
Mf-6	*17*	*10/0*	*2.970*	*0.529*	*0.836*	*0.392*	*20*	*9/0*	*3.161*	*0.800*	*0.850*	*0.084*
Gafu7	*17*	*8/0*	*4.000*	*0.588*	*0.804*	*0.297*	*20*	*13/1*	*2.941*	*0.600*	*0.871*	*0.334*
Gaaf9	17	4/0	3.000	0.529	0.455	-0.134	20	7/1	1.470	0.800	0.816	0.046
Gaaf11	*17*	*4/0*	*2.996*	*0.353*	*0.657****	*0.487*	*20*	*3/0*	*5.784*	*0.600*	*0.549*	*-0.068*
Central Park							Riverside Ditch					
Locus	N	N_A/N_P	A.R.	H_O	H_E	F_{IS}	N	N_A/N_P	A.R.	H_O	H_E	F_{IS}
Gafu2	*20*	*5/0*	*3.812*	*0.250*	*0.611****	*0.607*	*34*	*10/7*	*3.916*	*0.206*	*0.386****	*0.478*
Gafu3	20	17/3	5.000	0.750	0.846***	0.139	34	18/3	3.193	0.676	0.877**	0.243
Gafu5	*20*	*5/0*	*2.000*	*0.500*	*0.610***	*0.205*	*34*	*16/9*	*1.000*	*0.235*	*0.781****	*0.706*
Mf-6	*20*	*9/0*	*2.970*	*0.700*	*0.868**	*0.218*	*34*	*11/3*	*3.161*	*0.059*	*0.870****	*0.934*
Gafu7	*20*	*9/1*	*4.000*	*0.600*	*0.850*	*0.317*	*34*	*17/7*	*2.941*	*0.059*	*0.918****	*0.938*
Gaaf9	20	7/1	3.000	0.550	0.735***	0.276	34	8/2	1.470	0.529	0.839***	0.382
Gaaf11	*20*	*4/0*	*2.996*	*0.100*	*0.656****	*0.855*	*34*	*9/6*	*5.784*	*0.441*	*0.828****	*0.479*
University Oaks							Riverside Pond					
Locus	N	N_A/N_P	A.R.	H_O	H_E	F_{IS}	N	N_A/N_P	A.R.	H_O	H_E	F_{IS}
Gafu2	*21*	*7/1*	*5.351*	*0.429*	*0.705***	*0.413*	*19*	*10/4*	*5.351*	*0.263*	*0.551****	*0.542*
Gafu3	21	15/2	3.426	0.476	0.906***	0.493	19	13/2	3.426	0.316	0.742***	0.592
Gafu5	*21*	*4/0*	*2.000*	*0.190*	*0.681****	*0.732*	*19*	*9/0*	*2.000*	*0.053*	*0.755****	*0.934*
Mf-6	*21*	*9/0*	*3.085*	*0.714*	*0.795*	*0.125*	*19*	*8/0*	*3.085*	*0.263*	*0.663****	*0.62*
Gafu7	*21*	*12/0*	*3.999*	*0.571*	*0.885**	*0.376*	*19*	*8/0*	*3.999*	*0.579*	*0.792*	*0.294*
Gaaf9	21	6/0	1.991	0.524	0.714	0.289	19	3/0	1.991	0.684	0.532	-0.261
Gaaf11	*21*	*4/0*	*4.576*	*0.762*	*0.577*	*-0.298*	*19*	*6/2*	*4.576*	*0.474*	*0.769****	*0.407*

Table 2-4 Pairwise F_{ST}

Calculated by FSTAT All values are significant with a p<.00333

	Hensel Park	Autumn Circle	University Oaks	Central Park	Riverside Ditch	Riverside Pond
Hensel Park	0.0000					
Autumn Circle	0.0622	0.0000				
University Oaks	0.0506	0.0247	0.0000			
Central Park	0.0702	0.0599	0.0631	0.0000		
Riverside Ditch	0.1380	0.1012	0.0922	0.1064	0.0000	
Riverside Pond	0.0963	0.0962	0.0852	0.0760	0.1150	0.0000

Table 2-5 Bottleneck analysis

Observed and expected number of loci for each population that display excess of heterozygosity.

Population	# of loci with excess heterozygosity	# of loci with deficient heterozygosity	# of loci expected to show het excess	Probability
Hensel Park	6	1	4.1	.14034
Autumn Circle	7	0	4.12	.02443
University Oaks	7	0	4.17	.02677
Central Park	6	1	4.15	.14892
Riverside Ditch	5	2	4.23	.42855
Riverside Pond	3	4	4.15	.30489

In addition, the same distances were calculated following removal of the two loci that displayed potential stuttering. Again these genetic distances differed only at the third decimal place and resulted in nearly identical correlations when used for a Mantel test. These analyses seemed to indicate that the data were of sufficient quality for the analyses.

The results of the AMOVA on predator regime showed that 82% of the variance was within predator regimes ($P=5.24x10^{-17}$), while only 4% was found among regimes

and the remaining variance was within individuals (i.e., heterozygosity). My pairwise Mantel tests analyzed correlations between four distance matrices genetic (G), phenotypic (P), environmental (E) and geographic distances (L). Of the six pairwise Mantel tests, four resulted in non-significant correlations between the effects (Table 2-6). Only two correlations were significant: the correlation between genetic (G) and the geographic (L) matrices (p=0.041204) (figure 2-1). The second significant correlation was between the phenotypic (P) and environmental (E) matrices (p=0.03910).

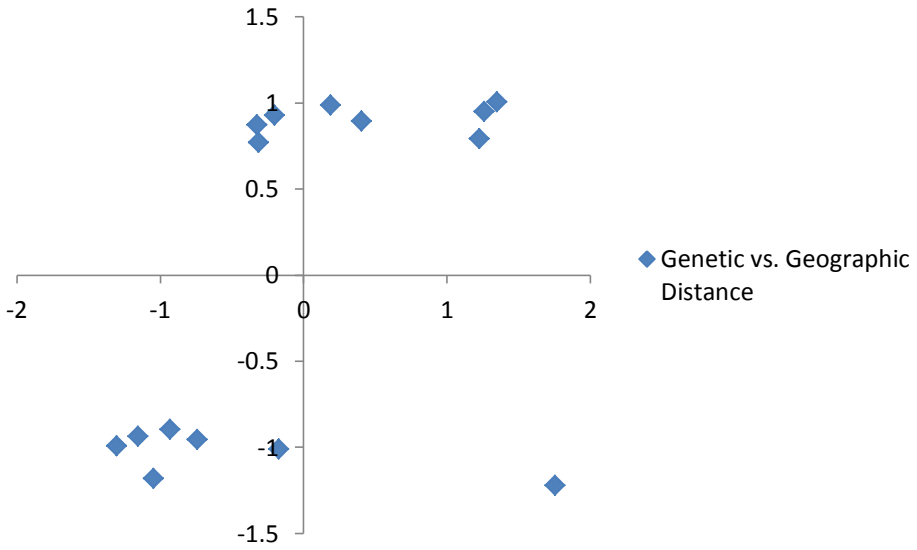

Figure 2-1 Correlation of genetic and geographic distance.

Table 2-6 Mantel tests correlation coefficients and p-values

Test Effects	Correlation Coefficient	P-value
rG-L	0.467053	0.041204
rP-G	-0.02375	0.448345
rG-E	-0.10627	0.655366
rP-L	-0.19384	0.75988
rL-E	-0.1978	0.69047
rP-E	0.4515	0.039104

I also conducted multivariate analysis of molecular variance (MAMOVA) and cluster analysis in JMP (version 5.0, SAS, Cary, N.C.) in order to generate a figure to visually represent the populations genetic relationship to one another. This analysis shows the genetic relatedness between the populations based upon the microsatellite data. My hypothesis was that groupings would be linked to location rather than predator regime. With the exception of the RD population, the cluster analysis seemed to cluster fairly tightly. This provides us with very little information about the genetic similarity of these populations. The RD population clustered separately from all other populations, (figure 2-2).

Discussion

The replicated pattern of phenotypic diversity across predatory and non-predatory sites did not appear to be due to a single historic divergence followed by differential colonization of habitats. Overall the six populations appear genetically

distinct based upon population differentiation tests performed in GenAlEx, and there was a pattern of increasing genetic distance with geographic distance (see Table 2-6). The significant correlation between marker genetic distance and geographic distance suggested that populations that are most genetically related are those that are spatially closer to one another. This result seems to support the hypothesis that phenotypic evolution in these populations is independent and not a result of a single evolutionary event. The only other significant relationship was between phenotype and environment which is not at all surprising given that this relationship has been previously established by Langerhans & DeWitt (2004). Thus, the main finding of this study is that predator-associated morphology seems to have evolved in genetically differentiated populations indicated by the lack of correlation between the genetic and phenotypic matrices, and significant correlations between the genetic and geographic matrices. These findings are consistent with independent and convergent evolution of morphology, representing repeated evolution.

The population genetic results are consistent with expectations for species inhabiting stochastic environments. A majority of the loci were out of Hardy-Weinberg equilibrium. This could result from any of several processes, including selection or sampling error caused by fluctuations in population size. Selection does not seem possible as microsatellite loci are not transcribed and the odds of all seven loci being closely linked with selected loci seem remote. However, *Gambusia* tend to be boom and bust species because many of their habitats are small ponds and drainages which dry seasonally during the summer, suggesting that sampling error is a likely cause for

disequilibrium. In particular the RD population persisted through many in which there appeared to be complete habitat drying. The fish may have persisted in small reserves in concrete culvert pipes, or cracks therein, much as other *Gambusia* populations have been found to persist in karstic fissures in habitats that seasonally dry (Kozba *et al.* 2004). As a result of such events populations often contract through periods of very small size. It is not at all uncommon for populations to go through these bottlenecks or to go extinct and then be recolonized when the rains return. The RD population is located in a drainage ditch, which dries nearly every year with the exception of a single culvert that maintains a small amount of water. As a result I would expect this to subject the population to intense and repeated bottleneck events. This may explain why the RD population clustered so far from all other populations in the cluster analysis. Because of these deviations from Hardy-Weinberg and the ephemeral nature of *Gambusia* populations we conducted a bottleneck analysis assuming the infinite allele model. This analysis revealed that five of the six populations displayed heterozygosity excess, which may be an indicator of recent reduction in effective population size or bottlenecking. However, other measures of heterozygosity such as the stepwise mutation model showed heterozygote deficiency. Despite these conflicting results, the IAM bottleneck analysis coupled with what is known about the biology of these systems seems to suggest that these populations may have experienced a recent bottleneck event. All of the

populations that appeared to have experienced bottlenecks were small ponds and drainages typical of ephemeral populations. The one population that did not appear to experience a bottleneck was Riverside Pond. Riverside Pond differs from the other habitats in that it is a fairly large lake that never completely dries up. As a result of this the Riverside Pond population is much more stable than the others.

I expected the cluster analysis to show populations that were geographically close to be more closely related on a genetic level. This would support the hypothesis of repeated independent evolution. If the populations grouped by predator regime, however it would indicate that the hypothesis is incorrect. The cluster analysis was not very conclusive. However, because of the relatively small number of populations and the fact that the Autumn Circle, Hensel Park and University Oaks populations are approximately equidistant from each other and group closely together in the cluster analysis, this is not sufficient evidence to conclude that the predation morphologies are the product of independent evolution.

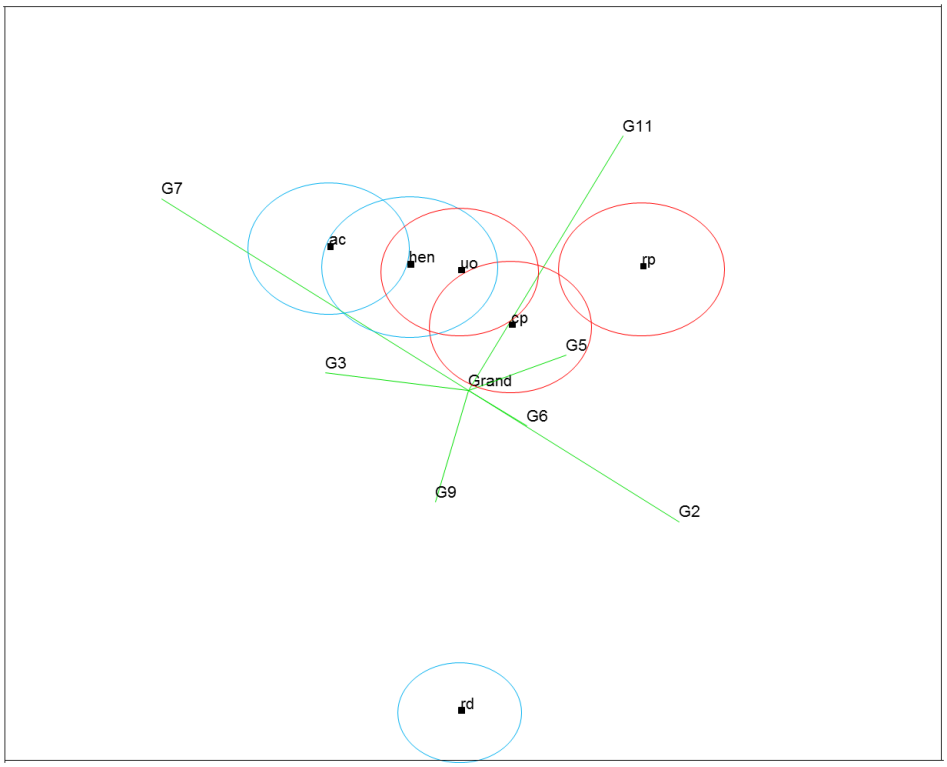

Figure 2-2 Cluster analysis

Centroid plot for six populations of Gambusia affinis in Brazos County. MAMOVA using the seven microsatellite loci produced this plot. Each axis represents one of the loci while each centroid circle represents a population. Predator populations are displayed in red and non-predator populations are displayed in blue.

While the cluster analysis does not provide sufficient evidence alone to conclude that evolution is independent, the Mantel results seem clearer. I saw no significant correlation between genetic distance and phenotypic distance, which would have indicated evolution that was not independent but rather stemmed from common ancestry. Instead the two relationships that exhibited the strongest support were genetic distance and geography, which would indicate independence of evolution, and phenotypic and

environment which has been established previously. This seems to supports the independence of evolution hypothesis and to indicate that these fish populations are indeed evolving independently and not as a result of ancestral variation and radiation.

The evidence provided in this study is somewhat conflicted because of the ambiguity of the cluster analysis; however, mantel tests seem to support the hypothesis that morphological evolution due to predation is independent in the populations of livebearers in this study. This indicates that evolution is occurring in the same way for each population separately rather than one ancestral evolutionary event followed by variable selection in the sites. While further study is necessary to be certain that convergent evolution is occurring, the evidence provided by this study and previous morphometric studies seems to provide strong support for this conclusion.

CHAPTER III

REPLICATED LAKE-ECOTYPE EVOLUTION DURING SPECIATION IN THREE FISH LINEAGES

Introduction

The determinism of evolution has been argued since before Darwin. In modern understanding, we know that evolution is driven by both chance and deterministic agents, and their interaction. Chance factors in evolution include mutation, random migration, genetic drift and accidents of fate (e.g., random mortality, bottlenecks). The only force that can change allele frequencies directionally is selection. However, selection does not always produce diversification in predefined pathways.

The path of diversification under selection is in part predictable by knowing the optima (i.e., relatively high-fitness trait combinations). Yet many factors complicate the path of diversification under selection. Such complicating factors include alternative genetic architectures and historical events arising in any given diversifying gene pool. Given that both chance and deterministic factors are at play in evolution, a classic question is whether evolution would repeat itself if somehow the time course of evolution could be set back to a given state and started anew. Would we still see dinosaurs evolve to predominantly flighted descendants? Would they have feathers? Though such a time-shifting experiment appears impossible, an analogous situation presents as replicate diversifying gene pools can be identified with similar recent

histories of selection. If lineages that experience similar environmental conditions evolve similar phenotypic solutions, this would suggest determinism wins the day. If instead radically different trait combinations emerge in similar environments, then chance factors overwhelm deterministic processes.

It is becoming increasingly common to partition the amount of evolutionary divergence across a given selection gradient into repeatable (i.e., shared, deterministic), and unique (lineage-specific) evolution by means of a simple statistical technique (Langerhans. & DeWitt 2004). For example, Langerhans *et al.* (2006) used this method to assess shared and unique elements of divergence in *Anolis* lizards. Studies of shared and unique variance have focused upon populations within a species (e.g. Langerhans *et al.* 2006), and many have focused on diversification of multiple populations for multiple species (e.g. Langerhans & DeWitt 2004; Ruehl *et al.* 2011). Intraspecific focus is important for elucidating the tempo and mode of divergence at the ecological and short-term evolutionary time scale (Futuyma & Moreno 1988; DeWitt *et al.* 2000). Presumably these processes (shared and unique diversification) may occur over long periods leading to speciation, if multiple incipient species experience the same or similar environments relative to the ancestral species (Haldane 1932; Schluter 1993, 2000).

Historically evolution was thought to occur through long periods of allopatry, but there is evidence that shows that it can also occur sympatrically (Johannesson, 2001; Schluter, 2000). This appears to be the case for many taxa. For sticklebacks and anolis lizards (Losos 1992, 1994; Rundle & Schluter 2004), it appears colonization by a single

gene pool promoted sympatric diversification into separate ecological niches, followed by ecological speciation (Rundel *et al.* 2000; Colismo *et al.* 2005). In these systems, each time a new colonization of the same ecological niche space occurs, "convergent divergence" follows. Convergent divergence is divergent evolution that evolves toward similar phenotypes in particular niches. In many instances of convergent divergence, it is unclear if a single gene pool diversified, or if multiple separate gene pools diverged. If separate species are exposed to a shared gradient of selection long enough, presumably speciation and convergence may happen simultaneously, a phenomenon we call "convergent speciation." Convergent speciation has only loosely been used in the literature, to mean at least two different things, and appears more often in common vernacular without formal reference. To be clear, we use the term as a simple extension of convergent evolution, but to mean convergent evolution by separate species during the process of speciation. Cases of convergent speciation would be most clear in cases where the same evolutionary crucible, such as environmental conditions during speciation were most likely shared by all species involved. With a shared evolutionary crucible, selection exerted upon the evolving lineages is not only similar, but potentially identical.

In the present study we document body shape diversification that took place in three species of livebearing fishes isolated for approximately 1.2 million years ago in a single lake, Lake Catemaco, Veracruz, Mexico, during which time each evolved into a new species endemic to the lake. We compared the derived lake endemics to their sister

lineages in rivers surrounding the lake, and addressed both the nature and magnitude of shared and unique divergence during evolution.

Based on geological and phylogenetic inference, Lake Catemaco appears to be approximately 0.75 to1.5 million years old, being formed from quaternary lava blockage (West 1964; Mateos *et al.* 2002). The lake is large (75 km²), shallow (7.7 m average depth) and is isolated from lower drainages by a 55 m tall waterfall. The lake has 12 native species, 10 of which are demonstrated or thought to be endemics (Miller and Conner 1997). The focal lake endemics in our work are *Poeciliopsis catemaco*, *Xiphophorus kallmani* and *Heterandria tuxtlaensis*, whose close relatives in the surrounding rivers are the cosmopolitan species *P. gracilis*, *X. helleri*, and *H. bimaculata*.

Methods

Fish were obtained from museum holdings including those of the Texas Cooperative Wildlife Collection, American Museum of Natural History (AMNH), Field Museum of Natural History (FMNH), Gulf Coast Research laboratory (GCRL), Tulane University Fish Collection (TU), Universidad Nacional Autónoma de México (UNAM), and University of Michigan Museum of Zoology (UMMZ). The specimens were collected in the late 1960s.

We used geometric morphometrics to characterize the two-dimensional multivariate shape of each species (Dryden & Mardia 1998). Fish were x-rayed in lateral perspective and x-ray film was scanned at a resolution of 31.5 pixels·mm^{-1} into

digital images. Sixteen landmarks were digitized on each fish image as indicated in Fig. 3-1 (McEachran & DeWitt 2008). We calculated centroid size of landmark conformations for use as a covariate in subsequent statistical analyses. Landmark configurations were then superimposed (centered, scaled to unit size, and rotated to minimize the sum of squared deviations relative to a target conformation). Superimposed landmarks were then entered into principal components analysis and null vectors were dropped, resulting in 28 shape variables for subsequent analysis. Landmark notation was performed in TpsDig 1.39 (Rohlf 2003). Centroid size calculation and superimposition was done with TpsRelw 1.46 (Rohlf 2008), with orthogonal projection and no adjustment to scale of variation (i.e. alpha=0).

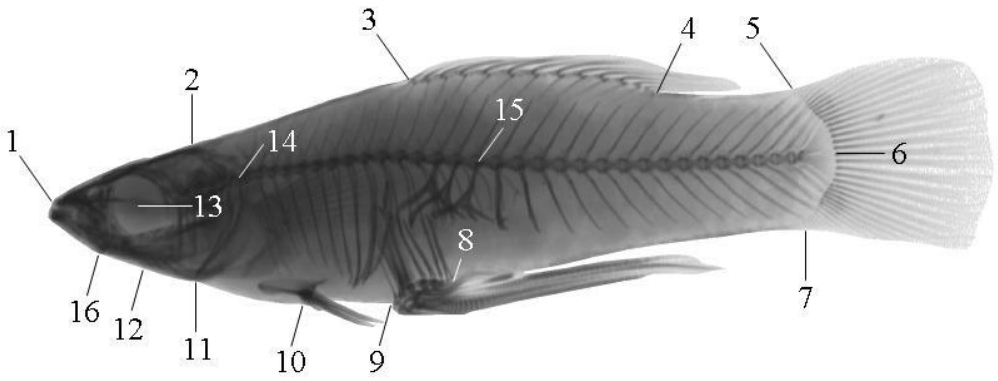

Figure 3-1 Radiograph negative of a male H. tuxtlaensis

Shows landmarks used for the body shape analysis. 1 – anterio-dorsal-most position on the snout; 2 – top of head where skull breaks away from the body outline; 3, 4 – origin and insertion of the dorsal fin, respectively; 5 – dorsal origin of the caudal fin (anterio-dorsal-most procurrent ray); 6 – middle of caudal fin base (between hypural plates); 7 – ventral origin of caudal fin (anterio-ventral-most procurrent ray; 8, 9 – insertion and origin of anal fin, respectively; 10 – anterior margin of pelvic fins; 11 – anterio-ventral corner of interoperculum; 12 – first branchiostegal ray at the body outline; 13 – center of orbit (eye position); 14 – junction between cranium and first vertebral centrum; 15 – vertebral centrum bearing third gonapophysis; 16 – reticular point of lower jaw.

Shared and unique divergence of shape was assessed with multivariate analysis of covariance (MANCOVA) following Langerhans & DeWitt (2004). Shape variables were assessed for association with genus, the environment (lentic or lotic), centroid size, and interactions of these effects. Non-significant interactions with the covariate were removed from the statistical models. Males and females were analyzed separately because of the marked sexual dimorphism in livebearers (Langerhans *et al.* 2004). Following MANCOVA we also conducted linear and quadratic discriminant functions to obtain intuitive (heuristic) information on how successfully we could predict fish habitats (lake versus river) based on canonical scores from our main analysis. To run the DFA's we ran first the respective MANCOVA minus the habitat effect and interactions with habitat. Residuals from these MANCOVAs were used in DFA. PCA, MANCOVAs and DFA were conducted in JMP version 5.0.1.2 (SAS Institute 2003).

Visualizations of shape effects were generated in TpsRegr 1.37 (Rohlf 2009) by entering the design matrix used by JMP as the independent variable, with raw coordinates as dependent variables.

Results

We used multiple analysis of covariance (MANCOVA), to determine which effects were significant sources of divergence and to estimate the strength of those effects. The three-way interaction with the covariate (sizexgenusxhabitat) was not significant and was removed from the model. All effects in the reduced model were significant, with the strongest effects generally being those due to genus and the shared

habitat effect (Table 3-1). Thus, despite differences in shape between genera, all converged on a similar morphology as they evolved to become lake endemics." We found that, in males, genus and habitat had equally strong effects, (η_p^2=.92). The interaction between genus and habitat was the third strongest effect (η_p^2=.82). In females, genus was slightly stronger than habitat (η_p^2=.93), whereas the partial eta squared value for habitat was .89. The interaction between genus and habitat was again the third strongest effect (η_p^2=.84). Visual representations of the shared and unique effects are shown below in (figure 3-2). In males, we see clear distinction between lentic and lotic fish, with no overlap in their morphologies. The female plot, however shows lake *Heterandria* overlapping with the river-specific morphologies.

Discriminant function analysis was conducted to predict ecotype for both males and females. 68% of males and 71% of females were correctly classified with regard to habitat, but with no regard to genus (Table 3-1). Correct classification across all six species, three lotic and three lentic shows that evolution due to habitat is not only repeatable but predictable. Repeatability of evolution was also detected within each sex, which is unusual for livebearers, which are subject to wide ranges of sexual dimorphism especially during stages of pregnancy (Langerhans *et al.* 2004). We were able to correctly predict which habitat a fish came from based upon the canonical axis for habitat. This means that simply by looking at the shape of a fish we can predict whether it comes from the lake or the surrounding rivers. This prediction has nothing to do with the genus of the fish tested; only its habitat, which shows that the habitat effect is a highly significant source of evolutionary pressure for Catemaco livebearers

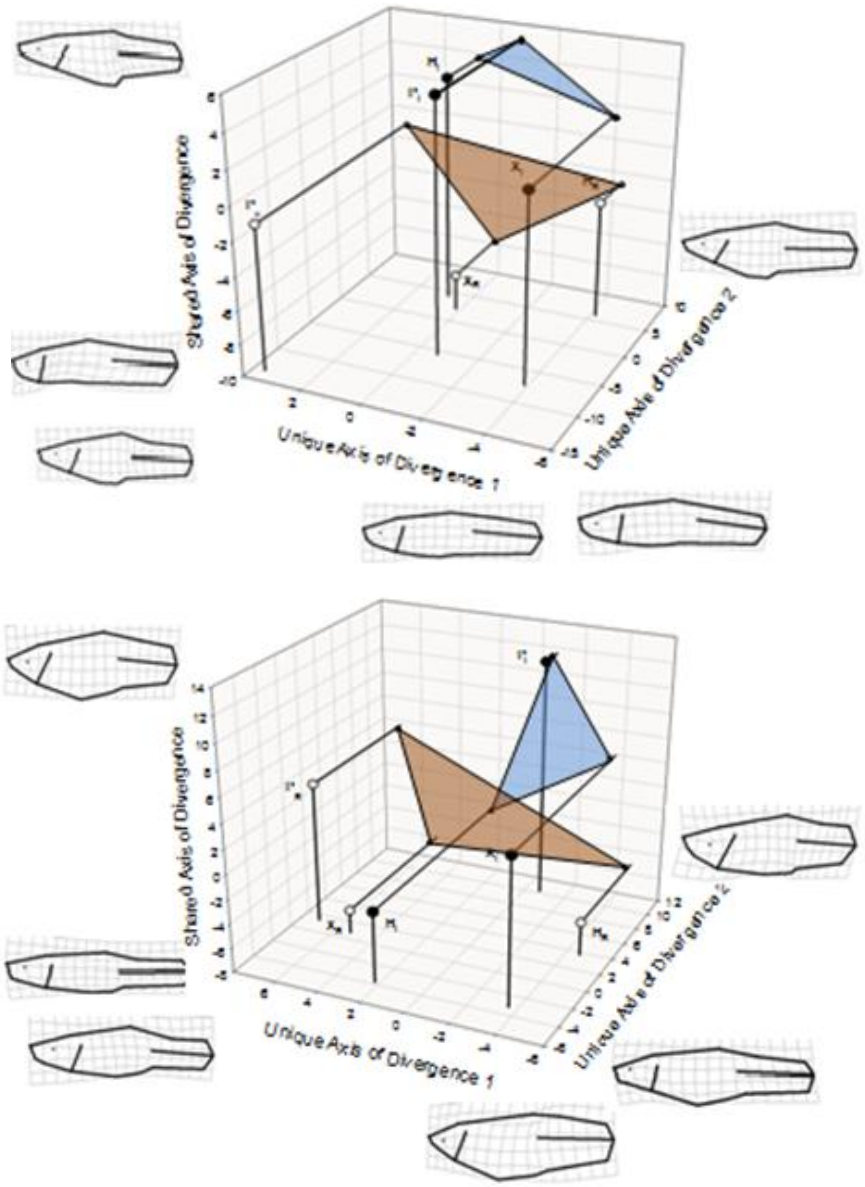

Figure 3-2 Shared and unique axes of morphological diversification

Shows diversification between environments for the three species by sex (males top) (females bottom). The vertical axis depicts the habitat canonical axis. Horizontal axes depict the two canonical axes derived from the interaction term of the MANCOVA. Genus and habitat type abbreviations are as follows: (L=Lake, R=River, H=heterandria, P=poeciliopsis, X=xiphophorous). Thin-plate spline transformation grids illustrate morphological differences described by each axis (magnified x3). Convex hulls (shaded triangles) were projected to help visualize the shared nature of divergence across habitat types, blue=Lake, brown=River.

Table 3-1. MANOVA and DFA

Shows effects of habitat, species, and size on diversification of the three focal species. Discriminant function analysis results showing the percentage of fish correctly classified to habitat type

Sex	Effect	DF			Λ	s	F	P	η_p^2	Correctly classified
		effect	num	denom						
Males	Genus	2	56	96	0.0067	2	19.2	1.3E-33	0.92	
	Habitat	1	28	48	0.0810	1	19.5	7.9E-18	0.92	68%
	Habitat × Genus	2	56	96	0.0324	2	7.8	1.8E-18	0.82	
	Size	1	28	48	0.2992	1	4.0	1.2E-05	0.70	
	Size × Genus	2	56	96	0.2788	2	1.5	0.0331	0.47	
	Error	75								
Females	Genus	2	56	96	0.0051	2	22.4	2E-36	0.93	
	Habitat	1	28	48	0.1059	1	14.5	3.5E-15	0.89	71%
	Habitat × Genus	2	56	96	0.0262	2	8.9	2E-20	0.84	
	Size	1	28	48	0.4027	1	2.5	0.00218	0.60	
	Size × Genus	2	56	96	0.2465	2	1.7	0.00864	0.50	
	Error	-9								

Discussion

Both shared and unique effects were observed to be strong, with shared effects being the larger part of phenotypic diversification in these lineages (Table 3-1). The nature of the shared response involved the evolution and speciation of endemic fish under lentic conditions, i.e. three species evolving in the same habitat. This effect involved lake fish evolving a shorter but centrally deepened body, medial fin displacement toward center, concomitantly shortened peduncles, and elongated heads. These transformations are standard lentic adaptations, wherein the body-plan is remolded from fusiform toward (but not fully achieving) planiform shape, to facilitate lateral maneuverability, and from compact to elongated heads/snouts to facilitate planktivory (Mittelbach *et al.* 1999; Ruehl & DeWitt 2005; Winemiller 1991). Lateral maneuverability and planktivory are functions of great use in lakes but of limited utility compared to the need for efficient steady swimming and low plankton availability of rivers. We did not measure trait function in this study and our morphological survey is rather gross, overall body shape, but the fit to ecomorphological expectations is clear. Decrease in dorsal fin insertion length, especially due to anterior advance of the posterior insertion, increase in body depth about the centroid, posteriorly displaced pelvic and anal fins, and longer and elongated head, involving longer and shallower head, particularly with elongated snout in which the mouth assumes a slightly smaller, more upturned mouth. Lake fish also have anterior procession of the hemophoesis bearing vertebra, and even more advancement of the caudal fin insertion, resulting overall in shorter peduncles (i.e. a shorter post hemal vertebral run). A good surrogate

for body cavity would be the polygon enclosed by landmarks 11, 14, 15, 9, and 10. Lake and river fish appear similar in body cavity size, at least in two dimensions (Figure 3-3).

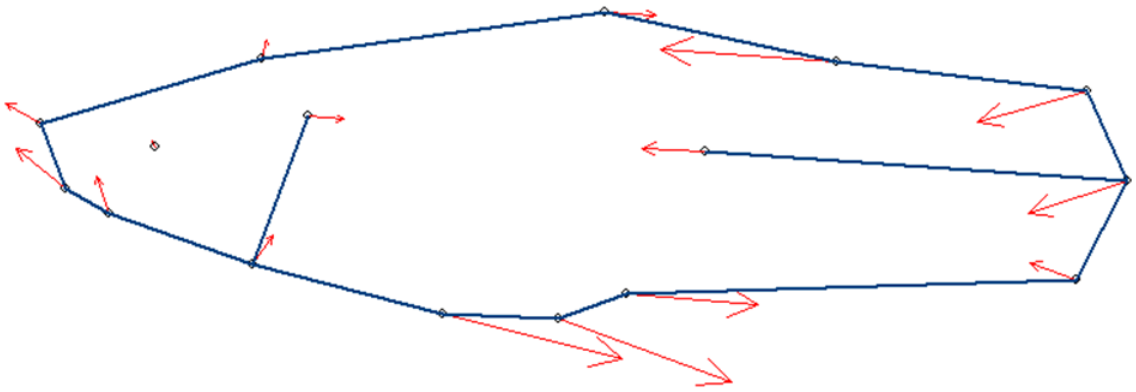

Figure 3-3 Evolutionary shift of landmarks

Landmark shifts affected by the differential habitat types. Arrows indicate the directionality and magnitude of the shift when river type fish were subjected to lake habitats resulting in speciation.

Unique effects largely involved different, species-specific magnitudes of the general response. Thus strict comparison of the partial etas would tend to inflate the unique effects. Adams & Collyer (2009) proposed a method to isolate interaction variance due to varied responses to selection by different species, but this approach was not employed here due to the weaker magnitude of the interaction effects. Unique effects that are more qualitative involved more complex elements of diversification, which are difficult to extract. However, the importance of this study is the convergence of evolution; therefore we are only concerned with the shared elements of divergence.

The functional significance of unique elements of diversification would be interesting to understand, but are not accessible at present. Functional understanding can

be achieved only through detailed comparison of all the species, and likely a microhabitat analysis, etc. Only part of these unique effects would be intelligible after such an effort, because much of the unique effects will be due to chance historical events that cannot be repeated or reconstructed. A more detailed description and illustrations of both the shared and unique aspects of diversification are available in Figures 3-4 and 3-5.

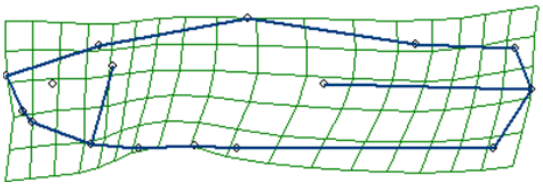

Figure 3-4 Thin-plate spline river morphology. Grids illustrating typical river type morphology

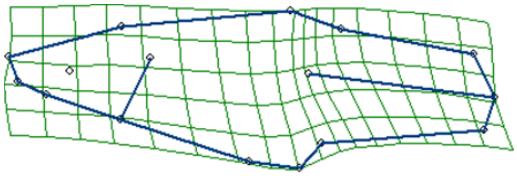

Figure 3-5 Thin-plate spline lake morphology. Grids illustrating typical lake type morphology

Male and female fish were analyzed separately due to historical findings that great sexual dimorphism exists. Female livebearers tend to display much weaker effects when analyzed using geometric morphometrics (Langerhans and DeWitt 2004). This is due largely to the high variability of body shape throughout stages of pregnancy. This variation tends to dilute effects of selective pressures. Because of this, we analyzed the sexes separately in order not to dilute the male results with the female noise. However, we found that in this case female effects were nearly as strong as male effects (Table 3-1).

All fish were subjected to discriminant function analysis using JMP to predict habitat (Table 3-1). The predictability of evolution shows the repeatability of evolution

via similar evolutionary pressures. We were able to correctly predict the ecotype of fish 68%-71% of the time. This indicates that evolution is most likely repeated and the morphological shifts are repeated, (i.e. all six species evolve similar morphologies with respect to their habitats).

The fact that in males, the genus and habitat effects are equally strong shows how strong selection due to habitat must be in this system. It is surprising that in the present study, variation in body shape within genera is as strong as variation between genera. By definition in traditional (morphological) systematics, taxa related at finer scales are more similar in morphology.

These results demonstrate strong repeatability of diversifying convergence, suggesting that deterministic elements of selection were stronger than all chance factors at work in both evolutionary mechanisms (e.g. mutation, drift), and unique phylogenetic historical factors. That determinism wins the day should not be surprising given the results of single lineage evolution resulting from selective pressures. This study addressed the topic at the level of speciation. Since the species in this case evolved together at one site, we deem the result convergent speciation. Convergence (i.e. the shared effect), however it manifests itself, is the hallmark of replicated natural selection (Langerhans & DeWitt 2004).

The mounting number of studies showing convergent divergence, and the likelihood of more studies to come on convergent co-speciation, and the many recent studies on ecological speciation, point to the great power to understand evolution

through ecology. For well resolved environmental gradients, we can, and our increasing understanding of the deterministic nature of selection gives us further insight into the mechanisms and direction of evolution.

CHAPTER IV

GENERAL SUMMARY

There is extensive variation involved in the evolution of natural systems. This variability often makes it difficult to understand the forces involved in selection and the directionality of evolution as a result of these forces. This thesis provides empirical examples showing that the forces affecting evolution are not only comprehensible but in some cases predictable. I showed that forces such as habitat and predation, which exert strong selection on organisms do so in such a way that the effects are quantifiable. Because of this we are able not only to observe the results of these evolutionary forces but can make comparisons with other systems. This thesis showed that evolutionary reactions to strong selective pressures can be highly consistent across taxa, which makes evolution in these systems both convergent and predictable. Predictability implies extremely high levels of convergence, which was shown in this thesis. This predictability of evolution shows that despite the complexity of natural systems evolution still occurs in a consistent manner when it is the result of strong selective pressures.

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