GEOGRAPHY, COLORATION AND SPECIATION
IN A GENUS OF NEOTROPICAL REEF FISHES
(GOBIIDAE: ELACATINUS)

A Dissertation

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

in

The Department of Biological Sciences

by
Michael S. Taylor
B.S., Central Missouri State University, 1986
December 2004
Acknowledgements

# Table of Contents

ACKNOWLEDGEMENTS ......................................................................................... ii

ABSTRACT ........................................................................................................... v

CHAPTER

1. INTRODUCTION ................................................................................................. 1

2. MARINE RADIATIONS AT SMALL GEOGRAPHIC SCALES ........................................ 3
   Materials and Methods .................................................................................. 4
   DNA Amplification ....................................................................................... 6
   Phylogenetic Analyses .................................................................................. 6
   Results ............................................................................................................ 7
   Geography ...................................................................................................... 8
   Habitat and Behavior ................................................................................... 12
   Coloration and Patterns ............................................................................ 12
   Discussion ...................................................................................................... 14
   The Geographic Scale of Speciation ........................................................... 14
   Radiation in Stages: Ecology and Color ...................................................... 15
   Conclusion ..................................................................................................... 17

3. GENETIC EVIDENCE FOR LOCAL RETENTION OF PELAGIC LARVAE .................... 18
   Materials and Methods ................................................................................ 18
   Otolith Analysis ......................................................................................... 18
   Genetic Analysis ......................................................................................... 19
   Coalescent Analysis ................................................................................... 20
   Results and Discussion ............................................................................. 20

4. COMPARATIVE PHYLOGEOGRAPHY AND BIOGEOGRAPHY ................................ 24
   Materials and Methods ................................................................................ 25
   DNA Amplification ....................................................................................... 25
   Genetic Analyses ......................................................................................... 27
   Results .......................................................................................................... 27
   Phylogeography of mtcyb .......................................................................... 27
   Geographic Variation of rag1 ...................................................................... 28
   Discussion ..................................................................................................... 30
   Biogeographic Barriers in the Caribbean .................................................... 31
   The Northwestern Caribbean Region ......................................................... 32
   Conclusion ..................................................................................................... 33

5. CONCLUSION ................................................................................................... 34

LITERATURE CITED ............................................................................................ 36

APPENDIX A: TABLE OF PRIMERS ................................................................... 46
APPENDIX B: LETTERS OF PERMISSION ................................................................. 47

VITA ....................................................................................................................... 50
Abstract

Studies of speciation in the marine environment have historically compared broad-scale distributions and presumed larval dispersal to infer the geographic barriers responsible for allopatric speciation. However, many marine clades show high species diversity in geographically restricted areas where barriers are not obvious and larval dispersal should bring sister taxa into contact. Genetic differentiation at spatial scales <1000 km could facilitate speciation by mechanisms other than the gradual accumulation of reproductive isolation during extended allopatry, such as ecological adaptation to local environmental conditions or the rapid evolution of genes tied to mate recognition. The role of each of these possibilities has not been simultaneously explored for any species-rich marine taxon. The most species-rich genus of Neotropical reef fishes is *Elacatinus* (Gobiidae), with 27 species. I examine potential mechanisms underlying this richness through analyses of three genetic markers to investigate genetic and ecological differentiation between closely related taxa and among island populations.

Phylogenetic analyses indicate that sister taxa of *Elacatinus* occur within the same oceans but occupy geographically separate ranges. Sister taxa usually differ by coloration, and distantly related sympatric species frequently differ by habitat. These differences suggest that some combination of coloration and ecological differences may facilitate assortative mating in sympatry or at range boundaries. The ranges of several *Elacatinus* taxa adjoin at Mona Passage and in the central Bahamas, both in the Caribbean Sea. These boundaries separate island populations by as few as 23 km, yet these populations are genetically distinct. Populations not separated by these breaks also show strong genetic structuring. Coalescent analyses suggest these populations have been demographically closed for up to 800,000 years. Such strong genetic structuring suggests that pelagic larvae are retained at natal populations, despite a three week larval duration (determined from otolith growth rings).

My results suggest that local retention of pelagic larvae, coupled with biogeographic breaks, has generated or maintained strong genetic population structure which may facilitate adaptation to local ecological conditions. Such adaptations may explain observed divergence along ecological and coloration gradients. Repeated radiations among allopatrically distributed sister taxa may explain much of the high diversity in *Elacatinus*. 
Chapter 1

Introduction

Coral reefs are renown for their species richness, yet the processes that have led to such high diversity remain a mystery. Speciation events underlying this diversity are routinely explained by allopatric speciation due to physical barriers to dispersal; however modern barriers are rarely apparent in oceans so other explanations must also be considered. Recent results suggest that speciation can result from ecological differentiation of geographically overlapping populations (Duffy 1996; Orr and Smith 1998). Interspecific morphological differences functionally tied to common resource utilization can reveal historical ecological separation when analyzed in a phylogenetic context. If sympatric sister species share ecological traits but differ morphologically, then character displacement due to resource competition might explain morphological differences and ecological differences may have contributed to speciation (Losos 1990). Alternatively, if species from different lineages share similar ecological and morphological traits but differ in geographic distributions, then classic allopatric speciation would be favored. Tests of these alternative historical hypotheses have yet to be applied in the marine environment.

To study such processes, I use gobies of the teleost family Gobiidae because, as the largest and most diverse family of marine fishes (Nelson 1994), gobies display a wealth of morphological, ecological and behavioral specializations. In the geographically compact region of the Caribbean Sea, gobies are the most diverse group of fishes found on coral reefs (Robertson 1998), yet they remain poorly studied. Gobies of the genus Elacatinus, the largest genus of any coral reef fish in the Caribbean, is an ideal model suited to address broad questions about speciation in the marine environment. The genus Elacatinus contains 27 described species in two subgenera: Elacatinus with 15 species and Tigrigobius with 12 species. The goal of my dissertation is to infer the phylogenetic relationships among the different species, then to use the phylogenetic information to explore how the diversity found in the genus Elacatinus may have arisen. Thus, my research has focused on genetic differentiation at both the species and the population level.

Chapter 1 focuses on mechanisms underlying the speciation process in the marine environment. Studies of speciation in the marine environment have historically compared broad-scale distributions and assumed larval dispersal potential to infer the geographic barriers responsible for allopatric speciation. However, many marine clades exhibit high species diversity in geographically restricted areas where barriers are not obvious and assumed dispersal potential should bring many sister taxa into contact. Genetic differentiation at spatial scales <1000 km could facilitate speciation by mechanisms other than the gradual accumulation of reproductive isolation during extended allopatry, such as ecological adaptation to local environmental conditions or the rapid evolution of genes tied to mate recognition, but the role of each these possibilities has not been simultaneously explored for any species-rich marine taxon. Thus, for this chapter, I develop a robust phylogenetic framework for 31 species and color forms from Elacatinus by using mitochondrial (cytochrome b) and nuclear (recombination-activating gene 1, rhodopsin) gene regions. I use this framework to explore the contributions of large- and small-scale geographic isolation, ecological differentiation, and coloration toward the formation and maintenance of species.
The phylogenetic relationships inferred in Chapter 1 suggest that species with multiple color forms are genetically distinct. Thus, Chapter 2 focuses on determining whether these differences correspond with geography or coloration, which can only be assessed by thorough sampling at the population level. This information is coupled with the determination of the potential capability of pelagic larvae to disperse among neighboring populations. I sampled multiple populations for the most widely distributed species, *E. evelynae*, which has three color forms. If genetic differentiation in *E. evelynae* is associated with geography, then common mitochondrial haplotypes should be shared independently of color form among geographically close populations. If differentiation is due primarily to coloration, then haplotypes should not be shared between different but geographically proximal color forms.

An important result that emerges from this study suggests that the genetic lineages associated with the three color forms of *E. evelynae* may be separated by biogeographic barriers to gene flow. These barriers had been previously hypothesized to be located at the Mona Passage in the Caribbean Sea and at the southern end of Exuma Sound in the Bahamas, but their presence has never been tested. In Chapter 3, I test whether the two proposed Caribbean biogeographic barriers separate discrete genetic lineages for nine taxa of *Elacatinus* whose distributions encompass the central Bahamas and the Mona Passage. For each taxon, I sequence one mitochondrial and one nuclear marker. Concordance of genetic lineages obtained from multiple taxa with independent genetic markers will allow me to make robust inferences about phylogeographic history of the Caribbean region with reference to previously proposed biogeographic breaks.

In total, the results from these three chapters of my dissertation begin to reveal how geography, ecology and coloration interact to contribute to high species diversity in the genus *Elacatinus*. 


Chapter 2

Marine Radiations at Small Geographic Scales

Following Mayr (1942), much of the literature on speciation has focused on identifying geographical barriers that can facilitate allopatric speciation. This search has been especially protracted in the marine literature because geographic isolating barriers are rarely obvious. Populations separated by several thousands of kilometers have been thought to be interconnected by pelagic larval dispersal, and thus could become isolated only by extreme distances or by extrinsic barriers that prevented dispersal (Mayr 1954; Briggs 1973; Springer 1982; Benzie 1998). One prominent barrier is the Isthmus of Panama, which separates closely related tropical marine taxa in the Atlantic and Pacific oceans (Bermingham and Lessios 1993; Marko 2002; Fukami et al. 2004). Other barriers, such as the Eastern Pacific Barrier (Ekman 1953) and land masses that emerge during lowered sea levels (e.g., Grigg and Hey 1992; Benzie 1998; Barber et al. 2002), were typically inferred by comparison of distributions for taxa believed to be closely related.

The presence of comparatively few known geographic barriers in the ocean, combined with the dispersal potential of larvae, does little to explain high species diversity in tropical regions such as the Caribbean Sea (e.g., Domeier 1994; Hastings 2000; Williams and Mounts 2003; Morrison et al. 2004). Furthermore, recent studies have found significant genetic structure, even reciprocal monophyly, at the scale of hundreds of kilometers (Planes et al. 2001; Riginos and Nachman 2001; Barber et al. 2002; Taylor and Hellberg 2003), as well as sister taxa with sympatric distributions (Duffy 1996; Hellberg 1998; Collin 2003). The absence of obvious geographic barriers in these regions, coupled with evidence of larval retention rather than dispersal (Jones et al. 1999; Swearer et al. 1999; Taylor and Hellberg 2003), suggests that population divergence and speciation may sometimes be mediated by mechanisms other than prolonged, broad-scale allopatry. Changes in climatic conditions or shifting ocean currents may isolate populations for a period sufficient for populations to diverge (Valentine and Jablonski 1983). Selection acting on differential resource utilization at localized geographic scales may play an important role in the speciation process (Duffy 1996; Orr and Smith 1998). Alternatively, the rapid evolution of reproductive traits may also result in reproductive isolation (Endler and Basolo 1998; Palumbi 1998; Hellberg and Vacquier 1999; Masta and Maddison 2002).

The role of geographical isolation, ecological differentiation (e.g., differences in habitat or behavior), and mate recognition to the formation and maintenance of new species can be evaluated in the context of a robust phylogenetic framework. If species from different lineages share similar ecological and morphological traits but differ in geographic distributions, then classic allopatric speciation would be favored. Alternatively, if sympatric sister species differ ecologically, then differences due to resource competition may have contributed to speciation (Lynch 1989; Losos 1990). Such inferences assume that species distributions have remained unchanged since their formation; however, when comparative phylogenetic inferences are coupled with independent evidence, such as common geographic distribution among different groups of sister taxa, the interpretation of historical processes may be reliable (Losos and Glor 2003).

To assess the historical contributions of geography, ecology and mate recognition as processes underlying speciation requires a suitable taxon. With nearly 2000 described species,
gobies (Gobiidae) constitute the largest family of marine fishes (Nelson 1994). In the Neotropical region, gobies are the most species-rich family of marine fishes (Robertson 1998). The Neotropical seven-spined gobies (Gobiosomatini) show particularly high levels of behavioral specializations and ecological differentiation, all of which have evolved over the last 40 million years (Rüber et al. 2003). Whether such specializations continue to influence patterns of speciation among recently formed taxa remains unknown.

Among the seven-spined gobies is the genus *Elacatinus*. With 27 nominal taxa (Table 1), *Elacatinus* is the largest genus of fishes found on Neotropical coral reefs. (I follow Hoese (1971) and Eschmeyer (1998) by recognizing the genus *Elacatinus* with two subgenera, *Tigrigobius* and *Elacatinus*. These subgenera are equivalent to those applied by Rüber et al. (2003) to the genus *Gobiosoma*. I will use *sensu lato* (s.l.) and *sensu stricto* (s.s.) to distinguish between the genus and subgenus *Elacatinus*, respectively.) The subgenus *Tigrigobius* contains 12 described species roughly equally divided between the Pacific and Atlantic Oceans. The subgenus *Elacatinus* has 15 described species, with only a single species found in the tropical eastern Pacific Ocean (Table 1). Several species of *Elacatinus* (s.s.) vary geographically by coloration but are otherwise morphologically indistinguishable (Colin 1975). Examining whether sister taxa of *Elacatinus* (s.l.) differ by geographical distribution, by ecological traits, by coloration differences, or by some combination of these will allow us to infer the mechanisms contributing to the origination of new species in this diverse and geographically restricted genus.

Here, I build a molecular phylogenetic framework using mitochondrial and nuclear markers to address mechanisms that potentially explain the observed diversity within the genus *Elacatinus*. Specifically, I address a series of related questions. First, did the Isthmus of Panama separate sister species of *Elacatinus* (s.l.)? If many sister species are so divided, then closure of the Isthmus would be implicated to have contributed greatly to species diversity in the genus. Second, if sister species occur together in the same ocean, do they have allopatric or sympatric distributions? Sympatric distributions for recently diverged sister species would suggest mechanisms other than gradual allopatric speciation. Alternatively, allopatric distributions would favor geographic speciation, albeit at smaller spatial scales than usually posited for marine taxa. Common distributional patterns for multiple species would suggest the presence of previously unrecognized geographic barriers. Finally, do sister taxa and sympatric taxa have consistent differences in ecological or behavioral traits, or in coloration? Such differences would suggest mechanisms that facilitate or maintain assortative mating at range boundaries and in sympatry.

**MATERIALS AND METHODS**

Two individuals from geographically distant populations were sampled for 21 of 29 ingroup taxa (Table 1); all other taxa were represented by two individuals sampled from the same population. I obtained samples of all currently described species and color forms in the genus except *E. (E.) tenox*, the white forms of *E. illecebrosus* and *E. xanthiprora*, and *E. (T.) zebrella*. Five putative outgroup taxa were selected based on previous morphological and molecular work (Van Tassell 1998; Rüber et al. 2003). In all, 67 individuals were analyzed. Specimens were collected and preserved in the field with 95-100% ethanol or a saturated salt-DMSO buffer (Amos and Hoelzel 1991). Specimens were subsequently stored in the laboratory at –80°C.
Table 1. List of species used in this study. Two additional species, *Elacatinus (E.) tenox* and *E. (T.) zebrella* were not available. Color refers to the lateral stripe color for Atlantic species of subgenus *Elacatinus*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Ocean</th>
<th>Color</th>
<th>Source of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Elacatinus (Elacatinus) atronasus</em></td>
<td>Atlantic</td>
<td>yellow</td>
<td>Cat Island &amp; Long Island, Bahamas</td>
</tr>
<tr>
<td><em>Elacatinus (E.) chancei</em></td>
<td>Atlantic</td>
<td>yellow</td>
<td>Barbados, Puerto Rico</td>
</tr>
<tr>
<td><em>Elacatinus (E.) evelynae</em></td>
<td>Atlantic</td>
<td>blue¹</td>
<td>Curaçao; St. Croix</td>
</tr>
<tr>
<td><em>Elacatinus (E.) evelynae</em></td>
<td>Atlantic</td>
<td>white</td>
<td>Navassa</td>
</tr>
<tr>
<td><em>Elacatinus (E.) evelynae</em></td>
<td>Atlantic</td>
<td>yellow</td>
<td>Lee Stocking Island, Bahamas</td>
</tr>
<tr>
<td><em>Elacatinus (E.) figaro</em></td>
<td>Atlantic</td>
<td>yellow</td>
<td>Brazil (2)</td>
</tr>
<tr>
<td><em>Elacatinus (E.) genie</em></td>
<td>Atlantic</td>
<td>white</td>
<td>Cat Island, Bahamas; Grand Turk, Turks &amp; Caicos</td>
</tr>
<tr>
<td><em>Elacatinus (E.) horsti</em></td>
<td>Atlantic</td>
<td>white</td>
<td>Jamaica; Navassa</td>
</tr>
<tr>
<td><em>Elacatinus (E.) horsti</em></td>
<td>Atlantic</td>
<td>yellow</td>
<td>Cat Island, Bahamas; Curaçao (2); Grand Cayman</td>
</tr>
<tr>
<td><em>Elacatinus (E.) illecebrosus</em></td>
<td>Atlantic</td>
<td>blue</td>
<td>Colombia (2)</td>
</tr>
<tr>
<td><em>Elacatinus (E.) illecebrosus</em></td>
<td>Atlantic</td>
<td>yellow</td>
<td>Panama (2)</td>
</tr>
<tr>
<td><em>Elacatinus (E.) lori</em></td>
<td>Atlantic</td>
<td>white</td>
<td>Belize (2)</td>
</tr>
<tr>
<td><em>Elacatinus (E.) louiseae</em></td>
<td>Atlantic</td>
<td>yellow</td>
<td>Lee Stocking Island, Bahamas; Grand Cayman</td>
</tr>
<tr>
<td><em>Elacatinus (E.) oceanops</em></td>
<td>Atlantic</td>
<td>blue</td>
<td>Belize (2); Florida Keys (2)</td>
</tr>
<tr>
<td><em>Elacatinus (E.) prochilos</em></td>
<td>Atlantic</td>
<td>white</td>
<td>Barbados, St. Croix</td>
</tr>
<tr>
<td><em>Elacatinus (E.) randalli</em></td>
<td>Atlantic</td>
<td>yellow</td>
<td>Curaçao (2)</td>
</tr>
<tr>
<td><em>Elacatinus (E.) xanthiprora</em></td>
<td>Atlantic</td>
<td>yellow</td>
<td>Belize (2)</td>
</tr>
<tr>
<td><em>Elacatinus (E.) puncticulatus</em></td>
<td>Pacific</td>
<td>blue</td>
<td>Gulf of California; Panama</td>
</tr>
<tr>
<td><em>Elacatinus (Tigrigobius) dilepis</em></td>
<td>Atlantic</td>
<td></td>
<td>Belize; Curaçao</td>
</tr>
<tr>
<td><em>Elacatinus (T.) gemmatus</em></td>
<td>Atlantic</td>
<td></td>
<td>Curaçao; Grand Cayman</td>
</tr>
<tr>
<td><em>Elacatinus (T.) macrodon</em></td>
<td>Atlantic</td>
<td></td>
<td>Bermuda, Florida Keys</td>
</tr>
<tr>
<td><em>Elacatinus (T.) multifasciatus</em></td>
<td>Atlantic</td>
<td></td>
<td>Cat Island, Bahamas; Puerto Rico</td>
</tr>
<tr>
<td><em>Elacatinus (T.) pallens</em></td>
<td>Atlantic</td>
<td></td>
<td>Curaçao; Grand Cayman</td>
</tr>
<tr>
<td><em>Elacatinus (T.) saucrus</em></td>
<td>Atlantic</td>
<td></td>
<td>Panama; Puerto Rico</td>
</tr>
<tr>
<td><em>Elacatinus (T.) dugueti</em></td>
<td>Pacific</td>
<td></td>
<td>Gulf of California (2)</td>
</tr>
<tr>
<td><em>Elacatinus (T.) inornatus</em></td>
<td>Pacific</td>
<td></td>
<td>Panama (2)</td>
</tr>
<tr>
<td><em>Elacatinus (T.) janssi</em></td>
<td>Pacific</td>
<td></td>
<td>Costa Rica (2)</td>
</tr>
<tr>
<td><em>Elacatinus (T.) limbaughii</em></td>
<td>Pacific</td>
<td></td>
<td>Gulf of California (2)</td>
</tr>
<tr>
<td><em>Elacatinus (T.) nesiotes</em></td>
<td>Pacific</td>
<td></td>
<td>Cocos Islands; Galapagos Islands</td>
</tr>
<tr>
<td><em>Aruma histrio</em></td>
<td>Pacific</td>
<td></td>
<td>Gulf of California</td>
</tr>
<tr>
<td><em>Gobiosoma bosc</em></td>
<td>Atlantic</td>
<td></td>
<td>Louisiana</td>
</tr>
<tr>
<td><em>Gobiosoma robustum</em></td>
<td>Atlantic</td>
<td></td>
<td>Florida</td>
</tr>
<tr>
<td><em>Ginsburgellus novemlineatus</em></td>
<td>Atlantic</td>
<td></td>
<td>Curaçao; Puerto Rico</td>
</tr>
<tr>
<td><em>Risor ruber</em></td>
<td>Atlantic</td>
<td></td>
<td>Belize; Curaçao</td>
</tr>
</tbody>
</table>

¹ Colin (1975) refers to this as the yellow-blue (YB) form in reference to the blue lateral stripe grading into yellow on the head. I refer to this as the blue form for simplicity.
DNA Amplification

Total genomic DNA was extracted from muscle tissue with a Qiagen (Valencia, CA) DNA Mini Kit by following the manufacturer’s instructions. The polymerase chain reaction (PCR) and the primers listed in Appendix A were used to amplify protein-encoding regions of three genetic markers: mitochondrial cytochrome $b$ (mtcyb), and nuclear recombination-activating gene 1 (rag1) and rhodopsin (rho). These three markers were chosen to provide independent estimates of phylogenetic relationships and to provide resolution at different hierarchical levels. Preliminary analyses of mtcyb revealed short branch lengths at some internal nodes within the subgenus Elacatinus. To increase resolution at these nodes, an additional 512 bp were amplified from the two mitochondrial tRNAs (tRNA$^{\text{Glu}}$ and tRNA$^{\text{Pro}}$) immediately following mtcyb and the 5’ region of the mitochondrial control region (displacement loop, $D$-loop).

The PCR was performed on a PTC-200 (MJ Research, Watertown, MA) with the following conditions: 94°C for three minutes for initial denaturing, followed by 35 cycles of 94°C for 15 sec, 48-58°C for 20 sec, and 72°C for 30-60 sec, depending on the primers used. Resulting amplicons were purified with a Strataprep PCR Purification Kit (Stratagene, La Jolla, CA), then sequenced in both directions with the amplification primers and Big Dye Terminators (V2.0, Applied Biosystems, Foster City, CA) on an ABI 377 automated sequencer.

Phylogenetic Analyses

Sequences for each gene region were assembled and edited with Sequencher 3.0, then aligned with an Internet implementation of ClustalW (http://www2.ebi.ac.uk/clustalw/) set to default parameters. The resulting dataset was analyzed with both maximum likelihood (ML) and Bayesian analyses using PAUP* v4.0b10 (Swofford 2000) and MrBayes 3.0b4 (Huelsenbeck 2000), respectively. Evolutionary models were inferred independently for each marker and for the combined data set with the aid of MrModeltest (J.A.A. Nylander, pers. comm.), a simplified version of ModelTest (Posada and Crandall 1998) that selects evolutionary models of nucleotide substitution applicable by both PAUP* and MrBayes. For the mtcyb, rag1 and combined generic analyses, the general time reversible model with a proportion of invariant sites and gamma distributed rate heterogeneity ($\text{GTR}+\Gamma$) was selected; the rho and $D$-loop and the combined subgeneric datasets were modeled similarly, except with a single transition:transversion ratio ($\text{HKY}+\Gamma$). The gamma distribution for each model was approximated with four discrete rate classes.

Each genetic marker was analyzed separately under both ML and Bayesian conditions and the appropriate model to determine whether they have similar phylogenetic histories and thus were suitable for combined analyses (Bull et al. 1993; Cunningham 1997). Confidence in combined analyses would be gained if individual markers showed evidence of similar phylogenetic histories. In contrast, questionable results would be obtained from a combined data set if the markers showed different phylogenetic histories. I considered the markers to have different histories by the presence of strongly supported but conflicting clades between markers (Wiens 1998). I considered a clade to be strongly supported only if the clade had both ML bootstrap (MLB) support of $\geq 70\%$ and Bayesian posterior probabilities (BPP) $\geq 95\%$ (Leaché and Reeder 2002), as these two values often correspond in simulations (Hillis and Bull 1993; Suzuki et al. 2002). I required both values as evidence for strong support because Bayesian analyses can occasionally assign high posterior probability values to incorrect clades (Huelsenbeck et al. 2002), but such clades were much less likely to receive high bootstrap values (Douady et al. 1998).
I considered the markers to have similar phylogenetic histories, and therefore suitable for combined analyses, only in the absence of conflicting clades.

All ML phylogenies were estimated with heuristic searches with tree bisection-reconnection (TBR) branch swapping. A starting phylogeny was derived from the model and associated parameters estimated by MrModelTest. The optimal ML phylogeny was then derived with a successive approach (Leaché and Reeder 2002). The initial phylogeny was used to reestimate model parameters, which were then used to derive a new phylogeny. This process was repeated until ML scores converged on a single value, suggesting the most likely phylogenetic hypothesis had been found. Support for each clade was estimated by performing 100 MLB replicates for each data set with the final estimated parameters for those data. ML analyses are computationally intensive, especially for large data sets. To conserve time, bootstrap analyses for the individual markers were performed with heuristic searches and nearest-neighbor interchange branch swapping on a starting neighbor-joining (NJ) tree. For the two combined datasets, a heuristic search and TBR branch swapping on a starting NJ tree was performed for 100 bootstrap replicates. ML bootstrapping of the combined dataset for the genus-level phylogeny took 4180 hours (nearly six months) of CPU time on a DEC Alpha 1 workstation (600 MHz EV67 21264A processor).

Bayesian analyses were performed by Markov Chain Monte Carlo sampling for 1.2 million generations. Four Metropolis-coupled chains were run simultaneously using uniform prior probabilities and appropriate model parameters estimated on a randomly generated starting phylogeny. Trees were sampled from the posterior-probability distribution once every one hundred generations. Bayesian analyses were repeated five times for each data set to reduce chances of selecting a local but not global optimum. All parameters were plotted to ensure each had reached stationarity and to determine the appropriate burn-in period. Burn-in occurred within the first 100,000 generations; I conservatively discarded the first 200,000 generations (2000 trees). The 10,000 sampled trees (after burn-in) from each of the five independent runs were combined to determine final posterior probabilities.

Comparisons between alternative phylogenetic topologies were analyzed a posteriori with the Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa 1999) and 100,000 RELL approximated bootstrap replicates as implemented in PAUP* (Swofford 2000). Associations between discrete color and ecological character states were tested with the concentrated changes test (Maddison 1990), as implemented in MacClade 4.06 (Maddison and Maddison 2003).

**RESULTS**

Sequences resulting from PCR amplification were trimmed to exclude ambiguous reads at each end, which yielded 1140 base pairs (bp) for mtcyb, 1290 bp for rag1, and 800 bp for rho, for a total of 3230 bp for all individuals. Additionally, 143 bp were amplified for the two tRNAs (144 bp for E. puncticulatus) and 357-366 bp of D-loop were amplified for all members of the subgenus Elacatinus, except for a single individual of E. xanthiprora for which D-loop could not be amplified. This individual was excluded from all subgeneric analyses. A single putative amino acid deletion (three nucleotides) was observed in mtcyb for both individuals of E. dilepis; no other indels were observed in mtcyb, rag1 or rho for any species. A single putative nucleotide insertion was observed in tRNAPro for E. puncticulatus. Several indels were observed in D-loop. Nucleotide positions containing indels were globally excluded from all phylogenetic analyses. Introns were absent from the sequenced regions of rag1 and rho.
I found no evidence for significant topological conflict among the three markers, for both ML (Fig. 1) and Bayesian (topologies not shown) analyses. Although differences for some clades are apparent among markers, none of the conflicting clades have strong support for both MLB and BPP. Most topological discrepancies are between mtcyb and the two nuclear markers. For example, the separate rag1 and rho phylogenies both place *E. gemmatus* in a clade with *E. pallens* and *R. ruber*, with 100% support for both MLB and BPP. In contrast, mtcyb places *E. gemmatus* basal to the Tigrigobius and Elacatinus (s.s.) clades with 100% BPP but with less than <50% MLB (Fig. 1). Given the lack of significant conflict, I concluded that the three genetic markers had a common phylogenetic history and were suitable for combined analyses (Bull et al. 1993; Cunningham 1997).

The ML phylogeny estimated from the combined dataset of mtcyb, rag1 and rho does not support the monophyly of the genus (Fig. 2). The topology obtained from Bayesian analysis is identical (not shown). *Tigrigobius*, as currently recognized (Böhlke and Robins 1968; Hoese 1971), is a polyphyletic grouping consisting of two clades. One of these clades consists of three Atlantic species (*E. macrodon*, *E. saucrus* and *E. dilepis*; hereafter, the “Tigrigobius” clade sensu Rüber et al. (2003)) and is sister to *Elacatinus* (s.s.) (Fig. 2). The Tigrigobius clade is strongly supported with 100% MLB and BPP support. The second, more species-rich clade (supported by 90% MLB and 100% BPP support) consists of the remaining Atlantic species, all of the Pacific species, and two putative outgroup taxa, *Ginsburgellus novemlineatus* and *Risor ruber* (Fig. 2; hereafter, the “Risor” clade sensu Rüber et al. (2003)). In addition to *Ginsburgellus* and *Risor*, Rüber et al. (2003) found that *Evermannichthys spongicola* (a species not sampled here) also fell within the Risor clade. *Elacatinus* (s.s.) is recovered as a monophyletic group by the analysis of the mtcyb, rag1 and rho data set (Fig. 2), and with the addition of D-loop and the two tRNAs (Fig. 3). The Bayesian phylogeny was again identical (not shown). The monophyly of Elacatinus (s.s.) is robust, with 100% support from both MLB and BPP. The Atlantic radiation in thus subgenus is also monophyletic (100% MLB and BPP), with the single Pacific species, *E. puncticulatus*, recovered basal to the Atlantic species (Fig 3). Although taxonomic revision of the genus Elacatinus appears necessary, current taxonomic alignment does not affect the conclusions drawn here.

**Geography**

Only two splits are associated with the Isthmus of Panama (Fig. 2). The first divides the Risor clade into Atlantic and Pacific subclades. Each subclade is supported by 75% MLB and 100% BPP. The second split, separating the basal *E. puncticulatus* from the Atlantic species of Elacatinus (s.s.), has maximum support of 100% for both analyses. No sister taxa are sundered by the Isthmus of Panama.

Instead, sister taxa occur within the same ocean (Fig. 2), with the degree of geographic overlap between sister taxa varying among the three major clades. Most sister species in the Tigrigobius and Risor clades overlap geographically. Within the Tigrigobius clade, *E. macrodon* is largely allopatric with respect to *E. saucrus* and *E. dilepis*, which have primarily a Bahamian and Caribbean distribution (based on museum collection records, data not shown). However, *E. macrodon* has been collected as far south as Grenada (Böhlke and Robins 1968), which overlaps the range of the other two species. Elacatinus saucrus and *E. dilepis* have primarily southeastern and northwestern Caribbean distributions, respectively, but may have some degree of overlap because both have been collected from the Bahamas, Jamaica, Haiti and the southern Lesser Antilles. In comparison, the five species that comprise the Atlantic Risor subclade (Fig. 2) are
Figure 1. Maximum likelihood phylogenies for the genus *Elacatinus* derived from three independent markers: *mtcyb*, *rag1* and *rho*. A single letter after a species name indicates blue (b), yellow (y), or white (w) lateral stripe color, as appropriate. The number two indicates where two identical sequences share a branch tip. Population name is indicated for species that do not cluster together. Asterisks indicate strongly supported branches, with both ML bootstrap proportions $\geq 70\%$ and Bayesian posterior probabilities $\geq 95\%$. None of the conflicting nodes are supported by high ML bootstrap and Bayesian posterior probabilities.
Figure 2. Maximum likelihood phylogeny (GTR+I+Γ) for the combined mtcyb, rag1 and rho dataset for the genus *Elacatinus*. Values above the branch or left of a slash are non-parametric ML bootstrap proportions. Values below the branch or right of a slash are Bayesian posterior probabilities. Asterisks represent 100% support for either analysis. Support values for the subgenus *Elacatinus* are shown in Figure 3. Ocean basins and clades are indicated by vertical bars. The *Tigrigobius* and *Risor* clades together comprise the subgenus *Tigrigobius* as presently defined. See text for details.
Figure 3. Maximum likelihood phylogeny (HKY+I+Γ) for the combined mtcyb, D-loop, two tRNAs, rag1 and rho data set for Atlantic species of subgenus Elacatinus. Tree was rooted with E. puncitculatus, E. limbaughi and Ginsburgellus novemlineatus. Support values indicated on branches as for Fig. 2. Ecological traits and mouth position are indicated by vertical bars. Plank. = schooling zooplankton feeder. Only species with inferior mouths placed below and behind snout tip are indicated; remaining species have mouths at snout tip (see text for details). Branch color corresponds to lateral stripe color.
broadly sympatric across the Bahamas and Caribbean Sea. For the Pacific Risor subclade, the range of *E. digueti* overlaps all species in this group except for *E. nesiotes*, which is endemic to the Galapagos and Cocos islands. The nominal sister taxa *E. nesiotes* and *E. inornatus* are allopatrically distributed but their limited genetic divergence (0.1-0.2% uncorrected pairwise distance) suggests that they may not be distinct species (see Hoese and Reader 2001).

In contrast to the two other clades, many sister taxa of *Elacatinus* (s.s.) are allopatrically distributed. For example, the three color forms of *E. evelynae* form a well-supported clade (91% MLB, 98% BPP; Fig. 3) and are allopatrically distributed across the Bahamas and Caribbean (Colin 1975; Taylor and Hellberg 2003). In turn, this clade is sister to *E. oceanops* (99% MLB, 100% BPP; Fig. 3), which is allopatric with respect to *E. evelynae* (Colin 1975). A similar distributinal relationship is found for *E. prochilos* and the color forms of *E. illecebrosus*, for *E. chancei*, *E. lori*, and the color forms of *E. horsti* (Fig. 2; Colin 1975). A white form is also known for *E. xanthiprora* (Colin 1975) but it was not obtained for this study. The two color forms of *E. xanthiprora* are also apparently allopatrically distributed (Colin 1975).

### Habitat and Behavior

Fully resolved sister taxa in *Elacatinus* (s.s.) all share similar ecological traits (Fig. 3). Sponge-dwelling (100% MBL and BPP) and cleaning behaviors (77% MLB, 99% BPP) are monophyletic clades (Fig. 3), that confirm earlier findings based on more limited taxon sampling by Rüber et al. (2003). The sole plankton-feeding species, *E. atronasus*, falls within the cleaner clade, although the node containing this species is not fully resolved (Fig. 3). The placement of *E. atronasus* as sister to a monophyletic clade of cleaners cannot be rejected (SH test, *P*>0.05).

Many sister species within the *Risor* clade also exhibit similar ecological traits. All species within the Pacific subclade (Fig. 4) are facultative cleaners except *E. janssi*, which is associated with sandy and rocky substrates (Humann 1993; Allen and Robertson 1994). Within the Atlantic *Risor* subclade (Fig. 4), both *G. novemlineatus* and *E. multifasciatus* associate with sea urchins, primarily *Echinometra* (Erdman 1956; Smith 1957) and *Diadema* (MST pers. obs., Humann 1994). *Elacatinus gemmatus* and *E. pallens* can both be found in holes drilled by the boring chiton *Choneplax lata* (Taylor and Van Tassell 2002). *Risor ruber* and the putative sister taxon *Evermannichthys* (Rüber et al. 2003) are both obligate sponge dwellers.

### Coloration and Patterns

Notably, within cleaner species belonging to *Elacatinus* (s.s.), sister taxa differ by the coloration of their lateral stripe (Fig. 3). The ancestral coloration appears to be yellow, with white and blue coloration evolving independently multiple times within the cleaners. No sister taxa share the derived white or blue coloration. White coloration has evolved at least twice in sponge-dwelling species (*E. horsti* and *E. lori*). If the unsampled white form of *E. xanthiprora* is sister to yellow form *E. xanthiprora*, then white coloration may have evolved three times in sponge-dwelling species. A monophyletic origin for each lateral stripe color within cleaners and within sponge-dwellers was strongly rejected (SH test, *P*<<0.001).

Coloration and patterns (e.g., vertical bands or spots) also distinguish between most sister species in the *Tigrigobius* and Atlantic *Risor* clades (Fig. 4). Most Pacific *Risor* species are similar, with subtle differences in coloration and pattern (Bussing 1990; Hoese and Reader 2001). Among Pacific *Risor*, *E. janssi* differs greatly from the other species by being spotted rather than banded (Bussing 1981).
Figure 4. Coloration and ecological traits of species belonging to the *Tigrigobius* and *Risor* clades. Phylogenetic relationships drawn from Fig. 2. Coloration is given as body ground color/features. Shared ecological traits indicated by vertical bars.
DISCUSSION

The results of my combined analysis of three gene regions suggest that species of the genus *Elacatinus* (s.l.) fall into three clades (Fig. 2). Within each of these well-supported clades, sister taxa always occur to the same side of the Isthmus of Panama, which suggests that this potential isolating barrier has played no important role in the most recent bouts of species formation in this genus. Instead, sister taxa show strong differences both in microgeographic (within-ocean) distribution and in coloration, although the degree to which this holds varies among the three clades. Sister species also tend to be ecologically similar, but more distantly related species often differ ecologically (Figs. 3, 4). Ecological differentiation by habitat, followed by diversification of behavior and habitat, has been previously demonstrated at higher hierarchical levels within the Neotropical gobies (Rüber et al. 2003), a pattern that fits a model of adaptive radiation in stages (Streelman and Danley 2003). This model also explains the phylogenetic pattern of *Elacatinus* (s.l.) demonstrated here. Together, this suggests that repeated radiations at small geographic scales, similar to that seen for terrestrial species on islands (Losos et al. 1998; Sato et al. 1999), may explain much of the gobiid diversity in the Neotropics.

The Geographic Scale of Speciation

That speciation in the marine environment may occur at much smaller geographic scales than previously believed has been suggested by many recent studies (Duffy 1996; Hellberg 1998; Riginos and Nachman 2001; Collin 2003; Taylor and Hellberg 2003; Mackenzie et al. 2004). Three lines of evidence support this conclusion. First, phylogenetic studies have revealed that sister taxa often have restricted distributions along the same coastline or occur sympatrically (Duffy 1996; Hellberg 1998; Marko 1998), which may be a common pattern for species-rich taxa (Collin 2003). Second, experiments with chemical tags provide direct evidence that larval individuals may not disperse away from their natal populations (Jones et al. 1999; Swearer et al. 1999). Finally, significant population genetic structure at spatial scales <1000 kilometers provides indirect evidence for larval retention (Riginos and Nachman 2001; Barber et al. 2002; Taylor and Hellberg 2003).

Most species of *Elacatinus* (s.l.) are restricted to the Caribbean Sea and Bahamas (20 species total, not including *Risor* or *Ginsburgellus*), with only 3-4 species regularly found around Florida and in the Gulf of Mexico (Colin 1975; Humann 1994). Additionally, four nominal species of *Elacatinus* (s.s.) have multiple, geographically separated color forms (Colin 1975) that are also genetically distinct (Chapter 4; Taylor and Hellberg 2003). This suggests that as many as 26 distinct taxa evolved within a geographic region spanning roughly 3000 km from Belize to Barbados and 3000 km from the north coast of South America to the northern Bahamas.

Contained within this region, however, are more than 1000 islands and thousands of km of coastline along Central and South America. The Bahamas alone contain over 700 islands spanning roughly 1225 km (Spalding et al. 2001). Most islands within the Bahamas and Caribbean are arranged in a stepping-stone arc enclosing this region, and are separated from neighboring islands by fewer than 100 km. The close proximity of the islands, coupled with strong currents and dispersal of planktonic larvae, may in some species facilitate the rapid spread of unique haplotypes throughout the Caribbean and Bahamas (Shulman and Bermingham 1995) which could render distant populations genetically identical. Yet, despite the potential ability of larvae to disperse up to 500 km in a single generation (Taylor and Hellberg 2003), the high
number of distinct *Elacatinus* (*s.l.*) taxa in this region suggests that gene flow among populations is minimal.

For example, *E. evelynae* has three allopatrically distributed color forms (yellow, blue, and white, Colin 1975) that are genetically distinct (Taylor and Hellberg 2003). A nearly identical distributional pattern (Colin 1975) is observed for a group containing *E. chancei* and genetically distinct color forms of *E. horsti* (Chapter 4). The different taxa share common distributional boundaries in the central Bahamas and at Mona Passage between Puerto Rico and Hispaniola (Colin 1975). This suggests a common evolutionary history underlying differentiation of these taxa that may be influenced by proposed biogeographic breaks (Colin 1975; Colin 2003). Yet, even within these regions, individuals from island populations are genetically distinct from other such populations, which suggests that larvae are not dispersing away from their natal populations (Taylor and Hellberg 2003). This lack of gene flow among populations may allow allopatric differentiation, and potentially speciation, to occur at geographic scales on the order of hundreds of kilometers.

A similar allopatric distribution is observed for the *Tigrigobius* clade (Fig. 2). Based on unpublished museum records, *E. saucrus* is found primarily in the southern Caribbean, *E. dilepis* in the northwestern Caribbean and Bahamas, and *E. macrodon* around Florida. Although all three species have been collected in close proximity to one another (e.g., western Hispaniola), the primarily non-overlapping distributions of these species suggests speciation in allopatry at the scale of 100s to 1000s of km. The allopatric distribution of sister taxa in *Elacatinus* (*s.l.*) suggests that geographic speciation at small spatial scales may be the most common mode of speciation in this genus.

Allopatric speciation at larger geographic scales, however, is evident for some species. *Elacatinus figaro* in the southwestern Atlantic Ocean may have been isolated from Caribbean species (Fig. 3) by the freshwater outflow of the Amazon and Orinoco rivers. This Amazon barrier has been implicated in the significant genetic differentiation and speciation of several coral reef fishes between the southwestern Atlantic Ocean and Caribbean Sea (Muss et al. 2001; Rocha et al. 2002; Rocha 2003). In the Pacific, *E. nesiotes* is endemic to the Galapagos and Cocos archipelagos and is separated by a vast expanse of open water from its mainland sister taxa of *E. inornatus* and *E. digueti* (Fig. 2). The Galapagos and Cocos archipelagos harbor a high percentage of gobiid endemics (Robertson 2001), suggesting their isolation generally proves beyond the dispersal ability of gobiid larvae.

Radiation in Stages: Ecology and Color

The rate at which allopatric populations give rise to new species may be enhanced by ecological differentiation and color-based mate choice (Turner and Burrows 1995; Allender et al. 2003; McKinnon et al. 2004). Both ecological and coloration differences have been implicated as forces that drive different stages of adaptive radiations (Streelman and Danley 2003). This evolutionary model predicts that divergence during adaptive radiations occurs in three intertwined stages: divergence by habitat, by morphological characters associated with trophic resource utilization, and by sensory communication. The order of these steps and the degree of diversification within each stage may vary among different taxa (Streelman and Danley 2003), however this overall pattern of radiation has been observed for tropical marine fishes (Streelman et al. 2002; Rüber et al. 2003).

Speciation in *Elacatinus* (*s.l.*) appears to match the pattern of a staged adaptive radiation. Among *Elacatinus* (*s.s.*), an initial ecological divergence in habitat occurred between cleaners
and sponge-dwellers (Fig. 3). The next stage appears to be diversification based on color. This is most notable among cleaner species but color changes are evident in both clades (Fig. 3). Finally, mouth position has changed among cleaner species (Fig. 3). Many cleaners (and all sponge-dwellers), have terminal mouths positioned at the tip of the snout, but *E. evelynae*, *E. oceanops*, *E. genie* and *E. illecebrosus* all have mouths placed inferiorly well below and behind the tip of the snout (Böhlke and Robins 1968). As a result, these four species have been treated previously as a complex of closely related species (Böhlke and Robins 1968; Colin 1975), but a monophyletic origin for inferior mouths is not supported by my molecular data (SH test, \( P<0.05 \)).

A morphological change to an inferior mouth position is significantly associated with a change to a blue lateral stripe color (concentrated changes test, \( P<0.05 \)). These associated morphological and coloration changes may be connected with cleaning behavior. The spectral reflectance of the blue lateral stripe of *E. oceanops* is similar to that of the Indo-Pacific cleaner wrasses and distinct from blues of most other reef fishes (Marshall 2000). The change to inferior mouth position may confer an advantage by facilitating removal of parasites from host fishes. Although speculative, this evidence suggests that cleaning behavior may in part be responsible for diversification of *Elacatinus* (s.s.).

The staged pattern of adaptive radiation is also evident for the *Risor* and *Tigrigobius* clades. Initial divergence again appears to be associated with ecological divergence (Fig. 4). Among Pacific *Risor*, initial divergence is between the rock-dwelling *E. janssi* and the remaining facultative cleaners. The facultative cleaners then differ by color pattern, although the differences are subtle for *E. inornatus*, *E. nesiotes* and *E. digueti* (Bussing 1990; Hoese and Reader 2001). Among the Atlantic *Risor* species, initial divergence appears to be between urchin-associated species and those associated with chiton burrows. This is followed by another habitat shift to obligate sponge-dwelling for *R. ruber*. Although Figure 4 suggests *E. pallens* and *R. ruber* are sister species that differ by habitat, other evidence suggests that *R. ruber* is sister to *Evermannichthys spongicola* (Rüber et al. 2003), another obligate sponge-dweller not included in this study. Habitat divergence is subsequently followed by divergence in color (between *E. gemmatus* and *E. pallens*, Fig. 4) or morphology (between short, stout *R. ruber* and long, slender *E. spongicola*). For *Tigrigobius*, initial divergence among species appears to be by color patterns, then by habitat between *E. macrodon* and *E. saucrus* (Fig. 4).

Coloration and habitat differences in *Elacatinus* (s.l.) may facilitate assortative mating between co-occurring species. Sympatric cleaner species of *Elacatinus* (s.s.) are most often differently colored, while similarly-colored sympatric sponge-dwelling species segregate by depth (Colin 1975). Similar color differences between sympatric Atlantic *Risor* taxa are also evident. *Elacatinus gemmatus* and *E. pallens* are frequently found in the same burrows (Taylor and Van Tassell 2002), but the former is dark-bodied and the latter is pale. *Ginsburgellus novemlineatus* and *E. multifasciatus* are both strongly banded, but differ greatly by color and the number of bands (Fig. 4). Although *Tigrigobius* species appear to be largely allopatric (see above), they occasionally occur sympatrically but differ by pattern, habitat, or both (Fig. 4). These ecological and coloration differences may simultaneously facilitate reproductive isolation between sister taxa (Domeier 1994; Seehausen et al. 1997; McMillan et al. 1999) and allow a greater number of species to coexist in the geographically compact region of the Neotropics.
Conclusion

Gobies are among the most species rich taxa of all fishes (Nelson 1994) and are the largest component of Neotropical reef fishes (Robertson 1998). My data suggest that, at least for *Elacatinus* (s.l.), speciation has occurred primarily in allopatry at small (separation <1000 km) geographic scales. If gobiid larvae, as well as larvae of other small reef dwellers such as blennies and snapping shrimp, tend to remain near their natal reefs rather than disperse (Leis 1991; Duffy 1996), this mechanism alone may explain their relatively high species diversity. However, populations that remain closed for thousands of generations, as demonstrated for *E. evelynae* (Taylor and Hellberg 2003), may be able to adapt to local ecological conditions (Warner 1997; Grosberg and Cunningham 2000). Such ecological adaptation may facilitate rapid divergence between transiently allopatric populations and increase the potential for speciation (Turner and Burrows 1995; Duffy 1996; Rüber et al. 2003). Much of the historical evolution in the Neotropical seven-spined gobies, which includes *Elacatinus* (s.l.), is based on major shifts in habitat, followed by diversification of behavior and habitat utilization (Rüber et al. 2003). My results suggest that more recent bouts of speciation follow a similar pattern. Thus, repeated stages of adaptive radiations among allopatrically distributed sister taxa may explain much of the high diversity of gobies in the Neotropics.
Chapter 3

Genetic Evidence for Local Retention of Pelagic Larvae

Many marine organisms have pelagic larvae that can potentially interconnect distant populations through dispersal on ocean currents. If these larvae disperse as passive propagules on advective current flow, they will be transported among both near and distant island populations (Roberts 1997). Species with such broadly dispersing larvae should be genetically homogeneous over large spatial scales, thus compromising their ability to adapt to local conditions (Warner 1997). If, however, pelagic larvae are retained near their natal populations by behavioral (Burton and Feldman 1982) or physical oceanographic (Cowen et al. 2000) mechanisms, then populations would have a greater opportunity for genetic differentiation and local adaptation. Should local retention persist over many generations, marine populations undivided by strong physical barriers might nonetheless form new species or at least differentiate to levels where different management or conservation strategies would be warranted for different populations.

Studies employing fluorescent tags and environmental trace elements as markers in otoliths—calcareous structures in the inner ear of fishes—from newly recruited juvenile fishes indicate that as many as 60% of a juvenile cohort may recruit to their natal populations, despite larval durations of 3-7 weeks (Jones et al. 1999; Swearer et al. 1999). However, exchange rates averaging just a single larval individual per generation among populations can be sufficient to hinder genetic differentiation due to drift or weak selection (Slatkin 1987). In the absence of biogeographic barriers, genetic analyses to date have failed to reveal significant population differentiation for species with broad larval dispersal potential (Shulman and Bermingham 1995; Lessios et al. 2001; Rocha et al. 2002), including one species (bluehead wrasse, Thalassoma bifasciatum) shown by trace element studies to have high larval retention (Swearer et al. 1999). Here, I test for genetic differentiation among island populations separated by hundreds of kilometers in a Caribbean reef fish with pelagic larvae.

Elacatinus (= Gobiosoma) evelynae, a reef-dwelling cleaner goby, is widely distributed throughout the Bahamas and Caribbean Sea (Fig. 5). It belongs to the most species-rich genus of fishes found on west Atlantic coral reefs, as well as to the largest family of marine fishes (Gobiidae) (Nelson 1994). While long recognized as a single species based on morphological criteria, E. evelynae has three brightly-colored forms: yellow, blue and white (Böhlke and Robins 1968; Colin 1975). Individuals of the different color forms rarely co-occur, however, despite geographic separation by as few as 23 km.

**MATERIALS AND METHODS**

**Otolith Analysis**

Left sagital otoliths were examined by transmission light microscopy from a subset of individuals representing all three color forms. Digital images of the otoliths were captured by a Diagnostic Instruments Spot RT imager and Spot v3.3 software. The resulting images were used to manually count daily growth rings from the first visible ring around the core to the settlement ring.
check. Each otolith was imaged through multiple focal planes to ensure all increments were visualized. Otoliths lacking an obvious settlement mark were not included.

Figure 5. Distribution of the yellow (circles), blue (diamonds), and white (triangles) color forms of *E. evelynae* across the Bahamas and Caribbean Sea. Green squares indicate localities where blue and yellow forms have both been reported. The 17 sampled populations are indicated with red lines. Northern Bahamas represents five sampled populations (north to south): Sweetings Cay, Eleuthera Island, Lee Stocking Island, Cat Island, and Long Island. Puerto Rico represents two sampled populations, Isla Desecheo (white form) and the main island (blue form). USVI represents two sampled populations, St. John and St. Croix. Distributions from Colin (1975, unpubl. data).

Genetic Analysis

I amplified DNA (extracted from muscle) using primers GLUDG-5' and CB2-3' (Palumbi 1996) and an annealing temperature of 48°C. Amplicons were directly sequenced in both directions on an ABI 377 sequencer, using the amplification primers. Neighbor-joining (NJ) analyses were performed with PAUP* (Swofford 2000) using Kimura two-parameter distances. Maximum likelihood analyses, using an HKY85 model of evolution, placed some Desecheo individuals basal to Jamaica, but topological arrangements were not significantly different from NJ results (Shimodaira-Hasegawa test, *P*>0.05). Trees were midpoint rooted; the position of the
root was confirmed using multiple outgroups. Analysis of molecular variation (Excoffier et al. 1992) was performed with Arlequin 2.000 (Schneider et al. 2000) using Kimura two-parameter pairwise distances. Sequences are deposited at Genbank (accession numbers AF543584-AF543681).

Coalescent Analysis

The maximum likelihood estimate of $\theta$ and its standard deviation were calculated for Barbados and Curaçao populations using Fluctuate (Kuhner et al. 1998). The analysis was performed five times, using randomly generated seeds, a search strategy of 10 short and 5 long Monte Carlo chains of 5000 and 20,000 steps, respectively, and a sampling increment of 20. The mean of $\theta$ and its standard deviation from the five runs were used to estimate the time to most recent common ancestor (Wares and Cunningham 2001), assuming a mutation rate of $2.0 \times 10^{-8}$ substitutions per site per year (Brown et al. 1979). The simultaneously estimated growth parameter was ignored.

RESULTS AND DISCUSSION

The potential for larval dispersal between geographically proximal populations can be assessed with knowledge of currents and pelagic larval duration (PLD). Current patterns in the Caribbean have been well studied; typical current speeds average 1-2 km/hr (Cowen et al. 2000). I determined PLD for *E. evelynae* by counting daily growth rings in the otoliths from the core (which forms as the planktonic stage begins after hatching) to the settlement check (that forms as the planktonic stage ends and individuals settle onto the reef). Larvae of all color forms had an PLD of about three weeks (Table 2); the mean PLD of the yellow form (25 days) was slightly longer than for blue or white forms (21 days; $P<0.001$). Assuming passive dispersal and a conservatively estimated current speed of 1 km/hr (Shulman and Bermingham 1995), an individual with a 21 day PLD could potentially disperse more than 500 km per generation (= one year). Dispersal, however, may be influenced by factors other than PLD. Ecological requirements or behavioral attributes may cause larvae to develop nearshore, rather than disperse (Riginos and Victor 2001). The pelagic larvae of gobies are typically found over or near reefs and not in open water (Leis 1991), suggesting limited dispersal.

To assess the realized extent of genetic exchange among populations, I sampled 246 individuals from 17 Caribbean and Bahaman island populations representing all color forms (Fig. 5), and amplified and sequenced 400 bp of the mitochondrial cytochrome *b* gene using the PCR. The different color forms of *E. evelynae* are genetically distinct and appear to be reproductively isolated: an analysis of molecular variation (Excoffier et al. 1992) indicates that 78.6% of the genetic variation is partitioned among color forms ($\Phi_{ST}$, Table 2), and none of the 79 unique haplotypes is shared among color forms except for three (Fig. 6). Notably, haplotypes are not shared between blue and white forms from Puerto Rico, where they are separated by only 23 km.

Within color forms, few haplotypes are shared among populations of either the blue or the white forms, indicating that haplotypes unique to each population are not spreading (via larval dispersal) to other populations. Of the 32 haplotypes found across blue form populations (separated by 60-2000 km), only six occurred in more than one population (five among Puerto Rico, St. John and St. Croix, and one between Barbados and Grenada). Of the 19 white form
haplotypes (populations separated by 250-750 km), only one occurred in more than one population (Jamaica and Navassa). The blue-form populations are strongly subdivided ($\Phi_{ST}=0.704$; Table 2); the white form also shows considerable subdivision ($\Phi_{ST}=0.584$). Furthermore, several populations within the blue form and the white form are reciprocally monophyletic (or nearly so) (Fig. 6), indicating that gene flow among populations has been absent or restricted over many generations. Using a coalescent model, I estimate that populations at Barbados and Curaçao (separated by 1000 km) have been isolated from each other for between 75,000 and 103,000 years.

### Table 2. Mean pelagic larval duration (PLD) in days, and genetic population subdivision ($\Phi_{ST}$) within and among color forms.

<table>
<thead>
<tr>
<th>Color form</th>
<th>N</th>
<th>PLD</th>
<th>Std. Err.</th>
<th>Pops</th>
<th>N</th>
<th>$\Phi_{ST}$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>48</td>
<td>21.7</td>
<td>0.46</td>
<td>8</td>
<td>156</td>
<td>0.704</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>White</td>
<td>24</td>
<td>21.1</td>
<td>0.66</td>
<td>4</td>
<td>49</td>
<td>0.584</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Yellow</td>
<td>20</td>
<td>25.2</td>
<td>0.72</td>
<td>5</td>
<td>41</td>
<td>0.038</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>All</td>
<td>92</td>
<td>22.3</td>
<td>0.37</td>
<td>17</td>
<td>246</td>
<td>0.786</td>
<td>P&lt;0.0001</td>
</tr>
</tbody>
</table>

Some of the geographic subdivision I found could be due to a “sweepstakes effect,” the genetic drift among larval cohorts that results from the random reproductive success of different small subsets of adults over time. If such sweepstakes effects are significant, then different larval cohorts should be genetically differentiated (Li and Hedgecock 1998). I sampled three populations repeatedly over as many as four generations, but found no evidence of temporal subdivision that would support a reproductive sweepstakes effect (Table 3). More detailed temporal sampling of marine species that are longer-lived and have overlapping generations (attributes most favorable for sweepstakes effects) have also failed to detect sweepstakes effects (Flowers et al. 2002).

My results show that strong phylogeographic structure can develop in the Caribbean Sea between marine populations separated by as few as 23 km for species that have potential for long-distance larval dispersal. The amount of genetic subdivision between populations of all three color forms (Table 2) is similar to that found between populations of an Indo-West Pacific stomatopod separated by a strong biogeographic barrier (Barber et al. 2000), where lineages with long separate histories meet; however, no such barriers are currently recognized for the Caribbean. Instead, the reciprocal monophyly of populations within the blue form and the close proximity of genetically distinct color forms observed here suggest that local larval retention generates the strong phylogeographic structure observed in *E. evelynae.*
Figure 6. Neighbor-joining tree of 79 mitochondrial cytochrome b haplotypes sampled from 246 E. evelynae individuals representing 17 populations across the Bahamas and Caribbean Sea. The three color forms are indicated on the branches. Average pairwise genetic distances (Kimura two-parameter) between color forms are white/yellow: 2.81%; blue/yellow: 3.04%; white/blue: 1.36%. Numbers at the branch tips indicate haplotypes shared by more than a single individual. † includes one blue individual from San Salvador. § includes two white individuals from Jamaica, one blue individual from Grand Turk, and one yellow individual from Long Island. ‡ includes two yellow individuals from Long Island. * includes a single individual from Grenada. Bold numbers indicate bootstrap support (100,000 replicates) for monophyletic populations.
Table 3. Genetic differentiation ($\Phi_{ST}$) among years for three populations. Analyses of molecular variation showed no evidence of temporal differentiation within populations ($P>0.05$).

<table>
<thead>
<tr>
<th>Population</th>
<th>$\Phi_{ST}$</th>
<th>Years Sampled (# individuals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbados</td>
<td>0.016</td>
<td>2000 (11), 2002 (13)</td>
</tr>
<tr>
<td>Curaçao</td>
<td>-0.038</td>
<td>1999 (13), 2002 (13)</td>
</tr>
<tr>
<td>St. Croix</td>
<td>-0.004</td>
<td>2000 (13), 2001 (12), 2002 (15)</td>
</tr>
</tbody>
</table>
Chapter 4  
Comparative Phylogeography and Biogeography

Geographic barriers that limit the movement of individuals between populations may lead to the evolution of phylogenetically discrete lineages, or maintain differences between lineages at range boundaries. Populations that remain distinct over evolutionary time scales may give rise to new species. Thus, a critical step toward understanding patterns of geographic speciation is the identification of barriers to dispersal (Avise 2000; Wiens 2004).

Many geographic barriers, such as mountains or desert expanses, are readily apparent; however, some barriers are less obvious, especially in the ocean. The lack of obvious oceanic barriers is especially acute in geographically compact regions such as the Caribbean Sea, which harbors a rich and diverse array of species (Briggs 1974; Robertson 1998) and several endemic radiations (e.g., Domeier 1994; Hastings 2000; Williams and Mounts 2003; Morrison et al. 2004), despite the potential for pelagic larvae to disperse long distances on Caribbean currents (Shulman and Bermingham 1995). Thus, the processes underlying the geographic origins of high biodiversity in this region remain enigmatic.

Increasingly, the biogeographic history of a region is studied with the aid of molecular phylogeographic analyses (Avise 2000; Barraclough and Vogler 2000). Biogeographic breaks may be inferred post-hoc by the observation of large genetic gaps between neighboring populations or cryptic sister species (Irwin 2002). Most commonly, the genetic markers employed in such phylogeographic studies are from mitochondrial DNA (mtDNA). mtDNA, however, has an effective population size that is four times smaller than autosomal nuclear DNA (Avise 2004). This property of mtDNA can lead to the random and comparatively rapid evolution of deep phylogenetic splits between lineages that are continuously distributed, especially for species that have small population size or low dispersal distance (Neigel and Avise 1993; Irwin 2002). The observation of a single deep division between mtDNA lineages may lead to the inference of a false barrier to gene flow (Neigel and Avise 1993; Irwin 2002). Thus, caution is required when proposing previously unrecognized biogeographic barriers based on mtDNA studies. Conclusions drawn from phylogeographic studies will be more robust if 1) phylogenies derived from independently segregating genetic markers are concordant, 2) phylogenies obtained for multiple taxa are concordant, and 3) the location of barriers are proposed a priori (Neigel and Avise 1993; Irwin 2002).

Few barriers have been proposed within the marine region that encompasses the Caribbean Sea, the Bahama Islands, and the lower Floridian peninsula. This mostly tropical region spans from a region of upwelling near Cape Canaveral, Florida south to the copious freshwater outflows of the Amazon and Orinoco rivers (Briggs 1974; Avise 2000). These delimiting boundaries coincide with distinct biogeographical break points (Avise 2000; Rocha 2003) and encompass broad faunal homogeneity, which suggests that the Caribbean region (in the broad sense) is a natural biogeographic province. Briggs (1974), however, recognized two provinces within this region, a coastal Caribbean Province (including peninsular Florida) and an insular West Indies Province (including the Bahamas and Bermuda). That Florida has many faunal differences from the remainder of the Caribbean region has been long recognized (e.g., Mayr 1954; Böhlke and Robins 1960; Böhlke and Springer 1961). However, the distinction between insular and coastal provinces (excluding Florida) may be unwarranted due to considerable faunal similarity between them (Greenfield 1979; Acero P. 1985).
Despite the ubiquity of many species across the Caribbean region, the presence of regional endemism (e.g., Böhlke and Robins 1968; Collette 1974; Johnson and Brothers 1989) and restricted distributions (e.g., Starck and Colin 1978; Domeier 1994) of some species suggests that biogeographic breaks may be present. One break has been inferred for the central Bahamas near Long Island, due to past isolation of the deep waters of Exuma Sound and the Tongue of the Ocean (Colin 1975) and potential for larval retention based on current flow in this area (Colin 1995). A second break may be present at Mona Passage between Puerto Rico and Hispaniola, based on distributions of some species of reef fishes that are found west but not east of Mona Passage (Colin 2003).

Genetic data supporting these biogeographic breaks have been lacking. Recently, however, I (Taylor and Hellberg 2003) found strong genetic structure in a Caribbean reef fish, *Elacatinus evelynae*, based on mitochondrial cytochrome *b* sequence. Genetic differentiation coincided with three recognized color forms of *E. evelynae*. The ranges of these color forms abut at Exuma Sound and at Mona Passage (Colin 1975), which suggests that these locations may be barriers to gene flow or determine the range limits of the individual color forms. Other *Elacatinus* species show distributions similar to that of *E. evelynae* (Colin 1975). Thus, these barriers may be a common factor underlying geographic speciation or constraining the distribution of these gobies.

Here, I test whether these two proposed Caribbean biogeographic barriers separate discrete genetic lineages for nine taxa belonging to the genus *Elacatinus* (Gobiidae) whose distributions encompass the central Bahamas and the Mona Passage. For each taxon, I sequenced one mitochondrial and one nuclear marker. Concordance of genetic lineages obtained from multiple taxa with independent genetic markers would allow us to make robust inferences about phylogeographic history of the Caribbean region with reference to previously proposed biogeographic breaks. My results strongly support the presence of a biogeographic break at the Mona Passage, with weaker support for a break in the central Bahamas.

**MATERIALS AND METHODS**

Species of *Elacatinus* with distributions encompassing Mona Passage and the Central Bahamas include a cleaner goby (*E. evelynae*) that removes parasites from other fishes, and a complex of sponge-dwellers (*E. chancei, E. horsti, E. lori, E. louisae*) that live primarily in tube sponges (Colin 1975). These species were chosen to represent the two major ecological radiations in this subgenus because different habitat preferences may affect their ability to transgress biogeographic barriers (Rocha et al. 2002). *Elacatinus lori*, which is restricted to the Gulf of Honduras (Colin 2002), may be more closely related to *E. horsti* from the Bahamas than to the geographically closer populations of Jamaica or Grand Cayman (Chapter 2). I therefore included *E. oceanops*, a cleaner goby that has a similar disjunct distribution between the Gulf of Honduras and Florida. For each species, I sampled 1-16 (usually 8; mean=7.3) individuals from populations that delimit the extent of their respective distributions, which includes representatives of color forms (cf. Colin 1975) for species that are otherwise morphologically indistinguishable (Fig. 7). In all, 100 cleaner and 83 sponge-dweller individuals were analyzed.

**DNA Amplification**

Total genomic DNA was extracted from muscle tissue with a Qiagen (Valencia, CA) DNA Mini Kit following the manufacturer’s instructions. The polymerase chain reaction (PCR) was used to amplify protein-encoding regions of two genetic markers: mitochondrial cytochrome *b*
(mtcyb) and nuclear recombination-activating gene 1 (rag1). mtcyb was amplified with primers GLUDG-5' and CB2-3' (Palumbi 1996), and TgrH15153 designed for this study. rag1 was amplified with primers rag1F623, rag1F626, rag1R1221 and rag1R, derived from rag1 sequences obtained from a phylogenetic study of the genus (Chapter 1). Primer sequences are listed in Appendix A.

![Figure 7. Map of the Caribbean region indicating hypothesized biogeographic barriers, island populations sampled, and taxa sampled from each population. Biogeographic barriers indicated by thick black lines with white text. MONA PSG = Mona Passage. CEN BHMS = Central Bahamas. Taxa sampled are indicated by three letter code except for E. lori: cha = chancei, eve = evelynae, hor = horsti, lou = louisae, oce = oceanops. Single letter in parentheses indicates color form: b = blue, w = white, y = yellow. Number in parentheses indicates number of sampled individuals.](image)

The PCR was performed on a PTC-200 (MJ Research, Watertown, MA) with the following conditions: 94°C for three minutes for initial denaturing, followed by 35 cycles of 94°C for 15 sec, 48-58°C for 20 sec, and 72°C for 30-60 sec, depending on the primers used. Resulting
amplicons were purified using a Strataprep PCR Purification Kit (Stratagene, La Jolla, CA), then sequenced in both directions on an ABI 377 automated sequencer, using Big Dye Terminators (V2.0 and V3.1, Applied Biosystems, Foster City, CA) and the amplification primers.

All mtcyb sequences for E. evelynae (80 individuals) are from my previous phylogeographic study (Taylor and Hellberg 2003). All other mtcyb and ragl sequences are presented here for the first time. Sequences are deposited at Genbank.

Genetic Analyses

Sequences for each gene region were assembled and edited with Sequencher 3.0, then aligned with ClustalW (http://www2.ebi.ac.uk/clustalw/) set to default parameters. The haplotype phase for ragl alleles was independently determined for each nominal species by Bayesian inference with PHASE v2.1.1 (Stephens et al. 2001; Stephens and Donnelly 2003). Alternative strategies that applied PHASE to taxa based on discrete mitochondrial lineages did not qualitatively affect haplotype inference for any taxon. Five separate PHASE runs of 100 iterations each and two further runs of 400 iterations each were performed to ensure convergence of haplotype estimation. Haplotypes for which phase could not be determined with ≥60% confidence (Sotka et al. 2004) were excluded from phylogenetic analyses. Collapse 1.2 (D Posada, http://darwin.uvigo.es) was used to reduce the datasets of each genetic marker to unique haplotypes within each population for phylogenetic analyses. The resulting datasets for each species was analyzed by neighbor-joining (NJ) analyses with either HKY85 (mtcyb) or uncorrected-p (ragl) distances using PAUP* v4.0b10 (Swofford 2000). Trees were rooted with multiple outgroup taxa, as determined by a fuller analysis of interspecific relationships within the genus (Chapter 2). Support for inferred relationships was obtained with 10,000 NJ bootstrap (NJB) replicates. Analysis of molecular variation (Excoffier et al. 1992) using Tamura-Nei pairwise distances was performed with Arlequin 2.000 (Schneider et al. 2000). Substitution model used in the phylogenetic analyses of mtcyb was inferred with the aid of ModelTest (Posada and Crandall 1998). Tamura-Nei distances were used with Arlequin because the HKY85 model is not available with this software.

RESULTS

Sequences resulting from PCR amplification were trimmed to exclude ambiguous reads at each end, which yielded 400 base pairs (bp) for mtcyb and 573 bp for ragl for all individuals. No indels were observed for either marker. Haplotypic phase was unambiguously inferred for ragl from E. oceanops. The inferred phase of ragl alleles for E. evelynae was unambiguous except for three of 80 individuals (one from Jamaica and two from the northern Bahamas). Similarly, haploptypic phase could not be inferred for ragl for three of 83 sponge-dwellers (one E. chancei from Grand Turk, and two E. horsti from Grand Cayman and Jamaica). These individuals were excluded from subsequent analyses of nuclear sequences. The number of unique haplotypes for mtcyb and ragl for each species is detailed in Table 4.

Phylogeography of mtcyb

Phylogenetic relationships inferred from mtcyb are well supported for both cleaning and sponge-dwelling species (Figs. 8a, 9a). Within cleaners, two primary monophyletic clades are evident (Fig. 8a). The first major clade contains E. oceanops and the yellow form of E. evelynae
The geographic range of the yellow form of *E. evelynae* lies in the northern Bahamas, which is supported as a monophyletic clade (72% NJB). Within *E. oceanops*, the Belize and Florida populations are reciprocally monophyletic (Fig. 8a, $\Phi_{ST}=0.810$) with robust support (74% and 99% NJB, respectively). A second principal clade, containing white and blue form *E. evelynae*, is also supported (78% NJB). Within this clade, several subclades are monophyletic, including the southern Bahamas (69% NJB), Curaçao (90%), Barbados (55%) and Grand Cayman (93%).

### Table 4. Number of individuals (N) and unique haplotypes for each genetic marker for each taxon.

<table>
<thead>
<tr>
<th>Species</th>
<th>mtcyb</th>
<th>N</th>
<th>rag1 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cleaners</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. oceanops</em></td>
<td>11</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td><em>E. evelynae</em></td>
<td>44</td>
<td>80</td>
<td>77</td>
</tr>
<tr>
<td>blue form</td>
<td>17</td>
<td>40</td>
<td>44</td>
</tr>
<tr>
<td>white form</td>
<td>17</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td>yellow form</td>
<td>10</td>
<td>16</td>
<td>23</td>
</tr>
<tr>
<td><strong>Sponge-Dwellers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. chancei</em></td>
<td>5</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td><em>E. horsti</em></td>
<td>13</td>
<td>32</td>
<td>15</td>
</tr>
<tr>
<td>white form</td>
<td>5</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>yellow form</td>
<td>8</td>
<td>24</td>
<td>9</td>
</tr>
<tr>
<td><em>E. lori</em></td>
<td>2</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td><em>E. louisae</em></td>
<td>6</td>
<td>18</td>
<td>12</td>
</tr>
</tbody>
</table>

1 Six individuals for which *rag1* allele phase could not be determined are not included.

Several monophyletic clades are also evident for sponge-dwellers, although these clades often do not coincide with current taxonomic delineations (Fig. 9a). One clade consists of *E. chancei* from Puerto Rico and Barbados, plus *E. horsti* from Curaçao (80% NJB). A second clade consists of white and yellow form *E. horsti* from the western Caribbean (70% NJB). A third clade consists of *E. louisae*, *E. lori* and *E. horsti* (yellow form) from the northern Bahamas. Monophyly of this clade is not supported by NJB (<50%), although several monophyletic subclades that correspond to regional differentiation are supported (Fig. 9a).

**Geographic Variation of rag1**

As with *mtcyb*, the Belize and Florida populations of *E. oceanops* shared no *rag1* alleles ($\Phi_{ST}=0.737$) and the two populations are reciprocally monophyletic (Fig. 10). For *E. evelynae*, however, the phylogeny inferred from *rag1* sequences is not well resolved (Fig. 8b). None of the four major clades are supported by >50% NJB (Fig. 8b). The geographic distribution of allelic haplotypes corresponding to these four clades shows significant genetic structure among the three regions ($\Phi_{ST}=0.099$), with especially sharp changes in allele frequencies between Puerto Rico and Isla Desecheo (Fig. 10).
Figure 8. Phylogeographic lineages inferred for cleaner species. A) mtcyb lineages for *E. oceanops* and *E. evelynae*. Shaded boxes highlight clades separated by biogeographic breaks, indicated by black arrows. Numbers above branches are bootstrap support values. Country codes at branch tips indicate sampled localities: Bar = Barbados, Bel = Belize, Cay = Grand Cayman, Cur = Curaçao, Des = Isla Desecheo, Gtu = Grand Turk, Jam = Jamaica, NB = northern Bahamas, PrR = Puerto Rico, SSI = San Salvador Island. Number after country code indicates number of individuals with that haplotype. B) rag1 lineage for *E. evelynae*. Shaded boxes denoted A-D represent four major allelic lineages. Open circles represent alleles from Puerto Rico. Filled circles represent alleles from Isla Desecheo. Open squares represent alleles from Barbados. Filled squares represent alleles from Curaçao. Number preceding circle indicates number of sampled alleles representing that haplotype. See text for details.

The rag1 phylogeny inferred for sponge-dwelling taxa shows considerable phylogeographic structure (Fig. 9b). Although the topology is similar to that derived from mtcyb (Fig. 9a), not all rag1 clades have NJB support >50%. The most notable discrepancy between the phylogenies obtained from mtcyb and rag1 is the placement of *E. lori* from Belize. For mtcyb, *E. lori* is placed in a clade with individuals of *E. horsti* from the northern Bahamas (58% NJB; Fig. 9ab). In contrast, the rag1 data place *E. lori* in a polytomy with eastern and western Caribbean taxa.
Similarly, *E. louisae* from Jamaica (based only on a single specimen) is placed in different clades between the two markers.

---

**DISCUSSION**

The results of my study of two independent genetic markers indicate that multiple monophyletic lineages have evolved within several species of *Elacatinus*, and that these lineages do not always correspond with current taxonomy. Instead, these lineages usually correspond to one of three geographic areas within the Caribbean region. The western Caribbean region spans from the Mona Passage westward to Jamaica and Grand Cayman. The eastern Caribbean region extends from the Mona Passage east and south throughout the Lesser Antilles, including the Netherlands Antilles northward toward the southern Bahaman archipelago. The third region
extends from near the southern end of Exuma Sound in the central Bahamas to the northern extent of the Bahaman archipelago (Fig. 7). These three regions are separated by biogeographic breaks that have been previously postulated based on current patterns and the distributions of species and color forms of reef fishes (Colin 1975; Colin 1995; Colin 2003).

Figure 10. Frequencies of \textit{rag1} alleles from \textit{E. evelynae}, distributed among the four major clades illustrated in Figure 8. Inset: Relationship of \textit{E. oceanops rag1} allelic haplotypes demonstrates reciprocal monophyly between Florida Keys and Belize.

Biogeographic Barriers in the Caribbean

My earlier work (Taylor and Hellberg 2003) provided the first genetic support for the presence of breaks at the Mona Passage and near Exuma Sound in the central Bahamas. These breaks separate distinct \textit{E. evelynae mtcyb} lineages (Fig 8a), with the most striking result being the lack of shared \textit{mtcyb} haplotypes between Puerto Rico and Isla Desecheo, islands that are separated by only 23 km (Taylor and Hellberg 2003). In the present study, I found that
haplotypes of nuclear \textit{rag1} also shifted suddenly between these two islands (Fig. 10), which provides additional support for a genetic discontinuity in \textit{E. evelynae} at the Mona Passage.

In addition to strengthening my previous observations for the parasite-cleaning \textit{E. evelynae}, my new data also support a coincident break in lineages of sponge-dwelling \textit{Elacatinus}. The Mona Passage divides \textit{mtcyb} lineages for all sponge-dwelling species studied here, with the separation of \textit{E. chancei} in the eastern Caribbean from \textit{E. horsti} in the western Caribbean especially evident (Fig. 9a). A similar pattern is observed for \textit{E. louisae}, with a western Caribbean \textit{mtcyb} lineage separated from eastern Caribbean and northern Bahama lineages (Fig. 9a). The corresponding \textit{rag1} lineages also suggest the presence of a break between \textit{E. chancei} and \textit{E. horsti} at the Mona Passage, although ongoing lineage sorting is likely (Fig. 9b).

The distribution of many other Caribbean fishes may also be influenced by the Mona Passage. Species not extending east of the passage include the serranids \textit{Gramma melacara} (Starck and Colin 1978; Colin 2003) and species of the \textit{Hypoplectrus} (hamlet) complex (Domeier 1994). Species as diverse as a tonguesole, \textit{Symphurus arawak} (Munroe 1998), and a pipefish, \textit{Anarchopterus tectus} (Dawson and Vari 1982), appear to be much more common west of the passage than to the east, while another Caribbean pipefish, \textit{Syngnathus dawsoni}, occurs only east of the passage (Dawson and Vari 1982). The widespread clinid fish, \textit{Malacoctenus triangulatus}, shows distinct morphometric differences between individuals sampled across this region (Springer and Gomon 1975). All of these species, along with the cleaner and sponge-dweller \textit{Elacatinus} species studied here, occupy very different habitats, which suggests that the distribution of these species is not determined by ecological differences across the Mona Passage.

The break at the Mona Passage extends to non-teleost species as well. Indeed, the Mona Passage features prominently in the genetic structure of \textit{Acropora palmata}, a reef building coral whose larvae develop in the plankton for about one week. Multi-locus genotyping reveals a genetic divide at the Mona Passage separating populations in the western and eastern Caribbean, with some western genotypes leaking into Puerto Rico (I. Baums, M. Miller, and M. Hellberg, unpubl. MS). The coincident genetic divergence for a coral and several fish species, coupled with distributional limitations and morphological changes for many ecologically diverse fishes, together provide robust support for the presence of a biogeographic barrier at the Mona Passage.

Another biogeographic break may be present in the central Bahamas, but my results are less conclusive than for the Mona Passage. The central Bahamas, near the southern end of Exuma Sound and Long Island, is where the ranges of the yellow and blue forms of \textit{E. evelynae} adjoin (Colin 1975). My earlier work demonstrated a large genetic gap (3.04\% for \textit{mtcyb}) between these forms (Taylor and Hellberg 2003) that is coincident with this break. However, my results here suggest that yellow form \textit{E. evelynae} is more closely related to \textit{E. oceanops} than to the other color forms of \textit{E. evelynae} (Fig. 8a), thus negating my earlier genetic evidence for the central Bahamas break. Genetic support for the break does come from \textit{mtcyb} lineages of \textit{E. louisae} (Fig. 3a), a species distributed throughout the Bahamian archipelago (Colin 1975). However, my sample size is small, and my \textit{rag1} data do not corroborate the presence of this barrier (Fig. 9b). The lack of clear genetic evidence for a central Bahamas barrier suggests that, if present, this break may be weaker or have developed more recently than the Mona Passage.

The Northwestern Caribbean Region

The northwestern Caribbean region, spanning from the Gulf of Honduras to the Bahamas, may represent a distinct biogeographic province within the larger Caribbean region. This is
supported by my mtcyb evidence for both cleaner and sponge-dweller species (Figs. 8a, 9a). Another sponge-dweller, *E. xanthiprora*, also has a distribution apparently restricted to this province (Colin 1975), as do other fishes such as the *Hypoplectrus* discussed above.

Within this province, genetic divergence is evident for several species (Figs. 8a, 9). Most notable is *E. oceanops*, which is reciprocally monophyletic between Belize and Florida for both mtcyb and rag1 (Figs. 8a, 10). Individuals from these two populations, geographically separated by about 1100 km, have an average mtcyb divergence of about 3% (2.5-4.2%). If a 2% divergence per million years for mtcyb (Brown et al. 1979) is assumed, a maximum likelihood analysis using Fluctuate (Kuhner et al. 1998) suggests that these two populations have been isolated for roughly 800,000 years. This isolation over evolutionary time scales may be facilitated by the apparent absence of intervening populations from Cuba (R. Claro, pers. comm., Colin 1975) and by local larval retention (Taylor and Hellberg 2003). Local retention of pelagic larvae in the Gulf of Honduras, due possibly to current gyres (Colin 2002), may explain not only the isolation of *E. oceanops*, but also the endemism of several other reef fishes in this region (e.g., Collette 1974; Johnson and Brothers 1989; Colin 2002).

The restricted distribution of many reef fishes to the northwestern Caribbean shows striking parallels to some freshwater and terrestrial vertebrates. Species as different as poeciliid fishes (Briggs 1984; Rauchenberger 1988) and anoline lizards (Guyer and Savage 1986; Losos et al. 1998) show considerable diversity extending from Central America, across the Greater Antilles, and into the Bahamas, but are absent or present at reduced diversity across the Lesser Antilles. This pattern is all the more striking due to the vastly different evolutionary ages of these groups: at least some of the lineages within *Elacatinus* likely split due to Pleistocene sea level fluctuations (Colin 1975), while divergences among anoline lizards in the Greater Antilles can date back to at least 20-33 million year ago (Polcyn et al. 2002).

**Conclusion**

Historical barriers to gene flow are sometimes inferred from a single mitochondrial marker from a single taxon, but such inferences may be problematic (Neigel and Avise 1993; Irwin 2002). My study of biogeographic breaks in the Caribbean avoids such problems in three ways. First, I used two independent genetic markers, one mitochondrial (mtcyb) and one nuclear (rag1). Second, I used nine taxa from two evolutionarily independent lineages. Finally, the biogeographic breaks that I test, the Mona Passage and the central Bahamas, have been hypothesized a priori (Colin 1995; Colin 2003). My results clearly support the presence of a biogeographic break at the Mona Passage, but are less conclusive for a similar break in the central Bahamas. The phylogenetic structure revealed by my study does not always correspond to existing taxonomic nomenclature, but it does indicate a general division between a northwestern Caribbean province, encompassing Belize, parts of the Greater Antilles and the northern Bahamas, and a southeastern province across the rest of the Caribbean region. Within each province, subsequent genetic division has generated several monophyletic lineages, especially for mtcyb, and reciprocal monophyly of rag1 between two populations is evident for one species. Thus, genetic subdivision generated or maintained by the biogeographic breaks studied here, may help to explain the radiations of *Elacatinus* in the Caribbean.
Chapter 5

Conclusion

Gobies are among the most species rich taxa of all fishes (Nelson 1994) and are the largest component of Neotropical reef fishes (Robertson 1998). Much of the historical evolution in the Neotropical seven-spined gobies, which includes Elacatinus, is based on major shifts in habitat, followed by diversification of behavior and habitat utilization (Rüber et al. 2003). Such ecological adaptation may facilitate rapid divergence between transiently allopatric populations and increase the potential for speciation (Turner and Burrows 1995; Duffy 1996; Rüber et al. 2003). My results (Chapter 2) suggest that more recent bouts of speciation in Elacatinus follow a similar pattern of divergence based on differential habitat utilization, followed by diversification of coloration and morphology. This diversification appears to have occurred primarily in allopatry at geographic scales <1000 km (Chapters 2, 3 and 4), and is probably facilitated by both larval retention (Chapter 3) and biogeographic barriers to dispersal (Chapter 4).

Many marine organisms have pelagic larvae that can potentially interconnect distant populations through dispersal on ocean currents. Species with such broadly dispersing larvae should be genetically homogeneous over large spatial scales, which may compromise their ability to adapt to local conditions (Warner 1997). If, however, pelagic larvae are retained near their natal populations by behavioral (Burton and Feldman 1982) or physical oceanographic (Cowen et al. 2000) mechanisms, then populations would have a greater opportunity for genetic differentiation and local adaptation. My results (Chapters 3 and 4) show that strong phylogeographic structure can develop in the Caribbean Sea in species that have potential for long-distance larval dispersal. The close proximity of genetically distinct populations suggest that local larval retention generates the strong phylogeographic structure observed for many Elacatinus species across the Caribbean. If gobid larvae, as well as larvae of other small reef dwellers such as blennies and snapping shrimp, tend to remain near their natal reefs rather than disperse (Chapter 3; Leis 1991; Duffy 1996; Leis et al. 2003), this mechanism alone may explain their relatively high species diversity. However, populations that remain demographically closed for thousands of generations, as demonstrated for E. evelynae (Chapter 3) and E. oceanops (Chapter 4), may be able to adapt to local ecological conditions (Chapter 2; Warner 1997; Grosberg and Cunningham 2000).

The ability of pelagic larvae to disperse among populations may also be hindered by biogeographic barriers to gene flow. Few barriers have been proposed within the marine region that encompasses the Caribbean Sea and the Bahama Islands. However, the presence of regional endemism (e.g., Böhlke and Robins 1968; Collette 1974; Johnson and Brothers 1989) and restricted distributions (e.g., Starck and Colin 1978; Domeier 1994) of some species suggests that biogeographic breaks may be present. Two such breaks have been inferred, one in the central Bahamas near Long Island (Colin 1995), and one at the Mona Passage between Puerto Rico and Hispaniola (Colin 2003). My results (Chapter 4) clearly support the presence of a biogeographic break at the Mona Passage, but are less conclusive for a similar break in the central Bahamas. Further, the phylogenetic structure revealed by my study does not always correspond to existing taxonomic nomenclature, but it does indicate a general division between a northwestern Caribbean province, encompassing Belize, parts of the Greater Antilles and the northern Bahamas, and a southeastern province across the rest of the Caribbean region. Within
each province, subsequent genetic division has generated several monophyletic lineages. Thus, genetic subdivision generated or maintained by the biogeographic breaks studied here (Chapter 4), coupled with larval retention at natal populations (Chapter 3), may help to explain the recurring radiations of *Elacatinus* (Chapter 2) in the Caribbean.

In the past, studies of marine organisms often assumed that extended larval duration will result in broad dispersal (e.g., Roberts 1997). My results (Chapters 3 and 4) clearly indicate that, for at least some taxa, this simple assumption is a faulty foundation for understanding the geographic context of speciation in the sea (Chapter 2). Allopatric populations, maintained by geographic barriers (Chapter 4) and persistent retention of larvae (Chapter 3) could allow rapidly evolving mate-recognition characters, such as the color differences seen here for many species of *Elacatinus* and for other fishes (Seehausen et al. 1997; Boughman 2001) or the specificity of fertilization proteins in free-spawning animals (Palumbi 1998), to follow independent, population-specific evolutionary trajectories that could leave such populations reproductively isolated upon subsequent contact. Whatever the mode of speciation for *Elacatinus*, my data suggest that the diversity of reef fishes, even for well-studied species, remains underestimated and that the bright colors that attract popular interest in coral reef fishes may in part be responsible for their remarkable biodiversity.
Literature Cited


Appendix A

Table of Primers

Table A. Primers used for the amplification of mitochondrial and nuclear markers used in this study. Primers lacking citations were developed as part of this study.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Name</th>
<th>Sequence (5'→3')</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtcyb</td>
<td>CBL 14724</td>
<td><strong>GCT TGA CTT GAA RAA CCA YCG TTG</strong></td>
<td>Palumbi 1996</td>
</tr>
<tr>
<td></td>
<td>(GLUDG-5')</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBH 15175</td>
<td><strong>CCC TCA GAA TGA TAT TTG TCC TCA</strong></td>
<td>Palumbi 1996</td>
</tr>
<tr>
<td></td>
<td>(CB2-3')</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBL 14915</td>
<td><strong>CCT ATT TCT TGC AAT ACA CTA CAC</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBH 15560b</td>
<td><strong>GCR TAA GCA AAC AAA AAG TAC CAC TC</strong></td>
<td>Modified from Palumbi 1996</td>
</tr>
<tr>
<td></td>
<td>CBH 15670a</td>
<td><strong>AGT CCT CGT ATT TTT GAG GTG TG</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBL 15560c</td>
<td><strong>CAC ATT AAG CCT GAG TGA TAC TT</strong></td>
<td>Modified from Palumbi 1996</td>
</tr>
<tr>
<td></td>
<td>CBH 15930a</td>
<td><strong>CGG GTG CCG GTT TAC AAG</strong></td>
<td>Modified from Palumbi 1996</td>
</tr>
<tr>
<td>tRNAs</td>
<td>TgrH15153</td>
<td><strong>GCW CCT CAR AAR GAY ATT TGT CC</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TRNA-F</td>
<td><strong>TAC AAG CTG CAY TGG TAG C</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TRNA-R</td>
<td><strong>TTG GAT CGA AAT GTT TCA CAT</strong></td>
<td></td>
</tr>
<tr>
<td>D-loop</td>
<td>CRL 15980</td>
<td><strong>CTA CCT CCA ACT CCC AAA GC</strong></td>
<td>Palumbi 1996</td>
</tr>
<tr>
<td></td>
<td>CRH 370</td>
<td><strong>GGA ACC AAA TGC CAG GAA ATA G</strong></td>
<td>Palumbi 1996</td>
</tr>
<tr>
<td>rag1</td>
<td>O1 1</td>
<td><strong>CTG AGC TGC AGT CAG TAC CAT AAG ATG T</strong></td>
<td>Holcroft 2004</td>
</tr>
<tr>
<td></td>
<td>Or2 1</td>
<td><strong>CTG AGT CCT TGT GAG CTT CCA TRA AYT T</strong></td>
<td>Holcroft 2004</td>
</tr>
<tr>
<td></td>
<td>rag1F</td>
<td><strong>TCC ACG ACT TTG AGT GGC AGC</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rag1R409</td>
<td><strong>AGG GTC TCG TGG TCG GAT T</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rag1F371</td>
<td><strong>GAG CCC AAG CCA AAC TCG GA</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rag1F623</td>
<td><strong>GTG TGC ACT CTC TGT GAC ACC AC</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rag1F626</td>
<td><strong>TTG CAC TCT CTG TGA CAC CAC TCG</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rag1R823</td>
<td><strong>TGG AAG ATT TTG TAG AAC TCT GTG GC</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rag1F785</td>
<td><strong>GCA AAG CCA TTC ATG GAG ACC CA</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rag1R1221</td>
<td><strong>GAC TCA TTC CCC TCG CTG GC</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rag1R</td>
<td><strong>GAC TCA TTC CCC TCG CTG GC</strong></td>
<td></td>
</tr>
<tr>
<td>rho</td>
<td>rhodF</td>
<td><strong>CCG TCA TGG GCG CCT AYA TGT TYY T</strong></td>
<td>G. Rosenthal, pers. comm.</td>
</tr>
<tr>
<td></td>
<td>rhodRi</td>
<td><strong>CAC AGC AGA CGG CCG TAG C</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rhodFi</td>
<td><strong>GTC GAC TAC TAC ACC CGC GC</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rhodR</td>
<td><strong>CAG CAC AGG GTG GTG ATC ATR CAR TG</strong></td>
<td>G. Rosenthal, pers. comm.</td>
</tr>
</tbody>
</table>

1 *rag1* from individuals representing each clade were amplified and sequenced. The remaining *rag1* primers were designed from these initial sequences and used for all subsequent amplifications.
Appendix B

Letters of Permission

22 September 2004

Ms. Elizabeth Sandler
Science Permissions Department
1200 New York Ave., NW
Washington, DC 20005

Dear Ms. Sandler:

I am a Ph.D. student at Louisiana State University. My major professor and I published a chapter of my dissertation in Science. Because Science holds the copyright, I am required by the University to obtain permission to include this chapter in my final dissertation. Thus, I seek permission to reprint an article that appeared in Science as a chapter of my dissertation. Below is the information requested by Science to process this request.

Michael S. Taylor, Ph.D. Student
Dept. of Biological Sciences
Louisiana State University
Baton Rouge, Louisiana 70803
Phone: (225) 578-9114
Fax: (225) 578-2597

The article appeared in the printed version.

Title: Genetic Evidence for Local Retention of Pelagic Larvae in a Caribbean Reef Fish
Authors: Michael S. Taylor and Michael E. Hellberg
Volume 299 (3 January 2003), pages 107-109

I need to include the entire text, Figures 1 & 2, and Tables 1 & 2, plus the supplementary online material in my dissertation. I will somewhat rearrange the article to conform with the dissertation consistency guidelines required by the University. The supplementary material will be inserted into the article as Materials and Methods. Consequently, the article will be divided into the traditional sections of Introduction, Materials and Methods, Results and Discussion, and Literature Cited. The literature cited will be rearranged for consistent style (alphabetical by author). The article will otherwise remain unaltered. A footnote is inserted at the start of the dissertation chapter acknowledging permission from Science to use the article.
The article will appear as the third chapter of my dissertation, the title of which will remain the same as the printed *Science* article. I will be the sole author of the dissertation, which will be submitted to the University as an electronic dissertation no later than 12 November 2004. The electronic dissertation will be archived by Middleton Library of LSU, in the National Digital Library of Theses and Dissertations, and with ProQuest®. The library will print a single copy that will be archived, unbound, in the Hill Memorial Library of LSU. The dissertation will be available to all users of these systems.

I will promptly address any issues that may arise. Thank you for your assistance with this matter.

Sincerely,

Michael S. Taylor
Monday, October 04, 2004

Michael S. Taylor  
Dept. of Biological Sciences  
107 Life Sciences Bldg.  
Louisiana State University  
Baton Rouge, Louisiana 70803  
mtayL22@lsu.edu

Dear Michael S. Taylor:

Thank you for your request. This letter is to inform you of *Science*’s policy on author use of his/her *Science* article(s) in a thesis or dissertation that he/she is writing:

1. After publication of a manuscript in *Science*, the author may reprint his/her manuscript, in print format, in a thesis or dissertation written by the author as part of a course of study at an educational institution. Credit must be given to the first appearance of the material in the appropriate issue of *Science*.

2. If the thesis or dissertation is to be published in electronic format, the accepted version of the work (the accepted version of the paper before *Science*’s copy-editing and production) should be used and a link to the work on the Science website included.

3. The author may not permit others to reproduce the *Science* manuscript in a thesis or dissertation. In these cases, requesting parties should be instructed to contact AAAS directly for permission.

If you have any questions regarding this policy, please feel free to contact me by telephone at +1 202 326 6765 or via email at esandler@aaas.org.

Thank you for writing.

Sincerely,

Elizabeth Sandler  
Rights and Permissions  
The American Association for the Advancement of Science  
1200 New York Ave., NW  
Washington, DC  20005  
+1-202-326-6765 (tel)  
+1-202-682-0816 (fax)  
esandler@aaas.org

Headquarters:  
1200 New York Avenue, NW, Washington, DC  2005 USA
Vita

Michael S. Taylor was born in St. Louis, Missouri, in September 1961. He grew up in South Florida, where he graduated from Cooper City High School in 1979. He received the degree of Bachelor of Science with a major in Biology and a minor in Chemistry from Central Missouri State University in Warrensburg, Missouri in 1986. After a brief graduate career at Auburn University, Alabama, Michael left academics in 1992 to work as Manager of Collections for the systematic research collections at the Tulane University Museum of Natural History, where he remained through 1999. In January 2000, Michael entered the Graduate School of Louisiana State University in Biological Sciences, where he was supported by a Louisiana State University Board of Regents Fellowship. He also taught laboratory classes for Introductory Biology for Non-Majors, Honors Zoology and Invertebrate Zoology.