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Ancient divergences and recent connections in two tropical Atlantic reef fishes *Epinephelus adscensionis* and *Rypticus saponaceus* (Percoidei: Serranidae)

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Abstract Reef habitats of the tropical Atlantic are separated by river outflows and oceanic expanses that may preclude larval dispersal or other population connections in shorefishes. To examine the impact of these habitat discontinuities on the intraspecific phylogeography of reef-associated species we conducted range-wide surveys of two amphi-Atlantic reef fishes that have dispersive pelagic larval stages. Based on 593 bp of mtDNA cytochrome *b* from the rock hind *Epinephelus adscensionis* and 682 bp from the greater soapfish *Rypticus saponaceus* ($n=109$ and 86 , respectively), we found evidence of relatively ancient separations as well as recent surmounting of biogeographic barriers by dispersal or colonization. Rock hind showed slight but significant population genetic differentiation across much of the tropical Atlantic Ocean ($\Phi_{ST}=0.056$), but deep divergence between the southeastern United States and seven other localities from the Bahamas to the south, central and east Atlantic (mean pairwise $d=0.040$, overall $\Phi_{ST}=0.867$). The geographic distribution of the two rock hind lineages is highly unusual in genetic studies of Caribbean Sea reef fishes, because those lineages are separated by less than 250 km of open water within a major biogeographic region. In contrast, highly significant population genetic structure was observed among greater soapfish from the SW Caribbean, Brazil, and mid-Atlantic ridge

($\Phi_{ST}=0.372$), with a deep evolutionary separation distinguishing putative *R. saponaceus* from West Africa (mean pairwise $d=0.044$, overall $\Phi_{ST}=0.929$). Both species show evidence for a potential connection between the Caribbean and Brazilian provinces. While widespread haplotype sharing in rock hind indicates that larvae of this species cross oceanic expanses of as much as 2000 km, such a situation is difficult to reconcile with the isolation of populations in Florida and the Bahamas separated by only 250 km. These findings indicate that populations of some species in disjunct biogeographic zones may be isolated for long periods, perhaps sufficient for allopatric speciation, but rare gene flow between zones may preclude such evolutionary divergence in other species.

Introduction

Studies of population genetic structure in marine organisms often reveal low levels of differentiation (Ward et al. 1994; Bucklin and Wiebe 1998; Graves 1998). In reef fishes, which are typically nonmigratory as juveniles and adults (Leis 1991), little or no genetic population structure has been observed among widely separated localities in the Caribbean Sea. Shulman and Bermingham (1995) examined mitochondrial DNA (mtDNA) from eight Caribbean species. Lacson (1992) surveyed 33 allozyme loci from five Caribbean species. Collectively these studies included six taxonomic families exhibiting a wide range of pelagic larval durations (PLD; from approximately 13 to 122 days). Estimates of divergence among localities within the Caribbean Sea and Gulf of Mexico were low ($\Phi_{ST}=0.000$ – 0.172 , Shulman and Bermingham 1995; $F_{ST}=0.004$ – 0.035 , Lacson 1992). On the same geographic scale, an allozyme survey of the damselfish *Stegastes partitus* showed no significant population structure (Lacson 1992; Lacson et al. 1989). The lack of divergence between distant localities is usually

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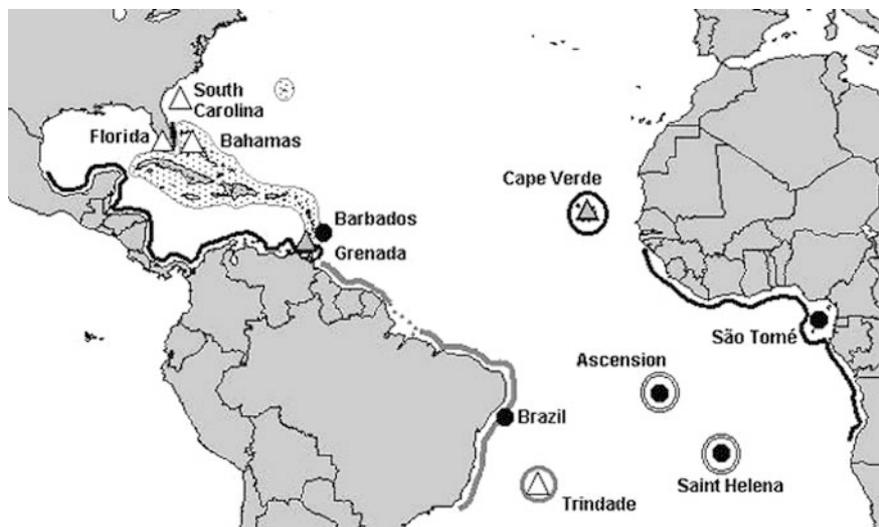
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attributed to egg and larval dispersal over hundreds or several thousand kilometers (see Johannes 1978; Brothers and Thresher 1985), a mechanism invoked to explain the presence of reef fish species on isolated islands (e.g., Allen and Robertson 1997; Robertson 2001). Shulman and Bermingham (1995, p 908) concluded that “understanding dispersal and consequent genetic differentiation among populations will require that we direct our attentions to the capabilities and behaviors of larvae that result in limitation or augmentation of dispersal distances.”

Briggs (1995) described two distinct biogeographic provinces within the tropical northwest Atlantic: a West Indian Province containing the islands from Cuba and the Bahamas to Grenada and the isolated northerly island of Bermuda (Fig. 1). The West Indies fauna are distinct from those of the Caribbean biogeographic province, which includes southern Florida, the southern Gulf of Mexico, and the coasts of Central America and northern South America. Because these provinces are separated by as little as 60 km, Briggs (1995) proposed that fast currents could act as vicariant barriers at the Florida Straits in the north, the Yucatán Channel in the west, and between Trinidad and Grenada in the south. However, to date, genetic studies have not indicated a restriction of gene flow between the Antilles and continental locations (Shulman and Bermingham 1995; Rocha et al. 2002).

Fig. 1 Biogeographic divisions of the tropical Atlantic with sample localities. Collection localities of greater soapfish *Rypticus saponaceus* (shaded triangles), rock hind *Epinephelus adscensionis* (open triangles), or both species (circles). The tropical biogeographic divisions discussed in the text are indicated as follows: the West Indian Province including Bermuda (gray stippled area); the Caribbean Province (western solid line); the Brazilian Province (gray line); the Ascension-St. Helena Province (double circles); and the East Atlantic Region including Cape Verde (eastern solid line and circle). The dotted gray line indicates the area of intrusion by Amazonian discharge, the Brazilian province north of this area is devoid of coral-type reefs. Modified from Figures 69 and 70 in Briggs (1995)



If a reef area as large as the Caribbean Sea and Gulf of Mexico can be connected by contemporary gene flow, then at what scale can one expect to find genetic isolation of reef populations? Many of the reef species surveyed in the Caribbean also occur in other biogeographic provinces of the Atlantic. If multiple biogeographic provinces are considered, evolutionary genetic partitions could be detectable despite a relatively lengthy PLD. Significant divergences have been observed between biogeographic provinces in antitropical species (e.g., Bowen and Grant 1997; Ball et al. 2000); and tropical species may also show genetic structure between discontinuous biogeographic zones (Planes et al. 1993; Banford et al. 1999; Colborn et al. 2001; Muss et al. 2001; Rocha et al. 2002).

For Atlantic reef species, all of the major biogeographic provinces are discontinuous (Briggs 1995). Coral reefs in the Gulf of Guinea (West Africa) are separated from Brazilian counterparts by an oceanic gap of 4,000 km, a distance postulated to be an effective barrier to dispersal (Rosen 1976). Caribbean reefs are separated from Brazilian reefs by 2,300 km of coastline dominated by soft bottoms and riverine outflows from the Amazon and Orinoco rivers. These outflows form coastally-trapped discharge zones of hyposaline water (Bonilla et al. 1993; Longhurst 1998), potentially decreasing effective latitudinal dispersal in the tropical western Atlantic. These barriers are invoked to explain the high endemism of the Brazilian and Caribbean provinces, and this level of isolation may be higher than is currently recognized. Recent research indicates a potentially large number of undescribed endemic reef fish species in the Brazilian province (Rocha and Rosa 1999; Bernardi et al. 2000; Floeter and Gasparini 2000; Floeter et al. 2001), and this finding also may be applicable to the poorly studied reefs in West Africa.

Vicariant barriers between Atlantic reef provinces may be sufficient to allow allopatric speciation in some cases (Bernardi et al. 2000; Muss et al. 2001). In other cases, there has clearly been some dispersal, gene flow,

or colonization in recent evolutionary time, as evidenced by tropical Atlantic reef fish species that occur on both sides of the Atlantic. One possible mechanism for mediating gene flow between the Brazilian and East Atlantic biogeographic provinces in particular is the presence of oceanic islands (e.g., Ascension, St. Helena, and Trindade Island) (Fig. 1). While small in size, the islands that interrupt the deep mid-Atlantic expanse may serve as stepping stones for transoceanic colonization (Briggs 1995).

With these issues in mind, the genetic variation in two amphiatlantic reef fishes was surveyed. The rock hind, *Epinephelus adscensionis* Osbeck (1971), is a serranid fish that occurs in shallow water from Bermuda to Brazil, on the mid-Atlantic ridge islands, and in the eastern Atlantic from the Gulf of Guinea to Morocco (Böhlke and Chaplin 1993). The greater soapfish, *Rypticus saponaceus* Bloch and Schneider (1801), is another amphiatlantic serranid species (as defined by Guimarães 1999) that has a distribution similar to the rock hind. Both species are demersal reef fishes (Thresher 1980), such that long-range dispersal is limited to the pelagic larval stage (Leis 1991). The PLD of rock hind and greater soapfish have not been reported, but a 40-day average is proposed for serranids (Lindeman et al. 2000). If barriers to dispersal by pelagic larvae exist in these amphiatlantic reef fishes, then sustained interruptions in gene flow may be evident on a broad geographic scale. Molecular genetic analysis of rock hind and greater soapfish from all major biogeographic zones within their ranges should therefore elucidate the population structure in these reef fishes; and may illuminate general biogeographic patterns for Atlantic reef species.

Materials and methods

Samples of *E. adscensionis*, *R. saponaceus*, and selected congeners were collected from multiple locations within the Caribbean, Brazilian, and mid-Atlantic biogeographic provinces, and eastern Atlantic region (Fig. 1). Northwest Atlantic specimens of *E. adscensionis* were collected from South Carolina, the Florida Keys, the Bahamas and Barbados. Brazilian rock hind specimens were collected from Trindade Island and the mainland states of Vitoria and Bahia. Mid-Atlantic ridge collections were made at Ascension Island and St. Helena. Eastern Atlantic *E. adscensionis* were obtained from São Tomé (Gulf of Guinea). Specimens of *R. saponaceus* were obtained from Grenada and Barbados (tropical NW Atlantic); Vitoria and Bahia (Brazilian province); Ascension and St. Helena (mid-Atlantic ridge province); and the eastern Atlantic localities of São Tomé and Cape Verde. Fish were collected during snorkel and SCUBA diving (using polespears and microspears) by D.R. Robertson and B.W. Bowen. In most locations, gill and/or muscle samples were taken and stored in a saturated-salt DMSO buffer (Amos and Hoelzel 1991).

Total genomic DNA was isolated with organic extraction (using phenol, chloroform, and isoamyl alcohol) and precipitated with sodium acetate in a 95% ethanol solution. Isolated DNA was resuspended in 100 μ l TE (10 mM Tris and 1 mM EDTA, pH 8.0). An approximately 750 base pair (bp) fragment of the mitochondrial cytochrome *b* gene was amplified via the polymerase chain reaction (PCR; Saiki et al. 1985) with primers Cyb-09H (5'-GTGACTT-GAAAACCCGTTG-3'; Song et al. 1994) and Cyb-07L

(5'-AATAGGAAGTATCATTCGGGTTTGATG-3'; Taberlet et al. 1992). The amplification reaction mix included 3 mM MgCl₂, 40 nM of each primer, 17.5 nM of each dNTP, 0.80 μ l of Taq DNA polymerase (Promega, Madison, Wis.) and 3.5 μ l of 10 \times PCR buffer (Promega) in 35 μ l total volume. Amplification temperature regime included an initial 3-min denaturation at 94°C and final extension at 72°C for 3 min, with 25 cycles of 30 s at 94°C, 30 s at 53°C, and 40 s at 72°C for *E. adscensionis*. Amplifications of *R. saponaceus* DNA used an identical protocol, except for an annealing temperature of 54°C and a reaction buffer with 1 mM MgCl₂. Oligonucleotide primers were removed by Ultrafree-MC centrifugal filter units (Millipore, Billerica, Mass.) and/or simultaneous digestion with exonuclease I and shrimp alkaline phosphatase (USB, Cleveland, Ohio).

Amplification products were denatured and DNA sequencing reactions were conducted with fluorescently labeled dideoxy terminators according to manufacturer's recommendations (ABI Model 800, Applied Biosystems, Foster City, Calif.). Labeled extension products were gel separated and analyzed with an automated sequencer (ABI Models 373A and 377) in the University of Florida DNA Sequencing Core. Resulting fragments were aligned and edited using Sequencher ver. 3.0 (Gene Codes, Ann Arbor, Mich.). All samples were sequenced in forward direction using Cyb-09H; or in the reverse direction using Cyb-19H (5'-CTCACTGCTCGGACTCTG-3') for some samples of *E. adscensionis* or using Cyb-22H (5'-GTGAARTTGTCTGGGTCTCCT-3') for some samples of *R. saponaceus*. Both forward and reverse sequences were obtained for at least one representative sample of each haplotype, to assure accuracy of haplotype assignments. Sequences were aligned with representative species of *Epinephelus* (20 spp.) and *Rypticus* (2 spp.) to confirm identification and provide outgroup comparisons. Haplotypes were also compared with the complete cytochrome *b* sequences from *Homo sapiens* (GenBank Accession V00662; Anderson et al. 1981) and *Epinephelus* sp. A (GenBank Accession AF143193; Allegrucci et al. 1999) as additional quality controls. For clarity of discussion, rock hind haplotypes are designated by letter abbreviations, while numerals are used for greater soapfish haplotypes.

Population genetic parameters were calculated with software package Arlequin ver. 2.0 (Schneider et al. 2000). Nucleotide diversity (π ; Equation 10.5 in Nei 1987) and haplotype diversity (h ; Equation 8.5 in Nei 1987) were calculated for each biogeographic province. Genetic distances (d) were calculated using the substitution model of Tamura and Nei (1993) corrected for unequal mutation rate ($\alpha=0.50$; Yang 1996) with a 3:1 transition to transversion (ti:tv) ratio. The distribution of pairwise differences among haplotypes generated a mismatch distribution (Rogers and Harpending 1992; Rogers 1995), with the sum of squared differences in frequency at each mismatch class, or raggedness index (r ; Harpending 1994). A resampling simulation (100 bootstraps) generated an expected distribution of pairwise differences using population-expansion parameters estimated by a least squares nonlinear regression (Schneider and Excoffier 1999).

The proportion of genetic diversity within and among localities was estimated with an analysis of molecular variance (AMOVA) in Arlequin (Excoffier et al. 1992). The total variance explained by population-level differences (Φ_{ST}) was estimated to infer degree of population subdivision. A population pairwise differentiation test was performed using a 10,000-step Markov chain simulation (Schneider et al. 2000). Significance (overall $\alpha=0.05$) was determined after full Bonferroni correction for multiple comparisons (individual $P=0.002$ for rock hind and $P=0.003$ for greater soapfish).

Both neighbor-joining (NJ) and maximum parsimony (MP) trees were constructed and evaluated using 500 bootstrap replicates. The NJ tree includes two congeners: *Epinephelus analogus* ($n=3$) and *E. labriformis* (Fig. 2). Rather than reporting only the most parsimonious phylogram(s), a consensus tree considering slightly less parsimonious solutions was constructed. This was reported only if there were few alternate solutions (i.e., <25 trees <5 steps longer than the most parsimonious cladogram). All phylograms were constructed using Paup* β ver. 4.0b (Swofford 2002).

Fig. 2 Distances among *E. adscensionis* haplotypes (A–HH) and selected congeners, as indicated by a neighbor-joining (NJ) tree. For those nodes with >70% retention after 500 bootstrap iterations, the bootstrap support is indicated above the node

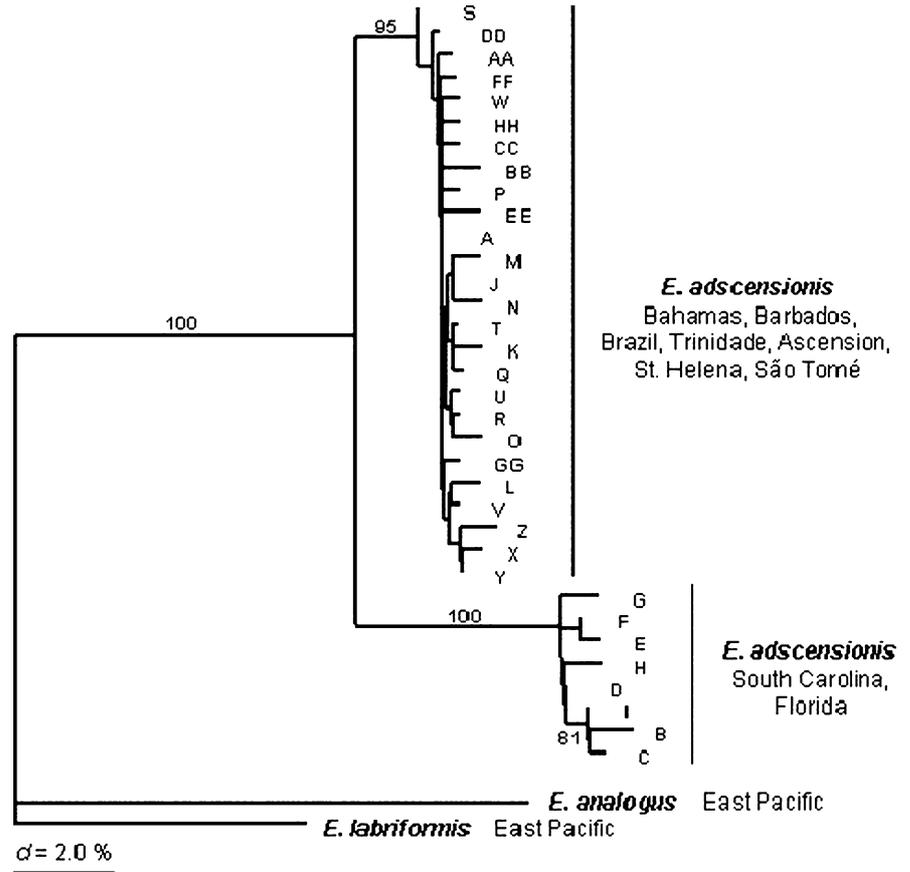


Table 1 Genetic variation in the rock hind *Epinephelus adscensionis* and greater soapfish *Rypticus saponaceus*. The number of specimens (*n*), the haplotype diversity (*h*) and nucleotide diversity (π) for

each locality and in total are listed. Some species were not collected (–) at some localities. Sample size at Trindade Island (*n*=2) precludes statistical treatment

Location	<i>E. adscensionis</i>			<i>R. saponaceus</i>		
	<i>n</i>	<i>h</i>	π	<i>n</i>	<i>h</i>	π
South Carolina	3	0.667 ± 0.314	0.002 ± 0.002	–	–	–
Florida	9	0.889 ± 0.091	0.003 ± 0.002	–	–	–
Bahamas	5	1.000 ± 0.127	0.004 ± 0.003	–	–	–
Grenada	–	–	–	5	0.900 ± 0.161	0.003 ± 0.002
Barbados	19	0.731 ± 0.109	0.001 ± 0.001	4	1.000 ± 0.177	0.003 ± 0.002
Brazil	16	0.625 ± 0.139	0.002 ± 0.002	23	0.913 ± 0.035	0.003 ± 0.002
Trindade	2	0.000 ± 0.000	0.000 ± 0.000	–	–	–
Ascension	21	0.186 ± 0.110	0.001 ± 0.001	11	0.873 ± 0.089	0.003 ± 0.002
St. Helena	17	0.228 ± 0.130	0.001 ± 0.001	16	0.242 ± 0.135	0.001 ± 0.001
Cape Verde	–	–	–	18	0.863 ± 0.042	0.004 ± 0.002
São Tomé	17	0.331 ± 0.143	0.001 ± 0.001	9	0.722 ± 0.097	0.003 ± 0.002
Total:	109	0.586 ± 0.058	0.008 ± 0.004	86	0.929 ± 0.016	0.019 ± 0.009

Results

Rock hind

A total of 109 *E. adscensionis* were surveyed, yielding 593 bp of resolved sequence per individual. No extra termination codons were observed in comparison with the Allegrucci et al. (1999) serranid sequence. Sequence comparisons revealed a transition-rich data set (ti:tv

ratio=23:5) that included a total of 34 haplotypes (*d*=0.002–0.044; Tables 1, 2). However, only seven haplotypes were observed in more than one specimen. These sequences revealed low nucleotide diversity (π =0.008) and moderate haplotype diversity (*h*=0.586), a common pattern in marine fishes (Grant and Bowen 1998). The AMOVA indicated very strong population structure for *E. adscensionis* (Φ_{ST} =0.867, *P*<0.001). Differentiation tests indicated significant population

Table 2 Haplotype occurrences and sample sizes in *E. adscensionis*

	South Carolina	Florida	Bahamas	Barbados	Brazil	Trindade	Ascension	St. Helena	São Tomé	Total
Haplotype	<i>n</i> = 3	9	5	19	16	2	21	17	17	109
A	–	–	–	10	10	2	19	15	14	70
B	2	–	–	–	–	–	–	–	–	2
C	1	–	–	–	–	–	–	–	–	1
D	–	3	–	–	–	–	–	–	–	3
E	–	2	–	–	–	–	–	–	–	2
F	–	1	–	–	–	–	–	–	–	1
G	–	1	–	–	–	–	–	–	–	1
H	–	1	–	–	–	–	–	–	–	1
I	–	1	–	–	–	–	–	–	–	1
J	–	–	1	–	–	–	–	–	–	1
K	–	–	1	–	–	–	–	–	–	1
L	–	–	1	–	–	–	–	–	–	1
M	–	–	1	–	–	–	–	–	–	1
N	–	–	1	–	–	–	–	–	–	1
O	–	–	–	2	–	–	–	–	–	2
P	–	–	–	1	–	–	–	–	–	1
Q	–	–	–	1	–	–	–	–	–	1
R	–	–	–	1	–	–	–	–	–	1
S	–	–	–	1	–	–	–	–	–	1
T	–	–	–	1	–	–	–	–	–	1
U	–	–	–	1	–	–	–	–	–	1
V	–	–	–	1	–	–	–	1	–	2
W	–	–	–	–	1	–	–	–	–	1
X	–	–	–	–	1	–	–	–	–	1
Y	–	–	–	–	1	–	–	–	–	1
Z	–	–	–	–	1	–	–	–	–	1
AA	–	–	–	–	1	–	–	–	–	1
BB	–	–	–	–	1	–	–	–	–	1
CC	–	–	–	–	–	–	1	–	–	1
DD	–	–	–	–	–	–	1	–	–	1
EE	–	–	–	–	–	–	–	1	–	1
FF	–	–	–	–	–	–	–	–	1	1
GG	–	–	–	–	–	–	–	–	1	1
HH	–	–	–	–	–	–	–	–	1	1

Table 3 Population pairwise differentiation in *E. adscensionis*. The pairwise Φ_{ST} values are given below the diagonal, while above the diagonal are significant (+) or not significant (–) pairwise differentiation as observed under Markov chain simulation, based on an

overall $P < 0.05$ after full Bonferroni correction. Trindade and South Carolina samples were not included because of low sample sizes

	Florida	Bahamas	Barbados	Brazil	Ascension	St. Helena	São Tomé
Florida		–	+	+	+	+	+
Bahamas	0.911		–	–	+	+	–
Barbados	0.947	0.165		–	–	–	–
Brazil	0.937	0.125	0.039		–	–	–
Ascension	0.973	0.363	0.043	0.055		–	–
St. Helena	0.962	0.238	0.018	0.025	0.007		–
São Tomé	0.962	0.246	0.028	0.030	0.007	0.000	

structure, with pairwise Φ_{ST} 's > 0.9 between Florida and all other localities except for comparisons with South Carolina and Trindade Island, for which low sample sizes ($n=3$ and 2, respectively) precluded testing (Table 3). The population structure is characterized by deep divergence between the southeastern United States haplotypes and those of all other locations and the abundance of haplotype A (64.2% of all rock hind samples) outside Florida/South Carolina. The mean sequence divergence between Florida/South Carolina haplotypes and all other haplotypes was $d=0.040$

(range: 0.034–0.044). Removing the highly divergent Florida/South Carolina samples from AMOVA decreased diversity measures of the remaining samples ($h=0.481$, $\pi=0.001$, $\Phi_{ST}=0.056$, $P < 0.001$). Significant differentiation was also found in comparing the Bahamas sample with the Ascension and St. Helena samples (Table 3), possibly reflecting the predominance of haplotype A in the mid-Atlantic localities, in contrast to its absence in Bahamas specimens ($n=5$) (Table 2, Fig. 2).

The MP tree had the same structure as the NJ tree: two primary branches in *E. adscensionis* with bootstrap

values ≥ 0.90 . All equally parsimonious trees had unresolved polytomies among those haplotypes found together in only one population (and usually differing by only one mutation). The close relationship among haplotypes within each branch of the rock hind phylogeny also created a bimodal mismatch distribution ($r=0.070$, $q_0=1.169$, $q_1=1.169$). When a distribution was estimated after removal of the Florida/South Carolina samples, no significant difference was found between the observed haplotype distribution and an equilibrium distribution simulated under a rapid population expansion model ($\tau=1.126$, $P=0.550$). Aside from haplotype A, only haplotype V (one specimen each from Barbados and St. Helena, 6,745 km apart), was found in multiple biogeographic provinces. The Caribbean province, however, is split by the presence of 21–29 nucleotide differences between the continental United States and the Bahamas and Barbados.

Greater soapfish

A total of 86 greater soapfish specimens were analyzed, including 27 specimens from the East Atlantic Region (Cape Verde and São Tomé) that diverged notably from samples in the remainder of the range ($d=0.044$, range: 0.037–0.052). These fish matched conventional

descriptions of *R. saponaceous* (Fischer et al. 1981) and were not consistent with the only other congeneric species in the East Atlantic (Guimarães 1999). Analyses of 682 bp of cytochrome *b* yielded a conventional pattern of mutations (ti:tv=27:6) and a total of 31 *R. saponaceous* haplotypes (Tables 1, 4) with somewhat higher genetic diversity ($\pi=0.019$, $h=0.929$) than the rock hind ($\pi=0.008$, $h=0.586$). As in rock hind, the dataset was influenced strongly by a bifurcation between samples in one portion of the range (eastern Atlantic for this species) and a branch containing all other collection sites (western and central Atlantic). The divergence between the eastern Atlantic haplotypes and all others yielded strong population structure ($\Phi_{ST}=0.876$, $P<0.001$). Large pairwise Φ_{ST} 's were calculated in comparisons between Cape Verde or São Tomé with all other localities ($\Phi_{ST}=0.906$ – 0.963 , Table 5); all but one of these comparisons were significant in differentiation tests after Bonferroni correction ($\alpha=0.003$).

Reanalysis of the *R. saponaceous* haplotypes, after excluding the divergent East Atlantic samples, reveals significant overall population structure ($\Phi_{ST}=0.372$, $P<0.001$). Nineteen specimens (22% of the total) possessed a haplotype unique within this study; haplotypes 1 and 5 are the only ones present in more than one biogeographic province. Within the West Atlantic samples, the low degree of haplotype sharing resulted in

Table 4 Haplotype occurrences and sample sizes in *R. saponaceous*

	Grenada	Barbados	Brazil	Ascension	St. Helena	Cape Verde	São Tomé	Total
Haplotype	$n=5$	4	23	11	16	18	9	86
1	2	1	4	–	–	–	–	7
2	1	–	–	–	–	–	–	1
3	1	–	–	–	–	–	–	1
4	1	–	–	–	–	–	–	1
5	–	1	3	–	1	–	–	5
6	–	1	–	–	–	–	–	1
7	–	1	–	–	–	–	–	1
8	–	–	5	–	–	–	–	5
9	–	–	3	–	–	–	–	3
10	–	–	1	–	–	–	–	1
11	–	–	1	–	–	–	–	1
12	–	–	1	–	–	–	–	1
13	–	–	1	–	–	–	–	1
14	–	–	1	–	–	–	–	1
15	–	–	1	–	–	–	–	1
16	–	–	1	–	–	–	–	1
17	–	–	1	–	–	–	–	1
18	–	–	–	4	14	–	–	18
19	–	–	–	2	–	–	–	2
20	–	–	–	1	–	–	–	1
21	–	–	–	1	–	–	–	1
22	–	–	–	1	–	–	–	1
23	–	–	–	1	–	–	–	1
24	–	–	–	1	–	–	–	1
25	–	–	–	–	1	–	–	1
26	–	–	–	–	–	5	2	7
27	–	–	–	–	–	3	4	7
28	–	–	–	–	–	3	3	6
29	–	–	–	–	–	3	–	3
30	–	–	–	–	–	2	–	2
31	–	–	–	–	–	2	–	2

Table 5 Population pairwise differentiation in *R. saponaceous*. The pairwise Φ_{ST} values are given below the diagonal, while above the diagonal are significant (+) or not significant (-) pairwise

	Grenada	Brazil	Ascension	St. Helena	Cape Verde	São Tomé
Grenada		-	-	+	+	-
Brazil	0.128		+	+	+	+
Ascension	0.456	0.327		-	+	+
St. Helena	0.695	0.424	0.005		+	+
Cape Verde	0.906	0.909	0.911	0.938		-
São Tomé	0.929	0.919	0.928	0.963	0.042	

differentiation as observed under Markov chain simulation, based on an overall $P < 0.05$ after full Bonferroni correction. Barbados specimens were not included because of low sample size

significantly different pairwise differentiation in comparisons between Brazil versus Ascension, Brazil versus St. Helena and Grenada versus St. Helena ($\Phi_{ST} = 0.327-0.695$, $P < 0.001$; Table 5). To examine the influence of small sample size in the Caribbean localities, an additional analysis was conducted considering the Barbados and Grenada as a single population ($h = 0.917$; $\pi = 0.003$; overall $\Phi_{ST} = 0.886$, $P < 0.001$; West Atlantic overall $\Phi_{ST} = 0.354$, $P < 0.001$). In this case only the comparison between the combined Caribbean sample and São Tomé changed in significance ($\Phi_{ST} = 0.927$, $P = 0.002$). The distribution of observed differences between all pairs of haplotypes ($r = 0.194$, $q_0 = 0.000$, $q_1 = 2.646$) was significantly different from the equilibrium model of rapid population expansion ($\tau = 4.430$, $P \geq 0.49$).

Analysis of three *Rypticus* species available in this study indicates that the two lineages of putative *R. saponaceous* described here may not be monophyletic (67% bootstrap support), and indeed form an unresolved polytomy with five specimens (three haplotypes) of *R. bicolor*, an eastern Pacific species, and *R. subbifrenatus*, another amphi-Atlantic species. Mean inter-specific (*R. saponaceous* vs. *R. bicolor*) pairwise sequence divergence was $d = 0.047$ (range: 0.043–0.055). The only other soapfish reported from the eastern Atlantic, *R. subbifrenatus*, was collected at the same locality in São Tomé as the putative *R. saponaceous* specimens. The mean genetic distance between *R. saponaceous* and *R. subbifrenatus* was $d = 0.115$ (range: 0.112–0.124), greater than that found between the East Atlantic and other samples of *R. saponaceous* ($d = 0.044$) (Fig. 3).

Discussion

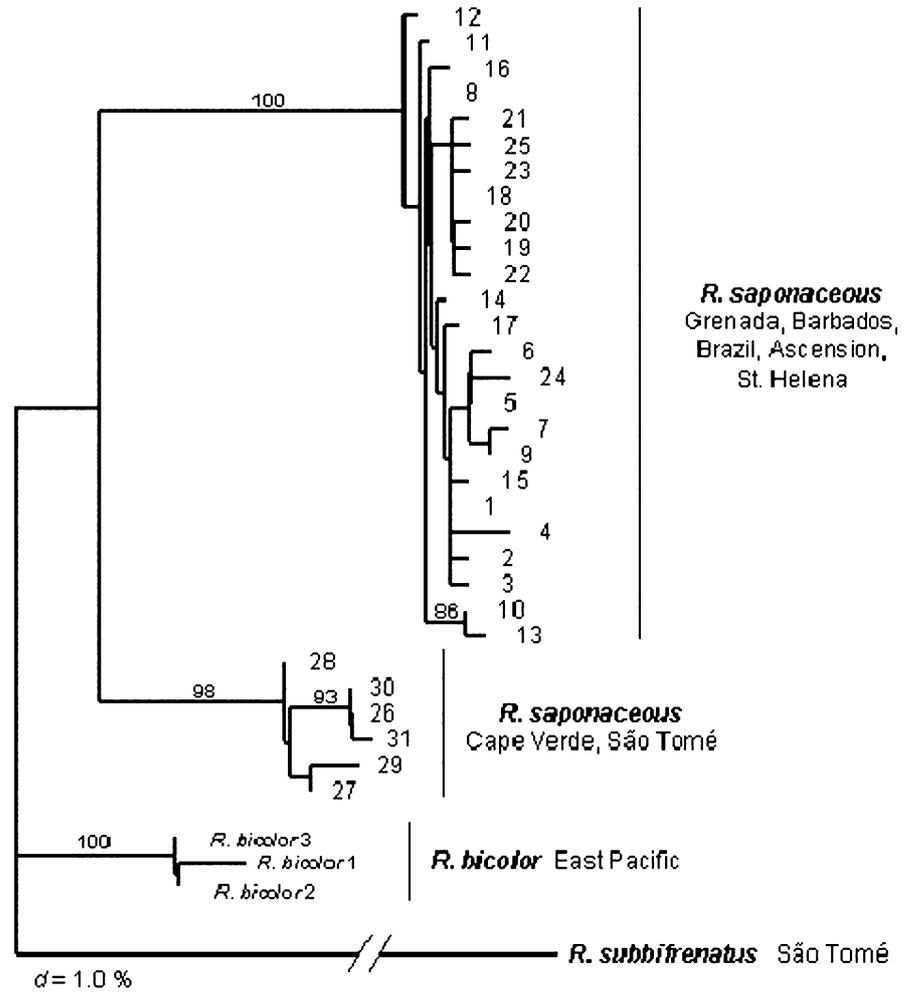
Several patterns concerning the phylogeographic histories of these two species can be recovered from these data. First, both rock hind and greater soapfish intraspecific phylogenies are characterized by a single major bifurcation, with one branch restricted to a single region while the other is widespread through multiple biogeographic provinces. Haplotypes within the divergent branches exhibit a shallow history and rapid coalescence to a common ancestor. For the rock hind, a single haplotype was shared over most of the species' distribution, and the large number of "twigs" is consistent with a population bottleneck and subsequent expansion

from two ancestral (and divergent) lineages (Avisé 2000). In contrast, greater soapfish exhibited less haplotype sharing among biogeographic provinces, and a null model of rapid population expansion was not consistent with the structure of the western and central Atlantic lineages. A reconstruction of the biogeographic and evolutionary history in greater soapfish and rock hind is attempted by first considering the prominent, relatively ancient, separations between phylogroups.

The greatest interlocality divergences found in rock hind and greater soapfish ($d = 4.4\%$ and 5.2% , respectively) are relatively deep for intraspecific comparisons of marine fishes (see reviews by Avisé 2000; Grant and Bowen 1998). However, descriptions of relatively ancient cytochrome *b* divergences in marine fishes are accumulating. Bernardi et al. (2000) observed a divergence of $d = 2.3\%$ in the parrotfish *Sparisoma rubripinne* between two eastern Atlantic (São Tomé) and two Caribbean specimens. Divergences of $d = 0.052-0.127$ were observed between five phylogroups in biogeographic provinces for the amphi-Atlantic reef blenny *Ophioblennius atlanticus* (Muss et al. 2001). The deep splits observed in rock hind, greater soapfish, parrotfish, and blenny are of an equivalent magnitude to the divergences observed in cytochrome *b* between Newfoundland and Norwegian samples of the capelin *Mallotus vilosus* ($d = 5.7\%$; Birt et al. 1995). These values approach divergences between sister species of other marine fishes (Johns and Avisé 1998), but are less than that described between cryptic sympatric species in another grouper, *E. marginatus* ($d = 0.158$; Gilles et al. 2000).

The deep divergences observed in *E. adscensionis* and *R. saponaceous* also invoke the possibility of cryptic species. This may be particularly likely for the greater soapfish, based on a Pliocene divergence between eastern and western Atlantic specimens. The molecular evolutionary rate of cytochrome *b* has been estimated to range from 1 to 3%/My (Canatore et al. 1994; Irwin et al. 1991; Fleischer et al. 1998). Although rates may vary among taxa (Mindell and Thacker 1996), a molecular clock rate of 1–2%/My is applied. This would place the earliest divergence date between Atlantic *R. saponaceous* and the East Pacific *R. bicolor* at 3.5 million years ago (Mya) (range = 2.1–5.5), a timeframe concordant with the last contact between East Pacific and West Atlantic fishes (about 3.1–3.5 Mya; Coates and Obando 1996).

Fig. 3 Distances among *Rypticus* haplotypes (1–31) and selected congeners, as indicated by a neighbor-joining (NJ) tree. For those nodes with >70% retention after 500 bootstrap iterations, the bootstrap support is indicated above the node



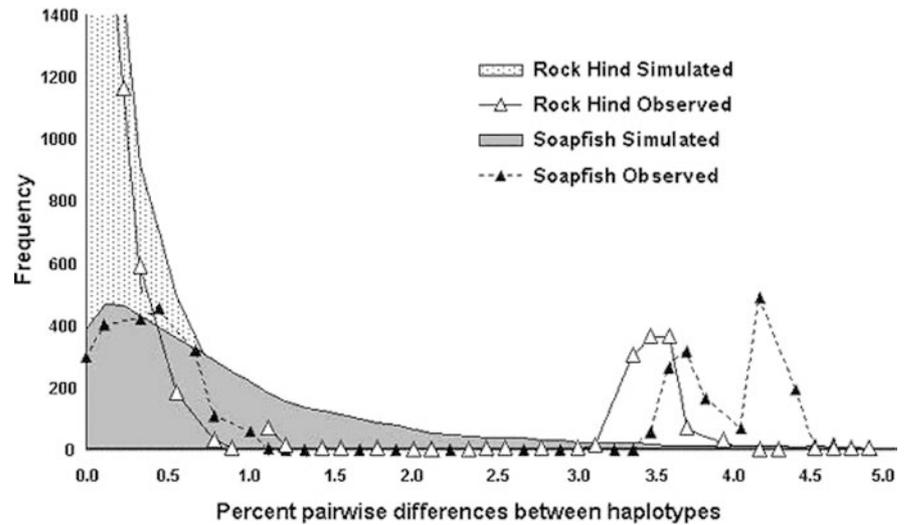
This benchmark is used to estimate other divergence dates in the evolutionary history of soapfishes, and provisionally apply this rate to rock hind, with the recognition that divergence dates for these species are not well-calibrated and must be interpreted with caution.

Based on the molecular clock rate of 1–2%/My, the deepest mtDNA lineages in *E. adscensionis* coalesce at approximately 2.7 My, and the deepest lineages in *R. saponaceus* coalesce at about 3.3 My. Evidence from oxygen isotope ratios in fossil foraminifera indicates the shoaling and separation of waters about the Isthmus of Panama from 5–8 Mya, even before the complete emergence of present-day Panama (Keigwin 1982; Collins et al. 1996). The resulting hydrostatic imbalance caused the major current systems of the Atlantic to increase in velocity, then slowed to present levels by ~2.5 million years before present to produce the general circulation patterns that persist today (Maier-Reimer et al. 1990; Haug and Tiedemann 1998). Thus, it is possible that divergence occurred among Atlantic *Rypticus* populations following the closure of the central American isthmus (and subsequent responses in Atlantic circulation) due to decrease in dispersal opportunities across the tropical Atlantic.

An obvious alternative is that the specimens collected in Cape Verde and São Tomé were misidentified members of another previously described *Rypticus* species. This possibility is unlikely for three reasons: first, our collection protocol was designed to distinguish *R. saponaceus* from the only other soapfish described from the East Atlantic (*R. subbifrenatus*; Courtenay 1970; Guimarães 1999); second, the phylogenetic analyses include *R. subbifrenatus* collected at São Tomé and show that it is highly divergent from both eastern and western Atlantic *R. saponaceus* as well as the East Pacific *R. bicolor*; and third, Cape Verde and São Tomé specimens had no gross departures from prior descriptions of *R. saponaceus*, the species with the greatest meristic variability in the genus (Courtenay 1967; Guimarães 1999). The molecular evidence, in combination with the field observation and geographic considerations, indicate the strong possibility of two species within the currently recognized *R. saponaceus*.

Regardless of taxonomic considerations, the cytochrome *b* gene tree for greater soapfishes indicates that eastern and western Atlantic lineages initially diverged at about the time of the closure of the Isthmus of Panama. Subsequently, or at nearly the same time (Fig. 4),

Fig. 4 Frequency distributions for pairwise haplotype distances for cytochrome *b* from *E. adscensionis* and *R. saponaceus*



the ancestor of *R. bicolor* (in the east Pacific) diverged from *R. saponaceus* (in the West Atlantic). This finding invokes the East Atlantic – East Pacific generalized track proposed by Rosen (1976) to explain the distribution of shorefishes. Under Rosen's hypothesis, the greatest vicariant separation in this region should be between East and West Atlantic, based on the opening of the Atlantic some 80 Mya. Subsequently, a more recent vicariant separation occurred between the West Atlantic and East Pacific, based on the closure of a central American seaway. Some aspects of fish phylogeography can support this theory (see Banford et al. 1999), and the *bicolor/saponaceus* split does seem to fit the closure of the West Atlantic/East Pacific connection. However, the East-West split in Atlantic greater soapfishes estimated at 3.3 Mya is much too shallow to be based on sea floor spreading. For this aspect of greater soapfish phylogeography, dispersal in an accelerated circulation system (see above) is a more likely explanation.

A similar divergence was observed among rock hind, with one mtDNA lineage restricted to the northwest Caribbean (Florida Keys) and the southeast United States. The other lineages were observed throughout the tropical Atlantic, and most notably at two Caribbean collecting sites (Barbados and the Bahamas). How can one explain this curious finding of a major genetic separation of lineages separated by < 250 km of open water? As above, the possibility of misidentification must be considered. This is especially pertinent for rock hind, because there are several similar congeners in the Caribbean. However, misidentification is unlikely for two reasons: first, the samples were collected by personnel specifically trained to distinguish rock hind from other epinephelines, and second, the Florida/South Carolina specimens were collected by several expeditions and by different trained persons and it is therefore unlikely that all parties collected the wrong species at 100% frequency.

What biogeographic process could explain the divergent mtDNA lineages within the tropical West Atlantic

rock hind? No obvious differences in size, coloration, or external morphology among rock hind specimens were observed, so it is possible that what is currently called one species consists of separate species: an insular species found in the eastern part of the greater Caribbean and a continental species with a currently unknown range. As in soapfish, the lineages appear to be allopatric, although Florida and South Carolina sample sizes are small. Fast-flowing currents in the Florida Straits have been proposed as vicariant barriers between the West Indian biogeographic province (including the Bahamas) and a Caribbean province containing the Florida Keys (Fig. 1; Briggs 1995). However, this strong divergence among rock hind is exceptional, because all the prior surveys of Caribbean Sea reef fishes revealed low or no population structure across such distances. In particular, Shulman and Bermingham (1995) showed that, for each of eight reef fish species (not including rock hind or soapfish), populations in Barbados and Florida shared haplotypes both with each other and with other continental and island locations in the Caribbean.

Another alternative is a long-term divergence between the divergent Caribbean lineages and those in the Brazilian province (as observed in other reef species; Bernardi et al. 2000; Muss et al. 2001), followed by recent dispersal into the West Indies from Brazil. Grenada, as one of the southernmost outposts of reef habitat in the Caribbean, is suitably located to encounter larvae drifting north on the Brazilian Current. Based on species distributions, geography, and ocean circulation patterns, Grenada is one of the gateways for occasional dispersal between Brazil and the Caribbean (Rocha et al. 2002). The genetic data support this hypothesis: while not found in abundance ($n=7$), greater soapfish haplotype 1 was found in specimens from Grenada, Barbados, and Brazil; and rock hind haplotype A was observed in both Brazil and Barbados. Genetic data from large population samples of both species at many sites scattered throughout the tropical northwest Atlantic will be

needed to (1) define the distributions of the two main lineages of rock hind and show how they relate to insular/continental biogeographic subdivisions of that region, (2) show whether those rock hind lineages are allopatric, and (3) show how the phylogeography of the greater soapfish within the Caribbean relates to that of the rock hind. As the latter species were sampled only in the southeastern Caribbean this study cannot say whether there are lineage subdivisions in that species within the tropical NW Atlantic.

Bowen et al. (2001) demonstrated that trumpetfish phylogroups, separated by geography for 3–4 My, hybridized in renewed contact in the South Atlantic. As noted by Knowlton (2000), reproductive isolation in sympatry is an acid test for speciation, and rock hind may be in the early stages of this test. The reproductive compatibility of the two main lineages of rock hind may determine whether they have speciated in isolation, or whether this is a single species characterized by deep divergence and subsequent admixture (see Veron 1995).

Against the backdrop of strong divergence, both species exhibit many slight differentiations (often by a single transition) within the major lineages. The distribution of these lineages among western and central Atlantic greater soapfish yields significant population genetic structure, but this is not the case in rock hind. The relative abundance of a widespread haplotype among biogeographic provinces and regions (rock hind haplotype A), accompanied by many closely related haplotypes, yielded a low level of population structure in rock hind samples ($\Phi_{ST} = 0.056$, $P \leq 0.001$) from the Bahamas to São Tomé. This may be due to extensive gene flow between biogeographic provinces and regions. Alternately, the shallow gene genealogy with widespread haplotype sharing could arise from selection or demographic processes, working either singly or in combination (Grant and Bowen 1998). Such demographic processes include the effects of temporal variation in recruitment success and large fluctuations in population size.

A shallow but diverse gene genealogy can be generated by extremes in recruitment success (Hedgecock 1994), where all recruits to a year-class were generated by a few successful mating events. While the population may have a large number of spawners, in sweepstakes reproduction successful cohorts of recruits are drawn from only the few propagules that meet a specific and stringent combination of abiotic and biotic factors. Typically, sweepstakes recruitment produces chaotic patchiness in allele distribution at small temporal and spatial scales (Avice 2000). While collections from single localities in multiple years were not attempted, the samples collected in this study span several years effort and (for Brazil and the southeastern United States) involved multiple localities. In contrast to chaotic patchiness, a consistency of pattern across years and samples were observed (e.g., the widespread abundance of haplotype A and the presence of the same haplotypes in South Carolina specimens collected in 1994 and Florida specimens collected in 1999).

Population fluctuations also eliminate mtDNA lineages, such that the female effective population size may be orders of magnitude lower than indicated by census size. A survey of haplotypes in such a species may therefore reveal a large number of closely related haplotypes. If rock hind represents a single species with significant population structure (in mtDNA), then pairwise comparisons between haplotypes would create an L-curve of similar shape to the expected Poisson distribution. The additional smaller peaks in the mismatch distributions (an abundance of large interhaplotype distances; Fig. 4) could be caused by rapid population expansion (Rogers et al. 1996). Overall, a waveform result may indicate a rapid recovery from a recent genetic bottleneck (Rogers and Harpending 1992). Similar patterns have been observed in other tropical fishes, such as the mullet (Rocha-Olivares et al. 2000) and parrotfishes (Dudgeon et al. 2000). These data support the growing body of evidence for large-scale instability of marine populations over recent evolutionary time (e.g., Lavery et al. 1996; Bowen and Grant 1997; Bucklin and Wiebe 1998; Grant and Bowen 1998; Zane et al. 1998).

The known species distribution of rock hind and greater soapfish implies a connection (either past or present) among reef habitats in multiple tropical biogeographic provinces and regions. There are considerable differences in the degree of genetic heterogeneity between species and between localities. Therefore an examination of the haplotype distribution in a geographic context may elucidate both potential differences in life history between these reef fishes as well as the effectiveness of biogeographic barriers.

Biogeographic barriers: Caribbean versus Brazil

The reef-building corals of Brazil are highly distinct from those of the Caribbean (see Fig. 49 in Veron 1995), providing longstanding evidence for recognition of those areas as separate, large-scale biogeographic regions (Briggs 1974). Recent studies have documented that Caribbean and Brazilian reef provinces are also highly distinct for fish species, in terms of their distributions (Floeter and Gasparini 2000; Floeter et al. 2001; Rocha and Rosa 2001), taxonomic distinctions (Rocha and Rosa 1999), and evolutionary genetics (Bernardi et al. 2000; Bowen et al. 2001; Muss et al. 2001; Rocha et al. 2002).

In contrast, comparisons in both species from western Atlantic localities did not reveal significant pairwise differentiations between the West Indian (i.e., not including the Florida/South Carolina rock hind) and Brazilian biogeographic provinces. However, this may be influenced by the smaller sample sizes in the West Indian samples (with the exception of 19 rock hind from Barbados). The sharing of haplotypes between the two provinces in both species studied here (Tables 2, 3) indicates recent or ongoing population connections. Are

these species capable of routinely crossing the Amazonian plume (described above)? One mechanism to accomplish this is that the extent of these species' PLD (presently unknown for both species) may allow recruitment of Brazilian haplotypes into the Caribbean (Fig. 1). However, a prolonged PLD alone does not explain the population genetic structure elsewhere in the range.

Perhaps larval transport is not the only method of dispersal between Brazilian and Caribbean provinces. Benthic (juvenile and adult) stages could occupy sparse hard-bottom habitat between the Caribbean and northeast Brazil, as they do in subtropical waters of the eastern United States (Randall 1967). Alternately, they could occupy certain soft-bottom habitats such as the sponge communities under the Amazon and Orinoco plumes (Collette and Rützler 1977).

The possibility of dispersal (or population connections) through alternative habitats is especially relevant for soapfishes. Effective dispersal between the Caribbean and Brazilian reef systems requires persistence across long distances without coral reef habitat, but also the capability to withstand or avoid the immense halocline imposed by the Amazon-Orinoco outflows (Longhurst 1998). The ability to exist in hyposaline waters is not unknown in the soapfishes, and is in fact a unifying character of *R. nigripinnus* and *R. bicolor* in the Pacific (Guimarães 1999; D.R. Robertson, STRI Panama, personal observation). Perhaps other soapfishes have wide salinity tolerances, as indicated by the presence of *R. randalli* in mangrove habitats in the Hawaiian archipelago (D. Greenfield, U. Hawaii, personal communication). Courtenay (1967) suggested that *R. saponaceous* might create shallow burrows in mud, although whether this actually occurs is not known (Guimarães 1999). Nonetheless, the genetic findings for *R. saponaceous* are consistent with a Caribbean-Brazil link via softbottom habitat.

Biogeographic barriers: West versus East Atlantic

The reef fishes of the East Atlantic have been understudied, and it is certain that additional species await discovery. In the comparisons of target species across the tropical Atlantic Ocean, the rock hind samples were characterized by the widespread distribution of haplotype A in Brazil, the mid-Atlantic Ridge, and East Atlantic. While this may be due to demographic processes unrelated to vicariance (see Discussion above), the nearly ubiquitous occurrence of haplotype A indicates that the rock hind has been able to colonize across long distances in the recent past.

In contrast, the greater soapfish had higher population structure ($\Phi_{ST}=0.327-0.424$; Table 5) between Brazil and the central Atlantic, and a strong (possibly species-level) distinction between the East Atlantic populations and elsewhere (pairwise Φ_{ST} 's > 0.90). The slight differentiations between haplotypes 1, 5, and 18

indicate a very rapid coalescence to an ancestor capable of colonization across the western-central Atlantic (Table 4). Collectively, these three haplotypes extend from Grenada and Barbados to Brazil to Ascension and St. Helena, and may support the 'stepping-stone' colonization pattern across the open ocean barrier of the central Atlantic. St. Helena exhibited a very low haplotype diversity relative to other greater soapfish samples: 14 of 16 specimens had haplotype 18, implying either highly differential survival of lineages, or too little time for divergence to occur in St. Helena soapfish in the cytochrome *b* region. Similarly, the lowest haplotype diversities in rock hind were in the Ascension and St. Helena samples.

The low haplotype diversity in the Ascension/St. Helena province, coupled with the restricted haplotype distributions of soapfish on São Tomé suggest that intermediate localities may be used as 'stepping-stones,' but the lack of widespread haplotypes in the greater soapfish indicates that this reef fish is not as able to cross the Atlantic as readily as the rock hind.

The reason for the difference in oceanic dispersal ability between rock hind and greater soapfish is not readily apparent. PLD, while a tempting potential predictor of population structure, is one of many factors influencing genetic differentiation. The roles of oceanographic currents, freshwater plumes, recruitment success, and niche competition surely play a part in the connections and divisions among Atlantic coral reef fauna. Although each species undoubtedly meets unique challenges the lack of concordance might be enhanced by stochastic processes such as rare long distance dispersal, sweepstakes recruitment, local extirpation, and genetic drift in small founder populations. This is especially relevant to the rock hind, in which there is evidence for an evolutionary separation (approximately 3.2 My) within the Caribbean Sea, but evidence of recent common ancestry between Brazil and West Indies populations. While this could be due to vicariance, other demographic and historical processes may also explain a pattern that appears to be very unusual among Caribbean reef fishes.

The distribution of mtDNA diversity in both rock hind and greater soapfish offers insight into how biogeography influences speciation in the marine realm. The geography and oceanography of the Atlantic basin may promote intraspecific (or intrageneric) partitions (Tringali and Wilson 1993; Graves 1995; Banford et al 1999; Muss et al 2001). The distances among biogeographic provinces and the durations of pelagic larvae are not the only relevant factors in shaping patterns of genetic structure in panoceanic species. Stochastic colonizations and utilization of isolated islands allow for contact between Atlantic regions. The different responses to the mid-Atlantic and the Amazon-Orinoco barriers by two confamilial and even congeneric species (e.g., Rocha et al. 2002) illustrate how complex interactions may be involved in "simple" predictors of population structure (e.g., dispersive larvae, adults restricted

in habitat type, modern surface current patterns, straight-line distances between habitats). Rock hind and greater soapfish thus provide examples of the dynamic evolutionary processes of population isolation and differentiation which may typify the superficially continuous distributions of marine organisms.

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