

PHYLOGEOGRAPHY OF *OPHIOBLENNIUS*: THE ROLE OF OCEAN CURRENTS AND GEOGRAPHY IN REEF FISH EVOLUTION

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Abstract.—Many tropical reef fishes are divided into Atlantic and East Pacific taxa, placing similar species in two very different biogeographic regimes. The tropical Atlantic is a closed ocean basin with relatively stable currents, whereas the East Pacific is an open basin with unstable oceanic circulation. To assess how evolutionary processes are influenced by these differences in oceanography and geography, we analyze a 630-bp region of mitochondrial cytochrome *b* from 171 individuals in the blennioid genus *Ophioblennius*. Our results demonstrate deep genetic structuring in the Atlantic species, *O. atlanticus*, corresponding to recognized biogeographic provinces, with divergences of $d = 5.2$ – 12.7% among the Caribbean, Brazilian, St. Helena/Ascension Island, Gulf of Guinea, and Azores/Cape Verde regions. The Atlantic phylogeny is consistent with Pliocene dispersal from the western to eastern Atlantic, and the depth of these separations (along with prior morphological comparisons) may indicate previously unrecognized species. The eastern Pacific species, *O. steindachneri*, is characterized by markedly less structure than *O. atlanticus*, with shallow mitochondrial DNA lineages ($d_{\max} = 2.7\%$) and haplotype frequency shifts between locations in the Sea of Cortez, Pacific Panama, Clipperton Island, and the Galapagos Islands. No concordance between genetic structure and biogeographic provinces was found for *O. steindachneri*. We attribute the phylogeographic pattern in *O. atlanticus* to dispersal during the reorganization of Atlantic circulation patterns that accompanied the shoaling of the Isthmus of Panama. The low degree of structure in the eastern Pacific is probably due to unstable circulation and linkage to the larger Pacific Ocean basin. The contrast in genetic signatures between Atlantic and eastern Pacific blennies demonstrates how differences in geology and oceanography have influenced evolutionary radiations within each region.

Key words.—Biogeography, cytochrome *b*, dispersal, mitochondrial DNA, ocean currents, population structure, reef fishes.

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The Isthmus of Panama has been a boon to evolutionary studies of marine organisms, providing a robust framework for evaluating morphological, ecological, and molecular divergences across a minimum span of 3.1 million years (Coates and Obando 1996). Many pairs of sister species are available for comparisons between the tropical Atlantic and eastern Pacific, and results to date have yielded a number of evolutionary insights (Knowlton et al. 1993; Bermingham et al. 1997; Lessios et al. 1999). However, the microevolutionary processes within each ocean basin have received far less attention (McCartney et al. 2000). Geographic and oceanographic conditions are quite different between the tropical Atlantic and eastern Pacific Oceans. For example, reef habitats are widely scattered in the Atlantic, but are arrayed along a continuous continental coastline (and a few offshore islands) in the eastern Pacific. The tropical Atlantic is a closed ocean basin, with relatively stable current systems since the shoaling of the Isthmus of Panama some 5 million years ago, whereas the tropical eastern Pacific is an open basin with unstable currents, cold water intrusions, and extensive mixing due to El Niño events and other Pacific-wide phenomena (Keigwin 1982; Haug and Tiedemann 1998). Here we examine a common reef fish (genus *Ophioblennius*) that inhabits both ocean basins to determine how differences in physical regime may influence evolutionary history.

Species of *Ophioblennius* (family Blenniidae) are algivorous fishes that inhabit shallow, rocky shores and reefs. Two species are recognized, the redlip blenny, *O. atlanticus*, in

the Atlantic and the Panamic fanged blenny, *O. steindachneri*, in the eastern Pacific (Springer 1962). *Ophioblennius* has a typical reef fish life history, including benthic eggs that hatch after 5 days (Marraro and Nursall 1983; Robertson et al. 1990) and a long planktonic larval phase of approximately 50 days (Labelle and Nursall 1992).

In the western Atlantic, *O. atlanticus* ranges throughout the greater Caribbean (see Fig. 1). To the south there is a range discontinuity that encompasses the freshwater outflows of the Amazon and Orinoco Rivers. The distribution resumes along the coast from northeast Brazil to approximately São Paulo, including the offshore islands of Fernando de Noronha, St Paul's Rocks, and Trindade; the mid-Atlantic ridge islands of Ascension and St. Helena; and the eastern Atlantic islands of São Tomé, Cape Verde, the Canary Islands, Madeira, and the Azores. *Ophioblennius atlanticus* occurs along the African coastline from Senegal to Angola, but the continuity of this distribution is uncertain. A sole specimen has been reported from Bermuda (Smith-Vaniz et al. 1999). Springer (1962) divided *O. atlanticus* into two subspecies, one in the Caribbean (*O. a. macclurei*) and another that occupies the remainder of the Atlantic range (*O. a. atlanticus*). However, he expressed hesitation about grouping Brazilian specimens with those of the eastern Atlantic (Springer 1962; pers. comm. 2000).

Ophioblennius steindachneri is distributed along the eastern Pacific coast from the Sea of Cortez to Peru and inhabits all the offshore islands in that region (Clipperton, the Revillagigedos, Cocos, the Galapagos, and Malpelo; Allen and Robertson 1994). Clipperton is the most geographically iso-

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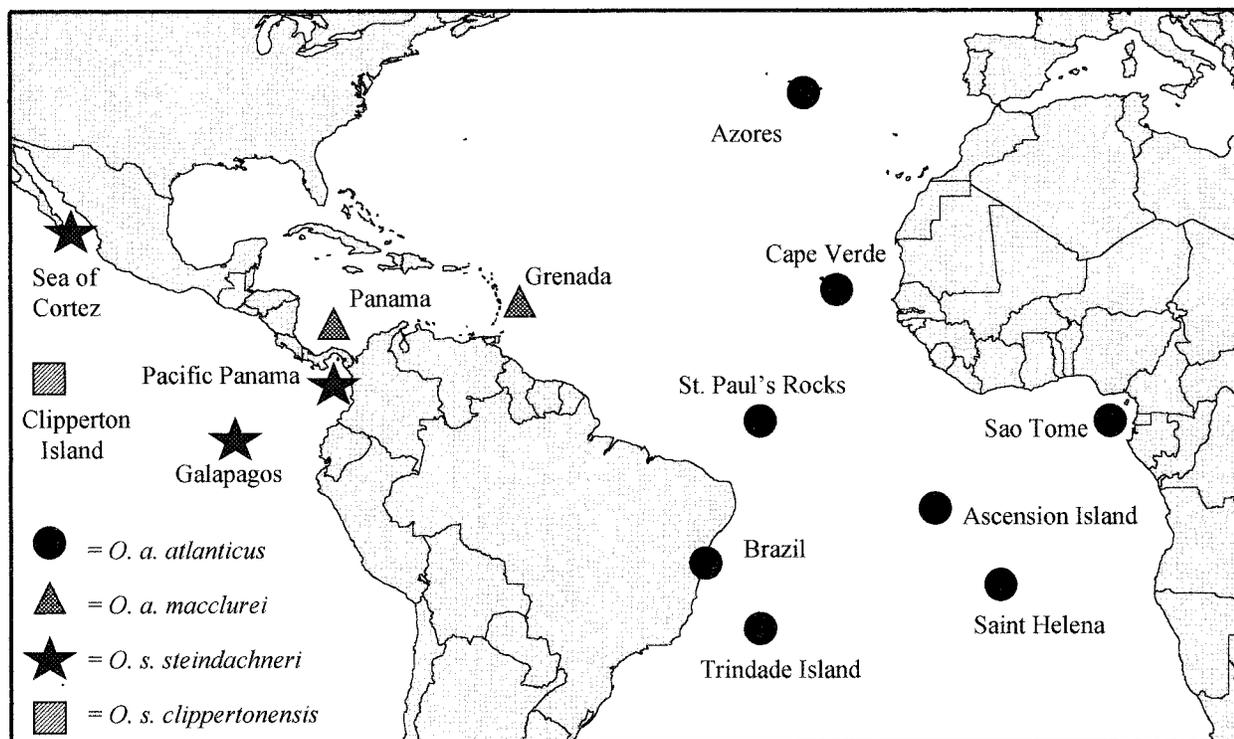


FIG. 1. Sample locations and taxonomic partitions in *Ophioblennius*.

lated island in the eastern Pacific, and the resident population is recognized as an endemic subspecies, *O. s. clippertonensis*. The other subspecies, *O. s. steindachneri*, occurs along the mainland and at the other offshore islands (Springer 1962).

In this study we assess evolutionary partitions in *Ophioblennius* with mitochondrial (mtDNA) cytochrome *b* sequences, which have proven informative in a variety of phylogeographic settings (Avice 2000). A prior mtDNA survey of *O. atlanticus* (and seven other reef fishes; Shulman and Bermingham 1995) revealed no population structure among six locations throughout the greater Caribbean (Belize, Panama, Curaçao, Barbados, St. Croix, Bahamas). Shulman and Bermingham (1995) also found no relationship between oceanographic patterns (northern vs. southern Caribbean current tracks), larval duration, and population genetic structuring. Thus, one conclusion from their study is that if larval dispersal and oceanic currents have detectable effects on population genetic structure, they likely operate on a larger geographic scale than the Caribbean. The present study tests this hypothesis by surveying the entire range of *Ophioblennius*, including island populations separated by thousands of kilometers. A principal objective of this study is to reconstruct the phylogeographic history of *Ophioblennius*, to potentially shed light on the influence of oceanic barriers, large-scale circulation systems, and habitat geography on processes of dispersal, isolation, and ultimately speciation in reef fishes. The genus *Ophioblennius* offers the advantage of looking at sister species in two regions with entirely different histories: the open ocean basin of the eastern Pacific and the closed Atlantic basin.

A second goal is to test for congruence between biogeographic provinces and intraspecific partitions. In the Atlantic,

Briggs (1974, 1995) described five biogeographic provinces that are relevant to this study: Caribbean (or West Indies), Brazil, St. Helena-Ascension (mid-Atlantic ridge islands, with somewhat controversial status), West Africa, and Lusitania (northeast subtropical Atlantic including the Azores). Cape Verde is designated as the boundary between the Lusitanian province and the tropical West African region, which extends along the African coastline south to Angola, including our sample site at São Tomé, Gulf of Guinea.

Three biogeographic provinces are sampled in the eastern Pacific: the Sea of Cortez (Gulf of California), the Panamanian province (from Oaxaca, Mexico to Ecuador), and the Galapagos Islands. Clipperton Island is not assigned to any of the provinces, but is geographically closest to the Mexican province, which lies between the Sea of Cortez and the Panamanian province.

Marine biogeographic provinces are delineated on the basis of faunal dissimilarity and/or percent endemism (Ekman 1953; Briggs 1974). If these faunal differences are due to oceanographic barriers to dispersal (rather than habitat requirements, competition, or other ecological considerations), then they should be consistently reflected in genetic partitions between provinces. Thus, this survey of the Atlantic and eastern Pacific distributions of *Ophioblennius* is designed to test for differences in the connectivity of biogeographic provinces. By surveying sister species, we hope to minimize the confounding factor of life-history variation among taxa.

Finally, we can use the mtDNA genealogy for *Ophioblennius* to evaluate vicariance hypotheses for relationships among biogeographic provinces. Rosen (1975) postulated the existence of an eastern Atlantic-eastern Pacific/Caribbean generalized distribution track, in which the most ancient sep-

TABLE 1. Sample size, haplotype diversity (h), and nucleotide diversity (π) for *Ophioblennius* populations of the Atlantic (*O. atlanticus*) and eastern Pacific (*O. steindachneri*).

Location	n	h	π
Atlantic			
Panama	9	1.000	0.0130
Grenada	11	1.000	0.0065
Northeast Brazil	15	1.000	0.0107
Trindade Is., Brazil	10	0.933	0.0110
St. Paul's Rocks	12	0.803	0.0053
Asension Is.	13	0.974	0.0071
St. Helena	12	0.970	0.0045
São Tomé	14	0.891	0.0054
Cape Verde	16	0.992	0.0122
Azores	9	0.417	0.0011
Eastern Pacific			
Clipperton Is.	17	0.846	0.0128
Galapagos Is.	9	0.972	0.0107
Panama	13	0.910	0.0080
Sea of Cortez	11	0.964	0.0126

arations occur between the eastern and western Atlantic due to the sea-floor spreading and widening of the Atlantic during the early Cenozoic (65–20 million years ago), well before the closure of the Isthmus of Panama 3.1–3.5 million years ago. In contrast, a dispersal hypothesis predicts that the most ancient separation should occur between the Pacific and Atlantic due to the Pliocene isthmus closure, and that amphiatlantic distributions reflect recent movement across the mid-Atlantic barrier (Briggs 1974).

MATERIALS AND METHODS

A total of 171 individuals from 10 Atlantic locations (in five biogeographic provinces) and four eastern Pacific locations (in three provinces) were surveyed (Table 1). Sample collections began in 1990, but the majority of samples were collected with polespears and microspears by D. R. Robertson between 1995 and 1999. Tissue samples (muscle and/or gill) were stored in a saturated salt-DMSO buffer (Amos and Hoelzel 1991).

Total genomic DNA was isolated with a lithium-chloride procedure (see Muss 1999). An 820-bp segment of the mtDNA cytochrome *b* gene was amplified using a heavy-strand primer (5'-GTGATCTGAAAACCACCGTTG-3'; Song 1994) and light-strand primer (5'-AATAGGAAGTAT-CATTGCGGTTTGTATG-3'; Taberlet et al. 1992). For population samples that could not be amplified using the above primers, 658 bp within the above sequence were amplified using the customized *Oph1* heavy-strand primer (5'-CGCCAACGACGCAGTCGTGG-3') and the *Oph2* light-strand primer (5'-GTGCAAGAGAAATAAGAGC-3'). Polymerase-chain-reaction (PCR) amplifications included an initial denaturing step at 94°C for 80 sec, then between 25 and 37 amplification cycles (42 sec 94°C, 30 sec 53°C, 55 sec 72°C), and a final extension at 72°C for 150 sec.

Single-stranded DNA sequencing reactions were conducted with fluorescently labeled dideoxy terminators according to the manufacturer's recommendations (Applied Biosystems, Inc., Foster City, CA), and labeled extension products were gel separated and analyzed with an automated DNA

sequencer (Applied Biosystems model 373A and 377) by the DNA Sequencing Core at the University of Florida. Fragments were aligned and edited with Sequencher version 3.0 (Gene Codes Corp., Ann Arbor, MI). All samples were sequenced in the forward direction, and problematic samples were resequenced in the forward or reverse direction. At least one representative individual from each population was sequenced in both directions to assure the accuracy of the nucleotide assignments.

Estimates of genetic variation within populations were obtained in the form of haplotype diversities (h ; eq. 8.5 in Nei 1987) and nucleotide diversities (π ; eq. 10.5 in Nei 1987) using Arlequin version 1.1 (Schneider et al. 1997) with uncorrected genetic distances. Many population statistics (e.g., tests of independence) are compromised in cases where most individuals have unique haplotypes (see Results). To facilitate comparisons between the populations, an exact test of population differentiation (Raymond and Rousset 1995) was conducted using haplotypes defined by transversion-differences only (see Bowen and Grant 1997). Using the full dataset, the proportion of genetic diversity within and among *Ophioblennius* populations (Φ_{ST}) and the number of migrants per generation among populations (Nm ; Slatkin 1985) were estimated with AMOVA using Arlequin.

Genetic distances were calculated for phylogenetic reconstruction with PAUP version 4.0b1 (Swofford 1998) using the Kimura two-parameter model. A neighbor-joining tree (Saitou and Nei 1987) was constructed using PAUP and evaluated with 500 bootstrap replicates. Results were also analyzed with unweighted parsimony and maximum-likelihood criteria. No reasonable outgroup could be found for phylogenetic analyses (the putative closely related taxa to the *Ophioblennius* genus are too divergent to be useful), yet the Pacific species (*O. steindachneri*) serves well as an outgroup for comparisons within the Atlantic species (*O. atlanticus*). Because this dataset included more than 100 haplotypes (see Results), phylogenetic analyses were conducted with a subset of three to five individuals per location. A hand-constructed parsimony network based on transversions was imposed upon the Atlantic/Pacific range of *Ophioblennius* to elucidate phylogeographic relationships among populations.

The ability to estimate the timing of divergence among lineages depends on an accurate molecular clock. For *Ophioblennius* cytochrome *b*, a geologically calibrated clock is unavailable due to the likelihood that the Atlantic and Pacific species diverged prior to the closure of the Isthmus of Panama (see Discussion). A molecular clock can thus only be estimated by proxy. Bermingham et al. (1997) measured separations across the Isthmus of Panama, and derived a rate of 1.7% per million years for mtDNA cytochrome oxidase. The two *Ophioblennius* species were assayed in that study, and the divergence between them was 12.4% (Kimura two-parameter distance). Because the cytochrome *b* divergence found in the present study is about 14.5% ($d = 0.133\text{--}0.159$; see Results), it appears that this operon evolves at an incrementally faster rate than cytochrome oxidase. Thus, the convention we will use for estimating divergence times will be the widely accepted benchmark of 2% per million year (Brown et al. 1979).

TABLE 2. Transversion haplotype distribution for *Ophioblennius atlanticus* (columns 2–11) and *O. steindachneri* (columns 12–15), based on 630 bp of the cytochrome *b* region. Haplotypes are designated by letters in the first column. CP, Caribbean Panama; GR, Grenada; BZ, Brazil; TI, Trindade Island; SP, St. Paul's Rocks; AS, Ascension Island; SH, St. Helena Island; ST, São Tomé; CV, Cape Verde; AZ, Azores; CI, Clipperton Island; GA, Galapagos; PP, Pacific Panama; SC, Sea of Cortez.

	CP	GR	BZ	TI	SP	AS	SH	ST	CV	AZ	CI	GA	PP	SC
A	1													
B	1													
C	1													
D	1													
E	5	10												
F		1												
G			10	5	12									
H			3	3										
I			1											
J			1											
K				2										
L						3								
M						10	12							
N								13						
O								1						
P									1					
Q									15	8				
R										1				
S											9			
T											1			
U											1			
V											1	1		1
W											5	8	12	10
X													1	

RESULTS

Sequence analysis was confined to 630 bp beginning 120 nucleotide sites from the tRNA glutamic acid region, which corresponds to sites H14850–H15480 in the human mitochondrial genome. Representative haplotypes are available in GenBank under accession numbers AF323030–AF323038, and the full dataset is available from the authors. Sequence comparisons revealed 176 variable sites with 272 transitions and 39 transversions, defining a total of 122 haplotypes in 171 individuals. Several Atlantic samples consisted entirely of unique haplotypes, and haplotype diversities (h) in both Atlantic and Pacific populations were very high (Table 1). Nucleotide diversities (π) within each population ranged from low to moderate (Table 1).

A high transition:transversion ratio (approximately 7:1) was observed, and many instances of suspected homoplasy were identified among the 272 observed transitions (consistency index = 0.707 in parsimony analysis). These transition homoplasies represent phylogenetic noise that result from multiple changes at single nucleotide sites, and the abundance of transitions contributes to the high haplotype diversities observed in *Ophioblennius*. Thus, only the transversion mutations were employed for the population differentiation tests and parsimony network.

The 39 observed transversions defined 25 haplotypes (Table 2), including from one to five transversion-haplotypes (tv-haplotypes) per location. There were no instances of tv-haplotype sharing between the Atlantic biogeographic provinces, but common tv-haplotypes were shared among sample sites within the Caribbean, Brazilian, mid-Atlantic, and Lusitanian biogeographic provinces. A test of tv-haplotype frequencies within Atlantic provinces (such as the Caribbean)

revealed no significant differences among locations (Table 3). For *O. steindachneri*, a significant difference was found between Clipperton Island and the other locations: Pacific Panama, the Sea of Cortez, and the Galapagos. There were no significant differences among the latter three sample sites ($P = 0.67$).

Φ_{ST} -values for comparisons within biogeographic provinces of the Atlantic range from 0.053 to 0.301, indicating low to intermediate levels of population structure (Table 3). Φ_{ST} -values in the eastern Pacific ranged from 0.000 to 0.396, with the former value characteristic for comparisons among coastal populations and the latter value describing comparisons with Clipperton Island. Nm -values for pairwise comparisons within biogeographic provinces of the Atlantic were generally high ($Nm > 1$), as were all comparisons among eastern Pacific sites, except the Clipperton-mainland comparisons (Table 3).

Ophioblennius atlanticus and *O. steindachneri* are separated by an average sequence divergence of $d = 0.145$ (range = 0.133–0.159). Fixed mutational differences occur in *O. atlanticus* among five biogeographic regions in the Atlantic, indicating deep population structure and ancient separations between the Caribbean, Brazil, mid-Atlantic ridge, São Tomé (Gulf of Guinea), and Cape Verde/Azores regions. Genetic distances among these regions were $d = 0.052$ – 0.127 , whereas two shallow mtDNA lineages ($d_{\max} = 0.027$) were observed in the eastern Pacific. These groupings are reflected in the topology of the neighbor-joining tree (Fig. 2), which is identical (for major lineages) to the branch orders based on unweighted parsimony and maximum-likelihood criteria. The full dataset (transitions and transversions) shows Brazil as the basal lineage in the Atlantic (Fig. 2). However, this

TABLE 3. Population isolation relative to distance in *Ophioblennius*. Shown are linear distances between pairs of sample locations, the among-population component of genetic variation (Φ_{ST}), a statistical test of population differentiation based on haplotype frequency shifts (exact test based on transversion haplotypes), and an estimate of gene flow (number of migrants per generation; Nm). Comparisons across distances of 686–1806 km show high gene flow ($Nm > 1$) and low levels of population differentiation. Comparisons across larger distances indicate moderate to low gene flow and significant population structuring, with one exception; the anomalous results for the Azores–Cape Verde comparison can be attributed to the presence of intermediate habitat along the Canary Current (Madeira, the Canary Islands).

Locations	Distance (km)	Φ_{ST}	Exact test	Nm
NE Brazil–St. Paul's Rocks	686	0.301	($P > 0.14$)	1.2
NE Brazil–Trindade	1098	0.134	($P > 0.34$)	3.2
Ascension–St. Helena	1277	0.198	($P > 0.22$)	2.0
Galapagos–Pacific Panama	1676	0.000	($P > 0.65$)	high
Grenada–Caribbean Panama	1806	0.062	($P > 0.10$)	7.6
Cape Verde–St. Paul's Rocks	1944	0.935	($P < 0.01$)	0.0
Clipperton–Sea of Cortez	1973	0.396	($P < 0.01$)	0.8
St. Helena–São Tomé	1988	0.949	($P < 0.01$)	0.0
Ascension–St. Paul's Rocks	2166	0.951	($P < 0.01$)	0.0
Grenada–NE Brazil	2275	0.937	($P < 0.01$)	0.0
Clipperton–Galapagos	2317	0.379	($P < 0.01$)	0.8
Azores–Cape Verde	2424	0.053	($P > 0.76$)	8.9
Ascension–São Tomé	2436	0.927	($P < 0.01$)	0.0
Trindade–St. Helena	2590	0.926	($P < 0.01$)	0.0
Cape Verde–São Tomé	3853	0.838	($P < 0.01$)	0.0

branch order is supported by bootstrap values of only 63% in the parsimony analysis and 55% in neighbor-joining analysis. Further, the transversions-only data (Fig. 3) indicate that the Caribbean may be the basal lineage; this ambiguity highlights how close in time the Caribbean–Brazil and western-eastern Atlantic partitions occurred.

DISCUSSION

The six distinct lineages identified in *Ophioblennius*, corresponding to the eastern Pacific and five Atlantic biogeographic provinces (Fig. 2), reflect evolutionary separations initiated during the Miocene or Pliocene. Based on our provisional molecular clock, *O. atlanticus* and *O. steindachneri*

have been separated for about 7 million years (average $d = 0.145$). The Caribbean and Brazil have been isolated for about 6 million years (average $d = 0.121$); the western Atlantic has been isolated from the eastern/mid-Atlantic for about 5.5 million years (average $d = 0.108$); the mid-Atlantic ridge islands of Ascension and St. Helena have been separated from the eastern Atlantic for about 4 million years (average $d = 0.078$); and the Gulf of Guinea was isolated from the Cape Verde/Azores region about 3 million years ago (average $d = 0.056$).

Population Structure and Biogeographic Provinces

The haplotype diversity (h) values of all populations except the Azores are high, likely a result of large population sizes; this blenny lives at notably high densities at most locations (Randall 1996). In contrast, nucleotide diversities (π) are moderate to low within regions (Table 1), a recurring pattern in marine fishes (Graves 1995; Grant and Bowen 1998).

The Azores population has low haplotype and nucleotide diversities. Given the high latitude of the Azores (38°N), and evidence that sea ice reached 50°N during the most recent (Wisconsin) glacial episode (Ruddiman and McIntyre 1981), it is very likely that warm-water elements of the Azorean fauna were extirpated during the late Pleistocene (Briggs 1995). Thus, the shallow diversity observed at the Azores is probably a vestige of recent colonization from elsewhere in the Lusitanian province.

The genetic partitions in *O. atlanticus* correspond exactly to the biogeographic provinces of the Atlantic described by Ekman (1953) and Briggs (1974), with the refinement that mid-Atlantic ridge islands are distinct from the West African province. The expanses of open ocean (and unsuitable coastal habitat) between these regions have evidently served as formidable barriers to dispersal.

In the eastern Pacific *O. steindachneri*, no genetic divergence was detected between the Cortez, Panamanian, and Galapagos provinces. Significant population differentiation

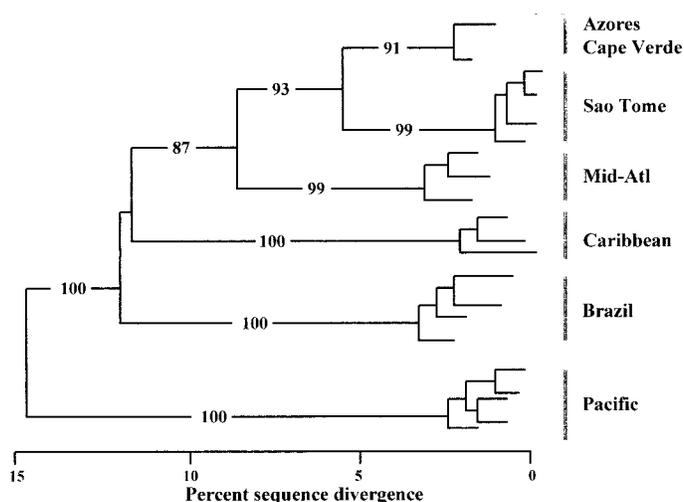


FIG. 2. Neighbor-joining tree of mtDNA cytochrome *b* lineages, based on representative specimens of *Ophioblennius*. Phylogenetic nodes were evaluated with 500 bootstrap replicates, and nodes with more than 80% support are indicated. Maximum-parsimony and maximum-likelihood algorithms gave an identical topology for the primary branches.

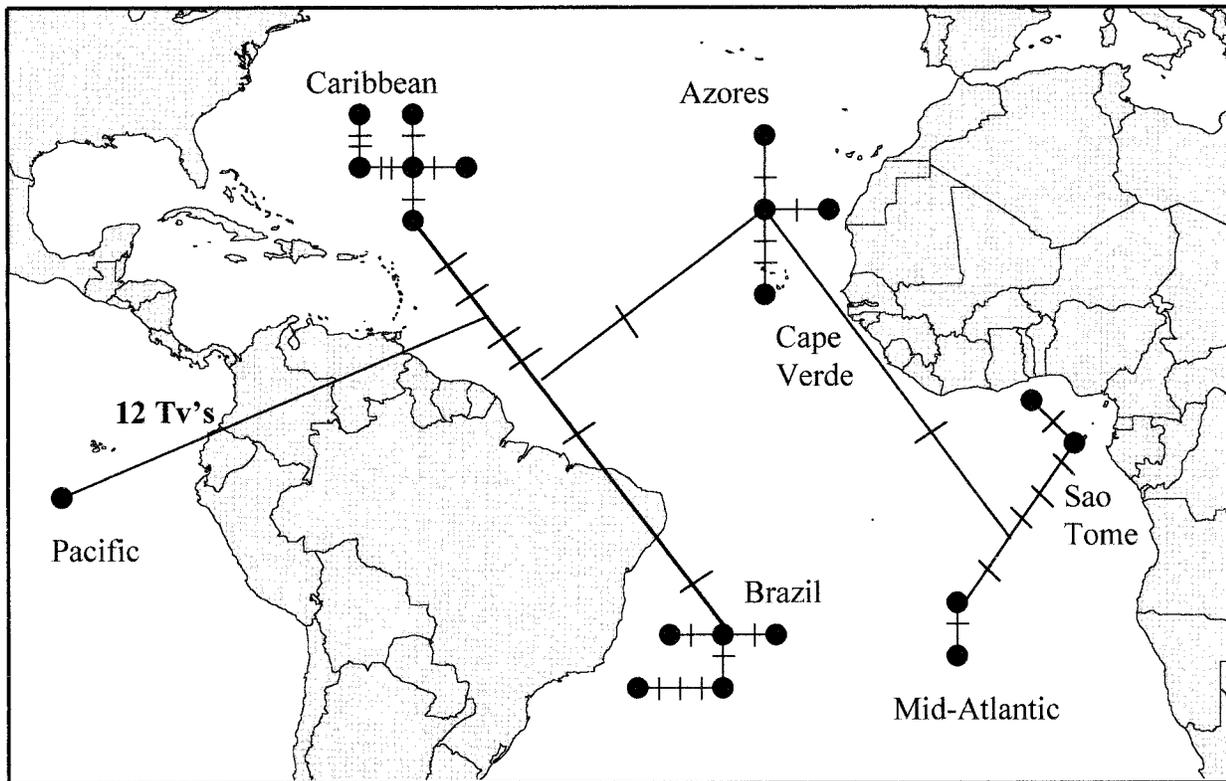


FIG. 3. Parsimony network of the six primary lineages detected in *Ophioblennius*, based on transversion mutations. Solid circles represent transversion haplotypes, solid lines show the relationships among haplotypes (based on the minimum number of mutation events), and cross-hatches indicate transversion mutations. Twelve transversions distinguish *O. atlanticus* and *O. steindachneri*. For economy, most of the transversion haplotypes for *O. steindachneri* are not shown.

(a strong haplotype frequency shift, Table 2) was observed only at Clipperton Island; thus, the oceanic stretch between the mainland and Clipperton Island evidently constitutes a partial barrier to gene flow. Lack of genetic structure in *O. steindachneri* may be due to a largely continuous habitat. With the exception of a distribution break of about 1000 km across the Central American Gap (between Oaxaca and Nicaragua; Springer 1958), suitable substrate exists without major interruptions (no more than a few hundred kilometers) along this coastline. The open water between the Galapagos and the South American mainland does not appear to prohibit gene flow for *O. steindachneri*. This observed connection can be attributed either to the seasonally enhanced Peruvian Current (Abbot 1966; Wyrki 1967; Briggs 1995) or to El Niño/Southern Oscillation (ENSO) events that periodically push water across this 1000-km stretch of open ocean (Richmond 1990).

Genetic differentiation within biogeographic provinces of the Atlantic is low overall (Table 3), which is consistent with the findings of Shulman and Bermingham (1995). Although the Φ_{ST} -values suggest there may be moderate population structure within provinces, the more conservative exact test of population differentiation (based on transversions only) indicates no significant structure within provinces. At these smaller scales, larval dispersal is sufficient to prevent strong genetic partitions.

The larval duration of *O. atlanticus* is variously estimated

to be approximately 50 days (based on spawning and settlement peaks; Labelle and Nursall 1992) and 38–39 days (based on otolith analyses; D. Wilson, pers. comm.), which is near the upper end of larval durations for tropical reef fishes (Brothers and Thresher 1985; Wellington and Victor 1989). Only the smallest larval size classes (3–7 mm) are found near the reef (Labelle and Nursall 1992; Brogan 1994), indicating that larvae move offshore following hatching. These life-history traits should enhance transoceanic dispersal. Nonetheless, oceanic stretches clearly influence genetic connectivity, as there is a strong link between linear distance and the level of population isolation in *Ophioblennius* (Table 3). Low (or no) population structure is detected between localities separated by less than 1800 km, restricted levels of gene flow are observed around 1900–2300 km (e.g., Clipperton–Sea of Cortez), and deeper evolutionary separations are generally apparent in the Atlantic beyond a distance of 2400 km (e.g. Ascension–Gulf of Guinea). This pattern indicates definite geographic limits for larval dispersal in *Ophioblennius*. It will be informative to test the 1800–2400-km yardstick in other reef fishes and in other settings (see Lessios et al. 1998).

Ophioblennius exhibits a distinct pattern of isolation by oceanic barriers, but what about the influence of riverine outflows? Passive larval transport across the Amazon-Orinoco outflows (via the Caribbean Current) could carry *O. atlanticus* larvae from Brazil to the Caribbean in 26–49 days, well within their larval duration. Yet, we observe an ancient

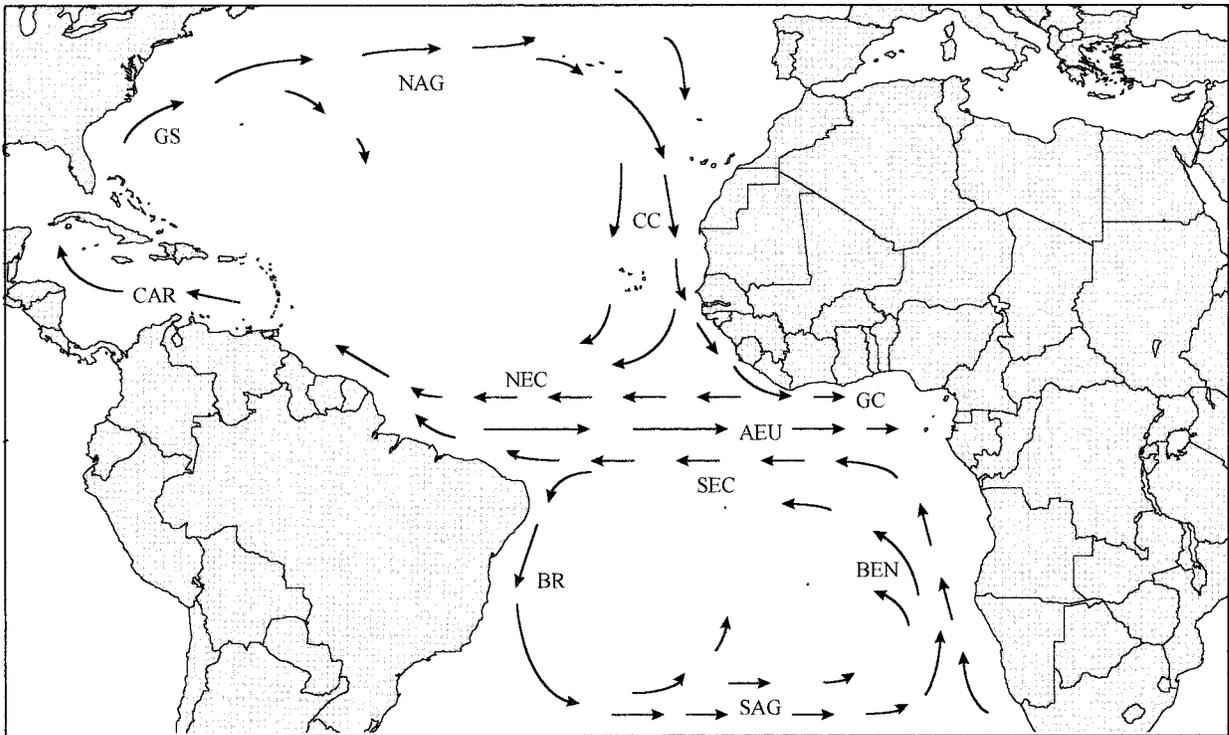


FIG. 4. Generalized circulation map depicting the major currents in the Atlantic, and possible colonization routes across the Atlantic Ocean. NAG, North Atlantic Gyre; AEU, Atlantic Equatorial Undercurrent; SAG, South Atlantic Gyre; CAR, Caribbean Current; GS, Gulf Stream; CC, Canary Current; GC, Guinea Current; NEC, North Equatorial Current; SEC, South Equatorial Current; BR, Brazil Current; BEN, Benguela Current.

separation between these areas, at approximately the same age as the Amazon River (about 6 million years; Nunan 1992; Hoorn 1994). Similarly, the genetic isolation of the Gulf of Guinea from Cape Verde is likely due to an absence of hard substratum along an expanse of approximately 1000 km encompassing the Niger and Volta Rivers. In contrast, the larvae of *O. steindachneri* exhibit high gene flow across the Central American Gap (~1000 km), a stretch that consists of inappropriate (soft bottom) habitat but that lacks large rivers. Thus, it is possible that riverine outflows are an impediment to larval dispersal in *Ophioblennius* and other reef species (but see Collette and Rutzler 1977).

Geography and Oceanography

In the tropical eastern Pacific, reef habitat extends from the Sea of Cortez to Ecuador along the mainland coast of Central and South America. In contrast, continental reefs of the Atlantic are highly discontinuous. Reef habitat exists along the coast of Brazil from the Manuel Luiz Reefs to Rio de Janeiro (Maida and Ferreira 1997; Rocha 1999; Floeter et al. 2000) and throughout the Caribbean, but between these regions no reefs exist for about 2300 km (Gilbert 1972; Briggs 1974). The mid-Atlantic barrier, an oceanic expanse of 3000–5000 km, separates the western and eastern Atlantic reefs. Bermuda, the Azores, St. Paul's Rocks, Ascension Island, and St. Helena harbor reef-associated fauna; collectively these oceanic islands are the only significant patches of habitat between the eastern and western Atlantic. At least for *Ophioblennius*, the distance between continental margins

and most of these oceanic islands evidently exceeds the limits of contemporary larval transport, fostering corresponding genetic partitioning. The ages of the oceanic islands mentioned above have been estimated (except Clipperton Island), and all were present by the late Miocene with the exception of Ascension Island, which is only about 1.5 million years old (Baker 1970; Mitchell-Thomé 1982). Given the Pliocene time frame of genetic partitions in *O. atlanticus*, most of these oceanic islands could have potentially acted as stepping-stones during range expansions.

The current patterns in the Atlantic are relatively well defined and dominated by major oceanic gyres. The Coriolis force drives easterly trade winds at the equator, pushing water westward in the North and South Equatorial Currents. At the northeast horn of Brazil the equatorial current bifurcates, becoming the Caribbean and Brazil Currents, which eventually feed into the North and South Atlantic Gyres that flow east across the Atlantic (Fig. 4). At the equator, the Equatorial Undercurrent flows east in the opposite direction of the two Equatorial Currents. Larval transport across the mid-Atlantic barrier would require entrainment in one of these major currents, however, the thermal cooling associated with the North and South Atlantic Gyres may prohibit higher latitude larval transport for many reef species. Because the Atlantic is a closed ocean basin, bound by the American and European/African continents, Atlantic circulation is shaped by the interaction of global geostrophic forces and the geography of the continental shorelines. Whereas the velocities of the major currents may shift in response to climatic changes, pat-

terns of epipelagic circulation in the Atlantic have remained fairly stable since the closure of the Isthmus of Panama (Maier-Reimer et al. 1990; Haug and Tiedemann 1998).

In the eastern Pacific, the currents are less stable. The cool Peruvian Current flows north along the South American coast, veering west near the equator and the Galapagos to become the South Equatorial Current. In the north, the California Current flows south along Baja California, and then veers west to form the North Equatorial Current. In between is an Equatorial Countercurrent. But unlike the circumscribed Atlantic, the eastern Pacific is conjoined to the much larger Pacific Ocean basin (Ekman 1953). The eastern Pacific is subject to variable upwelling, seasonal shifting of the Inter-tropical Convergence Zone (Pisias and Mix 1997), and episodic ENSO events. All of these phenomena are at least partly related to oceanographic processes in the larger Pacific Ocean basin. Paleooceanographic evidence reveals that the advection patterns and temperatures of the eastern Pacific boundary currents have changed repeatedly during the Pleistocene (Pisias and Mix 1997). This dynamic eastern Pacific circulation might act to facilitate periodic gene flow between the mainland and the offshore islands.

Phylogeography and Evolutionary History

The distribution of amphi-Atlantic reef species can be attributed either to vicariance, if populations became isolated as the ocean basin widened, or to dispersal events. The vicariant hypothesis, articulated by Rosen (1975) as the eastern Atlantic–eastern Pacific/Caribbean generalized track, is the sequential result of the early Cenozoic spreading of the Atlantic basin (65–20 million years ago), followed by the separation of Caribbean and eastern Pacific fauna due to the rise of the Panamanian isthmus (~3.1 million years ago). Rosen put this idea forth as an explanation for the faunal connections and geminate species pairs observed between the eastern Atlantic, western Atlantic, and eastern Pacific. An example that supports aspects of Rosen's hypothesis is the Spanish mackerel (*Scomberomorus regalis*) species group (Banford et al. 1999). In contrast to Rosen's model, Briggs (1974) hypothesized that disjunct amphi-Atlantic fish distributions are the result of jump dispersal events. Briggs suggested that the Caribbean has been functioning since the Miocene as a center of reef fish speciation, and that many western Atlantic species have dispersed to the eastern Atlantic. Thus, Briggs predicts that this dispersal occurs primarily in a west-to-east direction.

Given the diverse time frames associated with dispersal and vicariance models, these alternatives can be tested using molecular clocks. The splits between the populations of *O. atlanticus* ($d = 5.2\text{--}12.7\%$) correspond to isolations that began around 6 million years ago, a time when the geography of the Atlantic Ocean basin was largely the same as it is today. Thus, it is unlikely that *O. atlanticus* had an ancient distribution throughout the tropical Atlantic followed by vicariance-induced isolation resulting from the expansion of the Atlantic. The basal lineages in the neighbor-joining tree of *Ophioblennius* (Fig. 2) correspond to the eastern Pacific and western Atlantic, strongly indicating that the genus evolved in this region. It follows that *O. atlanticus* can only have achieved its present amphi-Atlantic distribution through

more recent dispersal events from the western to eastern Atlantic.

If dispersal from the west is responsible for the colonization of the eastern Atlantic, by what route was this achieved? The three possible routes are: (1) the North Atlantic Gyre; (2) the Equatorial Undercurrent; or (3) the South Atlantic Gyre (Fig. 4). In a network of transversion substitutions (Fig. 3), the most parsimonious connection links the western Atlantic with the Azores and Cape Verde. This network indicates that either the North Atlantic Gyre or the Equatorial Undercurrent could have been used, as the Azores lie in the path of the former, and the Cape Verde region (and adjacent African mainland) is likely affected by both current systems. The Southern Gyre is the least likely route, based on these findings as well as oceanographic considerations; this current system is colder and slower than the other routes (Reid 1989), and the distance between southern Brazil and the Gulf of Guinea is the largest trans-Atlantic gap.

The North Atlantic Gyre is fed by the warm, fast-flowing Gulf Stream, yet the estimated time of transport under contemporary conditions (120–300 days; see Scheltema 1971) is too long for *O. atlanticus* larvae to make it across from the western Atlantic to the Azores. Briggs (1970), Prud'homme Van Reine (1988), and Wirtz and Martins (1993) document a notable absence of western Atlantic species and genera at the Azores (which have an eastern Atlantic/Mediterranean faunal affinity), indicating that the North Atlantic Gyre has little effect as a vector for colonization of warm water species across the Atlantic. However, Vermeij and Rosenberg (1993) suggest that many western Atlantic mollusks colonized the eastern Atlantic via the Northern Gyre.

The eastward-flowing Atlantic Equatorial Undercurrent is the fastest-moving major current in the Atlantic, traveling at a rate of 1.8–5.4 km/h (Metcalf et al. 1962; Stalcup and Metcalf 1966). This current has a subsurface core (50–100 m deep) where water temperature drops to about 20°C (Scheltema 1971, 1995). Given that *Ophioblennius* survives winter temperatures at the Azores (14°C), their larvae likely are endowed with the thermal tolerance to survive in this current. Scheltema (1971) and Chesher (1966) estimated that the distance between Brazil and Africa could be traversed in 35–105 and 43–70 days, respectively, which are theoretically within the larval duration of *Ophioblennius*. This crossing time could be reduced even further, as the undercurrent is known to horizontally shear on its northern flank near the West African coast due to the influence of the North Equatorial Current (Fahrbach et al. 1986). Finally, amphi-Atlantic distributions are relatively common in tropical species (Briggs 1974; Laborel 1974; Lessios et al. 1999), highlighting the role of equatorial currents as vectors for colonization across the Atlantic.

Given these diverse bits of evidence, our proposed history for *O. atlanticus* is as follows. Prior to the closure of the Isthmus of Panama, *Ophioblennius* was split into ancestral populations in the eastern Pacific and western Atlantic. Oxygen isotope ratios in fossil foraminifera assemblages indicate a reduction in the mixing of Caribbean and Pacific waters 5–8 million years ago due to the uplift of the Panamanian shelf (Keigwin 1982; Collins et al. 1996). A standard mo-

lecular clock rate (2% per million years) indicates that ancestral *Ophioblennius* was separated during this initial shoaling (rather than the final closure) of the Panamanian shelf, approximately 7 million years ago. These results are concordant with other recent studies, indicating that many sister taxa in the Caribbean and eastern Pacific became isolated prior to the closure of the Isthmus of Panama, particularly taxa that inhabit reefs as opposed to estuarine habitats (Knowlton et al. 1993; Cronin and Dowsett 1996; Bermingham et al. 1997).

When the Pacific and Caribbean ceased effective exchange at the beginning of the Pliocene, the changes in circulation may have been abrupt (as fossil evidence suggests; Haug and Tiedemann 1998) and characterized by a period of instability due to an initial hydrographic imbalance. This imbalance may have been accentuated by a global sea-level transgression of 50–100 m during the beginning of the Pliocene (Vail and Hardenbol 1979; Haq et al. 1987). The reorganization of Atlantic circulation involved an initial period of high energy in major current systems (Berggren and Hollister 1974; Kaneps 1979; Haug and Tiedemann 1998) during which *O. atlanticus* larvae were able to traverse the mid-Atlantic barrier, via the enhanced Equatorial Undercurrent, to colonize the African mainland. Once regularized circulation patterns became established in the now closed Atlantic Ocean basin, larvae of *O. atlanticus* were not able to bridge the Amazon-Orinoco or mid-Atlantic barriers, as indicated by the mtDNA phylogeny (Fig. 2). The Brazil-Caribbean split and the colonization across the Atlantic were close in evolutionary time, consistent with the evidence that large-scale changes in circulation patterns were responsible for these events.

Following the establishment of *O. atlanticus* in continental Africa, range expansion occurred northward and southward along the African coast. Dispersal from the mainland to St. Helena occurred via the Benguela Current (which flows directly from the Angola region to St. Helena) approximately 4 million years ago. The Gulf of Guinea region then became isolated from the Cape Verde/Azores region around 3 million years ago, an event possibly related to the Niger/Volta River systems, which flow into the northern Gulf of Guinea. Sometime after the genesis of Ascension Island (~1.5 million years ago), it was colonized from St. Helena. Finally, colonization of the Azores probably occurred from northwestern Africa in the late Pleistocene (we have one individual from spatially intermediate Madeira that is identical to the dominant haplotype at the Azores). Although this route goes against the Canary Current, dispersal along this path has been demonstrated in a brooding bivalve (O'Foighil and Jozefowicz 1999).

The genetic signatures of *O. atlanticus* and *O. steindachneri* indicate entirely different evolutionary histories since they shared a common ancestor about 7 million years ago. In the eastern Pacific, *O. steindachneri* is composed of two shallow (average $d < 2\%$) geographically overlapping lineages, whereas in the Atlantic, *O. atlanticus* has five deeply divergent ($d = 0.052\text{--}0.127$), geographically discrete lineages. The shallow phylogeographic lineages of *O. steindachneri* provides historic resolution only for about the last million years, despite a much deeper evolutionary history. The only history that can be postulated for *O. steindachneri* is that Clipperton

Island was isolated for about 1 million years, followed by colonization from the mainland or the Galapagos in very recent evolutionary time, as indicated by a sharing of identical haplotypes (not transversion haplotypes) between mainland and island locations.

The lack of phylogeographic resolution in *O. steindachneri* can be attributed to the oceanographic instability of the eastern Pacific and connection to the larger Indo-Pacific system, which results in significant variation in circulation patterns on annual, interannual, and decadal scales. In contrast, the Atlantic is a closed ocean basin, with circulation patterns that are driven by a steady Gulf Stream and constrained by continental margins. Current regimes characterized by relative constancy would tend to maintain the status quo in terms of oceanographic barriers to dispersal, as compared to less stable regions where barriers between biogeographic regions may be penetrated by changes in circulation pattern. It should be noted that the shallow genetic structure found in the eastern Pacific may be due in part to the smaller tropical range in this basin. However, we observe ancient isolation events in the Atlantic across smaller distances relative to high gene flow observed across greater distances in the eastern Pacific (Table 3), thus corroborating our hypothesis that oceanographic factors foster genetic connectivity in the eastern Pacific.

The phylogeny for *O. atlanticus* is similar to a dendrogram of Atlantic biogeographic provinces based on the distributions of reef fishes (Floeter and Gasparini 2000). These findings indicate that the mtDNA genealogy for *O. atlanticus* was forged by the same forces that define overall reef-species composition. In other words, the distinctions between biogeographic provinces of the Atlantic are not attributable solely to ecological idiosyncracies of individual species, but to the presence of vicariant barriers that apply to most Atlantic reef fishes.

The phylogeographic histories of the *Ophioblennius* sister species, with their two greatly contrasting genetic architectures, highlight the influence that biogeographic (microevolutionary) processes have upon macroevolutionary processes. In the eastern Pacific, with low oceanographic stability and high gene flow among provinces, we found no evidence of independent evolutionary trajectories. In the more stable Atlantic, where disjunct reef habitats are beyond the dispersal limits of *Ophioblennius* larvae, we observed deep genetic partitions. If the five lineages of *O. atlanticus* prove to be species, as the genetic evidence indicates, then *Ophioblennius* is a case in which biogeographic processes clearly translate into macroevolutionary consequences.

Systematics and Taxonomy

The *O. atlanticus* genealogy reveals that five lineages in the Atlantic have evolved independently for approximately 3–6 million years. At least some of these lineages are likely at or beyond the transition between population differentiation and speciation. Given that blennioid fishes are one of the most diverse suborders of teleost fishes, comprising approximately 732 species, 127 genera, and six families (Nelson 1994; Stepien 1997), the phylogenetic picture for *O. atlanticus* resonates with the image of speciation in action.

The taxonomic division of *Ophioblennius* into two subspecies each in the Atlantic and eastern Pacific (Springer 1962) is partially supported by our study. In the eastern Pacific, Springer based a subspecies designation for Clipperton Island blennies (*O. s. clippertonensis*) on a significant frequency shift in fin-ray elements and nuchal cirri. Although we found a similar frequency shift in mtDNA haplotypes, evidence for recent gene flow (i.e., sharing of identical haplotypes) raises concerns about whether the Clipperton population is sufficiently distinct to warrant subspecies status. However, Clipperton Island blennies are the most genetically distinct group observed in our survey of eastern Pacific sites, and a fixed difference in mtDNA haplotypes is not a prerequisite for subspecies designation. The mtDNA data alone are not sufficient to revise taxonomic designations, and this issue requires further investigation. One intriguing possibility is that a major colonization event occurred in the 40 years between the morphological survey and the genetic survey, possibly facilitated by the vigorous ENSO events during this interval.

In the Atlantic, *O. a. macclurei* in the Caribbean represents a distinct lineage, but there are four other highly distinct lineages within *O. a. atlanticus*. The divergences among the five resolved Atlantic lineages are typical of species-level splits in other marine fishes (Grant and Bowen 1998; Johns and Avise 1998). Thus, the mtDNA data indicates that current taxonomy may not adequately reflect the evolutionary partitions within *O. atlanticus*. Although species designations based on mtDNA alone would be inappropriate, these data invoke the possibility of five *Ophioblennius* species in the Atlantic. Notably, Springer (1962) expressed concern about lumping Brazil with the eastern Atlantic under *O. a. atlanticus*. He raised the possibility of additional taxonomic partitions within the Atlantic, but could not justify formal descriptions based on the limited sample sizes available at the time. Thus, our Atlantic mtDNA data support and extend the conclusions based on morphological examinations. V. G. Springer noted recently that the Atlantic subspecies he described in 1962 may qualify as species on morphological grounds: "I think the five Atlantic taxa you suggest are probably valid, and . . . after 40 years another morphological study based on more extra-Caribbean material might corroborate your findings" (pers. comm.).

Conclusions

The concordance between the genetic structure of *O. atlanticus* and the biogeographic provinces of the tropical Atlantic indicates that the oceanic barriers separating these regions are formidable obstacles to gene flow. The emerging picture of Atlantic marine fishes supports the conclusion that genetic partitions are consistently found at the scale of these biogeographic provinces (Tringali and Wilson 1993; Graves 1995; Banford et al. 1999). The distance between provinces is likely not the only relevant factor; river outflows, the relatively stable circulation patterns in the Atlantic, and the thermal limitations of the North and South Atlantic Gyres probably play significant roles in shaping the patterns of genetic structure among Atlantic biogeographic provinces.

The contrast in genetic signatures between the Atlantic and

eastern Pacific *Ophioblennius* species offers insight into how biogeographic processes influence speciation in the marine realm. Genetic isolation and independent evolutionary trajectories are observed between Atlantic regions, due either to prohibitive distances or current patterns that inhibit gene flow. The geography and oceanography of the Atlantic Ocean basin may promote intraspecific (or intrageneric) partitions. This stands in marked contrast to the eastern Pacific, where coastal habitat continuity and oceanographic instability augment the potential for high gene flow. The two shallow, geographically overlapping lineages of *O. steindachneri* in the eastern Pacific and the five deep, geographically discrete lineages of *O. atlanticus* in the Atlantic illustrate how contrasting geographic and oceanographic characteristics translate into population isolation, differentiation, and speciation.

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