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Evolution, Vol. 49, No. 5 (Oct., 1995), 897-910.

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EARLY LIFE HISTORIES, OCEAN CURRENTS, AND THE POPULATION GENETICS OF CARIBBEAN REEF FISHES

MYRA J. SHULMAN¹ AND ELDREDGE BERMINGHAM^{2,3,4}

¹Department of Biology, University of California, Los Angeles, California 90024–1606

E-mail: shulman@biology.lifesci.ucla.edu

²Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Republic of Panama

Abstract.—Tropical reef fishes, along with many benthic invertebrates, have a life cycle that includes a sedentary, bottom-dwelling reproductive phase and a planktonic stage that occurs early in development. The adult benthic populations occupy disjunct, patchy habitats; the extent of gene flow due to dispersal of the planktonic life stage is generally unknown.

We investigated dispersal, gene flow, and endemism in eight species of Caribbean reef fishes that varied in two life-history traits that may affect dispersal ability: egg type (pelagic and nonpelagic) and length of planktonic (usually larval) life. Using restriction endonuclease analyses of mitochondrial DNA (mtDNA), we estimated the degree of genetic differentiation among six populations of each of the eight species; these populations came from widely separated locales, occupying both the northern and southern current tracks within the Caribbean. In addition, we calculated mtDNA divergence between two of the study species and their sister taxa in the eastern Pacific. The transisthmian taxa have been isolated from one another for approximately 3 million yr and thus provide a divergence measure against which to assess intra-Caribbean mtDNA distances.

Mean sequence divergence observed among conspecific Caribbean mtDNA haplotypes in each of the eight fish species was small, less than 0.7% for all but one species. This level of divergence is roughly one order of magnitude less than mtDNA divergence between Caribbean/eastern Pacific sister taxa. For each of the eight species of fishes, the predominant mtDNA haplotype was widespread in the Caribbean. Populations located in different major surface currents were no more differentiated from one another than populations occupying the same current track. These results suggest that there is considerable gene flow throughout the Caribbean, and that this gene flow has not been constrained by present-day ocean currents.

We found statistically significant population subdivision for three Caribbean fish species, though between-population variance accounted for only 8%–17% of the total. Fishes showing population structure were: *Stegastes leucostictus* (nonpelagic eggs; short planktonic life); *Gnatholepis thompsoni* (nonpelagic eggs; long planktonic life); and *Halichoeres bivittatus* (pelagic eggs; short planktonic life). These results suggest that neither egg type nor length of larval life is a simple predictor of geographic structure in reef fish populations.

Key words.—Coral-reef fishes, dispersal, gene flow, historical biogeography, life history, mitochondrial DNA, ocean currents.

Received September 3, 1993. Accepted July 6, 1994.

The dispersal abilities of organisms are likely to have strong effects on determining whether genetic connections exist between spatially segregated populations. Many benthic marine animals possess a dispersive life-history stage in which eggs and/or larvae are pelagic; in most of these species, the larvae are planktonic until metamorphosing into benthic juveniles. Planktonic eggs and larvae are more common among tropical groups than among their temperate counterparts (Thorson 1950; Mileikovsky 1971). For bony fishes inhabiting coral reefs, about 95 of the approximately 100 families have pelagic larvae and 59 of these families have pelagic eggs as well (Breder and Rosen 1966; Ehrlich 1975; Sale 1980; Leis 1991).

The distance that organisms travel during dispersal has important effects on (1) gene flow and genetic differentiation within a species; (2) the geographic extent and numerical size of an interbreeding population; (3) the number of effective barriers to gene flow and the potential for speciation; (4) the potential for adaptation by local populations to local physical and biotic conditions; (5) the spatial scale of ecological communities; and (6) the geographic distribution of species.

For tropical reef fishes, dispersal distances and the extent

of consequent gene flow are currently unknown. Restricted dispersal and genetic differentiation between populations could occur due to several processes. Larval movement may be constrained by major currents, with no dispersal occurring between populations in different current tracks; or, spawning patterns and larval life histories may have evolved to maximize return of larvae to the area of the parental reef. Johannes (1978), for example, has proposed that the seasonal, lunar, and diel timing of spawning has evolved to flush the eggs or larvae away from the reef but also to use currents and gyres that will bring larvae back to the reef. Emery (1972) suggested that fish larvae were being retained in a gyre in the wake of Barbados; Leis and Miller (1976) and Lobel and Robinson (1983) have proposed that similar mechanisms operate to retain larvae around islands in the Hawaiian Archipelago. If larvae are, in fact, returned to the parental population, then gene flow between geographically separated benthic populations would be restricted and genetic differentiation could occur.

Alternatively, dispersal of larvae between populations may be extensive enough to produce genetic homogeneity. Widespread dispersal could result from the relatively long larval life of many reef fishes; “stepping-stone” dispersal; travel by larvae in both major currents and coastal countercurrents; and/or changes in the direction of major currents over time scales of hundreds to thousands of years.

³ Mailing address: Smithsonian Tropical Research Institute, Unit 0948, APO, AA 34002–0948 USA.

⁴ Author order determined by an arm-wrestling competition.

TABLE 1. Life-history data for each of the eight species of coral-reef fishes.

	Egg location	Length of larval life in days			Reference
		Mean	Range	N	
<i>Stegastes leucostictus</i>	Benthic	20.1	19–21	10	Wellington and Victor 1989
		28.5	27–30	4	Thresher and Brothers 1989
<i>Ophioblennius atlanticus</i>	Benthic	28.6	28–29	5	E. Brothers pers. comm. 1991
<i>Abudefduf saxatilis</i>	Benthic	18.2	17–20	10	Wellington and Victor 1989
		27.2	25–29	7	Thresher and Brothers 1989
From drift algae*		33.9	30–55*	9	E. Brothers pers. comm. 1991
<i>Gnatholepis thompsoni</i>	Benthic	81.5	59–122	50	E. Brothers pers. comm. 1991
<i>Haemulon flavolineatum</i>	Pelagic	15	13–20	100	McFarland et al. 1985
<i>Halichoeres bivittatus</i>	Pelagic	24.1	22–26	10	Victor 1986
<i>Holocentrus ascensionis</i>	Pelagic	48.7	46–50	3	E. Brothers, pers. comm.
<i>Thalassoma bifasciatum</i>	Pelagic	49.3	38–78	1172	Victor 1986

* Collected from Sargassum in Florida: eight specimens had 30–32 otolith increments and one specimen had 55 increments with a transition mark at 29 increments.

Despite a great deal of interest, very little information regarding dispersal distances in coral-reef fishes is available, and hypotheses about mechanisms, distances, and functions remain untested and controversial (Ehrlich 1975; Sale 1980; Barlow 1981; Lobel and Robinson 1983; Leis 1991). There are two major factors that should determine whether disjunct populations of coral-reef fishes are genetically connected via planktonic larval dispersal: oceanographic conditions (i.e., current patterns and speeds), and the life history and behavioral traits of each species. There are a number of characteristics of spawning behavior and larval life that may affect the distance fish larvae travel while in the plankton. These are: (1) the timing and location of spawning relative to currents, gyres, and tides; (2) the type of eggs (pelagic and nonpelagic); (3) the length of planktonic larval life; (4) the swimming behavior of larvae, particularly its effect on vertical positioning in the water column; and (5) larval mortality caused by predation and unfavorable physical conditions.

We investigated dispersal, gene flow, and endemism in eight species of Caribbean reef fishes by examining the genetic structure of their populations. We performed restriction-enzyme analyses of mitochondrial DNA (mtDNA) from specimens collected from disjunct, widely separated populations around the Caribbean. We based this study in the Caribbean because both the currents and the geological history of this region are relatively well known. Furthermore, a number of Caribbean taxa, including two of the species that we investigated, are thought to have been separated from their sister taxa in the eastern Pacific by the rise of the Panamanian Isthmus roughly 3 million yr ago (Jordan 1908; Bermingham and Lessios 1993; Knowlton et al. 1993). We reasoned that divergence between allopatric eastern Pacific and Caribbean sister species would provide a good measure against which to assess intra-Caribbean mtDNA distances.

The species that we studied varied in two of the life-history traits that may affect a species' dispersal abilities: egg location (pelagic or nonpelagic) and length of planktonic life. Species with and without pelagic eggs were compared because there is some evidence that species falling into these two egg-type categories differ in the distribution of their larvae with respect to distance from the shore. Several studies have shown that the larvae of reef fishes that had pelagic eggs were found in offshore water; in contrast, larvae of fishes

with nonpelagic eggs were generally most abundant in in-shore waters (reviewed in Leis 1991). This difference could result in shorter dispersal distances for species with nonpelagic eggs compared with species with pelagic eggs. Similarly, the length of the pelagic-life phase in coral-reef fishes varies from several days to several months and it has frequently been hypothesized that this early life-history attribute might also have an important influence on dispersal distance (Goldman et al. 1983; Thresher and Brothers 1985; Wellington and Victor 1989; Victor 1991).

In this study we asked the following questions. (1) Do the mtDNA data provide evidence for genetic differentiation among disjunct benthic populations of Caribbean reef fishes? (2) Is the amount of genetic geographic differentiation correlated with length of planktonic life or the presence/absence of pelagic eggs? (3) Are populations within the same current track more similar to one another than those in different current tracks? (4) How do the levels of genetic differentiation among populations of Caribbean conspecifics compare with measures of genetic divergence between sister species (= geminate species; Jordan 1908) separated by the Isthmus of Panama? (5) How do the levels of geographic subdivision in reef fishes compare with other marine, terrestrial, and freshwater animals; and what are the resulting implications for evolutionary and ecological processes within these different groups?

MATERIALS AND METHODS

Study Species

Potential study species were identified by the following criteria: we knew them to be common in shallow reef and/or seagrass habitats; and information was available on reproductive mode (pelagic vs. nonpelagic eggs) and length of larval life. From species that met these criteria, we chose four species with pelagic eggs and four species with nonpelagic eggs (table 1). Within each of these two categories, we selected species that varied widely in length of planktonic life. Information on length of larval, and sometimes juvenile, pelagic life are available from otolith aging studies (see data and references in table 1). For two species (*Stegastes leucostictus* and *Abudefduf saxatilis*), the length of larval life differs between two published sources; both are listed in table

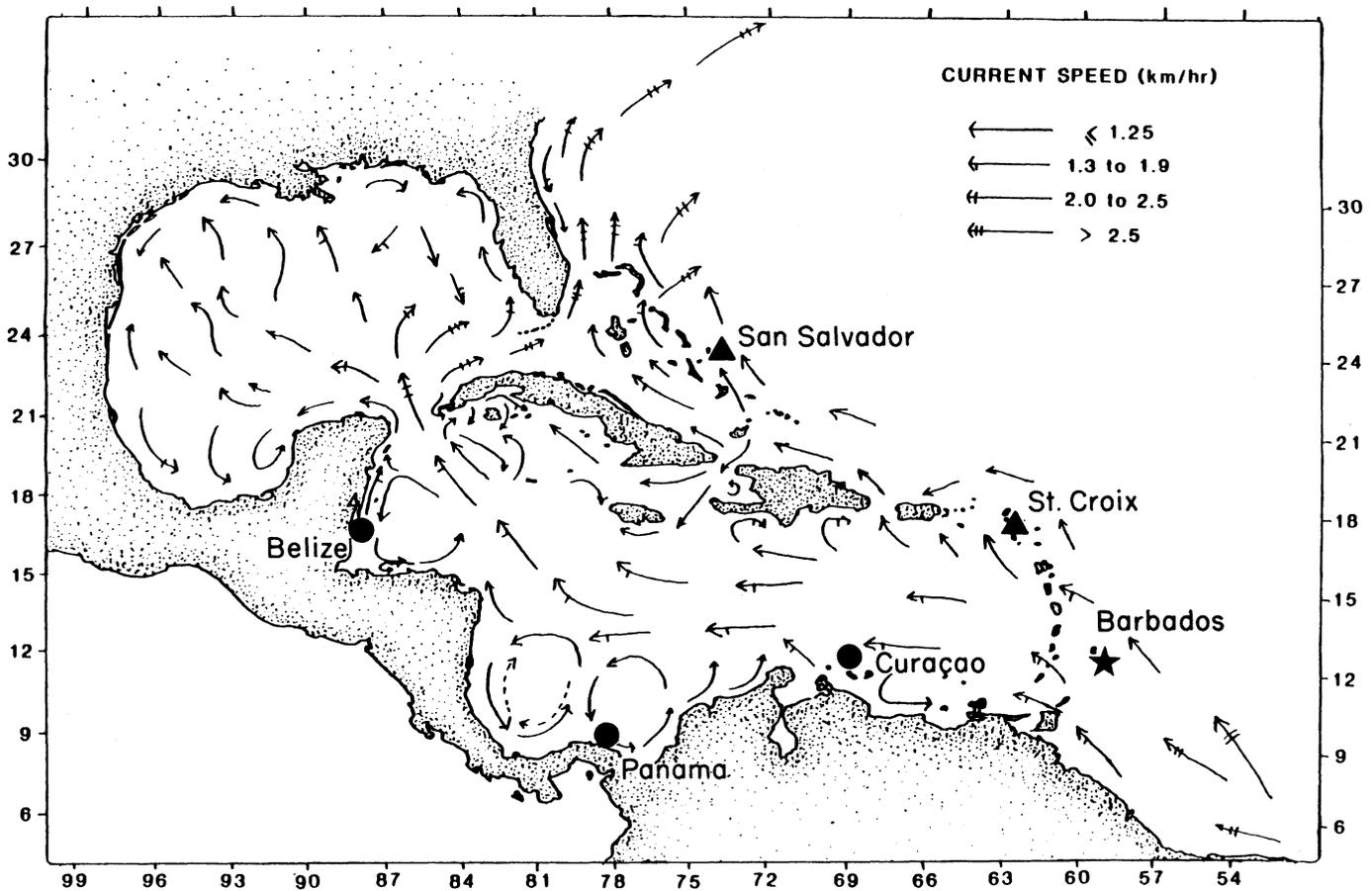


FIG. 1. Surface currents in the tropical Western Atlantic as summarized from the following sources: Wust 1964; Gordon 1967; Febres-Ortega and Herrera 1976; Hydrographer of the Royal Navy 1969, 1970, 1971; Molinari et al. 1980; Molinari et al. 1981; Roemmich 1981; U. S. Naval Oceanographic Office 1982; Kinder 1983; Lessios et al. 1984). Collection sites are indicated as: triangles, northern current track; circles, southern current track; star, Barbados.

1. The mean length of larval life for species with nonpelagic eggs ranges from a low of 18 or 27 d to a high of 82 d. For species with pelagic eggs, the length of planktonic larval life ranges from 15 d to 49 d. Owing to limited sampling, it is certainly possible that the range of larval life span is considerably larger than indicated in table 1. For example, *A. saxatilis* has a planktonic larval life that averages 18 or 27 d, but juveniles that are up to 55-d old have been found rafting under algae (E. Brothers pers. comm. 1991).

Collecting Locales

The prevailing currents in the Caribbean flow from the southeast toward the northwest (fig. 1; see legend for references). Given the current patterns, it is reasonable to hypothesize that the greatest degree of gene flow occurs in an east-to-west direction and that gene flow is restricted in the north-south direction if larvae are unable to cross the westwardly flowing currents. In addition, it is possible that populations within gyres along the Central American coast are relatively genetically isolated from populations outside.

We collected our study species from six locations within the Caribbean and Bahamas region (fig. 1). These locations were chosen to maximize the probability of finding geo-

graphic subdivision within the range of Caribbean fishes. Two of these sites are within the "northern Caribbean current track": San Salvador (Bahamas) and St. Croix. Three of the sites are within the "southern Caribbean current track": Belize, Panama, and Curacao. Two of these sites (Belize and Panama) are also located within gyres along the Central American coast. The sixth collection location, Barbados, is the easternmost island in the Caribbean and is upcurrent of the other Antillean islands and outside the dominant current tracks.

Field and Laboratory Methods

Tissue samples (muscle, liver, and/or ovaries) from freshly sacrificed fish were placed in ice-cold 70% ethanol (EtOH). Within 12 h, the EtOH was replaced with fresh ice-cold 70% EtOH and stored refrigerated for 2 d or more, after which the samples were typically transported in 70% EtOH at ambient temperatures. Upon arrival in the lab, the tissue samples were rehydrated through a series of diminishing EtOH concentrations (50%, 25%, 10%, 0%) and then lyophilized. Lyophilized tissues were stored at -70°C until analyzed. From some individuals of each species, liver and/or heart tissue samples were placed in ice-cold STEN buffer (100 mM NaCl,

10 mM Trizma base, 100 mM EDTA, pH 7.5) and stored refrigerated (1–3 d) until processed for probe mtDNA.

Total cellular DNA was extracted from each lyophilized sample. Samples were first ground in 500 μ liters of 2 \times CTAB buffer (Murray and Thompson 1980) and incubated at 50–55°C for 1–16 h with constant agitation in presence of 6–12 units of Proteinase K. The homogenate was then extracted with an equal volume of chloroform-isoamyl alcohol solution (CI; 24:1), then with phenol-chloroform-isoamyl alcohol solution (25:24:1), and a second time with CI. The DNA was recovered by cold-EtOH precipitation and redissolved in 200 μ liters of 1 \times TE (10 mM Tris, 1 mM EDTA, pH 8.0). In addition, ultrapure mtDNA to be ³²P nick-translated or random-primed for homologous probe mtDNA was isolated from the STEN-buffered tissues following the methods of Lansman et al. (1981) and twice purified by ultracentrifugation in cesium chloride-ethidium bromide density gradients.

Approximately 1 g of total genomic DNA extracted from the lyophilized tissue was digested with 20 units of restriction enzyme, following the manufacturer's recommendations. We used 15–19 restriction endonucleases to screen a subset of mtDNAs representing the full geographic range of our collections for each species. In turn, all conspecific individuals were analyzed with 11 restriction enzymes that our initial assay indicated would provide complete digests of the DNA. Thus, we did not use the same 11 enzymes across all species; we did use the same 11 restriction endonucleases to analyze all conspecific individuals. In sum, aliquots of DNA from each individual for all eight species of fishes were digested with the following five restriction endonucleases (and *r* values; see Nei 1987): *Hind*III (6.0), *Pvu*II (6.0), *Stu*I (6.0), *Hinc*II (5.3), and *Ava*II (4.6). Each individual DNA sample was also digested with an additional six enzymes from the following group of enzymes with an *r* value of 6.0 (unless otherwise noted): *Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*EII, *Cla*I, *Dra*I, *Eco*RI, *Eco*RV, *Nco*I, *Sac*I, *Xba*I, *Xho*I, and *Ava*I (5.3). Following digestion, restriction fragments were separated electrophoretically in 0.9%–1.5% agarose gels. After in-gel denaturation, the size-fractionated DNA samples were Southern blotted overnight by capillarity on ZetaBind[®] membranes. SCP buffer (10 \times : 1 M NaCl, 0.3 M Na₂HPO₄, 10 mM EDTA, pH 7.0–7.5) was used for transfers and post-transfer washing of the membranes.

Prehybridization and hybridization were performed at 65°C in either bags on a shaker or rotating canisters spinning at approximately 5 rpm. Membranes were prehybridized for several hours to overnight, in 25–40 ml of prehybridization solution (10% dextran sulfate, 0.5 M NaCl, 1% SDS; or 7.4% dextran sulfate, 4.4 \times SCP, 0.74% N-lauryl-sarcosine, 0.4 mg/ml heparin). Membrane-bound DNA from each species of fish was then hybridized to species-specific, ultrapurified mtDNA that was either random-primed or nick-translated in the presence of [α -³²P]dXTP. Blots were hybridized for 12–36 h, and then washed with three 15-min washes of increasing stringency at 65°C (2 \times SCP, 1% SDS; 0.2 \times SCP, 0.1% SDS; 0.1 \times SCP, 0.05% SDS). Scorable bands on autoradiographs were obtained, using one or two intensifying screens, in 20–120 h on Kodak XAR[®] film at –70°C.

Data Analysis

For each species, each distinctive mtDNA fragment pattern (Restriction Fragment Length Polymorphism, RFLP) produced by a given restriction enzyme was given an alphabetic label. The labels for each of the 11 restriction patterns of an individual's mtDNA defined the composite haplotype for each specimen. In some cases, the fragment patterns on the autoradiographs could not be determined. Specimens missing data for two or more enzymes were eliminated from the data set. We included 35 individuals that were missing information for one enzyme.

In almost all cases, the fragment patterns could be readily related to each other in terms of the gain or loss of one or more restriction sites, thus allowing site data to be inferred with little ambiguity (Bermingham 1990). Composite haplotype designations were therefore represented by the presence or absence of restriction sites. All analyses were performed with this restriction-site information as the raw data. (These data are available from the authors upon request.)

Data for each species were summarized using indices of haplotype diversity, nucleotide diversity, and nucleotide divergence described by Nei and Tajima (1983), Nei (1987), and Nei and Miller (1990). The expected number of nucleotide substitutions per nucleotide site (d_{xy}) was calculated from the restriction-site data following Nei and Miller (1990, eq. 4). Individual d_{xy} values for each enzyme class were weighted as per Nei and Tajima (1983). Genetic differences between individuals within the same populations (π) and in different populations (d_{xy}) were summarized by calculations of the mean nucleotide divergence performed by the program REAP (version 4.0; McElroy et al. 1992).

Relationships among the haplotypes were described phenetically using the UPGMA and Neighbor-joining programs in NTSYS (versions 1.5–1.7, Rohlf 1990), and cladistically using PAUP (version 3.0s, Swofford 1990) and MacClade (versions 2.1–3.0, Maddison and Maddison 1987, 1990).

The development of statistical techniques for evaluating population-level mtDNA data is in a rapidly growing, but still preliminary, stage. Thus, we employed a variety of indices to determine the presence and extent of genetic differentiation between the geographically separated populations; multiple tests indicating genetic differentiation were taken as strong evidence that population subdivision does exist. The statistical significance of each index was determined by 500–1000 random permutations of individuals among populations, producing a null distribution of the statistic against which the observed value could be compared. The seven indices used are described below.

Z^* .—This statistic, described in Hudson et al. (1992), involves ranking the d_{xy} values for all pairs of individuals sampled within a species. A population Z^* value is then determined by averaging the logarithm of 1 plus the rank of the d_{xy} value for all possible pairs of individuals within the population. The overall Z^* statistic is calculated as the mean of the population Z^* 's, each weighted by that population's proportion of the total sample size. The null hypothesis of no significant differences between populations is rejected if the actual Z^* is smaller than 95% of the null distribution.

K_{st} .—The K_{st} statistic, also developed by Hudson et al.

TABLE 2. Summary of sample size, restriction site, and haplotype information for each of the eight species of coral-reef fishes.

	Individuals <i>N</i>	Haplotypes <i>N</i>	No. haplotypes per location	% Individuals (no. locations) for most common haplotype	Mean no. restriction sites per individual	Mean no. polymorphic restriction sites per individual
<i>Stegastes leucostictus</i>	61	7	1–3	72.1 (6)	67.1	4.1
<i>Ophioblennius atlanticus</i>	64	55	8–14	6.3 (4)	58.5	18.5
<i>Abudefduf saxatilis</i>	67	18	4–9	31.3 (6)	54.9	7.0
<i>Gnatholepis thompsoni</i>	61	42	8–11	14.8 (5)	62.8	15.8
<i>Haemulon flavolineatum</i>	65	17	3–7	38.5 (6)	62.6	12.6
<i>Halichoeres bivittatus</i>	57	23	4–7	50.9 (6)	56.5	12.5
<i>Holocentrus ascensionis</i>	61	34	7–9	27.9 (6)	53.2	12.2
<i>Thalassoma bifasciatum</i>	89	20	4–10	67.4 (6)	61.0	12.0

(1992), is one minus the average difference between pairs of individuals in the same population, divided by the average difference between all pairs of individuals sampled. The K_{st}^* statistic differs from K_{st} by using a logarithmic form of the sequence-difference measure. Our calculation of K_{st}^* follows the formula in equation (11) of Hudson et al. (1992), using d_{xy} values as the measure of sequence difference. There are significant differences between the populations if the actual K_{st}^* statistic is larger than 95% of the null distribution.

G_{st} .—Takahata and Palumbi's (1985) analogue to Nei's (1973) G_{st} calculates the fraction of genetic variation within the species that is due to genetic variation between populations. In a method similar to K_{st} (Hudson et al. 1992), G_{st} is based on estimates of the probability that individuals will be identical at each restriction site and compares those estimates for individuals within the same and between different populations. Our calculations of G_{st} were performed with a program supplied by S. Palumbi.

AMOVA.—Excoffier et al. (1992) have developed an ANOVA method for partitioning genetic variance among categories: (1) within populations; (2) between populations within a region; and (3) between regions. Populations were considered significantly different from one another if the actual within-population variance was lower than 95% of the within-population variances in the null distribution. We performed two AMOVAs to examine between-population differences: (1) using the number of restriction-site differences between haplotypes as a Euclidean distance measure; and (2) assuming that all haplotypes are equidistant from one another (this is equivalent to simply examining the haplotype distribution without reference to the haplotypes relationships to one another).

χ^2 .—Two different χ^2 tests were performed. (1) A test of the distribution of all haplotypes across locales. The null hypothesis was that the distributions were the same for all haplotypes. (2) Because differences between species in the results of this first chi-square test might be due to the different numbers of haplotypes per species, a second test on haplotype "clusters" was performed. The lowest number of haplotypes observed for any species was seven (*S. leucostictus*); thus our aim in the second χ^2 analysis was to compare a like number of haplotype "clusters" across all species. Haplotypes were clustered using the UPGMA program of NTSYS. All trees were found and a consensus tree based on the majority rule method was constructed. We used the consensus trees to

choose equally divergent consensus groups that approached 7 as closely as possible (range 4–8) and, in turn, tested the distribution of haplotype "clusters" across locales.

Of the seven different tests for genetic differences between populations, two were "haplotype statistics" (sensu Hudson et al. 1992), four were "sequence statistics" (i.e., they employed information regarding the degree of mtDNA sequence difference between haplotypes), and 1 was mixed. The haplotype statistics are: AMOVA using the assumption of equidistance between haplotypes; and the χ^2 test of the distribution of haplotypes across locales. The four sequence statistics are: Z^* ; K_{st}^* ; G_{st} ; and AMOVA using restriction-site differences as the distance metric. The χ^2 test of clustered haplotypes is a mixed haplotype-sequence statistic: it uses sequence information to determine the clusters, but the sequence differences between the clusters does not enter into the statistic.

Our sampling design was established to examine the evolutionary pattern, as opposed to the demographic pattern, of population relationships in Caribbean coral reef fishes. The numbers of individuals sampled and the geographic distribution of these samples were sufficient to determine the degree and evolutionary significance of phylogeographic structure among conspecific populations. As a second step in our analytical process, we examined the mtDNA haplotype data for evidence of *demographically* significant population structure. Although results demonstrating population subdivision can be interpreted at face value, a failure to detect geographic heterogeneity may be due to the relatively weak power of the test at small sample size.

RESULTS

We used 11 restriction enzymes to analyze the mitochondrial genomes of 525 individuals representing 8 species of coral-reef fishes (table 1) that were collected from 6 widely separated locations in the Caribbean (fig. 1). Information on numbers of sampled individuals, haplotypes, and restriction sites for each species are summarized in table 2. An average of 60 restriction sites (range over the 8 species: 53.2–67.1) were assayed per individual, of which an average of 12 (range: 4.1–18.5) were polymorphic. Our estimate of mtDNA molecular weight in these species range from 16.4 kilobase pairs (kbp) in *Ophioblennius atlanticus* to 18.0 kbp in *Stegastes leucostictus*. The size of the mtDNA genome varied

by as much as 1 kbp within *Gnatholepis thompsoni*, *S. leucostictus*, *Thalassoma bifasciatum*, and *Holocentrus ascensionis*, and individual mtDNA size heteroplasmy was sometimes noted in these species.

Our restriction-enzyme analyses identified a considerable range in the numbers of mtDNA haplotypes across the eight reef fish species studied (table 2). For example, assays of 61 *S. leucostictus* revealed only seven haplotypes, whereas in *O. atlanticus* we observed 55 mtDNA haplotypes across the 64 fish surveyed. The species also differed enormously in the extent to which they were predominantly represented by a single haplotype (fig. 2). The most common haplotype observed in *S. leucostictus* accounted for 72% of the total sample; in *O. atlanticus*, the common haplotype accounted for only 6% of the total (table 2). These differences were not solely a function of the number of haplotypes found. For example, *T. bifasciatum*, with 20 haplotypes, had 67% of the individuals belonging to the most common haplotype (table 2). In contrast, *Abudefduf saxatilis*, with 18 haplotypes, had only 31% of the individuals in the most abundant haplotype class (table 2). *Abudefduf saxatilis* was also unusual in having two mtDNA haplotypes in moderately high frequency (fig. 2).

The eight species also differed in the number of haplotypes that were represented by only a single individual (fig. 2), leading to variation in haplotype diversity across species and geographic locales (table 3). Unique individuals constituted a low of 5% of the specimens in *S. leucostictus* to a high of 59% of *O. atlanticus*. As would be expected, species with very large numbers of haplotypes had high percentages of unique individuals. However, a high proportion of unique individuals was also observed in *H. bivittatus*, in which 21 of 23 haplotypes were singletons and comprised 37% of the individuals surveyed (fig. 2).

In general, conspecific mtDNA haplotypes in each of the 8 species were very closely related. With the exception of *O. atlanticus*, the mean mtDNA sequence divergence observed between haplotypes within a species did not exceed 0.7% (table 4). In contrast, on average, *O. atlanticus* haplotypes were about 1% diverged from one another. Considering all species, haplotypes differed from one another, on average, by 3.8 restriction sites (range over the 8 species: 2.3–6.8; table 4).

For most of the eight species studied, we obtained a large number of rival, minimum-length trees in each of the phylogenetic-analysis programs used. This situation resulted from the fact that many mtDNA haplotypes are each one mutation step (= 1 restriction site) removed from a "central" haplotype, thus yielding a star phylogeny. There was no apparent phylogeographic structure revealed for any of the eight species by either cladistic or phenetic analyses of the restriction-site data (fig. 2). The different estimation procedures resulted in sets of trees that were largely congruent across analytical methods. In other words, the consensus UPGMA trees presented in figure 2 are very similar to those resulting from analyses using neighbor-joining and Wagner parsimony methods. We chose to present UPGMA trees in order to permit: (1) mtDNA sequence divergence levels to be easily compared across the eight species herein (fig. 2); and (2) com-

parisons to the large number of UPGMA-based mtDNA trees in the literature.

A survey of the geographic distribution of haplotypes (fig. 2) reveals that the common haplotype in six of the species was found in all locations. This was not the case for *O. atlanticus* and *G. thompsoni*. Even for these two species, however, the most common mtDNA haplotype was quite widespread. The most common *O. atlanticus* mtDNA haplotype was found in four individuals collected from four different sites; the most abundant *G. thompsoni* mtDNA haplotype was found in nine individuals collected from five locations (fig. 2).

Tests for significant genetic variation between populations revealed differences between species. The two haplotype statistics (χ^2 and AMOVA assuming equidistant haplotypes) both indicated that only *S. leucostictus* had a nonrandom distribution of haplotypes across locales (table 5). An examination of the distribution of haplotypes within this species (fig. 2) showed that the second most common haplotype was patchily distributed, being present in Belize, Panama, and San Salvador, and absent from the other locations. Additionally, the frequency of the most common haplotype was highly variable.

When sequence differences between the haplotypes were included in the test statistics (table 5), *S. leucostictus* again showed significant differences between populations. All four of the sequence statistics indicated highly significant population structure ($P < 0.01$) as did the mixed haplotype/sequence statistic (clustered χ^2 test). The AMOVA results allocated 17.2% of the total variance to between-population variance with the remainder being within-population variance.

With the addition of the sequence information to the test statistics, two other species also showed significant between-population variance. *Gnatholepis thompsoni* had highly significant ($P \leq 0.01$) results for the four sequence statistics and $P = 0.02$ for the clustered χ^2 test. The sequence AMOVA indicated that 8.2% of the total variance was partitioned between populations. Results for *Halichoeres bivittatus* were mixed. K_{st}^* , sequence AMOVA, and clustered χ^2 all indicated highly significant between-population differences ($P \leq 0.002$). However, G_{st} had a P value of only 0.1, and the Z^* statistic P value was 0.38. The sequence AMOVA allocated 7.9% of the variance to between-population differences.

None of the other five species showed significant results for any of the tests for between population differences (table 5). The sequence AMOVA allocated nearly 100% of the variance to within-population differences in these species. In addition, none of these species show larger between-than within-population mtDNA sequence divergence values (table 4).

Comparisons of statistical methods indicate that sequence statistics are much more sensitive measures of population differentiation than are haplotype statistics. For *S. leucostictus*, *G. thompsoni*, and *H. bivittatus*, the three species evidencing geographic differentiation, Z^* appears to be the least sensitive of the sequence statistics. Because Z^* is a rank-order statistic, we would expect that this should be the case. K_{st}^* and sequence AMOVA produced consistent and significant measures of population subdivision for all three species,

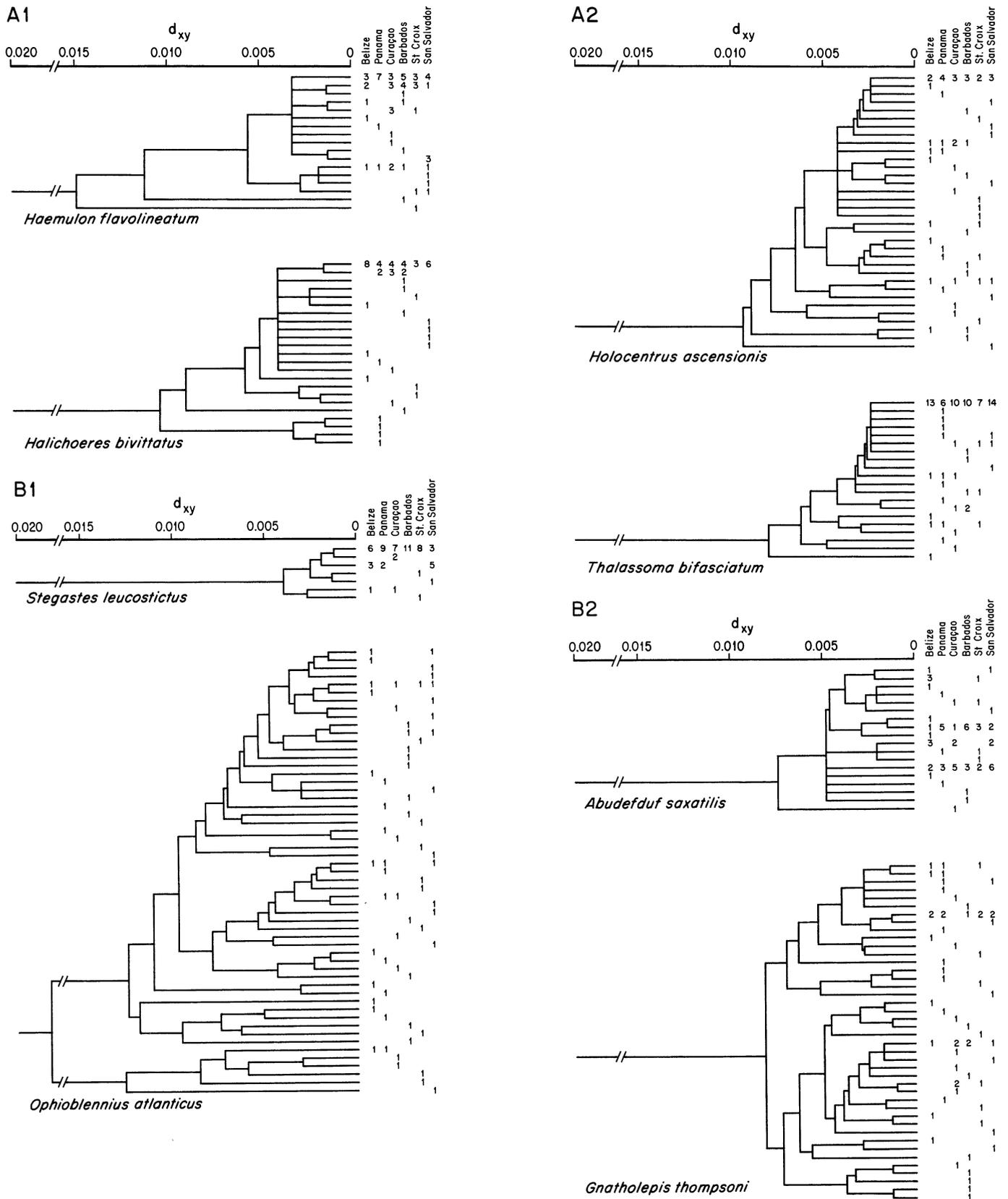


FIG. 2. UPGMA majority-rule consensus trees summarizing relationships among mtDNA haplotypes observed within each of eight coral-reef fish species. All dendrograms are plotted on the same scale of mtDNA sequence divergence (d_{xy}). The frequency and collection locale of each haplotype is shown to the right of the dendrogram. A1 and A2: Species with pelagic eggs; *Haemulon flavolineatum*, *Halichoeres bivittatus*, *Holocentrus ascensionis*, *Thalassoma bifasciatum*. B1 and B2: Species with nonpelagic eggs; *Stegastes leucostictus*, *Ophioblennius atlanticus*, *Abudefduf saxatilis*, *Gnatholepis thompsoni*. Within each reproductive mode, species are listed in order of increasing time spent as pelagic larvae.

TABLE 3. mtDNA haplotype diversity (and number of haplotypes) by geographic locale.

	Belize	Panama	Curacao	Barbados	St. Croix	San Salvador	Average haplotype diversity (no.)
<i>Stegastes leucostictus</i>	0.60 (3)	0.33 (2)	0.51 (3)	0.00 (1)	0.38 (3)	0.64 (3)	0.41 (2.5)
<i>Ophioblennius atlanticus</i>	1.00 (11)	1.00 (10)	1.00 (8)	1.00 (11)	1.00 (10)	1.00 (14)	1.00 (10.7)
<i>Abudefduf saxatilis</i>	0.92 (9)	0.76 (5)	0.76 (5)	0.67 (4)	0.89 (6)	0.74 (5)	0.79 (5.7)
<i>Gnatholepis thompsoni</i>	0.97 (8)	0.99 (11)	0.96 (9)	0.98 (10)	0.97 (8)	0.97 (8)	0.98 (9)
<i>Haemulon flavolineatum</i>	0.86 (5)	0.42 (3)	0.87 (6)	0.82 (7)	0.83 (5)	0.86 (7)	0.78 (5.5)
<i>Halichoeres bivittatus</i>	0.49 (4)	0.87 (7)	0.75 (4)	0.84 (6)	0.80 (4)	0.67 (5)	0.74 (5)
<i>Holocentrus ascensionis</i>	0.98 (9)	0.87 (7)	0.91 (7)	0.95 (9)	0.98 (9)	0.93 (8)	0.94 (8.2)
<i>Thalassoma bifasciatum</i>	0.43 (5)	0.86 (10)	0.57 (6)	0.56 (5)	0.53 (4)	0.33 (4)	0.55 (5.7)

as did the mixed haplotype/sequence statistic, the chi-square test of clustered haplotypes. The G_{st} statistic was consistent with the other sequence-based statistics for *S. leucostictus* and *G. thompsoni*, but not for *H. bivittatus*.

We also tested hypotheses of regional variation. First, we tested the a priori hypothesis that populations in different current tracks are more different from one another than populations in the same current track. This comparison was accomplished by nesting populations in a sequence AMOVA. Assigning populations to a "northern current track" (St Croix and San Salvador), "southern current track" (Curacao, Panama, and Belize), and eastern upcurrent isolate (Barbados) produced no significant differences among these three regions; populations within the same current track are as different from one another as populations in different current tracks.

For *S. leucostictus*, *G. thompsoni*, and *H. bivittatus*, the three species that showed significant between-population variation, additional tests were made of several a posteriori hypotheses of regional affinities. (1) Do populations closest to one another have the least sequence divergence (d_{xy}) between them? A statistical test of this hypothesis showed no significant correlation between geographical and genetic distance between populations for any of the three species (all $r^2 < 0.01$; all $P > 0.9$). (2) Do populations in the eastern Caribbean differ from those in the western Caribbean? A sequence AMOVA comparing an eastern region consisting of Barbados and Curacao with a western region of the remaining four locales showed significant regional variation in *G. thompsoni* but not *S. leucostictus* or *H. bivittatus*. A similar test comparing an eastern/central region (Barbados, Curacao, Panama, and St. Croix), the Bahamas region (San Salvador), and Belize showed significant regional variation in *S. leu-*

costictus but not in either of the other two species. No tests of additional regional groupings produced any evidence for regional structure in *H. bivittatus*.

DISCUSSION

Disjunct geographic distributions provide the opportunity for the evolution of genetically distinct populations within a species. Genetic differentiation would be expected to occur if physical conditions (geologic and/or oceanographic) separating populations continue to exist over a relatively long period of time, and the dispersal abilities of the organisms are limited. We would expect to find genetic homogeneity if physical conditions isolating populations vary, or break down over time, or if the dispersal distances are long. In this study we compared mtDNA distribution patterns across species in order to investigate underlying causal mechanisms. We explicitly investigated three possible causes that could underlie congruent patterns of mtDNA haplotype relationship in Caribbean coral-reef fishes: (1) shared geologic histories, (2) shared oceanographic features, and (3) shared life-history attributes.

Historical Biogeography

In the extreme case of no dispersal, we might have anticipated finding that the genealogical relationships among fish populations reflected the Caribbean's geological history. Although the geological history of the region is complicated, it appears clear that by the beginning of the Miocene (18 mya) most Caribbean geographic features showed their present-day relationships (Rosen 1985; Buskirk 1985). By that time, most of the areas that we sampled were already separated by deep water from one another: Central America (Be-

TABLE 4. Mitochondrial DNA sequence variation within populations, sequence divergence between populations and haplotypes, and unweighted numbers of restriction-site differences between haplotypes.

	Within population π		Between population d_{xy}		Between haplotype d_{xy}		Number of restriction site differences between haplotypes	
	Range	Mean	Range	Mean	Range	Mean		
<i>Stegastes leucostictus</i>	0.0000–0.0011	0.0007	0.0002–0.0015	0.0008	0.0012–0.0053	0.0030	1–4	2.3
<i>Ophioblennius atlanticus</i>	0.0076–0.0129	0.0104	0.0087–0.0118	0.0104	0.0015–0.0237	0.0109	1–15	6.8
<i>Abudefduf saxatilis</i>	0.0023–0.0035	0.0029	0.0023–0.0034	0.0029	0.0015–0.0106	0.0049	1–6	2.9
<i>Gnatholepis thompsoni</i>	0.0041–0.0070	0.0057	0.0053–0.0072	0.0062	0.0013–0.0149	0.0068	1–10	4.6
<i>Haemulon flavolineatum</i>	0.0011–0.0047	0.0029	0.0016–0.0039	0.0029	0.0013–0.0174	0.0062	1–10	3.7
<i>Halichoeres bivittatus</i>	0.0017–0.0051	0.0028	0.0018–0.0049	0.0030	0.0015–0.0157	0.0065	1–9	3.8
<i>Holocentrus ascensionis</i>	0.0034–0.0052	0.0044	0.0038–0.0049	0.0044	0.0016–0.0154	0.0062	1–9	3.6
<i>Thalassoma bifasciatum</i>	0.0005–0.0026	0.0015	0.0007–0.0021	0.0014	0.0014–0.0117	0.0048	1–7	2.9

TABLE 5. Summary of the results for the geographic-structure statistics. Statistically significant results ($P < 0.05$) are underlined.

	Haplotype statistics			Sequence-based statistics						Evidence for geographic structuring
	AMOVA†		χ^2	G _{st}		Z	K _{st}		AMOVA†	
	P-value (% variation between populations)	All haplotypes P-value		"Clustered" P-value	P-value		P-value	P-value		
<i>Stegastes leucostictus</i>	<u>0.005</u> (15.5)	<u>0.00</u>		<u>0.00</u>	<u>0.008</u>	<u>0.000</u>	<u><0.001</u> (17.2)	very strong		
<i>Ophioblennius atlanticus</i>	0.989 (-0.8)	0.94		0.80	0.790	0.512	0.347 (0.3)	none		
<i>Abudefduf saxatilis</i>	0.069 (4.0)	0.21		0.38	0.420	0.614	0.595 (-1.1)	none		
<i>Gnatholepis thompsoni</i>	0.082 (-0.9)	0.85		0.01	0.010	0.000	0.009 (8.2)	very strong		
<i>Haemulon flavolineatum</i>	0.192 (2.2)	0.15		0.36	0.242	0.494	0.455 (0.1)	none		
<i>Halichoeres bivittatus</i>	0.447 (0.38)	0.20		0.10	0.378	0.002	0.002 (7.9)	strong		
<i>Holocentrus ascensionis</i>	0.978 (-2.7)	0.99		0.18	0.874	0.632	0.561 (-0.3)	none		
<i>Thalassoma bifasciatum</i>	0.322 (1.1)	0.74		0.56	0.378	0.848	0.920 (-1.2)	none		

† In the AMOVA model, when the true variance is zero its estimation can be slightly negative.

lize and Panama); Curacao; Barbados; St. Croix; and the Bahamas. With the exception of the two sites in Central America, all of these areas have remained separated from one another up through the present.

If populations had been genetically sequestered from one another since the formation and isolation of their associated geological features, and given empirically estimated rates of mtDNA evolution (Brown et al. 1982; Martin et al. 1992), we would expect to find: (1) geographically restricted mtDNA haplotypes (endemism); and (2) high sequence divergence between mtDNA haplotypes found in allopatric populations. These predictions were not met. For each of the eight species of fishes considered, the predominant mtDNA haplotype was widespread in the Caribbean. In fact, for six species, the most common haplotype was found at all six collecting sites and even relatively rarer mtDNA haplotypes tended to be broadly distributed (fig. 2).

Comparisons to sister taxa separated by the Central American Isthmus allowed us to exclude the possibility that mtDNA similarity across Caribbean regions was due to reduced rates of mtDNA evolution. Two of the species studied, *A. saxatilis* and *O. atlanticus*, are thought to have been separated from their eastern Pacific sister taxa (*A. troschelii* and *O. steindachneri*, respectively) by the Pliocene rise of the Isthmus of Panama roughly 3 mya (Jordan 1908; Rubinoff 1963). Mitochondrial DNA sequence divergence between the eastern Pacific/Caribbean sister species is roughly one order of magnitude greater than that observed within our eight Caribbean study species (Bermingham et al. in prep; Lessios and Bermingham in prep). This finding suggested that the mtDNA molecules of *Abudefduf* and *Ophioblennius* are evolving at rates consistent with those observed for other vertebrates (1%–2% per million yr; Brown et al. 1982; Martin et al. 1992) and indicated that the mtDNA haplotypes surveyed in each species probably coalesce in the Pleistocene.

The maternal lineages represented by the assayed mtDNA haplotypes of Caribbean reef fishes are considerably less divergent than mtDNA lineages observed among conspecifics in each of three freshwater fish species (*Lepomis* spp.) broadly distributed across the southeastern United States (Bermingham and Avise 1986). In the freshwater fishes, some mtDNA lineages were presumably buffered against loss as a result of genetic isolation in discontinuous riverine habitats. It is apparent that an analogous process has not acted across discontinuous coral-reef habitats. In fact, the limited genetic distances observed between conspecific mtDNA haplotypes suggest that mtDNA lineage extinction in these coral-reef fishes is especially rapid.

High rates of mtDNA lineage extinction might result from at least two demographic processes relevant to coral-reef fishes. First, Pleistocene reductions in reef area might have led to decreased effective population sizes of coral-reef fishes. We have made rough measurements of shallow water habitat (0–60 m depth) at present sea-level and at –100 m sea level; these measurements suggested that reef area in the Caribbean may have been reduced by at least one order of magnitude during Pleistocene low sea-level stands. This reduction in habitat probably led to decreased population sizes in most reef-associated organisms. Second, high variance in reproductive success among females has been theoretically shown

to cause rapid pruning of mtDNA trees leading to decreased times to coalescence (Avise et al. 1984). The extremely low, and presumably highly stochastic, survival rate of pelagic larvae (Leis 1991) makes it very likely that female reef-fish vary considerably with regard to the number of daughters that replace them.

It is not only maternally inherited genes that evidence significant gene flow across the Caribbean. For example, Lacson (1992) has studied allozyme variation in six species of coral-reef fishes collected from Puerto Rico and Jamaica. He found no evidence for population subdivision in any of the species and estimated that a mean of 5.1–11.6 migrants per generation crossed the 1000 km separating the two locales.

Ocean Currents

The widespread distribution of common mtDNA haplotypes across the Caribbean also argued against the possibility that current tracks in the Caribbean have acted as barriers to gene flow through evolutionary time. Statistical analyses of haplotype distributions demonstrated that the differences between northern and southern current tracks were no greater than differences within current tracks and permitted us to reject the hypothesis that gene flow is constrained within these major present-day currents. An important point is that this was true for all of our eight species, despite the wide variation in reproductive mode and length of pelagic life. Clearly, the path of gene flow is not solely described by the major surface current patterns within the Caribbean region.

Analyses of current patterns in the Caribbean basin led to additional predictions regarding the genetic isolation of regional fish populations. Owing to the upcurrent location of Barbados, we posited that novel mtDNA haplotypes originating elsewhere would not be carried to Barbados, resulting in reduced mtDNA haplotype diversity within that population. Our data did not support this hypothesis. Barbados had the lowest number of haplotypes and below average haplotype diversity for only two of the eight species (fig. 2, table 3), which does not statistically differ from random expectation. This result suggests that larvae are moving from other locales within the Caribbean to Barbados, in the direction counter to that described by the major surface currents. Although it seems unlikely to us, an alternative explanation is that Barbados is receiving an influx of haplotypes from outside of the Caribbean region; possible sources would include the coast of Brazil, Ascension Island in the central Atlantic, and islands in the tropical eastern Atlantic. This possibility of course raises a question of scale: What are the genetic relationships among the populations of reef fishes throughout the tropical Atlantic Ocean?

We also considered the possibility that regional current gyres along the coasts of Panama and Belize might lead to increased mtDNA endemism in these regions. Both visual and statistical examination of the data counter this hypothesis. Finally, the data were examined for geographical congruence of mtDNA distribution patterns that might indicate regional sources or sinks of mtDNA haplotypes and none were found.

What might be the reasons that genetic relationships of coral-reef fishes do not reflect the geographic differences pre-

dicted from present-day currents? We can suggest several that encompass ways in which the currents that larvae experience may vary on both spatial and temporal scales.

1. Over the time scales influencing mtDNA divergence and distribution, current patterns may periodically reverse themselves and gyres may break down. Recent studies of the El Niño-Southern Oscillation (ENSO) events indicate that they can have major effects on current directions at many locations within the Pacific Ocean; similar kinds of events may have occurred in the tropical Atlantic (Philander 1990). On a more local scale, reversals of gyre direction have been noted along the coast of Central America (Wust 1964; Kinder 1983). It also appears that currents may vary on very short and very long time scales. Hurricanes can produce localized, short-lived changes in current direction. On a longer time scale, it appears that there were major interruptions of water flow within the Atlantic during glacial periods, with the most recent occurring during the Younger Dryas, 10,000 yr ago (Broecker and Denton 1990). These major changes in Atlantic circulation may have significantly altered currents within the Caribbean. Changes of current direction within the Caribbean could explain the distribution of haplotypes in Barbados as well as the movement of larvae between the major current tracks.

2. Measurements by drogues of the direction and speed of surface currents may not accurately describe the speed and/or direction of all the vertical strata of water within the top 100 m (Pickard and Emery 1990), the vertical range of most reef-fish larvae (Leis 1991). As a result, larvae occupying different depths may be experiencing different current speeds and direction, such that dispersal distances and direction may vary widely both within and between species.

3. The currents affecting reef fish larvae are only partially described by the major current patterns: meso- and microscale currents might be of importance. Reef-fish eggs or larvae are first released into the water within or very near the reef. The first currents experienced by the larvae (or pelagic eggs) are complex, and strongly influenced by the topography of the individual reef (Hamner and Wolanski 1988; Wolanski and Hamner 1988). They include very local gyres and tidal fronts that may confine larvae, depending on larval location (Kingsford et al. 1991). Once escaping the influence of the reefs, larvae then enter coastal currents, often tidal in nature, whose patterns are not well-described by the major current tracks. These local, secondary currents may be moving in the same or different directions than the larger currents. Thus, larvae of the same species may move in different directions depending on whether they remain within local, reef or island/shore influenced currents, or enter the major currents.

Our results clearly indicate that present-day current patterns have not produced evolutionary significant differentiation between regions separated by different major currents. Uncovering more subtle influences of currents on the demographic relationships of populations will require genetic studies that compare larger numbers of individuals across populations or direct studies of larval dispersal. However, our study would suggest that the low levels of population subdivision that may be found, though of potential ecological importance, are relatively insignificant in an evolutionary context.

Life History

A major focus of this study was to determine the effects of two early life-history attributes on the genetic architectures of coral reef fishes. The species included in this study were chosen specifically because they varied with respect to egg type (nonpelagic vs. pelagic) and length of pelagic (usually larval) life. Despite this variation in life histories, all eight species were similar in having extremely widespread distributions of the most common haplotype; this pattern provides strong evidence that all the species were experiencing significant gene flow among the Caribbean locales. If larvae behave as passive particles, the potential dispersal distances are large: with a minimum pelagic duration of 15d and a very conservative estimate of average current speed as 1 km/h (fig. 1), a larva traveling in the major Caribbean currents would be carried at least 360 km. Given the "stepping stone" geography of the Caribbean and a total distance along a current track of 4500 km, it could take as few as 13 generations for a novel haplotype to spread throughout the Caribbean. Despite this potential for high dispersal, we found significant population subdivision for *S. leucostictus*, *G. thompsoni*, and *H. bivittatus*, though between population variance accounted for only 8%–17% of the total variance. In these species it appears that gene flow, though extensive, has been restricted enough to allow some genetic differences to develop between populations.

Can we explain the differences among species in population structure by variation in egg type? The passivity of eggs relative to larvae might make egg type an important predictor of dispersal distances. Of the eight species we studied, two (*S. leucostictus*; *G. thompsoni*) out of the four species with nonpelagic eggs and one (*H. bivittatus*) of the four with pelagic eggs showed evidence of population subdivision. These mixed findings negate the hypothesis that only species with nonpelagic eggs show genetic differentiation over the spatial scale and geographic layout of the Caribbean. However, our results do not eliminate the possibility that nonpelagic-egg species, on average, have more restricted dispersal abilities and greater genetic differentiation than pelagic-egg species. Some support for the above hypothesis has come from studies that have examined the relationship between reproductive mode and geographic distributions, under the assumption that distribution is correlated with dispersal ability (Rosenblatt 1963; Thresher 1991).

We also designed this study to investigate the relationship between genetic structure and pelagic life span, with the hypothesis that dispersal distance would be correlated with pelagic duration. Other researchers have examined the relationship between dispersal and length of pelagic life by examining biogeographic distributions of species within a family. Studies on Pacific angelfishes (Thresher and Brothers 1985), damselfishes (Thresher et al. 1989; Wellington and Victor 1989), and wrasses (Victor 1986) have uncovered few relationships between these variables (Victor 1991). The problem may be methodological. No study has yet employed a comparative method incorporating genealogical information (Felsenstein 1985; Harvey and Pagel 1991); such a method might reveal the pelagic duration-dispersal relationship

by mapping the very complicated, present-day distributional data onto a phylogenetic framework.

In our study, the three species showing population subdivision nearly spanned the range in length of planktonic life demonstrated by the eight study species. In fact, *G. thompsoni*, the species in our study with the longest pelagic life (mean = 82 d) showed strong evidence for mtDNA differentiation, as did *S. leucostictus* and *H. bivittatus* with mean pelagic durations of 20–29 d and 24 d, respectively. If the otolith data are correctly measuring pelagic duration, then, clearly, length of pelagic life is not a simple predictor of movement between disjunct, benthic populations of coral-reef fishes and other traits that differ between species, such as adult spawning or larval behaviors, must strongly influence dispersal distances.

One important larval behavior is vertical positioning within the water column; due to variations in current speed with depth, larvae at different depths will be carried different distances and possibly directions. Vertical distribution surveys of reef-fish larvae have shown that daytime depth distributions appear to be species-specific, and generally similar between related species (Leis 1991). If vertical distribution has a confounding effect on the relationship between larval duration and dispersal, then it would be worthwhile to compare related species that might be expected to have similar depth distributions. The same argument can be made for other taxon-specific behaviors that might influence dispersal.

In our study, we included two members each of the families Pomacentridae (*S. leucostictus* and *A. saxatilis*) and Labridae (*H. bivittatus* and *T. bifasciatum*). *S. leucostictus* and *A. saxatilis* share several reproductive characteristics [nonpelagic eggs; semilunar spawning cycles (Robertson et al. 1990)] but differ in the duration of pelagic life. Mean length of pelagic life in *S. leucostictus* is 20–29 d; *A. saxatilis* has a mean pelagic larval duration of only 18–27 d, but pelagic life can extend to at least 55 d through association with floating algae (table 1). The two labrids are similar to one another in spawning patterns: both have pelagic eggs and spawn year-around (Robertson and Hoffman 1977; Warner and Robertson 1978). The two species differ in length of larval life, with a mean pelagic duration of 24 d for *H. bivittatus* and 49 d for *T. bifasciatum*. In each of the within-family comparisons, the species with the shorter pelagic duration showed significant mtDNA differences between populations while the species with the longer pelagic duration did not. These comparisons, though severely limited in number, suggest that the length of the pelagic life might influence dispersal distance and consequent gene flow, but that its effects are confounded by the influence of other behaviors.

Comparisons with Other Taxa

Coral-reef fishes share a similar range of life-history features with coastal marine invertebrates and fishes. High levels of gene flow, such as we have found in the Caribbean reef-fishes, have also been observed in the majority of allozyme studies on benthic invertebrates and fishes with long-lived pelagic larvae (e.g., gastropods, Gooch et al. 1972; bivalves, Levinton and Suchanek 1978; sea urchins, Britten et al. 1978) and coastal marine fishes (Rosenblatt and Waples 1986; Wa-

ples 1987). Of particular relevance is a study showing that the Queen conch (*Strombus gigas*), with a pelagic larval duration of 12–35 d, had high levels of gene flow throughout the Caribbean (Mitton et al. 1989). Recent studies of geographic patterns in mtDNA have also demonstrated extensive gene flow in marine animals with long-lived planktonic larvae or juvenile stages (e.g., sea urchins; Palumbi and Wilson 1990; Palumbi and Kessing 1991; McMillan et al. 1992; and catadromous American eels, Avise et al. 1986).

Counterexamples are provided by studies of blue mussels, *Mytilus edulis*, and American oysters, *Crassostrea virginica*, both of which have fairly long-lived pelagic larvae. In blue mussels significant allozyme differentiation between populations has resulted from very strong selection gradients acting on genetically uniform larval recruits (Koehn et al. 1976, 1980). Phylogeographic structure of mtDNA haplotypes in the American oyster, on the other hand, has been attributed to historical factors (Reeb and Avise 1990). Phylogeographic differentiation in species with long-lived larvae might be anticipated when populations have been separated by oceanographic “barriers” of unsuitable planktonic conditions: inappropriate water temperatures and/or salinity or inadequate food supply for the larvae.

Geographic differentiation in mtDNA has also been found in species in which the planktonic larval stage is presumed short or absent (e.g., horseshoe crab, Saunders et al. 1986). The Australian sea urchin, *Heliocidaris erythrogramma* (McMillan et al. 1992), which has no planktonic feeding stage, showed a large phylogenetic break between western coast and eastern/southeastern coast populations. These sea urchins also showed significant population differentiation, but no phylogeographic structure, over smaller geographic scales. This pattern is similar to that observed for three of the reef fish species described in our study.

There are only a few studies on coastal invertebrates and fishes that have directly compared species with different life-history characteristics that would presumably be correlated with dispersal ability. In the intertidal gastropod genus *Littorina*, species with planktonic larvae have, on average, about one-third the genetic differentiation found in species lacking a planktonic larval stage (Berger 1973; Janson and Ward 1984; Ward 1989). A qualitatively similar result was found for two temperate sea urchins in the genus *Heliocidaris* (see above), which vary with regard to planktonic feeding ability (McMillan et al. 1992). A comparative study of ten species of fishes on the west coast of North America found that, though there was considerable gene flow among all populations studied, differences in allozyme frequencies were inversely correlated with rankings of presumed dispersal abilities (Waples 1987).

Concluding Remarks

What are the implications of high gene flow in many marine organisms with a dispersive pelagic life-phase? Coral reefs around the Caribbean are highly variable in topography, tidal regimes, local currents, and relative abundance of community components. Thus, we might not expect to see fine-tuned adaptations to these locally varying conditions. Instead, we expect selection to be “averaging” over the environmental

conditions of the entire Caribbean, and/or to be favoring responses to cues, such as tidal currents, that produce adaptive responses in each locality.

The amount of gene flow, though considerable in all species, is clearly not uniform across reef fishes or benthic invertebrates with dispersive pelagic stages. Our results suggest that length of pelagic life contributes to these differences but is strongly confounded by other traits. 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.

Finally, however, we must reconcile the apparent paradox of extensive gene flow among coral-reef fishes with their striking species richness. Perhaps differentiation of demes at a species margins (Bush 1975) can explain why many highly dispersed species belong to species-rich clades in which speciation must, at times, have been frequent. An interesting and somewhat heterodox view suggests that speciation in marine animals might frequently result from incompatible gamete interactions between partially isolated populations (Palumbi 1992). Possibly, patterns of marine species richness can be explained by orthodox models of allopatric speciation, suggesting that present-day sea levels, currents, and patterns of gene flow may not be representative of the past marine environments in which much of the species richness observed today developed (Vermeij 1978). Thus, speciation in the marine realm may ebb and flow with high and low sea level, respectively.

ACKNOWLEDGMENTS

We thank J. Morin, O. McMillan, D. Goulet, and A. Cohen for their help in obtaining and processing samples, P. Morin and L. Beal for their assistance with the laboratory analyses, and L. Excoffier and S. Palumbi for providing computer programs for data analyses. T. Tostafson and D. Shapiro graciously made their laboratories at La Parquera Marine Laboratory, University of Puerto Rico available to us. E. Brothers generously provided his unpublished otolith-aging data. We gratefully acknowledge Recursos Marinos and the Kuna people for their support of our research in Panama. We thank R. Emler, N. Knowlton, H. Lessios, J. Morin, S. Palumbi, D. Buth, and D. R. Robertson for comments on the research and manuscript. M.J.S. was partially supported by a University of California President's Fellowship. Our research was supported by NSF (BSR-8607403), the Smithsonian Molecular Evolution program, and the National Geographic Society.

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Corresponding Editor: W. Eanes