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INCIPIENT SPECIATION ACROSS A DEPTH GRADIENT IN A SCLERACTINIAN CORAL?

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Abstract.—A few marine cases have demonstrated morphological and genetic divergence in the absence of spatial barriers to gene flow, suggesting that the initial phase of speciation is possible without geographic isolation. In the Bocas del Toro Archipelago of the Atlantic Coast of Panama, we found two morphotypes of the scleractinian coral *Favia fragum* with opposing depth distributions. One morphotype fit the classical description of *F. fragum* and was most abundant at 3 m depth. A second morphotype was distinguished by raised corallites and was restricted to ≤ 1 m depth. The two morphotypes overlapped in distribution at 1 m depth. Multivariate analysis of polyp-level characters (shape and distribution of septa within corallites) divided samples into two groups corresponding to initial qualitative observations of colony shape and corallite relief. To determine whether reduced gene flow maintains morphological variation, we measured the frequencies of alleles at five allozyme loci in both morphotypes at three sites 1–2 km distant. While there were significant differences in allele frequencies between morphotypes within sites, there were also frequency differences among sites at most loci, with the exception of nearly fixed alleles at the *PGM* locus. Extremely low heterozygosity permitted us to use haplotypes to compare genetic distance between morphotypes and among sites. Comparisons between haplotype data and a null model assuming gene flow between morphotypes showed that the two morphotypes shared significantly fewer haplotypes than expected, and average genetic distance between morphotypes was significantly greater than expected. Partitioning haplotype variation with analysis of molecular variance demonstrated that 35% of the variation was explained by morphotype, whereas 28% of the variation was explained by site. Two *PGM* heterozygotes and several individuals homozygous for rare *PGM* alleles are consistent with hybridization, and perhaps introgression by selfing within morphotypes. We consider three hypotheses for this morphological and genetic divergence in *F. fragum*: (1) intraspecific polymorphism, (2) incipient species, (3) biological species; and discuss the role of reproductive characters in a divergence-with-gene flow mechanism of speciation.

Key words.—Assortative mating, inbreeding, morphometrics, parapatric, philopatry, speciation, sympatric.

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How common is speciation without geographic isolation? The crux of this question rests on whether genetic differences can accumulate between populations in the presence of gene flow. The ''divergence-with-gene flow'' model of speciation (Rice and Hostert 1993) includes a continuum of geographic modes of speciation (Endler 1977). At one end of the continuum is parapatric speciation. In this mode an ancestral species expands in range over a spatially heterogeneous area, populations adapt to local environmental conditions, but gene flow occurs between the contiguous borders of populations. At the other end of the continuum is sympatric speciation. Here, an ancestral species does not increase in range and divergence occurs by ecological or temporal partitioning of habitat. Since geographic ranges completely overlap each other in sympatric speciation, gene flow potentially occurs throughout the range. Geography has played an important role in models of speciation because geography will determine the magnitude of potential gene flow between candidates for new species.

Initial theoretical studies showed that speciation via divergence-with-gene flow is unlikely. Felsenstein's influential model (Felsenstein 1981) illustrates the salient points. In this model two loci control fitness in two habitats and a third locus controls behavior so that individuals that share the same behavior genotype are more likely to mate than those that do not. Natural selection favors genotypes that have high fitness in each habitat and mate assortatively, leading to linkage disequilibrium between the fitness loci and the assortative mating loci. However, recombination between fitness loci and the assortative mating locus breaks down favorable allelic combinations and the resulting linkage equilibrium opposes the evolution of reproduction isolation. According to this, and similar models, the evolution of assortative mating in the face of gene flow is restricted to special cases, such as when fitness loci and assortative mating loci do not recombine. However, the restrictions of recombination are sidestepped when strong disruptive selection has pleiotropic effects on assortative mating (Slatkin 1982; Rice 1984, 1987; Diekman and Doebeli 1999). For example, disruptive selection on a trait that influences fitness on different hosts, habitats, or at different points along an environmental gradient, can automatically lead to assortative mating when dispersal is philopatric. Pleiotropic effects of selection can explain assortative mating and genetic divergence in one of the strongest cases of sympatric speciation: host-race speciation in the apple maggot fly, *Rhagoletis* (Feder 1998). In this case, mating is more likely to occur on the natal host plant than on non-natal plants (Feder et al. 1994). Other pleiotropic mechanisms that do not rely on philopatry may also lead to assortative mating. For example, a recent monophyletic radiation of *Tilapia* cichlids in a tiny African lake has resulted in two different-sized morphotypes with adaptations to benthic and planktonic habitats. These two morphotypes overlap in depth distribution, but mate assortatively within their respective size categories (Schliewen et al. 2001). The first

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stage of speciation has been documented in an increasing number of sympatric populations that combine strong disruptive selection and pleiotropic mechanisms of assortative mating (Via 2001).

In contrast to the study of speciation on land, the divergence-with-gene flow model has received less attention in marine environments. It is indeed common for benthic marine invertebrates and fish to have a planktonic larval phase that can last for weeks (Sale 1980; Strathmann 1985). Not surprisingly, there is typically little genetic structure among populations of marine organisms with broadly dispersing planktonic larvae at the scale of tens to hundreds of kilometers (Palumbi 1992; Grosberg and Cunningham 2001). Given these population structures the diversifying effect of natural selection must be extremely strong to overcome the homogenizing effects of high gene flow among populations (Rice and Hostert 1993). Nonetheless, there are at least three cases of morphological and behavioral divergence without geographic isolation in the sea. In the best-studied case, the rocky intertidal snail *Littorina saxatallis* has diverged in shell characters over a gradient of tidal height (Johannesson et al. 1993, 1995). Assortative mating is driven by aggregation of like morphotypes within microhabitats (Rolan-Alvarez et al. 1997) and females rejecting nonsimilar morphotypes (sexual selection, Rolan-Alvarez et al. 1999). A second example comes from a phylogeographic analysis of morphological variation in *Littorina subrotundrata* along the west coast of North America (Kyle and Boulding 1998). Two ecotypes are found in salt marsh and rocky intertidal habitats, and the characters that distinguish the two ecotypes are heritable. However, the majority of genetic differentiation in this case occurs among geographic locations, rather than between ecotypes. This appears to be an example of repeated and directed morphological divergence in independent lineages: termed ''parallel evolution'' when morphotypes interbreed and ''parallel speciation'' if the same mechanism of reproductive isolation evolves in each lineage (Schluter and Nagel 1995). Finally, Duffy (1996) found genetic and behavioral divergence in snapping shrimp (*Synalpheus*) that occupy different hosts (sponges) that exist sympatrically. The first stage of speciation has occurred in all these marine examples without any obvious barriers to gene flow.

Are these examples unique? These cases share two conditions that make divergence-with-gene flow likely. First, in contrast to the long-lived planktonic larvae of many marine organisms, development is direct and juvenile dispersal is either known (snails) or inferred (shrimp) to be limited to within meters of birth. Second, these species are distributed across ecological landscapes with the potential for strong gradients or ecotones of selection (for a snail example, see Rolan-Alvarez et al. 1997). The consequence of divergent selection is ecological segregation of populations within the landscape, which in turn increases assortative mating when dispersal is philopatric. These two conditions are likely to be met in other taxa, and in other marine environments as well. All of the major marine phyla have representatives that either lack a larval phase (development occurs within the parent or capsule) or have larvae that spend a brief time in the water column before settlement (Knowlton and Jackson 1993). Moreover, intertidal and subtidal marine environments are renowned for their intensity and diversity of physical and biological gradients (see part II of Bertness et al. 2001). The fact that many marine populations meet these two conditions of the divergence-with-gene flow model (i.e., strong philopatry and distributions over environmental gradients) suggests that speciation without geographical isolation may be more common in marine environments than presently appreciated.

In tropical environments throughout the world, the coral family Faviidae has the second largest number of species (Veron and Stafford-Smith 2000). Within the Faviidae, the genus *Favia* contains an estimated 30 species worldwide (Veron 1995) and has the longest fossil record (Wells 1956). The majority of species are found in the western Pacific, with only three extant species in the tropical Atlantic. *Favia leptophylla* is endemic to Brazil and *F. gravida* is found in Brazil and western Africa (Laborel 1967, 1974). The third species, *F. fragum* (Esper), exists in Bermuda, the Bahamas, Florida, and throughout the Caribbean Sea (Glynn 1973). A species of similar morphology has been described from the Cape Verde Islands of western Africa (Laborel 1974). Compilations of stratigraphic data (Budd et al. 1994a; Johnson 1998; Budd and Johnson 1999a,b) show a total of nine recorded *Favia* species in the Caribbean since the middle Eocene (~50 million years ago); three of these species persisted until the end of the Miocene (5.3 million years ago) and a fourth became extinct in the earliest Pleistocene (~1.8 million years ago). *Favia fragum* is known from latest early Pliocene deposits dated as 3–3.5 million years old.

Favia fragum is found in a variety of intertidal and shallow subtidal (generally <5-m depth) environments. It exists in intertidal splash pools, on unconsolidated rubble in turtle grass stands, and on consolidated reef crests and forereef slopes (D. Carlon, pers. obs.). This species is a simultaneous hermaphrodite capable of self-fertilization (Brazeau et al. 1998; Carlon 2002). Fertilization is internal, and embryos are brooded within the parent for an approximately two-week interval (Szmant-Froelich et al. 1985). Larvae are released over several days beginning 7–10 days before the full moon of each month (Szmant-Froelich et al. 1985; Soong 1991). Larvae swim toward the bottom when released by divers (Carlon and Olson 1993) and may settle and metamorphose immediately after release if presented with a suitable cue (Duerden 1902; Lewis 1974).

In this paper we examine the ecological, morphological, and genetic boundaries of two morphotypes of *Favia fragum* in Panama. We found discrete differences in polyp-level characters that defined two morphotypes, which were found at opposite ends of the depth gradient. Using allelic information in protein allozymes, we show repeated patterns of genetic divergence among three sites. We consider three scenarios that could explain these patterns: (1) intraspecific polymorphism with no reproductive barriers between morphotypes, (2) partial reproductive isolation between two incipient species, and (3) two biological species (sensu Mayr 1963) with incomplete lineage sorting. Our data are consistent with either partial reproductive isolation between morphotypes, or complete reproductive isolation and a recent divergence. The ecological distribution and reproductive characters of *Favia fragum* are consistent with the divergence-with-gene flow model.

Materials and Methods

Study Location and Morphotype Descriptions

We conducted fieldwork at the Smithsonian Tropical Research Institute's (STRI) Bocas del Toro Field Station on the Atlantic Coast of Panama. The reefs and surrounding habitats have been described in detail (Guzman and Guevara 1998a,b, 1999). On the protected side (Bahia Almirante) of the Bocas del Toro Archipelago, there is reduced circulation and high runoff from coastal rain forests, which have strong effects on the subtidal environment. In shallow habitats, rapid changes in water clarity, salinity, and temperature are common during the rainy season (April–December). The shallow habitats on the protected side of islands are characterized by small patch reefs of the scleractinians *Porites furcata* and *Agaricia tenuifolia* amid unconsolidated coral rubble and turtle grass (*Thallassia testudinum*). Initial trips to Bocas del Toro yielded what appeared to be two discrete morphotypes of *Favia fragum* in the Bahia Almirante. The first morphotype (Morph 1) fits the original description of *Favia fragum* (Esper): colonies are small (<5 cm diameter), morphology is massive, and individual corallites rarely protrude a few millimeters above the colony surface (Fig. 1A). The second morphotype (Morph 2) is smaller in size (<3 cm), colonies are typically hemispherical (a few encrusting colonies were also found), and corallites extend outward from the colony surface, creating valleys between the corallites > 3 mm deep (Fig. 1B). In subsequent trips, we made additional observations on the broader distribution of the two morphotypes. To determine if both morphotypes existed in both protected and exposed localities, we searched shallow habitat in two additional protected localities: Hospital Bight (32, 34), and Isla Solarte (35, 36); and three exposed localities: Bastimentos (42), Isla Popa (45), and Cayos Zapatillos (30, 31). Numbers in parentheses correspond to sites surveyed by Guzman and Guevara (1998a,b, 1999).

Depth Distribution

To quantify the vertical distribution of Morphs 1 and 2, we counted the number of each morphotype in 1-m^2 quadrats at four depths (0.5, 1.0, 3.0, and 5.0 m depth) at two sites (Sites 2 and 3, Fig. 2) along the Isla Colon. At each site, we counted corals in 22 quadrats at each depth by laying out a 60-m line transect and positioning quadrats at 2-m intervals over the tape. Exposed and vertical surfaces were searched thoroughly for the two morphotypes. Colonies ≥ 1 cm in diameter could easily be identified and counted. At each site, we analyzed abundance data with a two-factor ANOVA, with morph and depth as fixed factors. Our expectation was that the pattern of abundance with depth would change for each morphotype. A significant interaction between morph and depth would support this expectation. Data were transformed ($x' = \log [x + 1]$) to meet the assumption of homogeneity of variance among levels. We used Systat (Systat Software 1998) for these analyses.

Morphometrics

To test whether morphological variation was continuous or discrete, we conducted a morphometric analysis of corallites, a collection of skeletal structures that define individual polyps within the coral colony. Each corallite consists of a tube (corallite wall) with vertical plates that radiate inward and outward from the corallite wall (Fig. 3). These vertical plates are termed septa inside the corallite wall, and costa outside the corallite wall. A complete vertical plate is termed a costosepta. Measurements were related to the height of corallites from the colony surface (calical relief) and the development of costosepta. Measurements were made from analyzing 3-D landmark data on corallite surfaces using a Reflex (Somerset, England) microscope. We obtained Cartesian coordinates (x-y-z) for 20 landmarks (Table 1) along three adjacent costosepta of six mature corallites from each of 66 colonies. We randomly chose 10–11 colonies of each morphotype per site from the total sample used in electrophoresis (see Electrophoresis and Genetic Analyses, below). In addition, we included two individuals that were heterozygous

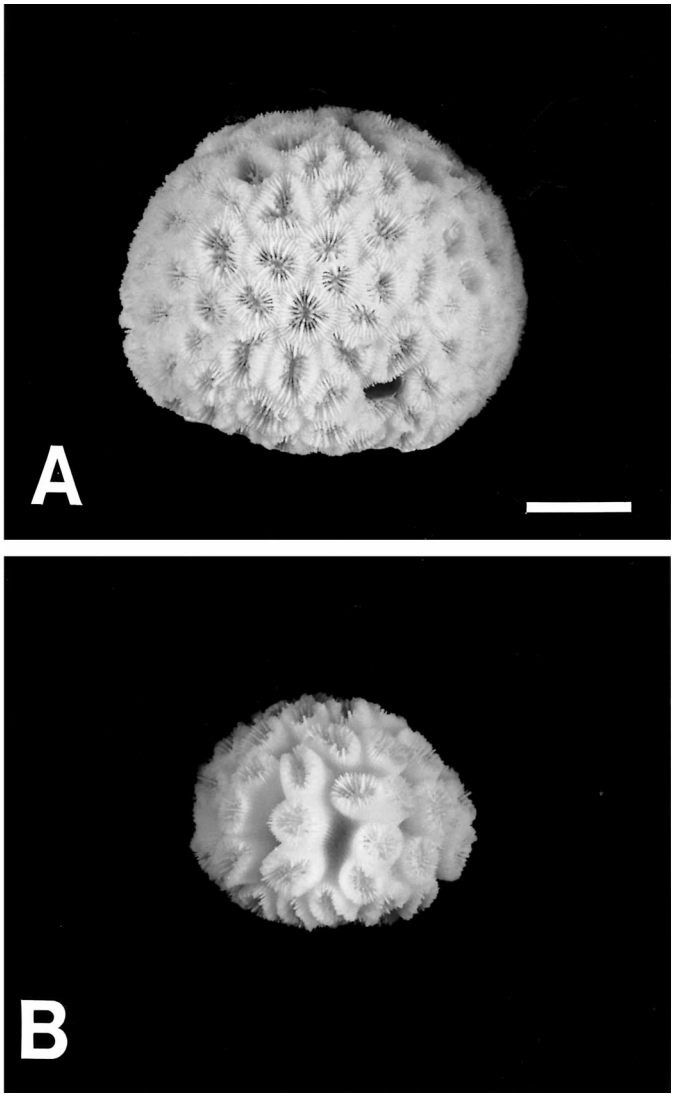

FIG. 1. Photographs of dried skeletons of the two *Favia* morphotypes from the Bocas del Toro, Panama. Scale bar is 1 cm. (A) *Favia fragum* (Morph 1). (B) Undescribed *Favia* morphotype (Morph 2).

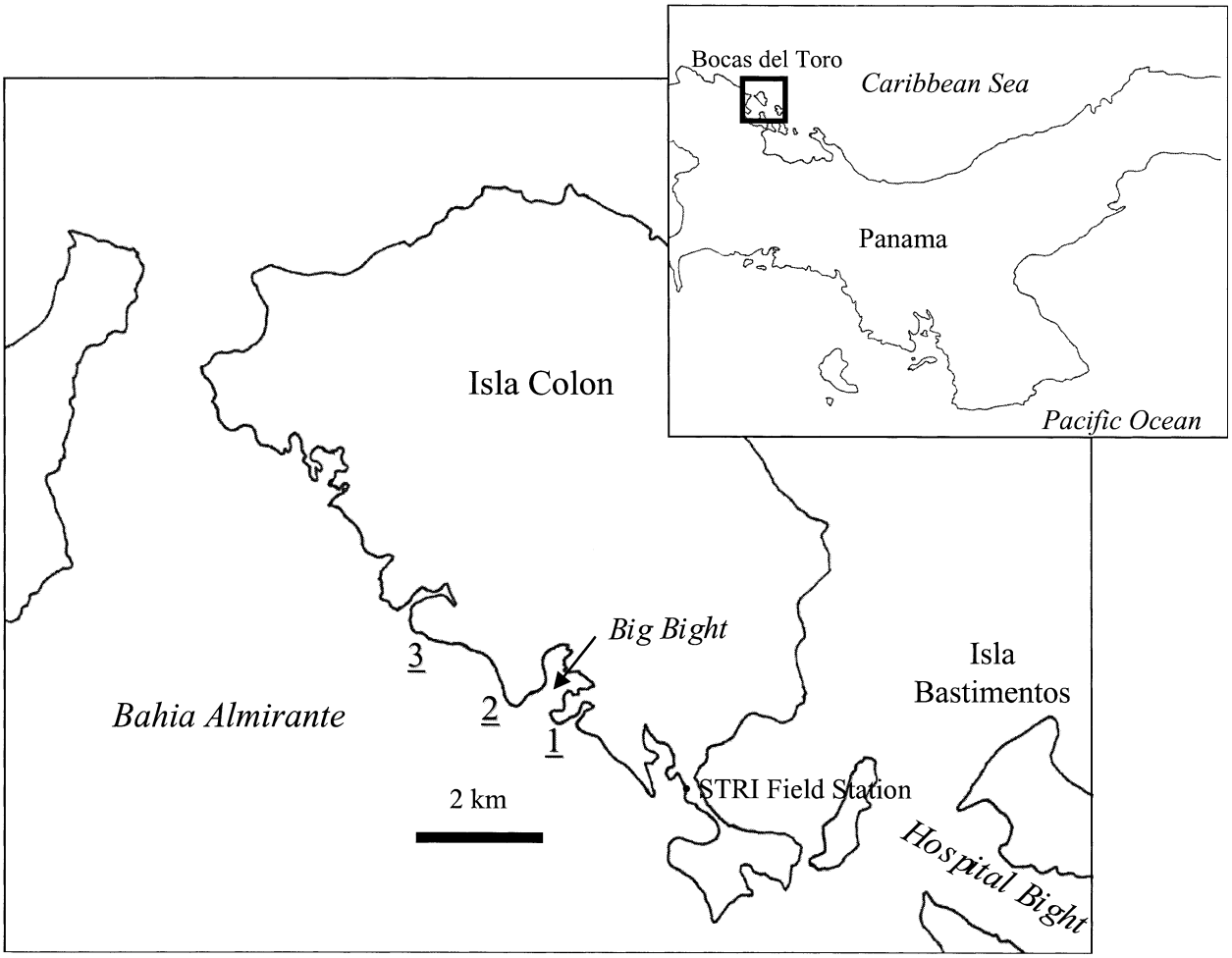

FIG. 2. Inset: location of the Bocas del Toro region on the Atlantic side of Panama. Larger map shows the location of study sites (indicated by numbers) along the Isla Colon.

at the *PGM* locus to determine if the morphological characters of these putative hybrids were intermediate from the ''pure'' morphotypes. The landmarks consist of spatially homologous points designed to reflect the shape of the septal margin (the uppermost growing edge) and costal extensions between corallites. Size and shape coordinates (Bookstein 1991) were determined using the computer program GRF-ND (Generalized rotational fitting of n-dimensional landmark data, 1994, written by Dennis E. Slice available at http://life.bio.sunysb.edu/morph/). Centroid size was calculated in three dimensions by summing the squared distances from each of the 20 landmarks to a common centroid. Shape coordinates were calculated by rotating and transforming each landmark relative to a plane defined by points 5 (0, 0, 0); 18 (1, 0, 0); and 11 (z = 0). The resulting x-y-z coordinates for 17 points (all except points 5, 18, 11); and x-y coordinates for point 11, termed ''shape coordinates,'' were used as variables in subsequent statistical analyses (Budd et al. 1994b; Budd and Johnson 1996). In general, x-values are related to spacing between septa, y-values are related to costal and septal elevation, and z-values are related to costal and septal lengths. Preliminary statistical analyses based on qualitative field identifications of the two morphotypes showed that x-values did not differ between morphotypes. We therefore performed cluster analysis using a total of 28 variables consisting of y-values for 16 points (all except points 6 and 17), z-values for 11 points (all except points 6, 7, 9, 11, 12, 17, 19), and centroid size. Size and shape coordinates were used to calculate Mahalanobis distances among colonies, which were input into an average linkage cluster analysis. As in Budd et al. (1994b) and Budd and Johnson (1996), we used Mahalanobis distances instead of the more commonly used squared Euclidean distances, in an effort to maximize between-group relative to within-group variation (Klecka 1980). Support for nodes on the resulting dendrogram was estimated using cross-validation classification percentages determined by canonical discriminant analysis. Multivariate differences between the two morphotypes were evaluated using canonical discriminant analysis; univariate differences are evaluated using nonparametric tests. All statistical analyses were performed using SPSS (SPSS Science 1997).

Electrophoresis and Genetic Analyses

To determine whether there were genetic differences between morphotypes, we sampled three sites along Isla Colon

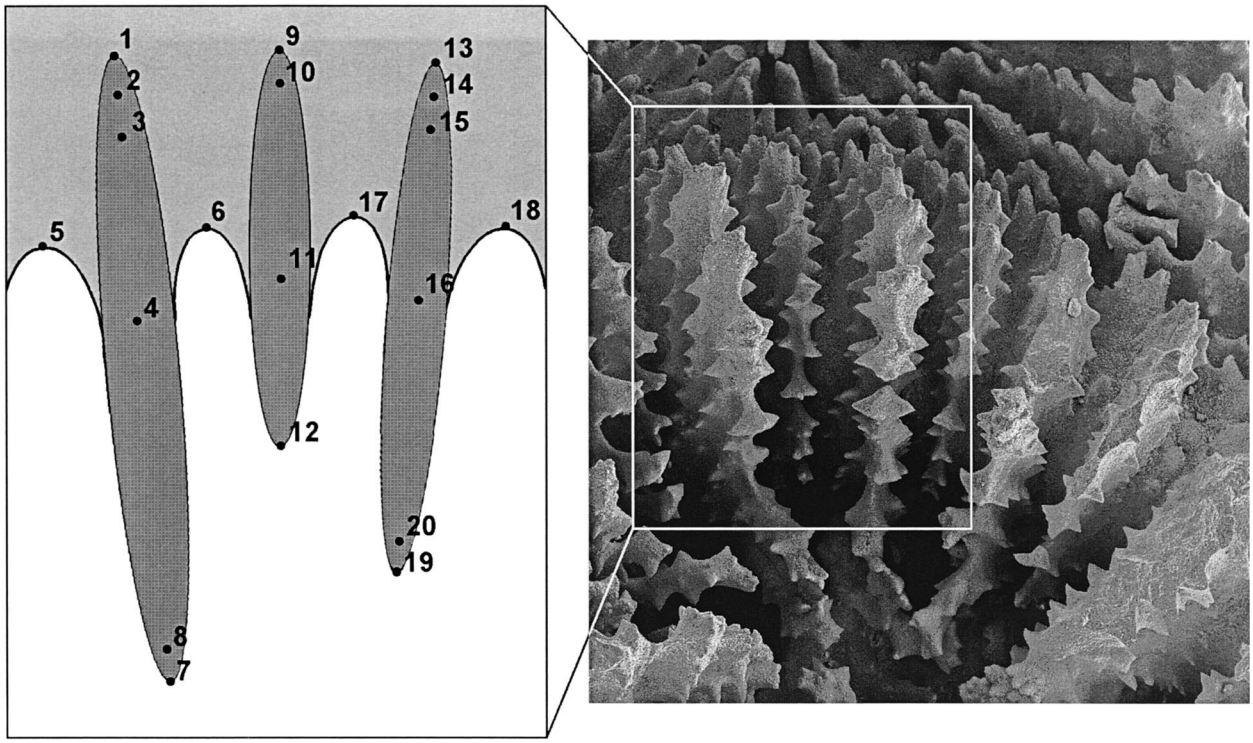

FIG. 3. Scanning electron micrograph of costosepta within a corallite and the location (inset) of three-dimensional Cartesian coordinates collected for 20 landmarks along costosepta. Photograph shows a side view of the inner edges (septa) of costosepta from the corallite center. The inset depicts a two-dimensional representation of the costosepta in a top view of the corallite surface. Landmarks 1–7 and 13–19 are located on major, and 9–12 are located on minor, septa. Landmarks 5, 6, 17, and 18 are located on the inner edge of the corallite wall. Shape coordinates were calculated by rotating and transforming each landmark relative to a plane defined by points 5 (0, 0, 0), 18 (1, 0, 0), and 11 ($z = 0$). These landmarks were selected to characterize the shape of the septal margin (the uppermost growing edge) and the development of costal extensions between calices.

in 1998 (Fig. 2). To confirm genetic patterns obtained from the Isla Colon sites, we collected additional samples of Morph 2 from Hospital Bight in 1999. Morphotypes were collected throughout their respective distributions between the shallowest portions of the reef (0.25–0.5 m depth) near the edge of mangrove stands down to 5 m depth. At the STRI field station, tissue samples were extracted from colonies by inserting an X-Acto (Hunt Corporation, Statesville, NC) hobby knife into a single corallite and removing a cone-shaped plug of tissue. This procedure was repeated for 5–10 polyps, or half of the colony, depending on colony size. Tissue samples were placed in 0.5 ml ependorf tubes with a drop of grinding buffer (Stoddart 1983) and immediately snap frozen in liquid nitrogen. Samples were transported in liquid nitrogen or on dry ice to Los Angeles, and stored at −80°C until electrophoresis.

We prepared samples for horizontal starch gel electrophoresis by adding an additional drop of grinding buffer and sonicating the sample on ice for 5 sec. Sonicated samples were centrifuged for 30 sec at 10,000 rpm, and a paper wick (no. 2 Whatman, Ann Arbor, MI) was soaked in the supernatant. Wicks were loaded onto 13% starch gels (cat. no. S-4501, Sigma, St. Louis, MO). From a screening of *Favia fragum* from the San Blas Islands of Panama, 20 enzyme systems had good expression, and seven were polymorphic (D. Carlon, unpubl. data). Five of these enzyme systems, representing five putative enzyme-encoding loci, could be reliably scored. These same loci were scored in samples from Bocas del Toro: aspartate aminotransferase (*AAT,* EC 2.6.1.1), leucyl-valine-peptidase (*LVP-2,* EC 3.4.11/13, substrate leucyl-valine, Sigma L-1377), mannose-6-phosphate isomerase (*MPI,* EC 5.3.1.8), phosphoglucomutase (*PGM,* EC 5.4.2.2), and triose-phosphate isomerase (*TPI,* EC 5.3.1.1). We ran *AAT* and *MPI* on the tris-citrate II buffer; and *TPI* and *LVP* on the lithium-borate/tris-citrate buffer described in Murphy et al. (1996). We ran *PGM* on a SAC/TC tris-citrate buffer (Waycott and Sampson 1997). Stain recipes follow Murphy et al. (1996) with two exceptions. For *TPI* we used the substrate dihydroxyacetone phosphate (lithium salt), Sigma D-7137. The *AAT* stain recipe was as follows: 80 mg α-Ketoglutaric acid, 270 mg l-aspartic acid, 1.4 g sodium phosphate (dibasic), 1 g of polyvinylpyrrolidone, and 100 mg EDTA were added to 100 ml of deionized water and mixed thoroughly. The gel slice was incubated in this solution at 37°C for 20 min. Following incubation, 200 mg of Fast Garnet GBC (salt; cat. no. F8761, Sigma) was added and mixed with the gel/substrate. We typed a total of 136 individuals of Morph 1 and 167 individuals of Morph 2.

To eliminate the possibility that enzyme expression was due to the genome of symbiotic zooxanthellae rather than that of the animal, we ran zooxanthellae control lanes with coral samples for each enzyme-encoding locus. Zooxanthellae were *Symbiodinium microadriaticum* isolated from *Cassiopeia xamachana* (LB 2282, UTEX, Culture Collection of

TABLE 1. Landmarks on corallites of *Favia*. Numbers correspond to Figure 3. Types (x-y) are: 1, juxtaposition of structures; 2, maximum of curvature; 3, external points. In the z-, all are maximum of curvature.

Number	Type (x-y plane)	Description
1	3	Outermost point on left major costa (low)
2	3	Outermost point on left major costa (high)
3	1	Outer junction of left major costoseptum with wall (high)
4	1	Inner junction of left major costoseptum with wall (high)
5	2	Point of maximum wall curvature between septum left of left major septum (low)
6	2	Point of maximum wall curvature between septum right of left major septum (low)
7	3	Inner margin of left major septum (low)
8	3	Inner margin of left major septum (high)
9	3	Outermost point on minor costa (low)
10	3	Outermost point on minor costa (high)
11	1	Outer junction of minor costoseptum with wall (high)
12	3	Inner margin of minor septum (high)
13	3	Outermost point on right major costa (low)
14	3	Outermost point on right major costa (high)
15	1	Outer junction of right major costoseptum with wall (high)
16	1	Inner junction of right major costoseptum with wall (high)
17	2	Point of maximum wall curvature between septum left of right major septum (low)
18	2	Point of maximum wall curvature between septum right of right major septum (low)
19	3	Inner margin of right major septum (low)
20	3	Inner margin of right major septum (high)

Algae, Department of Botany, University of Texas at Austin). Although this *Symbiodinium* isolate is only distantly related to those that form symbioses with *F. fragum*. (symbionts from *Cassopeia* are in RFLP clade A and symbionts from *F. fragum* are in clade B; Rowan and Powers 1991), the dinoflagelate proteins migrated at much different rates than those of the animal. Cultured algal cells were washed with deionized water, mixed with grinding buffer, and sonicated in the same way as coral tissue. In only one case (*TPI*) was any zooxanthellae expression detected, but dinoflagelate bands had much higher electrophoretic mobility than those of coral samples. Thus, we were confident that we were scoring polymorphisms from the animal, rather than symbiont, genome.

If gene flow is reduced between morphotypes, we expect significant genetic distance between morphotypes within sites at each locus. We tested this hypothesis at individual allozyme loci by comparing allele frequencies between morphotypes at each site. We used exact tests (Raymond and Rousset 1995) with the program Genepop (available via http://wbiomed.curtin.edu.au/genepop) to test for significant differences between allele frequencies. Since each comparison (five allozyme loci and three sites) was used to test the same hypothesis, we used the Bonferroni correction (Weir 1996) to adjust significance levels (α).

Inbreeding leads to deviations from single- and multi-locus Hardy-Weinberg expectations (Hartl and Clark 1997). At an individual locus, inbreeding will reduce heterozygosity, and among loci, will increase linkage disequilibrium. We had two interests in these effects. First, since *F. fragum* is a self-fertile hermaphrodite, we wanted to know potential rates of selfing in our Panamanian populations. To measure effects of inbreeding on heterozygosity, we calculated observed levels of heterozygosity, expected levels of heterozygosity, and Wright's fixation index (F) with the program Genetic Data Analysis (GDA; Lewis and Zaykin 2001). Significant reductions in observed heterozygosity compared to expected heterozygosity were tested with exact tests. For each morph and site combination there were five potential comparisons (e.g., five loci), and we adjusted α with the Bonferroni correction. We used F to estimate the amount of selfing within populations (S), with the model: $S = 2F/1 + F$. The value of S ranges from 0.0 in populations where offspring are all outcrossed to 1.0 in populations where all offspring are all self fertilized. This model assumes genotypic equilibrium and that no other microevolutionary forces are affecting heterozygosity. We used GDA to estimate linkage-disequilibria coefficients (D) and compare them to null expectations with exact tests. Significance levels were Bonferroni corrected for the total number of comparisons (12 for Morph 1 and nine for Morph 2). Second, associations among loci (linkage disequilibrium) have consequences on measures of genetic distance and our interpretation of genetic differences between the morphotypes. This means we could not use standard multilocus genetic distance measures (i.e., Nei's or Roger's distance metrics) because the loci were not independent. Instead, we took advantage of the low heterozygosity in the dataset and used the allelic identity of the gametic phase (haplotype) to compare genetic distances within and between morphotypes. Some individuals were excluded from this analysis because either haplotype could not be known with certainty (two individuals were doubly heterozygous) or genotype information was incomplete ($n = 26$). The remaining dataset for haplotype analyses consisted of 538 haplotypes. The dataset included two haplotypes each from 259 individuals that were homozygous at all loci, and two haplotypes each from 10 individuals that were heterozygous at one locus.

To determine genetic distance between morphotypes we constructed a distance matrix for the entire sample of haplotypes (Appendix). The distance between two haplotypes is equal to the total number of loci that do not share the same allele. Thus distances ranged from zero (same allele at all five loci) to a potential maximum of five (no shared alleles

at five loci). Genetic distance between haplotypes may evolve by mutation, which is assumed to be rare, or by recombination, which in selfing populations will depend on the frequency of outcrossing and its effects on heterozygosity. We cannot estimate probabilities of recombination between haplotypes because map distances among loci are unknown. However, given that most scleractinian corals have a haploid chromosome number of 14 (Heyward 1985; Kenyon 1997), it is likely that at least some of the allozyme loci are located on different chromosomes. To test whether the observed genetic distance between morphotypes was greater than expected with gene flow between the two morphotypes, we constructed a null model based on random sampling. The model consisted of two populations constructed by randomly sampling (with replacement) *n* haplotypes from the total sample, where $n = 252$ for Morph 1 and $n = 286$ for Morph 2. For each pair of populations we calculated four parameters: (1) the average genetic distance between haplotypes, (2) the number of shared haplotypes, (3) the number of unique haplotypes in Morph 1, and (4) the number of unique haplotypes in Morph 2. Sampling of pairs of populations was repeated 1000 times. We used Matlab (ver. 5.3, The Mathworks Inc., Natick, MA) for the randomization. Finally, to determine whether there was significant variation in haplotypes among sites, we conducted an analysis of molecular variation (AMOVA; Excoffier et al. 1992) with Arlequin software (Schneider et al. 2000) to partition haplotype variation between morphotypes and among sites. The significance of fixation indices was determined by permutation.

Results

Geographic and Depth Distribution of Morphotypes

We found both morphotypes in sympatry at two protected localities: Isla Colon and Hospital Bight, but only *Favia fragum* (Morph 1) at an additional protected locality: Isla Solarte, and all of the exposed localities: Bastimentos, Isla Popa, and Cayos Zapatillos.

At Isla Colon, we found differences in the distribution of the two morphotypes between 0.5 and 5.0 m of depth (Fig. 4). Morph 2 was restricted to the shallowest regions of both sites (1.0 m depth or less), whereas Morph 1 was rare at 0.5 m at both sites, but was found at higher densities at 3.0, and 5.0 m at Site 2. Two-way ANOVAs revealed a significant interaction between morph and depth at Site 2 ($F_{3,168} = 13.980$, $P < 0.001$) and Site 3 ($F_{3,168} = 49.296$, $P < 0.001$).

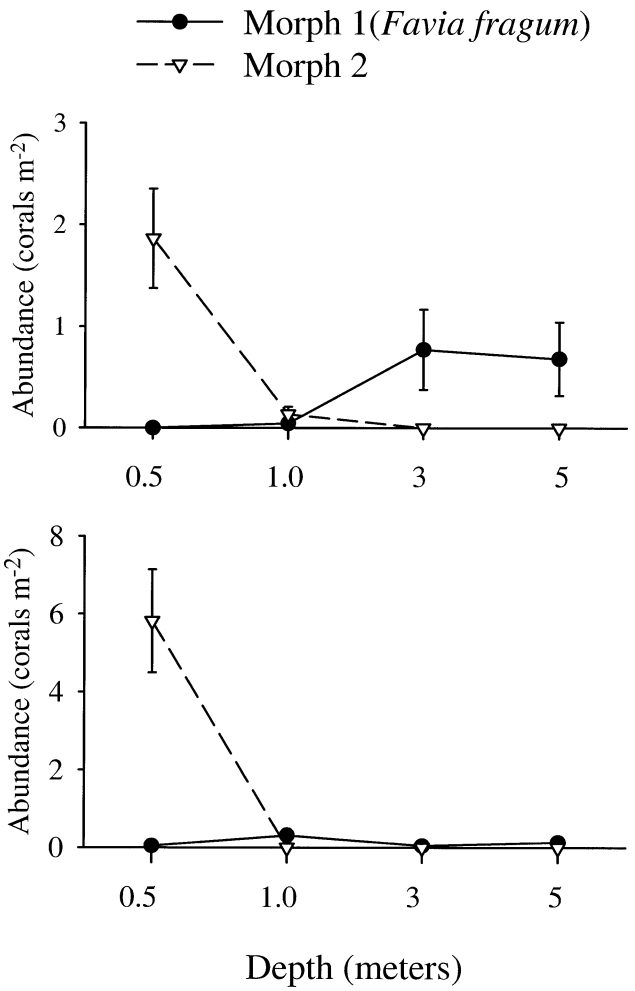

FIG. 4. Abundance of the two morphotypes with depth at two sites at Isla Colon. Error bars are 1 SE. Upper panel, Site 2; lower panel, Site 3.

Morphometrics

Multivariate analyses of skeletal features support the qualitative observations of the differences between the morphotypes. The cluster analysis results show that Morphs 1 and 2 form two distinct groups (Fig. 5), strongly supported by crossvalidation values (> 94% of the dendrograms grouped the two morphotypes separately). All colonies belong to the same group in the dendrogram as they did in a priori identifications. Discriminant analysis indicates that the most important variables distinguishing the morphotypes consist of morphological characters related to the elevation (Y1–3, Y13–15, Y9–10) and development of the costae (Z2, Z14, Z10), and depth of the calical pit (Y8, Y20). Morph 1 has significantly higher major (Y1–3, Y13–15) and minor (Y9–10) costae than Morph 2; however, differences in height between major and minor septa (Y4, Y16) are more pronounced in the latter (Table 1). Major (Z2, Z14) and minor (Z10) costae are longer in Morph 2, and the calical pit (Y8, Y20) is deeper.

Genetic Analyses

Comparison of allele frequencies between morphotypes within sites revealed 10 instances (of 12 possible tests) where there were significant differences in allele frequencies in coexisting morphotypes (Table 2). There was a nearly fixed difference at the *PGM* locus between the two morphotypes. Among sites, the PGM^{δ} allele was sampled at frequencies ≥ 0.980 in Morph 2, while the PGM^{β} allele was sampled at frequencies ≥ 0.870 in Morph 1. The six additional Morph 2 individuals from Hospital Bight (8 km distant from Isla Colon) had the PGM^{δ} allele (Table 2). All of these individuals were homozygous for PGM^{δ}. We sampled two individuals with the $PGM^{\beta/\delta}$ phenotype, and some cases that were ho-

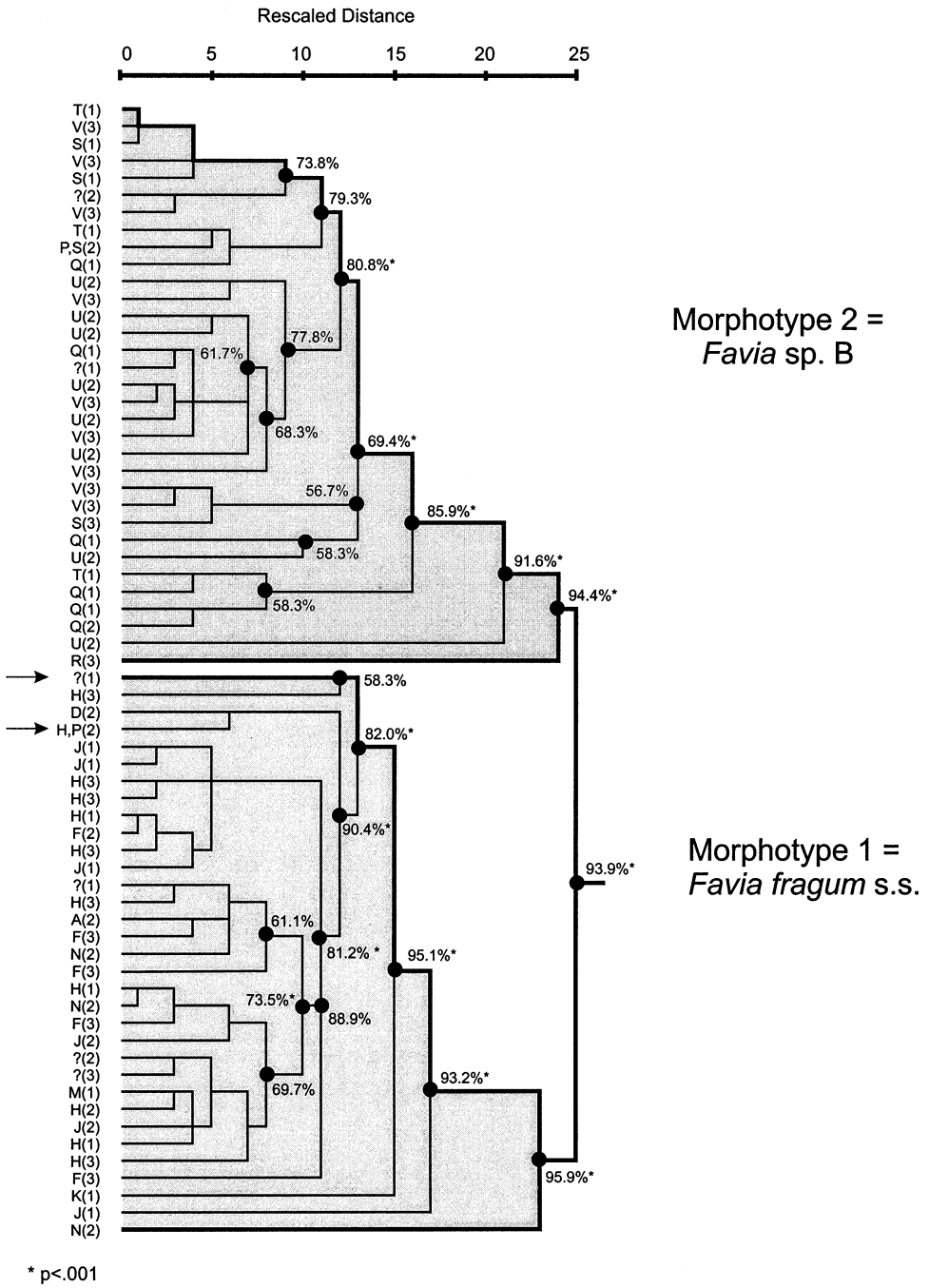

FIG. 5. Cluster analysis (UPGMA) of 3-D landmark data on corallites of two morphotypes of *Favia fragum*. Each branch of the dendrogram represents one individual. Letters on branches indicate haplotype(s) and numbers in parentheses indicate site of collection as in Figure 2. Individuals with two haplotypes were heterozygous at one of five loci. A question mark indicates individuals which either were heterozygous at two loci or could not be completely typed at all loci. The percentages given for selected nodes are cross validation classification percentages determined by canonical discriminant analysis. Asterisks are given for nodes with highly significant *P*-values ($P < 0.001$) determined using Wilks' lambda values and their corresponding chi-square values.

mozygous for rare *PGM* alleles. Three Morph 1 individuals had the $PGM^{\delta/\delta}$ phenotype, and one Morph 2 had the $PGM^{\beta/\beta}$ phenotype.

For both morphotypes, heterozygosity was extremely low across all loci (Table 3), although Wright's inbreeding coefficients (*F*) ranged from 0.0 to 1.0 depending on the locus and site. For both morphs there were significant departures from Hardy-Weinberg expectations at every locus where tests were possible, with one exception: *MPI* in Morph 1 at Site 1. In this population the MPI^{δ} allele was sampled in one heterozygous individual ($MPI^{\beta/\delta}$). Excluding this value, the lowest estimate of *F* within sites was 0.6635 (*PGM* in Morph

TABLE 2. Allele frequencies of five allozyme loci at four sites and two morphotypes of *Favia* at the Bocas del Toro, Panama. Alleles are labeled with increasing electrophoretic mobility ($\alpha < \beta < \delta$). Probability values are from exact tests of allele frequencies between morphotypes. Asterisks indicate Bonferroni-corrected significance. ns, not significant; nv, no variation for the test.

Locus allele	Site 1		Site 2		Site 3		Hospital Bight
	Morph 1	Morph 2	Morph 1	Morph 2	Morph 1	Morph 2	Morph 2
PGM							
N	37	49	46	51	49	56	6
α	0.027	0.000	0.076	0.000	0.000	0.000	0.000
β	0.959	0.020	0.870	0.000	0.980	0.000	0.000
δ	0.014	0.980	0.054	1.000	0.020	1.000	1.000
P	< 0.00001***		< 0.00001***		< 0.00001***		
MPI							
N	38	49	47	49	50	55	6
α	0.000	0.000	0.128	0.000	0.500	0.000	0.000
β	0.947	0.633	0.447	0.214	0.460	0.309	1.000
δ	0.053	0.367	0.442	0.786	0.040	0.691	0.000
P	< 0.00001***		< 0.00001***		< 0.00001***		
TPI							
N	38	46	44	48	49	56	6
α	0.908	0.000	0.773	0.750	0.633	0.893	0.500
β	0.092	1.000	0.227	0.250	0.367	0.107	0.500
P	< 0.00001***		0.726 ns		< 0.00001***		
AAT							
N	38	49	46	45	49	56	6
α	0.895	0.327	0.870	0.800	1.000	1.000	0.500
β	0.105	0.673	0.130	0.200	0.000	0.000	0.500
P	< 0.00001***		0.243 ns		nv		
LVP-2							
N	37	48	46	47	49	56	6
α	1.000	1.000	1.000	0.277	1.000	1.000	1.000
β	0.000	0.000	0.000	0.723	0.000	0.000	0.000
P	nv		< 0.00001***		nv		

*** $P < 0.001$.

1 at Site 1). Correspondingly, estimates of selfing (*S*) within populations were high: greater than 0.79 in every case. There were significant linkage disequilibria between some of the loci, however the magnitude of these associations varied among sites (Table 4). For example, in Morph 1 linkage disequilibrium was detected between *MPI* and *TPI* at Site 3, but this association was not significant at Sites 1 and 2 (Table 4A). Similarly, in Morph 2 *MPI* and *TPI* alleles were significantly associated at Site 2 but not at Site 3 (Table 4B).

A total of 22 allozyme haplotypes were sampled, but only three were shared between morphotypes (Table 5). The three shared haplotypes (H, P, and R) were distributed asymmetrically within the two morphotypes. For example, R is fairly common in Morph 2 (12%) but rare in Morph 1 (<2%). The observed number of shared haplotypes was significantly less than expected if gene flow occurs between morphotypes. In the null model, the mean number of shared haplotypes was 16.25 and the minimum value was 11 (Table 6). Morph 1 had higher haplotype diversity (17 haplotypes) than Morph 2 (8 haplotypes); however, only four haplotypes in Morph 1 were sampled at frequencies >2%. Haplotype diversity in Morph 1 was similar to that expected in the null model, however haplotype diversity in Morph 2 was much less than expected in the null model (Table 6). The observed average genetic distance between morphotypes was much greater than that calculated from the null model (Table 6).

There was clearly structure in the distribution of haplotypes among sites (Fig. 6). Analysis of haplotypes between morphotypes and among sites with AMOVA revealed that morphotype explained 35% of the total variation, and site explained 28% of the total variation (Table 7). Both the between morphotype (F_{CT}), and among site (F_{SC}) fixation indices were significant.

When we mapped haplotype and site on to the morphometric dendrogram (Fig. 5), there was no association between morphology and haplotype, or morphology and site of collection. Two samples that were putative hybrids ($PGM^{\beta/\delta}$ phenotype) had the morphology of Morph 1 and are indicated by arrows on the morphometric dendrogram (Fig. 5).

DISCUSSION

On the protected side of the Bocas del Toro, we found discrete morphological variation in the coral *Favia fragum* across a depth gradient of 5 m. Morphologies with projecting corallites (Morph 2) are found at the shallow end of this depth gradient, and morphologies with flat corallites (Morph 1) at the deep end of the gradient. Analyses of protein variation at three sites revealed strong differences in allele frequencies between morphotypes, particularly at the *PGM* locus, but at four other loci as well. Furthermore, there were significantly fewer shared haplotypes and greater genetic distance between

TABLE 3. Heterozygosity at five loci and in two morphotypes of *Favia*. H_e, Expected heterozygosity; H_o, observed heterozygosity; *F*, Wright's fixation index; *S*, fraction of the population due to selfing; *P*, probability of deviation from Hardy-Weinberg expectations by exact tests. Asterisks indicate Bonferroni-corrected significance; ns, not significant; nv, no variation for the test.

Morph	Site	Locus	H_e	H_o	*F*	*S*	*P*
Morph 1	1	*PGM*	0.0796	0.0270	0.6635	0.7977	0.0119*
		MPI	0.0263	0.0263	0.0000	0.0000	1.0000 ns
		TPI	0.1695	0.0263	0.8465	0.9169	0.0003**
		AAT	0.1909	0.0000	1.0000	1.0000	<0.0001***
		LVP-2	nv				
Morph 1	2	*PGM*	0.2377	0.0434	0.8187	0.9003	<0.0001***
		MPI	0.6076	0.0000	1.0000	1.0000	<0.0001***
		TPI	0.3737	0.0000	1.0000	1.0000	<0.0001***
		AAT	0.2293	0.0000	1.0000	1.0000	<0.0001***
		LVP-2	nv				
Morph 1	3	*PGM*	0.0404	0.0000	1.0000	1.0000	0.0084**
		MPI	0.5422	0.0400	0.9269	0.9621	<0.0001***
		TPI	0.4696	0.0408	0.9139	0.9550	<0.0001***
		AAT	nv				
		LVP-2	nv				
Morph 2	1	*PGM*	0.0381	0.0000	1.0000	1.0000	0.0078*
		MPI	0.4571	0.0385	0.9166	0.9565	<0.0001***
		TPI	nv				
		AAT	0.4302	0.0000	1.0000	1.0000	<0.0001***
		LVP-2	nv				
Morph 2	2	*PGM*	nv				
		MPI	0.3578	0.0200	0.9446	0.9715	<0.0001***
		TPI	0.3737	0.0000	1.0000	1.0000	<0.0001***
		AAT	0.3182	0.0000	1.0000	1.0000	<0.0001***
		LVP-2	0.3991	0.0000	1.0000	1.0000	<0.0001***
Morph 2	3	*PGM*	nv				
		MPI	0.4379	0.0545	0.8764	0.9341	<0.0001***
		TPI	0.1931	0.0000	1.0000	1.0000	<0.0001***
		AAT	nv				
		LVP-2	nv				

** $P < 0.01$, *** $P < 0.001$.

morphotypes than predicted if substantial gene flow occurred between morphotypes. We consider three hypotheses that could explain these patterns.

Intraspecific Polymorphism

Polymorphisms maintained by balancing selection are well-documented in a number of marine systems. These include mussels (Koehn et al. 1980), barnacles (Schmidt et al. 2000; Schmidt and Rand 2001), and estuarine fish (Powers and Schulte 1998). In all these cases genes coding proteins involved in metabolic function are favored at opposite ends of environmental gradients such as temperature or salinity. In Panama, the association of *PGM* alleles with depth could be the result of direct selection on the *PGM* locus, or linkage of this locus with the trait(s) under selection. If selection favors the PGM^{δ} allele at shallow depths and the PGM^{β} allele at deeper depths, and the two morphotypes freely interbreed, we would expect regions of the genome not linked to *PGM* to reflect this gene flow. We found no evidence for linkage disequilibrium between *PGM* and three of the four remaining loci: *MPI*, *TPI*, and *AAT* (Table 4). Assuming these loci to be freely recombining in regard to *PGM*, then gene flow between morphotypes will result in similar allele frequencies in each morphotype within sites. This was clearly not the case. We found significant differences in allele frequencies at all three loci, in at least one of the three sites. It appears that large components of the genome are not freely exchanged between the morphotypes.

Incipient Species with Partial Reproductive Isolation

Incipient species, morphologically diverged populations in which partial but not complete reproductive barriers have evolved, are consistent with our morphological and genetic data. We sampled two individuals that were heterozygous at the nearly fixed *PGM* locus, and several others that were homozygous for *PGM* alleles that were rarely sampled within their respective morphotype, but common in the alternative morphotype (PGM^{δ} in Morph 1 and PGM^{β} in Morph 2). Such patterns are predicted by occasional gene flow between pure morphotypes. Self-fertilization in the F_1 of intermorph crosses would result in progeny that are homozygous for introgressed alleles. Additional evidence for some gene flow between morphotypes comes from the distribution of haplotypes in the two morphotypes. The three haplotypes sampled in both morphotypes had asymmetric frequencies. Interestingly, the morphology of two putative hybrids ($PGM^{\beta/\delta}$ phenotypes) was more similar to Morph 1 (indicated by arrows on Fig. 5) rather than forming a third intermediate clade. It is known from other coral species that hybrids typically express the morphology of one of the parental species rather

TABLE 4. Probability values for exact tests of two-locus linkage disequilibrium for two *Favia* morphotypes at each of three sites. Boldface denotes changes in linkage disequilibrium among sites. Number of asterisks indicate Bonferroni-corrected significance. ns, not significant; nv, no variation for the test.

A. Morph 1

	Loci pair					
Site	*PGM/MPI*	*PGM/TPI*	*PGM/AAT*	*MPI/TPI*	*MPI/AAT*	*TPI/AAT*
1	nv	0.0509 ns	0.1594 ns	**0.1034 ns**	**0.1028 ns**	0.0400 ns
2	0.0903 ns	0.3091 ns	0.6562 ns	**0.0403 ns**	**0.0003****	0.1244 ns
3	nv	nv	nv	**<0.0001****	**nv**	nv

B. Morph 2

	Loci pair								
Site	*PGM/MPI*	*PGM/TPI*	*PGM/AAT*	*MPI/TPI*	*MPI/AAT*	*MPI/LVP*	*TPI/AAT*	*TPI/LVP*	*AAT/LVP*
1	nv	nv	0.3088 ns	**nv**	<0.0001***	nv	nv	nv	nv
2	nv	nv	nv	**<0.0001*** **	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***
3	nv	nv	nv	**0.2184 ns**	nv	nv	nv	nv	nv

** $P < 0.01$, *** $P < 0.001$.

than form intermediates (M. J. H. van Oppen, pers. comm. 2001). Perhaps relatively few genes determine the heritable component of coral morphology.

Ecology may play a role in the morphological divergence within *Favia* across depth. The protected sides of the islands of the Bocas del Toro are heavily influenced by terrestrial runoff. A lens of freshwater, high in organic matter, forms in the surface layer at the protected sides of these islands (Guzman and Guevara 1998a). The effects of terrestrial runoff create a gradient of salinity and sedimentation with distance from the shore. Near shore and at shallow depths, there are episodic reductions in salinity and high rates of sedimentation as detritus rains out of the surface layer. Farther from shore and at deeper depths, salinity is more stable and sedimentation rates will be lower. At exposed sites, this gradient is much weaker, as high wave energy mixes surface waters and resuspends sediments. Reductions in salinity (Coles and Jokiel 1992; Moberg et al. 1997; Ferrier-Pages et al. 1999) and high sediment loads (Staffordsmith and Ormond 1992; Staffordsmith 1993; Wesseling et al. 1999) have been shown to negatively affect physiological processes and survivorship of scleractinian corals. Of special relevance to the morphological variation we have documented here, Lasker (1980) found increased convexity of the colony surface and higher corallite relief increased passive sedimentation removal (removal of sediment particles without the use of mucous or tentacles) relative to colonies with flatter surfaces and low corallites in a coral with similar morphological characters (*Montastrea cavernosa*: Faviidae). These corallite characters define Morph 2, which exists in the very shallow, near shore habitats of the Bocas del Toro region. A cost of this morphology would be a weaker attachment site and increased drag in wave-exposed sites, which would explain why we failed to sample Morph 2 at exposed sites on the outer islands with higher wave energy. Gradients in sedimentation across depth or distance from shore may have played a functional role in the morphological divergence.

Ecological segregation of the two morphotypes combined with reproductive and larval traits is likely to increase population subdivision. Clearly, the amount of selfing within populations is an important parameter with regard to gene flow, because of the trade-off between selfing and outcross-

TABLE 5. The frequency (%) of haplotypes sampled in two morphotypes of *Favia fragum.*

Haplotype	Morph 1	Morph 2
A	0.79	0.00
B	0.79	0.00
C	0.79	0.00
D	1.59	0.00
E	0.40	0.00
F	19.84	0.00
G	0.79	0.00
H	18.65	0.70
I	0.79	0.00
J	36.11	0.00
K	0.79	0.00
L	1.59	0.00
M	1.59	0.00
N	12.70	0.00
O	0.79	0.00
P	0.40	3.15
Q	0.00	25.17
R	1.59	11.89
S	0.00	11.54
T	0.00	4.20
U	0.00	20.28
V	0.00	23.08
n	252	286

TABLE 6. Comparison of haplotype data and the null model. Parameters for the null model were generated from constructing 1000 population pairs with random sampling from the total haplotype sample.

Parameter	Null model	Observed
Average genetic distance between haplotypes	2.04 (Max. = 2.13)	2.62***
Number of shared haplotypes between morphotypes	16.25 (Min. = 11.0)	3***
Number of unique haplotypes within Morph 1	18.20 (Min. = 13.0)	17^{ns}
Number of unique haplotypes within Morph 2	18.67 (Min. = 14.0)	8***

*** $P < 0.001$.

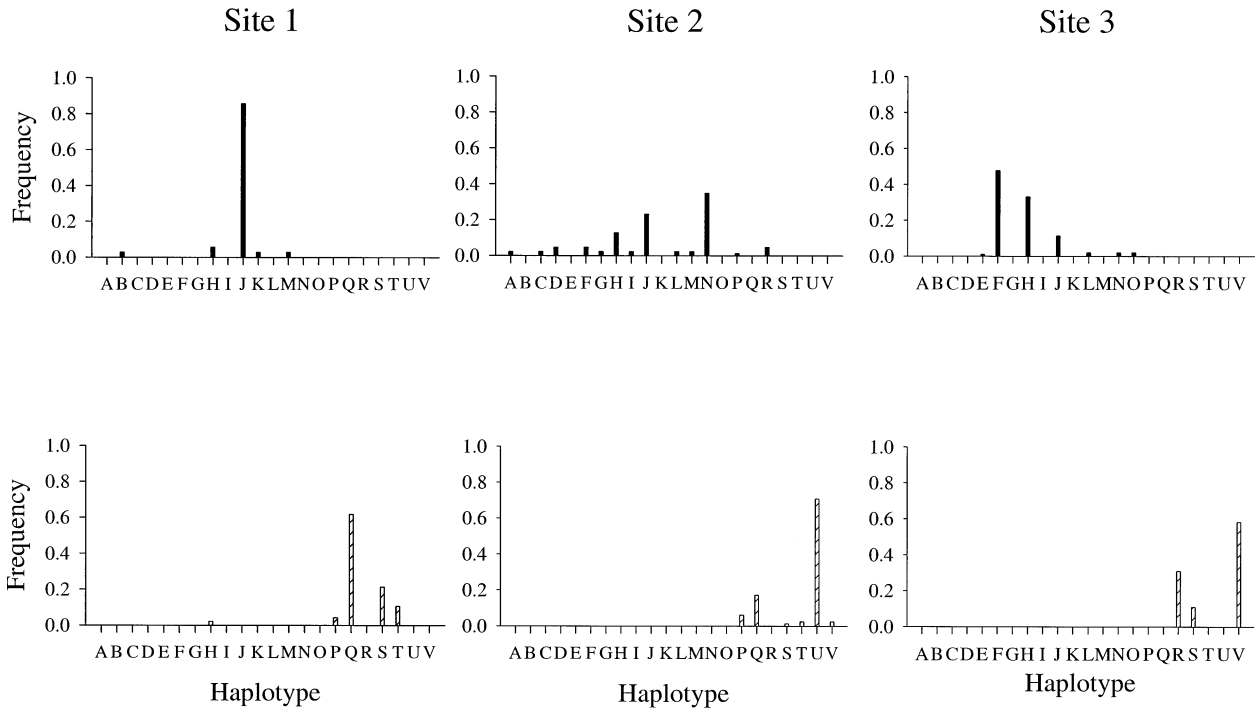

FIG. 6. Frequency of haplotypes at three sites for the two morphotypes. Upper panels and solid bars are for Morph 1. Lower panels and hatched bars are for Morph 2.

ing. Our single-locus estimates of selfing (S) approached 1.0 at all sites for both morphotypes, suggesting that nearly all offspring were the consequence of self-fertilization rather than outcrossing. These estimates assume that genotypic frequencies are at equilibrium, that there are no differences in survivorship between selfed and outcrossed progeny, and that no other microevolutionary forces affect levels of heterozygosity. Our estimates of selfing are likely to be biased towards higher selfing, because philopatric dispersal leads to mating between relatives (biparental inbreeding), which also reduces heterozygosity within populations (Carlon 1999). On a single reef in Florida, Brazeau et al. (1998) report a lower population selfing rate ($S = 0.49$) than we found at any site in Panama. However, in that same study 94% of the progeny of one individual were selfed. Even considering a potential bias in our estimates, a moderate amount of selfing will ultimately limit the amount of gene flow between neighboring individuals and populations. A second trait that will affect the amount of population subdivision in sessile marine organisms like *F. fragum* is the behavior of planktonic larvae. The brooded larvae of *F. fragum* remain near the benthos when tracked by divers (Carlon and Olson 1993) and will settle readily in response to a variety of natural cues associated with shallow habitats (Carlon and Olson 1993; Carlon 2002). These larval traits will limit the distance that larvae are transported in currents near the benthos. Consistent with the reproductive traits of this species, there was obvious genetic structure in both morphotypes among sites 1–2 km distant as indicated by variation in the frequency of alleles and haplotypes across these sites. Although selection could also cause these patterns within morphotypes, the three sites were essentially replicates of the same type of habitat. Thus the reproductive and larval traits of *F. fragum* will reduce gene flow between individuals by selfing and between sites by limited larval dispersal.

Biological Species with Incomplete Lineage Sorting

The two morphotypes could be biological species that have evolved complete reproductive isolation if the speciation event is recent. Individuals that were heterozygous at *PGM* or homozygous for rare alleles may reflect an ancestral polymorphism that has not completely sorted between lineages. The probability that two copies of a gene reach fixation (i.e., the gene genealogy is reciprocally monophyletic with respect to the two populations) increases as a function of the number of generations since the interruption of gene flow and the effective population size (Harrison 1998). The fact that none

TABLE 7. Molecular analysis of variance of haplotype data.

Variance component	Variance component	Percentage of variation	F-statistic	P
Between morphotype	$V_a = 0.465$	35.27	$F_{CT} = 0.353$	<0.001
Among sites within morphotype	$V_b = 0.369$	28.04	$F_{SC} = 0.633$	<0.001
Within sites	$V_c = 0.484$	36.69	$F_{ST} = 0.433$	<0.001

of our populations were completely fixed for allozyme alleles at five loci could reflect the incomplete sorting of ancestral polymorphism in two reproductively isolated species with a recent divergence. The first fossil record of *F. fragum* (Morph 1) is in the Pinecrest Sandstone of Florida, dated between 3 and 3.5 million years old (Budd et al. 1994a). There is no fossil record of Morph 2. This absence could be interpreted as a relatively recent divergence between the two morphotypes. However, it seems just as likely that the absence of Morph 2 reflects the poorly preserved and rarely sampled shallow habitats where Morph 2 exists. Other historical events documented in the Caribbean fossil record can be used to infer the time since speciation between morphotypes. Compilations of stratigraphic data for the Caribbean region show a sharp increase in both the extinction and speciation rate between 4 and 1.5 million years ago (Budd and Johnson 1999b). Further analyses of these data show that extinction was highest in small attached or free-living coral genera such as *Favia*, *Thysanus*, and *Manicina*, and occurred primarily between 2 and 1.5 million years ago (Johnson et al. 1995; Johnson 1998). Much of the modern Caribbean reef coral fauna originated between 1 and 2 million years before the peak in extinction. Assuming a generation time of two years (Szmant-Froelich et al. 1985), then 500,000 generations would have passed if reproductive isolation evolved 1 million years ago. Since the effective population size in this case is likely to be small (due to the effects of inbreeding and population subdivision discussed in the previous section), this number of generations provides ample time for at least some of the allelic variation sampled here to reach fixation. Thus, if *F. fragum* and Morph 2 are completely reproductively isolated, the onset of isolation must have been extremely recent.

In the absence of a precise date for this divergence, we must consider the possibility that the divergence is considerably older (approximately 3 to 3.5 million years ago) and that the two morphotypes behave as ''good'' biological species. In terrestrial plants, species boundaries between distantly related species remain permeable to gene flow. For example, in cottonwood trees (*Populus*) hybridization occurs between species of different sections, yet morphological boundaries are maintained because hybridization and recombination act as a filter, allowing some genes to introgress but preventing most of the genome from crossing the species boundary (Martinsen et al. 2001). In this context, our genetic evidence for hybridization between *Favia* species does not necessarily indicate an incipient speciation event.

An older divergence has implications for inferring the geography of speciation from present-day distributions. A recent analysis comparing the amount of geographic overlap versus age of divergence (using a molecular clock) has shown that in many groups of animals, the degree of sympatry tends to increase with age (Barraclough and Vogler 2000). Thus it is possible that ancestral populations had greater spatial isolation and what we observe in the Bocas del Toro region is a zone of secondary contact following a range expansion, rather than the site of primary differentiation (Endler 1977). It is not inconceivable to construct an historical scenario in which the two populations had greater spatial isolation than they do today. In general, species of the genus *Favia* are limited to shallow tropical environments; there are no deep-water species (Veron and Stafford-Smith 2000), therefore the potential for greater isolation by depth between ancestral populations compared to present seems limited. In contrast, there is potential for geographic isolation of ancestral populations. *Favia fragum* primarily exists on consolidated hard substrata in reef environments, while Morph 2 was only sampled in sea-grass beds near mangrove stands. Coral reef, mangrove, and sea-grass habitats are not always regionally associated. It is possible that significant divergence of Morph 2 occurred in mangrove or sea-grass habitat, isolated by geographic distance from an ancestral species that was restricted to the hard substrata of coral reefs.

Conclusions

Regardless of the status of this speciation event, the morphological and genetic divergence in *Favia* within shores in the Bocas del Toro region of Panama adds a scleractinian coral to the number of marine examples that fit the divergence-with-gene flow model of speciation. Other examples include the snapping shrimp *Synalpheus* and two intertidal snails of the genus *Littorina*. While these previous examples have reproductive traits that limit gene flow between neighboring populations, our coral example is unique in that the genetic and morphological divergence has evolved in the absence of behavior that can increase assortative mating by sexual selection. Corals free-spawn either sperm (brooding species) or eggs and sperm (broadcasting species) into the water column where fertilization success is determined by an interaction between the proximity of mates and the physical properties of water flow (e.g., velocity, turbulence, etc.) at the time of spawning (Levitan and Petersen 1995; Yund 2000). Without behavior to reject potential mates, prezygotic isolation must evolve by characters that limit fertilization in the water column, such as the phenology of gamete production or perhaps gamete recognition systems (Palumbi 1994; Vacquier 1998). A mechanistic understanding of the role of these processes in reproductive isolation is emerging in broadcasting corals (Knowlton et al. 1997; Szmant et al. 1997; van Oppen et al. 2001). Further study of this divergence would provide insights into the parallels and differences in the evolution of reproductive isolation between broadcasting and brooding marine invertebrates.

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APPENDIX

Distance matrix of haplotypes.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
A																						
B	2																					
C	2	1																				
D	2	4	4																			
E	3	3	3	1																		
F	2	2	2	2	1																	
G	1	3	3	1	2	1																
H	4	2	3	2	1	2	3															
I	3	3	4	1	2	3	2	1														
J	3	1	2	3	2	1	2	1	2													
K	2	2	3	2	3	2	1	2	1	1												
L	4	3	2	2	1	2	3	1	2	2	3											
M	2	3	2	2	3	2	1	3	2	2	1	2										
N	3	2	1	3	2	1	2	2	3	1	2	1	1									
O	2	2	2	3	2	1	2	3	4	2	3	3	3	2								
P	4	2	3	3	2	3	4	1	2	2	3	2	4	3	2							
Q	3	3	4	2	3	4	3	2	1	3	2	3	3	4	3	1						
R	3	1	2	4	3	2	3	2	3	1	2	3	3	2	1	1	2					
S	4	3	2	3	2	3	4	2	3	3	4	1	3	2	2	1	2	2				
T	3	4	3	2	3	4	3	3	2	4	3	2	2	3	3	2	1	3	1			
U	4	3	2	5	4	3	4	4	5	3	4	3	3	2	2	3	4	2	2	3		
V	3	2	1	4	3	2	3	3	4	2	3	2	2	1	1	2	3	1	1	2	1	