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Variation in Morphology vs Conservation of a Mitochondrial Gene in *Montastraea cavernosa* (Cnidaria, Scleractinia)

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Skeletal morphology of many scleractinian corals may be influenced by environmental factors and may thus result in substantial intraspecific phenotypic plasticity and, possibly, in overlapping morphologies between species. Environmentally induced variation can also mask phenotypic variation that is genetically based. Morphological analyses and DNA sequence analyses were performed on *Montastraea cavernosa* from the Flower Garden Banks, Texas, and from the Florida Keys in order to assess variation within and between geographic regions. Skeletal characters, including corallite diameter, columella width, theca thickness, nearest-neighbor distance, length of first septa cycle, and width of first septa cycle, varied within colonies, among colonies, and between the Flower Garden Banks and the Florida Keys. Morphological variation may be controlled by environmental and genetic influences at different levels. If phenotype is under genetic control, it is not influenced by the mitochondrial cytochrome oxidase subunit I gene, because analysis of a 708 base pair fragment revealed identical sequences of *M. cavernosa* from these geographic regions. This high level of nucleotide sequence similarity may result from functional constraints, efficient DNA repair mechanisms, or other processes. This gene was not found to exhibit any variation in association with that observed in the morphology, and we suggest that it is an inappropriate genetic marker to use to assess intraspecific variation within this species and possibly other scleractinian species as well. Analysis via other molecular techniques will be necessary in order to assess the factors that influence morphological variation and that distinguish populations within this species.

Extensive morphological variation within scleractinian coral species has in some cases led to difficulty in defining taxonomic boundaries. The direct influence of environmental factors (light, wave action, current patterns, sedimentation, and biological interactions) on skeletal characters may result in a wide range of morphological variation within a single species from a single locality (Lang, 1971; Dustan, 1975, 1979; Land et al., 1975; Foster, 1979; Lasker, 1981; Willis, 1985; Ayre and Willis, 1988). These factors are known to have even greater effects across wide environmental gradients and geographic scales. Thus, genetically based morphological variation can be masked by that which is environmentally induced, making it difficult to distinguish between morphologically similar species via skeletal characters alone.

For this reason, allozyme electrophoresis, behavior, distribution and abundance, and timing of reproduction have been used in conjunction with skeletal features to assist in taxonomic identification (Lang, 1971; Ayre, 1991; McMillan et al., 1991; Weil, 1992; Fenner, 1993; Weil and Knowlton, 1994; Knowlton et al., 1997). For example, *Montastraea annularis* has recently been identified as a complex of three sympatric cryptic species—*M. annularis, M. faveolata,* and *M. franksi*—based on allozyme electrophoresis, aggressive behavior, skeletal morphology, isotopic ratios, and timing of reproduction (Knowlton et al., 1992; Weil and Knowlton, 1994; Knowlton et al., 1997).

Montastraea cavernosa (Faviidae) exhibits a wide degree of environmentally induced morphological variation (Foster, 1985; Budd, 1988, 1993; Amaral, 1994; A. Acosta, pers. comm.). The existence of at least two distinct coexisting morphotypes, diurnal and nocturnal (Lehman and Porter, 1973; Lasker, 1976, 1978, 1981; Budd, 1988, 1993), suggests that genetic variation is an important influence on the behavior, and perhaps on the morphology, of this species. Variation within the nocturnal morphotype of *M. cavernosa* was investigated in this study.

Mitochondrial DNA (mtDNA) analyses have been used on a variety of organisms to distinguish between populations and to identify cryptic species. Sequence variation of the cytochrome oxidase subunit I (COI) gene has helped to successfully distinguish between populations and species of several closely related or morphologically similar organisms, such as horseshoe crabs (Avise et al., 1994), copepods

(Burton and Lee, 1994), gastrotrichs (Todaro et al., 1996), barnacles (VanSyoc, 1994), vesicomyid bivalves (Kojima et al., 1995), fruit flies (Spicer, 1995), mussels (Baldwin et al., 1996), and pseudoscorpions (Wilcox et al., 1997). In these species, COI exhibits a range of variation at different taxonomic levels. Relatively few DNA sequences of scleractinian corals have been published. We raise the question of whether the mitochondrial COI gene exhibits similar variation within a scleractinian coral.

Here we assessed morphological and genetic variation in *M. cavernosa* colonies within and between reefs at the Flower Garden Banks and the Florida Keys. Our null hypothesis was that both morphology and the COI nucleotide sequence would not vary between these distant locations. Our intention here was not necessarily to identify the specific causes for variation but rather to determine whether there was congruence between these two independent data sets.

Methods

Collection of samples.—*Montastraea cavernosa* samples were collected from colonies in the Flower Garden Banks National Marine Sanctuary (FGB; n = 4) and from four reefs in the Florida Keys (FK): Bahia Honda (n = 6), Tennessee Reef (n = 5), Pickles Patch (n = 7), and Carysfort Reef (n = 6). Samples were collected in July and Aug. 1996. Within each geographic location (FGB and FK), samples were collected at the same general depth; thus, depth was not a contributing source of intrasite variation. The depth range of sampling at the Florida Keys was 3–6 m and at the Flower Garden Banks it was 21–24 m (the minimum depth at this site).

Differences in sample size were due to limits set by permits and to local coral abundance. Sampled colonies were ≥ 0.3 m in diameter and ≥ 5 m apart in order to minimize the chance of sampling clonemates. In most cases, samples were chipped from the basal edge of each colony with a hammer and chisel in order to minimize damage to the remainder of the colony. The samples were ~10 cm^2 in area and possessed ≥ 10 mature polyps. Because of the requirements of the regional administering agency, some samples were taken with a 2-inch core mounted in a hydraulic drill adapted for underwater use. Samples designated for morphological analysis were submersed in 50% sodium hypochlorite solution in order to dissolve the coral tissue without damaging the skeletal elements. Tissue samples designated for DNA analysis were frozen in liquid nitrogen and stored at −80 C.

Morphological analysis.—Seven characters—number of septa, corallite diameter, columella width, theca thickness, nearest-neighbor distance, length of first septa cycle, and width of first septa cycle (described in Foster, 1985)—from 10 corallites per colony were counted or measured to the nearest 50 μm using vernier calipers. Photographs were taken of each sample and were enlarged to facilitate measurement. These characters were selected based on their use in previous morphological studies of *Montastraea* (Amaral, 1994; Weil and Knowlton, 1994; Foster, 1985). The measurements were analyzed using univariate, multivariate, and principal component analyses. Intracolony variation was quantified using these data. The colony mean for each character was used to determine variation at the intercolony, intrareef, interreef, and interregional levels.

DNA analysis.—Samples of five *M. cavernosa* were prepared by extraction of total DNA from frozen coral tissue. Approximately 50 mg of soft tissue was removed from a single polyp with a sterile razor and was homogenized with a plastic pestle in 200 μl sodium chloride-Tris ethylenediamine tetraacetic acid (EDTA) with 0.2% sodium dodecyl sulfate (SDS). DNA was isolated from the crude homogenate using the manufacturer-recommended methods of the Prep-A-Gene DNA Purification Kit (BioRad).

Amplifications of a segment of the COI gene were performed in 100-μl volume solutions containing 0.5 mM deoxynucleoside triphosphate (dNTPs) 1× concentration of 10× buffer (Promega), 2.5 mM $MgCl_2$ (Promega), 10 mM each primer, ~50 ng genomic DNA, and 1 unit *Taq* DNA polymerase (Promega). One primer was a shortened version of the Folmer et al. (1994) LCOI1490 universal primer (1490 refers to the corresponding position of the *Drosophila yakuba* mtDNA sequence; Clary and Wolstenholme, 1985): 5′-CAACAAATCATAAAGATATTGG-3′. The other was a degenerate primer designed for amplification of echinoderm COI (Hrincevich et al., in press). These primers flanked ~868 base pairs (bp) of the coral COI gene.

The DNA was amplified via 38 cycles of the polymerase chain reaction (PCR) (0.5 min at 95 C, 1.5 min at 47 C, and 1.5 min at 72 C). Amplification products were visualized on 2.0% agarose gels stained with ethidium bromide. The absence of amplification products in the negative controls (distilled water was

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TABLE 1. Proportion of total variation (as percentages, in each morphological character examined) that is attributable to each nested source of variation, based on measurements of 10 corallites per colony.[a]

Source of variation	DF	NS	CD	CLW	NND	TT	LEN	WID
Within colonies	252	67.5	33.0	41.9	16.8	20.8	31.7	35.8
Between FK colonies	23	16.7	14.8	46.0	6.8	7.7	23.9	38.0
Between FK reefs	3	15.89	2.7	4.4	0.0	0.0	0.0	0.0
Between FGB and FK	1	0.0	49.5	7.7	76.4	71.5	44.4	26.2
Total	279	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] DF = degrees of freedom; NS = number of septa; CD = corallite diameter; CLW = columella width; NND = nearest-neighbor distance; TT = theca thickness; LEN = length of first septa cycle; and WID = width of first septa cycle; FK = Florida Keys; FGB = Flower Garden Banks.

added to the reaction mixture rather than genomic DNA) indicated that there was no contamination. Amplified DNA was purified by the addition of polyethylene glycol (20% final concentration) to the PCR product for 1 hr, which was followed by ethanol washing. The DNA pellet was dried and resuspended in an appropriate volume of sterile filtered water (10–30 μl). Concentrated amplified DNA of each individual was sequenced through the Interdisciplinary Center for Biotechnology Research at the University of Florida in Gainesville. The shortened version of the LCOI1490 universal primer described above was used as the sequencing primer.

Nucleotide sequences similar to the coral sequences were identified via a search using BLAST (National Center for Biotechnology Information; Altschul et al., 1990), which resulted in a sequence similarity of 76% to the azooxanthellate sea anemone *Metridium senile* (GenBank Accession #U36783; Beagley et al., 1996). This suggested that the coral DNA was successfully amplified, whereas that of the symbiotic dinoflagellates associated with scleractinian corals was not.

RESULTS

Morphological analysis.—A nested analysis of variance of the character measurements for each of the 10 corallites (Snell, 1997) indicated that most characters varied widely within colonies. Number of septa accounted for 67.5% of overall intracolony variation (Table 1). Nearest-neighbor distance and theca thickness exhibited low intracolony variation. For comparative purposes, means and standard deviations were determined for each character of each colony within FGB and FK populations, between Floridian populations, and between geographic regions (Table 2).

TABLE 2. Means ($\bar{Y}$) and standard deviations (s) for the morphological characters of *Montastraea cavernosa* by population and location.[a]

Character		BH	TR	PP	CR	All FK populations	FGB
NS	$\bar{Y}$	46.167	42.000	42.286	42.167	43.167	42.500
	s	±1.472	±2.915	±2.059	±1.472	±2.582	±2.887
CD	$\bar{Y}$	5.710	5.758	5.361	5.757	5.630	6.501
	s	±0.314	±0.274	±0.354	±0.428	±0.372	±0.396
CLW	$\bar{Y}$	2.774	2.802	2.546	2.981	2.765	3.075
	s	±0.199	±0.459	±0.392	±0.432	±0.391	±0.277
NND	$\bar{Y}$	3.452	3.501	3.656	3.323	3.490	5.474
	s	±0.548	±0.507	±0.385	±0.439	±0.454	±0.441
TT	$\bar{Y}$	1.776	1.747	1.846	1.738	1.781	2.776
	s	±0.257	±0.229	±0.161	±0.282	±0.223	±0.386
LEN	$\bar{Y}$	0.914	0.823	0.891	0.859	0.875	1.154
	s	±0.147	±0.067	±0.166	±0.121	±0.130	±0.229
WID	$\bar{Y}$	0.326	0.320	0.353	0.351	0.339	0.404
	s	±0.021	±0.048	±0.066	±0.074	±0.055	±0.024
n		6	5	7	6	24	4

[a] BH = Bahia Honda; TR = Tennessee Reef; PP = Pickles Patch Reef; CR = Carysfort Reef; FK = Florida Keys; FGB = Flower Garden Banks. Means of all Florida colonies are included in "All FK populations." Data represent means of each colony collected at each location. NS = number of septa; CD = corallite diameter; CLW = columella width; NND = nearest-neighbor distance; TT = theca thickness; LEN = length of first septa cycle; WID = width of first septa cycle; and n = sample size.

TABLE 3. Correlation coefficient matrix of morphological characters in *Montastraea cavernosa*. Values indicate the R coefficient with significance levels indicated by ** ($P < 0.01$) and *** ($P < 0.001$).[a]

	NS	CD	CLW	NND	TT	LEN	WID
NS		0.00466	0.09259	−0.00070	0.06401	−0.05929	0.09136
CD			0.63221***	0.58427**	0.57808**	0.59057***	0.27038
CLW				0.17744	0.22639	0.22021	0.16160
NND					0.96944***	0.59234***	0.37251
TT						0.55410**	0.35007
LEN							0.61299***

[a] NS = number of septa; CD = corallite diameter; CLW = columella width; NND = nearest-neighbor distance; TT = theca thickness; LEN = length of first septa cycle; and WID = width of first septa cycle.

In general, intracolony variation in the characters was high with respect to intercolony variation. The highest percentage of total variation was exhibited in columella width and in width of the first septa cycle (Table 1). With respect to interreef variation in the FK, other characters showed little variation (0–15.8%). There was a high degree of interregional morphological variation, which was primarily due to nearest-neighbor distance, theca thickness, corallite diameter, and length of first septa cycle characters. There was no interregional variation in the number of septa (Table 1).

In order to determine the degree of independence between morphological characters, the means for all colonies were used to calculate a correlation coefficient matrix (Table 3). Number of septa did not correlate with any other character; however, many correlation coefficients were significant, indicating nonindependence of characters. For example, a significantly high correlation was exhibited between theca thickness and nearest-neighbor distance ($R = 0.969$, $P < 0.0001$).

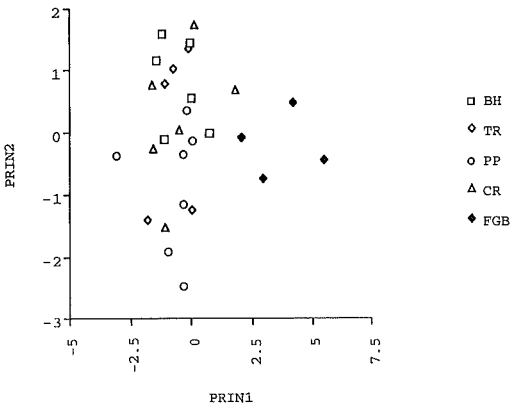

Fig. 1. Plot of the principal components analysis, performed on morphological characters of *Montastraea cavernosa* colonies collected from FGB and FK. BH = Bahia Honda; TR = Tennessee Reef; PP = Pickles Patch; CR = Carysfort Reef; and FGB = Flower Garden Banks.

A principal component analysis, using within-colony character means (Fig. 1), revealed that the first principal component score explained 48.5% of the total variation. Nearest-neighbor distance, theca thickness, corallite diameter, and length of first septa cycle contributed heavily to this component. In most morphometric studies, the first principal component is strongly influenced by the size of the organism because of the allometric growth of some characters (Quicke, 1993). The second through fourth principal component scores each explained approximately the same proportion of the remaining variance (11.7–16.9%). Columella width and number of septa contributed heavily to the second principal component.

DNA analysis.—Cytochrome oxidase subunit I sequences ranging from 721 to 786 bp were trimmed to 708 corresponding bp and were aligned by eye. All 708 nucleotides of the sequenced COI gene fragment were identical for the five *M. cavernosa* samples from the FGB and the FK (GenBank Accession numbers AF051094, AF108710, AF108711, AF108713, and AF108714).

DISCUSSION

Morphological analysis.—*Montastraea cavernosa* exhibited generally high degrees of intracolony and interregional variation. Intercolony variation was moderate, and interreef variation was low. Although there was a high degree of intracolony variation, it was not indicative of genetic polymorphism; all polyps within a colony were assumed to be genetically identical. As observed in other studies of this species (Amaral, 1994; Foster, 1985), intracolony skeletal characters commonly exhibit plasticity due to differences in microenvironmental condi-

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tions. These conditions may cause significant differences in the growth rate of corallites between sections within *M. cavernosa* colonies (Foster, 1985). For example, corallites at the top of the colony have higher growth rates than do basal corallites (Foster, 1985).

Variation among colonies within a reef was most likely the result of specific environmental influences, including, for example, shading or competition for space, which affects colony growth. This is consistent with the intercolony variation in the growth of the Brazilian *M. cavernosa,* observed by Amaral (1994). The small amount of additional variation between the Floridian populations sampled here suggests that the sampled reefs are exposed to the same general environmental conditions at a macroscale level. Since all samples were collected at the same depth within regions, depth was not a potential influence on intercolony, intrareef, or interreef variation.

Our analyses may have yielded overestimates of the morphological variation between the FGB and FK populations, since the skeletal characters were not independent. This is a common problem in morphological studies and may be addressed by using multiple criteria for differentiating between populations or species when identities are in question.

Interregional variation may be affected by environmental influences; [e.g., water depth (~7 m vs ~22 m depth, respectively)] and by the concomitant variation in light intensity and/or spectrum. The gross morphology of colonies sampled from the FGB was flattened relative to that of the FK colonies. Such gross morphological variation in general colony form is commonly found in conspecific corals from different depths (Kawaguti, 1937; Dustan, 1975; Land et al., 1975; Fricke and Meischner, 1985).

Variation in microcalical characters, however, may not necessarily be affected in the same manner by environmental factors at this scale. Genetic polymorphism may well contribute to differences observed in the fine structure of the calices between the two regions. This would be an indication of restricted gene flow between the two regions, which is consistent with Gulf of Mexico circulation patterns (Science Applications International Corporation, 1989), distance between the two regions (~2,000 km), larval longevity (≤ 100 d; Richmond 1987, 1988; see Harrison and Wallace, 1990, for review), and time required for larvae to travel from one region to the other. We believe that interregional differences in gross colony morphology are due to environmental differences, and that differences in microcalical morphology are due primarily to genetic differences and possibly secondarily to environmental differences. Clearly, additional work is required to identify the degree to which these two factors are influencing morphology. Regardless of the cause of interregional calical variation, it may be concluded that the COI gene is not associated with morphological variation at any level in *M. cavernosa,* since this gene exhibited no sequence variation at this or higher levels of spatial resolution.

Genetic variation.—Lack of sequence variation in the COI gene fragment of *M. cavernosa* clearly indicated that this gene is not associated with the variation in morphology observed between FGB and FK populations. The COI gene appears to be an inappropriate marker for assessing intraspecific variation within this species, despite its successful use in a variety of other invertebrates (see references above). The same COI fragment has been sequenced for two *M. cavernosa* colonies from Bermuda (GenBank Accession numbers AF108712 and AF108715; Snell, 1997), and nucleotide sequences were found to be identical to those observed in this study from the FGB and FK. An independent analysis of other *M. cavernosa* samples from Florida also yielded an almost identical corresponding nucleotide sequence (two nucleotide differences; M. Medina, pers. comm.). This reinforces our findings of a high level of conservation within this gene region.

The conservation of the COI nucleotide sequence may be the result of number of factors, including functional constraints or DNA repair mechanisms, which are potentially active in some cnidarians. The COI gene is an essential component of the respiratory pathway in mitochondria. Because of its functional importance, evolutionary changes in the amino acid sequence of this gene may be expected to occur at a slow rate (Brown et al., 1979; Palumbi, 1996). This mitochondrial gene does not undergo recombination; thus, nucleotide variation is most likely the result of mutation events. Although changes in the amino acid composition because of nucleotide substitutions may result in reduced fitness of the colony, this does not explain the absence of silent third-position substitutions in these coral nucleotide sequences.

The recent identification of a *mutS* homologue in the mitochondrial genome of an octocoral (*Sarcophyton glaucum*; Pont-Kingdon et al., 1995) may suggest the presence of a DNA-mismatch repair system in some cnidarians. It

has been indicated that a functional repair mechanism may have contributed to unexpectedly low levels of nucleotide sequence variation observed in the mitochondrial cytochrome *b* gene among *Acropora* species (van Oppen et al., 1999) and in the mitochondrial large subunit rRNA within octocorallians (France et al., 1996).

Low rates of evolution have been observed in conspecific, congeneric, confamilial, and distantly related cnidarians. *Diploria strigosa* (GenBank Accession numbers AF108716 and AF108717) and *Agaricia agaricites* (GenBank Accession numbers AF112120 and AF112121) exhibited no intraspecific sequence variation in this COI fragment (Snell, 1997). Best and Thomas (1994) presented evidence of intraspecific sequence identity within 486 bp of the COI fragment of *Acropora* species possessing different reproductive modes (sequences unpublished at this time). The nuclear ribosomal ITS1 sequences in populations of the corallimorpharian *Rhodactis* sp. (Anthozoa) were highly conserved (97.5–100%) over a wide geographic distribution (Chen and Miller, 1996). A 615 bp region of the COI nucleotide sequences of *M. faveolata* and *M. franksi* were determined to be identical, while *D. strigosa* and *Favia fragum* varied by only a single third-position transition (Snell, 1997). Romano and Palumbi (1996) also found that sequences of the mitochondrial 16S ribosomal RNA region were highly conserved in 34 scleractinian species. Confamilial sequences varied up to 2.5–3% and up to 9% between sister families. In several instances, identical 16S sequences were observed in multiple genera within a family (e.g., *Pectinia alcicornis, Caulastraea furcata,* and *Echinopora lamellosa*). Identical sequences were found in *Fungia fragilis* and *F. vaughani,* but these varied from the corresponding sequence of *F. scutaria.* France et al. (1996) also described slow evolution within the cnidarian mitochondrial 16S region, particularly in octocorals.

Given the general conservation of mtDNA in many cnidarians, the monomorphism of the *M. cavernosa* nucleotide sequences is not necessarily incongruent with the polymorphism observed in the skeletal analysis. The genetic makeup of an individual is not usually altered by normal external factors; morphological characters often are. In scleractinian corals, it is common to have genetically identical colonies with varying morphological characteristics due to different environmental conditions.

Identity of this COI gene region does not indicate overall genetic identity between colonies (i.e., clonemates), nor does it indicate inheritance from a single recent ancestor (i.e., founder effect). This gene region may exhibit an unusually high degree of conservation relative to other organisms, whereas other regions of the genome are variable.

At first glance, the lack of sequence variation observed would suggest that there is gene flow between the FGB and FK, but we cannot reach any definitive conclusions regarding this issue. Variation (or lack thereof) in the DNA is created primarily by genetic mutation (or lack thereof), whereas gene flow is responsible for the distribution of these sequences throughout the geographic range of the population or species. In this case, there was no detection of mutation. It is possible that a small sample size may have contributed to the apparent homogeneity of the COI fragment, but this would not explain the observed homogeneity between these samples and those from Bermuda (Snell, 1997).

In summary, there was no congruence between morphological and genetic data in this study of *M. cavernosa* from the FGB and the FK. The reason for this variation or lack thereof is not yet known. The fact that lack of variation in the genetic data was not paralleled by the morphological data indicates that identification of a coral population or species using a single criterion may result in unexpected or unexplainable results.

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