Similar modifications of RNA's have been detected in other systems. For example, the 3′ ends of guide RNA's that participate in RNA editing of mitochondrial transcripts in kinetoplastid protozoa are post-transcriptionally elongated by UMP addition (*31*). The two other known types of RNA's with cyclic phosphates at their 3′ ends are both metabolically active, being intermediates in RNA cleavage and ligation events: the 5′ splicing intermediates of intron-containing tRNA's of mammalian cells (*32*) and the cleavage products of hammerhead and hairpin ribozymes (*33*). In a similar fashion, the cyclic phosphate of U6 RNA may participate directly in one of the steps of pre-mRNA splicing by forming a transient, covalent linkage with another spliceosomal component.

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Sibling Species in *Montastraea annularis*, Coral Bleaching, and the Coral Climate Record

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Measures of growth and skeletal isotopic ratios in the Caribbean coral *Montastraea annularis* are fundamental to many studies of paleoceanography, environmental degradation, and global climate change. This taxon is shown to consist of at least three sibling species in shallow waters. The two most commonly studied of these show highly significant differences in growth rate and oxygen isotopic ratios, parameters routinely used to estimate past climatic conditions; unusual coloration in the third may have confused research on coral bleaching. Interpretation or comparison of past and current studies can be jeopardized by ignoring these species boundaries.

MONTASTRAEA ANNULARIS (ELLIS and Solander, 1786) is the most abundant, wide-ranging, and intensively studied reef-building coral of the tropical western Atlantic (*1, 2*). Skeletal characters in this species (*3, 4*) are routinely used to assess local and global environmental change (*5–7*), but temporal and spatial comparisons involving multiple colonies are problematic if the enormous variation in colony morphology shown by this species (*1, 8*) has a genetic basis. No systematic examination of genetic influences on colony morphology has been attempted, however.

We recognized three often sympatric, discrete morphotypes of *M. annularis* on Panamanian and Venezuelan reefs using the following criteria in the field: Morphotype 1 (Fig. 1A) has small polyps, and large colonies form groups of columns that widen distally. Living tissue is found mainly on tops of columns, and margins are senescent. Morphotype 2 (Fig. 1B) also has small

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Table 1. Morphometric comparisons of the three morphotypes [means ($\bar{x}$) and standard deviations (SD)]. *P* values indicate significance for differences among morphotype means (ANOVA) and groups show where significant ($P < 0.05$) pair-wise differences between morphotype means occur (Newman-Keuls). All characters with statistically significant pair wise differences also showed significant differences among morphotypes by nonparametric tests (Kruskal-Wallis, all *P*'s ≤ 0.033). All characters except number of septa entered into the stepwise discriminant function analysis ($P < 0.0001$ in all cases).

Morph	*n*	Diameter (mm)				Septa dimensions (mm)						Number of septa		Intercalical spacing (mm)			
		Calice		Columella		Width 1°		Length 1°		Length 3°				Maximum		Minimum	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
1	10	2.34	0.15	1.02	0.09	0.20	0.02	0.81	0.07	0.28	0.03	24.30	0.79	1.89	0.30	1.16	0.11
2	10	2.35	0.13	0.90	0.10	0.17	0.02	0.80	0.08	0.23	0.06	23.83	0.40	1.69	0.21	0.78	0.16
3	10	2.60	0.25	1.14	0.12	0.20	0.02	0.83	0.10	0.33	0.07	24.92	1.44	2.55	0.36	1.29	0.22
P		0.0043		0.0001		0.0008		0.6115		0.0014		0.0597		0.0000		0.0000	
Groups		3>2,1		3>1>2		3,1>2				3,1>2		3>2		3>1,2		3,1>2	

polyps, but large colonies form massive heads or sheets with actively growing, sometimes skirt-like edges. Conical or keel-like protrusions are common, and sheets may cascade down reef faces. Morphotype 3 (Fig. 1C) has bigger, unevenly exsert polyps, giving a bumpy appearance. Edges and prominent polyps are often pale but appear to be actively growing. Large colonies are usually irregularly massive, although columns that narrow distally and plates are seen.

Morphotype 2 has the shallowest distribution and morphotype 3 the deepest, but all three commonly occur together in 5 to 10 m depth, where all but the smallest colonies can be unambiguously assigned to morphotype. The sympatric occurrence of discrete morphotypes suggests the presence of unrecognized sibling species. Species boundaries in corals are best defined by multiple independent methods (*9*), so we distinguished the morphotypes using behavioral, biochemical, and micromorphological criteria.

Rapid, contact-induced mortality (here termed "aggression") between paired colonies provides a sensitive assay for detecting sibling species in corals (*9, 10*). Pairings (*11*) between colonies of different morphotypes always resulted in conspicuous damage to the subordinate (Fig. 1D), typically within one day, a response never observed between clearly conspecific corals in other studies (*10, 12*). Dominance was hierarchical, with morphotype 3 the most aggressive and morphotype 1 the least aggressive without exception. In contrast, intramorph aggression was usually absent and, when present, was significantly delayed and less severe (*13*).

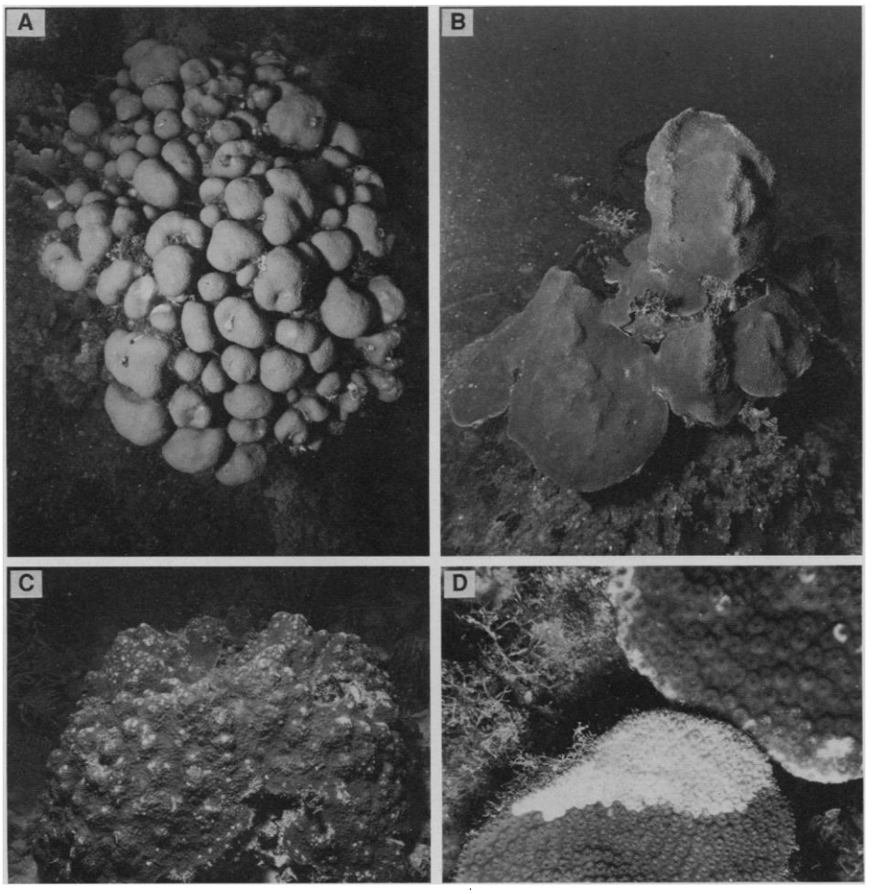

Fig. 1. Examples of colony morphology and aggressive interaction. (**A**) Morphotype 1, showing tissue capped columns, with algae growing on nonliving sides. (**B**) Morphotype 2, showing keels and skirt-like edges. (**C**) Morphotype 3, showing irregular form, bumpy surface, and scattered, pale polyps. (**D**) Aggressive interaction between morphotypes 3 (upper right) and 2 (lower left), showing recently killed part of morphotype 2 (seen as white skeleton). Note conspicuous differences in polyp size between the two morphotypes and pale growing edge of morphotype 3. [Smithsonian photographs, Carl Hansen]

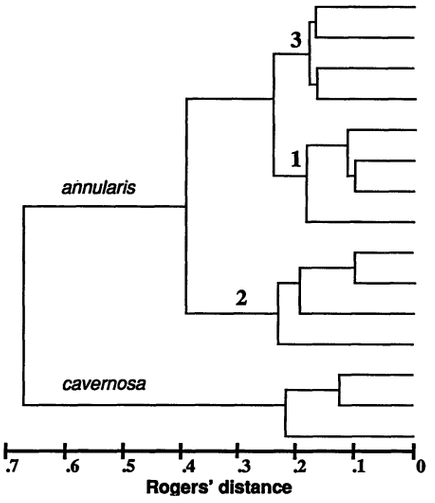

Fig. 2. Dendrogram from average linkage cluster analysis (unweighted pair group method) (*15, 16*) for populations of the three morphotypes of *M. annularis* and its congener *M. cavernosa* (species and morphotypes indicated on branches). Each of the morphotypes was sampled at four reefs (*11*), three of which also provided samples of *M. cavernosa*. Standard deviation, 10.4%, cophenetic correlation, 0.99. Wagner trees with the same coefficient had identical topologies at the species level.

Table 2. Growth rate, banding, and isotopic comparisons of the three morphotypes. Data and analyses as for Table 1, except proportion with scorable bands was analyzed by Fisher's exact test (morphotypes 1 and 2 combined). There was no relation between sites or depths and growth or isotopic ratios. Isotopic ratios are expressed by standard [δ (per mil)] notation relative to the PDB-1 standard (*4*).

Morph	Banding (scorable)		Vertical growth (mm)						Isotopic ratios				
			1987		1988		1989		$\delta^{13}C$			$\delta^{18}O$	
	Yes	No	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	*n*	$\bar{x}$	SD	$\bar{x}$	SD
1	11	0	7.4	2.4	7.7	2.4	8.3	2.5	10	−2.4	0.3	−4.6	0.2
2	13	1	4.9	1.9	4.9	2.1	4.5	1.7	10	−2.0	0.6	−4.1	0.3
3	10	5	4.4	1.0	4.6	1.5	4.0	1.3	10	−2.1	0.6	−4.1	0.3
P	0.0209		0.0018		0.0019		0.0000			0.1506		0.0006	
Groups	1+2>3		1>2,3		1>2,3		1>2,3					1<2,3	

Protein electrophoresis is routinely used to recognize sibling species (*14, 15*). We reliably scored nine loci (*16*), all polymorphic, for the morphotypes of *M. annularis* and their only sympatric congener, *M. cavernosa*. These two taxa, thought to have diverged at least 20 million years ago (*17*), were readily distinguished at five loci and had an average Nei's unbiased genetic distance of 1.10. Within *M. annularis*, morphotype 2 was also clearly distinct, with Nei's unbiased genetic distances of 0.24 (for morphotype 2 versus 1) and 0.26 (2 versus 3); this level of divergence is consistent with that reported for congeneric pairs of invertebrates (*18*). At reefs and depths where all morphotypes occurred in close proximity, the ME-1 locus was diagnostic (*14*) (P = 0.054, for 2 versus 1, and P = 0.002, for 2 versus 3). These results are not due to cloning, as we found no individuals with identical genotypes. Morphotypes 1 and 3 were much more similar, with a Nei's unbiased genetic distance of only 0.06. Nevertheless, standard clustering algorithms using Rogers' modified genetic distances grouped the four sampled populations of each morphotype before joining morphotypes 1 and 3 (Fig. 2). At sites of sympatry, the probability of misidentification (*14*) between morphotypes 1 and 3 is low (P = 0.056) when four loci (GDH-2, PGM-1, PEP A-1, and PEP D-1) are considered simultaneously.

Systematists have traditionally used skeletal characters of individual corallites to define species of *Montastraea* (*17*). Standard measures (*17*) (Table 1) were taken from colonies collected from the same reef and overlapping depth ranges (*19*). Discriminant analyses (*17*) readily separated the morphotypes, even though most differences (including all those separating morphotypes 1 and 2) were not initially apparent. The morphotypes were significantly different (all pairwise P's < 0.001; group covariance matrices not significantly different by Box's M, P > 0.12), and 90%, 100%, and 90% of individual colonies of morphotypes 1, 2, and 3, respectively, were correctly assigned. When canonical discriminant function scores were displayed in two dimensions, polygons enclosing all individuals of the same morphotype did not overlap, and there was no tendency for colonies from similar depths to have similar morphologies. Earlier morphologic studies (*17*) failed to detect these species because analyses were based primarily on morphotype 2 (*20*); the only explicit attempt to determine the relation between colony and corallite morphology used a measure of colony shape (*21*) that would not distinguish these morphotypes efficiently.

The standard, independent techniques we used provide completely consistent and therefore unambiguous confirmation of the specific distinctiveness of the three shallow-water morphotypes of *M. annularis* (*22*). Past failure to distinguish them reflects long-standing emphasis on the importance of nongenetic sources of variation in colony form (*8, 23, 24*). Marked sympatric variation in shallow-water colony form, although sometimes noted (*1, 8, 24*), has rarely been pursued (7). The unrecognized species are probably widespread, as they can be identified in photographs from elsewhere [for example, Jamaica (*1, 8*)]. We did not study platey forms characteristic of deeper water (*25*), where additional species may be found.

The morphotypes also differ significantly in growth rates, banding patterns, and isotopic ratios (Table 2) (*26*). Morphotype 1 showed higher growth rates than the others, whereas morphotype 3 was more often unscorable due to irregular growth. Highly significant differences in average $\delta^{18}O$ values are equivalent to those produced by major oceanographic temperature differences of approximately 2.5°C (*5*). Both the $\delta^{18}O$ pattern and the nonsignificant trend for $\delta^{13}C$ are consistent with predictions based on growth rate differences (*27*).

Past analyses of growth and isotopic ratios have been primarily based on sections of columns from morphotype 1 or cores from morphotype 2, with morphotype 3 being lumped with either. Thus recognition of differences among morphotypes clarifies poorly understood variation and patterns formerly attributed to environmental effects, such as depressed shallow-water growth rates, unpredictable banding, and regional differences in growth and oxygen isotopic ratios (*3, 4, 24*). Studies of bleaching in *M. annularis* are similarly affected. The scattered pale polyps of morphotype 3 (Fig. 1C) have been classified as bleached, but are probably a natural color pattern (*28*). In general, environmental interpretations of differences between colonies, sites and times are suspect without confirmation that only one morphotype was sampled. The methods provided above can be used to identify specimens still available in the field or in collections.

Acceptable signal-to-noise ratios in bioassays depend on accurate species identifications, because substantial biological differences among morphologically similar, sympatric species are common (*29*). Applied research is not the only area compromised by failure to recognize sibling species. Much ecological and evolutionary research is also meaningless without reliable taxonomy at the species level, which even well-studied taxa may lack.

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pairings. Each class had a minimum of five pairs from the same reef and five pairs from different reefs (one 1-3 interreef pair was later lost). Pairs were distributed in ten groups at a 10-m depth along Aguadargana Reef, with different types of pairings evenly distributed among the groups to avoid confounding location with treatment. Initially healthy areas of paired corals were separated by 1 to 2 mm, and monitored daily. Corals were subsequently collected within a 3-day interval for laboratory analyses.
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28. Bleaching of Jamaican *M. annularis* occurs in two forms, and that resembling morphotype 3 is temporally stable and unrelated to sea temperature [R. D. Gates, *Coral Reefs* **8**, 193 (1990)]; see also A. M. Szmant and N. J. Gassman, *ibid.*, p. 217. If morphotypes differ in propensity to bleach, a morphotype difference in $\delta^{18}O$ could be mistaken for a temperature signal, because the differences are comparable in magnitude [J. W. Porter, W. K. Fitt, H. J. Shapiro, C. S. Rogers, M. W. White, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 9342 (1989)].
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Induction of Broadly Cross-Reactive Cytotoxic T Cells Recognizing an HIV-1 Envelope Determinant

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An immunodominant determinant for cytotoxic T lymphocytes (CTLs) exists in the hypervariable portion of human immunodeficiency virus-1 (HIV-1) gp160. Three mouse CTL lines (specific for isolates MN, RF, and IIIB) were examined for recognition of homologous determinants from distinct isolates. Only MN-elicited CTLs showed extensive interisolate cross-reactivity. Residue 325 played a critical role in specificity, with MN-elicited CTLs responding to peptides with an aromatic or cyclic residue and IIIB-induced cells recognizing peptides with an aliphatic residue at this position. CTL populations with broad specificities were generated by restimulation of IIIB-gp160 primed cells with MN-type peptides that have an aliphatic substitution at 325. This represents an approach to synthetic vaccines that can generate broadly cross-reactive CTLs capable of effector function against a wide range of HIV isolates.

THE ENVELOPE GLYCOPROTEIN GP160 has been used in numerous prototype vaccine preparations designed for prophylaxis against or immunotherapy of infection by HIV-1 or its close simian lentivirus relatives (*1–4*). Studies in humans and mice have revealed a small region of this protein, called the V3 loop, between cysteine residues 303 and 338, that evokes the major neutralizing antibodies to the virus (*5–7*) and stimulates both helper and cytotoxic T cell responses in both species (*8–11*). This same region is one of the most variable in sequence among different clonal isolates (*12, 13*); this variation may arise by selection of mutant virus as a result of the intense immune pressure directed against this region of the molecule (*14–17*). Thus, this segment of gp160 is both an attractive candidate for a major component of an acquired immunodeficiency syndrome (AIDS) vaccine because of its known antigenic properties, and a problem for the design of useful vaccines because of the extensive diversity in its structure. Because an effective anti-HIV vaccine strategy must anticipate to the greatest extent possible such potential changes in viral antigenicity, we have examined in detail the specificity of CTL recognition of diverse HIV-1 isolates and describe a method for immunization that generates broadly reactive CTLs with an enhanced capacity to respond to variant sequences at this critical immunodominant site.

We could elicit from BALB/c (H-2^d) mice CTL specific for the peptide SITKGPGRVIYATGQ (18RF), the segment of the HIV-1 RF isolate corresponding to the 315 through 329 region of gp160 IIIB previous-

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