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Intraspecific variation of a dominant Caribbean reef building coral, *Montastrea annularis*: genetic, behavioral and morphometric aspects

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ABSTRACT: We investigated intraspecific variation of the dominant Caribbean reef building coral *Montastrea annularis* (Ellis & Solander) in terms of genetic variation (protein electrophoresis), intraspecific interaction and micro/macro morphometry. Our study included 3 sympatric morphotypes, 'Bumpy', 'Massive' and 'Columnar', distinguishable within *M. annularis* populations on the leeward coasts of Curaçao and Bonaire (Netherlands Antilles). The genetic study demonstrated 8 polymorphic and 1 monomorphic loci. The mean number of alleles over all loci was 4.7, and the average heterozygosity (H) over all loci examined was high (0.36). One out of 9 taxonomic units showed a significant heterozygote deficiency; the others matched expectation. The *M. annularis* morphotypes showed a significant variation in allele frequencies but no fixed differences were found. The 'Columnar' and 'Bumpy' morphotypes were more similar with a genetic distance of 0.07. The 'Massive' morphotype demonstrated larger genetic distances: 0.13 with 'Columnar' and 0.16 with 'Bumpy' The 'Bumpy' morphotype was dominant over the other 2 morphotypes in the intraspecific interaction experiments, and 'Massive' was dominant over 'Columnar' The percentage of interactions was lower in intra-morphic experiments. Of the 22 micro-morphometric parameters examined, 14 showed significant differences between the 3 morphs. In addition the mean number of polyps per cm^2 was very different: values ranged from 28.55 for 'Bumpy' to 40.97 for 'Columnar'

INTRODUCTION

That corals display a high morphological plasticity is well known (Wood-Jones 1907, Stephenson & Stephenson 1933, Barnes 1973, Foster 1979). However importance of this variation in relation to evolutionary and life history tactics is not well understood. More understanding is required on relations between genotypic and phenotypic variation as well as phenotypic variation and fitness in different environments.

The factors reported to cause intraspecific variation are abiotic factors (Roos 1967, 1971, Barnes 1973, Dustan 1975, Foster 1977, 1979, Graus & Macintyre 1982) and genetic variation (Ohlhorst 1979, Stoddart 1984a, b, Willis & Ayre 1985, Ayre et al. 1991). Understanding colony morphology in relation to environmental parameters is very difficult because of lack of understanding of ecological strategies of the taxa, for example feeding and sediment removal mechanisms (Wijsman-Best 1974, Foster 1977, 1979).

As a taxonomic tool at interspecific and intraspecific levels, enzyme electrophoresis is well known (Thorpe 1983). This technique has been used in coral research (Ohlhorst 1979, Stoddart 1984a, b, Ayre et al. 1991), sometimes combined with histocompatibility experiments (Willis & Ayre 1985). The shortage of data sets combining genetic variation with morphological and environmental data, however, makes comparisons between morphologically variable taxa difficult. More genetic analyses are required to achieve a better understanding of breeding systems and population biology of corals (Stoddart 1983), e.g. heterozygosity values, Hardy-Weinberg equilibria and genetic distances.

Montastrea annularis (Ellis & Solander) and *Acropora palmata* (Lamarck) are recognized as major reef framework builders of Atlantic reefs (Van Duyl 1985).

Since the recent decline of *A. palmata* throughout much of its range due to disease and effects of pollution (Gladfelter 1982, Van Duyl 1985), *M. annularis* may be the most important species in terms of reef structure and reef habitat building. It is probably the most frequently investigated Atlantic coral species. Aspects investigated include growth and form (Dustan 1975, Hudson 1981a, b, Graus & MacIntyre 1982, Goenaga 1988), carbon budgets (Porter 1985), eutrophication (Tomascik & Sander 1987), regeneration (Bak et al. 1977, Lester & Bak 1985), survival and mortality (Bak & Engel 1979, Bak & Luckhurst 1980, Hughes & Jackson 1980), morphometrics (Foster 1977, 1979), genetics (Ohlhorst 1979, Knowlton et al. 1992), interspecific interactions (Lang 1973, Bak et al. 1982), reproduction (Szmant-Froelich 1985, Szmant 1986), sediment rejection (Szmant-Froelich et al. 1981, Dodge 1982, Parker et al. 1984), black band disease (Ramos-Flores 1983), and bleaching (Hayes & Bush 1990, Szmant & Gassman 1990, Meesters & Bak 1993). Barnes (1973) previously reported several growth forms of *M. annularis*, but until recently (Tomascik 1990, Knowlton et al. 1992) most studies neglected to describe the morphotype studied. If *M. annularis* is to be used as a biological monitor in Atlantic reefs, as suggested (Ogden & Gladfelter 1986, Tomascik 1990), a proper description of differences in life history aspects in relation to growth form variability is required. This is because repudiating the variability of growth form may lead to higher variability in research outcome and misinterpretation of the data when data sets of different places and researchers are compared.

This study presents our first results on intraspecific variation in the *Montastrea annularis* communities at Curaçao. We distinguished 3 different sympatric morphotypes: 'Bumpy', 'Massive' and 'Columnar', and will treat differences in (1) genetic variation (protein electrophoretic), (2) intraspecific interactions, and (3) micro/macro morphometry.

MATERIAL AND METHODS

Morphotypes. The 3 morphotypes of *Montastrea annularis* are illustrated in Fig. 1. These sympatric growth forms are strikingly different and easily distinguishable underwater:

(1) 'Bumpy' (B): Colonies are massive. Polyps are irregularly oriented and usually larger than those in other morphotypes. The tissue is usually brown, though white or discolored spots are often present. This morphotype is found from intermediate to deep water (10 to 45 m). Collections were made between 11.5 and 27 m. Equivalent names are 'Irregular-massive' (Barnes 1973), 'Lumpy-massive' or 'Flat plates with lumpy surfaces' (Dustan 1975), or 'Morphotype 3' (Knowlton et al. 1992).

(2) 'Massive' (M): Massive colony whose surface can be smooth, raised with knobs, or extended in ridges. The tissue is green or brown. Often, the oral disk is colored lighter. The polyps are uniformly arranged. This morphotype is abundant between 1 and 30 m. In deeper water (10 to 30 m) colonies usually form rosettes of separate plates on the lower sides of the colony. Collections were made between 3.5 and 20.5 m. In earlier studies this morphotype is reported as 'Plate-like' or 'Rounded colonies' (Barnes 1973), 'Round-bulbous', 'Skirted massive hemispherical' or 'Flat-plate colonies' (Dustan 1975), 'Lobate' (Tomascik 1990), or 'Morphotype 2' (Knowlton et al. 1992).

(3) 'Columnar' (C): Colonies consist of pillar-like columns with a smooth surface. Tissue is only found over the apex of columns. Polyps are usually brown, including the oral disk, and uniformly arranged. This morphotype occurs between 1 and 30 m deep but is more abundant in shallow waters. For our study, it was collected between 3.5 and 20 m. 'Columnar-lobate' (Barnes 1973), 'Knobby-massive', 'Columnar-lobate' (Dustan 1975), 'Columnar' (Tomascik 1990), or 'Morphotype 1' (Knowlton at al. 1992) may be considered as equivalent.

Material. Specimens of the *Montastrea annularis* morphotypes were collected at 3 localities on the leeward coast of Curaçao (Fig. 2): Awa Blancu (AB), CARMABI buoy 1 (B1) and Slangenbaai (SB). The distances between the sites are 20 and 4 km, respectively. At each locality corals were collected within an area of 400 m. For description of the sites, see Van Duyl (1985). Parts of colonies were collected haphazardly, avoiding intermediate forms, using hammer and chisel. All collections and field observations were made using SCUBA.

Electrophoretic analyses. ***Sample collection and preparation:*** For each of the 3 study sites, 25 individuals per morphotype were sampled. After removing epiphytic organisms, corals were transported in seawater to a laboratory running seawater system. In the laboratory the living coral tissue and underlying skeleton were scraped off with a chisel, placed in a cryovial containing a few drops of grinding buffer (Stoddart 1983), frozen, and immediately stored in liquid nitrogen.

Electrophoresis: Electrophoretic procedures were performed upon 15 individuals of each population following the methods and terminology of the *Montastrea annularis* data by Knowlton et al. (1992). Our investigation was performed at the same laboratory (STRI, Panama).

Samples were ground by hand on cold plates with several drops of grinding buffer (Stoddart 1983). Before soaking filter paper in the protein solution, a

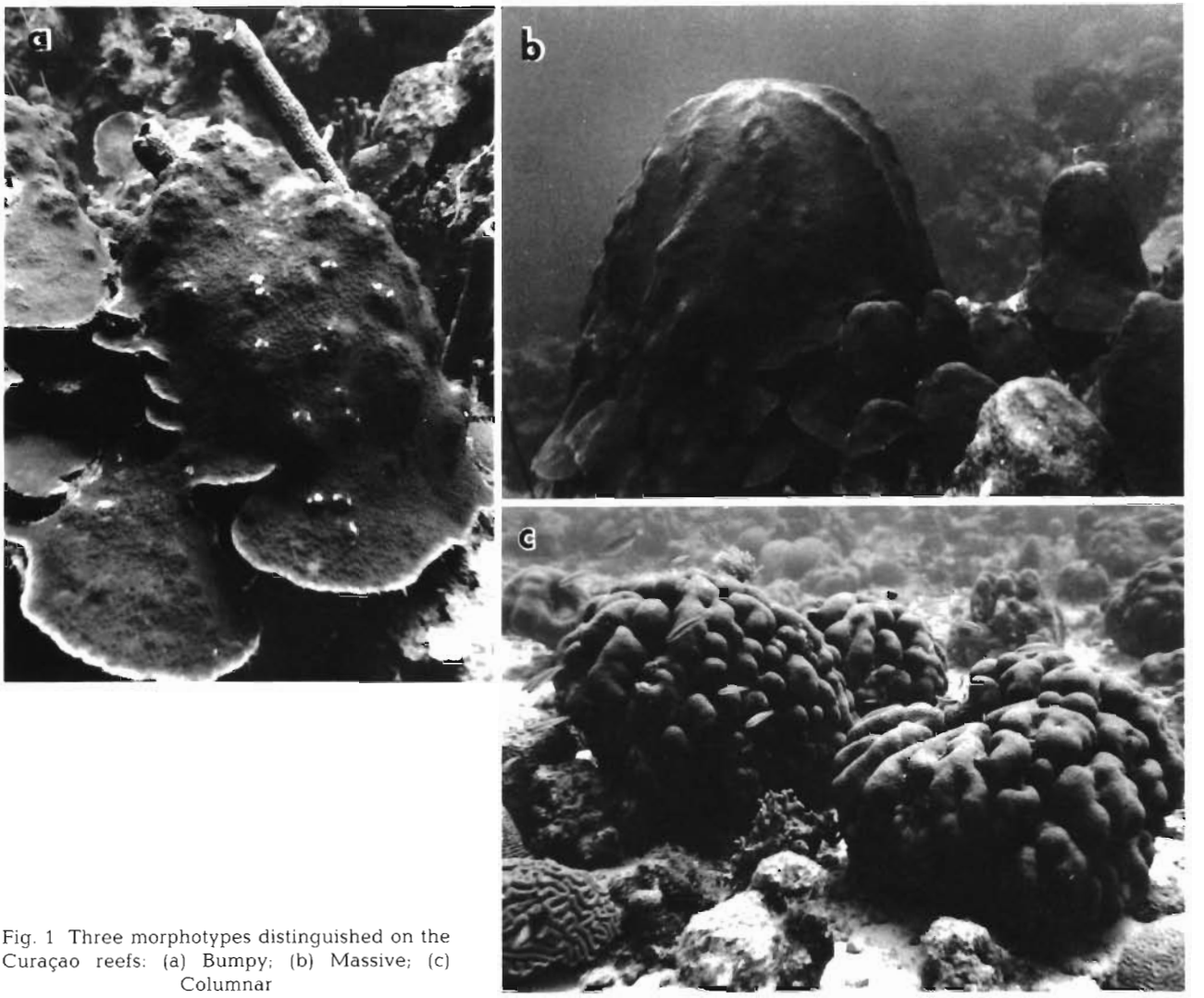

Fig. 1 Three morphotypes distinguished on the Curaçao reefs: (a) Bumpy; (b) Massive; (c) Columnar

Miracloth filter was placed on top of the homogenized sample. Starch gels containing 15 % potato starch (Sigma, S-4501) were used. Three different continuous buffers were used: Tris-citrate (bridge: Tris/Citric acid, pH = 8.0; gel:Tris/Citric acid, pH = 8.0 1:29 dilution), Lithium Hydroxide (bridge: Tris/Citric acid/NaOH pH = 8.4; gel: LiOH/boric acid pH = 8.1 and bridge solution 9:1 dilution) and Ridgeway (bridge: LiOH/boric acid pH = 8.5; gel: Tris/Citric acid pH = 8.5 1:100 dilution). In total 17 different enzyme stainings were initially screened: Isocitric Dehydrogenase (ICD), Aspartate Aminotransferase (AAT), 6-Phosphogluconate Dehydrogenase (6-PGDH), Malate Dehydrogenase (MDH), Esterase (EST), Octopine Dehydrogenase (ODH), Non-Specific Protein (NSP), Catalase (CAT), Leucyl-amino Peptidase (LAP), Mannose Phosphate Isomerase (MPI), Glucose Phosphate Isomerase (GPI), Phosphoglucomutase (PGM), Malic Enzyme (ME), Glutamate Dehydrogenase (GDH), Triosephosphate Isomerase (TPI), Leucyl-Tyrosine Peptidase (LTP), Leucyl-Proline Peptidase (LPP). The products of the last 7 stainings showed a consistent and usable pattern. In the final screening process 2 different buffer systems and 7 stainings were used: the TC 8.0 buffer for GPI, ME, GDH and PGM and the LiOH buffer for TPI, LTP and LPP. As a control we used one colony of *Tubastrea coccinea* (Lesson), which showed a consistent and clear pattern in all of the stainings; *T. coccinea* was chosen because it is easy to collect and to grind.

Allele frequencies, heterozygosity (H), Nei's unbiased genetic distance (D) and identity (I) indices (Nei 1978) were computed. The hypotheses that each population is in Hardy-Weinberg equilibrium was tested with Chi-square tests. Analyses were performed using the BIOSYS-1 (release 1.7) computer program of Swofford & Selander (1981).

Intra-colony variance: In order to insure that isolated parts of the same colony are ramets, 2 isolated

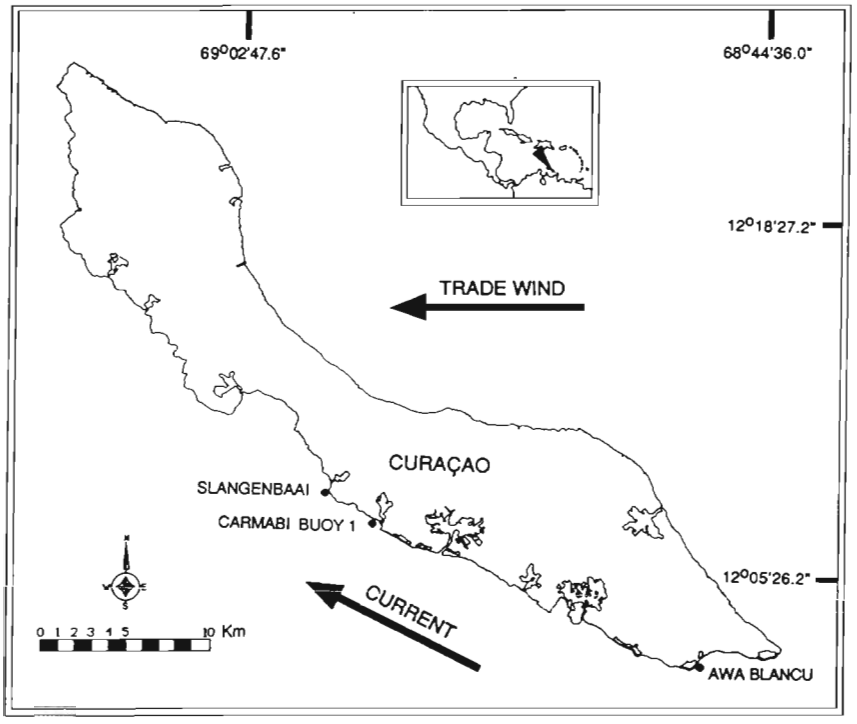

Fig. 2. Location of sampling stations on Curaçao, Netherlands Antilles

parts from one colony were collected and electrophoretically screened running next to each other (total: 5 Massive and 3 Columnar).

Interaction experiments. ***Sample collection and setup:*** To study aggressive interaction corals were collected and placed in contact at a depth of 4 to 6 m (Location B1). Interaction pairs were examined at intervals over a period of up to 30 d. We carried out 3 different interaction experiments:

(1) Isogenic contacts: 2 parts of the same colony were placed in contact (n = 240).

(2) Intra-morphic contacts: 2 parts of the same morphotype, but from different colonies, were placed in contact (n = 176).

(3) Inter-morphic contacts: coral parts of different morphotypes were placed in contact (n = 179).

To avoid possible complications due to the 1990 coral bleaching event (Meesters & Bak 1993), we performed our experiments between July and September 1990 and January and February 1991.

Interpretation of results: At each survey of the interaction series all interactions for each coral were scored and used as data points in the analysis. The maximal length of damage and length of the contact area were measured with calipers to the nearest 0.1 mm. Categories were: (1) no interaction: coral tissue undamaged; (2) equally aggressive: coral tissue of both interacting colonies damaged, the ratio between the damaged areas being less than 2; (3) dominant: one colony not damaged or slightly damaged (ratio between damaged areas greater than 2). When a pair of experimental colonies was disturbed , i.e. not in contact, it was not included in the analysis. Chi-square tests for 2 independent samples (Siegel 1956) were performed to compare experiments.

Comparisons of electrophoretic and interaction experiments. Altogether 47 non-isogenic interaction pairs were included in the electrophoretic analyses. The percentage of alleles shared by each pair was correlated with the results of interactions. Correlations were tested using a single classification ANOVA with unequal sample size (Sokal & Rohlf 1981).

Morphometrics. ***Micro-morphometrics:*** Twelve micro-morphometric corallite measurements (Table 1, Fig. 3) were taken, of which 10 were measured for minimum and maximum values. Ten specimens of each morphotype collected at B1 (Fig. 2) were measured. On each of these, 10 haphazardly chosen mature

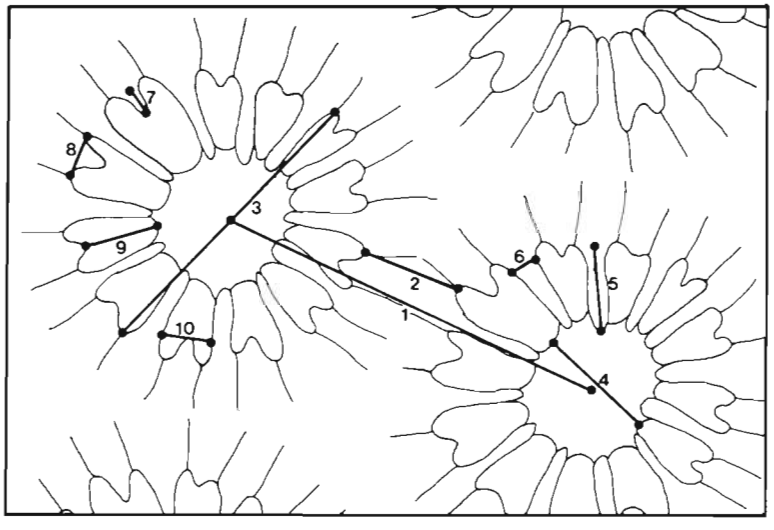

Fig. 3. Corallite showing 10 of the 12 micro-morphometric measurements

Table 1. List of morphometric measurements of *Montastrea annularis* as illustrated in Fig. 3

No.	Character	Abbr.	Description
1	Columella distance	CuD	Linear measure from columella centre to neighboring columella centre
2.	Corallite spacing	CaS	Linear measure between theca/coenosteum margins of neighboring corallites
3.	Corallite diameter	CaD	Linear measure between theca/corallite cavity margins across the columella
4.	Columella width	CuW	Linear measure between outer columella/corallite cavity margins
5.	Primary septa length	PsL	Linear measure between theca/corallite cavity margin and outer columella/corallite cavity margin
6.	Primary septa thickness	PsT	Linear measure of exoseptum thickness; measured at the outer theca/corallite cavity
7.	Secondary septa length	SsL	Linear measure between entoseptum tip and theca/columella margins
8.	Secondary septa thickness	SsT	Linear measure of exoseptum thickness; measured at the outer theca corallite cavity
9.	Theca length	ThL	Linear measure from entoseptum tip to outer columella/corallite margins
10.	Theca width	ThW	Linear measure of theca thickness, measured just above the entoseptum tip
11.	Primary septa count	PsC	Number of 1st and 2nd cycle septa
12.	Secondary septa count	SsC	Number of highest cycle septa

corallites, located at least 2 cm from the colony edge, were examined using the JAVA V1.20 program (Jandel Scientific, Corte Madera, CA). In Columnar morphotypes, 5 corallites at the apex and on the side were examined. Mean and standard deviation of all corallite characters were computed for each colony and each morphotype. Single classification ANOVA's with 2 and 3 groups were used to test significant differences between the measurements (Sokal & Rohlf 1981).

Macro-morphometrics: In 268 coral fragments, the number of calices in a standard circle of 7.6 cm^2, located at least 1 cm off the colony edge, were counted. Columnar colonies were surveyed at the apex as well as on the side. A 2-way ANOVA (Sokal & Rohlf 1981) was used to test if there was a significant difference between localities and morphotypes.

RESULTS

Electrophoresis

The electrophoretic analyses were encoded by 8 polymorphic and one monomorphic loci for 3 *Montastrea annularis* morphotypes (total n = 135) at the 3 localities. Isozymes were identified by their anodal mobility, where A migrated further than B. An exception was made with the *Tpi-2* locus allele J which migrates further than the A allele on this locus. After screening the allelic composition of all samples, we can conclude that all individuals showed a unique genotypic pattern. The 8 colonies tested for intra-colony variation showed the same allele pattern.

Allelic frequencies. Table 2 shows the allele frequencies for the 9 examined loci. Although the frequency distributions of the morphotypes were significantly different (G-test, $p < 0.001$), none of the loci was found to be diagnostic. The *Me-1* locus, with 6 alleles, was found to be most variable; the C (0.35, 0.06 and 0.25), D (0.55, 0.11 and 0.51), and E (0.06, 0.71 and 0.08) alleles showed the highest variation for Bumpy, Massive and Columnar respectively. The mean number of alleles per locus was calculated at 4.7.

Heterozygosity. The mean observed heterozygosity (H) values per locus ranged from 0.290 for Columnar (Location SB) to 0.44 for Massive (Location AB). The mean observed heterozygosity, H, (standard error) values per morphotype were: Bumpy = 0.34 (0.08); Massive = 0.38 (0.09); and Columnar 0.37 (0.07).

Hardy-Weinberg equilibrium. Chi-square tests demonstrated that 25 % of the polymorphic loci tested departed highly ($p < 0.06$); and 17 % significantly

Table 2. *Montastrea annularis*. Allele frequencies at 9 loci in 3 morphotypes of coral from sampling sites in Curaçao

Locus, (sample size), allele	Morphotypes Bumpy	Massive	Columnar
Tpi-1			
(N)	43	45	45
A	.012	.044	.156
B	.988	.944	.833
C	–	.011	.011
Tpi-2			
(N)	45	45	45
A	.333	.033	.022
B	–	–	–
C	.289	.489	.200
D	–	–	–
E	–	–	.011
F	.356	.467	.744
G	–	–	.011
H	.022	–	.011
I	–	–	–
J	–	.011	–
Gpi-1			
(N)	45	43	40
A	–	–	–
B	–	–	–
C	.044	.012	.013
D	.044	.012	–
E	.856	.965	750
F	.022	–	.050
G	.033	.012	188
Me-1			
(N)	41	42	38
A	–	–	–
B	.037	–	145
C	.354	.060	.250
D	.549	107	.513
E	.061	714	.079
F	–	119	.013

Locus, (sample size), allele	Morphotypes Bumpy	Massive	Columnar
Gdh-1			
(N)	45	45	45
A	–	–	–
B	1.00	1.00	1.00
Gdh-2			
(N)	41	45	43
A	.037	133	–
B	.329	.611	.547
C	.085	.067	.174
D	.024	–	–
E	.500	.189	.279
F	.024	–	–
Pgm-1			
(N)	37	36	31
A	.027	–	0.032
B	.176	.069	.016
C	176	.333	.306
D	.514	.389	.516
E	.108	139	.129
F	–	.069	–
Ltp-1			
(N)	43	26	43
A	.012	–	.047
B	.663	173	.512
C	.302	.635	.337
D	.023	192	.105
Lpp-1			
(N)	29	39	41
A	.034	–	–
B	.017	.026	–
C	.310	.385	.037
D	.224	.333	.549
E	.414	.256	.415

($p < 0.05$); from the Hardy-Weinberg equilibrium (Table 3). For the loci *Pgm-1* and *Lpp-1*, this was 56 %. Although there was a general deficiency in the observed number of heterozygotes, only one taxonomic unit, B/SB (*G*-test, $p < 0.005$), was significantly different from the expected value. Only the taxonomic unit M/AB showed a heterozygote excess.

Genetic distance and similarity. Coefficients of unbiased genetic distance and similarity were calculated and clustered comparing the 3 morphotypes at 3 locations: a total of 9 operational taxonomic units. The dendogram (Fig. 4) shows the genetic differentiation between these taxonomic units. These results demonstrate that the morphotypes Columnar and Bumpy are more similar, with values of 0.07 (0.93), compared to the Massive morphotype, with values of 0.13 (0.88) and 0.16 (0.86) average genetic distance and similarity, respectively. Samples from the same morphotype clearly cluster together. Clustering is not consistent in terms of the geographic position of the different locations.

Interaction experiments

Tissue lesions, formed as a result of digestive mesenterial filaments, were observed the day after the beginning of the experiment. Over a 30 d period no repeated reversals occurred (Chornesky 1989).

Dominance did not occur in isogenic experiments (Table 4). Dominance was observed in the intramorphic contacts but was outnumbered by the total of non- and equal-aggression contacts. Comparing the results of the isogenic contacts with those from the

Table 3. *Montastrea annularis*. Probabilities from Chi-square tests for deviation from Hardy-Weinberg equilibrium for all polymorphic loci of the morphotype complex on Curaçao. sf = small frequencies; – = monomorphic; B = bumpy; C = Columnar; M = Massive; AB = Awa Blancu; B1 = Buoy 1; SB = Slangenbaai. Significant values in bold type

Locus	Morphotype/Location								
	B/AB	B/B1	B/SB	M/AB	M/B1	M/SB	C/AB	C/B1	C/SB
Tpi-1	sf	–	–	.847	sf	–	sf	**.058**	sf
Tpi-2	.908	.467	.886	sf	.886	.744	**.034**	.681	sf
Gpi-1	**.051**	.194	.611	.847	–	sf	.517	.516	sf
Me-1	sf	.814	**.053**	.963	.073	**.054**	**.053**	.081	.239
Gdh-1	–	–	–	–	–	–	–	–	–
Gdh-2	.371	.599	.296	**.026**	.908	.599	154	.168	.687
Pgm-1	.901	**.000**	**.040**	.089	**.043**	**.014**	.905	sf	.646
Ltp-1	.098	.358	.371	.530	.073	.311	.273	.339	.905
Lpp-1	**.036**	.095	.380	.959	.278	.814	**.003**	**.002**	**.001**

intra-morphic contacts, only the Bumpy morphotype showed a significant difference: an increase in dominant interactions (Chi-square test, $p < 0.01$).

The frequency distribution in the isogenic experiments shows that the Columnar morphotype only scored in the category 'equal aggression'; this is a significant difference (Chi-square test, $p < 0.001$) when tested against the 2 other morphotypes.

The number of dominant scores in the inter-morphic contacts is high, and the proportions differed significantly from the intra-morphic contacts (Chi-square tests, $p < 0.001$). In a hierarchical ranking, Bumpy was dominant over the other 2 morphotypes and Massive was dominant over Columnar.

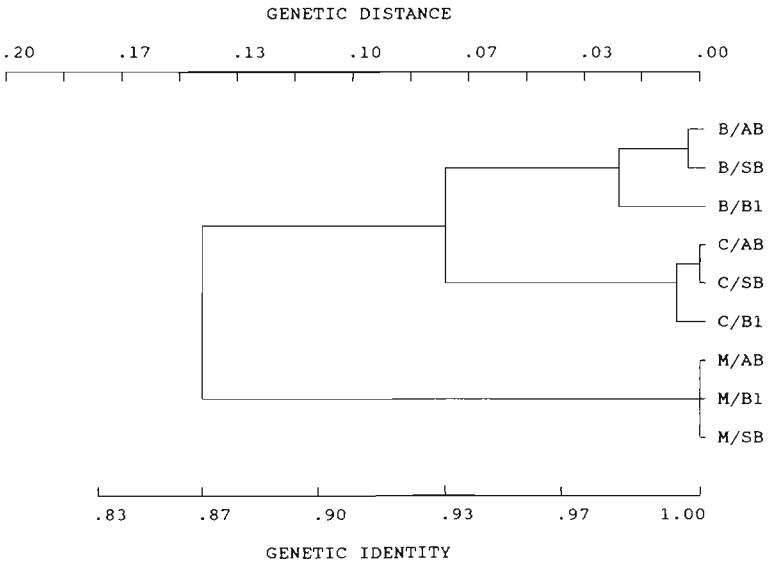

Fig. 4. Cluster dendogram based on Nei's (1978) unbiased genetic distance (upper *x*-axis) and genetic identity (lower *x*-axis) coefficients for comparisons of 9 loci in the Caribbean coral *Montastrea annularis* from 3 sampling sites on the leeward coast of Curaçao. (Abbr.: B = Bumpy; C = Columnar; M = Massive; AB = Awa Blancu; B1 = Boei 1; SB = Slangenbaai)

Electrophoresis versus interaction results

The mean number of alleles shared by 1 of the 3 possible outcomes of interaction ranged from 62.01 to 66.22 % for no interaction and dominance, respectively. No significant differences were found by a 2-way ANOVA ($F_{[2,43]} = 0.02$) between the interaction outcome and the percentage of alleles shared between the 2 opponents.

Morphometrics

Micro-morphometrics. A single classification ANOVA was carried out to test for significant differences between means of micro-morphological measurements (Table 5). For the Columnar morphotype, data of the apex of the colony and side measurements differed significantly only for one character, corallite spacing (min) (Table 6; ANOVA, $p < 0.05$). Consequently these data were pooled in further comparisons. Bumpy tested against Massive and Columnar resulted in 12 and 11 significantly different characters respectively (Table 6; ANOVA, $p < 0.05$). Only 6 were different when testing Massive against Columnar. The character CaS-min was the only one differing significantly when comparing all test possibilities.

Macro-morphometrics. Within the Columnar morphotype the number of polyps on the apex of the colony was significantly higher than that on the side (Table 6; ANOVA, $p < 0.001$). To facilitate comparison with the 2 other morphotypes, only the apex measurements were used in further testing. The number of polyps per

Table 4. *Montastrea annularis*. Scores in interaction experiments. B = Bumpy; M = Massive; C = Columnar; N = total of scored observations. Observed scores see text

Experiment	Observed scores None	Equal	Dominant	N
Isogenic contacts				
B - B	60	7	0	67
M - M	46	3	0	49
C - C	0	51	0	51
Intra-morphic contacts				
B - B	13	9	11	33
M - M	35	4	7	46
C - C	4	43	2	49
Inter-morphic contacts				
1st 2nd			1st 2nd	
B - M	7	2	31 - 2	42
B - C	0	6	35 - 0	41
M - C	4	9	33 - 3	49

surface unit was significantly different between the morphotypes (Table 6; 2-way ANOVA, $p < 0.001$), but there was no significant difference between localities.

DISCUSSION

Our experiments on intraspecific variation in the coral species *Montastrea annularis* showed significant differences in genetical, behavioral and morphometric aspects of the 3 morphotypes from Curaçao and Bonaire.

There is evidence that the banding pattern of *Montastrea annularis* in electrophoretic analysis is polymorphic (Ohlhorst 1979). In our study *M. annularis* displays a high genetic variability, indicated by a high mean heterozygosity (0.36) and mean of alleles per locus (4.7), which can be correlated with morphological characters. Olhorst (1979), in her pioneering study, found no correlation between allelic composition and colony shape or tissue color, but thought allelic composition to be related to locality.

We show that genetic variation is not a local phenomenon; all localities on Curaçao and Bonaire (Van Veghel unpubl.) display a comparable pattern in allelic frequencies. From a total of 46 alleles examined in this study and in Panama (Knowlton et al. 1992), 5 were not found in Panama and 4 were not found in this study. These were all alleles with a mean frequency of 7% or lower. Common alleles were not restricted to local populations.

No fixed or nearly fixed differences were found in the allelic composition. In contrast, Knowlton et al. (1992) found 5 (nearly) fixed differences using the same loci comparing *Montastrea annularis* with its

Table 5. *Montastrea annularis*. Average values (in mm) and standard deviations of morphometric measurements in the 3 different morphotypes. N polyps = number of polyps per 7.6 cm^2 a = colony apex; s = colony side. For other abbreviations and descriptions see Table 1

Measurement		Bumpy	Massive	Columnar
1. CuD	min	3.84 (0.66)	3.40 (0.46)	3.32 (0.53)
	max	5.33 (1.20)	4.43 (0.87)	4.23 (0.71)
2. CaS	min	1.36 (0.44)	1.10 (0.38)	0.98 (0.34)
	max	2.17 (0.64)	1.77 (0.41)	1.83 (0.54)
3. CaD	min	2.38 (0.33)	2.35 (0.18)	2.34 (0.21)
	max	2.62 (0.34)	2.54 (0.22)	2.52 (0.24)
4. CuW	min	1.04 (0.21)	0.96 (0.16)	1.01 (0.13)
	max	1.22 (0.28)	1.13 (0.18)	1.16 (0.16)
5. PsL	min	0.67 (0.11)	0.70 (0.08)	0.65 (0.11)
	max	0.83 (0.15)	0.81 (0.09)	0.79 (0.12)
6. PsT	min	0.22 (0.09)	0.23 (0.03)	0.21 (0.07)
	max	0.31 (0.10)	0.30 (0.04)	0.30 (0.09)
7. SsL	min	0.20 (0.05)	0.17 (0.05)	0.17 (0.05)
	max	0.31 (0.08)	0.30 (0.08)	0.25 (0.07)
8. SsT	min	0.16 (0.03)	0.15 (0.03)	0.14 (0.03)
	max	0.24 (0.04)	0.24 (0.05)	0.21 (0.04)
9. ThL	min	0.40 (0.09)	0.42 (0.09)	0.42 (0.09)
	max	0.58 (0.11)	0.58 (0.09)	0.58 (0.10)
10. ThW	min	0.31 (0.06)	0.30 (0.05)	0.31 (0.07)
	max	0.42 (0.07)	0.39 (0.06)	0.39 (0.06)
11. PsC		11.92 (0.58)	11.95 (0.44)	12.06 (0.55)
12. SsC		11.92 (0.58)	11.95 (0.44)	12.05 (0.59)
13. N polyps		28.55 (5.98)	38.54 (6.60)	a 40.97 (5.88)
				s 35.52 (5.43)

sympatric congener *Montastrea cavernosa* (Linnaeus). The Nei's unbiased genetic distances for Curaçao and Panama are respectively: Massive - Columnar = 0.13, 0.24; Massive - Bumpy = 0.16, 0.26; and Columnar - Bumpy = 0.07, 0.06. Values for Panama are appreciably higher than in Curaçao morphs. These differences are probably due to geographic variation, since the environmental parameters and species history of the populations are quite different.

Although a strict relation between systematic divergence and genetic measures is not generally accepted (Menken & Ulenberg 1987), Thorpe (1983) suggests that a genetic identity value (Nei 1978) between most conspecific populations should be above 0.9, where congeneric species fall within the range between 0.25 and 0.85. The genetic identity we found points to levels characteristic for conspecific populations. Coral taxonomy is based on the morphological distinctness of species, but at present the biological species concept is the most widely used (Coyne et al. 1988). We think the reproductive biology should be studied before deciding on the taxonomic status of *Montastrea annularis* morphotypes.

Montastrea annularis shows a high mean heterozygosity, $H = 0.36$, compared with a mean for marine invertebrates of $H = 0.15$, and an overall mean of $H =$

Table 6. *Montastrea annularis*. Single Classification ANOVA with 2 and 3 groups of morphometric measurements concerning the 3 different morphotypes. Significance: ••• $p < 0.001$; •• $0.01 < p < 0.001$; • $0.01 < p < 0.05$; ns = not significant. B = Bumpy; M = Massive; C = Columnar; Ca = columnar apex; Cs = columnar side. N polyps = number of polyps per square unit. For abbreviations and descriptions of characters see Table 1

Character		B–M	B–C	M–C	B–M–C	Ca–Cs
CuD	min	•••	•••	ns	•••	ns
	max	•••	•••	ns	•••	ns
CaS	min	•••	•••	•	•••	••
	max	•••	•••	ns	•••	ns
CaD	min	ns	ns	ns	ns	ns
	max	•	•	ns	•	ns
CuW	min	••	ns	•	••	ns
	max	••	ns	ns	•	ns
PsL	min	•	ns	•••	••	ns
	max	ns	•	ns	•	ns
PsT	min	ns	ns	ns	ns	ns
	max	ns	ns	ns	ns	ns
SsL	min	•••	•••	ns	•••	ns
	max	ns	•••	•••	•••	ns
SsT	min	••	•••	ns	•••	ns
	max	ns	•••	•••	•••	ns
ThL	min	ns	ns	ns	ns	ns
	max	ns	ns	ns	ns	ns
ThW	min	•	ns	•	ns	ns
	max	•••	•••	ns	•••	ns
PsC		ns	ns	ns	ns	ns
SsC		ns	ns	ns	ns	ns
N polyps		•••	•••	••	•••	•••

0.10 (Ferguson 1980). In general, heterozygosity and fitness are thought to be positively correlated (Mayr 1970). Nevo et al. (1984) summarized the various ways that high heterozygosity can be established or maintained. However, they did not find direct cause-effect relationships between biotic and genetic factors. The longevity of *M. annularis* colonies, which makes a multi-generation gamete exchange possible, may be responsible for the high heterozygosity.

Despite the high heterozygosity, we found that the number of heterozygotes observed is consistent with expectations. An exception is taxonomic unit B/SB, showing a significant (*G*-test, $p < 0.005$) deficiency in the number of expected heterozygotes.

The Hardy-Weinberg equilibrium is based on conditions of random mating if the genes are of equal selective value (Mayr 1970). A high percentage, 26 %, of the polymorphic loci tested departs (Chi-square, $p < 0.06$) from the Hardy-Weinberg equilibrium. Stoddart (1983) also found high disequilibria resulting from proliferation of relatively few genotypes, indicated by identical genotypes. In our case, all colonies sampled were genotypically unique. *Montastrea annularis* eggs are externally fertilized (Szmant 1986, Van Veghel pers. obs.), so mating presumably will be random and self fertilization is not to be expected. A preliminary explanation for this high percentage of Hardy-Weinberg deviations could lie in an unequal selective value of the alleles.

The genetic variability could have been affected by sampling from a broad range of generations. As it is not possible to determine genetic age in corals, the genetic age of a sample may vary from several years to hundreds of years. If the genetic sampling involved in transmission of genes between generations is random, any extant population is a random representative of all the replicate populations that may arise under the same set of conditions (Weir 1990). For this analysis we assumed that the 9 examined loci are representative for the overall genomic variation.

The results of the isogenic interaction experiments demonstrated dominant interactions to be absent within morphotypes. This is in contrast to intra-morphic and inter-morphic experiments where dominance was found in all morphotypes. The inter-morphic contact experiments show a clear polymorphic distinction between the morphotypes in behavior. The hierarchical ranking from more to less dominant, Bumpy > Massive > Columnar, is the same as between these morphotypes in Panama (Knowlton et al. 1992).

No relationship was found between interaction outcomes and the percentage of alleles shared between 2 individuals. This could mean that the interactive response is a phenotypic characteristic or, more likely, that the fraction of the genotype examined electrophoretically is not representative of the genotypic differences determining interaction outcomes (Stoddart et al. 1985).

Foster (1977) showed that there are small-scale variations in skeletal morphology within *Montastrea annularis* in relation to the environment. She did not find a corallite character that could be used for a direct prediction of colony shape or vice versa, because measured ranges overlapped (Foster 1983). We demonstrate significant differences between micro-morphological characteristics of the morphotypes, but these cannot be used as a taxonomic characteristic. When comparing Massive and Columnar corallites, fewer differences were observed than when comparing these with the Bumpy morphotype. This contrasts with the genetic variance where the Massive morph is the one differing most.

The differing number of polyps per square unit of area (ANOVA, $p < 0.01$) is explained by the differences found in corallite spacing (ANOVA, $p < 0.05$), because the corallite diameter was not very different between the morphotypes.

Skeletal variation between the apex and side of the columnar morph within a colony was also found by

Land et al. (1975). At the side, this showed as slower rates of calcification, more widely separated calices, scarcely distinguishable theca, less exsert septa and highly developed dentations. We found significant differences in the minimum spacing of the corallite and the number of polyps per square unit. Foster (1977) claimed this is due to micro-environmental factors. Tissue is found only over the top of the columns; die-off of the polyps on the side of the columns must be related to unfavorable conditions on the side of the column.

In conclusion, we demonstrate that the present *Montastrea annularis* population in Curaçao is comprised of at least 3 recognizable morphotypes. Characters which define genetic, behavioral and morphological aspects show significant differences. However, none of these characters are fixed for all 3 morphotypes, and so far they cannot be used as a diagnostic tool. Studies on reproductive biology and ecological characteristics are necessary to define the status of the *M. annularis* morphotypes.

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