



Morphological and genetic variation across reef habitats in a broadcast-spawning octocoral

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Abstract

Octocorals, like many other colonial benthic invertebrates, exhibit remarkable levels of morphological variability. The basis for this variability has been largely unexplored. *Plexaura flexuosa* Lamouroux, a common Caribbean octocoral, is found in virtually all reef habitats, and exhibits habitat-related differences in growth rates and fecundity. Population genetic structure and morphological variability in branch and sclerite characters were examined in nine populations from back reef, shallow forereef, and forereef habitats in the Florida Keys. Discriminant analysis of morphological characters correctly classified 89, 88, and 100% of samples from back, shallow fore, and forereef habitats, respectively. Analysis of randomly amplified polymorphic DNA profiles (RAPDs) showed no evidence of genetic differentiation between shallow back, shallow fore, and the majority of forereef colonies. However, there were two distinct genetic groups of forereef colonies. Much of the habitat-related morphological variability among *Plexaura flexuosa* is due to plasticity, which is likely an important feature contributing to the broad distribution of this species. However, there may be two genetic subgroups on forereefs.

Introduction

Although morphological features are the primary means of species identification among colonial anthozoans such as octocorals, for close to a century, workers have commented on the extraordinary morphological and physiological variability exhibited by these taxa (Wood-Jones, 1907). Some studies evaluating the basis for this variability, particularly those using transplant experiments, have pointed to the importance of phenotypic plasticity in response to environmental factors such as light, water flow, and depth (Table 1). Thus, phenotypic plasticity may play an important role in broadening species distributions (Bruno & Edmunds, 1997).

But, as Knowlton (1993) argues, what is interpreted as phenotypic plasticity may sometimes

represent ecotypic (genetic) variation (Tureson, 1922). For instance, *Montastraea annularis*, an important Caribbean reef-building coral, was thought to have a broad depth distribution, with depth-related morphotypes (Goreau, 1959) resulting from differences in light availability (Graus & Macintyre, 1976). However, subsequent studies have provided genetic, morphological, and reproductive evidence to separate *M. annularis* into a complex of at least three species (Weil & Knowlton, 1994; Rowan & Knowlton, 1995; Knowlton et al., 1997).

Thus, one challenge for reef ecologists is to assess whether species that are broadly distributed, and for which widespread variation and/or distinct morphotypes are known, represent complexes of sibling species. The Caribbean gorgonian *Plexaura flexuosa* Lamouroux presents a good candidate

Table 1. Studies examining phenotypic plasticity in corals

I. Correlation with habitat or environmental cline	
Plasticity present	
	<i>Agaricia</i> , d (Helmuth et al., 1997)
	<i>Alcyonium</i> , f (Sebens, 1984)
	<i>Eunicella</i> , f (Velimirov, 1976)
	<i>Faviinae</i> , h, d (Wijsman-Best, 1974)
	<i>Montastraea</i> , d (Dustan, 1975)
	<i>Pocillopora</i> , f (Lesser et al., 1994)
	<i>Porites</i> , d (Brakel, 1983)
Plasticity absent	
	<i>Montastraea</i> (Barnes, 1973)
	<i>Platygyra</i> (Miller, 1994)
	<i>Porites</i> (Brakel, 1977)
II. Transplant experiments	
Plasticity present	
	<i>Acropora</i> , d, h (Potts, 1978; Oliver et al., 1983)
	<i>Briareum</i> , d (West et al., 1993)
	<i>Montastraea</i> , d, l, h (Graus & Macintyre, 1976; Foster, 1979; Hudson, 1981)
	<i>Pocillopora</i> , f (Lesser et al., 1994)
	<i>Sidastraea</i> , h (Foster, 1979)
	<i>Turbinaria</i> , d (Willis & Ayre, 1985)
Plasticity absent	
	<i>Acropora</i> (Potts, 1984)
	<i>Pavona</i> (Willis & Ayre, 1985)
	<i>Pocillopora</i> (Takabayashi & Hoegh-Guldberg, 1995)
III. Molecular genetic analysis	
Plasticity present	
	<i>Plexaura</i> (this study)
Plasticity absent (i.e. genetic differentiation)	
	<i>Alcyonium</i> (McFadden, 1983)
	<i>Briareum</i> (Brazeau & Harvell, 1994)
	<i>Favia</i> (Carlson & Budd, 2002)
	<i>Montastraea</i> (Knowlton et al., 1997, Van Veghel & Bak, 1993)
	<i>Montipora</i> (Stobart & Benzie, 1994)
	<i>Pavona</i> (Willis & Ayre, 1985, Ayre & Willis, 1988)

For each study, genus and type of environmental gradient studied (where appropriate) are given. Abbreviations: f = flow, h = habitat, d = depth, l = light.

species for testing whether phenotypic plasticity or ecotypic variation underlie broad species distributions. *Plexaura flexuosa* is common throughout the Caribbean, inhabiting virtually all reef habitats and a wide depth range (Goldberg, 1973; Kinzie, 1973;

Lasker & Coffroth, 1983; Beiring, 1997). Moreover, a recent study found habitat-related differences in growth rates and fecundity (Beiring, 1997; E. Kim et al., submitted). In this study, we examine morphometric and genetic variation among *Plexaura flexuosa* collected across various habitat types.

Materials and methods

Study sites and sampling

Plexaura flexuosa was collected from nine reefs in the upper Florida Keys comprising three habitat types: back reef (≤ 5 m; Three Sisters, Triangles, and Cannon Patch); shallow forereef (5–6 m; Conch, Molasses, and Pickles) and forereef (15–16 m; Conch, Molasses, and French). Female colonies were used so that egg production also could be measured. Samples were collected approximately 1 week before spawning. On each reef, two primary branches, each ~ 100 mm long, were taken from colonies taller than 300 mm (the height at which most colonies are reproductive: Beiring, 1997; E. Kim et al., submitted). The sampled colonies were chosen haphazardly from at least two areas of each reef. Branches for morphological analyses were preserved in 10% formalin in seawater and later rinsed in tap water for ~ 24 h and transferred to 70% ethanol. Branches for DNA analysis were frozen in liquid N_2 and stored at -70 °C until DNA was extracted.

Morphological analysis

An overview of morphometric characters used in this study is given in Table 2. Branch thickness and polyp measurements were made with a binocular dissecting microscope fitted with an eyepiece micrometer. Branch diameter was measured at 20 and 30 mm below the branch tip, and the average was used to estimate surface area (cm^2 per linear cm) of the branch. Polyyps between 20 and 30 mm below the tip were counted to determine polyp density (polyyps/ cm^2). Twenty randomly chosen calyces were cleared of surface sclerites so that calyx diameters and intercalyx distances could be measured.

For each colony sample, the diameters of all eggs in 10 haphazardly selected polyyps located

Table 2. Overview of characters examined

Character	Description	Sampling
(a) Colony		
(1) height (cm)	Height from base to tip	1 per colony
(b) Branch		
(2) surface area (cm ²)	Estimated from branch diameters at 2 and 3 cm	1 per colony
(c) Polyps		
(3) polyp density (cm ⁻²)	Number of polyps in 1 cm band of branch	1 per colony
(4) calyx diameter (μ m)	Diameter of polyp opening	20 per colony
(5) intercalyx distance (μ m)	Rim to rim between calyces	20 per colony
(d) Reproductive status*		
(6) fecundity (no. of mature eggs)	Number of eggs $\geq 500 \mu$ m per polyp	10 polyps/colony
(e) Sclerites		
(7–8) club length and width (μ m)	Maximum sclerite length and width	20 per colony
(9–10) spindle length and width (μ m)	Maximum sclerite length and width	20 per colony
(11–12) capstan length and width (μ m)	Maximum sclerite length and width	20 per colony

* Data from Kim et al. (submitted).

Data were collected from a total of 53 colonies. Six were collected from each of three back reefs and each of three forereefs; six were collected from each of two shallow forereefs, and five were collected from a third shallow forereef (Molasses).

between 20 and 30 mm from the branch tip were measured (Beiring, 1997; E. Kim et al., submitted). Fecundity refers to the number of mature eggs per polyp. Previous work (Beiring & Lasker, 2000) had shown that *Plexaura flexuosa* eggs released during spawning are $\geq 500 \mu$ m in diameter; therefore, fecundity was determined as the number of eggs $\geq 500 \mu$ m.

Sclerites were collected from a 10-mm segment between 35 and 45 mm from the branch tip. Sclerites were prepared according to the methods outlined by Bayer (1961). Briefly, using 1–2 drops of bleach (i.e. commercial hypochlorite solution) and a scalpel, the three sclerite layers (surface clubs, middle spindles, inner capstans) were separated from whole dried samples and placed in microfuge tubes with bleach to dissolve any remaining tissue. Sclerites were then rinsed 3 \times with distilled water and 1 \times with 70% ethanol, dried 24 h, and mounted on glass slides. Clubs and capstans were measured with the image analyzer Optimas (BioScan Inc., Version 4.10). Images were obtained using a 28 \times petrographic microscope and a Kodak Megaplus video camera control unit (Model 1.4). Spindles, which were too large to be measured using the image analyzer system, were measured using a binocular dissecting microscope fitted with an eyepiece micrometer. For each

sclerite type, maximum length and width of 20 sclerites were measured from each colony. Sclerites were chosen by systematically but blindly moving across the slide and choosing the sclerite closest to the center of each new field of view.

MANOVA was used to determine if there were habitat-related differences in morphology. Discriminant analysis was performed to determine if colonies could be categorized into habitat types on the basis of these morphological characters. All data were tested for, and transformed as needed to meet assumptions of, normality (Kolmogorov test) and homogeneity of variance (Levene test).

Genetic analysis

From each habitat type, 13–14 colonies were examined genetically using Randomly Amplified Polymorphic DNA markers (RAPDs), a technique that has been used successfully in the past in the genetic identification of gorgonians, including *Plexaura flexuosa* (Coffroth et al., 1992; Coffroth & Mulawka, 1995; Coffroth & Lasker, 1998). DNA was extracted from 5 mm pieces of frozen branch by grinding in 0.6 ml 2 \times CTAB (hexadecyltrimethyl ammonium bromide) buffer (1.4 M NaCl, 20 mM EDTA [ethylenediaminetetraacetic acid], 100 mM Tris-HCl pH 8.0, 2% CTAB, and

0.2% 2-mercaptoethanol), and 0.5 ml phenol (modification of method described in Coffroth et al., 1992; phenol was used to prevent degradation of DNA). After the phenol was removed, 3.6 μ l of proteinase K was added to the aqueous phase, the sample was incubated for 1 h at 65 °C, and the extraction was continued according to the methods in Coffroth et al. (1992). RNA was digested by adding 0.5 μ l RNase (0.01 μ g/ μ l) and incubating for 30 min at 56 °C. DNA was quantified spectrophotometrically and adjusted to a concentration of 5 ng/ μ l.

In order to identify primers that produce polymorphisms, nine colonies (one per reef) were screened using 45 primers from University of British Columbia (UBC) Nucleic Acid-Protein Service Unit or from Operon Technologies Inc. Most of the primers produced some polymorphic markers, but none produced markers that were specific to one habitat type. Four primers from UBC that produced polymorphic markers and amplified well (UBC 504, 512, 528, and 554) were chosen and used to amplify DNA from each of the 41 colonies.

As RAPDs can be sensitive to reaction conditions, RAPD profiles were produced as described in Coffroth and Mulawka (1995), where the polymerase chain reaction (PCR) conditions (Mg^{2+} , primer, and DNA template concentration) were optimized to obtain reliable and reproducible patterns in gorgonians. Controls with no DNA were run to check for contamination. One colony was arbitrarily chosen as a positive control for each primer and run twice on each gel. Samples were amplified with one cycle of 2.5 min at 94 °C, 1 min at 35 °C, and 2 min at 72 °C; 45 cycles of 1 min at 94 °C, 1 min at 35 °C, and 2 min at 72 °C; and one cycle of 8 min at 72 °C. Samples were eluted on a 1% Synergel (Diversified Biotech)/0.6% agarose gel for 7–8 h with recirculating 0.5 \times TBE buffer (0.045 M Tris-borate; 0.001 M EDTA), stained with ethidium bromide, and visualized by UV light. Each gel contained five individuals from each habitat type, two samples of the positive control, and two 123-bp markers.

To verify the reliability of the RAPD markers, RAPD profiles of all samples were replicated at least once. In every case the overall banding pattern in replicate runs was the same, but the intensity of the bands differed in some. Gels were scored conservatively by including only bands that

were strong and distinct and repeated in each gel. With the large number, 108, of markers identified in this study, it is likely that any random 'noise' due to non-reproducible markers or PCR artifacts would be overwhelmed by the true variation. Using this approach, RAPDs have been found to be reliable markers (Kjolner et al., 2004).

All colonies were coded for the presence or absence of each polymorphic RAPD marker produced by the primers. These data were used to calculate similarity indices between all pairs of individuals ($2 \times$ number bands shared/total number of bands in each individual; Nei & Li, 1979) and average similarities within and between habitat types. RAPD data were also used in a cluster analysis (complete linkage) based on the presence/absence of bands and in an Analysis of Molecular Variance (AMOVA; Excoffier et al., 1992) using the software Arlequin (Schneider et al., 2000) to detect evidence of population differentiation.

Results

Morphological variation

There was significant morphological variability among *Plexaura flexuosa* from different habitat types (Fig. 1). However, there did not appear to be any consistent trends among individual characters across habitat type. For instance, colonies on the shallow forereef had the thickest branches but shared similarities in calyx size with forereef colonies and polyp density with back reef colonies. Colonies in the back and shallow forereefs were taller than forereef colonies, but surface area among shallow fore colonies was higher than in both back and forereef colonies. Shallow fore colonies had the largest surface clubs, but among the smallest spindles.

While individual characters did not vary consistently among habitat types, there was systematic morphological variation among colonies from the three reef types. Discriminant analysis using all of the morphological characters produced two linear functions that correctly categorized 89, 88, and 100% of samples from back, shallow fore, and forereef habitats, respectively (Fig. 2). The functions were highly correlated with habitat type (0.88 and 0.76, respectively: Table 3). The parameters that loaded most heavily on the

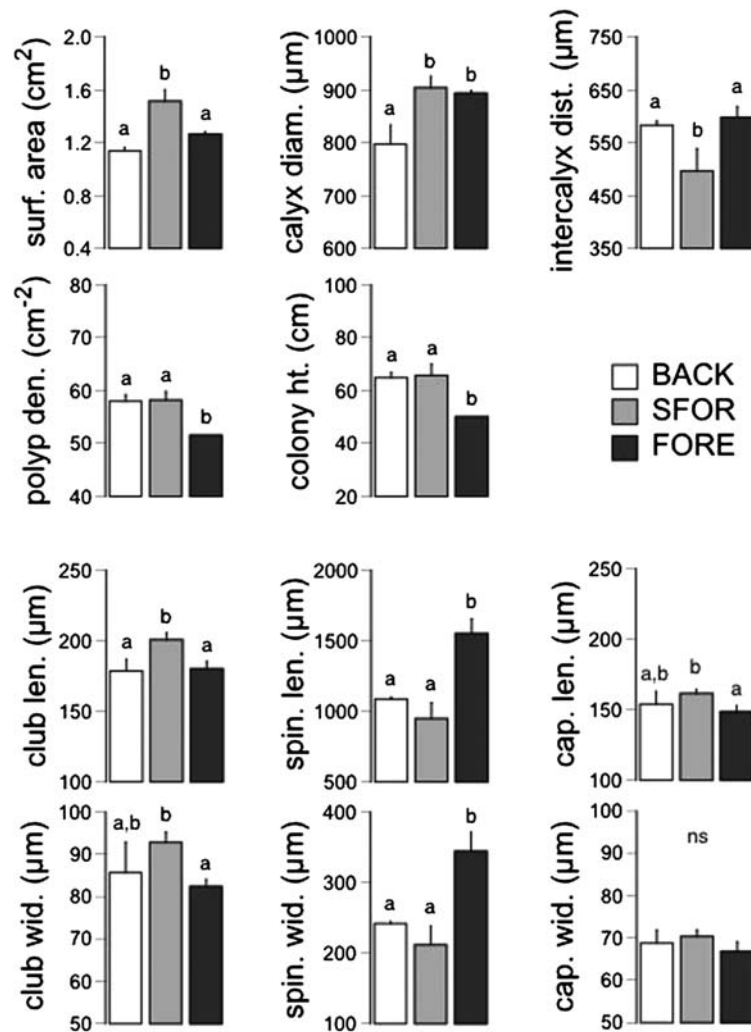


Figure 1. Summary of *Plexaura flexuosa* morphological characters as a function of habitat type. All values represent means \pm SE. Letters above bars identify groups with similar means. See Table 2 for character descriptions. BACK = back reef, SFOR = shallow forereef, FORE = forereef.

first function were spindle length and width and colony height. The parameters that loaded most heavily on the second function were branch surface area, calyx diameter, and club length (Table 3).

Genetic variation

The four 10-mer primers (UBC 504, 512, 528, and 554) generated a total of 108 polymorphic RAPD markers across all colonies. The average number of markers per colony was 21 ± 0.58 (mean \pm SE). There was no significant difference

in the number of markers per individual based on habitat type (ANOVA, $F = 1.21$, $p = 0.31$). No single marker was unique to a habitat, i.e. present in all colonies of one habitat and not present in any other habitat.

Average similarity (Nei & Li, 1979) within shallow back reefs and within shallow forereefs (0.55 and 0.56, respectively) was the same as the average similarity between these two habitats (0.56), indicating no genetic differentiation between the two habitats (Table 4). Average similarity among the forereef colonies was lower

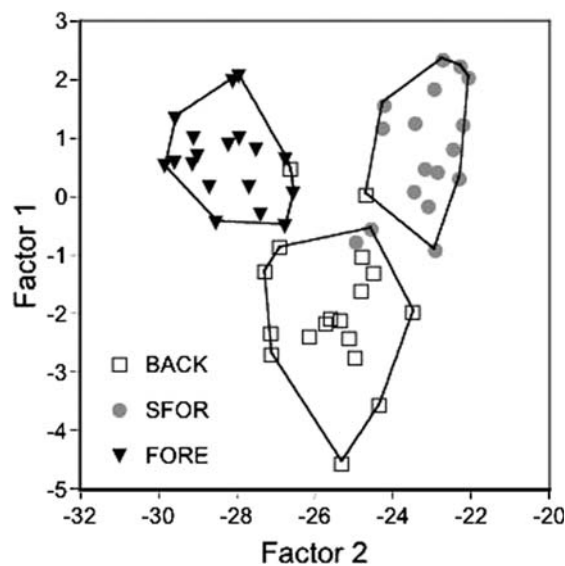


Figure 2. Discriminant analysis of morphological characters (see Fig. 1). Polygons represent groups predicted by the analysis. Abbreviations as for Figure 1.

(0.42), as was similarity between forereefs and the other habitat types (0.46 with shallow back reefs; 0.45 with shallow forereefs).

Complete linkage cluster analysis showed general similarity of colonies from all habitat types, except for one cluster of five forereef colonies

Table 3. Canonical correlations and factor loadings for discriminant analysis of *Plexaura flexuosa* morphometric characters ($n = 53$ colonies)

	Function 1	Function 2
Canonical correlations	0.876	0.760
Variables		
Spindle length	-0.460	0.207
Spindle width	-0.352	0.162
Colony height	0.294	-0.202
Fecundity	0.256	0.183
Polyp density	0.232	-0.174
Club width	0.231	0.086
Intercalyx distance	-0.219	-0.159
Capstan length	0.190	0.043
Capstan width	0.142	-0.017
Surface area	0.302	0.622
Calyx diameter	0.007	0.338
Club length	0.254	0.288

Variables are ordered by correlation within function.

(Fig. 3). The group comprised representatives from each of the three forereefs. When this 'out-group' of five forereef colonies was removed from the similarity matrix, the average similarity among colonies within the forereef habitat rose to 0.52, and the similarity between forereef and the other habitat types rose comparably (Table 4). Average similarity among the five outgroup forereef colonies was also higher (0.57), while average similarity between these colonies and the other groups was low (0.32 for shallow back reefs; 0.29 for shallow forereefs; 0.31 for the remaining forereef colonies). This indicates that the majority of colonies in the forereef habitats were genetically similar to the shallower colonies, while there was a small group of forereef colonies that differed from all the others.

AMOVA revealed significant variation in RAPD profiles among habitats and within reef (Table 5). However, habitat explained only 4.2% of the genetic variation whereas more than 93% was due to differences among colonies within reefs. We tested whether the habitat effect was due to the cluster of five forereef colonies (Fig. 3). When the AMOVA was re-run without them, the habitat effect was no longer detectable ($p = 0.4047$).

Discussion

Corals and other colonial organisms show extraordinary morphological variability. Understanding the basis of this variability is essential for examining species interactions and speciation, assessing changes in community structure (e.g. species diversity), and formulating effective management and conservation plans. Over the last several decades, much effort has focused on differentiating geographic populations, particularly in the context of testing the 'openness' of marine populations (McFadden, 1999; Ayre & Hughes, 2000; Bastidas et al., 2001; Ridgway et al., 2001). These and other studies (Table 1) have shown that both plasticity and genetics contribute to a species' morphological variability, but more importantly that the relative roles of these mechanisms differ among species. Our work suggests the presence of two groups of *Plexaura flexuosa*. The more common group is present in all habitats, is genetically homogeneous (suggesting gene flow among reefs),

Table 4. Average similarities of *Plexaura flexuosa* colony RAPD profiles within and between habitats using Nei and Li (1979) similarity indices

	All colonies	Without deep outgroup
<i>Within habitat</i>		
Shallow Back	0.55 ($n = 14$)	0.55 ($n = 14$)
Shallow Fore	0.56 ($n = 13$)	0.56 ($n = 13$)
Fore	0.42 ($n = 14$)	0.52 ($n = 9$)
<i>Between habitat</i>		
Shallow Back/Shallow Fore	0.56	0.56
Shallow Back/Fore	0.46	0.53
Shallow Fore/Fore	0.45	0.54

and varies phenotypically across habitats. The second group is restricted to the deeper sites and is genetically distinct from the common group.

Habitat-related differences

In *Plexaura flexuosa*, we documented strong habitat-related morphological variation (Fig. 1). The majority of colonies from each habitat type were classified correctly based on their morphology using two canonical functions (Fig. 2), suggesting a strong role of habitat in determining form. The major morphological differences among habitat types include branch thickness, size of spindles and clubs, size and spacing of polyps, and colony height (Fig. 1). Each of these characters could be hypothesized to affect fitness differentially across the three habitats, but no single aspect of the environment appears to correlate with the observed distributions of these characters. For instance, branches were thickest on colonies in the shallow forereefs, where wave action is likely the greatest. This is consistent with observations of thicker branches in sea fans (Grigg, 1972; Velimirov, 1976) and *Briareum asbestinum* (see West et al., 1993) from wave-exposed environments. On the other hand, polyp density was lowest in the forereef colonies (Fig. 1). A decrease in polyp density with depth is common in gorgonians (West et al., 1993) and scleractinian corals (Wijisman-Best, 1974; Davies, 1977; Lasker, 1981; Oliver et al., 1983). This decrease may be due to a decrease in resource availability at greater depths (Kim & Lasker, 1998) or could simply be an epi-

phenomenon of differential changes in growth rates (i.e. rates of polyp formation and branch extension).

Analysis of RAPD data also revealed habitat-related differences, with colonies on the forereefs genetically distinct from colonies on the back and shallow forereefs. However, this difference appears to be driven by the presence of two groups of colonies on the forereefs (Fig. 3, Table 4). The possibility that the smaller group of forereef colonies is a distinct subpopulation or cryptic species is supported by the absence of mature gonads in these colonies, despite the fact that they were collected shortly before *Plexaura flexuosa* spawning (Beiring, 1997). However, some of the colonies in the larger group of forereef colonies also lacked mature gonads at the time of collection. Reproductive compatibility experiments, transplant studies, and a more thorough genetic survey would help to fully assess the population status of the forereef colonies.

Knowlton & Jackson (1994) have discussed the importance of discerning the population structure of reef organisms for understanding the ecology of reefs as well as their past and future resistance to environmental change. Understanding population structure is also critical for developing management programs. Marine reserves are being established around the world as a means of protecting biodiversity and enhancing stocks of fishes and invertebrates both within and beyond reserve boundaries (Halpern & Warner, 2002; Palumbi, 2002). Protection of species complexes with marked population structure will require careful assessment of candidate populations to ensure that a given refuge contains viable populations of each of the member subpopulations/species.

In the present study, although *Plexaura flexuosa* colonies varied morphologically across habitats, the bulk of the colonies belong to a genetically homogeneous subgroup. This suggests larval mixing across all habitat types and the possibility that any single habitat could, and perhaps does, act as a source for all habitats. Identification of recruitment patterns and larval sources may be necessary to ensure that appropriate areas are chosen for protection, but the genetic data suggest that genetic diversity of *Plexaura flexuosa* can be maintained using any of

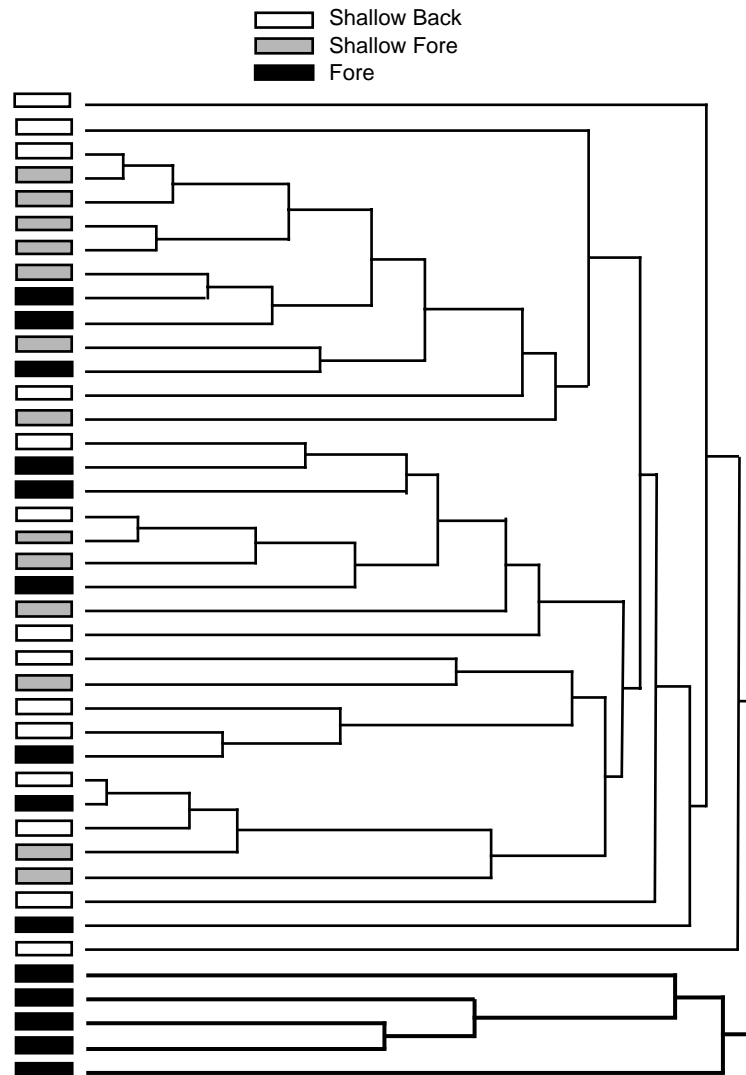


Figure 3. Complete linkage cluster analysis of RAPD data, with cluster of forereef colonies indicated by thicker lines.

Table 5. Analysis of molecular variance of RAPD profile data

Source	d.f.	Sum of squares	Variance	Percent of variation	<i>P</i>
Among habitats	2	34.89	0.4565	4.19	0.0127
Among reefs within habitats	6	67.17	0.2117	1.94	0.1906
Within reefs	32	327.6	10.27	93.9	0.0332

the habitats as a source area. In contrast, the genetic distinctness of the forereef morphotype suggests that maintenance of this subpopulation will depend on recruitment from other forereef habitats only.

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