Marine Biology (1997) 129: 561–572

A. M. Szmant · E. Weil · M. W. Miller · D. E. Colón

Hybridization within the species complex of the scleractinan coral *Montastraea annularis*

Received: 16 April 1997 / Accepted: 17 June 1997

Abstract The morphologically variable reef coral previously known as *Montastraea annularis* (Ellis and Solander, 1786) has recently been separated into three species based on differences in morphology, behavior, allele frequencies and some life-history traits of Panamanian specimens. To further investigate the proposed reclassification and its conformity to the biological species concept we conducted reciprocal intra- and interspecific fertilization experiments with gametes from each of the three species on Florida reefs. With one exception, self-fertilization rates were very low or zero. Within-species crosses resulted in production of planulae, as did all inter-species (hybrid) crosses, but there was much variation in fertilization success within each type of cross. In an experiment with separated gametes, hybrid crosses between *M. annularis* and *M. franksi* produced more larvae than within-species crosses for each species. Hybridization crosses between *M. faveolata* and the other two species produced fewer larvae than did within-*M. faveolata* crosses in the experiment with separated gametes, but many larvae resulted when the hybridizations were performed by mixing entire gamete bundles. Additional observations showed that *M. franksi* had 20% larger eggs and fewer eggs per gamete bundle than did the other two species and that it consistently spawned 1 to 1.5 h before the others, a potential temporal barrier to hybridization. These results indicate that there is no inherent pre-zygotic barrier to cross-fertilization among the three morphological species, although post-zygotic survival and fertility remain to be determined. The adherence of the proposed reclassification to the biological species concept requires further examination.

Communicated by N. H. Marcus, Tallahassee

A.M. Szmant (✉) · E. Weil[1] · M.W. Miller · D.E. Colón
Rosenstiel School of Marine and Atmospheric Science,
Division of Marine Biology and Fisheries,
University of Miami, 4600 Rickenbacker Causeway,
Miami, Florida 33149, USA

[1] *Present address*:
Department of Marine Sciences, University of Puerto Rico,
P.O. Box 908, Lajas 00667, Puerto Rico

Introduction

The biologic species concept is based on the precept that there is minimal to no gene flow (= reproductive isolation) between defined subsets of co-existing morphologically similar individuals (= species) (Mayr 1968, 1970; see also discussion in Mayr 1982). For many types of organisms, the criteria for defining the subsets of similar individuals are mainly morphological, but more recently behavioral, soft-tissue and genetic characteristics have been used for multi-character classifications (Lang 1984; Weil 1992, 1993). For sympatric marine invertebrates, reproductive isolation can be the result of different reproductive characteristics, such as the mode or timing of reproduction, or spawning patterns, that prevent gametes of different species from crossing. Thus, reproductive characteristics can be important diagnostics of species identity (e.g. Van Moorsel 1983). On the other hand, post-zygotic barriers, such as non-viable embryos or sterile adults, could lead to reproductive isolation even when hybridization events do occur. Therefore, concurrent reproductive events and evidence for hybridization do not necessarily indicate lack of reproductive isolation. A species' status could still be valid even if an occasional hybrid is of high fitness. There are known examples of morphological species complexes that commonly inter-breed, and then the question becomes one of defining species boundaries: How much inter-breeding between morphotypes should be accepted before the species designation is abandoned in favor of sub-species or lower taxon status? (see discussions in Mayr 1982; Wallace and Willis 1994).

Until recently, the reef coral *Montastraea annularis* (Ellis and Solander, 1786) sensu lato, an important Caribbean reef-building species (Goreau 1959), was thought to be a single morphologically highly variable

species. However, significant differences in morphological, molecular, and behavioral characters have been reported between three common and widespread colony morphologies (Knowlton et al. 1992; Van Veghel and Bak 1993; Van Veghel 1994; Van Veghel and Kahmann 1994), which prompted the re-description of *M. annularis* sensu stricto and the resurrection of two older taxa, *M. faveolata* (Ellis and Solander, 1786), and *M. franksi* (Gregory, 1895) (Weil and Knowlton 1994). Table 1 summarizes many of the morphological, biological and ecological characteristics of these three morphological species and their Caribbean congener *M. cavernosa*.

The validity of these three species is not universally accepted (e.g. Van Veghel and Bak 1993) because there is much overlap in characters and no fixed diagnostic genetic differences were found with allozymes. Furthermore, while each of the species' morphotypes in its "pure" form is easy to distinguish, there are variants that appear intermediate in characteristics between the described species (Fig. 1). Finally, all of the colonies used for the morphological and genetic work came from a small area (Panamá, Curaçao) within these taxa's distributional range (Caribbean Sea and western Atlantic as far north as Bermuda). There is some suggestion that the abundance of intermediate morphologies may be greater in the Greater Antilles, Florida and Bahamas. Given the structural and ecological importance of these particular corals to Caribbean/Atlantic coral reef communities and the fact that *Montastraea annularis* sensu lato is the most studied coral in this region, it is important to resolve this controversy and clarify the species boundaries among the various colony morphologies.

Reproductive traits in corals are, in general, less well studied and understood than other aspects of coral biology. However, much progress has been made during the past two decades in learning about the reproductive biology and ecology of reef corals. A major breakthrough occurred with the discovery of mass spawning events, and the correlation of spawning episodes for many corals to certain phases of the lunar cycle (Harrison et al. 1984; reviewed in Harrison and Wallace 1990). The ability to reliably predict spawning has opened the door for more experimental approaches to the study of coral reproductive biology, especially aspects such as fertilization and larval biology.

The ability and opportunity (contemporaneous spawning) for cross-fertilization in corals may not necessarily imply conspecificity. Experimental cross-breedings done by Australian researchers between species within the same genus and even across genera have produced viable larvae and juvenile colonies (Willis et al. 1993; Wallace and Willis 1994; Miller and Babcock 1997; Willis et al. 1997), pointing out the difficulties in reconciling the biological species concept in corals with morphology and phylogeny-based systematics (discussions in: Willis 1990; Wallace and Willis 1994; Veron 1995). Indeed, in most all scleractinian cases examined, there is a common finding that morphological and ecological distinctness do not correlate with genetic or reproductive distinctness [e.g. various groups of *Acroporas* (Willis et al. 1997), *Platygyras* (Miller and Babcock 1997), *Montastraeas* (Knowlton et al. 1992; Weil and Knowlton 1994; present study). Moreover, long overlapping generation times with interbreeding between generations coupled with the potential for hybridization may confuse the genetic signal, adding to the difficulty of checking for hybrid viability. Veron (1995) proposed that these life history and reproductive characteristics result in a pattern of reticulate evolution in corals, with a consequent overlap in species boundaries. On the other hand, Knowlton (1993) pointed out the importance of recognizing sibling and cryptic species where they occur.

The main object of this study was to determine whether the three morphological species of *Montastraea* could hybridize given their similar annual reproductive cycles and their general synchrony of spawning (Szmant 1991). This is a first step in testing the adherence of the proposed reclassification to the biological species concept. Additional observations on timing of spawning and gamete characteristics were collected to further characterize species' similarities or differences and the potential for hybridization to occur in nature.

Materials and methods

These studies were conducted on coral reefs in the Florida Keys, where the three distinct morphologies of *Montastraea annularis* (Ellis and Solander, 1786) as well as many intermediate morphologies are present. Only distinct morphotypes of the three species were used for the hybridization experiments. The three species are hermaphrodites that spawn with lunar periodicity during the third-quarter moons of August through October, depending on latitude and the timing of the August full moon within the annual calendar (Szmant 1986, 1991; Wyers et al. 1991; Van Veghel 1994). Preliminary results of this study have been reported by Szmant et al. (1995).

The cross-fertilization experiments were carried out on a cruise to Key Largo Dry Rocks, Florida Keys, USA (~25°10′N; 80°20′W) on board the R.V. "Calanus", during late August 1994; the spawning observations were made during several summer sessions in the Bahamas (1990 to 1991) and Florida Keys (1993 to 1995).

Cross-fertilization experiments

Subsamples of live coral tissue were drilled with a 5 cm-diam corer from three large colonies of each of *Montastraea annularis* and *M. faveolata* and six colonies of *M. franksi* 2 d before spawning was expected. The individual cores (two per colony) were kept separated in small buckets (one colony per bucket) in a flow-through seawater system aboard the ship. Normal diel light-regimes were maintained by covering the seawater system at night with black plastic to shade the corals from the ship's lights. Several carboys of seawater were collected 1 to 2 d before spawning, filtered (0.45 μm GF/A), and stored at room temperature for later use. Before dusk on the first evening of spawning (27 August 1994), individual cores were transferred to 1-liter beakers filled with the filtered seawater. Within minutes of spawning, egg-sperm bundles from each colony were transferred into 100 μm sieve cups, and gently agitated in a small beaker of clean filtered water until the bundles broke down. The water in these beakers constituted the sperm solutions for each colony, and sperm counts were conducted on subsamples of each

Table 1 *Montastraea* spp. Summary of morphological, biological, and ecological characteristics of each of currently described species of *Montastraea* in the Caribbean. In "Ecology" data, *Physical disturbance* refers to wave energy, light exposure and predation as described in Weil and Knowlton (1994) (environmental conditions are extrapolated from distributional trends); in "Biology" data, *Genetic markers* represent either loci differing significantly in genetic frequencies from the other species, or combination of loci that give significant low probability of misidentification (Ayala 1983) when used to separate species [triosephosphate isomerase (*TPI2*), glutamate dehydrogenase (*GTDH2*), leucyl-tyrosine-peptidase (*LTY1*), malate dehydrogenase (*MDHP1*), phosphoglucomutase (*PGM1*), and leucyl-proline-peptidase (*LPP1*)] (– no data)

Character	*M. annularis*	*M. faveolata*	*M. franksi*	*M. cavernosa*	Source[a]
Macromorphology					
Colony shape	columnar-bulbous round	massive mounds plates, shingles	crustose, massive plates	massive, round crustose, mounds	1, 2, 3, 4, 5, 6, 7
Colony diameter (m)	up to 8	up to 10	up to 5	up to 2	1, 2, 5, 7
Colony height (m)	up to 2.5	up to 4–5	up to 2	up to 1–2	1, 2, 5, 7
Colony surface	even–smooth	smooth with keels, bumps and ridges	uneven–irregular, bumpy	slightly uneven, smooth	3, 5, 6, 7, 8
Calice distribution	even	even	uneven	even	5, 6, 7
Skeletal density	medium	low	high	medium–high	3, 5
Coloration variability	low	medium	high	high	2, 7
Common colors	golden, tan, greenish	gray, green, brownish	green, gray, brown	green, gray, pink, pale reds, brown	2, 7
Micromorphology					
Calice diameter (mm)	2.29–2.43	2.39–2.44	2.5–2.57	5.8–7.2	5, 6, 7, 8
Columella diameter (mm)	1.02	0.96	1.13	–	5, 7
Calice spacing (mm)	1.40–1.53	0.98–1.43	1.76–1.88	2.58–3.32	5, 6, 7 ,8
Primary septa-thickness (mm)	0.20–0.26	0.12–0.18	0.21–0.27	0.12–0.17	5, 6, 7, 8
Primary septa height	low	exsert	low	low	5, 7
Number of septa	24	24	24	29–42	5, 6, 7, 8
Number polyps/area	high	high	medium	low	6, 7, 8
Ecology					
Depth range (m)	0–20	0–25	1–50	2–70	1, 3, 7, 9, 10, 11, 12, 13
Peak abundance (m)					
protected reef	3–9	1–6	10–20	10–25	7
semi-exposed	3–9	3–9	12–20	10–20	7
exposed reef	5–10	3–10	10–20	15–30	7
Light exposure	high	high	low	low	2, 7, 12
Physical disturbance	medium	high	low	low	7
Predation (snails)	high	medium	low	low	14
Intra-specific aggression	absent	low	high	low	5, 6, 7
Inter-specific aggression	low	medium	high	medium	5, 6, 7
Biology					
Upward growth	high	medium	low	medium	2, 3, 12, 15
Lateral growth	low–absent	medium–high	high	medium	2, 3, 12, 15
Growth rates	high	medium	low	low	2, 3, 5, 12, 16, 17
Tissue regeneration	low	medium	high	low	7, 11
Fragmentation	high	medium–low	low–absent	low	4, 5, 7, Ob
Sexual mode	hermaphroditic	hermaphroditic	hermaphroditic	gonochoric	18, 19, 20
Gametogenesis	annual	annual	annual	annual	18, 19, 20
Minimum colony reproductive size (cm^2)	$\geq$50	$>$100	$\geq$100	$>$150	20, 21, 22
Eggs/polyp (histology)	72–120	72–120	72–120	$>$200	19, 22, Ob, P
Egg size (μm) (SD)	308 (14)	318 (16)	325 (16)	350	18, 19, 22, P
Spawning	broadcaster	broadcaster	broadcaster	broadcaster	19, 20, 22
Spawning frequency	1–2/yr	1–2/yr	1–2/yr	1–2/yr	19
Spawning time (h after sunset)	2–3	2–3	1–2	1–2	P
Recruitment/survival[b]	low/low	medium-low/low	high/medium	high/high	7, Ob
Genetic markers	TPI2, GTDH2, LTY1	MDHP1 ($P > 0.99$)	PGM1, LPP1	TPI2, GTDH1, GTDH2, LTY1	5, 7

[a] *1* Goreau (1959); *2* Dustan (1975); *3* Graus and Macintyre (1982); *4* Coates and Jackson (1985); *5* Knowlton et al. (1992); *6* Van Veghel and Bak (1993); *7* Weil and Knowlton (1994); *8* Budd (1993); *9* Roos (1964); *10* Bak and Engel (1979); *11* Weil (1980); *12* Lasker (1981); *13* Reed (1985); *14* Hayes (1990); *15* Tomascik (1990); *16* Hubbard and Scaturo (1985); *17* Huston (1985); *18* Szmant (1986); *19* Szmant (1991); *20* Van Veghel (1994); *21* Szmant-Froelich (1985); *22* Van Veghel and Kahmann (1994); *Ob* our own observations; *P* present study

[b] Unpublished data from Florida and personal observations (Weil) from many reefs around the Caribbean

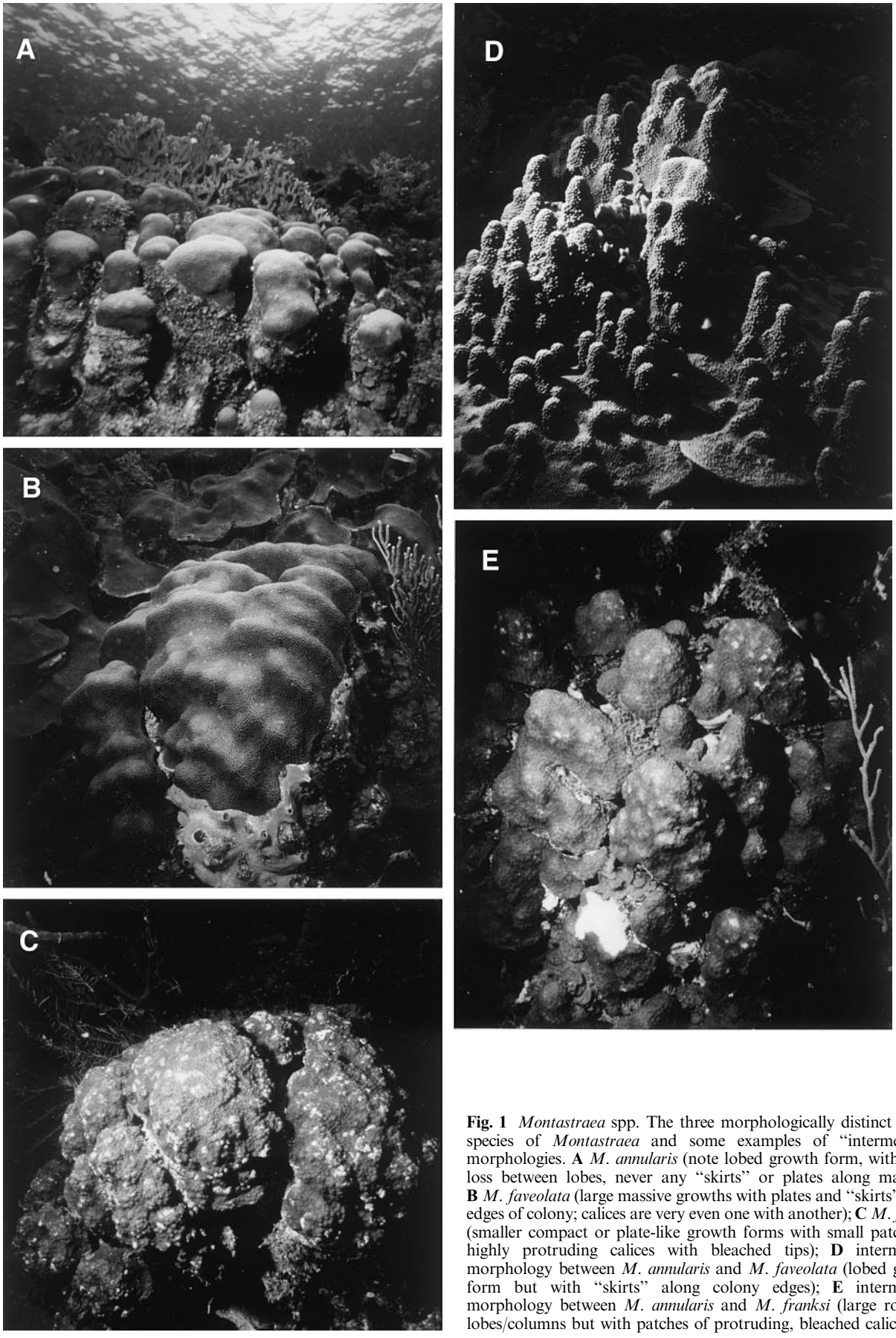

Fig. 1 *Montastraea* spp. The three morphologically distinct sibling species of *Montastraea* and some examples of "intermediate" morphologies. **A** *M. annularis* (note lobed growth form, with tissue loss between lobes, never any "skirts" or plates along margins); **B** *M. faveolata* (large massive growths with plates and "skirts" along edges of colony; calices are very even one with another); **C** *M. franksi* (smaller compact or plate-like growth forms with small patches of highly protruding calices with bleached tips); **D** intermediate morphology between *M. annularis* and *M. faveolata* (lobed growth form but with "skirts" along colony edges); **E** intermediate morphology between *M. annularis* and *M. franksi* (large rounded lobes/columns but with patches of protruding, bleached calices)

solution. The eggs in the sieve cups were serially transferred through five more beakers of filtered seawater to remove sperm. Great care was taken to not cross-contaminate samples. Any equipment used for more than one coral was rinsed with copious amounts of hot tap water before re-use.

An estimated 150 to 200 eggs from each individual colony were transferred into each of ten 50 ml disposable culture tubes. To each tube was added either no sperm solution (= no sperm-fertilization control), or between 0.5 and 2 ml of sperm solution (1.6 to 2.3 $\times$ 10^6 sperm per tube) from one of the nine corals (three corals per species; only three of the six colonies of *Montastraea franksi* were used), including itself (= self-fertilization control). This yielded a total of 90 culture tubes, 9 containing eggs only (one from each individual coral), and the remaining 81 tubes containing one of the reciprocal crosses (e.g. *M. annularis-1*-egg $\times$ *M. annularis-1*-sperm, *M. annularis-1*-egg $\times$ *M. annularis-2*-sperm, ... *M. annularis-1*-egg $\times$ *M. franksi-3*-sperm, *M. annularis-2*-egg $\times$ *M. annularis-1*-sperm, *M. annularis-2*-egg $\times$ *M. annularis-2* sperm ... etc.: Fig. 2). Due to the unfortunate but inevitable limitation of time, material and personnel, it was not possible to conduct replicates of each individual cross. Culture tubes were left at room temperature (26 to 28 °C) overnight. The number of planulae in each tube was estimated after 12, 36 and 56 h by examining the contents of each culture tube in a small petri dish with a Wild dissecting microscope. For the first two observation periods, the number of planulae in each culture tube was estimated by assigning a rank to each sample: tubes with no live planulae were assigned a rank of 1, those with <10 planulae were assigned a rank of 2, those with between 10 and 50 planulae were assigned a rank of 3, those with between 50 and 100 planulae were assigned a rank of 4, and those with >100 planulae were assigned a rank of 5 (see key in Fig. 2). At the 56 h examination, the planulae in each dish were carefully counted. Culture water was replaced with fresh filtered water after each count. After the 56 h count, all samples from the same type of species cross (e.g. *M. annularis* $\times$ *M. annularis*, *M. annularis* $\times$ *M. faveolata* etc., six types in total excluding the self and no sperm controls which were discarded) were pooled, and the cultures were maintained for up to 2 wk in plastic containers and trays previously washed and soaked in salt water.

On the second night of spawning (28 August 1994), a different procedure was followed because most of the coral cores had spawned the first night. Egg-sperm bundles were collected in the field from three individual colonies of *Montastraea annularis* and *M. faveolata*, and one of *M. franksi*. The samples were obtained by suspending cone-shaped "spawn-collectors" made of fine fabric over the corals the afternoon before spawning. The buoyant bundles floated up into small plastic containers placed in the cod-end of each collector. Divers retrieved the containers, closing them underwater, and returned them to the ship-based laboratory within minutes. One of the cores of *M. franksi* spawned on 28 August and was used in this second experiment. Sets of two bundles from each of the eight individual colonies (only two colonies of *M. franksi* including the core) were placed into eight test-tubes per individual. Two additional bundles from each of the other seven colonies were added to each of seven tubes from each coral; the eighth tube of each coral received additional bundles from itself (= self-fertilization control). Therefore, each tube (except the self-fertilization controls) contained both eggs and sperm from two individuals.

The number of viable planulae in each test-tube was estimated by ranks (as explained above) after ~18 and 36 h. Water was changed in the tubes after each count. After 56 h, all samples from the same types of crosses (e.g. *Montastraea annularis* $\times$ *M. annularis*, *M. annularis* $\times$ *M. faveolata*, etc., six in total) were pooled and the cultures maintained for up to 13 d in plastic trays and bins previously washed and soaked in seawater. They were observed and counted daily.

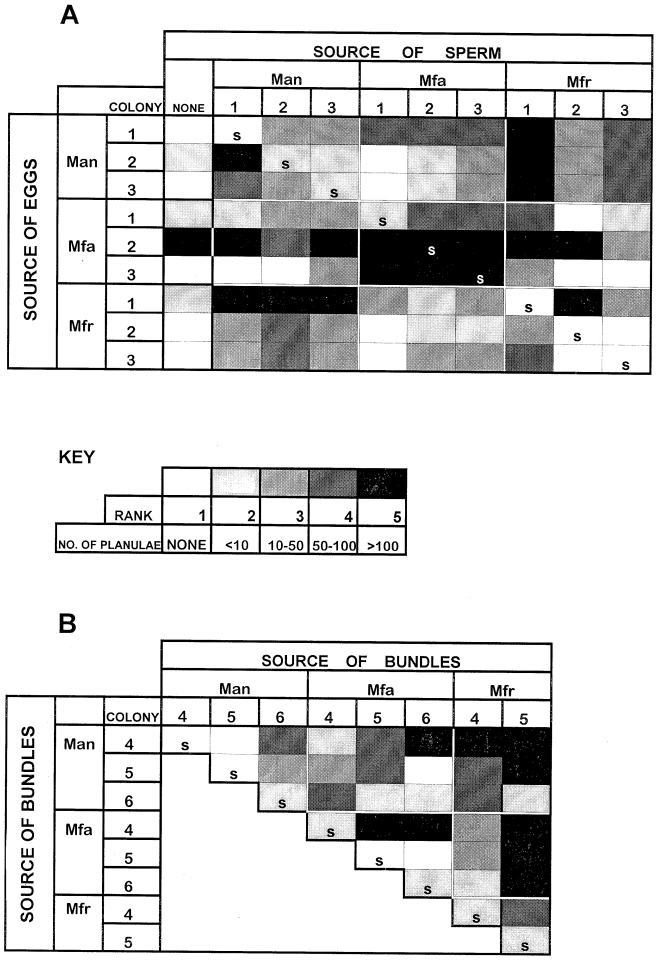

Fig. 2 *Montastraea* spp. Fertilization trials on 27, 28 August 1994. Matrix of fertilization results estimated 36 h after fertilization, expressed as ranks (see "key" and "Materials and methods – Cross-fertilization experiments") for all crosses made on the two dates. **A** Fertilization trials made with separated eggs and sperm preparations from 27 August 1994 spawn [*Man M. annularis*, *Mfa M. faveolata*, *Mfr M. franksi*; *arabic numerals* designate individual colonies within each species; *column heads* colony providing sperm for each cross in that column; *left-hand labels* colonies providing eggs; each box in matrix plot represents individual culture tube ($n = 1$ for each cross)]. **B** Fertilization trials with intact bundles collected from field on 28 August 1994. Selfing trials (*s*, *boxes along diagonal*) included four bundles from single colony in each culture tube ($n = 1$ per colony); out-crosses and hybrid crosses included two bundles from each of two colonies as designated in column heads and left-hand labels (*abbreviations* as in **A**) in each culture tube ($n = 1$ per cross)

Gamete-bundle characteristics

Samples of gametes were collected during the 1994 cruise and during the 1995 spawning from corals in Biscayne National Park (25°23.201′N; 80°0.9779′W). Intact gamete-bundles from each species were collected with clean Pasteur pipettes, placed individually in 1 ml microcentrifuge tubes, and preserved with 100 µl Lugol's solution. They were later carefully broken open; the numbers of eggs per bundle were counted, and egg diameters were measured by a computerized image-analysis system consisting of an NEC video camera (NEC Technologies) attached to an Olympus dissecting scope and the MOCHA (Jandel Scientific, USA) image-analysis software (Table 2). Egg volumes were calculated from the mean diameter for each egg. Egg sizes measured on the preserved eggs were similar to sizes measured on a smaller number of freshly spawned eggs.

Table 2 *Montastraea* spp. Means ($\bar{x}$) and standard deviations of reproductive characteristics of three sibling species of *Montastraea* in Florida measured in 1994 and 1995 (*Egg size* average between maximum and minimum diameters; *p* significance for differences among species). Statistical tests for differences between species used either Kruskal–Wallis and Dunn's tests [*1*] or one-way ANOVA and Student–Newman–Keuls test [*2*]

Species	*M. annularis* (= a)			*M. faveolata* (= b)			*M. franksi* (= c)			*p*	Pair-wise tests
	($\bar{x}$)	SD	(*n*)	($\bar{x}$)	SD	(*n*)	($\bar{x}$)	SD	(*n*)		
1994											
Eggs/bundle	96.5	(21.2)	(6)	122.4	(81.2)	(9)	67.0	(6.5)	(3)	–	
Egg size (μm)	308	(14)	(120)	318	(16)	(180)	325	(16)	(60)	< 0.0001 [1]	NS
Egg vol ($mm^3 \times 10^{-2}$)	1.54	(0.3)	(120)	1.72	(0.3)	(180)	1.80	(0.6)	(60)	< 0.0001 [1]	c > b > a
No. of colonies		2			3			1			c > b > a
1995											
Eggs/bundle	110.9	(21.0)	(7)	117.7	(41.0)	(7)	51	(33.2)	(6)	< 0.0052 [2]	b > a > c
Egg size (μm)	316	(20)	(170)	321	(20)	(155)	345	(32)	(150)	< 0.0001 [1]	c > b = a
Egg vol ($mm^3 \times 10^{-2}$)	1.67	(0.3)	(170)	1.75	(0.3)	(155)	2.21	(0.6)	(150)	< 0.0001 [1]	c > b = a
No. of colonies		3			4			3			

Spawning timing and behavior

In 1991, 1993, 1994 and 1995, spawning behavior and times of spawning were recorded by divers in the water in the Bahamas and the Florida Keys, and in 1991 and 1994 were compared to core samples kept in the running seawater system aboard the ship. Additional observations were derived from analysis of histological time series (Szmant 1991). Divers were in the water from ~21:00 to 01:00 hrs. In 1990, observations were made on the nights of 13 and 14 August (Days 7 and 8 after the full moon) in the Joulters Keys, Bahamas (no spawning was observed). The exact date and place for each spawning observation are given in Table 3.

In 1991, sets of replicate cores taken from three large *Montastraea faveolata* colonies from Andros Island, Bahamas (25°18.99′N; 78°05.13′W), were kept in flow-through seawater onboard the R.V. "Calanus", with the cores from each colony split between two separate aquaria. Two of the colonies were from the same depth from the same reef (10 m apart); the third was from the same depth on a different reef. To emulate differences in light exposure along the surfaces of a colony, light levels in the aquaria containing "side" cores were reduced compared to that of the aquaria containing the "top" cores, using extra layers of shade cloth. An additional colony of *Montastraea annularis* was in another aquarium.

Data analysis

Statistical analysis was done with both the 56 h planula counts from the crosses laboratory-spawned on 27 August and the 36 h rank data from both crossing experiments. Kruskal–Wallis one-way analyses of variance on ranks were run with Sigma Stat (Jandel Scientific) to determine whether crosses between species were significantly different from crosses within species. For analysis of the crosses from 27 August (separated eggs and sperm), statistical tests were done using subsets of the data with the same source of eggs, by comparing fertilization success of the within-species crosses (excluding self-fertilization ones) with those of out-crosses using the same sets of eggs. For example, for *Montastraea annularis* crosses, larval numbers or ranks obtained by fertilizing *M. annularis* eggs with *M. annularis* sperm (within-species) were compared with larval numbers or ranks obtained when crossing *M. annularis* eggs with *M. faveolata* and *M. franksi* sperm (hybrid crosses). For ANOVAs with significant differences, a posteriori multiple-comparison tests (Dunn's test) were used to identify the group(s) that differed from the other groups, but the number of cases was generally too small to obtain a statistically significant pairwise difference. Means and medians of egg diameter and the number of eggs/bundle were compared within species and between species using parametric and non-parametric one-way ANOVAs and their a posteriori tests.

Results

Self-fertilization and control trials

In the first experiment (27 August 1994), control unfertilized cultures had few (<10) or no planulae in them, except for the culture with eggs from colony *Montastraea faveolata-2* (Fig. 2A), indicating that little self-fertilization had taken place during the gamete separation processing. Because the control for colony *M. faveolata-2* failed to perform as expected, data for crosses using egg preparations from this colony were not included in any of the analyses.

In both fertilization experiments, larval production in self-fertilized cultures was low compared to that of most out-crosses (Fig. 2). In the 27 August experiment, four

Table 3 *Montastraea* spp. Spawning observations (*Man*, *M. annularis*; *Mfa*, *M. faveolata*; *Mfr*, *M. franksi*) (– no data)

Year, locality	Reef	Species (no. of colonies)	Date (night)	Days after full moon	Time of day (hrs)	Duration (min)
1991						
Bahamas, Andros Island	Joulters Keys	*Man*, *Mfa*	30 Aug	7	22:30	–
		Man, *Mfr*	1 Sep	7	22:30	60
1993						
Florida	Key Largo Dry Rocks	*Man*, *Mfa*	8,9 Sep	7–8	22:30	60
1994						
Florida	Key Largo	*Man*, *Mfa* (>20 each)	27 Aug	7	22:00	60
	Dry Rocks	*Mfr* (>10)		7	20:30	35
		Man, *Mfa* (>10 each)	28 Aug	8	23:30	45
		Mfr (> 5)		8	22:30	45
1995						
Florida	Biscayne National Park Alina's Reef	*Man* (>15)	16–18 Aug	6–8	23:30	45
		Mfa (>20)		6–8	23:00	45
		Mfr (4)		6–8	21:30	30
	Key Largo	*Man* (1)	14 Sep	7	23:20	15
		Mfa (3)	15 Sep	8	23:00	30

colonies produced no larvae when self-fertilized, and three produced <10 planulae. Only two colonies of *Montastraea faveolata* (*Mfa-2* noted above and *Mfa-3*) had high levels of self-fertilization (note that the no-sperm control of *M. faveolata-3* performed as expected with no planulae found). In the 28 August experiment, three out of eight selfing samples had no larvae, and five had <10 larvae (Fig. 2B). The slightly higher incidence of selfing success in this second experiment may be because all samples except one (*M. franksi-1*) were field-collected gamete bundles, and thus there is the possibility that these bundles were contaminated with stray sperm from other colonies at the time of collection. However, only one of these selfed cultures still had any live planulae when examined 60 h after fertilization.

Intraspecific vs interspecific crossings

Fertilization success varied greatly within similar types of crosses, with some crosses within a group yielding more than 100 larvae and others yielding none (Figs. 2 and 3). In the first experiment with separated eggs and sperm (27 August), within-species crosses for *Montastraea faveolata* produced significantly more larvae than did within-species crosses for the other two species, but this was not the case during the second experiment using egg bundles (28 August) (Figs. 2 and 4). Crosses between *M. annularis* and *M. franksi* yielded a higher number of larvae than did their respective intraspecific crosses, but these differences were only statistically significant for *M. franksi* in the first experiment (Kruskal–Wallis ANOVA on ranks, $p < 0.05$). In the second experiment, inter-specific crosses between *M. franksi* and the other two species yielded more larvae than on the previous night, especially for the hybridization with

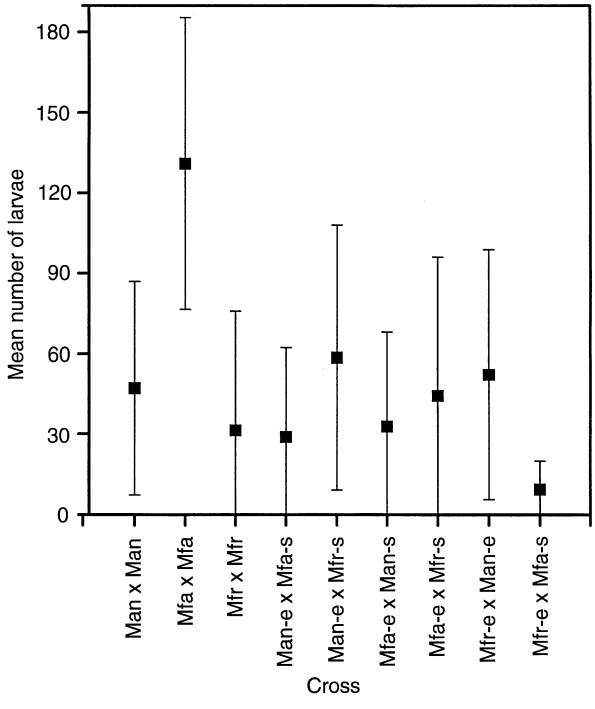

Fig. 3 *Montastraea* spp. Fertilization trials on 27 August 1994. Mean and standard deviations of number of planula larvae counted in each type of cross (excluding control and selfing crosses) 56 h after fertilization (*species abbreviations* as in Fig. 2; *e* eggs; *s* sperm) $n = 6$ for *Man* × *Man* and *Mfr* × *Mfr* crosses; $n = 4$ for *Mfa* × *Mfa* crosses because of exclusion of data from trials with eggs from Colony *Mfa-2*; $n = 9$ for each of hybrid crosses except those using *Mfa* eggs and other species sperm, where $n = 6$ because of exclusion of data from cultures with eggs from Colony *Mfa-2*

M. faveolata. Overall, the crosses between *M. annularis* and *M. franksi* were the most successful of the hybridization crosses and those between *M. faveolata* and

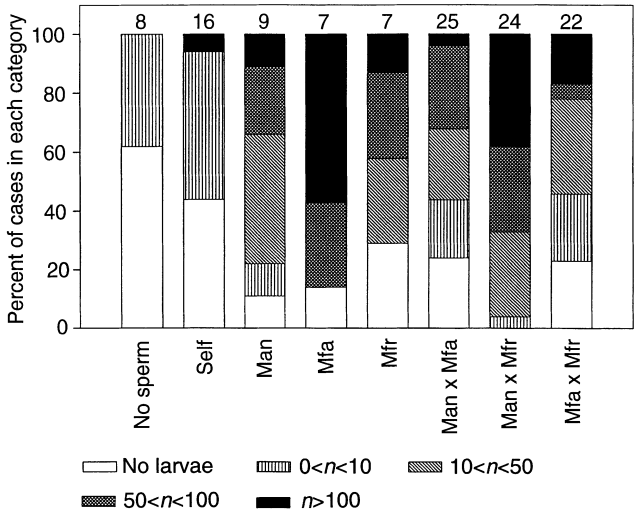

Fig. 4 *Montastraea* spp. Overall fertilization success of each type of cross expressed as percentage of total number of cases (individual crosses) in each rank class (see "Materials and methods – Cross-fertilization experiments" for explanation of ranks) [*Species abbreviations* as in Fig. 2; *numbers above each column* total number of cases for that particular cross; data pooled from both fertilization trials, excluding crosses with eggs of *Mfa-2* in first trial; *No sperm* control trials where no sperm was added to eggs]

either *M. franksi* or *M. annularis* the least, but even in the latter cases, 20 to 35% of the crosses produced >50 larvae, respectively (Fig. 4).

Gamete characteristics

None of the gamete characteristics measured differed significantly between colonies within species (one-way ANOVA ($p < 0.001$) and Kruskall–Wallis ($p < 0.005$) although there were small differences between years (Table 2).

Average egg volume of *Montastraea franksi* was 20 to 30% larger than those of the other two species, and eggs of *M. faveolata* were 6% larger than those of *M. annularis* ($p < 0.001$). There were also significant differences in the number of eggs per gamete bundle, with *M. franksi* having the fewest, and *M. faveolata* the most. The resulting total egg volume per bundle was dramatically different between the species, with *M. franksi* gamete bundles having 43% less egg volume than those of *M. faveolata* and 30% less than those of *M. annularis*. Average egg volume per *M. faveolata* gamete bundle was 24% greater than that for *M. annularis*.

Sperm in the freshly spawned bundles showed no sign of motility. Motility began ~30 min after breaking open the bundles.

Spawning timing and behavior

All observations indicate that *Montastraea franksi* begins spawning ~1 to 1.5 h (beginning ~2 h after sunset) earlier than *M. annularis* and *M. faveolata* (Table 3), but that the end of its spawning period overlaps the initiation of spawning by the latter two. In August 1995, *M. annularis* was observed to begin spawning ~30 min after *M. faveolata* on the reef in Biscayne National Park. The cores observed in the ship-board aquaria in 1991, collected ~1 wk before spawning, began to spawn at approximately the same time as field colonies, but there were small differences in the timing of spawning between the three colonies. Cores from the same colony in the two aquaria spawned at the same time, and spawning time differed by 10 to 15 min between colonies. This suggests a genetic component to the inter-colony variation in spawning time.

Discussion

Interspecific hybridization

In the present experiments, hybridization tests between the three morphological *Montastraea* species produced significant numbers of larvae, and in fact hybrid crosses between *M. annularis* and *M. franksi* produced as many or more larvae than did intra-specific crosses for each species. The somewhat greater hybridization success of crosses between *M. annularis* and *M. franksi* is consistent with the higher genetic similarity between these two species compared to *M. faveolata* (Knowlton et al. 1992; Van Veghel and Bak 1993; Weil and Knowlton 1994). In the first experiment, *M. faveolata* produced fewer larvae in hybrid than in intra-specific crosses, but in the second experiment it hybridized well with both of the other species. Given the low total number of crosses made, the lack of replication of individual crosses, and the high variability in successfulness within each type of cross, we must be cautious to not over-interpret the present results, but high variabilty in fertilization success seems to be common for corals (Wallace and Willis 1994; Miller and Babcock 1997; Willis et al. 1997). It is clear from these experiments that there are minimal inherent prezygotic barriers to fertilization between these species. Whether the hybrid larvae are capable of settlement, long-term survival, and eventually producing viable gametes remains to be determined. Two other studies (Knowlton et al. 1997; D. Hagman et al. unpublished data) have also tested hybridization success between these species with corals from Panamá and the Texas Flower Gardens, respectively. However, in contrast to our study, they both report reduced fertilization success between *M. faveolata* and *M. franksi* (crosses between *M. faveolata* and *M. annularis* were not done by D. Hagman et al.). Differences between the results from the three areas could represent true differences in hybridization potential between the regions, or be a consequence of the lower replication or methodology of the other studies.

Self-fertilization

The first studies of fertilization in broadcasting hermaphroditic corals indicated that a majority of the species studied had poor ability to self-fertilize, but that for some species, self-fertilization potential increased with time after spawning (Kojis and Quinn 1981; Heyward and Babcock 1986; Wallace and Willis 1994). The present study also found that the three species of *Montastraea* have very low ability to self-fertilize, and similar results were obtained by Knowlton et al. (1997) and D. Hagman et al. (unpublished data). We have no explanation for the high self-fertilization rates of the two colonies of *M. faveolata* in our first fertilization experiment. It is unlikely that contamination during processing of the gamete bundles could account for such high rates of fertilization given the methods used. More work will be necessary to determine whether there is significant variability between individual colonies in this trait, or whether eggs of some colonies are capable of parthenogenesis (e.g. Brazeau and Lasker 1989). Another intriguing possibility would be that chimeric colonies form from fusion of closely settled larvae (Harrison and Wallace 1990; T. Hayashibara personal communication; and unpublished data from our laboratory), and that such colonies can produce two sets of gametes without gamete incompatibility for each other.

The delay of sperm activation until after bundle breakdown, observed in this and other studies, is one mechanism of minimizing self-fertilization in corals (Heyward and Babcock 1986; Heyward et al. 1987; Oliver and Babcock 1992). This and other mechanisms provide time for gametes from different colonies to mix at the water surface, an important requirement for species that cannot self-fertilize (Babcock and Heyward 1986; Heyward and Babcock 1986; Willis et al. 1993). Such mechanisms also increase the chances of hybridization with gametes of other species with similar spawning patterns.

Gamete characteristics

The gamete bundles of *Montastraea annularis* and *M. faveolata* collected during the 1994 and 1995 spawning seasons contained the full number of eggs estimated to be produced by individual polyps each year (Table 2: 96 to 122 eggs per bundle; 12 gonads per polyp $\times$ 7 to 10 eggs per gonad; Szmant 1991). The bundles of *M. franksi*, however, had only half as many eggs as would be expected were all eggs per polyp included. One of the possible explanations for this latter result could be that *M. franksi* has fewer developed gonads per polyp or fewer eggs per gonad than the other two species; however this was ruled out by examination of histological sections of $\sim$20 colonies of *M. franksi* collected over the 1993 to 1995 period. Van Veghel and Kahmann (1994), working in Curaçao, also found as many or more gonads and eggs per polyp in *M. franksi* as in *M. annularis* and *M. faveolata*. The remaining possibility is that *M. franksi* partitions its annual egg production into more than one gamete bundle per polyp each spawning season, either by spawning multiple nights in a given month, or by spawning multiple months per season (split-spawning). Split-spawning (observed for these species) requires either that entirely different colonies spawn during the different nights and months of an annual spawning season, or that individual colonies or polyps split up their annual gamete production into different spawning packets. Such spawning behavioral characteristics may be different for each species, and thus serve as a diagnostic character for differentiating between species, or may vary annually depending on environmental cues and other factors. In 1996, a year in which there was split-spawning, gamete bundles collected during the second spawn (3 and 4 September) from *M. annularis* had only 28 $\pm$ 8 eggs per bundle ($n = 8$), and those from *M. faveolata* only 86 $\pm$ 35 ($n = 3$) (cf. with Table 2). Thus, the species differences in eggs/bundle observed in the 1994 and 1995 samples should not be interpreted as indicating species differences until confirmed with much larger sample sizes over multiple years.

Spawning timing and behavior

The annual cycle of spawning appears to be similar over much of the geographic range of these species, even though annual temperature ranges and light cycles differ significantly over the latitudinal range (Szmant 1991; Van Veghel 1994). Based on the available history of spawning observations, (Szmant 1991; Wyers et al. 1991; Van Veghel 1994; Knowlton et al. 1997; D. Hagman et al. unpublished data) the timing of spawning of Caribbean/Atlantic *Montastraea* should occur each year over Nights 6 to 9 after the full moon (third-quarter moon) of one or more months from July through August, depending on latitudinal location. The main calendar month for spawning over most of the range appears to be August, unless the full moon falls before the first week in August. When the full moon occurs late in July or too early in August, gametes in most colonies will not be ripe for spawning during that third-quarter moon. In such case, spawning will follow on the next full moon in late August or early September. Furthermore, there is good evidence for split-spawning, with a minor spawning after the early August full moon and a more intense spawning the next month (Szmant 1991 from histological data; Hagman et al. unpublished data). In 1995, the August full moon occurred early in the second week in August, and this resulted in an unusual geographic pattern of spawning. Florida corals spawned heavily after the August full moon (spawning in the middle of August), while most observations from the Gulf of Mexico, Bahamas and Caribbean (Jamaica, Panamá, Roatan) indicated that few corals spawned. Corals from these latter areas were observed to have a

major spawning after the September 1995 full moon when our observation of over 200 colonies from five sites in Key Largo, Florida, showed only five that contained ripe gonads. At present, the few observations available do not indicate any major differences in the monthly-scale temporal patterns of spawning between the three *Montastraea* species (Van Veghel 1994; D. Hagman et al. unpublished data), but this is a topic that merits further study.

There were, however, consistent differences in the daily time of onset of spawning between *Montastraea franksi* and the other two species in both 1994 and 1995. In both years, *M. franksi* began spawning ~1.5 h before the first *M. annularis* and *M. faveolata* released their bundles. A similar difference in spawning time was observed by Knowlton et al. (1997) in Panamá and D. Hagman et al. (unpublished data) in the Gulf of Mexico. These observations contrast with the lack of temporal differences between *M. franksi* and the other two species reported for Curaçao (Van Veghel 1994).

Implications

For marine organisms that broadcast their gametes, dilution factors, the duration of gamete viability, and sperm–egg contact time are three important factors affecting fertilization success (Levitan et al. 1991; Oliver and Babcock 1992). Spawning aggregations and synchronous release of gametes during a short period of time, presumably when environmental conditions are most favorable, are thought to represent evolutionary adaptations aimed at enhancing fertilization success (i.e. Babcock et al. 1986; Pearse et al. 1986). For sessile invertebrates such as corals, synchronous spawning is more critical, since they cannot move around to aggregate with conspecifics. However, when dozens of closely related species of corals spawn simultaneously (Harrison et al. 1984; Willis et al. 1985; Babcock et al. 1986; Gittings et al. 1992; Van Veghel 1993), high concentrations of gametes in surface slicks make it likely that some hybridization will occur. Species-specific sperm attractants have been found in coral eggs (Miller 1985; Coll et al. 1994), but it is not known how effective they are in enhancing intraspecific fertilization under such concentrated conditions.

Hybridization reports for corals are mostly from experimental matings where gametes do not have the option of selecting for conspecifics (Willis et al. 1993; Miller and Babcock 1997; present study). The frequency of natural hybridization remains to be determined. Near-simultaneous spawning periods and lack of prezygotic barriers to cross-fertilization within the species complex of *Montastraea* present the possibility of hybridization under natural conditions, and could explain what appear to be "intermediate" colony morphologies (Fig. 1D, E). There is no general agreement as to how much hybridization should be accepted before species are considered subspecies or races, and this is a subject matter that will remain a topic of debate for some time to come (Mayr 1982). Molecular techniques will hopefully help clarify this problem. However, both genetic and hybridization studies of *Platygyra* spp. have failed to differentiate between morphologically distinct species of this genus (Miller and Babcock 1997).

In the present case, occasional hybridization among the three morphological species would not necessarily invalidate their species designation as long as gene flow between them is limited. The allozyme studies characterizing genetic differences between the three species were conducted over only a limited portion of their distribution range (Panamá and Curaçao) in localities where few intermediate morphologies are encountered. Results of sequencing the rDNA fragment that embraces both the ribosomal internal transcribed spacers (ITS1 and ITS2) and the 5.8 S ribosomal gene, and mtDNA cytochrome oxidase non-transcribed regions [both regions used regularly to discriminate between sub-populations within species (Hillis and Dixon 1991; Hillis et al. 1991)] of three to five specimens of each *Montastraea* species (including *M. cavernosa* as an out-group) collected in Florida, do not indicate divergence between the species in these DNA regions (Medina et al. 1997). Morphological intermediates are abundant in the U.S. Virgin islands, Greater Antilles, Florida and Bahamas. While congruent suites of independent characters (morphological, behavioral, ecological and molecular) represent a compelling argument for species separation, clearly more work is required to verify the breeding and genetic isolation of these species over their broader range. On the other hand, this study provides information on two additional characters, timing of spawning and egg size, thought to separate these species.

Acknowledgements This project was made possible by Florida Keys National Marine Sanctuary biologist J. Halas, who helped yearly with permits, logistical support, and advice, and R. Curry, Science Director, Biscayne National Park, who similarly assisted our work during the 1995 spawning season. D. Anderegg, V. Cornwell, E. McKinley and the staff of Biscayne National Park helped with field and laboratory work, and the crew of the R.V. "Calanus" provided excellent logistical support. Comments from three anonymous reviewers greatly improved the paper. This research was partially supported by NSF Grants OCE89-00095 and OCE92-17993 to AMS.

References

Ayala FJ (1983) Enzymes as taxonomic characters. In: Oxford GS, Rollinson T (eds) Protein polymorphism: adaptive and taxonomic significance. Academic Press, London, 3–26

Babcock RC, Bull GD, Harrison PL, Heyward AJ, Oliver JK, Wallace CC, Willis BL (1986) Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef. Mar Biol 90: 379–394

Babcock RC, Heyward, AJ (1986) Larval development of certain gamete spawning scleractinian corals. Coral Reefs 5: 111–116

Bak RPM, Engel MS (1979) Distribution, abundance and survival of juvenile hermatypic corals (Scleractinia) and the importance of life history strategies in the parent coral community. Mar Biol 54: 341–352

Brazeau DA, Lasker HR (1989) The reproductive cycle and spawning in a Caribbean gorgonian. Biol Bull mar biol Lab, Woods Hole 176: 1–7

Budd AF (1993) Variation within and among morphospecies of *Montastraea*. CFS-Courier 164: 241–254 (Forschungsinstitut, Senckenberg: Sonderdruck)

Coates AG, Jackson JBC (1985) Morphological themes in the evolution of clonal and aclonal marine invertebrates. In: Jackson JBC, Buss LW, Cook RE (eds) Population biology and evolution of clonal organisms. Yale University Press, New Haven, Connecticut, pp 67–106

Coll JC, Bowen BF, Mechan GV, Konig GM, Carrol AR, Tapiolas DM, Aliño PM, Heaton A, De Nys R, Leone PA, Maida M, Aceret TL, Willis RH, Babcock RC, Willis BL, Florian Z, Clayton MN, Miller RL (1994) Chemical aspects of mass spawning in corals. I. Sperm-attractant molecules in the eggs of the scleractinian coral *Montipora digitata*. Mar Biol 118: 177–182

Ellis J, Solander D (1786) The natural history of many curious and uncommon zoophytes. Benjamin White & Son, London

Dustan P (1975) Genecological differentiation in the reef-building coral *Montastrea annularis*. PhD dissertation. State University of New York, Stony Brook

Gittings SR, Boland GS, Deslarzes KJP, Combs C, Holland BS, Bright TJ (1992) Mass spawning and reproductive viability at the East Flower Garden Bank, Northern Gulf of Mexico. Bull mar Sci 51: 420–428

Gregory JW (1895) Contributions to the paleonthology and physical geology of the West Indies. Q Jl geol Soc Lond 51: 255–312

Goreau TF (1959) The ecology of Jamaican coral reefs. I. Species composition and zonation. Ecology 40: 67–90

Graus RR, Macintyre IG (1982) Variation in growth form of the coral *Montastrea annularis* (Ellis and Solander): a quantitative evaluation of growth response to light distribution using computer simulation. Smithson Contr mar Sci 12: 441–459

Harrison PL, Babcock RC, Bull GD, Oliver JK, Wallace CC, Willis BL (1984) Mass spawning in tropical reef corals. Science, NY 223: 1186–1189

Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z (ed) Ecosystems of the world. Vol 25. Coral reefs. Elsevier, Amsterdam, pp 133–207

Hayes JA (1990) Distribution, movement and impact of the corallivorous gastropod *Coralliophila abbreviata* (Lamarck) on a Panamanian patch reef. J exp mar Biol Ecol 142: 25–42

Heyward AJ, Babcock RC (1986) Self- and cross-fertilization in scleractinian corals. Mar Biol 90: 191–195

Heyward AJ, Yamazato K, Yeemin T, Minei M (1987) Sexual reproduction of coral in Okinawa. Galaxea 6: 331–343

Hillis DM, Dixon MT (1991) Ribosomal DNA: molecular evolution and phylogenetic inference. Q Rev Biol 66: 409–451

Hillis DM, Moritz C, Porter CA, Baker RJ (1991) Evidence for biased gene conversion in concerted evolution of ribosomal DNA. Science, NY 251: 308–310

Hubbard DK, Scaturo D (1985) Growth rates of seven species of scleractinian corals from Cane Bay and Salt River, St. Croix, USVI. Bull mar Sci 36: 325–338

Huston M (1985) Variation in coral growth rates with depth at Discovery Bay, Jamaica. Coral Reefs 4: 19–25

Knowlton N (1993) Sibling species in the sea. A Rev Ecol Syst 24: 189–216

Knowlton N, Maté JL, Guzmán HM, Rowan R, Jara J (1997) Direct evidence for reproductive isolation among the three species of the *Montastraea annularis* complex in Central America (Panamá and Honduras). Mar Biol 127: 705–711

Knowlton N, Weil E, Weigt LA, Guzmán HM (1992) Sibling species in *Montastraea annularis*, coral bleaching, and the coral climate record. Science, NY 255: 330–333

Kojis Bl, Quinn NJ (1981) Aspects of sexual reproduction and larval development in the shallow water hermatypic coral *Goniastrea australensis* (Edwards and Haime, 1857). Bull mar Sci 31: 558–573

Lang JC (1984) Whatever works: the variable importance of skeletal and of non-skeletal characters in scleractinian taxonomy. Palaeontogr am 54: 18–44

Lasker HR (1981) Phenotypic variation in the coral *Montastraea cavernosa* and its effects on colony energetics. Biol Bull mar biol Lab, Woods Hole 160: 292–302

Levitan DR, Sewell MA, Chia F (1991) Kinetics of fertilization in the sea urchin *Strongylocentrotus franciscanus*: interaction of gamete dilution, age, and contact time. Biol Bull mar biol Lab, Woods Hole 181: 371–378

Mayr E (1968) Illiger and the biological species concept. J Hist Biol 1: 163–178

Mayr E (1970) Populations, species, and evolution. Harvard University Press, Cambridge, USA

Mayr E (1982) The growth of biological thought. Diversity, evolution and inheritance. Belknap Harvard University Press, Cambridge, USA

Medina M, Weil E, Szmant AM (1997) The *Montastraea annularis* species complex from a molecular approach. Proc 8th int coral Reef Symp (In press) [Lessios HA, Macintyre I (eds) Smithsonian Tropical Research Institute, Panamá]

Miller K, Babcock R (1997) Conflicting morphological and reproductive species boundaries in the coral genus *Platygyra*. Biol Bull mar biol Lab, Woods Hole (In press)

Miller RL (1985) Sperm chemo-orientation in the metazoa. In: Metz CB Jr, Monroy A (eds) The biology of fertilization. Vol 2. Acad Press, New York, pp 275–337

Oliver J, Babcock PL (1992) Aspects of the fertilization ecology of broadcast spawning corals: sperm dilution effects and in situ measurements of fertilization. Biol Bull mar biol Lab, Woods Hole 183: 409–417

Pearse JS, Eernisse DJ, Pearse VB, Beauchamp KA (1986) Photoperiodic regulation of gametogenesis in sea stars, with evidence for an annual calendar independent of fixed daylength. Am Zool 26: 417–431

Reed JK (1985) Deepest distribution of Atlantic hermatypic corals discovered in the Bahamas. Proc 5th int coral Reef Congr 6: 249–254 [Gabrié C et al. (eds) Antenae Museum-EPHE, Moorea, French Polynesia]

Roos PJ (1964) The distribution of reef corals in Curaçao. Stud Fauna Curaçao 20: 1–51

Szmant AM (1986) Reproductive ecology of Caribbean reef corals. Coral Reefs 5: 43–54

Szmant AM (1991) Sexual reproduction by the Caribbean reef corals *Montastraea annularis* and *M. cavernosa*. Mar Ecol Prog Ser 74: 13–25

Szmant AM, Weil E, Jones DE, Miller M (1995) Cross-fertilization tests between sibling species of an Atlantic reef coral. J cell Biochem (Suppl) 19B: p. 343

Szmant-Froelich AM (1985) The effect of colony size on the reproductive ability of the Caribbean coral *Montastraea annularis* (Ellis and Solander). Proc 5th int coral Reef Congr 4: 295–300 [Gabrié C et al. (eds) Antenne Museum-EPHE, Moorea, French Polynesia]

Tomascik T (1990) Growth rates of two morphotypes of *Montastrea annularis* along a eutrophication gradient, Barbados, W.I. Mar Pollut Bull 21: 376–380

Van Moorsel GWNM (1983) Reproductive strategies in two closely related stony corals (*Agaricia*, Scleractinia). Mar Ecol Prog Ser 13: 273–283

Van Veghel MLJ (1993) Multiple species spawning on Curacao reefs. Bull mar Sci 52: 017–1021

Van Veghel MLJ (1994) Reproductive characteristics of the polymorphic Caribbean reef building coral *Montastraea annularis*. I. Gametogenesis and spawning behavior. Mar Ecol Prog Ser 109: 209–219

Van Veghel MLJ, Bak RPM (1993) Intraspecific variation of a dominant Caribbean reef building coral, *Montastraea annularis*: genetic, behavioral and morphometric aspects. Mar Ecol Prog Ser 92: 255–265

Van Veghel MLJ, Kahmann MEH (1994) Reproductive characteristics of the polymorphic Caribbean reef building coral

Montastraea annularis. II. Fecundity and colony structure. Mar Ecol Prog Ser 109: 211–227

Veron JEN (1995) Corals in space and time: Biogeography and evolution of the Scleractinia. Cornell University Press, Ithaca, New York

Wallace CC, Willis BL (1994) Systematics of the coral genus *Acropora*: implications of new biological findings for species concepts. A Rev Ecol Syst 25: 237–262

Weil E (1980) Papel del erizo *Diadema antillarum* Philippi en la regulación de la estructura de las comunidades coralinas. MSc thesis. Universidad Central de Venezuela, Caracas

Weil E (1992) Genetic and morphological variation of *Porites* (Anthozoa, Scleractinia) across the Isthmus of Panama. PhD dissertation. University of Texas at Austin, Austin, Texas

Weil E (1993) Genetic and morphological variation in Caribbean and Eastern Pacific *Porites* (Anthozoa, Scleractinia). Preliminary results. Proc 7th int coral Reef Symp 2: 643–656 [Richmond RH (ed) University of Guam, Mangilao, Guam]

Weil E, Knowlton N (1994) A multi-character analysis of the Caribbean coral *Montastraea annularis* (Ellis and Solander 1786) and its two sibling species, *M. faveolata* (Ellis and Solander 1786) and *M. franksi* (Gregory 1895). Bull mar Sci 55: 151–175

Willis BL (1990) Species concepts in extant scleractinian corals: considerations based on reproductive biology and genotypic population structures. Syst Bot 15: 136–149

Willis BL, Babcock RC, Harrison PL, Oliver JK, Wallace CC (1985) Patterns in the mass spawning of corals on the Great Barrier Reef from 1981 to 1984. Proc 5th int coral Reef Congr 4: 343–348 [Gabrié C et al. (eds) Antenne Museum-EPHE, Moorea, French Polynesia]

Willis BL, Babcock RC, Harrison PL, Wallace CC (1993) Experimental evidence of hybridization in reef corals involved in mass spawning events. Proc 7th int coral Reef Symp 1: p.504 [Richmond RH (ed) University of Guam, Mangilao, Guam]

Willis BL, Babcock RC, Harrison PL, Wallace CC (1997) Hybridization and breeding incompatabilities within the mating systems of mass spawning reef corals. Coral Reefs (In press)

Wyers SC, Barnes HS, Smith SR (1991) Spawning of hermatypic corals in Bermuda: a pilot study. Hydrobiologia 216/217: 109–116