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Geographic differences in vertical connectivity in the Caribbean coral *Montastraea cavernosa* despite high levels of horizontal connectivity at shallow depths

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Abstract

The deep reef refugia hypothesis proposes that deep reefs can act as local recruitment sources for shallow reefs following disturbance. To test this hypothesis, nine polymorphic DNA microsatellite loci were developed and used to assess vertical connectivity in 583 coral colonies of the Caribbean depth-generalist coral *Montastraea cavernosa*. Samples were collected from three depth zones (≤10, 15–20 and ≥25 m) at sites in Florida (within the Upper Keys, Lower Keys and Dry Tortugas), Bermuda, and the U.S. Virgin Islands. Migration rates were estimated to determine the probability of coral larval migration from shallow to deep and from deep to shallow. Finally, algal symbiont (*Symbiodinium* spp.) diversity and distribution were assessed in a subset of corals to test whether symbiont depth zonation might indicate limited vertical connectivity. Overall, analyses revealed significant genetic differentiation by depth in Florida, but not in Bermuda or the U.S. Virgin Islands, despite high levels of horizontal connectivity between these geographic locations at shallow depths. Within Florida, greater vertical connectivity was observed in the Dry Tortugas compared to the Lower or Upper Keys. However, at all sites, and regardless of the extent of vertical connectivity, migration occurred asymmetrically, with greater likelihood of migration from shallow to intermediate/deep habitats. Finally, most colonies hosted a single *Symbiodinium* type (C3), ruling out symbiont depth zonation of the dominant symbiont type as a structuring factor. Together, these findings suggest that the potential for shallow reefs to recover from deep-water refugia in *M. cavernosa* is location-specific, varying among and within geographic locations likely as a consequence of local hydrology.

Keywords: climate change, cnidarians, conservation genetics, deep reef refugia hypothesis, great star coral, mesophotic, population genetics—empirical

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Introduction

The most devastating impacts of climate change on coral reef ecosystems have occurred at shallow depths (Glynn *et al.* 2001; West & Salm 2003; Bak *et al.* 2005), where the combination of intense light and high temperature has resulted in the most severe episodes of mass coral 'bleaching' (loss of symbionts from corals and other reef invertebrates or loss of photopigments from symbionts) and mortality. Recent evidence suggests that mesophotic coral ecosystems (MCEs) in the range of 30–150 m fared better than their shallow water counterparts, due to lower heat-induced photoinhibition (Glynn 1996; Glynn *et al.* 2001; Baker *et al.* 2008; van Oppen *et al.* 2011; T. B. Smith, unpublished data), less severe storm impacts (Bongaerts *et al.* 2010 and van Oppen *et al.* 2011; but see Bak *et al.* 2005), reduced loss of major herbivores (Bongaerts *et al.* 2010; van Oppen *et al.* 2011) and less diseases (Bongaerts *et al.* 2010; but see Calnan *et al.* 2008). These observations support the notion that deep reefs may act as refugia for shallow reefs (deep reef refugia hypothesis; Bongaerts *et al.* 2010), but whether they can provide a viable larval supply for shallow reefs following disturbance has not yet been established.

One way to determine the capacity of deep coral populations to replenish shallow reefs following disturbance is to measure the extent and direction of gene flow among these habitats. For example, the brooding coral *Seriatopora hystrix* (Dana, 1846) exhibits different patterns of vertical connectivity depending on location: at Scott Reef (northwest Australia), recruitment of deep-water larvae into shallow habitats was inferred from cluster-based analyses, but migration rates and direction of gene flow (shallow to deep vs. deep to shallow) were not assessed (van Oppen *et al.* 2011). Conversely, at Yonge Reef (northeast Australia), little connectivity among these habitats was observed. A reciprocal transplantation experiment later carried out at Yonge reef (Bongaerts *et al.* 2011a) revealed that the lack of vertical connectivity at this location could be the result of strong selective pressures along the depth gradient. More recently, Prada & Hellberg (2013) measured survivorship in reciprocally transplanted colonies of the Caribbean octocoral *Eunicea flexuosa* (Lamouroux, 1821) and showed that native colonies (i.e. colonies transplanted to the same depth of origin) had a selective advantage over non-native colonies that originated from a different depth. In addition, genetic differentiation supported the presence of two depth-segregated lineages, providing the first evidence of higher migration rates from shallow to deep habitats for a coral species. Together, these studies suggest that genetic structure with depth originates from local adaptation of corals to the different environmental conditions in shallow vs. deep habitats.

Montastraea cavernosa (Linnaeus, 1767) is a gonochoric broadcast spawner considered an 'extreme' depth-generalist (Bongaerts *et al.* 2010), as it inhabits depths from 3 to 100 m (Reed 1985; Lesser *et al.* 2010). In addition, this species is found throughout the Atlantic region, extending from Bermuda to Brazil to the West African coast (Veron 2000; Nunes *et al.* 2009; Goodbody-Gringley *et al.* 2011). In Caribbean and Bermudian reefs, spawning takes place approximately 1 week after the full moon during the months of July through September (Szmant 1991), and recent observations have shown that deep and shallow colonies can spawn in synchrony (Vize 2006). The large eggs of *M. cavernosa* are thought to increase larval survival time and dispersal capability, as well as increase postsettlement survival (Nunes *et al.* 2009). In addition, algal symbionts are not present in the eggs (Szmant 1991), which suggest that corals might be capable of colonizing habitats over a broad depth range by acquiring the appropriate local symbionts from the water column. Overall, these characteristics might facilitate wide-scale dispersal, as evidenced by the moderate to high gene flow documented among shallow sites in the Caribbean–North Atlantic (Nunes *et al.* 2009 and Goodbody-Gringley *et al.* 2011 but see Brazeau *et al.* 2013) and within the region of Brazil (Nunes *et al.* 2009). However, whether *M. cavernosa* comprises a single panmictic population or various isolated populations across its depth distribution remains poorly understood. To date, only Brazeau *et al.* (2013) has assessed patterns of vertical connectivity in a subset of *M. cavernosa* colonies ($N = 105$) from Cayman Islands and Bahamas (within the Caribbean region), providing the first evidence of significant genetic differentiation with depth for this species.

In this study, we aimed at understanding the role deep reefs may play in shallow reef recovery for *M. cavernosa*. Using high-throughput (454) sequencing, we developed nine DNA microsatellite loci and assessed vertical connectivity in 583 coral colonies collected from three depth zones (shallow ≤10 m, mid 15–20 m and deep ≥25 m) at sites in Florida (within the Upper Keys, Lower Keys and Dry Tortugas), Bermuda and the U.S. Virgin Islands (USVI). We evaluated patterns of connectivity at three levels: among geographic locations (long-distance *horizontal* connectivity), among reefs within a geographic location (intraregional comparisons in the Florida Reef Tract) and among depths at each region (short-distance *vertical* connectivity). Migration rates were estimated among depths at each region to determine whether migration is more likely to occur from deep to shallow reefs, from shallow to deep reefs or in both directions

symmetrically. Finally, we assessed algal symbiont (*Symbiodinium* spp.) diversity in a subset of corals to determine the presence of symbiont zonation, if any.

Methods

Sample collection

Field activities were focused on 'shallow' (≤10 m), 'intermediate' (15–20 m) and 'deep' (≥25 m) coral communities along the (i) Florida Reef Tract (within sites in the Upper Keys, Lower Keys and Dry Tortugas), (ii) Bermuda and (iii) USVI (Table 1, Fig. 1). These different locations provided an opportunity to assess geographic differences in vertical connectivity. In addition, the comprehensive sampling design along the Florida Reef Tract (~400 km) allowed for intraregional scale comparisons of patterns of connectivity. Finally, 'deep' reefs were defined as those ≥25 m because there is very little coral cover information available for Florida at depths ≥30 m (Murdoch & Aronson 1999; Smith *et al.* 2011), and because this approximates the lower 1% attenuation depth (i.e. compensation depth) for visible radiation in the Lower Keys (~27 m, Barron *et al.* 2009).

Table 1 *Montastraea cavernosa* samples (N = 583). Given are the total sample size (N), number of unique multilocus genotypes (Ng) and ratio of genets over samples collected (Ng/N). GPS locations are in decimal degrees (WGS84)

Region	Subregion	Population	Site name or location	Site in map	Estimated depth (m)	N	Ng	Ng/N	Latitude	Longitude
Florida	Upper Keys	UK shallow	Conch reef	UK1	5	13	13	1.00	24.9465	−80.50207
			DL patch	UK2	5	6	6	1.00	25.0136833	−80.41387
			Little Conch reef	UK3	5	11	11	1.00	24.9511167	−80.4614
			Marker 39	UK4	5	12	12	1.00	25.0094333	−80.45792
			Sand island	UK5	5	12	12	1.00	25.0178667	−80.36823
			Tavernier Rocks	UK6	5	3	3	1.00	24.9389833	−80.56272
			Hens and Chickens	UK7	5	4	4	1.00	24.9341333	−80.54952
			Wolf reef	UK8	5	9	9	1.00	25.02185	−80.39623
			Behind Conch reef	UK9	5	6	6	1.00	24.9575833	−80.45603
		UK mid	SW of Molasses reef	UK10	16	14	14	1.00	25.0042333	−80.38757
			NE of Conch reef SPA	UK11	17	16	16	1.00	24.9465333	−80.45687
		UK deep	Pickles deep	UK12	25	7	7	1.00	24.97095	−80.43075
			Conch reef	UK13	29	12	12	1.00	24.9580667	−80.45243
			N of Molasses reef	UK14	37	4	4	1.00	25.0041333	−80.37987
	Lower Keys	LK shallow	Western Sambo reef	LK1	8	6	6	1.00	24.4784833	−81.7302
			Marker 32	LK2	8	10	10	1.00	24.4741667	−81.74268
			Near Key West	LK3	9	11	11	1.00	24.4687667	−81.82217
		LK mid	American Shoal mid1	LK4	16	21	20	0.91	24.5158167	−81.54315
			American Shoal mid2	LK5	14	20	19	1.00	24.5138167	−81.54248
		LK deep	American Shoal	LK6	25	30	30	1.00	24.5042167	−81.58197
	Dry Tortugas	DT shallow	Dry Tortugas National Park	DT1	8	38	38	1.00	24.6107833	−82.87133
		DT mid	Near Dry Tortugas	DT2	15	31	31	1.00	24.72225	−82.78715
		DT deep	Outside Dry Tortugas	DT3	25	44	44	1.00	24.62875	−83.10167
Bermuda		BDA shallow	Castle Harbour 4 m inshore	BDA1	4	25	23	0.92	32.3598833	−64.69243
			Castle Harbour 4 m offshore	BDA2	4	25	25	1.00	32.3367167	−64.65738
		BDA mid	Castle Harbour 18 m	BDA3	18	43	43	1.00	32.3354167	−64.65405
		BDA deep	Castle Harbour 26 m	BDA4	26	16	15	0.94	32.3252667	−64.65423
			Castle Harbour 35 m	BDA5	35	1	1	1.00	32.3253667	−64.65287
			Castle Harbour 40 m	BDA6	40	9	9	1.00	32.3246167	−64.65308
			Castle Harbour 44 m	BDA7	44	7	7	1.00	32.3255833	−64.6661
			Castle Harbour 49 m	BDA8	49	4	4	1.00	32.3251833	−64.65173
			Castle Harbour 53–58 m	BDA9	53–58	9	9	1.00	32.3253167	−64.6623
USVI		USVI shallow	Flat Cay	USVI1	7	42	42	1.00	18.5303667	−65.65172
		USVI mid	Buck Island	USVI2	20	12	12	1.00	18.4647167	−65.49722
		USVI deep	College Shoal	USVI3	30–33	50	49	0.98	18.3098167	−65.12772
Total						583	577	0.99		

USVI = U.S. Virgin Islands.

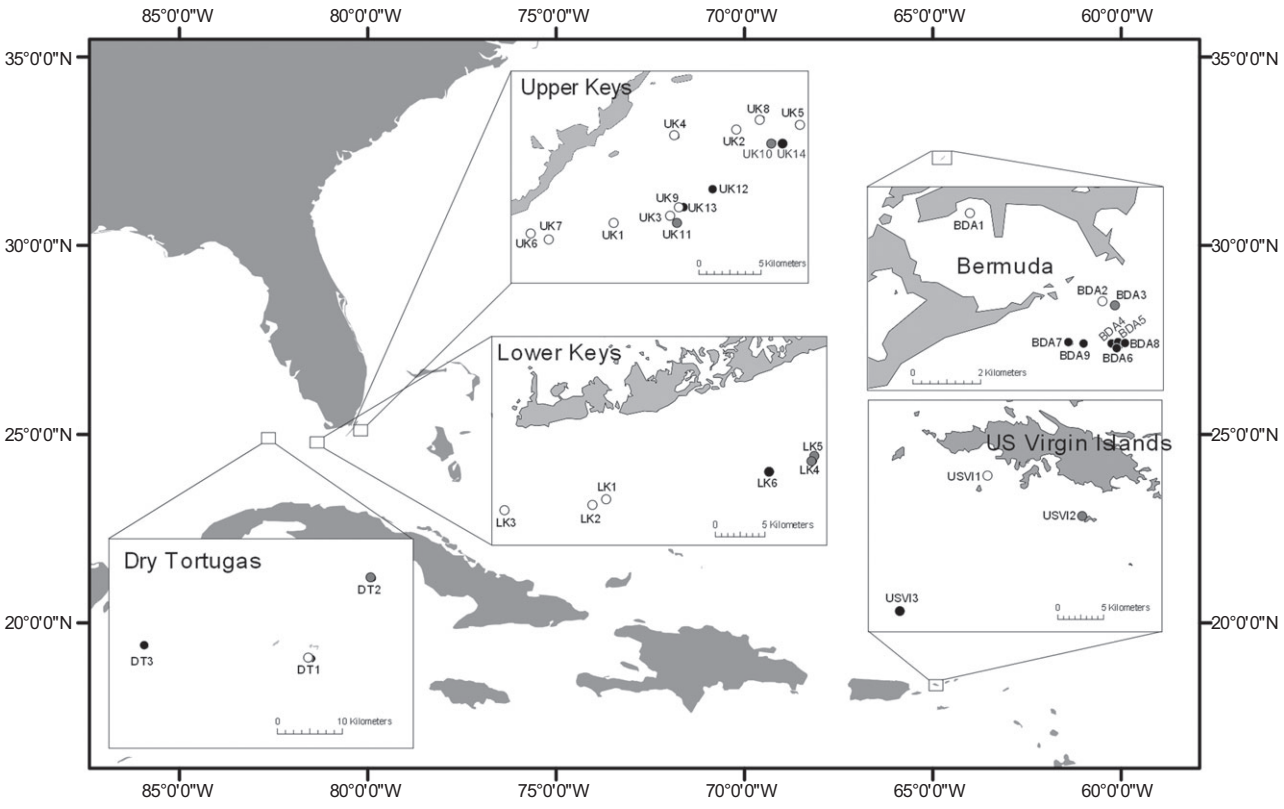

Fig. 1 Sampling locations in the Caribbean and western Atlantic. Individual sites are labelled as designated in Table 1. White circles denote 'shallow' (≤10 m) sites, grey circles denote 'intermediate' (15–20 m) sites and black circles denote 'deep' (≥25 m) sites.

At each geographic location, corals were sampled using SCUBA along depth transects. A haphazard approach was used to collect samples from colonies at least 1 m apart to minimize the likelihood of sampling clones. Samples were collected from the top of the colony (from a polyp facing upwards) to minimize intra-colony variation in the symbiont community. Two different sampling methods were used, per the requirements of the respective permitting agencies. Where allowed, samples were removed from colonies as small tissue biopsies (0.25 cm^2) using a 4-mm internal diameter hollow steel punch, and preserved in 95% ethanol until laboratory analyses. When destructive sampling was not permitted, small polyp biopsies were collected using a modification of the syringe method described in Correa *et al.* (2009a). The contents of each syringe were then expelled into a 15 mL-Falcon tube at the surface and centrifuged for 5 min at 500 *g*. The supernatant was removed, the pellet transferred to a 2-mL tube with 500 μL of DNAB + 1% SDS (Rowan & Powers 1991) and heated to 65 °C for 1.5–2 h, then preserved in the laboratory at room temperature. Finally, genomic DNA was extracted using a modified organic extraction protocol (Baker *et al.* 1997).

Microsatellite development

Cnidarians with algal symbionts are particularly difficult candidates for microsatellite development because DNA extraction protocols typically extract both host and symbiont DNA (Shearer *et al.* 2005). Therefore, to minimize contamination by symbiont DNA, the coral colony used for microsatellite development was bleached using the photosynthetic inhibitor DCMU as described in Jones (2004). Sampling occurred when the colony appeared visibly pale and genomic DNA was extracted prior to 454 sequencing on a Roche GS 454 FLX+ sequencer, and subsequent library construction (Genomics Core Facility, Penn State University).

A total of 47 968 single sequence reads (i.e. not assembled into contigs) generated from the 454 sequencing were trimmed with PipeMeta (Vera *et al.* 2008) and assembled with the GS De Novo Assembler (Roche Diagnostics Corporation, Indianapolis, IN), keeping the default settings and a minimum sequence length of 45 base pairs. Cnidarian sequences were then imported to the Tandem Repeat Finder (TRF) database (Benson 1999) and processed using the default alignment parameters as follows: Match: 2; Mismatch: 7; Indels: 7. Primers

were designed for a subset of sequences with a minimum of six tri-, tetra-, penta- or hexanucleotide repeats ($N = 104$) using the web-based program Primer 3 (Untergrasser *et al.* 2012). Primers were screened and optimized by visually inspecting bands on 2% agarose gels. Candidate markers ($N = 14$) were then screened against the algal symbionts isolated from a healthy portion of the colony used for microsatellite development (identified as *Symbiodinium* type C3) and other preexisting algal cultures in clades B, C and D isolated from the coral species *Orbicella faveolata* (Ellis and Solander, 1786), to confirm specificity to host DNA. Primers that amplified any of the cultured *Symbiodinium* ($N = 4$) were considered to be derived from the symbiont and thus were excluded from further development.

Microsatellite genotyping

A total of 10 microsatellite loci were developed for scoring on an ABI 3730 automated sequencer by fluorescently labelling forward primers with NED, VIC or 6-FAM (Applied Biosystems, CA). One of the 10 markers exhibited strong deviations from Hardy–Weinberg equilibrium (HWE) expectations (data not shown) and was excluded from further analysis. PCRs for the remaining nine loci are described in Table 2 and were performed in four multiplex reactions (11 μL total volume) and one singleplex reaction (10 μL total volume) using 1 μL of 50–100 ng of template DNA. Reactions were performed using primer concentrations specific to each locus (Table 2), 5× PCR Reaction Buffer (Promega), 2.75 mM of $MgCl_2$ (Promega), 0.8 mM of dNTPs and 0.5 U of Taq polymerase (Promega). Thermal cycling for all reactions was performed with an initial denaturation step of 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min; 57 °C (annealing temperature) for 1 min; 74 °C for 1 min; and a final extension at 74 °C for 7 min. PCR products were visualized with an automated sequencer (ABI 3730) using an internal size standard (Genescan LIZ-500, Applied Biosystems). Electropherograms were visualized and alleles scored using the software GeneMapper 4.0 (Applied Biosystems). Samples that failed to amplify more than two of the nine loci were excluded from further analysis ($N = 160$). In this data set, there was a per locus failure rate of <10% (except for marker MC49 which had a failure rate of 13.7%), and a per sample failure rate of 2.7%.

Analysis of multilocus genotype data

Identical *multilocus genotypes* (MLGs) were identified in GenAlEx v.6.41 (Peakall & Smouse 2006) by requiring complete matches at all loci. The same number of unique MLGs ($N = 577$) was found whether missing data were considered or not. Unique MLGs were then used for subsequent analyses. Tests for conformation to HWE expectations were performed using the program

Table 2 Nine microsatellite loci developed for *Montastraea cavernosa*, amplified in four multiplexes (plex A-D) and one singleplex reaction. Given are the locus name, primer sequences, motif type and the size range of the alleles amplified in base pairs (bp). Locus-specific primer concentrations are also given. All reactions had the same annealing temperature (57 °C). Forward primers were fluorescently labelled with one of three dyes (6-FAM, VIC or NED; Applied Biosystems, CA)

Locus	Primer sequence (5′-3′)	Motif type	Allele size range (bp)	Forward primer (μM)	Reverse primer (μM)	Plex
MC4	F: 6-FAM-ACGATCAAGACTCCAACGA R: GCTCTTCGTGAACACTGAGG	(TTA)7 T (TTA)2	97–222	0.4	0.4	A
MC18	F: VIC-GGAGAACTGGATACCATGTC R: TATGGTCCTGGGACAACTT	(AAT)2 TAT (AAT)9	218–260	0.4	0.4	A
MC29	F: 6-FAM-CTCCTTGGTCACCCTACAA R: GGTGAAGAAGCAGCCATTGG	(AAAC)7	155–194	0.08	0.08	B
MC41	F: 6-FAM-AATTACGCAACACTGTGCA R: TCGACTGACCGAAGTACCT	(GGTA) imperfect	344–448	0.4	0.4	B
MC46	F: VIC-CGGTGTAGCTCTAGCAGGA R: ACTGAGTCGCAGCATTTGG	(TTTTGT) imperfect	124–163	0.08	0.08	C
MC49	F: VIC-ATTCCTCCAGTGATGTACCT R: CTGAGTTCCTGCCATTAGG	(TGT)10	192–384	0.5	0.5	D
MC65	F: NED-TTTGTGATTGGCCAGGGTG R: TTGTGCTGTGAAGCATGAT	(TTTGGT)6	112–172	0.35	0.35	D
MC97	F: 6-FAM-ACATGTGGCCTTGTTACCA R: CGAACATCAGTGACAACCT	(ACAA)6 ACAG (ACAA)	163–187	0.08	0.08	C
MC114	F: VIC-ACTGTAGATCGAGGCGTTTC R: TCTGTTCCTCTGACTCTTTCG	(TTG)10 [15 bp insert] (TTG)6	152–230	0.55	0.55	Single

Genepop (Raymond & Rousset 1995). The *R*-package FDRtool was then used to adjust *P*-values for multiple testing (Strimmer 2008). As large heterozygote deficits are common in marine invertebrates (Addison & Hart 2005; Baums 2008), the program INEST (Chybicki & Burczyk 2009) was used to distinguish among some of the possible causes for departures for HWE by estimating null allele frequencies while accounting for inbreeding. Population-level pairwise F_{ST} comparisons were performed in GenAlEx v.6.41. Finally, principal component analysis (PCA) was performed on a matrix of covariance values calculated from population allele frequencies in the program GenoDive v.2.20 (Meirmans & Van Tienderen 2004).

Population structure was investigated using a Bayesian clustering approach performed in STRUCTURE v.2.3.3 (Pritchard *et al.* 2000), on the web-based Bioportal server from the University of Oslo. Correlated allele frequencies and admixed populations were assumed. Because sampling location information set as prior information can assist clustering for data sets with weak structure (Hubisz *et al.* 2009), the LOCPRIOR option was used. Preliminary runs were conducted to assess whether individuals within a same region and depth could be pooled to increase statistical power (none of these runs introduced additional structure, see 'Supporting Information'). Values of K (hypothesized number of populations) from 1 to 20 were tested by running three replicate simulations per K with 10^6 Markov chain Monte Carlo repetitions and 10^3 burn-in iterations. Two different approaches were then used to estimate the 'optimal' K based on the STRUCTURE outputs of the final data set. The most likely value for K was first determined by plotting the log probability [$L(K)$] of the data over multiple runs and comparing that with delta K (Evanno *et al.* 2005), as implemented in the web-based program STRUCTURE HARVESTER (Earl 2009). An alternative approach, implemented in the program ObStruct (Gayevskiy *et al.* 2014), was used to statistically analyse STRUCTURE ancestry profiles and determine whether the inferred population assignment and the factor of interest (e.g. origin of individuals) were significantly correlated. Finally, results of the three STRUCTURE runs assuming $K = 2$ or $K = 3$ clusters were merged with CLUMPP (Jakobsson & Rosenberg 2007) and visualized with DISTRUCT (Rosenberg 2004).

Migration patterns were estimated using MIGRATE v.3.4.2 (Beerli & Felsenstein 2001). MIGRATE uses a Bayesian approach to estimate the likelihood of complex uni- or multidirectional population geneflow models including that of panmixia (Beerli & Palczewski 2010) using coalescence theory. In this study, we used results of STRUCTURE (Fig. 2) and preliminary MIGRATE runs (described in the results section) as prior information to compare four different geneflow models at each geographic location: (A) a full model with three populations (shallow, mid and deep) and symmetrical gene flow; (B) a model with two populations (shallow and mid/deep pooled) and one migration rate from shallow to mid/deep; (C) a model with two populations (shallow and mid/deep pooled) and one migration rate from mid/deep to shallow; and (D) a model where all three populations (shallow, mid and deep) were considered part of the same panmictic population. Upper bounds for M (mutation-scaled immigration rate) and θ (mutation-scaled population size) were adjusted to a maximum value of 100, as these values are considered appropriate for microsatellite-based analyses (P. Beerli, personal communication). All other values were

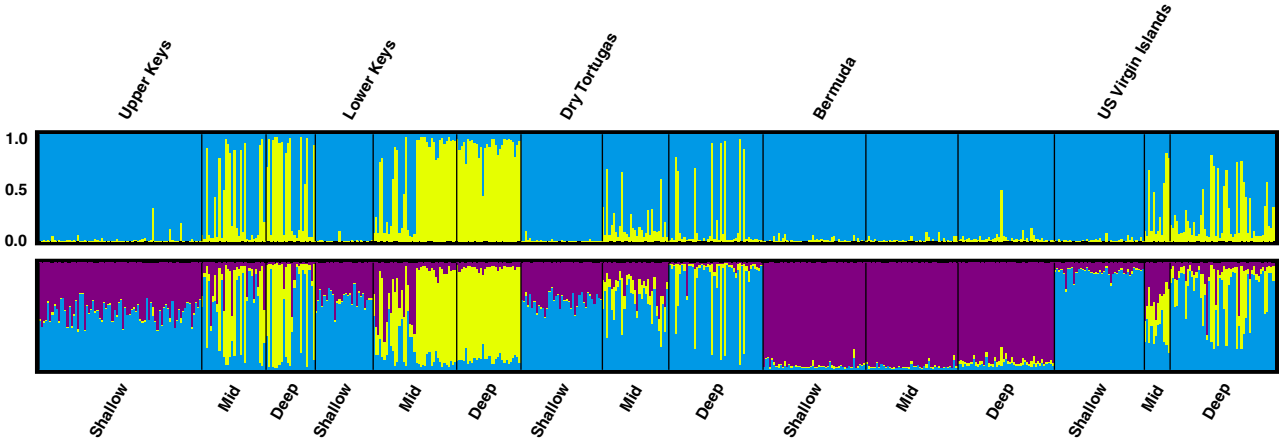

Fig. 2 *Montastraea cavernosa* population structure across regions [Upper Keys, Lower Keys and Dry Tortugas (within Florida), Bermuda and the U.S. Virgin Islands] and depths [shallow (≤10 m), intermediate (15–20 m) and deep (≥25 m)]. Bar graphs show the average probability of membership (y-axis) of individuals ($N = 577$, x-axis) in $K = 2$ (top panel) and $K = 3$ (bottom panel) clusters as identified by STRUCTURE. Samples were arranged in order of increasing depth within region.

kept default. The most complex model (A) was used to experiment with run conditions until convergence was achieved and posterior distributions were acceptable [final parameter settings: long-inc 100, long-sample 15 000, replicates 20, burn-in 20 000 and four heated chains (1, 1.5, 3, 100 000)]. Models A–D were then compared and ranked using the thermodynamic integration framework as described in Beerli & Palczewski (2010).

Algal symbiont characterization

A subset of the corals used for microsatellite analyses was selected haphazardly to assess the diversity of symbiont populations and potential patterns of depth zonation. *Symbiodinium* types were identified by denaturing gradient gel electrophoresis (DGGE) and sequencing of ITS-2 rDNA. This gene region was amplified using the primers ITSintfor2 and ITS2clamp (LaJeunesse & Trench 2000), and amplification products were separated by DGGE using a 35–75% gradient. Dominant bands on the gel were excised, reamplified and sequenced using the BigDye Terminator v3.1 cycle sequencing kit and an automated sequencer (ABI 3730). Sequences were then identified via BLAST in GenBank (accession numbers are given in 'Supporting Information').

Quantitative PCR (qPCR) assays were used in addition to DGGE to better understand patterns of depth zonation and detect the presence of background symbiont types not detectable by DGGE (Mieog *et al.* 2009). The assay for *Symbiodinium* clade B targeted the large subunit of the nuclear rDNA and was carried out as described in Correa *et al.* (2009b). Assays targeting specific actin loci in *Symbiodinium* clades C and D, however, were carried out in multiplex as described in Cunning & Baker (2013). All qPCRs were performed using a StepOnePlus Real-Time PCR System (Applied Biosystems), and reaction volumes were 10 μL, using 1 μL of genomic DNA template. Detection levels were established via a standard curve generated using known concentrations of target DNA. Finally, two replicates per sample were used per clade assayed.

Results

Multilocus genotyping and tests of Hardy–Weinberg equilibrium

Our analysis of 583 samples yielded 577 unique MLGs (Table 1), suggesting virtually no asexual reproduction in this species at sampling distances >1 m. Repeated MLGs were always confined to a single sampling location (within <1 km). Tests of HWE for each of the 15 combinations of region/depth (Table 1, population column) individually revealed that all nine loci were largely in HWE, as only 3.7% of 135 tests showed significant deviations from HWE after FDR correction ('Supporting Information'). Individual inbreeding values were generally low (mean F_i = 0.01; 95% confidence interval = 0–0.07), as well as null allele frequencies (ranging between 0.04 and 0.12 across loci and populations, see 'Supporting Information').

Assessment of vertical vs. horizontal connectivity

Overall, patterns of genetic subdivision showed strong support for two clusters that correlate with depth in both Florida and the USVI (Fig. 2 top panel). At these locations, shallow colonies were all assigned to a 'shallow' cluster (depicted in blue), while intermediate and deep colonies were assigned to this shallow cluster or to a 'deep' cluster (depicted in yellow), with high probabilities of membership to either cluster (>80%). Fifty-three individuals (9%) had similar probabilities of membership to both clusters (50–79%), probably as a result of admixture (i.e. interbreeding between shallow and deep colonies). In the case of Bermuda, however, all individuals across depths were either assigned to the 'shallow' cluster common to Florida and the USVI (Fig. 2 top panel) or as a separate 'local' cluster (depicted in purple, Fig. 2 bottom panel). Interestingly, whereas the Evanno method suggested that the most likely number of populations present in the data set is two (K = 2; 'Supporting Information'), analyses using ObStruct further partitioned the data by geographic location, separating Bermuda from individuals in Florida and the USVI, and suggesting that the most likely number of populations present in the data set is three (K = 3; Fig. 2 bottom panel and 'Supporting Information').

The degree of genetic differentiation with depth also varied among regions (Fig. 2). The Upper and Lower Keys displayed the largest genetic structure with depth, with ~57% and ~90% of all colonies at deep depths (≥25 m) assigned to the deep population, respectively. Conversely, Dry Tortugas samples consisted of a single panmictic population, with 81–99% of all colonies assigned to the common shallow cluster, despite being collected at depths from 8 to 25 m and in close proximity to the Lower Keys (~130 km apart). Similarly, the USVI and Bermuda were dominated by one cluster across all depths (67–98% in the USVI and 95–98% in Bermuda, respectively).

When restricting comparisons to the shallow depth category across regions, no genetic structure was evident at K = 2 clusters (Fig. 2 top panel), suggesting a high degree of horizontal connectivity among sites separated by up to 1992 km. These findings were also

Table 3 *Montastraea cavernosa* pairwise F_{ST} values for each population

Population	UK shallow	UK mid	UK deep	LK shallow	LK mid	LK deep	DT shallow	DT mid	DT deep	BDA shallow	BDA mid	BDA deep	USVI shallow	USVI mid
UK mid	0.016													
UK deep	0.035	0.005												
LK shallow	0.007	0.025	0.045											
LK mid	0.033	0.009	0.015	0.041										
LK deep	**0.064**	0.022	0.015	**0.070**	0.011									
DT shallow	0.000	0.020	0.040	0.003	0.034	**0.063**								
DT mid	0.020	0.006	0.027	0.012	0.025	0.042	0.017							
DT deep	0.012	0.005	0.008	0.017	0.024	0.035	0.013	0.009						
BDA shallow	0.013	0.019	0.028	0.029	0.037	**0.070**	0.017	0.035	0.020					
BDA mid	0.013	0.019	0.025	0.032	0.036	**0.066**	0.016	0.039	0.021	0.003				
BDA deep	0.013	0.018	0.031	0.018	0.038	**0.071**	0.018	0.023	0.018	0.000	0.003			
USVI shallow	0.014	0.026	0.046	0.007	0.048	**0.079**	0.012	0.022	0.020	0.028	0.032	0.019		
USVI mid	0.035	0.037	0.047	0.026	0.037	**0.060**	0.019	0.031	0.026	0.029	0.035	0.022	0.032	
USVI deep	0.015	0.016	0.022	0.013	0.025	0.043	0.011	0.021	0.004	0.020	0.019	0.017	0.017	0.007

Statistically significant values ($P < 0.05$) after FDR correction are highlighted in bold.
UK = Upper Keys; LK = Lower Keys; DT = Dry Tortugas; BDA = Bermuda; USVI = U.S. Virgin Islands.

supported by pairwise F_{ST} comparisons (Table 3), where the greatest F_{ST} values were observed among locations separated by depth rather than horizontal distance. The best example are shallow and deep sites in the Lower Keys, which showed one of the largest F_{ST} values (0.07; Table 3) even though the distance between these sites is only ~20 km. Finally, PCA on allele frequency differences among *M. cavernosa* populations (Fig. 3) separated sites by depth (PC1 = 34% of variance) and geographic location (PC3 = 15% of the variance), consistent with analyses of ObStruct ('Supporting Information').

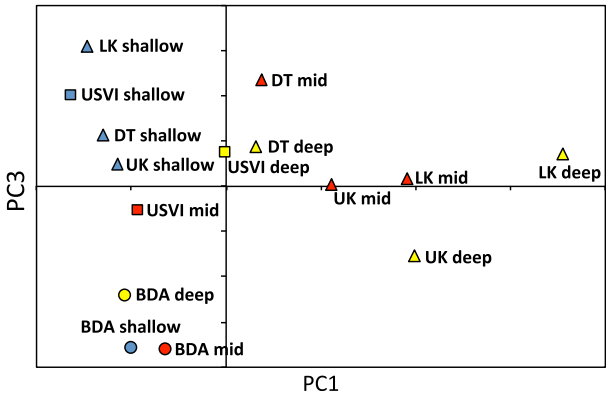

Fig. 3 Principal component analysis (PCA) of allele frequency covariance in *Montastraea cavernosa* populations. Eight of 163 axes were retained, explaining 100% of the cumulative variance. Plotted are the first and third axes explaining 33.70% ($P < 0.01$) and 14.49% ($P < 0.01$) of the variance, respectively. Axes cross at 0. The different shapes denote each of the three geographic locations sampled in this study (triangles: Florida, circles: Bermuda, squares: U.S. Virgin Islands), whereas the different colours denote each of the three depths under comparison [blue: shallow (≤10 m), red: intermediate (15–20 m), yellow: deep (≥25 m)]. UK = Upper Keys, LK = Lower Keys, DT = Dry Tortugas, BDA = Bermuda and USVI = U.S. Virgin Islands.

Migration rates among shallow and deep habitats

Preliminary runs were performed to determine which (if any) depth intervals could be pooled to reduce the parameters estimated in the geneflow models under comparison. MIGRATE was thus performed (i) pooling shallow and intermediate individuals or (ii) pooling intermediate and deep individuals. These runs consistently ranked the geneflow model in which intermediate and deep individuals were pooled as the best (data not shown). Therefore, all subsequent analyses were performed pooling individuals from intermediate and deep depths as described in the methods section.

Table 4A summarizes the marginal log-likelihood differences (log-Bayes factors) and ranking of each of the four geneflow models compared (A = full, B = shallow to mid/deep, C = mid/deep to shallow and D = panmixia) using the thermodynamic integration approximation as described in Beerli & Palczewski (2010). Overall, this method consistently ranked the geneflow model with migration from shallow to mid/deep as the best model across all regions. This ranking occurred even for regions where STRUCTURE suggested panmixia (Dry Tortugas, Bermuda and the USVI), although the panmixia model was ranked second in two of these locations (Dry Tortugas and Bermuda). Furthermore, in regions with the largest degree of genetic differentiation with depth (Florida's Upper and Lower Keys, Fig. 2),

Table 4 *Montastraea cavernosa* (A) Comparison of log-Bayes factors (marginal log-likelihood differences, LBF) approximated by thermodynamic integration for four different geneflow models (A = full, B = shallow to mid/deep, C = mid/deep to shallow and D = panmixia). (B) Estimated mutation-scaled population sizes (θ), mutation-scaled migration rates (M) and number of migrants per generation (Nm = θ*M/4) between source and receiving populations for model best supported (B = shallow to mid/deep). Numbers in parenthesis indicate the 95% confidence interval (CI) for parameters θ and M

(A)

	LBF for model				Rank of model			
Region	A	B	C	D	A	B	C	D
UK	−409 905	0	−3845	−13985	4	1	2	3
LK	−20 701	0	−765	−6320	4	1	2	3
DT	−25 438	0	−219775	−8971	3	1	4	2
BDA	−26 545	0	−214437	−13043	3	1	4	2
USVI	−29 678	0	−800	−4121	4	1	2	3

(B)

		Parameter and 95% CI		
Source population	Receiving population	θ	*M*	Nm
UK shallow	UK mid/deep	3.87 (0.60–7.40)	14.77 (6.40–24.26)	14.28
LK shallow	LK mid/deep	6.82 (2.60–11.2)	2.24 (0.07–4.33)	3.82
DT shallow	DT mid/deep	25.01 (4.40–67.27)	7.49 (2.73–12.53)	46.83
BDA shallow	BDA mid/deep	13.30 (2.40–40.27)	11.65 (4.33–20.47)	38.73
USVI shallow	USVI mid/deep	6.16 (0.80–11.33)	9.68 (4.40–15.33)	14.90

UK = Upper Keys; LK = Lower Keys; DT = Dry Tortugas; BDA = Bermuda; USVI = U.S. Virgin Islands.

the panmictic geneflow model was ranked third, suggesting it was less likely. Finally, as expected, the smallest number of migrants per generation (Nm) from shallow to deep was observed in the Lower Keys, region that exhibited the largest degree of genetic structure (Table 4B).

Algal symbiont characterization

M. cavernosa samples predominantly housed *Symbiodinium* type C3 across depths and regions, as shown via ITS-2 DGGE (N = 109, upper column in each depth category in Fig. 4). Two other *Symbiodinium* types were identified (D1a and B1, Fig. 4) in 11% of the shallow colonies assessed from Florida, but not in any other location (Bermuda or the USVI) or depth (intermediate or deep colonies). Further analysis with qPCR (lower column in each depth category in Fig. 4) detected additional background symbiont types in clade D in only four of the samples previously assessed with DGGE, confirming the DGGE results.

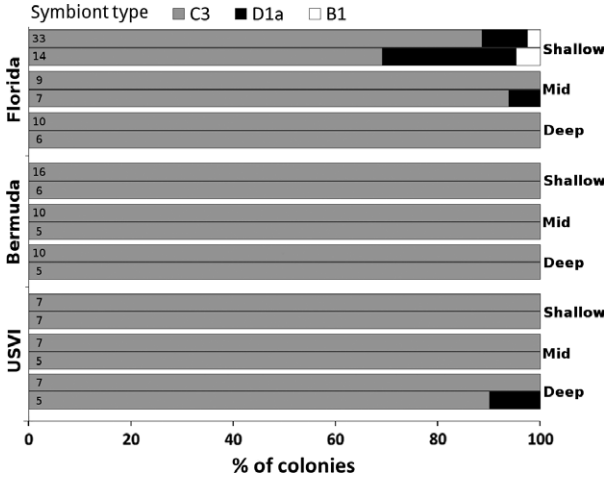

Fig. 4 *Symbiodinium* types detected in a subset of *Montastraea cavernosa* samples from each depth and geographic location, using either denaturing gradient gel electrophoresis (upper bar in each depth category) or quantitative PCR (lower bar in each depth category). The numbers of individual coral colonies sampled at each depth are indicated in the left corner of each bar. Colonies hosting mixed symbiont communities (i.e. more than one type) were partitioned into each appropriate category. USVI = U.S. Virgin Islands.

Discussion

We developed nine new microsatellite loci to undertake the most comprehensive analysis of population genetic structure to date for the Caribbean reef-building coral *Montastraea cavernosa* in both horizontal (long-distance) and vertical (short-distance) directions. We show that this species (i) exhibits patterns of genetic subdivision that correlate with depth, (ii) exhibits panmixia across

shallow sites separated by >1700 km, (iii) exhibits regional differences in vertical connectivity, (iv) exhibits migration rates that are higher from shallow to intermediate/deep habitats and (v) predominantly hosts the same symbiont type across regions and depths. These findings suggest that the potential for shallow reefs to recover from deep-water refugia in *M. cavernosa* is location-specific, varying among and within geographic locations likely as a consequence of local hydrology.

Genetic subdivision is correlated with depth

Overall, the small percentage of admixed individuals observed in this study (<10%), particularly at deeper sites, suggests that these individuals may be less fit and selected against as a consequence of outbreeding depression. Alternatively, migration between deep and shallow habitats could have occurred, but little interbreeding among colonies has taken place. These findings are consistent with observations from van Oppen *et al.* (2011) and Prada & Hellberg (2013) for *S. hystrix* and *E. flexuosa*, respectively. van Oppen *et al.* (2011) suggested that one possible explanation for the lack of interbreeding among shallow and deep individuals is reproductive isolation. This might result if, for example, populations at different depths become locally adapted, and immigration to the 'wrong' habitats results in poor performance (immigrant inviability, sensu Nosil *et al.* 2005). Interestingly, Budd *et al.* (2012) showed no significant genetic differentiation between two different *M. cavernosa* morphotypes present at depths of 10–30 m, suggesting a high degree of polymorphism and phenotypic plasticity in this species. Conversely, Brazeau *et al.* (2013) assessed *M. cavernosa* colonies in the range of 3–90 m and found significant differentiation with depth in shallow/intermediate colonies (3–25 m) when compared to deep (≥30 m) colonies. It is possible, however, that the two nuclear loci used in Budd *et al.* (2012) had insufficient resolution to detect differentiation by depth or that the depths at which differentiation occurs were deeper than those assessed.

Finally, the potential for interbreeding between shallow and deep colonies can be reduced if there are temporal differences in spawning times (Levitan *et al.* 2004; Prada & Hellberg 2013). In the case of *M. cavernosa*, although mass spawning of deep-water colonies has been observed to be synchronized with their shallow water counterparts in the Gulf of Mexico (Vize 2006), it is possible that they could spawn at different times in other locations, especially those where strong genetic differentiation was observed. Furthermore, even if spawning is synchronized, little fertilization can occur if deep gametes arrive late to the surface (Levitan *et al.* 2004).

High connectivity among shallow sites

At $K = 2$ clusters (Fig. 2 top panel), we found little evidence for geographic differentiation between shallow sites of all three geographic locations examined (Florida, Bermuda and the USVI). These findings confirm results of Nunes *et al.* (2009) and Goodbody-Gringley *et al.* (2011), both of which showed high levels of gene flow within the Caribbean region and no significant differentiation between Bermuda and the Caribbean. However, our analyses at $K = 3$ clusters (Fig. 2 bottom panel) further divided genetic diversity according to geographic location. These findings suggest lower levels of gene flow between Bermuda and the Caribbean than those reported in previous studies, but these differences can be explained by the use of molecular markers with higher resolution in our study. Regardless, findings suggest that long-distance horizontal connectivity is much greater than short-distance vertical connectivity at all locations examined, with the exception of Bermuda, where shallow sites may be actually more dependent on the local deeper population. Furthermore, based on the patterns of connectivity observed here, we hypothesize that it is more likely that shallow reefs rely on more distant, unimpacted shallow reefs to provide a viable source of new recruits following disturbance.

Regional differences in vertical connectivity

Even though there was strong evidence to support the presence of two populations separated by depth, the depth at which different populations make this transition, and the degree of genetic differentiation, vary among regions (Fig. 2). For example, changes in genetic structure were observed at a shallower depth in the Upper and Lower Keys compared to the Dry Tortugas or the USVI, implying that either (i) the corresponding deep habitat may be at greater depths than those assessed, or (ii) that deep reefs could potentially reseed nearby shallow reefs at these latter two locations, even if migration occurs asymmetrically (i.e. higher migration rates from shallow to deep). Interestingly, the deep cluster appears to be absent in Bermuda: despite sampling a broad depth gradient (4–58 m), only one individual was found showing signs of admixture. It is possible that this location only receives larvae from the closest upstream shallow population (Florida), due to its high latitude. Alternatively, the lack of genetic differentiation observed could be due to the small horizontal distance between the shallow and deep sites at this location (~1 km) compared to other locations in this study (generally >10 km). We think this is unlikely as slope does not appear to explain the variance in genetic distance (F_{ST}) ($P > 0.05$; see 'Supporting Information').

However, the close proximity of sites, coupled with strong wave action at this location, could facilitate high levels of genetic exchange among shallow and deep sites. Overall, our findings are consistent with those from Billinghurst *et al.* (1997), which showed no genetic subdivision for this species in Bermuda from depths of 2–30 m.

Surprisingly, the largest genetic differentiation observed in this study occurred between the Lower Keys shallow and deep sites, while the lowest degree of differentiation occurred in the Dry Tortugas, even though these regions are only ~130 km apart. These differences could be due by significantly greater downwelling attenuation coefficients (Kd) in the Lower Keys compared to the Dry Tortugas or Upper Keys, as well as lower 1% attenuation depths for visible radiation in the Lower Keys (~27 m) compared to the other two regions (~40–43 m; Barron *et al.* 2009). Alternatively, these findings could be due to site-specific differences in local hydrology. Florida represents a unique case for testing the deep reef refugia hypothesis, as larvae are subject to complex current patterns driven primarily by the interannual variability in the Florida Current and associated frontal eddies. The region near Dry Tortugas, for example, has a well-established spatial pattern of more mesoscale eddy activity compared to the Lower or Upper Keys (Hitchcock *et al.* 2005; Kourafalou & Kang 2012). These mesoscale eddies, extending down to >100 m (Kourafalou & Kang 2012), can act as important retention mechanisms for larvae spawned in the Dry Tortugas (Hitchcock *et al.* 2005; C. B. Paris, unpublished data), and may therefore facilitate genetic mixing of individuals from shallow and deep habitats, as evidenced by the lack of genetic structure at this location (Fig. 2, Table 3) and highest number of migrants from shallow to deep (Table 4B). Conversely, the general decrease in mesoscale eddies west from the Dry Tortugas (Kourafalou & Kang 2012) suggests that in the Lower and Upper Keys, the strong currents produced by the Florida Current could quickly advect the deep larvae away from adjacent shallow habitat. These reefs, however, could be important larval sources for shallow reefs further downstream (e.g. the northern Florida reef tract). Therefore, future work should aim at elucidating the role of deep reefs as refugia for nearby vs. more distant, downstream shallow reefs.

Higher migration from shallow to deep

The geneflow model with migration from shallow to mid/deep was ranked as the best model in all five regions (Table 4A). Contrary to expectations, this ranking was consistent even in regions where STRUCTURE suggested panmixia (Dry Tortugas, Bermuda and the USVI). This could be explained by the fact that while the framework implemented in MIGRATE explicitly models a migration process that can take into account asymmetric situations, STRUCTURE does not (P. Beerli, personal communication). MIGRATE can thus detect gene flow even under situations where STRUCTURE may fail to differentiate between 1 or 2 populations, and in our case, it is not surprising that STRUCTURE reported panmixia given the number of migrants per generation (Nm) observed (Table 4B) (P. Beerli, personal communication).

Overall, findings from MIGRATE suggest that there is asymmetrical gene flow among shallow and deep habitats. Thus, genetic mixing between shallow and deep populations might be maintained by the supply of larvae down the slope (from shallow to deep), rather than by migration in both directions. This is further evidenced by the lack of individuals in shallow habitats assigned with high probability to the deep cluster (Fig. 2). Therefore, although we cannot rule out the possibility that recovery of shallow reefs might be aided by nearby deeper reefs at these locations, it might be insufficient to promote rapid recovery. Deep reefs, however, may still serve as important resources of local genotypic diversity.

To date, only Prada & Hellberg (2013) have estimated migration rates between shallow and deep habitats for a coral species. In the octocoral species studied, the authors also found that migration was higher from shallow to deep, suggesting that this might be a consistent pattern for many coral species. One possibility is that larger population sizes in shallow habitats result in higher migration from shallow to deep. Alternatively, there could be a higher gamete production in shallow environments because this is where coral growth rate and coral cover tend to be highest (Riegl & Piller 2003). As rates of photosynthesis and calcification tend to decline significantly with depth (Mass *et al.* 2007; Slattery *et al.* 2011), shallow coral colonies tend to grow faster to sexual maturity due to higher light availability. In addition, shallow colonies might be capable of allocating more energy reserves to gamete production than deep colonies due to a greater translocation of photosynthetic products from algal symbionts (Muscatine *et al.* 1989; Mass *et al.* 2007). Although *M. cavernosa* might supplement this deficit with heterotrophic feeding (particularly at depths >45 m, Lesser *et al.* 2010) and nitrogen-fixing symbiotic cyanobacteria (Lesser *et al.* 2007; Olson & Lesser 2013), total reef accretion is considered to be negatively correlated with depth (Grigg 2006). Furthermore, a higher polyp density per area recorded in shallow (3 m) vs. deep (18 m) sites in *O. faveolata* suggests a significant reduction in fecundity (egg production per cm^2) at deep sites, implying a depth-related fecundity cost arising from a change in colony architecture (Villinski 2003). Finally, deeper reefs may be less environmentally harsh than shallow reefs, promoting higher survivorship of migrants in a downward direction. Shallow colonies might also fragment, fall down the

slope and subsequently reattach in deeper habitats resulting in higher migration from shallow to deep; however, this is unlikely for *M. cavernosa*, as fragmentation would result in clone mates and we only observed ~1% clonality in our data set.

Single symbiont type across regions and depths

The coral–symbiont interaction implies that studies of larval dispersal and connectivity in corals need to be supplemented by investigations of symbiont dispersal (Baums 2008), because processes of symbiont transfer may impose limitations on the colonization and postsettlement survival of coral offspring (Bongaerts *et al.* 2010). However, such limitations are not expected for species that acquire their *Symbiodinium* from the environment (i.e. horizontally). These corals are able to acquire locally abundant symbionts and so might be capable of colonizing habitats over a broad depth range.

Depth-generalist coral species often harbour a single symbiont type over their entire depth range but can also show a cladal or subcladal shift in symbiont types (e.g. Rowan & Knowlton 1995; Warner *et al.* 2006; Sampayo *et al.* 2007; Frade *et al.* 2008; Bongaerts *et al.* 2011b). Overall, we found that *M. cavernosa* predominantly hosts a single *Symbiodinium* type (C3) across all regions and depths assessed, regardless of the method used (DGGE or qPCR). It is possible that these corals host the same *Symbiodinium* type, yet the symbionts are physiologically distinct, as shown by Howells *et al.* (2012) for *Symbiodinium* type C1 associated with corals from two different thermal environments. *Symbiodinium* type C3 is known to be a pandemic, host-generalist symbiont (LaJeunesse 2005), because it occurs in a wide range of Pacific genera at both shallow and intermediate (<20 m) depths (LaJeunesse *et al.* 2003, 2004), as well as in mesophotic samples of at least 5 of the 10 Pacific species assessed in Bongaerts *et al.* (2011b). However, our findings contrast with those from Lesser *et al.* (2010), which showed a subcladal shift in symbiont types to 'deep reef' types at depths >60 m for *M. cavernosa*. These differences may arise from (i) using different methods for the identification of symbiont types and/or (ii) assessing shallower depths in our study compared to Lesser *et al.* (2010). Regardless, given the homogeneity of results found for *M. cavernosa* in this study, it is unlikely that the coral–symbiont association observed limits the vertical connectivity of the holobiont.

Implications: the role of deep reefs in shallow reef recovery

Overall, our findings were surprising for a broadcast spawning coral with documented high gene flow throughout the Caribbean. *M. cavernosa* is one of a few Caribbean species inhabiting a broad depth range between 3 and 100 m (Reed 1985; Lesser *et al.* 2010), making it an exceptional candidate for testing the deep reef refugia hypothesis. However, we found significant genetic structure with depth in this species within regions in Florida, suggesting that the extent of vertical connectivity highly depends on local conditions that may facilitate genetic exchange among shallow and deep sites. Furthermore, our findings support those from Caribbean broadcast spawners in the genus *Oculina* (Eytan *et al.* 2009), the Pacific brooding coral *Seriatopora hystrix* (van Oppen *et al.* 2011), the Caribbean brooding coral *Madracis pharensis* (Frade *et al.* 2010) and the Caribbean octocoral *Eunicea flexuosa* (Prada & Hellberg 2013), despite different study locations, coral species and reproductive strategies. Therefore, although a broader survey of species and larger sample sizes are needed to obtain a better understanding of the role of deep reefs in shallow reef recovery, the expectation that MCEs may be refugia for shallow water taxa appears reduced.

What if we were to lose all shallow reefs?

It has been suggested that, while reefs would suffer badly in a scenario of total coral mortality at depths <10 m, only about 50% of the total area occupied by reef-building corals would actually disappear (Riegl & Piller 2003). However, loss of the upper 20 m would result in a reduction in area of >80% (Riegl & Piller 2003). If we assume that coral reefs will still exist in intermediate and deep habitats (≥15 m) partly due to lower thermal stress and more stable conditions, then our findings suggest that some locations might be able to recover via larval supply from nearby deep reefs (e.g. Bermuda). However, other locations (e.g. Lower Keys) might be unable to recover from their deep-water counterparts and will have to rely on the supply of larvae from distant deep reefs. Even if impacted shallow reefs are recolonized by larvae originating from deep water, selective forces (Bongaerts *et al.* 2011a; Prada & Hellberg 2013) might result in reduced or little postsettlement survival. Alternatively, more substrate availability in shallow habitats might reduce competition for space and potentially increase the likelihood of deep-water migrants (van Oppen *et al.* 2011), considered inferior competitors in areas with high irradiance (Kahng *et al.* 2010). Nevertheless, our findings suggest that shallow reefs (<15 m) should be of high priority for managers and measures should be taken to reduce local and global anthropogenic impacts that might further accelerate their loss rate.

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X.S. performed statistical analysis and wrote the paper. X.S., I.B. and A.B. developed methods. X.S. and K.O. performed laboratory analysis. X.S., A.B., T.S., R.J., T.S. and F.N. collected samples. All authors read, edited and approved the manuscript. X.S. and A.B. obtained funding and designed the study.

Data accessibility

Montastraea cavernosa multilocus genotypes, sampling locations for each colony and *Symbiodinium* typing results from DGGE and quantitative PCR are available in Dryad (doi:10.5061/dryad.47dk8). Structure and Migrate analysis input files and settings are also available in Dryad (doi:10.5061/dryad.47dk8).

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Summary of statistics per locus and population of *Montastraea cavernosa*.

Table S2 Mean null allele frequencies and mean inbreeding coefficients per locus and population of *Montastraea cavernosa*.

Table S3 *Symbiodnium* types identified in figure 4 and corresponding GenBank accession numbers for the ITS-2 marker.

Table S4 R^2 values calculated using ObStruct for STRUCTURE analysis of *Montastraea cavernosa* samples.

Fig. S1 STRUCTURE runs performed prior to analyses to assess whether we could pool *Montastraea cavernosa* individuals from different sites within a same region and depth to increase statistical power.

Fig. S2 *Montastraea cavernosa* population structure by site (as designated in Table 1).

Fig. S3 *Montastraea cavernosa* population structure across regions [Upper Keys, Lower Keys and Dry Tortugas (within Florida), Bermuda and the U.S. Virgin Islands] and depths [shallow (≤10 m), mid (15–20 m) and deep (≥25 m)].

Fig. S4 Mean log-likelihood LN of (A) K (hypothesized number of populations) and delta K (B) values for STRUCTURE analysis of *Montastraea cavernosa* samples using the Evanno method.

Fig. S5 Canonical discriminant analysis calculated using ObStruct for *Montastraea cavernosa* samples. ObStruct was run assuming two populations ($K = 2$).

Fig. S6 Canonical discriminant analysis (CDA) calculated using ObStruct for *Montastraea cavernosa* samples.

Fig. S7 Relationship between genetic distance (F_{ST}) and slope for all geographic locations examined in this study.