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Vectored dispersal of *Symbiodinium* by larvae of a Caribbean gorgonian octocoral

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Abstract

The ability of coral reefs to recover from natural and anthropogenic disturbance is difficult to predict, in part due to uncertainty regarding the dispersal capabilities and connectivity of their reef inhabitants. We developed microsatellite markers for the broadcast spawning gorgonian octocoral *Eunicea* (*Plexaura*) *flexuosa* (four markers) and its dinoflagellate symbiont, *Symbiodinium* B1 (five markers), and used them to assess genetic connectivity, specificity and directionality of gene flow among sites in Florida, Panama, Saba and the Dominican Republic. Bayesian analyses found that most *E. flexuosa* from the Florida reef tract, Saba and the Dominican Republic were strongly differentiated from many *E. flexuosa* in Panama, with the exception of five colonies from Key West that clustered with colonies from Panama. In contrast, *Symbiodinium* B1 was more highly structured. At least seven populations were detected that showed patterns of isolation by distance. The symbionts in the five unusual Key West colonies also clustered with symbionts from Panama, suggesting these colonies are the result of long-distance dispersal. Migration rate tests indicated a weak signal of northward immigration from the Panama population into the lower Florida Keys. As *E. flexuosa* clonemates only rarely associated with the same *Symbiodinium* B1 genotype (and vice versa), these data suggest a dynamic host–symbiont relationship in which *E. flexuosa* is relatively well dispersed but likely acquires *Symbiodinium* B1 from highly structured natal areas prior to dispersal. Once vectored by host larvae, these symbionts may then spread through the local population, and/or host colonies may acquire different local symbiont genotypes over time.

Keywords: Caribbean, dispersal, octocoral, population genetics, specificity, *Symbiodinium*

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Introduction

The temporal and spatial scales over which coral reef ecosystems are connected are fundamental to understanding their evolutionary history (Hellberg 2007) and resilience to natural and anthropogenic stressors (Jones *et al.* 2009). The extent of dispersal of planktonic larval stages, whether by large or small-scale oceanographical features, and/or differential larval behaviour, largely determines the connectivity of coral reef habitats (Roberts 1997; Cowen *et al.* 2000, 2006). Because coral reefs worldwide have experienced ecological degradation, both chronic and acute, over the last few decades (Gardner *et al.* 2003; Hughes *et al.* 2003; Bellwood *et al.* 2004; Bruno & Selig 2007; De'ath *et al.* 2012), there is a growing need for information regarding coral reef connectivity that will help to better understand the natural history of reef invertebrates (Hedgecock *et al.* 2007) and maximize conservation efforts (Palumbi 2003; Van Oppen & Gates 2006; Jones *et al.* 2007).

Octocorals, together with a variety of other reef-inhabiting invertebrates, form symbiotic relationships

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with dinoflagellates in the genus *Symbiodinium*. This mutualistic partnership benefits both members, and its maintenance is critical to their survival (Baker 2003; Coffroth & Santos 2005). Worldwide, octocorals are host to *Symbiodinium* in clades A, B, C, D and G (*sensu* Rowan & Powers 1991; Pochon & Gates 2010; Van Oppen *et al.* 2005; Goulet *et al.* 2008), while in the Caribbean, most octocoral species (~88%) host only *Symbiodinium* in clade B [genotyped as B1 using internal transcribed spacer 2 (ITS-2), *sensu* LaJeunesse (2001); and B184 using chloroplast large subunit ribosomal DNA (cp23S-rDNA), *sensu* Santos *et al.* (2003a); referred to here as '*Symbiodinium* B1'] (Goulet & Coffroth 2004). However, although most octocorals in the Caribbean only host *Symbiodinium* B1, at least as the dominant symbiont, ITS haplotype diversity within this subgroup is disproportionately high compared with other *Symbiodinium* clades (Van Oppen *et al.* 2005), and microsatellite analyses show substantial genotypical diversity within *Symbiodinium* B1 among different octocoral hosts (Santos *et al.* 2003b; Kirk *et al.* 2009; Andras *et al.* 2011). Among some octocorals, specificity at the level of *Symbiodinium* clade is low during early ontogeny, and larvae and newly settled polyps can acquire *Symbiodinium* from at least three different clades (A, B and C; Coffroth *et al.* 2001). Yet, over several months, cladal diversity is winnowed to only clade B, and adult gorgonian octocorals are always dominated by members of this clade. However, adult colonies of the octocoral *Briarium* are able to acquire different *Symbioninium* B1 types from the external environment after experimental bleaching, suggesting some level of flexibility among adult octocorals to acquire different *Symbiodinium* B1 types in response to environmental change (Lewis & Coffroth 2004). Despite this, at the level of the individual, adult octocoral colonies appear to maintain an acute genetic specificity to their symbionts at not only the level of clade, but also at the level of genotype over space and time (Goulet & Coffroth 2003a,b; Kirk *et al.* 2005; Hannes *et al.* 2009).

Gorgonian (or branching) octocorals are ecologically important and abundant members of tropical reef environments in the Caribbean/tropical western Atlantic (e.g. Goldberg 1973; Lasker & Coffroth 1983; Sanchez *et al.* 1997). Genetic studies from the region show different patterns of population structure among gorgonian octocorals, and the *Symbiodinium* they host. For example, *Pseudopterogorgia elisabethae* exhibits significant genetic structure in the Bahamas among sites separated by distances up to 100 km (Gutierrez-Rodriguez & Lasker 2004). However, the dominant symbiont of this species, *Symbiodinium* B1, shows more extreme population subdivision among similar sites in the region separated by 10s of kilometres (Santos *et al.* 2003b). Similarly, populations of another Caribbean/western Atlantic gorgonian octocoral, *Gorgonia ventalina* and its *Symbiodinium* B1 symbionts are structured differently across their geographical range (Andras *et al.* 2011, 2013).

The Florida reef tract (FRT) is a ~260-km-long system of coral reefs located at the northern latitudinal limit of coral reef formation in the Caribbean (Spalding *et al.* 2001). Because much of the FRT has experienced widespread ecological and habitat deterioration (Pandolfi *et al.* 2005; Wilkinson & Souter 2008), larval replenishment of degraded sites from intact sites upstream may be critical for the persistence and sustainability of this reef system. Studies of genetic connectivity along the FRT, comprising taxonomically diverse invertebrates from different phyla (e.g. Porifera, Cnidaria, Arthropoda and Echinodermata) with a variety of life history characteristics, indicate a generally well-mixed system with only modest population subdivision, at least using markers available to date (e.g. Richards *et al.* 2007; Vollmer & Palumbi 2007; Baums *et al.* 2010; Debiasse *et al.* 2010; Hemond & Vollmer 2010). One exception to this is *Symbiodinium* B1, which microsatellite analysis has shown to be strongly subdivided over small spatial scales (10s of km) along the FRT over horizontal (similar depths) and vertical (different depths) distances, although intermittent gene flow and limited dispersal are also likely to occur (Kirk *et al.* 2009). The gorgonian octocoral *Eunicea* (*Plexaura*) *flexuosa* is a common species in reef and hard-bottom environments throughout the FRT and greater Caribbean/western Atlantic (Bayer 1961; Jaap 1984). Like most gorgonian octocorals of this region, it only hosts *Symbiodinium* B1 as its dominant dinoflagellate symbiont (Goulet & Coffroth 2004). As a gonochoric broadcast spawner, *E. flexuosa* has the potential for long-distance larval dispersal, with male and female colonies releasing their gametes after the summer full moons (Beiring & Lasker 2000). In this study, microsatellite markers were developed for both *E. flexuosa* and its dinoflagellate symbiont, *Symbiodinium* B1, and were used for analyses of population structure, spatial correlation, migration rates and genotypical specificity among host and symbionts of the same colonies to (i) quantify their genetic structure (and therefore evaluate ecologically relevant dispersal and directionality), (ii) assess their resilience capacity and (iii) measure host/symbiont specificity among *E. flexuosa* and *Symbiodinium* B1 genotypes.

Materials and methods

Sample collection and microsatellite development

Samples of *Eunicea flexuosa* were collected from nine sites along the Florida Keys and Biscayne Bay reef

tracts; three sites within the Bocas del Toro Province, Panama; one site from Punta Cana, Dominican Republic; and Saba Bank (Fig. 1; Table 1). Only host colonies >30 cm were collected, and sampling occurred over a period of 3 years and 7 months. As growth rates in *E. flexuosa* average ~1–2 cm/year (Beiring & Lasker 2000), all sampled colonies were likely >10 years old. Individual colonies were sampled by clipping a ~3-cm branch tip from one of the apical branches of the colony and preserved in 95% ethanol or saline DMSO (Seutin *et al.* 1991). To reduce the possibility of sampling the same colony twice, a 15-m transect line was used on sites with sufficient densities of the target species. On sites with low colony densities, a random collecting approach was used. If two divers were collecting on the same site, they examined the area in opposite directions.

In order to increase the ratio of host (*E. flexuosa*) to symbiont (*Symbiodinium* B1) DNA in total (host + symbiont) DNA extractions (Shearer *et al.* 2005), live *E. flexuosa* branch clippings (~7 cm) were first bleached using a photosynthetic inhibitor (DCMU; Jones 2004), combined with increased temperature and irradiance. Microsatellite isolation followed the approach of Glenn & Schable (2005), which employs Dynabeads® (Life Technologies, Carlsbad, CA, USA) to sequester DNA fragments with microsatellite regions with tri- or tetranucleotide repeats. Sequences with microsatellite regions were identified, and forward and reverse primers were designed using PRIMER3 (v. 0.4.0; Rozen & Skaletsky 2000) and GENEIOUS PRO 4.8.5 (Drummond *et al.* 2008).

For screening of *E. flexuosa*- and *Symbiodinium* B1-specific markers, tissue from the donor colony used for microsatellite isolation was also harvested to isolate and culture its symbionts. Denaturing gradient gel electrophoresis analysis (LaJeunesse 2001) of amplified ITS-2 region of rDNA genotyped the cultured symbiont as clade B1 (see below for PCR protocol). Other *Symbiodinium* types were also screened against the microsatellite marker candidates from existing cultures (provided by Scott Santos, Auburn University www.auburn.edu/~santosr/index.php), including two from clade B (culture ID: PurPflex and 703) and one each from clades A (ID: 719), C (ID: PtBr) and D (ID: 013).

Molecular analyses and marker validation

Total genomic DNA was extracted from each field-collected sample using a modified organic protocol

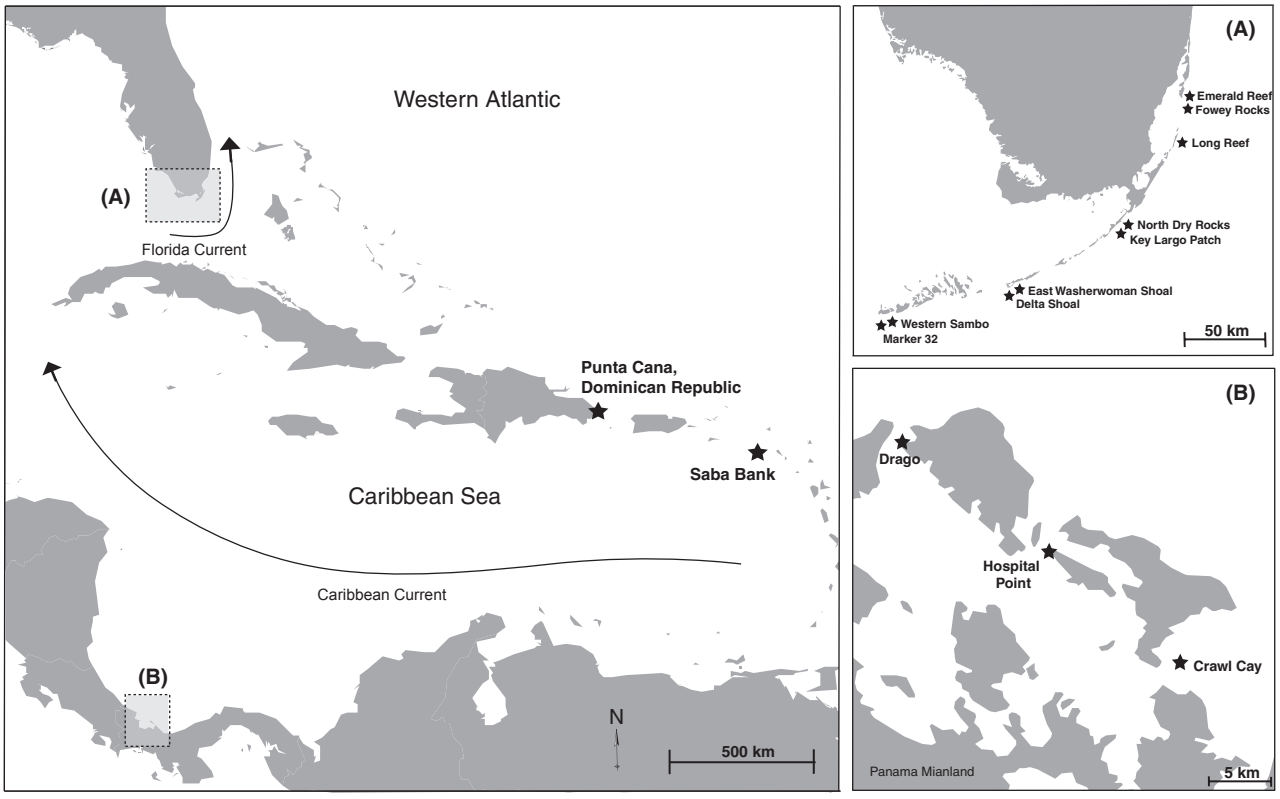

Fig. 1 Map of 14 sites across the Caribbean and western Atlantic where *Eunicea flexuosa* samples were collected. Shaded boxes are enlarged for (A) the Florida reef tract and (B) Bocas del Toro, Panama sites. Arrows indicate the direction of the prevailing sea surface currents discussed in the text.

Table 1 Collection sites, their geographical regions and subregions, GPS position, total numbers collected and collection date

Region	Subregion	Site	GPS		*n*	Date collected
			Latitude	Longitude		
Biscayne Bay, Florida	Offshore reefs	Emerald Reef	N25 40.450	W080 05.920	54	July 2010
		Fowey Rocks	N25 35.417	W080 05.800	65	August 2009
		Long Reef	N25 24.734	W080 07.642	76	November 2009
Florida Keys	Upper Keys	North Dry Rocks	N25 08.180	W080 17.359	52	May 2010
		Key Largo Patch	N25 05.580	W080 19.380	82	May 2010
	Middle Keys	East Washerwoman Shoal	N24 40.000	W081 04.437	70	August 2010
		Delta Shoal	N24 38.048	W081 05.342	77	August 2010
	Lower Keys	Western Sambo	N24 28.709	W081 43.812	54	August 2010
		Marker 32	N24 28.450	W081 44.561	42	August 2010
Panama	Bocas del Toro	Drago	N09 24.833	W082 19.997	9	August 2007
		Hospital Point	N09 19.798	W082 13.264	25	August 2007
		Crawl Cay	N09 15.696	W082 07.389	21	August 2007
Saba Bank		Conch Valley	N17 20.000	W063 14.844	12	October 2008
Dominican Republic		Punta Cana	N18 31.953	W068 21.074	22	March 2011

(Baker *et al.* 1997). Host- and symbiont-specific primers were fluorescently labelled on the forward primer. PCR (10 μL volume) was conducted with 10 pmol of each primer, 200 μM dNTPs, 2 mM $MgCl_2$ and 1 μL of genomic DNA using 0.6 U of GoTaq® (Promega Inc.) polymerase and the manufacturer's buffer. Thermal cycling consisted of an initial denaturing step of 94 °C for 3 min, followed by 35 rounds of 94 °C for 1 min, 57 °C for 1 min and 74 °C for 1 min, and a final extension of 72 °C for 7 min. PCR products were analysed using an ABI 3730 DNA Analyzer (Applied Biosystems) from the Core Laboratories Center at Cornell University. Sizing was achieved using an internal standard (Gene Scan 500-Liz; Applied Biosystems), and alleles were scored using GENEMAPPER software 4.0 (Applied Biosystems).

For host-specific diploid microsatellite markers, Hardy–Weinberg equilibrium (HWE) exact tests (Guo & Thompson 1992) were implemented using ARLEQUIN v.3.5.1.2 (Excoffier & Lischer 2010). For this test, we used a Markov chain method, using the default parameter options, to determine the level of significance. Additionally, chi-square tests for HWE, following the methods of Hedrick (2000), were performed using GENEALEX v.6.41 (Peakall & Smouse 2006). MICRO-CHECKER (Van Oosterhout *et al.* 2004) was used to test for the presence of null alleles using Bonferroni-adjusted 95% confidence intervals. We tested for linkage disequilibrium for host- and symbiont-specific markers using GENEPOP v.4.0 (Rousset 2008) with default parameters for the Markov chain options.

Population structure, spatial analyses and specificity

Population structure was investigated using a Bayesian clustering approach performed in STRUCTURE v.2.3.3 (Pritchard *et al.* 2000) using the Web-based Bioportal server from the University of Oslo (www.bioportal.uio.no). We employed ARLEQUIN v.3.5.1.2 (Excoffier & Lischer 2010) to calculate pairwise F_{ST} values, using Slatkin's (1995) genetic distance with 1000 permutations to determine significance, and analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) to examine genetic partitioning among regions, sites within regions and individuals within sites as defined in Table 1 (but with the Florida Keys and Biscayne Bay pooled as one region). For STRUCTURE, model options were set to allow admixture, assumed allele frequencies to be correlated (Falush *et al.* 2003), and did not prespecify populations of origin. Because sampling location information set as prior information can assist clustering for data sets with few markers, few individuals or very weak structure (Hubisz *et al.* 2009), the LOCPRIOR option was used. The three sites from Biscayne Bay and each of the two sites from the upper keys, middle keys and the lower keys (Table 1) were pooled into separate 'locations'.

For *E. flexuosa* ('eflex' data sets), analyses were initially performed using only those markers found to be in HWE with duplicate genotypes removed. For comparison, additional runs were performed that included a marker found to deviate from HWE (Plfl7, see Results). As gorgonian octocoral hosts may contain more than one haploid *Symbiodinium* B1 genotype (Kirk *et al.* 2009; Andras *et al.* 2011), analyses for *Symbiodinium* B1 were performed using two separate data sets. One data set, 'singlehaps', contained only those individuals genotyped with a single allele for each marker. This allowed analyses based on allele size. A second data set, 'totalmatrix', contained individuals with both single alleles and those genotyped with multiple haplotypes with at least one marker. This data set was scored

as a presence/absence matrix (1s and 0s) of all possible alleles found at each locus for each individual, similar to analyses of amplified fragment length polymorphisms. STRUCTURE options for the 'totalmatrix' data set were the same as the 'singlehaps' data set, but included the RECESSIVEALLELES=1 option, and '0s' set as the recessive allele (Pritchard 2010). Clonal (duplicate) haplotypes were not removed from either *Symbiodinium* B1 data set, as their inclusion does not alter STRUCTURE analyses, and more accurately represents *Symbiodinium* diversity among individual colonies (Andras *et al.* 2011). For both *E. flexuosa* and *Symbiodinium* B1, Markov chain runs consisted of an initial 'burn-in' (values discarded) of 2×10^6 steps followed by a final 2×10^6 iterations. Three independent runs were performed for 1–12 Ks. The number of population clusters (K) was chosen using the ΔK method (Evanno *et al.* 2005), as implemented in STRUCTURE HARVESTER v0.6.8 (Earl & vonHoldt 2011), and a 'standard' approach (Pritchard 2010) in which the most likely Ks are chosen among the least negative plots of the estimated log probability of the data vs. each of the 12 Ks. Among the least negative plots, the most likely K is then chosen as the one that most robustly recovers structure in the data and is biologically reasonable. CLUMPP v.1.1.2 (Jakobsson & Rosenberg 2007) was used to fuse the results of the three independent runs for the chosen K, and DISTRUCT v1.1 (Rosenberg 2004) was used to visualize the results as the probability of each individual's membership to K populations.

Correlations between geographical and genetic distance (i.e. isolation by distance) and between genetic distances of the host and symbiont were tested using Mantel tests (Mantel 1967) performed with GENALEX v.6.41 (Peakall & Smouse 2006). BAYESASS v1.3 (Wilson & Rannala 2003a) was used to determine directionality of gene flow among the sampling locations by estimating recent migration rates of individuals (over the last few generations). Default settings were used for number of iterations (3 000 000), 'burn-in' (999 999) and sampling frequency (2000).

To examine host/symbiont genotypical specificity, genetic clones for *E. flexuosa* and *Symbiodinium* B1 were identified and assigned a genotype label using GENEALEX v.6.41 (Peakall & Smouse 2006). *Eunicea flexuosa* clones were compared with the genotypes of their *Symbiodinium* B1 partners, and vice versa. Probability of identity (PI; the average probability of randomly drawing two individuals from a population that, by chance, have identical genotypes) analyses were performed in GENALEX v.6.41 to evaluate the ability of the *E. flexuosa* markers to reliably identify clones. Fisher's exact tests (Fisher 1944) were used to test for nonrandom associations among *Symbiodinium* B1 and *E. flexuosa* genotypes using 2×4 contingency tables calculated with *In-Silico* Online (Joosse 2011). Structure outputs were used to identify the most genetically dissimilar host and symbiont genotypes.

Results

Microsatellite markers for E. flexuosa and Symbiodinium B1

Fifty-two sequenced clones were obtained with microsatellite regions containing either tri- or tetra-nucleotide repeats. After screening for *Symbiodinium* (see Materials and Methods), four were *E. flexuosa* specific (~8%) (Table 2); 17 (~33%) were specific to *Symbiodinium* B1; 18 (~35%) robustly PCR-amplified the host *Symbiodinium* B1 culture plus at least one of the other *Symbiodinium* cultures; and the remaining clones performed poorly with PCR. Of the *Symbiodinium* B1-specific candidates that were variable and amplified well across all sampled locations (data not shown), five were used for analyses (Table 3).

For *E. flexuosa*, exact tests for heterozygote deficiencies (Table S1, Supporting Information) and chi-square tests (Table S2, Supporting Information) performed for each marker and sampling location (which represent arbitrarily chosen populations) yielded departures from HWE at some sites. Only Plfl7 showed consistent and highly significant departures from HWE ($P < 0.001$) for all sites using both tests. Positive F_{IS} values were notably greater (0.40–0.79) with Plfl7 compared with the other three markers. Tests for null alleles were significant at all sampling sites for Plfl7, indicating missed alleles likely influenced the homozygote excess found with this marker. In addition, Marker 32 and Panama were significant for null alleles for all of the markers (except Plfl67 at Marker 32). However, the bias towards null alleles at these sites may have been influenced by individuals whose genotypes were strongly assigned to separate clusters (see Fig. 2A). Tests of linkage disequilibrium for each *E. flexuosa* marker revealed no significant deviations from linkage equilibrium for all sites except Panama (Table S3, Supporting Information). At this site, three of the six marker pairs showed significant departures from linkage equilibrium.

For *Symbiodinium* B1, 53% ($n = 326$) contained single alleles, and 47% ($n = 284$) contained at least one multiple allele at any locus. Tests of linkage disequilibrium ('singlehaps' data set) found significant deviations from linkage equilibrium for all, or most, marker pairs at six of the 12 sites (Table S4, Supporting Information). However, when individuals within each sampling site were pooled into their population clusters as determined by STRUCTURE (see below), only one site (Panama)

Table 2 Locus name, primer sequence and marker characteristics of four microsatellite loci in *Eunicea flexuosa*

Locus Name	Primer Sequence	Repeat motif	Ta (°C)	Size range (bp)	Number of alleles	H_O	H_E
Plfl19	F- FAM_CAA CAT CGT CAC CAG TCA CC R- TGG ATT GTG GTT GGA CAG TG	$(TCA)_2CCA(TCA)_3CCAA(TCA)_4ATAA(TCA)_3ATCA(TCA)_2ATAA(TCA)_2ATAA(TCA)_2A(TCA)_3$	57	136–298	25	0.46 (SE 0.017)	0.50 (SE 0.033)
Plfl67	F- VIC_ATT TAA CGT AAT TCA GCC TCT GG R- CCA CAA ATC ATT TAG TCA TAT TGC	$(TATC)_6$	57	179–251	18	0.50 (SE 0.015)	0.60 (SE 0.025)
Plfl199	F- NED_GCG TTT CGT TCA GGC TTT AG R- TGC AGC ATG GTC AAG ATA CC	$(TTG)_5$	57	134–296	13	0.22 (SE 0.055)	0.29 (SE 0.058)
Plfl7	F- PET_TAG TGG GAA TGC ACA TCT CG R- GCT TCC GAG ATA GTT TGT AGG G	$(AAC)_6AAG(ACC)_2AAG(AAC)_2AAG(AAC)_2$	57	139–226	19	0.32 (SE 0.025)	0.80 (SE 0.012)

Table 3 Locus name, primer sequence and marker characteristics of five *Symbiodinium* type B1 microsatellite loci (host—*Eunicea flexuosa)*

Locus name	Primer sequence	Repeat motif	Ta (°C)	Size range (bp)	Number of alleles (single haplotypes)	Number of alleles (multiple haplotypes)
Plflsym17	F- PET_AGG CTG CAG ACA CAA ATG C R- TTT GTC TCA ATG GCA TCA GC	$(AAC)_{24}$	57	150–291	33	36
Plflsym21	F- FAM_AAT CAT TTT GGA AGG CGA TG R- TGA GTG GGA ATG AAC TTG TGA	$(AAC)_{20}(AAG)_5$	57	147–258	22	25
Plflsym196	F- VIC_CTT GAT CGC ATG TGC ATC TC R- CCG GAT TCG TGT TTC AAG AT	$(TTG)_{23}$	57	145–214	20	23
Plflsym211	F- NED_GCG GAT ATG GTT TCT TGG AG R- CCC CCT TTT GAA AGT GAA CA	$(ATC)_{15}ACC(ATC)_{12}$	57	177–300	27	31
Plflsym71/72	F- PET_GAC CTT GCC AAT TCA TGT CC R- GAC ATG ACA TGA CAT GAA ATG C	$(TACA)_9TTCA(TACA)_2TTCA(TACA)_5$	57	125–237	14	21

T_a, annealing temperature.

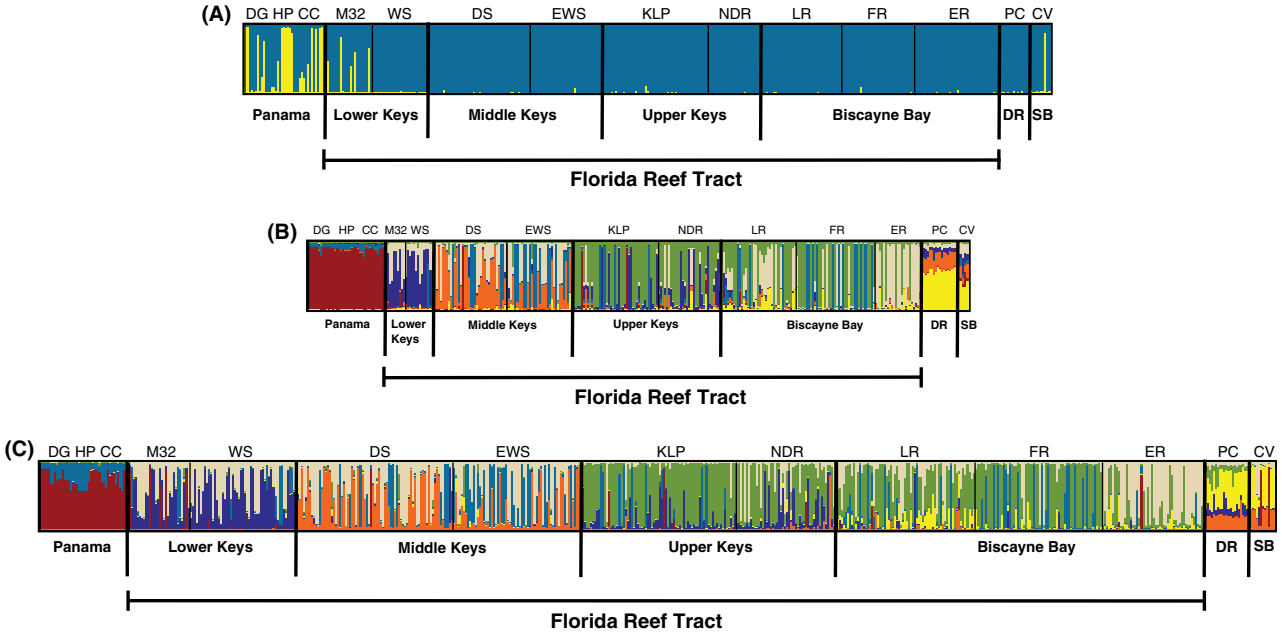

Fig. 2 Graphical depiction of population structure for (A) *Eunicea flexuosa* (K = 2), (B) *Symbiodinium* B1 for the 'singlehaps' (K = 7) and (C) 'totalmatrix' (K = 7) data sets as inferred by Bayesian clustering analyses. Each vertical line represents one individual, with that individual's assignment fraction to each of K population clusters.

demonstrated significant departures from linkage equilibrium, with seven of the 10 primer pairs (Table S5, Supporting Information).

Population structure and spatial analyses

For *E. flexuosa*, duplicate genotypes (i.e. clones) were found within and among collection sites and were removed from each sampling location. As a result, a particular genotype could only be found once per sampling location, but could also occur once in other sampling locations. For Bayesian analyses in STRUCTURE of the 'eflex' data sets, ΔK plots and a 'standard' approach (see Materials and Methods) for determining the number of K population clusters indicated K to be 2 for data sets run with markers in HWE and with an additional marker (Plfl7; ΔK method only) found to deviate from HWE. Population assignments were the same for both data sets at this value of K (Fig. 2A). All of the Florida Keys/Biscayne Bay sites, in addition to Punta Cana, Dominican Republic, were strongly assigned to the same population cluster (colour = blue). Panama contained individuals with high membership probabilities (>0.90) to a second yellow population cluster, in addition to the blue population cluster. Five individuals from Marker 32 (lower keys) were also assigned to the yellow population cluster, but with lower membership probabilities (0.40–0.83). Saba individuals showed strong membership to the blue cluster (>0.90) except for one individual with a robust assignment to the yellow cluster (0.89).

Eunicea flexuosa estimates of pairwise F_{ST} for all locations ranged from 0.000 to 0.149 (overall = 0.020; Table S6, Supporting Information) and revealed a similar pattern of population differentiation to that of STRUCTURE. For example, paired FRT sites showed no significant F_{ST} values with the exception of Marker 32 (lower keys) with three other sites (Fowey Rocks, F_{ST} 0.048; Delta Shoal, F_{ST} 0.033; Western Sambo, F_{ST} 0.072; Ps < 0.05), and Panama F_{ST}s differed significantly from all other sites (F_{ST}s 0.056–0.119, $P < 0.05$) except Marker 32 (F_{ST} 0.003, $P > 0.05$). However, two FRT sites (Fowey Rocks and Western Sambo) showed significant population structure with the Dominican Republic (F_{ST}s 0.049, 0.045; $P < 0.05$). Similarly, AMOVAs indicated a significant partitioning among regions (e.g. FRT and Panama; $P < 0.000$) and individuals within sites ($P = 0.001$), but not among sites within regions (e.g. sites within the FRT and Panama; $P = 0.093$; Table 4).

For *Symbiodinium* B1, the 'singlehaps' and 'totalmatrix' data sets revealed the value of K populations to be 2 and 3, respectively, for the ΔK method and 7 for the 'standard' approach with both data sets. At K = 2, *Symbiodinium* B1 group into two principal clusters comprised of Panama differentiated from most of the FRT, Dominican Republic, and Saba (Fig. S1, Supporting Information). However, at each increasing value of K from 2 to 7, both data sets show individuals with strong membership probabilities (>0.90) to new, and more subtle, population clusters up to K = 7. At K = 8, admixture begins to confound the results. The difference

Table 4 AMOVA results for *Eunicea flexuosa* ('eflex' data set with marker Plfl7 and duplicates removed) and *Symbiodinium* B1 ('singlehaps' and 'totalmatrix' data sets)

Source of variation	d.f.	Sum of squares	% Of variation	Fixation indices	*P* value
'eflex'					
Among regions	3	4.54	5.41	0.061	0.000*
Among sites within regions	10	4.02	0.73	0.008	0.093
Among individuals within sites	782	209.91	93.85	0.054	0.001*
'singlehaps'					
Among regions	3	7.95	8.56	0.099	0.000*
Among sites within regions	8	12.55	9.01	0.176	0.000*
Among individuals within sites	305	117.8	82.42	0.086	0.025*
'totalmatrix'					
Among regions	3	32.18	2.15	0.104	0.000*
Among sites within regions	8	126.1	10.15	0.123	0.000*
Among individuals within sites	599	1209.44	87.7	0.022	0.166

Regions and sites are as defined in Table 1 except with all Florida sites pooled as one region.
*Significance ($P < 0.05$).

between the values of K for the ΔK and the 'standard' approach may be the result of population structuring in *Symbiodinium* B1 that is not effectively hierarchical. The ΔK method largely captures the highest hierarchical level among populations (Evanno *et al.* 2005) and may therefore underestimate population structure in groups that do not conform to this type of subdivision (Kalinowski 2011), including octocorals (Aurelle *et al.* 2011; Andras *et al.* 2013). Therefore, despite the ΔK method identifying K = 2 or 3 as the most likely number of populations, we believe that K = 7, as predicted by the 'standard' approach, more robustly captures the complexity of the genetic signal of *Symbiodinium* B1 for both 'singlehaps' and 'totalmatrix' data sets (Fig. 2B, C). For the 'singlehaps' data set (Fig. 2B), each sampling location (i.e. reef) along the FRT contained *Symbiodinium* B1 strongly assigned (>0.90) to up to four population clusters. Saba and the Dominican Republic showed moderate signs of admixture, indicating a weaker signal, but were principally assigned to the yellow cluster (>0.50). No population subdivision was detected among *Symbiodinium* B1 from three sites at Bocas del Toro, Panama (red cluster). A separate analysis consisting of only the Bocas del Toro sites similarly did not detect any subdivision (data not shown). Population assignments based on the 'totalmatrix' data set (Fig. 2C) were virtually identical to those of the 'singlehaps' data set (Fig. 2B). An exception was the assignment of five individuals from the lower keys site, Marker 32, to the red cluster (>0.50). Both host and symbiont of these five colonies were assigned to population clusters that were primarily assigned to samples from Panama (yellow and red, respectively; Fig. 3). Symbionts of three other individuals spanning the FRT and two individuals from Saba were also assigned to the red cluster.

Symbiodinium B1 pairwise F_{ST} values were largely significant (Table S6, Supporting Information). With the 'singlehaps' data set, F_{ST} values ranged from 0.000 to 0.431 (overall = 0.129) and showed high population subdivision with 77.3% of paired sites exhibiting significant differentiation. The 'totalmatrix' data set F_{ST} values ranged from 0.010 to 0.298 (overall = 0.109), and 93.9% of paired sites were significant. AMOVA analyses of both *Symbiodinium* B1 data sets also indicated acute genetic partitioning with significant differences found among regions ($P < 0.000$), sites within regions ($P < 0.000$) and individuals within sites ($P = 0.025$, 'singlehaps' only; Table 4).

The robustness of population assignments for both *E. flexuosa* and *Symbiodinium* B1 data sets was visualized by pooling only those individuals with assignment probabilities >0.90 (with a few exceptions) at each sampling location and mapping them over their geographical location (Figs 4 and 5; Tables S7 and S8, Supporting Information). Mantel tests for pairwise comparisons among all of the sampling locations in the data set (FRT, Panama, Saba and Dominican Republic) were not significant for *E. flexuosa*, although the trend was positive ($R_{xy} = 0.272$, $P = 0.150$). Similarly, *E. flexuosa* comparisons among sites exclusive to the FRT were also nonsignificant ($R_{xy} = -0.103$, $P = 0.250$). In contrast, for *Symbiodinium* B1, Mantel tests among all of the sampling locations revealed a significant trend of increasing genetic distance with geographical distance ($R_{xy} = 0.691$, *P*-value = 0.010). Sites within the FRT also displayed a significant positive trend ($R_{xy} = 0.459$, *P*-value = 0.010). In addition, Mantel test comparing pairwise genetic distances of *E. flexuousa* with *Symbiondinium* B1 by reef site across the FRT showed no significant correlation ($R_{xy} = -0.222$, *P*-value = 0.160)

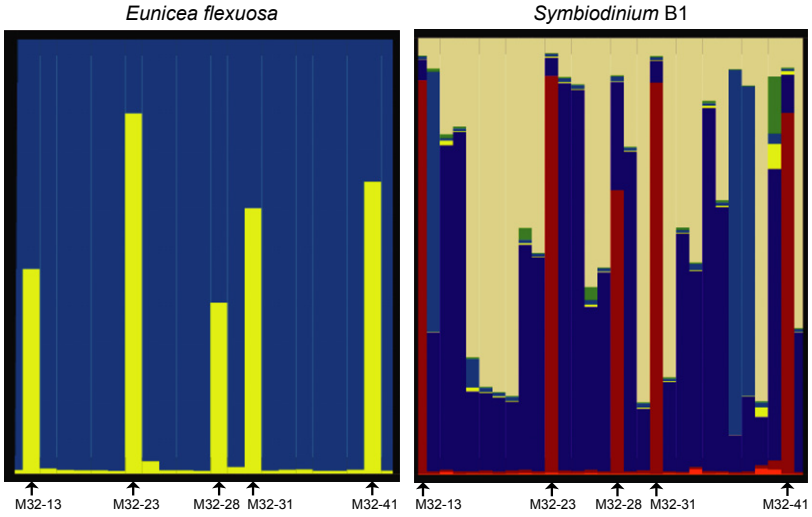

Fig. 3 Assignment probabilities of *Eunicea flexuosa* (from Fig. 2A) and *Symbiodinium* B1 (from Fig. 2C) of individuals from Marker 32 (M32) in the lower Keys. Both host and symbiont of five colonies were assigned to population clusters (yellow and red, respectively) that were primarily assigned to samples from Panama. Sample identification numbers are located beneath each individual.

and suggests no relationship among the genetic structure of *E. flexuosa* and *Symbiodinium* B1.

Migration rates

For *E. flexuosa*, outputs from BAYESASS were used to determine directionality of recent gene flow among three principle regions, namely Panama (SW Caribbean), eastern Florida (western Atlantic) and Saba/Dominican Republic (NE Caribbean; Table 5). Of the nine sampling locations across the FRT, seven contained ~67% nonimmigrants (i.e. self-seeding). This is the minimum amount allowed by BAYESASS and suggests that there is not enough genetic differentiation among these sites (with these markers) to robustly assess migration (i.e. the sites are genetically well mixed; Wilson & Rannala 2003b). However, Emerald Reef and North Dry Rocks were 99% and 95% self-seeding (CIs straddled the upper null CI limit), respectively, which suggests sufficient resolution to detect migration, although less reliably for North Dry Rocks than Emerald Reef. These two locations were the most likely source of ~8–29% of immigrants to 8 reefs within the FRT (six reefs with CIs outside the null, and two reefs with CIs that straddled the upper null CI limit). The overall directionality within the FRT was from north to south. Unexpectedly, FRT sites were also the source of 6–15% of immigrants to sites in Panama, Saba and Dominican Republic, although their CIs indicated low reliability. This may represent an artefact of limited sampling from other candidate sites in the region and/or insufficient marker resolution. Conversely, Saba and the Dominican Republic were not a significant source of immigrants to either Panama or the FRT (≤1%). Immigrants from Panama into all other locations were <1%, with the exception of Marker 32 (3%) and Saba (2%), but contained unreliable CIs. Although the signal was weak, the trend of migration from Panama into the FRT via Marker 32 (3%) was greater than an inverse migration of Marker 32 to Panama (0.0%).

Host and symbiont genotype specificity

For a conservative approximation of specificity, only *Symbiodinium* B1 samples with single alleles at all five microsatellite loci were compared with host genotypes that amplified at all 4 host-specific markers. For *E. flexuosa*, 1–5 clonal host genotypes were found (each comprising 2–6 individuals) within a given sampling location (i.e. reef), and they rarely associated with the same symbiont genotype (Table 6A). Conversely for *Symbiodinium* B1, comparisons of symbiont clonal genotypes to host genotypes mostly contained cases of the same symbiont genotype being found in different host genotypes (Table 6B). Tests for PI for *E. flexuosa* from each sampling location revealed a low probability of two individuals having the same genotype by chance using this suite of markers (0.000–0.010). Fowey Rocks contained the highest PI (0.010), and the expected number of individuals at that site to contain the same genotype is 0.432, or less than one-half of an individual. All other sites contained lower PIs and therefore lower expected numbers of individuals to contain the same genotype by chance. Consequently, the identical genotypes found at each location are not likely to be the result of inadequate marker resolution, but more likely represent clonal individuals.

Fisher's exact tests for nonrandom associations between host and symbiont genotypes revealed a random association among three of the four sites examined along the FRT (Emerald Reef, $P = 0.087$; Key Largo Patch, $P = 0.178$; Delta Shoal, $P = 0.092$). However, Marker 32 demonstrated a significant nonrandom

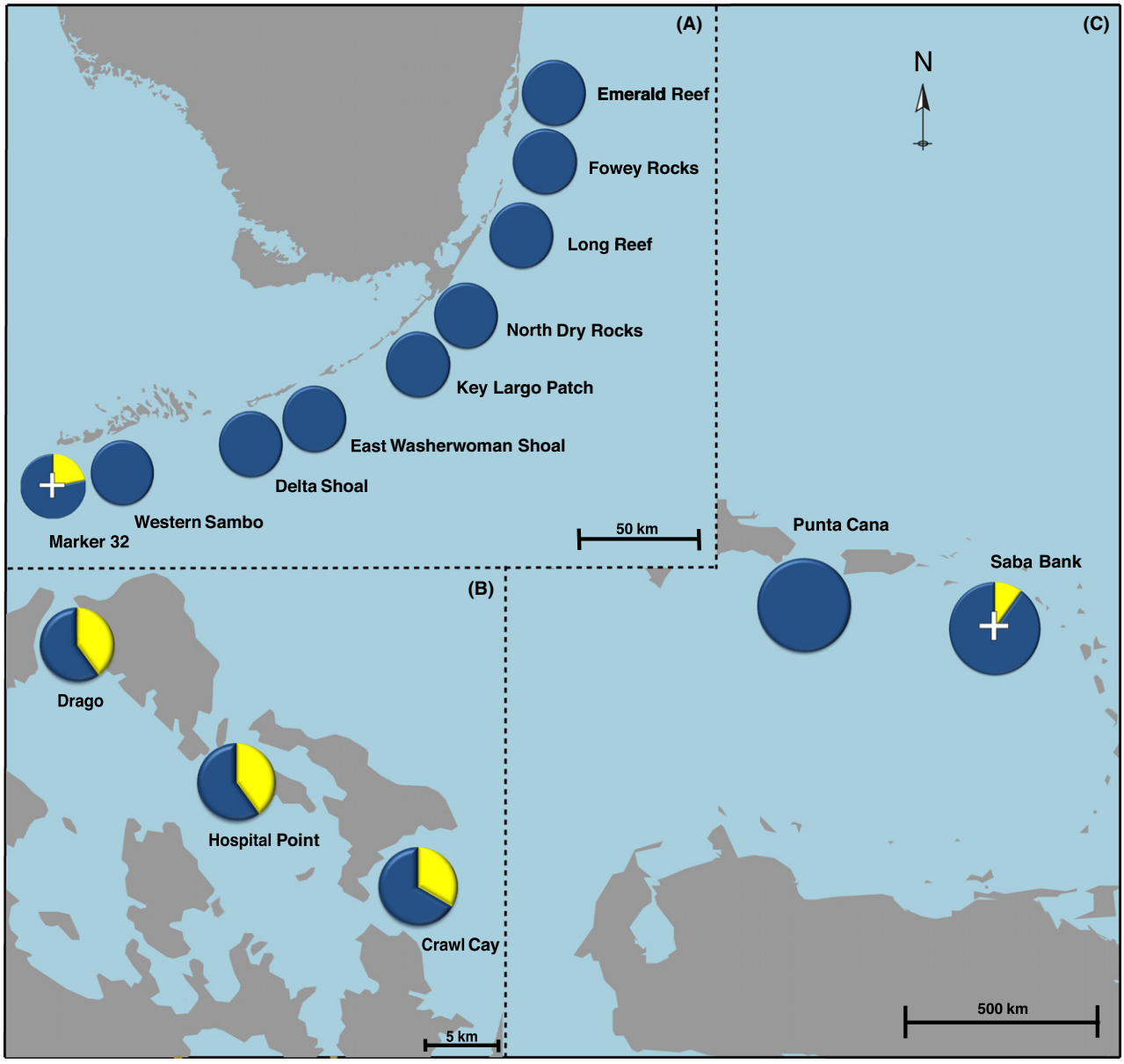

Fig. 4 The fraction of *Eunicea flexuosa* colonies with >0.90 assignment to either blue or yellow population clusters from the (A) Florida reef tract, (B) three sampling locations within the Bocas del Toro Province, Panama, and (C) Punta Cana, Dominican Republic and Saba Bank, mapped over their geographical location. A cross indicates individuals for that location with <0.90 assignment probabilities (see Table S8, Supporting Information for data).

pattern of host/symbiont genotypes ($P < 0.000$; i.e. it is unlikely that the association of the five colonies from Marker 32 to 'Panamanian' clusters for both host and symbiont occurred by chance).

Discussion

Hardy–Weinberg and linkage equilibrium tests

It is not uncommon for both scleractinian corals and gorgonian octocorals to display significant departures from HWE, particularly heterozygote deficits, with microsatellite markers (e.g. Gutierrez-Rodriguez & Lasker 2004; Underwood *et al.* 2007; Baums *et al.* 2010; Ledoux *et al.* 2010; Starger *et al.* 2010; Mokhtar-Jamaï *et al.* 2011). These deficiencies are generally attributed to biological characteristics, such as overlapping generations and nonrandom mating due to inbreeding. Three of the four microsatellite loci developed for *E. flexuosa* were in HWE (Plfl19, Plfl199 and Plfl67) for most sampling locations using exact and chi-square tests. The deficiency found at some of the sites may be attributed

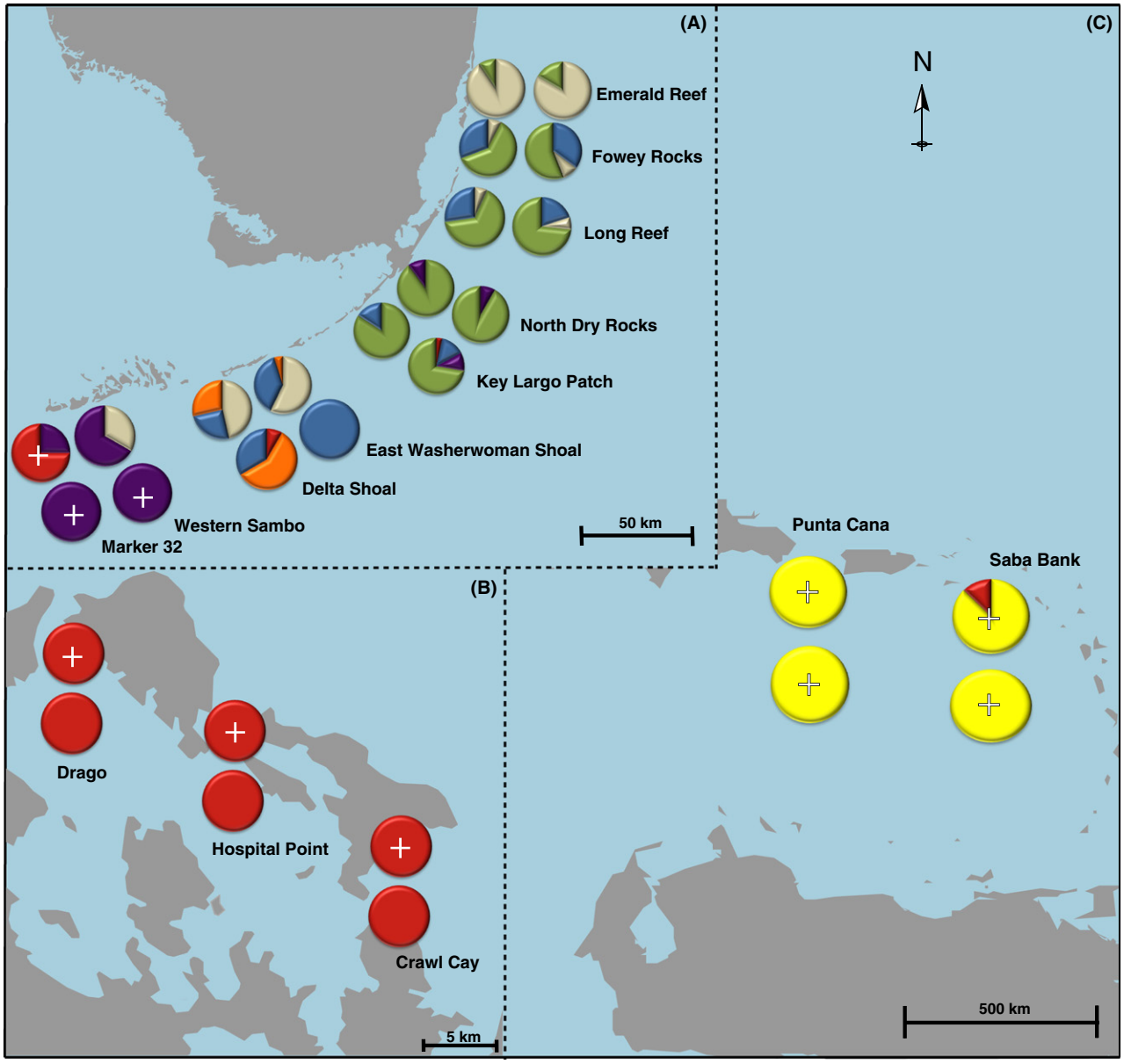

Fig. 5 The fraction of Symbiodinium B1 with >0.90 assignment to their respective population cluster with the 'singlehaps' and 'totalmatrix' data sets from (A) the Florida reef tract ['singlehaps' (right/bottom, 'totalmatrix' (left/top)], (B) three sampling locations within the Bocas del Toro Province, Panama, and (C) Punta Cana, Dominican Republic and Saba Bank ['singlehaps' (bottom), 'totalmatrix' (top)], mapped over their geographical location. A cross indicates individuals for that location with <0.90 assignment probabilities (see Table S7, Supporting Information for data).

to the inability to capture the true allelic diversity of the likely large FRT-wide population of *E. flexuosa* at a single reef. Locus Plfl7 in *E. flexuosa* showed extreme and consistent departures from HWE in all sampling locations and displayed the highest difference between observed/expected heterozygote frequencies compared with the other loci. However, it did not significantly alter the estimation of K populations using Bayesian analyses. Although Plfl7 may not conform to HWE expectations among the collection sites of this study, sampling from other locations across the Caribbean/western Atlantic may reveal this marker to be more useful.

For *Symbiodinium* B1, pairwise tests for linkage disequilibrium based on sampling location revealed significant levels of linkage disequilibrium within sites and among locus pairs. High linkage disequilibrium is likely a consequence of asexually reproducing organisms that do not sexually recombine (Istock *et al*. 1992), and it is

Table 5 *Eunicea flexuosa* immigration matrix. Percentage of immigrants is listed from the source locations ('From' column) to their end location ('Into' row). Bold/boxed values in diagonal represent nonmigrants (self-recruitment) for each location. Dark shaded cells contain immigration rates >5%. Among the bold/boxed and dark shaded cells, underlined values contain confidence intervals (CI; listed in parentheses) outside of the upper bound of the 'null' CI—nonmigrants (0.675–0.992), and migrants (0–0.110). Those not underlined contained a CI that straddled the upper 'null' CI limit. Two light-shaded cells show the biased directionality of immigrants to Panama from Marker 23 (and not vice versa), although their CIs indicate low reliability.

		Biscayne			**Upper Keys**		**Middle Keys**		**Lower Keys**				
	Into ➔ From ↓	Emerald Reef	Fowey Rocks	Long Reef	North Dry Rocks	Key Largo Patch	East Washerwoman Shoal	Delta Shoal	Western Sambo	Marker 32	Panama	Saba	Dominican Republic
Biscayne	Emerald Reef	**0.99 (0.97-0.99)**	0.01	0.01	0.02	0.21 (0.12-0.31)	0.01	0.29 (0.22-0.33)	0.01	0.03	0.01	0.06 (0.00-0.16)	0.10 (0.00-0.23)
	Fowey Rocks	0.00	**0.68**	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01
	Long Reef	0.00	0.00	**0.68**	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01
Upper Keys	North Dry Rocks	0.00	0.28 (0.22-0.32)	0.28 (0.22-0.32)	**0.95 (0.84-0.99)**	0.08 (0.00-0.17)	0.29 (0.22-0.32)	0.02	0.28 (0.20-0.32)	0.19 (0.09-0.27)	0.15 (0.09-0.21)	0.11 (0.02-0.24)	0.13 (0.02-0.27)
	Key Largo Patch	0.00	0.00	0.00	0.00	**0.68**	0.00	0.00	0.00	0.03	0.01	0.02	0.01
Middle Keys	East Washerwoman Shoal	0.00	0.00	0.00	0.00	0.00	**0.68**	0.00	0.00	0.01	0.00	0.01	0.01
	Delta Shoal	0.00	0.00	0.00	0.00	0.00	0.00	**0.67**	0.00	0.01	0.00	0.01	0.01
Lower Keys	Western Sambo	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.68**	0.01	0.00	0.01	0.01
	Marker 32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.69**	0.00 (0.00-0.03)	0.01	0.01
	Panama	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03 (0.00-0.10)	**0.80**	0.02	0.01
	Saba	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	**0.70**	0.01
	Dominican Republic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	**0.69**

Table 6 Comparison of clonal host genotypes (4 markers) to their symbiont genotypes (5 markers) (A), and replicate symbiont genotypes to their host genotypes (B). Genotypes are arranged by location (i.e. reef)

(A)

Sampling location	Sample ID	Host genotype label	Symbiont genotype label	% Host colonies with same genotype that have the same *Symbiodinium* genotype
Emerald Reef (No duplicate host genotypes)				
Fowey Rocks	**FR_07**	**O**	**R**	100% (n = 2)
Fowey Rocks	**FR_50**	**O**	**R**	
Fowey Rocks	FR_02	OO	178	33% (n = 6)
Fowey Rocks	FR_22	OO	41	
Fowey Rocks	FR_40	OO	151	
Fowey Rocks	FR_30	OO	145	
Fowey Rocks	**FR_12**	**OO**	**F**	
Fowey Rocks	**FR_31**	**OO**	**F**	
Fowey Rocks	FR_03	P	T	0% (n = 2)
Fowey Rocks	FR_36	P	F	
Long Reef (No duplicate host genotypes)				
North Dry Rocks	NDR_06	GG	M	0% (n = 3)
North Dry Rocks	NDR_30	GG	50	
North Dry Rocks	NDR_34	GG	117	
North Dry Rocks	NDR_31	JJ	173	0% (n = 2)
North Dry Rocks	NDR_38	JJ	172	
North Dry Rocks	NDR_15	OO	127	0% (n = 2)
North Dry Rocks	NDR_40	OO	142	
Key Largo Patch	**KLP_28**	**FFF**	**D**	100% (n = 2)
Key Largo Patch	**KLP_64**	**FFF**	**D**	
East Washerwoman Shoal	EWS_18	JJ	80	0% (n = 2)
East Washerwoman Shoal	EWS_65	JJ	132	
East Washerwoman Shoal	EWS_31	LL	12	0% (n = 2)
East Washerwoman Shoal	EWS_40	LL	8	
East Washerwoman Shoal	EWS_26	NN	E	0% (n = 2)
East Washerwoman Shoal	EWS_50	NN	H	
East Washerwoman Shoal	EWS_01	O	E	67% (n = 3)
East Washerwoman Shoal	**EWS_09**	**O**	**B**	
East Washerwoman Shoal	**EWS_22**	**O**	**B**	
East Washerwoman Shoal	EWS_24	OO	C	0% (n = 3)
East Washerwoman Shoal	EWS_35	OO	K	
East Washerwoman Shoal	EWS_63	OO	66	
Delta Shoal	DS_55	III	135	0% (n = 2)
Delta Shoal	DS_65	III	105	
Delta Shoal	DS_59	JJJ	64	0% (n = 2)
Delta Shoal	DS_66	JJJ	107	
Western Sambo (No duplicate genotypes)				
Marker 32 (No duplicate genotypes)				
Panama	PA_40	SSS	7	0% (n = 2)
Panama	PA_57	SSS	4	
Saba (No duplicate genotypes)				
Dominican Republic	DR_16	III	130	0% (n = 2)
Dominican Republic	DR_20	III	100	
Dominican Republic	**DR_12**	**UUU**	**G**	100% (n = 2)
Dominican Republic	**DR_21**	**UUU**	**G**	

(B)

Sampling location	Sample ID	Symbiont genotype label	Host genotype label	% Symbiont with the same genotype found with the same host genotype
Emerald Reef (No duplicate symbiont genotypes)				
Fowey Rocks	FR_18	F	74	25% (n = 8)
Fowey Rocks	FR_58	F	89	

Table 6 *Continued*

(B)

Sampling location	Sample ID	Symbiont genotype label	Host genotype label	% Symbiont with the same genotype found with the same host genotype
Fowey Rocks	FR_39	F	105	
Fowey Rocks	FR_49	F	257	
Fowey Rocks	FR_48	F	J	
Fowey Rocks	**FR_12**	**F**	**OO**	
Fowey Rocks	**FR_31**	**F**	**OO**	
Fowey Rocks	FR_36	F	P	
Fowey Rocks	**FR_07**	**R**	**O**	100% (n = 2)
Fowey Rocks	**FR_50**	**R**	**O**	
Fowey Rocks	FR_03	T	P	0% (n = 2)
Fowey Rocks	FR_41	T	QQ	
Long Reef	LR_50	I	94	0% (n = 2)
Long Reef	LR_60	I	309	
North Dry Rocks	NDR_06	M	GG	0% (n = 2)
North Dry Rocks	NDR_08	M	55	
Key Largo Patch	**KLP_28**	**D**	**FFF**	100% (n = 2)
Key Largo Patch	**KLP_64**	**D**	**FFF**	
Key Largo Patch	KLP_24	F	101	0% (n = 2)
Key Largo Patch	KLP_35	F	174	
Key Largo Patch	KLP_23	P	314	0% (n = 2)
Key Largo Patch	KLP_62	P	311	
Key Largo Patch	KLP_27	U	126	0% (n = 2)
Key Largo Patch	KLP_47	U	125	
East Washerwoman Shoal	EWS_20	B	33	50% (n = 4)
East Washerwoman Shoal	EWS_39	B	291	
East Washerwoman Shoal	**EWS_09**	**B**	**O**	
East Washerwoman Shoal	**EWS_22**	**B**	**O**	
East Washerwoman Shoal	EWS_01	E	O	0% (n = 2)
East Washerwoman Shoal	EWS_26	E	NN	
East Washerwoman Shoal	EWS_05	H	217	0% (n = 2)
East Washerwoman Shoal	EWS_50	H	NN	
East Washerwoman Shoal	EWS_33	K	48	0% (n = 2)
East Washerwoman Shoal	EWS_35	K	OO	
Delta Shoal	DS_48	F	269	0% (n = 2)
Delta Shoal	DS_74	F	22	
Delta Shoal	DS_36	J	315	0% (n = 2)
Delta Shoal	DS_53	J	171	
Western Sambo (No duplicate symbiont genotypes)				
Marker 32 (No duplicate symbiont genotypes)				
Panama	PA_10	A	47	0% (n = 2)
Panama	PA_11	A	HH	
Saba (No duplicate symbiont genotypes)				
Dominican Republic	**DR_12**	**G**	**UUU**	100% (n = 2)
Dominican Republic	**DR_21**	**G**	**UUU**	
Dominican Republic	DR_08	O	UU	0% (n = 2)
Dominican Republic	DR_09	O	20	

Individuals in bold highlight clones with matching host and symbiont genotypes. Genotype labels assigned by GENEALEX v.6.41 (Peakall & Smouse 2006).

possible that the linkage disequilibrium observed here is a result of the asexual mode of reproduction in *Symbiodinium* when in symbiosis. However, when *Symbiodinium* samples were pooled according to assigned population clusters as predicted by STRUCTURE, the amount of significant linkage disequilibrium among locus pairs dropped dramatically. This suggests that haploid *Symbiodinium* B1 undergo considerable asexual reproduction, but may also experience recombination (LaJeunesse 2001; Santos *et al.* 2003b), possibly by

sexual reproduction in a free-living stage (Andras *et al.* 2011) within these population clusters.

Population structure and dispersal

All analyses indicated a single population of *E. flexuosa* among nine sampling locations covering the north-eastern and south-western boundaries of the FRT (~225 km). Only the south-westernmost location, Marker 32 (Key West), contained a few individuals with assignment profiles to a second population using clustering analysis. The population homogeneity observed for *E. flexuosa* across the FRT is consistent with other studies that examined the connectivity of scleractinian corals (e.g. Baums *et al.* 2010; Hemond & Vollmer 2010) and other invertebrates (e.g. Richards *et al.* 2007; but see Debiasse *et al.* 2010). However, additional markers may reveal further population subdivision among *E. flexuosa* along the FRT not detected with this suite of markers. Nonetheless, gene flow and therefore larval dispersal in *E. flexuosa* in the FRT appears to be high and suggests a strong potential for resilience in this species following disturbance events.

Symbiodinium B1 on the FRT showed strong population structure among sites and at individual collection sites (i.e. reefs) over distances on the scale of metres. Each site contained individuals assigned to 2–4 different population clusters with high (>0.90) assignment probabilities. This acute population structuring suggests some level of dispersal among *Symbiodinium* B1 genotypes to different reef locations of the FRT, but with minimal sexual recombination. However, the large number of samples with multiple symbiont genotypes (47%), and the robust levels of admixture found among many samples using Bayesian analyses, also suggests some level of mixing among *Symbiodinium* B1, likely in the form of mixed symbiont communities. As gorgonian octocorals have not been recorded to routinely shuffle their symbiont communities, especially in the absence of any stress-induced bleaching (Kirk *et al.* 2005; Goulet 2006; Baker & Romanski 2007; Hannes *et al.* 2009), it is unlikely that the observed genetic pattern of *Symbiodinium* was caused by seasonal or synchronous changes in symbiont communities during the 3.6 years over which sampling occurred. The operational timescales of this data set are likely much longer (>10 years).

Unexpectedly, *E. flexuosa* from the FRT was not differentiated from either Saba or the Dominican Republic (distances > 1000 km) with Bayesian analyses. However, the Dominican Republic did show significant differentiation from three sites of the FRT with F_{ST} analyses. This mixed signal contrasts with the scleractinian coral, *Acropora cervicornis* (also a broadcast spawner), which was found to be genetically well connected along the FRT, but which showed clear population subdivision at sites from St. Thomas and Honduras (Baums *et al.* 2010), which are as distant from the FRT as Saba and the Dominican Republic. The lack of a strong population signal in *E. flexuosa* from these three regions could be the result of insufficient resolution based on the number of loci used for analyses (3 or 4 in this study compared with 7 and 8 in Baums *et al.* 2010). However, for broadcast spawning corals, it is possible that gene flow may be maintained over long geographical distances (Nunes *et al.* 2009). In contrast, *Symbiodinium* B1 from the FRT were differentiated from those in Saba and Dominican Republic using Bayesian analyses, with the latter two sites sharing a similar, albeit admixed, population signal. A single-population cluster at Saba and the Dominican Republic, although only weakly supported, is plausible because of their relatively close proximity to one another.

Migration rate analyses in BAYESASS indicated a primarily southerly dispersal of *E. flexuosa* along the FRT, in contrast to the northerly flow followed by the Florida Current (Fig. 1). Although tests indicated a well-mixed system along the FRT (nonmigrant values of ~68%) among most sampled sites, this suite of markers contained sufficient resolution to assess migration from two sites (Emerald Reef and North Dry Rocks), both of which signified a predominately southerly flow of migrants along the FRT. Conversely, none of the middle keys (East Washerwoman Shoal and Delta Shoal) or lower keys (Western Sambo and Marker 32) sites showed migration rates >1% to any of the upper keys or Biscayne Bay sites, although many immigrant values were at the maximum allowed by BAYESASS (~30%), suggesting a weak signal. Richards *et al.* (2007) and Debiasse *et al.* (2010) found a similar southerly migration pattern among amphipods and a reef sponge, respectively, along the FRT and attributed the pattern to inshore counter currents running north to south, west of the Florida Current (Lee & Williams 1999; Yeung & Lee 2002). The *E. flexuosa* samples in this study were collected primarily from inshore patch reefs, which could be influenced by inshore counter currents. Furthermore, smaller-scale (both spatial and temporal) oceanographical features, such as mesoscale eddies, may potentially affect larval dispersal across the FRT, counter to generalized current patterns (D'Alessandro *et al.* 2007; Parks *et al.* 2009). Migration rate tests also suggest the immigration of *E. flexuosa* to sites outside the FRT (Saba, Dominican Republic and Panama) from Emerald Reef and North Dry Rocks, although the CIs for these sites indicate a tenuous signal. The pattern of gene flow (and low migration rate reliability) observed among these sites is likely an artefact of inadequate

sampling. Additional sampling between these sites, together with the addition of other markers, may reveal different patterns of dispersal.

A second-population cluster of *E. flexuosa* was assigned predominantly to individuals sampled from three sites at Bocas del Toro, Panama, in addition to five individuals from a site off Key West (Marker 32). These individuals were found sympatrically (on the same reef site) among other individuals that were assigned to a separate population, sometimes separated by distances of only a few metres. This extreme within-reef population subdivision could be the result of fine-scale niche partitioning and adaptation to microreef environments (Finke & Snyder 2008), or cryptic speciation (Bickford *et al.* 2007). *Eunicea flexuosa* is highly plastic and can vary morphologically in different areas of a reef (Kim *et al.* 2004; Prada *et al.* 2008). Whether the genetic subdivision of *E. flexuosa* within these reefs corresponds to morphological variation needs to be examined further.

Migration rate tests indicated very weak directionality of immigrants from Panama to the FRT (Marker 32, 3%), but not vice versa (Marker 32 to Panama, 0%). Although tenuous, this result agrees with the major oceanographical currents of the western Caribbean (Fig. 1; Roberts 1997) and suggests that the individuals from Marker 32 (Key West) that were assigned to the second (yellow) population are immigrants from Panama (excluding ghost populations not sampled). However, the dispersal of individuals from this second population, for example, from Marker 32 to more northern regions of the FRT was not detected using assignment tests. This suggests that successful recruitment into the FRT from Panama may not only be limited, but migrants from the second population are not dispersing further into the FRT. Instead, these individuals may be mating with local individuals, not of the same population, as indicated by the admixture of the blue and yellow populations among some individuals of Marker 32.

Vectored dispersal of Symbiodinium B1 by E. flexuosa larvae

The high level of population subdivision among *Symbiodinium* B1 along the FRT contrasts markedly with that of its host, *E. flexuosa*, which formed a single-population cluster. The discrepancy between *E. flexuosa* and its symbiont populations is in agreement with the life history of *E. flexuosa* (i.e. as a broadcast spawner, its larvae obtain symbionts from the external environment) and suggests that adult colonies acquire (and maintain) *Symbiodinium* B1 found in local environmental pools after settlement. Analyses of *E. flexuosa* and *Symbiodinium* B1 indicate high dispersal of the host across the FRT and Caribbean, but not in the symbiont. Therefore, if symbiont acquisition occurs at, or close to, settlement, *E. flexuosa* larvae that disperse long distances will likely be exposed to genetically different symbionts compared with those found in their natal symbiont habitat. However, as gorgonian octocoral larvae typically obtain their symbionts quickly (during the first few days) and are nonspecific during early symbiont uptake (Coffroth *et al.* 2001), *E. flexuosa* larvae may initially incorporate *Symbiodinium* B1 from their natal site and only later acquire local symbionts after settlement. Another possibility is that *E. flexuosa* can acquire natal symbionts, transport them to new settlement sites and retain them over time. For example, five samples from the southwesternmost site in the lower keys (Marker 32) were assigned to the red *Symbiodinium* B1 population cluster, to which mostly individuals from Panama were assigned (Fig. 3). Moreover, the hosts in which these five *Symbiodinium* samples were found were assigned to the yellow *E. flexuosa* population, which was also principally assigned to individuals from Panama. This pattern of association among host/symbiont genotypes in Marker 32 was nonrandom (Fisher's exact test) and is not likely to have occurred by chance. Therefore, as the direction of *E. flexuosa* immigrants is likely to move from Panama to the lower keys (Marker 32), and not vice versa, it is hypothesized that the larvae of these five *E. flexuosa* colonies originated in Panama (disregarding unsampled locations) and acquired natal symbionts there. They were then subsequently transported to the lower keys (perhaps via intermediate populations that were not sampled), where they eventually settled and, in this case, retained their natal symbionts. Similarly, one individual from Saba was also assigned to both the yellow host and red symbiont 'Panamanian' populations, unlike the other samples of that site, and likely exhibited a similar pattern of symbiont acquisition, dispersal and retention.

As previously discussed, it does not appear that the red 'Panamanian' symbiont genotypes are specific to the yellow 'Panamanian' host genotypes. Excluding the five individuals from Marker 32, the red 'Panamanian' symbiont population cluster was also strongly assigned to at least three other individuals (and weakly to another four) in the FRT whose hosts were assigned to the blue cluster (Delta Shoal, Key Largo Patch, and Emerald Reef). This suggests that *Symbiodinium* B1 from Panama, after arriving in the FRT with *E. flexuosa* larvae, can then infect local *E. flexuosa* hosts in the FRT and mix with other (local) *Symbiodinium* B1 genotypes within these hosts. This may explain why the red 'Panamanian' symbiont genotype was strongly admixed with local (FRT) symbiont genotypes in four of the FRT hosts.

Symbiodinium from three sites from Bocas del Toro, Panama, were principally assigned to a single (red)-population cluster, with minimal admixture. In contrast to most of the FRT, *E. flexuosa* within these sites were assigned to separate population clusters (blue and yellow). This suggests that two genetically distinct groups of *E. flexuosa* occur sympatrically at these sites, but are nonspecific to the *Symbiodinium* B1 genotypes with which they associate. If *E. flexuosa* were strictly specific to a particular *Symbiodinium* B1 genotype (Santos *et al.* 2004), then individuals from the two *E. flexuosa* populations would be expected to host different populations of *Symbiodinium* B1. This was not the case and is contrary to what has been suggested regarding host/symbiont specificity in gorgonian octocorals to date (Coffroth *et al.* 2001; Goulet & Coffroth 2003a,b; Santos *et al.* 2004). In addition, Fisher's exact tests comparing groups of the most genetically dissimilar *E. flexuosa* genotypes along the FRT with the population cluster of *Symbiodinium* B1 genotypes with which they associate showed no significant nonrandom relationship. This suggests that *E. flexuosa* genotypes of the FRT are not specific to a particular *Symbiodinium* B1 genotype, nor do they continually maintain them over time.

A comparison of clonal host genotypes to their symbiont genotypes revealed very few instances of genotypical matches, and PI tests suggest that it is unlikely the clonal genotypes found are a result of insufficient marker resolution. This host/symbiont flexibility contrasts with other studies that have shown strict (100%) specificity of clonemates of a particular host genotype to a single symbiont genotype (Goulet & Coffroth 2003a,b). Conversely, Andras *et al.* (2013) did not find duplicate genotypes of the gorgonian octocoral *G. ventalina* using 10 microsatellite loci. However, *G. ventalina* is not known to regularly fragment asexually and is phylogenetically unrelated to *E. flexuosa* at the family level. The extent to which *E. flexuosa* routinely fragments is unknown, although fragmentation among a more closely related gorgonian octocoral, *Plexaura kuna*, can be extensive (Lasker 1984). Replicate *Symbiodinium* B1 genotypes were not found to be restricted to a particular *E. flexuosa* genotype. Instead, in most cases, the same *Symbiodinium* B1 genotype was found in different *E. flexuosa* genotypes. This suggests that *Symbiodinium* B1 genotypes, reproducing asexually within hosts, may not be restricted to that individual, but may be transferred and shared among different *E. flexuosa* individuals. Alternatively, many *Symbiodinium* B1 with the same genotype may each initially associate with a different *E. flexuosa* genotype and remain in those specific symbioses. Future work using temporal data (e.g. Goulet & Coffroth 2003b) may be necessary to accurately distinguish these two possibilities.

It is likely that the relatively complex population structure observed among *Symbiodinium* B1 in *E. flexuosa* is the result of a passive process of stochastic dispersal and host acquisition, although a selection-mediated process of local adaptation cannot be ruled out. To understand these processes more clearly, a thorough understanding of the relationship between the genetic diversity of *Symbiodinium* B1 and ecological/physiological adaptation is needed. Nevertheless, environmental pools of *Symbiodinium* B1 appear to be genetically diverse, and both host and symbiont likely exhibit short- and long-range dispersal over ecological time periods. This could, in turn, contribute to the spread of locally viable genetic combinations of host and symbiont in response to environmental change.

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References

Andras JP, Kirk NL, Drew Harvell CD (2011) Range-wide population genetic structure of *Symbiodinium* associated with the Caribbean Sea fan coral, *Gorgonia ventalina*. *Molecular Ecology*, **20**, 2525–2542.

Andras JP, Rypien KL, Harvell CD (2013) Range-wide population genetic structure of the Caribbean sea fan coral, *Gorgonia ventalina*. *Molecular Ecology*, **22**, 56–73.

Aurelle D, Ledoux JB, Rocher C, Borsa P, Chenuil A, Feral JP (2011) Phylogeography of the red coral (*Corallium rubrum*): inferences on the evolutionary history of a temperate gorgonian. *Genetica*, **139**, 855–869.

Baker AC (2003) Flexibility and specificity in coral-algal symbiosis: diversity, ecology and biogeography of *Symbiodinium*. *Annual Review of Ecology and Systematics*, **34**, 661–689.

Baker AC, Romanski AM (2007) Multiple symbiotic partnerships are common in scleractinian corals, but not in octocorals: comment on Goulet (2006). *Marine Ecology Progress Series*, **335**, 237–242.

Baker AC, Rowan R, Knowlton N (1997) Symbiosis ecology of two Caribbean acroporid corals. *Proceedings of the 8th International Coral Reef Symposium, Panama*, **2**, 1295–1300.

Baums IB, Johnson ME, Devlin-Durante MK, Miller MW (2010) Host population genetic structure and zooxanthellae diversity of two reef-building coral species along the Florida Reef Tract and wider Caribbean. *Coral Reefs*, **29**, 835.

Bayer FM (1961) *The Shallow-Water Octocorallia of the West Indian Region: A Manual for Marine Biologists*. Martinus Nijho, The Hague.

Beiring EA, Lasker HR (2000) Egg production by colonies of a gorgonian coral. *Marine Ecology Progress Series*, **196**, 169–177.

Bellwood D, Hughes T, Folke C, Nyström M (2004) Confronting the coral reef crisis. *Nature*, **429**, 827–833.

Bickford D, Lohman DJ, Sodhi NS *et al.* (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, **22**, 148–155.

Bruno JF, Selig ER (2007) Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. *PLoS ONE*, **2**, e711.

Coffroth M, Santos S (2005) Genetic diversity of symbiotic dinoflagellates in the genus *Symbiodinium*. *Protist*, **156**, 19–34.

Coffroth MA, Santos SR, Goulet TL (2001) Early ontogenetic expression of specificity in a cnidarian-algal symbiosis. *Marine Ecology Progress Series*, **222**, 85–96.

Cowen RK, Lwiza KMM, Sponagule S, Paris CB, Olson DB (2000) Connectivity of marine populations: open or closed? *Science*, **287**, 857–859.

Cowen RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. *Science*, **311**, 522–527.

D'Alessandro E, Sponaugle S, Lee T (2007) Patterns and processes of larval fish supply to the coral reefs of the upper Florida Keys. *Marine Ecology Progress Series*, **331**, 85–100.

De'ath G, Fabricius KE, Sweatman H, Puotinen M (2012) The 27–year decline of coral cover on the Great Barrier Reef and its causes. *Proceedings of the National Academy of Sciences*, **109**, 17995–17999.

Debiasse MB, Richards VP, Shivji MS (2010) Genetic assessment of connectivity in the common reef sponge, *Callyspongia vaginalis* (Demospongiae: Haplosclerida) reveals high population structure along the Florida reef tract. *Coral Reefs*, **29**, 47–55.

Drummond AJ, Ashton B, Cheung M *et al.* (2008) Geneious v4.8.5. Available from http://www.geneious.com/

Earl DA, vonHoldt BM (2011) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes, application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure: extensions to linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.

Finke DL, Snyder WE (2008) Niche partitioning increases resource exploitation by diverse communities. *Science*, **321**, 1488–1490.

Fisher RA (1944) *Statistical Methods for Research Workers*. Oliver and Boyd, Edinburgh, section 21.02.

Gardner TA, Cote IM, Gill JA, Grant A, Watkinson AR (2003) Long-term region-wide declines in Caribbean corals. *Science*, **301**, 958–960.

Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. *Methods in Enzymology*, **395**, 202–222.

Goldberg W (1973) Ecological aspects of salinity and temperature tolerances of some reef-dwelling gorgonians from Florida. *Caribbean Journal of Science*, **13**, 465–488.

Goulet TL (2006) Most corals may not change their symbionts. *Marine Ecology Progress Series*, **321**, 1–7.

Goulet TL, Coffroth MA (2003a) Genetic composition of zooxanthellae between and within colonies of the octocoral *Plexaura kuna*, based on small subunit rDNA and multilocus DNA fingerprinting. *Marine Biology*, **142**, 233–239.

Goulet TL, Coffroth MA (2003b) Stability of an octocoral-algal symbiosis over time and space. *Marine Ecology Progress Series*, **250**, 117–124.

Goulet TL, Coffroth MA (2004) The genetic identity of dinoflagellate symbionts in Caribbean octocorals. *Coral Reefs*, **23**, 465–472.

Goulet TL, Simmons C, Goulet D (2008) Worldwide biogeography of *Symbiodinium* in tropical octocorals. *Marine Ecology Progress Series*, **355**, 45–58.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics*, **48**, 361–372.

Gutierrez-Rodriguez C, Lasker HR (2004) Microsatellite variation reveals high levels of genetic variability and population structure in the gorgonian coral *Pseudopterogorgia elisabethae* across the Bahamas. *Molecular Ecology*, **13**, 2211–2221.

Hannes AR, Barbeitos M, Coffroth MA (2009) Stability of symbiotic dinoflagellate type in the octocoral *Briareum asbestinum*. *Marine Ecology Progress Series*, **391**, 65–72.

Hedgecock D, Barber PH, Edmands S (2007) Genetic approaches to measuring connectivity. *Oceanography*, **20**, 70–79.

Hedrick PW (2000) *Genetics of Populations*, 2nd edn. Jones and Bartlett, Boston, Massachusetts, USA.

Hellberg ME (2007) Footprints on water: the genetic wake of dispersal among reefs. *Coral Reefs*, **26**, 463–473.

Hemond EM, Vollmer SV (2010) Genetic diversity and connectivity in the threatened staghorn coral (*Acropora cervicornis*) in Florida. *PLoS ONE*, **5**, e8652.

Hubisz M, Falush D, Stephens M, Pritchard J (2009) Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, **9**, 1322–1332.

Hughes TP, Baird AH, Bellwood DR *et al.* (2003) Climate change, human impacts, and the resilience of coral reefs. *Science*, **301**, 929–933.

Istock CA, Duncan KE, Ferguson N, Zhou X (1992) Sexuality in a natural population of bacteria-*Bacillus subtilis* challenges the clonal paradigm. *Molecular Ecology*, **1**, 95–103.

Jaap WC (1984) *The Ecology of the South Florida Coral Reefs: A Community Profile*. US Department of the Interior, Fish and Wildlife Service, Minerals Management Service, Washington, DC, 138 pp.

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switch-

ing and multimodality in analysis of population structure. *Bioinformatics*, **23**, 1801.

Jones RJ (2004) Testing the 'photoinhibition' model of coral bleaching using chemical inhibitors. *Marine Ecology Progress Series*, **284**, 133–145.

Jones GP, Srinivasan M, Almany GR (2007) Population connectivity and conservation of marine biodiversity. *Oceanography*, **20**, 100–111.

Jones GP, Almany GR, Russ GR *et al.* (2009) Larval retention and connectivity among populations of corals and reef fishes: history, advances and challenges. *Coral Reefs*, **28**, 307–325.

Joosse SA (2011) Fisher's exact test. Available from http://in-silico.net/statistics/fisher_exact_test.

Kalinowski ST (2011) The computer program STRUCTURE does not reliably identify the main genetic clusters with species: simulations and implications for human populations structure. *Heredity*, **106**, 625–632.

Kim E, Lasker H, Coffroth M, Kim K (2004) Morphological and genetic variation across reef habitats in a broadcast-spawning octocoral. *Hydrobiologia*, **530**, 423–432.

Kirk NL, Ward JR, Coffroth MA (2005) Stable *Symbiodinium* composition in the sea fan *Gorgonia ventalina* during temperature and disease stress. *Biological Bulletin*, **209**, 227–234.

Kirk NL, Andras JP, Harvell CD, Santos SR, Coffroth MA (2009) Population structure of *Symbiodinium* sp. associated with the common sea fan, *Gorgonia ventalina*, in the Florida Keys across distance, depth, and time. *Marine Biology*, **156**, 1609–1623.

LaJeunesse TC (2001) Investigating the biodiversity, ecology and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS region: In search of a "species" level marker. *Journal of Phycology*, **37**, 866–880.

Lasker H (1984) Asexual reproduction, fragmentation, and skeletal morphology of a plexaurid gorgonian. *Marine Ecology Progress Series*, **19**, 261–268.

Lasker H, Coffroth M (1983) Octocoral distributions at Carrie Bow Cay, Belize. *Marine Ecology Progress Series*, **13**, 21–28.

Ledoux J-B, Mokhtar-Jamai K, Roby C *et al.* (2010) Genetic survey of shallow populations of the Mediterranean red coral [*Corallium rubrum* (Linnaeus, 1758)]: new insights into evolutionary processes shaping nuclear diversity and implications for conservation. *Molecular Ecology*, **19**, 675–690.

Lee T, Williams E (1999) Mean distribution and seasonal variability of coastal currents and temperature in the Florida Keys with implications for larval recruitment. *Bulletin of Marine Science*, **64**, 35–56.

Lewis CL, Coffroth MA (2004) The acquisition of exogenous algal symbionts by an octocoral after bleaching. *Science*, **304**, 1490–1492.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.

Mokhtar-Jamaï K, Pascual M, Ledoux J-B *et al.* (2011) From global to local genetic structuring in the red gorgonian *Paramuricea clavata*: the interplay between oceanographic conditions and limited larval dispersal. *Molecular Ecology*, **20**, 3291–3305.

Nunes F, Norris R, Knowlton N (2009) Implications of isolation and low genetic diversity in peripheral populations of an amphi-Atlantic coral. *Molecular Ecology*, **18**, 4283–4297.

Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications*, **13**, S146–S158.

Pandolfi J, Jackson J, Baron N *et al.* (2005) Are US coral reefs on the slippery slope to slime? *Science*, **307**, 1725–1726.

Parks AB, Shay LK, Johns WE, Martinez-Pedraja J, Gurgel K-W (2009) HF radar observations of small-scale surface current variability in the Straits of Florida. *Journal of Geophysical Research Oceans*, **114**, C08002.

Peakall R, Smouse P (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Pochon X, Gates RD (2010) A new *Symbiodinium* clade (Dinophyceae) from soritid foraminifera in Hawai'i. *Molecular Phylogenetics and Evolution*, **56**, 492–497.

Prada C, Schizas N, Yoshioka P (2008) Phenotypic plasticity or speciation? A case from a clonal marine organism. *BMC Evolutionary Biology*, **8**, 47.

Pritchard JK (2010) Documentation for structure software: Version 2.3.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Richards VP, Thomas JD, Stanhope MJ, Shivji MS (2007) Genetic connectivity in the Florida reef system: comparative phylogeography of commensal invertebrates with contrasting reproductive strategies. *Molecular Ecology*, **16**, 139–157.

Roberts CM (1997) Connectivity and management of Caribbean coral reefs. *Science*, **278**, 1454.

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes*, **4**, 137–138.

Rousset F (2008) genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, **8**, 103–106.

Rowan R, Powers DA (1991) A molecular genetic classification of zooxanthellae and the evolution of animal-algal symbiosis. *Science*, **251**, 1348–1351.

Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. *Methods in Molecular Biology*, **132**, 365–386.

Sanchez J, Diaz J, Zea S (1997) Gorgonian communities in two contrasting environments on oceanic atolls of the southwestern Caribbean. *Bulletin of Marine Science*, **61**, 453–465.

Santos SR, Gutiérrez-Rodríguez C, Coffroth MA (2003a) Phylogenetic identification of symbiotic dinoflagellates via lengthheteroplasmy in domain V of chloroplast large subunit (cp23S)-ribosomal DNA sequences. *Marine Biotechnology*, **5**, 130–140.

Santos S, Gutierrez-Rodriguez C, Lasker H, Coffroth M (2003b) *Symbiodinium* sp. associations in the gorgonian *Pseudopterogorgia elisabethae* in the Bahamas: high levels of genetic variability and population structure in symbiotic dinoflagellates. *Marine Biology*, **143**, 111–120.

Santos S, Shearer T, Hannes A, Coffroth M (2004) Fine-scale diversity and specificity in the most prevalent lineage of symbiotic dinoflagellates (*Symbiodinium*, Dinophyceae) of the Caribbean. *Molecular Ecology*, **13**, 459–469.

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology*, **69**, 82–90.

Shearer TL, Gutierrez-Rodriguez C, Coffroth MA (2005) Generating molecular markers from zooxanthellate cnidarians. *Coral Reefs*, **24**, 57–66.

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, **139**, 457–462.
Spalding MD, Ravilious C, Green EP (2001) *World Atlas of Coral Reefs*. University of California Press, Berkeley, Los Angeles, London.
Starger CJ, Barber PH, Ambariyanto Baker AC (2010) The recovery of coral genetic diversity in the Sunda Strait following the 1883 eruption of Krakatau. *Coral Reefs*, **29**, 547–565.
Underwood JN, Smith LD, Van Oppen MJH, Gilmour JP (2007) Multiple scales of genetic connectivity in a brooding coral on isolated reefs following catastrophic bleaching. *Molecular Ecology*, **16**, 771–784.
Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.
Van Oppen MJH, Gates RD (2006) Conservation genetics and the resilience of reef-building corals. *Molecular Ecology*, **15**, 3863–3883.
Van Oppen MJH, Mieog JC, Sánchez CA, Fabricius KE (2005) Diversity of algal endosymbionts (zooxanthellae) in octocorals: the roles of geography and host relationships. *Molecular Ecology*, **14**, 2403–2417.
Vollmer SV, Palumbi SR (2007) Restricted gene flow in the Caribbean staghorn coral *Acropora cervicomis*: implications for the recovery of endangered reefs. *Journal of Heredity*, **98**, 40–50.
Wilkinson C, Souter D (2008) *Status of Caribbean Coral Reefs After Bleaching and Hurricanes in 2005*. Global Coral Reef Monitoring Network, and Reef and Rainforest Research Centre, Townsville, 152 p.
Wilson G, Rannala B (2003a) Bayesian inference of recent migration rates using multilocus genotypes. *Genetics*, **163**, 1177–1191.
Wilson GA, Rannala B (2003b) Documentation for BayesAss 1.3.
Yeung C, Lee T (2002) Larval transport and retention of the spiny lobster, *Panulirus argus*, in the coastal zone of the Florida Keys, USA. *Fisheries Oceanography*, **11**, 286–309.

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Data accessibility

Microsatellite genotype data sets for *Eunicea flexuosa* 'eflex' and *Symbiodinium* B1 'singlehaps' and 'totalmatrix': DRYAD, doi: 10.5061/dryad.82ms3.

Supporting information

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

Fig. S1 Estimation of K populations for the 'singlehaps' (A) and 'totalmatrix' (B) datasets.

Table S1 Exact tests (*Eunicea flexuosa*) for Hardy–Weinberg equilibrium for each locus and sampling location with F_{IS} estimates using Weir & Cockerham (1984) [W&C] and Robertson & Hill (1984) [R&H] methods, observed and expected heterozygosity, *P*-value, and significance.

Table S2 Chi-square tests (*Eunicea flexuosa*) for Hardy–Weinberg equilibrium (H1 = significant deviations from HWE frequences) for each locus and sampling location, degrees of freedom, chi-square value, *P*-value, and significance.

Table S3 Tests for linkage disequilibrium (*Eunicea flexuosa*) for each primer pair in each sampling location, standard error, *P*-value, and significance.

Table S4 Tests for linkage disequilibrium for each *Symbiodinium* primer pair in each sampling location, *P*-value, and significance for the 'singlehaps' dataset.

Table S5 Linkage disequilibrium tests for individuals grouped by within-site population clusters as determined by STRUCTURE for each primer pair, *P*-value, and significance for the 'singlehaps' dataset.

Table S6 Pairwise F_{ST} values among all sampled locations (reefs) for *Eunicea flexuosa* ('eflex' dataset without marker Plfl7 and duplicates) and *Symbiodinium* B1 ('singlehaps' and 'totalmatrix' datasets).

Table S7 Numbers of host individuals with *Symbiodinium* genotypes with >90% assignment to a particular population including the total number of individuals collected at each sampling location, total number of individuals with >90% assignment per location, the percent of individuals with >90% assignment for each location, and individuals with >90% assignment and their predicted population.

Table S8 Numbers of *Eunicea flexuosa* colonies with >90% assignment to a particular population including the total number of individuals collected at each sampling location, the total number of individuals with >90% assignment per location, the percent of individuals with >90% assignment for each location, and individuals with >90% assignment and their predicted population (data set excludes marker Plfl7).