

Supporting Information

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SI Materials and Methods

Molecular–Genetic Identification. Nucleic acid extractions were conducted using a modified Promega Wizard genomic DNA extraction protocol (54). The dominant resident symbiont was initially identified by denaturing gradient gel electrophoresis (DGGE) fingerprinting of the partial 5.8S and internal transcribed spacer region 2 (*ITS2*) (55, 56). All samples used in this study for multilocus genotyping were verified by this protocol to contain high abundances of *S. trenchii* (20–100% of the total symbiont population). For colonies used in our physiological measurements, only those with homogenous populations of a particular symbiont were selected (Fig. S3).

Microsatellite Allele Calling. Twelve microsatellite loci (Table S1) developed for clade D *Symbiodinium* were used to determine the genetic diversity of *S. trenchii* populations (57, 58). Many of these loci were used previously for other species of clade D *Symbiodinium* (e.g., *Symbiodinium boreum* and *Symbiodinium eurythalphos*) (15) and *S. “glynni”* (nomen nudum) (22); however, *S. trenchii* has experienced a partial or whole genome duplication making these loci diallelic (15, 57, 58). Although *S. trenchii* exists predominantly in the haploid stage, like other dinoflagellates, we treated these loci as diploid in our analyses, unless otherwise noted. Microsatellite fragments were amplified and analyzed according to published methods, and multilocus genotypes (MLGs) were constructed according to previously described methods (22, 59).

Population Genetic Data from Other Species of *Symbiodinium*. To investigate the allelic diversity and pairwise genetic differences among MLGs for other *Symbiodinium* and contrast them with those of *S. trenchii* in the Greater Caribbean, three previously published datasets representing symbionts within clades A, B, and C were analyzed. Populations of these symbionts were collected from the Greater Caribbean; *Symbiodinium “fitti”* (nomen nudum = A3) in *Acropora palmata* from across this coral’s entire Atlantic distribution ($n = 652$) (23), *Symbiodinium B7* in corals of the genus *Madracis* spp. ($n = 35$), also from locations around the Greater Caribbean (60), and *Symbiodinium C7* in *Orbicella* spp. ($n = 47$) from Belize, western Caribbean (61). These three symbiont lineages (formal species descriptions are pending) have experienced similar environmental conditions in their recent evolutionary past, and their population data provide a method to investigate the demographics and genetic variation among populations of *Symbiodinium* species within this region. A fourth *Symbiodinium* sp. was also included, *S. “glynni”* (nomen nudum = type D1) in *Pocillopora* type I from the Gulf of California ($n = 109$) (22) and represents another species of clade D *Symbiodinium* and a sibling lineage to *S. trenchii*, and thus provides a comparison of diversity and divergence among MLGs between species within the same clade based on the same microsatellite loci.

Microsatellite Data Analysis. Populations of organisms that commonly reproduce asexually tend to have a proportion of individuals with identical MLGs (i.e., a clonal cell lineage equivalent to an individual), and, therefore, statistical calculations based on allele frequencies can be negatively biased (discussed in ref. 59). For this reason, all summary statistics and analyses (unless stated otherwise) were conducted with duplicated MLGs in each sampling location removed from datasets. The probability of identity (PI) was calculated to determine the power to resolve genetically distinct individuals, which estimates the probability that two unrelated

individuals drawn at random will by chance have the same MLG (62). PIs were calculated for each locus, and then the overall PI for discerning individual clones was determined from the product of all loci. Because these values are affected by population substructure (63), PI was calculated for each regional location. Along with PI, additional summary statistics were calculated for each regional location using GenAlEx (version 6.4) (62) and included observed and expected heterozygosity (H_o and H_e), the information index (I), and the number of private alleles.

Clonal richness (R) is the frequency of unique MLGs and was calculated to give an indication of the contribution of asexual reproduction within each region. Pairwise genetic difference (PWD) was calculated in the statistical program R (R Core Team) (64) by determining the number of nonidentical alleles between genotypes, and the observed distribution of PWD was compared with that expected in a panmictic population. The expected distribution was calculated by permuting the alleles of the observed genotypes to yield datasets of unlinked multilocus genotypes of equal diversity and size as the observed dataset. One thousand permuted MLG datasets were created, and the PWDs of the permuted genotypes, along with the summary statistics of variance in pairwise genetic difference and the index of association (I_a) (65), were calculated for each of the permuted datasets. The same summary statistics were calculated from the observed data to determine whether there was significant departure from panmixia (R code written for this task is available at doi:10.5061/dryad.d4152). PWD was further used to construct a principal coordinate analysis (PCoA), using GenAlEx, to graphically depict the relatedness between individuals within and between regional populations of *S. trenchii*. Lastly, a dendrogram of genetic divergence among unique MLGs of *S. trenchii* was generated using an unweighted pair group method with arithmetic mean (UP-GMA) in the Neighbor routine from the PHYLIP package (66) and based on pairwise differences in alleles at each locus.

Photosynthesis and Calcification Measurements. To investigate the potential implications of *S. trenchii* in the Greater Caribbean, the physiology of *Orbicella faveolata* colonies with either *S. trenchii*, A3, B17, or C7 were examined by measuring rates of photosynthesis and calcification. Fragments ($\sim 11 \text{ cm}^2 \text{ SD} \pm 3.3$) from four to six healthy colonies of *O. faveolata* per symbiont were collected at depths ranging between 4 m and 7 m from La Bocana and La Pared reef sites off Puerto Morelos, Mexico in October 2010. To compensate for possible variations in the photo-physiological state of individual samples derived from differences in the relative position in the colony and depth, fragments were maintained in outdoor flowing seawater tables for 2 mo before being analyzed at constant $28 \pm 0.5^\circ \text{C}$ under neutral density shade cloth providing a maximal midday irradiance of $\sim 400 \mu\text{mol quanta m}^{-2}\text{s}^{-1}$. Acclimation in the photophysiology of corals generally requires 1–4 wk (67). Temperature settings were close to the yearly average for the reef whereas the 30% attenuation of the neutral density filters provides irradiance levels similar to the collection sites. Photosynthesis to irradiance curves (P-E) were performed to estimate photosynthetic functioning of each symbiont and according to published methods (68). Briefly, coral fragments were incubated in airtight water-jacketed acrylic chambers equipped with Clark-type O_2 electrodes (Hansatech) and magnetic stir bars to provide water circulation. The temperature within the chambers was maintained at $28^\circ \text{C} (\pm 0.5)$, and the irradiance was supplied using 6-W pure white LED bulbs. Coral fragments were sequentially exposed to 0, 6, 20, 40, 51, 96, 604,

and $1,392 \mu\text{mol quanta m}^{-2}\text{s}^{-1}$ to obtain the rate of oxygen evolution ($\mu\text{mol O}_2 \text{ h}^{-1}$) at each irradiance. P-E curve parameters were obtained by a nonlinear fitting to a hyperbolic tangent function. Three to five replicate fragments representing each host-symbiont combination were used to obtain an average rate of maximal photosynthesis (P_{max}) for each of the four host-symbiont combinations. P_{max} was normalized to fragment surface area obtained by imaging each coral specimen and applying the trace function using ImageJ (69).

To estimate instantaneous rates of calcification, fragments were incubated in 200-mL acrylic chambers containing filtered reef water ($0.45 \mu\text{m}$), each fitted with a stir bar to provide adequate water circulation. Samples were incubated for 1 h at constant temperature ($\pm 0.5^\circ\text{C}$) and irradiance ($400 \mu\text{mol quanta m}^{-2}\text{s}^{-1}$) using the same lighting described above. At the end of 1 h, incubation seawater was collected and measured for total alkalinity ($\pm 2.5 \mu\text{Eq kg}^{-1}$) using an Ocean Optics USB4000 spectrophotometer following published procedures (70). Instantaneous rates of calcification (G) were calculated following the alkalinity anomaly principle on the basis that, for each mole of CaCO_3 precipitated, total alkalinity is reduced by two molar equivalents (71). Calcification was measured over a range of ecologically relevant temperatures (24, 26, 28, 30, and 32°C) for all host-symbiont combinations. Four to six *O. faveolata* fragments (from different parent colonies) representing each partner combination (A3, $n = 6$ colonies; B17, $n = 4$; C7, $n = 6$; *S. trenchii*, $n = 5$) were used to measure calcification at each temperature treatment. The same fragments were used across temperature treatments so that, after incubation at one temperature, the fragments were placed in tanks at the next highest temperature and allowed to acclimate >2 d between incubations. Instantaneous rates of calcification ($\mu\text{mol CaCO}_3 \text{ h}^{-1}$) were normalized to coral surface area as described above.

Physiological Data Analyses. Statistical analyses were performed in the program R V 3.1.1 (R Core Team) using the packages lme4 (72) and lmerTest (73). To test the effect of symbiont identity on coral calcification across all four temperatures, we performed a nested linear mixed effects model, modeling temperature and symbiont identity as fixed effects and coral colony as a random effect nested in symbiont identity. We then calculated the significance ($\alpha = 0.05$) of pairwise differences of the levels of the fixed effects in lmerTest. Pairwise *t* tests (data not transformed) were conducted in R to determine significant differences in symbiont photosynthetic rates.

SI Results

ITS2-DGGE Fingerprinting and Sequencing. DGGE-ITS2 fingerprinting allowed for the rapid detection of *S. trenchii* in host specimens where this symbiont species was abundant. Its profile is characterized by two codominant ITS2 sequences (sequence “1” and sequence “a,” or “4”; thus, this symbiont was originally called type D1a and later D1-4) (15) that are numerically dominant in the ribosomal array and appear as two bands (plus two heteroduplexes) on a denaturing gel (13, 55, 74). Samples with this fingerprint alone or in mixture with another species of *Symbiodinium* were used for multilocus genotyping. The significant genetic divergence among major *Symbiodinium* clades means that microsatellites developed for clade D do not work on *Symbiodinium* from other clades and thus samples with mixed symbiont assemblages can be analyzed without complications.

Colony fragments of *O. faveolata* collected for physiological measurements were analyzed before measurements of photosynthesis and calcification. Fragments exhibiting high homogeneity in their symbiont population were selected for physiological analyses (Fig. S1).

Microsatellite Allele Diversity. The number of alleles per locus ranged from 4 to 29 (Table S1), with the allele frequencies for each location given in Table S2. The frequencies of putative null alleles were rare among loci ranging from 0 for loci D1Sym9 to a value of 0.109 for D1Sym14. Although all of the alleles in the Greater Caribbean were shared with the Indo-Pacific locations, certain alleles were found only in populations from the Indian Ocean or the West Pacific (Table 1). In Palau there were 16 alleles across nine loci (D1Sym9, -17, -34, -66, -67, -82, -87, -88, and -93) that were unique to *S. trenchii* populations from that region whereas the Andaman Sea populations possessed 16 other alleles distributed across seven loci that were distinct to that geographic location (D1Sym14, -17, -34, -67, -82, -87, and -93). Allele frequency distributions for the Indo-Pacific populations show a dominance in the proportion of rare alleles (0.1 or less) (Fig. S3). In contrast, the proportion of rare alleles was substantially lower in the Greater Caribbean relative to the Indo-Pacific, with a small number of alleles found at higher frequencies (Fig. S3). A total of 147 different genotypes were scored among the 245 MLGs obtained from the entire sample set (Indo-Pacific and Atlantic). Twenty-nine of these MLGs were from the Greater Caribbean, 52 from the Pacific Ocean (Palau), and 66 from the Indian Ocean (Andaman Sea; Table 1).

Observed heterozygosity ranged from 0.65 to 0.78 and was lowest in the Greater Caribbean (Table 1). Observed heterozygosity was higher than expected in every region. Similar to heterozygosity, the information index (I) was higher in the Indo-Pacific (1.24–1.42) than in the Caribbean (0.70). These high values are expected under the assumption that *S. trenchii*'s genome was recently duplicated and that its “diploid” condition frequently results in two different alleles per locus (15). The probability that two samples with the same MLGs may not have originated from the same clone lineage (PI) was exceedingly low for most locations and ranged from 6.1×10^{-4} (within populations from the Atlantic) to 2.7×10^{-7} (within populations from the Andaman Sea). All of these values were at or well below the 0.0001 criteria recommended by ref. 63 and are therefore adequate for population-level investigations.

Genotypic Diversity, Prevalence, and Distribution. Clone richness (R), or genotypic richness, was high at both Indo-Pacific locations, ranging from 0.87 to 0.91 (Table 1). The most abundant clone, or MLG, found in the Indo-Pacific was observed in just four samples (Andaman Sea), and no clones were shared between sample locations (Figs. 1B and 1C). By contrast, R was low in populations from the Atlantic Ocean (0.25) where distinct clones were found multiple times within and between sampling locations as far as 3,000 km apart (Table 1 and Fig. 1E). In the most extreme case, genotype “ α ” was found in 47 of the 112 samples from around the Greater Caribbean and at every location except the Flower Garden Banks (Figs. 1E and 2A). A list of all *S. trenchii* MLGs is provided in Table S1, organized by region, location, and host taxon.

The mean pairwise genetic difference between individuals was high from locations in the Andaman Sea (Indian Ocean) and Pacific Ocean (15.16 and 12.03 differences, respectively) but was much lower in the Atlantic Ocean (4.32) (Fig. 2C). The Andaman Sea and Palau showed a bell-shaped distribution around the mean pairwise genetic difference for each population, and their observed distribution closely matched that expected in sexually recombining populations. In contrast, pairwise genetic difference from the Atlantic Ocean was skewed toward zero and was substantially different from the distribution expected from that region's allele frequency data (and higher H_o than H_e). The skewed distribution of the Atlantic Ocean is attributed to the high similarity between individuals within this region, many of which ($\sim 96\%$) differ by only four alleles, or fewer, and involving one or two loci.

Fig. S2. The distribution of pairwise genetic difference (PWD) among individual multilocus genotypes (MLGs) of typical *Symbiodinium* populations. Frequency of pairwise genetic difference typical of MLGs found for native *Symbiodinium* spp. [types A3 (A), B7 (B), and C7 (C)] from the Greater Caribbean (GC), and *S. "glynni,"* a member of clade D and sibling species to *S. trenchii*, from the Eastern Pacific (D). The frequency distributions depict pairwise genetic differences between MLGs among individuals for observed data (black bars) and those expected for a panmictic (randomly mating) population (white bars). Gray bars indicate the overlap between expected and observed. All observed pairwise genetic difference were significantly different ($\alpha < 0.05$) than the expected pairwise genetic difference, determined by comparing the variance in pairwise genetic difference of observed and expected values in a permutation test, indicating that all lineages do undergo significant asexual reproduction. However, the low allelic diversity and significantly lower average pairwise genetic difference than expected average pairwise genetic difference (from our permutation tests) exhibited by *S. trenchii* in the Atlantic Ocean seems unique in this basin to this lineage, and not simply a characteristic of *Symbiodinium* populations from the Caribbean in general (Fig. 2C).

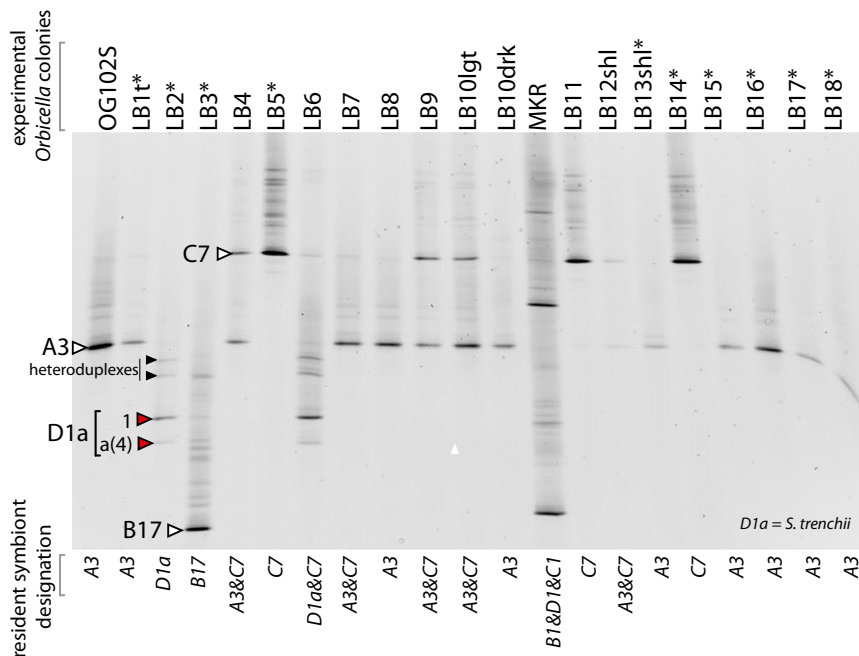


Fig. S3. Example of ITS2-DGGE fingerprints diagnostic of *Symbiodinium* spp. living in small colonies of *O. faveolata*. Colonies designated by an asterisk were used for photosynthesis and calcification measurements.

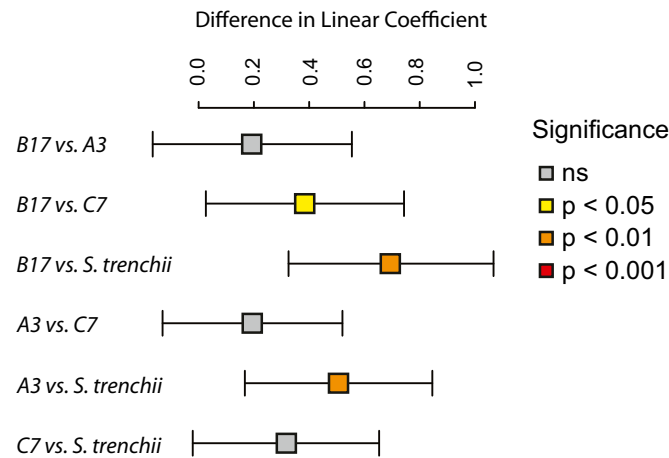


Fig. S4. The statistical assessment of pairwise differences in instantaneous calcification rates based on symbiont identity. Colonies with *S. trenchii* calcified significantly slower than colonies with either *Symbiodinium* A3, or B17, when integrated in the linear mixed model over the five temperature treatments (24, 26, 28, 30, and 32 °C). Boxes depict the estimated differences between symbiont types (based on differences in the coefficient of the linear model as assessed by least square means), with error bars depicting 95% confidence intervals around this estimate. Boxes were colored by significance (*P* values) for each pairwise comparison.

Table S1. Description and summary data on microsatellite loci

Locus	Primer sequence (5'–3')	Repeat motif	Size range, bp	No. of alleles
D1Sym9	F-CAGAAGCCCAATTATATGCGGCA (FAM) R-AGGATGATGAGCATGCCGACG	(GTT) ₆	106–112	3
D1Sym11	F-TGAAATCTCACTCAGAGTCGGAC (FAM) R-GCAGACAGTGATTTCAGTTCCGA	(AC) ₁₃	149–161	7
D1Sym14	F-TCTCAGTGGAAAGCATGTGG (FAM) R-TCGTCTGAATCAGGATCTGACG	(CT) ₁₁ AT (CT) ₄	167–191	7
D1Sym17	F-TGTGAATGCTTCTTGGGGTG (HEX) R-TCATGCTTGTCGGTGAGCAG	(CA) ₈	137–149	7
D1Sym34	F-ACCTGAGACCTGAGTGTGC (FAM) R-ATCATGGGCAGAGCTCCTGG	(CAAA) ₉ CACA (CAAA) ₄ (GAAACAAA) ₂ (CAAA) ₁₃	350–433	29
D1Sym66	F-CTCTGGGATGGACCCGAA (PET) R-GACAGTGTTTCACTGGTCGCA	(TCT) ₈	288–303	6
D1Sym67	F-GAATCCAGATGGTGCTGC (VIC) R-CAAAGGTAGCCGATTGTCTC	(ATC) ₈	125–140	6
D1Sym82	F-AAGACTTCCGCAAGCACAC (HEX) R-CGCATCTACTGGTGGTCAGAC	(ACT) ₁₂	219–285	22
D1Sym87	F-CCTATGACTCCAAGGTGACG (FAM) R-AGACATACCTCGGTCTTGTC	(GAAG) ₇	232–256	7
D1Sym88	F-TTGTCAGACTGAATGCTCCA (NED) R-GTGTTCAAGCGACATCCCA	(CTTT) ₃ G (TTTC) ₇ (TCTCTTTC) ₂ TCTTTT (CT) ₃ (TCTT) ₂ (TTTC) ₂ T (CTT) ₄	232–250	6
D1Sym92	F-GCGTTTGACACAAGGATCCCT (FAM) R-TTGGGATGCTCTTGGCGAC	(CCTA) ₆ (CCTG) ₃	124–136	4
D1Sym93	F-GCTCAAAGGAGCTCTAGGGGT (NED) R-TGTCAAGGTAGAGAGCCTGGT	(AATC) ₆	141–173	8

Subscript numbers after the repeat motif indicate the number of repeats in the initial cloned sequence that was used to develop locus primers.

Table S3. *P* values for assessment of significance in all pairwise comparisons in mean P_{\max} involving different host-symbiont combinations

	A3	B17	C7
A3	—	—	—
B17	0.61	—	—
C7	0.17	0.82	—
<i>S. trenchii</i>	0.61	0.91	0.82

Table S4. *P* values for assessment of significance in all pairwise comparisons in mean instantaneous calcification rates

	A3	B17	C7
A3	—	—	—
B17	0.282	—	—
C7	0.234	0.039	—
<i>S. trenchii</i>	0.006	0.001	0.068

Values in bold font indicate rates that were significantly different.

Other Supporting Information Files

[Dataset S1 \(DOCX\)](#)