Ecological factors rather than barriers to dispersal shape genetic structure of algal symbionts in horizontally-transmitting corals

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Abstract

Many reef-building corals acquire their algal symbionts (*Symbiodinium* sp.) from the local environment upon recruitment. This horizontal transmission strategy where hosts pair with locally available symbionts could serve to increase coral fitness across diverse environments, as long as hosts maintain high promiscuity and symbionts adapt locally. Here, we tested this hypothesis in two coral species by comparing host and symbiont genetic structures across different spatial scales in Micronesia. Each host species associated with two genetically distinct *Symbiodinium* lineages, confirming high promiscuity in broadly dispersing hosts. However, contrary to our initial expectation, symbiont genetic structure was independent of physical barriers to dispersal between islands, unlike genetic structure of their hosts that was nearly perfectly explained by ocean currents. Instead, *Symbiodinium* consistently demonstrated genetic divergence among local reefs and between the two host species at each island, although not necessarily between distant islands. These observations indicate that *Symbiodinium* lineages disperse much more broadly than previously thought and continuously adapt to specific hosts and reef environments across their range, following the classical Baas Becking's hypothesis: "Everything is everywhere, but the environment selects". Overall, our findings confirm that horizontal transmission could be a mechanism for broadly dispersing coral species to enhance their local fitness by associating with locally adapted symbionts. Dramatic differences in factors driving the genetic structures of horizontally-transmitting corals and their *Symbiodinium* imply that viewing their combined genomes as a single entity ('hologenome') would not be useful in the context of their evolution and adaptation.

Introduction

Many well-known symbioses involve the passing of symbionts from parents to offspring (vertical transmission), fully aligning the evolutionary trajectories of symbiotic partners and typically lead to their deep integration at biochemical and genomic levels (i.e. Buchnera in aphids (1, 2)). The result of such symbiosis is essentially a novel composite organism. In other types of symbioses, the association between partners must be newly established in every generation (horizontal transmission), which allows for the maintenance of each partner's species identity. In theory, this kind of relationship should generate novel ecological opportunities for both symbiotic partners through their mixing and matching across environments. For example, association with ecologically specialized algal photobionts can lead to distinct ecological guilds of lichens (3) or allow a fungal partner to expand its geographic range across a broader climatic envelope (4). Similarly, in aphids, association with various horizontally transmitted bacterial symbionts allows these insects to colonize novel host plants across climatic zones (5). Considering these and other examples of ecological adaptation based on varying symbiotic associations, it has been argued that the joint genomic content of symbiotic systems should be studied as a single unit of evolution, the 'hologenome' (6, 7). However, the usefulness of this concept depends on whether the evolutionary trajectories of both symbiotic partners are sufficiently aligned to present a unified target of selection (8). Here, we explore this question in the symbiosis between a horizontally transmitting reef-building coral and dinoflagellate algae of the genus *Symbiodinium* (9).

Association with *Symbiodinium* is obligatory for many coral hosts that rely on algal photosynthesis for energy, while the algae benefit from protected and light-exposed

residence as well as inorganic nutrients and CO_2 concentration regulatory mechanisms provided by the host (10-13). Given the obligatory nature of this symbiosis for the host, it is somewhat surprising that in the majority of coral species (~85%) *Symbiodinium* are not transmitted vertically, but rather must be acquired by the juvenile coral from its local environment (14, 15). One possible explanation is that dispersal ranges of aposymbiotic coral larvae typically extend over hundreds of kilometers (i.e. (16), while the environmental variation corals must deal with exists on much smaller spatial scales: reef environments with varying light, thermal and nutrient conditions can be separated by meter-scale distances (i.e. (17). Under such circumstances, coral hosts would benefit from the mixing and matching strategy, improving their fitness by associating with the locally available, and putatively ecologically specialized, algal strains (18-20). Establishing the relative roles of these symbiotic partners in adaptation to variable environments is essential for better prediction of coral reefs' future under climate change (i.e. (21).

The working hypothesis for this study, the 'global host, local symbiont' hypothesis, posits that (i) coral host disperses widely and is able to establish symbiosis with diverse algal strains across locations; (ii) *Symbiodinium* algae are poor dispersers (i.e., 21), which results in strong divergence among locations aligning with physical barriers and facilitates their local environmental specialization; (iii) local *Symbiodinium* strains also diverge with respect to the host species as a result of selective pressure towards higher host specificity (22, 23). To validate all three components of our hypothesis, we examined multi-locus genotypes (MLG) of clade C *Symbiodinium* in two species of *Acropora* – *A. hyacinthus* and *A. digitifera* – collected from the same reef

locations across the Micronesian Pacific (Fig. 1A). Our previous work has shown that both host species exhibit extensive genetic connectivity and their genetic structure is nearly perfectly explained by the patterns of regional surface currents (16). By using two coral species that co-occur across the same locations as well as local reef environments we aimed to disentangle the roles of environmental specialization, host specialization, and physical barriers to dispersal in driving the fine-scale genetic structure of *Symbiodinium*.

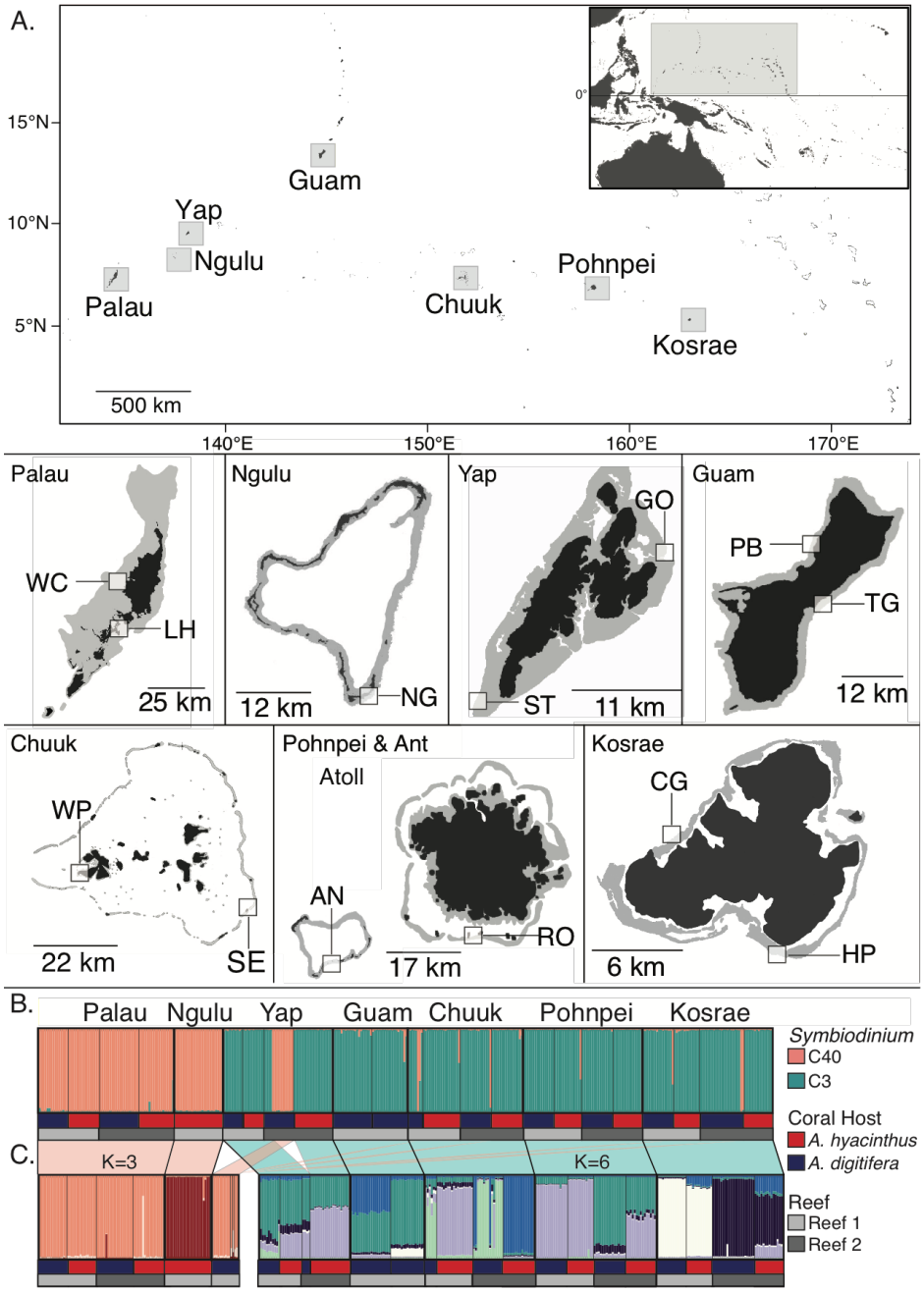

Figure 1: Geographic location of the Micronesian islands where *Acropora hyacinthus* and *A. digitifera* coral samples were collected (A). Top: Map of the Micronesian Pacific with an inset of the Pacific Ocean for reference. Islands where samples were collected and analyzed for *Symbiodnium* genetics are designated with grey boxes. Detailed information on each sampling site is located in Table S3. No *A. hyacinthus* were present in Guam and *A. digitifera* were not collected in Ngulu. Analyses of microsatellite loci data for *Symbiodinium* hosted by *Acropora hyacinthus* and *Acropora digitifera* using multilocus genotyping data. B. STRUCTURE population assignment for *Symbiodinium* from two *Acropora* host species across greater Micronesia at an optimal population number (K=2), corresponding to C40 (pink) and C3 (turquoise) clade C subclades. Colors in the bottom panels correspond to host species and shades of grey correspond to different sites within each island. C. Within-subclade STRUCTURE analysis.

111 **Results**

112 **Two *Symbiodinium* clade C lineages**

113 To enable standard population genetic analysis, data analysis was restricted to corals
114 hosting a single diploid *Symbiodinium* clone, which encompassed the majority (69% *A.*
115 *digitifera* and 64% *A. hyacinthus*) of our samples. Across the two coral species in
116 Micronesia (Fig. 1A), two distinct *Symbiodinium* lineages were observed, which were
117 most clearly discriminated by two of the six microsatellite loci (Fig. S1). Sequencing of
118 the $psbA_{ncr}$ gene for a subset samples classified these lineages as C40 and C3 (39, (24)
119 (Fig. S2). Corals of both species from Palau and Ngulu hosted exclusively C40 (Fig. 1B,
120 pink bars). This lineage was also prevalent in *A. digitifera* at one reef site on Yap and was
121 rarely found in *A. digitifera* across the rest of Micronesia. All other coral hosts of either
122 species associated with C3 (Fig. 1B, turquoise bars). Both *Symbiodinium* lineages
123 possessed high genetic diversity, with a total of 70 unique alleles across three islands in
124 C40 (Table S1A) and 130 unique alleles across five islands in C3 (Table S1B). Mean
125 numbers of alleles per island for each locus for C40 ranged from 3.00-4.67 with the
126 highest numbers of private alleles in Palau (N=7) (Table S1A). Mean allele numbers per
127 island for each locus for C3 ranged from 3.83-5.17 and numbers of private alleles ranged
128 from 1-4 (Table S1B).

129

130 ***Symbiodinium* genetic structure**

131 All pairwise between-island F_{ST}'s for C40 were significant (Table 1A). C3 had one non-
132 significant F_{ST} (Yap-Pohnpei) while all others were significant and ranged from 0.058
133 (Guam-Chuuk) to 0.078 (Yap-Kosrae) (Table 1B). Notably, *Symbiodinium* C3

differentiation did not show the isolation-by-distance (IBD) pattern observed in both coral hosts (Fig. 2). IBD could not be computed for C40 due to too few between-island comparisons. C3 F_{ST} values generally exceeded F_{ST} values for the host (Fig. 2A), however, this result did not imply stronger symbiont genetic differentiation: higher F_{ST} in the symbionts was a consequence of higher mean heterozygosity in *Symbiodinium* markers (e.g. (25). Indeed, an alternative measure of genetic differentiation controlling for mean heterozygosity - Jost's D (26) - demonstrated that symbiont genetic divergence was in fact intermediate between the two hosts (Fig. 2B).

Table 1. Summary of pairwise F_{ST} values between all islands for *Symbiodinium* C40 (A) and C3 (B). Permutations were run 9999 times. All significant comparisons are shaded in grey.

A. *Symbiodinium* C40

	PAL	**NGU**	**YAP**
PAL	0.000	***	0.004
NGU	0.279	0.000	***
YAP	0.056	0.326	0.000

B. *Symbiodinium* C3

	YAP	**GUA**	**CHU**	**POH**	**KOS**
YAP	0.000	***	***	0.059	***
GUA	0.062	0.000	***	***	***
CHU	0.066	0.058	0.000	***	***
POH	0.009	0.071	0.063	0.000	***
KOS	0.078	0.077	0.076	0.067	0.000

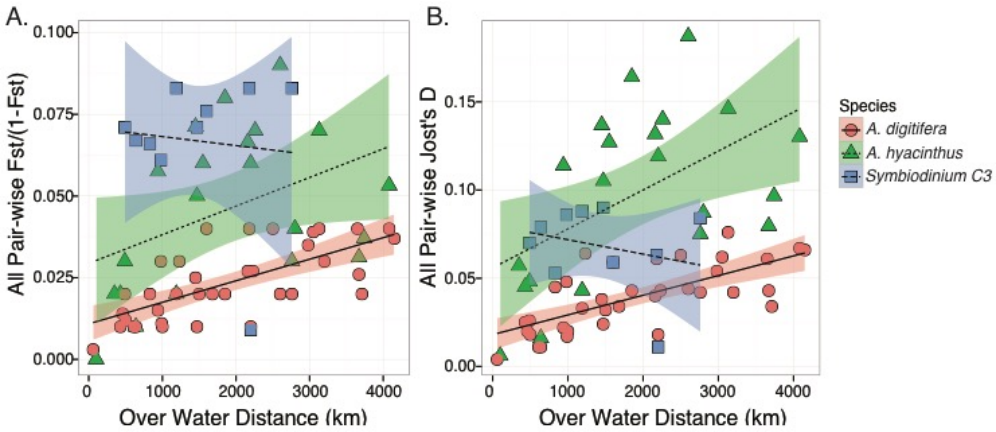

Figure 2: Comparison of two host species and *Symbiodinium* C3 differentiation. A. Pairwise genetic differentiation [(F_{ST} /(1-F_{ST})] of two species of *Acropora* coral and *Symbiodinium* C3 across linear distances (km) demonstrating significant isolation by distance for the two host species but no correlation for the symbiont. B. Pairwise Jost's D for the same two host species and *Symbiodinium* C3 across linear distances (km) demonstrating no isolation by distance and no difference in overall divergence between the host and symbiont.

Host specificity and environmental specialization

Discriminant analysis of principal components (DAPC) strongly differentiated between host species for both *Symbiodinium* C40 and C3 (Table S2, Fig. S3A, B), suggesting that host specificity drives symbiont diversity. In addition, DAPC demonstrated consistent differences among islands for each *Symbiodinium* lineage irrespective of host species: strong per-island assignment proportions were observed for C40 (Fig. S3C, 82-100%) and C3 (Fig. S3D, 51-98%), consistent with both STRUCTURE (Fig. 1B) and F_{ST} results (Table 1). Moreover, nearly all pairwise F_{ST} values between different reef sites within islands were significant for both lineages (Table 2), suggesting environmental partitioning of symbionts. In accord with these results, of the two top eigenvalues in DAPC of individual within islands, one explained divergence by host species (host specificity) and the other explained differences between reef sites (environmental specialization) (Fig. 3).

Table 2. Summary of pairwise F_{ST} values for *Symbiodinium* C40 (A) and C3 (B) between all sites pooled across host species. Permutations were run 9999 times. All significant comparisons are shaded in grey.

A. *Symbiodinium* C40

	PAL1	PAL2	NGU	YAP2
PAL1	0.000	0.009	***	0.007
PAL2	0.031	0.000	***	0.007
NGU	0.283	0.303	0.000	***
YAP2	0.057	0.074	0.326	0.000

B. *Symbiodinium* C3

	YAP1	YAP2	GUA1	GUA2	CHU1	CHU2	POH1	POH2	KOS1	KOS2
YAP1	0.000	0.139	0.002	0.009	***	***	0.002	0.055	***	***
YAP2	0.011	0.000	***	0.003	***	***	0.027	0.064	***	***
GUA1	0.059	0.107	0.000	0.045	***	***	***	***	***	***
GUA2	0.045	0.062	0.025	0.000	0.002	***	***	0.008	***	***
CHU1	0.071	0.073	0.104	0.065	0.000	***	***	***	***	***
CHU2	0.079	0.112	0.070	0.086	0.068	0.000	***	***	***	***
POH1	0.053	0.028	0.126	0.111	0.085	0.126	0.000	***	***	***
POH2	0.018	0.018	0.093	0.039	0.067	0.092	0.063	0.000	***	***
KOS1	0.154	0.146	0.170	0.110	0.163	0.176	0.168	0.119	0.000	***
KOS2	0.092	0.121	0.116	0.131	0.129	0.090	0.136	0.100	0.183	0.000

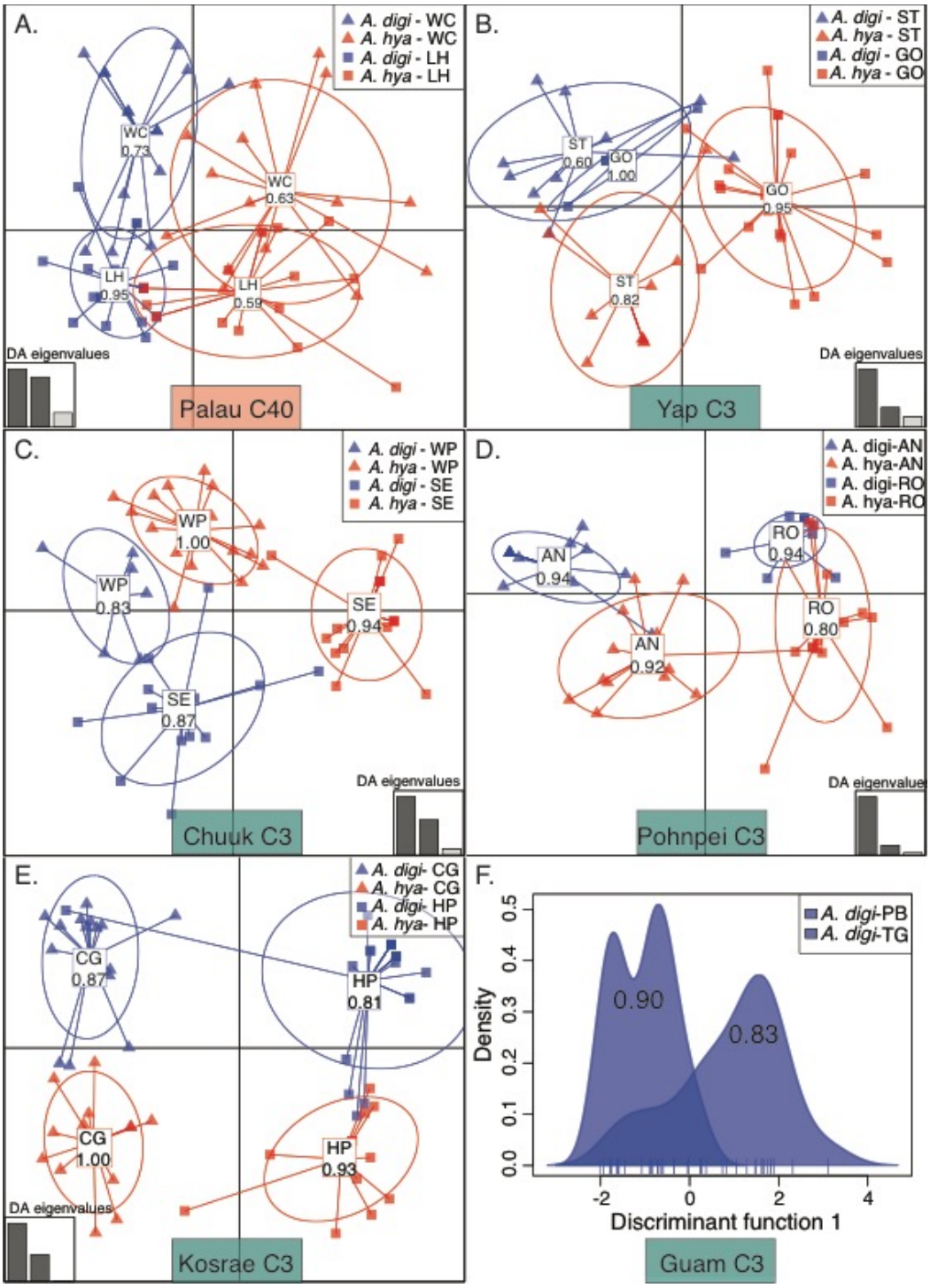

Figure 3: Discriminant analysis of principal components (DAPC) of MLG data for *Symbiodinium* C40 and C3 hosted by *Acropora hyacinthus* and *Acropora digitifera* at twelve sites across six islands (Ngulu not included) in Micronesia. DAPC analysis on two discriminant functions demonstrating host species assignments and site assignments. DAPC scatter plots for individual samples from within A. Palau for *Symbiodinium* C40. B. Yap for *Symbiodinium* C3, C. Chuuk for *Symbiodinium* C3, D. Pohnpei for *Symbiodinium* C3, E. Kosrae for *Symbiodinium* C3, and F. Guam for *Symbiodinium* C3 for *A. digitifera* hosts only (1 DF axis). Proportions of assignments are indicated in the clusters. Information on the DAPC models can be found in Table S2.

182 **Discussion**

183 *Promiscuity of the coral host*

184 Clade C *Symbiodinium* are considered to be the most derived lineage within the genus
185 *Symbiodinium* and exhibit significantly higher within-clade diversity when compared to
186 other, more basal, clades (9, 27, 28). Across the Micronesian Pacific (Fig. 1A), both coral
187 hosts associated exclusively with clade C *Symbiodinium*, which was nevertheless
188 represented by two distinct lineages– C40 and C3 (Fig. S2). This observation confirms
189 the first prediction of the 'global host, local symbiont' hypothesis: coral hosts show
190 considerable flexibility in their symbiotic association across their range and within their
191 habitat.

192

193 *Lack of dispersal limitation in* Symbiodinium *across Micronesia*

194 Initially, we expected to find strong isolation by distance in *Symbiodinium*, since the
195 prevailing view of the *Symbiodinium* life cycle involves symbiotic existence in sedentary
196 hosts alternating with a free-living form that largely exists in the benthos where dispersal
197 by ocean currents must be limited (29-32). In contrast, we observed that genetic
198 differentiation between islands of *Symbiodinium* C3 across Micronesia did not exceed
199 $F_{ST} = 0.078$, which contrasts studies from other locations reporting symbiont F_{ST} as high
200 as 0.54 in clade A (33) and Φ_{ST} as high as 0.468 in clade C (34). This lack of physical
201 dispersal limitation is best demonstrated by comparing *Symbiodinium* C3 divergence to
202 divergence of their coral hosts, which has been shown to nearly perfectly follow
203 biophysical barriers (16). If *Symbiodinium* dispersal were limited by the same physical
204 barriers and to a greater extent, as our hypothesis posited, we would have observed
205 steeper increases in divergence with distance relative to the host; instead, no increase of

206 F_{ST} with distance was observed (Fig. 2A). Moreover, after correcting for mean
207 heterozygosity, genetic divergence (Jost's D) in *Symbiodinium* C3 was found to be
208 intermediate between *A. hyacinthus* and *A. digitifera* (Fig. 2B), once again demonstrating
209 that symbiont dispersal is less physically limited than their hosts. This finding contradicts
210 the second prediction of our 'global host, local symbiont' hypothesis and is contrary to
211 our expectations based on earlier F_{ST}-based reports of stronger *Symbiodinium*
212 differentiation compared to hosts (20-22, 34).
213
214 *Host specificity*
215 The majority of reef-building coral species associate with a specific strain (termed
216 "clade" or "subclade") of *Symbiodinium* broadly defined based on ribosomal and/or
217 chloroplast markers (22, 35-37). Previous *Symbiodinium* multilocus genotyping
218 studies revealed that each of these strains harbors greater diversity, both in terms of
219 genetic and functional diversity (20, 34, 38). Our data indicate that the local association
220 of hosts and symbionts of the same genotypic cluster is due to pervasive evolution of host
221 specificity in *Symbiodinium* (Fig. S3 & 3). As our study includes two coral species, we
222 also observe that this specificity is not perfect: at every location there were symbionts
223 that would have been assigned to another coral host based on their multilocus genotype
224 (Fig. 3). This suggests that host specialization in *Symbodinium* arises in the face of
225 considerable exchange between symbiont communities hosted by different coral species
226 at the same location.
227
228

229 *Environmental partitioning*

230 The most ecologically relevant postulate of the 'global host, local symbiont' hypothesis is
231 that locally available symbionts are also locally adapted, giving rise to a locally adapted
232 holobiont. Recurrent genetic divergence of symbionts between reef sites within the same
233 island (Fig. 3) despite the lack of physical dispersal limitation across much larger
234 distances (Fig. 2) suggests that symbiont genetic divergence is likely due to poor survival
235 of immigrants rather than to physical barriers to migration. Given that hosts are available
236 at every site, this leaves other ecological parameters of local reef environment as the most
237 likely barrier-forming force, preventing survival of immigrants adapted to a different
238 environment– a situation termed "phenotype-environment mismatch" (39). This in turn
239 implies environmental specialization (i.e., local adaptation) in the symbionts, which
240 supports our hypothesis. Interestingly, just like host specificity, this environmental
241 specialization is imperfect: there are several *Symbiodinium* genotypes that appear to be
242 successful migrants between reef sites (particularly clearly visible for Kosrae, Fig. 3E),
243 indicating that environmental partitioning also arises in the face of considerable gene
244 flow.

245

246 **Conclusions**

247 Both coral host species associated with two divergent *Symbiodinium* lineages, indicating
248 that coral hosts are promiscuous in their symbiont associations across the seascape. In
249 contrast, within each *Symbiodinium* lineage, strong associations with particular host
250 species were observed, suggesting that host-specificity is an important driver of
251 *Symbiodinium* diversification. Unexpectedly, *Symbiodinium* genetic divergence did not

252 correlate with physical distance between islands, but was driven by the combined effects
253 of local (within-island) environment and host identity. This indicates that *Symbiodinium*
254 assemblages across Micronesia are comprised by broadly dispersing lineages
255 dynamically adapting to specific coral hosts and reef environments across their range.
256 Assuming that host species can be regarded as part of *Symbiodinium*'s environment, this
257 pattern clearly follows the classical hypothesis of Baas Becking (originally proposed for
258 soil microbes): "Everything is everywhere, but the environment selects" (40). Overall,
259 our observations support the view that horizontal transmission might be a strategy for
260 broadly dispersing coral hosts to improve their local fitness by associating with locally
261 available, environmentally specialized, *Symbiodinium*. Finally, dramatic differences in
262 factors that structure genetic diversity in the coral host and *Symbiodinium* indicate that
263 viewing their combined genomes as a single 'hologenome' would not be useful in the
264 context of their evolution and adaptation.
265

266 **Materials and Methods**

267 ***Sampling:*** This study comprised a subset of samples previously analyzed for coral host
268 genetics in Davies *et al.* (16) (Table S3, Fig. 1A). Twenty-five individuals of each coral
269 host species (*Acropora hyacinthus* and *A. digitifera*) were examined at two reef sites
270 within seven islands, with the exception of Ngulu (only collected *A. hyacinthus*) and
271 Guam (no *A. hyacinthus* found), for a total of thirteen sites.
272

273 ***Laboratory Procedures:*** Holobiont DNA was isolated following Davies *et al.* (41).
274 Microsatellite primers consisted of six previously published clade C loci (42, 43) and one

275 novel locus mined using Msatcommander (44) from nucleotide EST data for
276 *Symbiodinium* sp. clade C3 (45) (Table S4). Loci were multiplexed in 20 µl polymerase
277 chain reaction (PCR) mixtures containing 10 ng of template, 0.1 µM of each forward and
278 reverse primers, 0.2 mM dNTP, 1 µl 10X *ExTaq* buffer, 0.025 U *ExTaq* Polymerase and
279 0.0125 U *Pfu* Polymerase. Amplifications began at 94°C for 5 min, followed by 35 cycles
280 of 94°C for 40 s, annealing temperature for 120 s, and 72°C for 60 s and a 10 minute
281 72°C extension period. Molecular weights were analyzed using the ABI 3130XL
282 capillary sequencer. Data were binned by repeat size and individuals failing to amplify at
283 ≥3 loci were excluded from analyses.
284
285 ***Data Analysis:*** *Symbiodinium* were scored as diploid based on available information on
286 their ploidy (22, 43, 46) Since each host could potentially contain populations of
287 genetically distinct *Symbiodinium*, multi-locus genotypes (MLG) were only considered if
288 they contained two or fewer alleles across all loci, suggesting that all alleles originate
289 from a single, clonally replicated, genome (46), which was the case for 190 of 277 *A.*
290 *digitifera* hosts and 178 of 278 *A. hyacinthus* hosts. STRUCTURE v2.3.3 (47) assigned
291 similar MLGs to populations (10^6 iterations, burn in = 300,000) across ten replicate runs
292 for each K (1-10) using an admixture model with no location prior. Following (48), ΔK
293 was calculated in STRUCTURE Harvester (49) and CLUMPP (50) and DISTRUCT (51)
294 produced all graphics. We observed high percent assignment to two clusters among
295 sympatric individuals. Following (46), we investigated the relationship between these
296 assignments and allele identities and found strong relationships between assignments and
297 allele identity, particularly at Sgr_21 and Spl_33 (Fig. S1), and we concluded that two

298 distinct *Symbiodinium* lineages were present. MLG data were then split into lineages and
299 additional STRUCTURE runs were completed.

300 Partitioned lineage data were investigated for genetic divergences among islands
301 and sites using F_{ST} (AMOVA, 9999 permutations in GENALEX v6.5, (52). Pairwise
302 differentiations (F_{ST} and Jost's D) and pairwise island distances were correlated to test
303 for isolation by distance (IBD) in *Symbiodinium* C3 (insufficient C40 data) and these
304 trends were compared to results from the host species (53). Mean allelic diversities per
305 island and numbers of private alleles per island were also calculated in GENALEX v6.5.

306 To resolve differences between islands, between host species and between sites
307 and host species within each island, assignment of samples to genetic clusters using
308 discriminant analysis of principal components (DAPC) was performed in R (54) using the
309 ADEGENET package (55, 56). Data were converted into principle components (PCs) and
310 then a-scores were used to determine trade-offs between power of discrimination and
311 model over-fitting. Relationships were examined by DAPC and model information are
312 listed in Table S2.

313

314 **Sequencing Analysis of *Symbiodinium* psbA^{ncr}**

315 To confirm individual assignments to different *Symbiodinium* lineages, the non-coding
316 region of the circular plastid (psbA^{ncr}) was amplified in a subset of samples using the
317 methods described by (24). Amplified products were directly sequenced and aligned with
318 ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) to clade C psbA^{ncr} sequences
319 from common Indo-Pacific *Symbiodinium* types (Table S5). The phylogeny was
320 reconstructed using the default settings of Phylogeny.fr (57) and identities

of *Symbiodinium* types were interpreted based on percent sequence identity with previously identified *Symbiodinium* types.

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Supporting Information

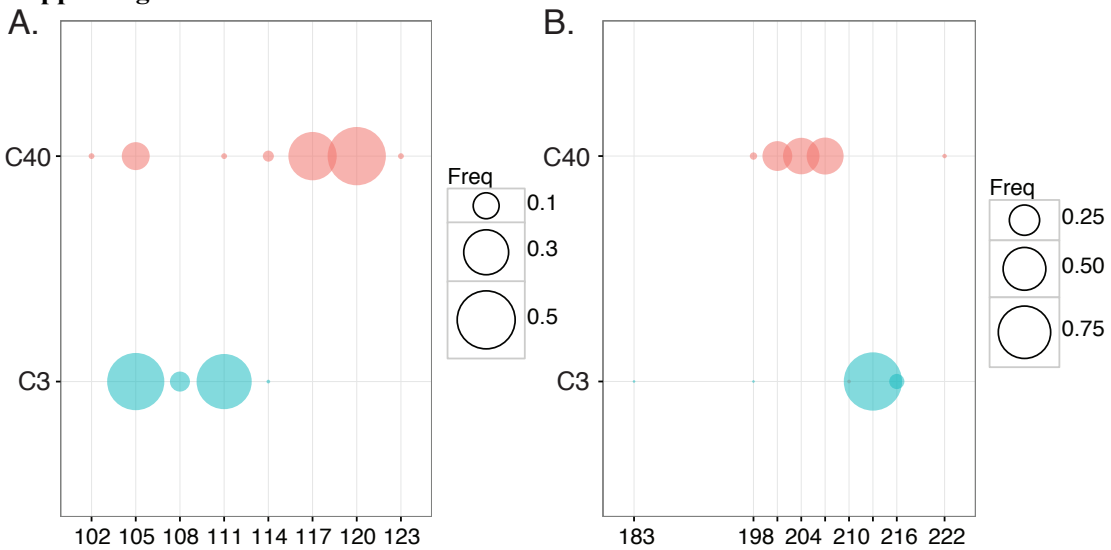

Figure S1: Bubble plot of the SSR allele frequencies of C3 (red) and C40 (blue) at the two loci that showed the largest discrimination power, Sgr_21 (A) and Spl_33 (B). The size of each bubble is proportional to the allele frequency within each *Symbiodinium* type.

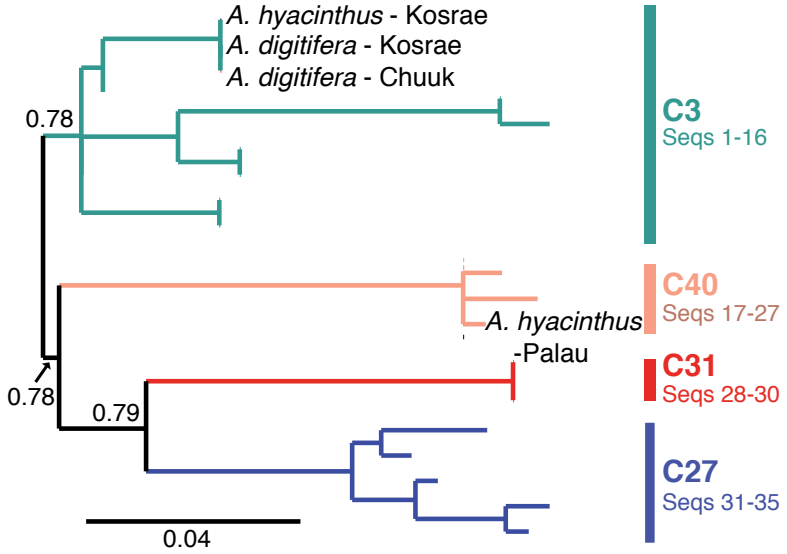

Figure S2. Phylogenetic analysis of psbA^{ncr} sequences from representative samples from this study (labeled branch tips) along with publically available psbA^{ncr} sequences from known *Symbiodinium* subclades identifies two lineages present (C3 and C40). Bootstrap support values are shown at the partitions that define known subclades. Scale bar: replacements per nucleotide site. Sequence accession numbers for reference sequences are given in Table S1.

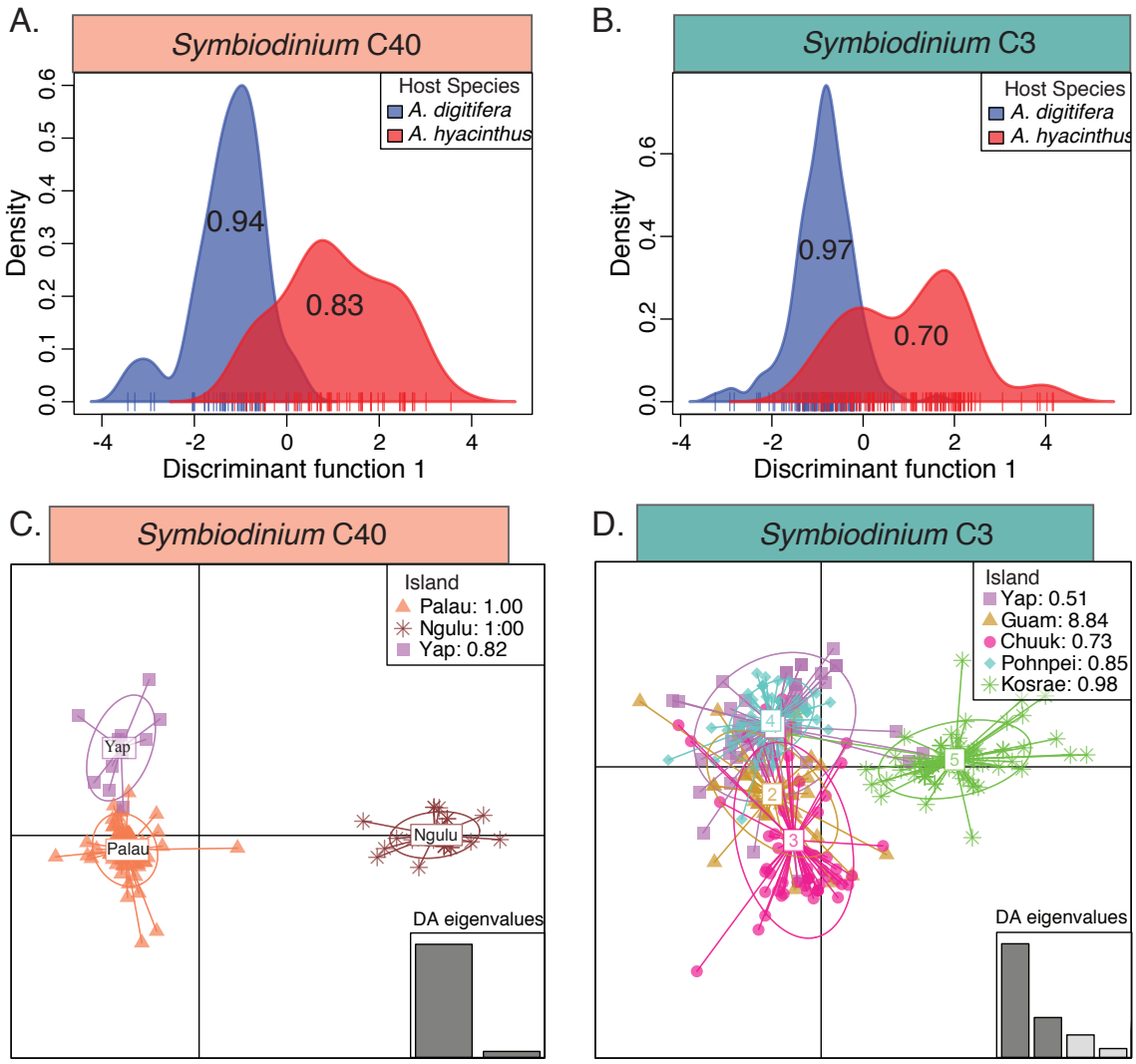

Figure S3: Discriminant analysis of principal components (DAPC) of MLG data for *Symbiodinium* C40 and C3 hosted by *Acropora hyacinthus* and *Acropora digitifera* at thirteen sites across seven islands in Micronesia. A. DAPC analysis on two discriminant functions demonstrating strong host species assignments across all islands for *Symbiodinium* C40 and B. *Symbiodinium* C3. Numbers overlaying the curves indicate successfully assigned fraction of samples. C. DAPC scatter plot for individual samples from *Symbiodinium* C40 represented by colored dots clustered by islands. D. DAPC scatter plot for individual samples from *Symbiodinium* C3 represented by colored dots clustered by islands. Proportions of assignments are indicated in the clusters or in the legends. Information on the DAPC models can be found in Table S2.

Table S1: Summary of allelic diversity measures for *Symbiodinium* C40 (A) and *Symbiodinium* C3 (B). Na: number of alleles, He: expected heterozygosity, Ho: observed heterozygosity, PA: number of private alleles

A. *Symbiodinium* C40

	Na	He	Ho	PA
PAL	4.67	0.52	0.64	7
NGU	4.00	0.42	0.55	6
YAP	3.00	0.43	0.51	2

B. *Symbiodinium* C3

	Na	He	Ho	PA
YAP	4.33	0.47	0.56	1
GUA	3.83	0.42	0.46	3
CHU	5.17	0.58	0.51	3
POH	4.33	0.47	0.56	1
KOS	4.00	0.48	0.51	4

Table S2: Discriminant analysis of principle component (DAPC) model information including the number of principle components ("PC") and discriminant functions ("DF") retained, the proportion of conserved variance by the clustering model ("var"), and the overall assignment proportions across the model ("assign"). A. DAPC information for *Symbiodinium* from different host species, B. islands, and C. sites and host species within each island.

Model Information

A. Host Species	**PC**	**DF**	**var**	**assign**
Symbiodinium C40	7	1	0.814	0.879
Symbiodinium C3	14	1	0.922	0.846
B. Islands	**PC**	**DF**	**var**	**assign**
Symbiodinium C40	16	4	0.970	0.981
Symbiodinium C3	19	4	0.966	0.796
C. Within Island	**PC**	**DF**	**var**	**assign**
Palau C40	14	3	0.984	0.735
Yap C3	7	3	0.844	0.778
Guam C3	3	3	0.647	0.860
Chuuk C3	12	3	0.933	0.927
Pohnpei C3	8	3	0.906	0.900
Kosrae C3	7	3	0.878	0.888

529 Table S3: Reef Site Collections. GPS coordinates, main island group, number of *A. digitifera* and
530 *A. hyacinthus* hosts genotyped. The numbers in brackets are individuals hosting a single
531 *Symbiodinium* MLG, which were included in all analyses presented here. Site letter corresponds
532 to island insets in Figure 1A.

Site	Island	GPS	*A. digitifera*	*A. hyacinthus*
WC. West Channel Reef	Palau	7°31'55.7 N, 134°29'42.8 E	24 (20)	25 (17)
LH. Lighthouse Reef	Palau	7°16'62.4 N, 134°27'61.9 E	23 (15)	24 (16)
NG. Ngulu	Ngulu Atoll	8°18'12.0 N, 137°29'18.7 E	0[1]	39 (25)
ST. South Tip Reef	Yap	9°26'05.4 N, 138°02'10.4 E	25 (10)	25 (11)
GO. Goofnuw Channel	Yap	9°34'26.4 N, 138°12'19.2 E	24 (15) [m]	25 (20)
PB. Pago Bay	Guam	13°25'66.6 N, 144°47'94.3 E	26 (20)	0*
TG. Tanguisson	Guam	13°32'61.1 N, 144°48'52.6 E	21 (17)	0*
WP. West Polle	Chuuk	7°19'69.7 N, 151°33'21.1E	15 (6) [m]	24 (18)
SE. South East Pass	Chuuk	7°14'60.3 N, 152°01'29.1 E	21 (17) [m]	22 (16)
AN. Ant Atoll (East)	Pohnpei	6°47'42.3 N, 158°01'20.7 E	24 (16)	22 (13)
RO. Roj	Pohnpei	6°46'37.7 N, 158°12'24.1 E	24 (16)	23 (15)
CG. Coral Garden	Kosrae	5°18'47.2 N, 162°53'01.8 E	25 (15) [m]	24 (13)
HP. Hiroshi Point	Kosrae	5°15'88.0 N, 162°59'01.8 E	25 (23) [m]	25 (14)
TOTAL			**277 (190)**	**278 (178)**

533 * indicates that no individuals of this species were found
534 [1] indicates that individuals were not collected from this site but are likely present
535 [m] indicated sites where multiple *Symbiodinium* species were detected

536 Table S4: Summary of six polymorphic microsatellite loci used to assess genetic variation
537 *Symbiodinium* clade C hosted by *A. hyacinthus* and *A. digitifera* and their corresponding
538 multiplexing groups.

Multiplex Group	Locus (Repeat)	Primer Sequence 5'-3'	Repeat	Annealing Temperature	Source
A	*SgrSpl_30*	F: FAM-ccgaactacctttggtcaac R: aaaagacaaggacatctcgg	TA	53	Wham et al., 2014
B	*SgrSpl_78*	F: FAM-tgaaattcggtgttcattgt R:ctcagatgtttccgacgagt	TA	54	Wham et al., 2014
	Sgr_21	F: HEX-tgctgagtggcgtgtatatc R: tgatggtacttgatggtg	TCA	54	Wham et al., 2014
	Spl_33	F: HEX-acttgcaaagtccaagatcg R: gaacggtgaaaggaaaatga	CAT	54	Wham et al., 2014
C	*C784*	F: Hadp-ctccttaggactggactcgc R: agaagtcaaatcgtcaccatcg	ATC	60	This Study
D	*C105*	F: FAM-tttcgttgttggacattgttatg R: ggactgaaaggtgcttgagg	complex	55	Bay *et al.,* 2009

539 Fadp- labeled primers were indirectly labeled in each PCR reaction with an additional FAM labeled adapter
540 tag sequence: FAM: agcagcgaactcagtacaaca
541 Hadp- labeled primers were indirectly labeled in each PCR reaction with an additional FAM labeled
542 adapter tag sequence: HEX: tcgtcgcttgagtcatcgtta
543

544 Table S5: Accession number and corresponding Clade C *Symbiodinium* lineage for each unique
545 sequence identifier from Figure S2.

Sequence ID	Accession Number	Lineage
1	JQ043642	C3
2	KF572372	C3
3	JQ043643	C3
4	JQ043641	C3
5	JQ043640	C3
6	JQ043644	C3
7	JQ043635	C3
8	JQ043638	C3
9	JQ043637	C3
10	JQ043636	C3
11	KF572331	C3
12	KF572332	C3
13	KF572318	C3
14	KF572319	C3
15	KF572373	C3
16	KF572374	C3
17	KF572358	C3
18	KF572368	C40
19	KF572359	C40
20	KF572367	C40
21	KF572366	C40
22	KF572360	C40
23	KF572357	C40
24	KF572363	C40
25	KF572365	C40
26	KF572364	C40
27	KF572370	C40
28	JQ043603	C31
29	JQ043599	C31
30	JQ043604	C31
31	JQ043671	C27
32	JQ043673	C27
33	JQ043670	C27
34	JQ043669	C27
35	JQ043676	C27

546