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

Davies SW[1,2*], Wham D[3], Kanke MR[1,4] and MV Matz[1]

[1] The University of Texas at Austin, Department of Integrative Biology, Austin, TX

[2] The University of North Carolina at Chapel Hill, Department of Marine Sciences, Chapel Hill, NC

[3] Pennsylvania State University, Department of Biology, University Park, PA

[4] The University of North Carolina at Chapel Hill, Department of Genetics, Chapel Hill, NC

* Corresponding author:
Sarah W. Davies, 512-609-9134. daivessw@gmail.com
Department of Marine Sciences, 123 South Road, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3300

KEYWORDS: coral, symbiosis, *Symbiodinium,* clade C, population structure, divergence, dispersal, local adaptation, host-specificity, ecological partitioning, ecology

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 the host maintains high promiscuity and symbionts adapt locally. Here, we tested this hypothesis in two coral host 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 oceanic 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* 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 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 evolving entity ('hologenome') would be misleading.

Introduction

Symbioses are ubiquitous in nature and the interactions between symbiotic partnerships have been implicated in eukaryote evolutionary diversification (1-4) and in the origin of eukaryotes (5). 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 (6, 7)). The result of this 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 (8) or allow a fungal partner to expand its geographic range across a broader climatic envelope (9). Similarly, in aphids, association with various horizontally transmitted bacterial symbionts allows these insects to colonize novel host plants across climatic zones (10). 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' (11, 12). 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 (13). Here, we explore this question in the symbiosis between a horizontally transmitting reef-building coral and dinoflagellate algae of the genus *Symbiodinium* (14).

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 (15-18). 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 (19, 20). One possible explanation is that dispersal ranges of aposymbiotic coral larvae typically extend over hundreds of kilometers (i.e. 21), 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. 22). 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 (23-25). 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. 26).

Although symbiont-free coral larvae show practically unrestricted flexibility in symbiont uptake (27-30), it nevertheless results in strong specificity of the resulting coral-*Symbiodinium* association: the majority of coral species are found hosting a single *Symbiodinium* subclade (26, 31-38). This specificity putatively arises from within-host competition between algal strains after initial uptake (32). Selection for a better match with the host is therefore expected to be another major evolutionary force affecting *Symbiodinium* populations (3), in addition to environmental specialization.

92 The third force that would influence the dynamics of coral-*Symbiodinium*
93 symbiosis is dispersal. It is reasonable to hypothesize that local environmental
94 specialization of the symbionts would be most easily achieved if the symbionts were
95 dispersal-limited compared to the coral host. Baums *et al.,* (26) investigated within-
96 subclade genetic variation of Caribbean *Symbiodinium* in the larger context of the
97 genetics of the host (*Acropora palmata*) and determined that *Symbiodinium* exhibited
98 stronger genetic structure than the coral host, suggesting limitation of symbiont dispersal
99 relative to the host.

100 To integrate these three evolutionary forces, we formulated a working hypothesis
101 for this study, the 'global host, local symbiont' hypothesis. It posits that (i) coral hosts
102 disperse widely and are able to establish symbiosis with diverse algal strains across
103 locations; (ii) *Symbiodinium* algae are poor dispersers, which results in strong divergence
104 among locations aligning with physical barriers and facilitates their local environmental
105 specialization; (iii) local *Symbiodinium* strains also diverge with respect to the host
106 species as a result of selective pressure towards higher host specificity. To validate all
107 three components of our hypothesis, we examined multi-locus genotypes (MLG) of clade
108 C *Symbiodinium* in two species of *Acropora* – *A. hyacinthus* and *A. digitifera* – collected
109 from the same reef locations across the Micronesian Pacific (Fig. 1). Our previous work
110 has shown that both host species exhibit extensive genetic connectivity and their genetic
111 structure is nearly perfectly explained by the patterns of regional surface currents (21).
112 By using two coral species that co-occur across the same locations as well as local reef
113 environments we aimed to disentangle the roles of environmental specialization, host
114 specialization, and physical barriers to dispersal in driving the fine-scale genetic structure

of *Symbiodinium*.

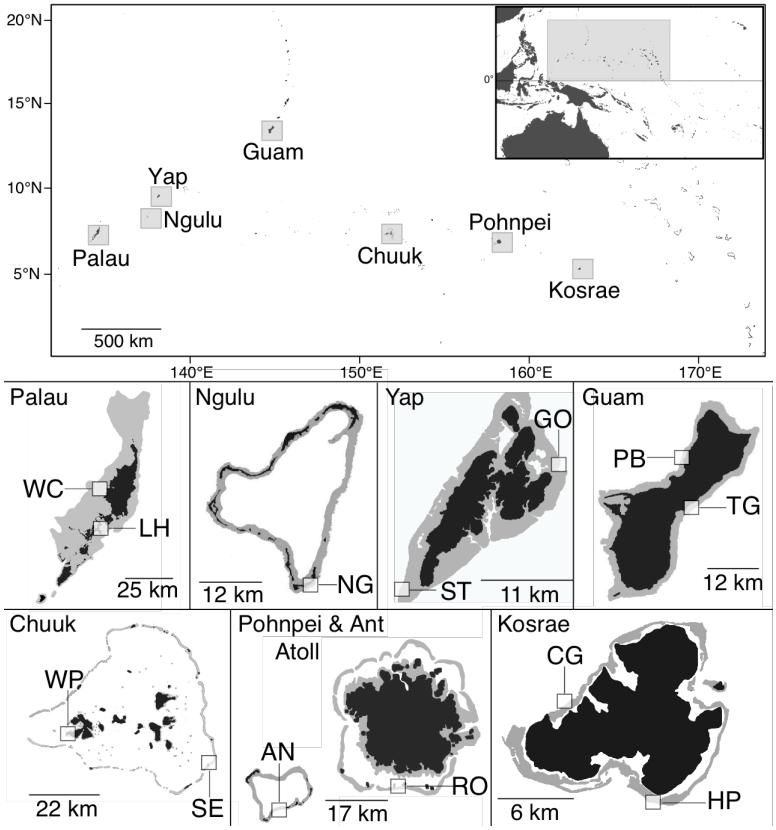


Figure 1: Geographic location of the Micronesian islands where *Acropora hyacinthus* and *A. digitifera* coral samples were collected. Top: Map of the Micronesian Pacific with an inset of the Pacific Ocean for reference. Islands where samples were collected and analyzed for *Symbiodnium* genetics are designated with grey boxes. Detailed information on each sampling site is located in Table 5. No *A. hyacinthus* were present in Guam and *A. digitifera* were not collected in Ngulu.

Results

Two *Symbiodinium* clade C lineages

To enable standard population genetic analysis, we restricted our study to only corals that hosted a single diploid *Symbiodinium* clone (i.e., yielded a unique diploid *Symbiodinium* genotype across six analyzed microsatellite loci), which encompassed the majority (69% *A. digitifera* and 64% *A. hyacinthus*) of our samples. Across the two coral species in Micronesia (Fig. 1), two distinct *Symbiodinium* lineages were observed, which were most

130 clearly discriminated by two of the six microsatellite loci (Fig. S1). Sequencing of the
131 psbA$_{ncr}$ gene for a subset samples classified these lineages as C40 and C3 *sensu*
132 Lajeunesse et al. (39) and LaJeunesse and Thornhill (40) (Fig. 2). Corals of both species
133 from Palau and Ngulu hosted exclusively C40 *Symbiodinium* (Fig. 3A, pink bars). This
134 lineage was also prevalent in *A. digitifera* at one reef site on Yap and was rarely found in
135 *A. digitifera* across the rest of Micronesia. All other coral hosts of either species
136 associated with *Symbiodinium* C3 (Fig. 3A, turquoise bars). Both *Symbiodinium* lineages
137 possessed high genetic diversity, with a total of 70 unique alleles across six SSR loci
138 observed in C40 across three islands (Table 1A) and 130 unique alleles across the same
139 six SSR loci in C3 across five islands (Table 1B). Mean numbers of alleles per island for
140 each locus for C40 ranged from 3.00-4.67 with the highest numbers of private alleles in
141 Palau (N=7) (Table 1A). Mean allele numbers per island for each locus for C3 ranged
142 from 3.83-5.17 and numbers of private alleles ranged from 1-4 (Table 1B).

143

144 Table 1. Summary of allelic diversity measures for *Symbiodinium* C40 (A) and *Symbiodinium* C3
145 (B). Na: number of alleles, He: expected heterozygosity, Ho: observed heterozygosity, PA:
146 number of private alleles

147 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

148 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

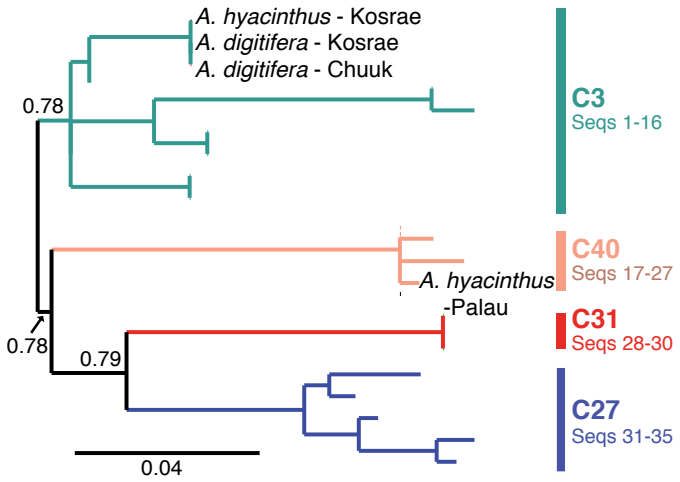


Figure 2. Phylogenetic analysis of psbA^{ncr} sequences from representative samples from this study (labeled branch tips) along with publically available psbA^{ncr} sequences from known *Symbiodinium* subclades identifies two lineages present (C3 and C40). Bootstrap support values are shown at the partitions that define known subclades. Scale bar: replacements per nucleotide site. Sequence accession numbers for reference sequences are given in Supplementary Table 1.

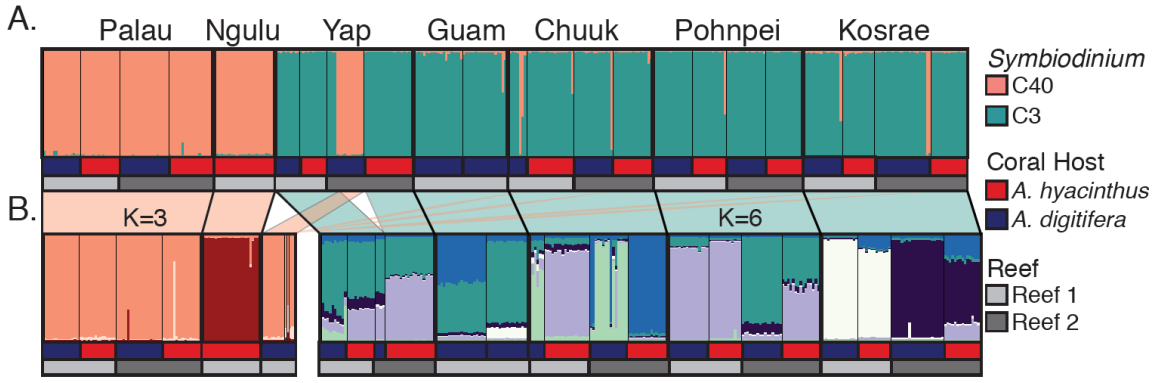


Figure 3: Analyses of microsatellite loci data for *Symbiodinium* hosted by *Acropora hyacinthus* and *Acropora digitifera* at thirteen sites across seven islands in Micronesia, using multilocus genotyping data. A. STRUCTURE population assignment for *Symbiodinium* from two *Acropora* host species across 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. B. Within-subclade STRUCTURE analysis.

Symbiodinium **genetic structure**

All pairwise between-islands F_{ST} for *Symbiodinium* C40 were significant (Table 2A). *Symbiodinium* C3 had one non-significant F_{ST} (Yap-Pohnpei) while all others were

169 significant and ranged from 0.058 (Guam-Chuuk) to 0.078 (Yap-Kosrae) (Table 2B).
170 Notably, *Symbiodinium* C3 differentiation did not show the isolation-by-distance pattern
171 observed in both coral hosts (Fig. 4). The correlation of F_{ST} with distance could not be
172 computed for *Symbiodinium* C40 due to few between-island comparisons available. For
173 *Symbiodinium* C3 F_{ST} values generally exceeded F_{ST} values for the host (Fig. 4A),
174 however, this result did not imply stronger genetic differentiation in the symbionts:
175 higher F_{ST} in the symbionts was a consequence of higher mean heterozygosity in
176 *Symbiodinium* markers (e.g. 41). Indeed, an alternative measure of genetic differentiation
177 controlling for mean heterozygosity - Jost's D (42) - demonstrated that *Symbiodinium* C3
178 genetic divergence was in fact intermediate between the two hosts (Fig. 4B).

179

180 Table 2. Summary of pairwise F_{ST} values between all islands for *Symbiodinium* C40 (A) and C3
181 (B). Permutations were run 9999 times. All significant comparisons are shaded in grey.
182 A. *Symbiodinium* C40

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

183 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

184

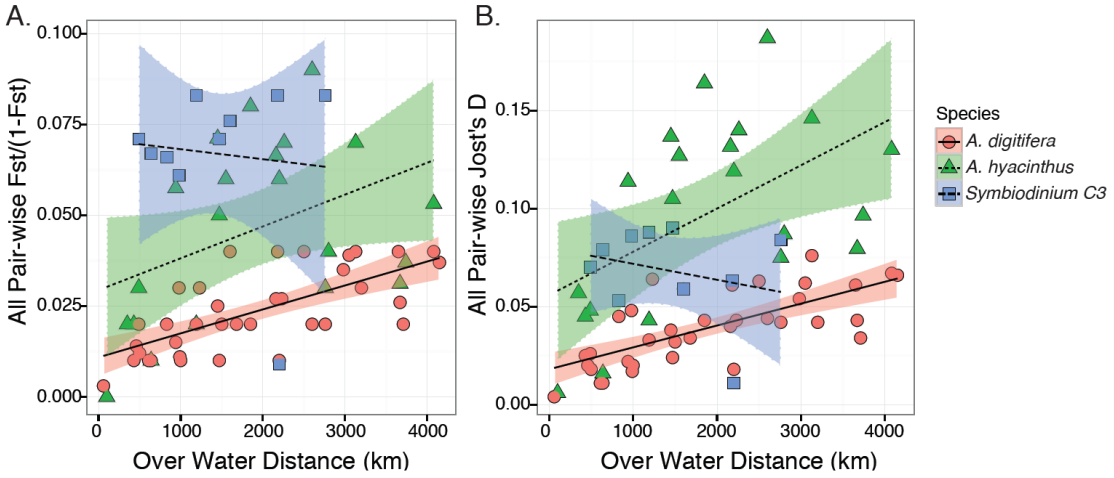


Figure 4: Comparison of two host species and *Symbiodinium* C3 differentiation. A. Pairwise genetic differentiation [(F_{ST} /(1-F_{ST})] of two species of *Acropora* coral and *Symbiodinium* C3 across linear distances (km) demonstrating significant isolation by distance for the two host species but 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 3, Fig. 5A, B), suggesting that host specificity is an effective driver of symbiont diversity. In addition, DAPC analysis also demonstrated consistent differences among islands for each *Symbiodinium* type irrespective of host species: strong per-island assignment proportions were observed for C40 (Fig. 4C, 82-100%) and C3 (Fig. 5D, 51-98%), consistent with both STRUCTURE (Fig. 3) and F_{ST} results (Table 1). Moreover, nearly all pairwise F_{ST} values between different reef sites at the same island were significant for both *Symbiodinium* lineages (Table 4), suggesting environmental partitioning of symbionts. In accord with these results, of the two top eigenvalues in DAPC analyses within individual islands, one explained *Symbiodinium* genetic divergence by host species (host specificity) and the other corresponded to differences between reef sites (environmental specialization) (Fig.

6).

Table 3: 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

Table 4. 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

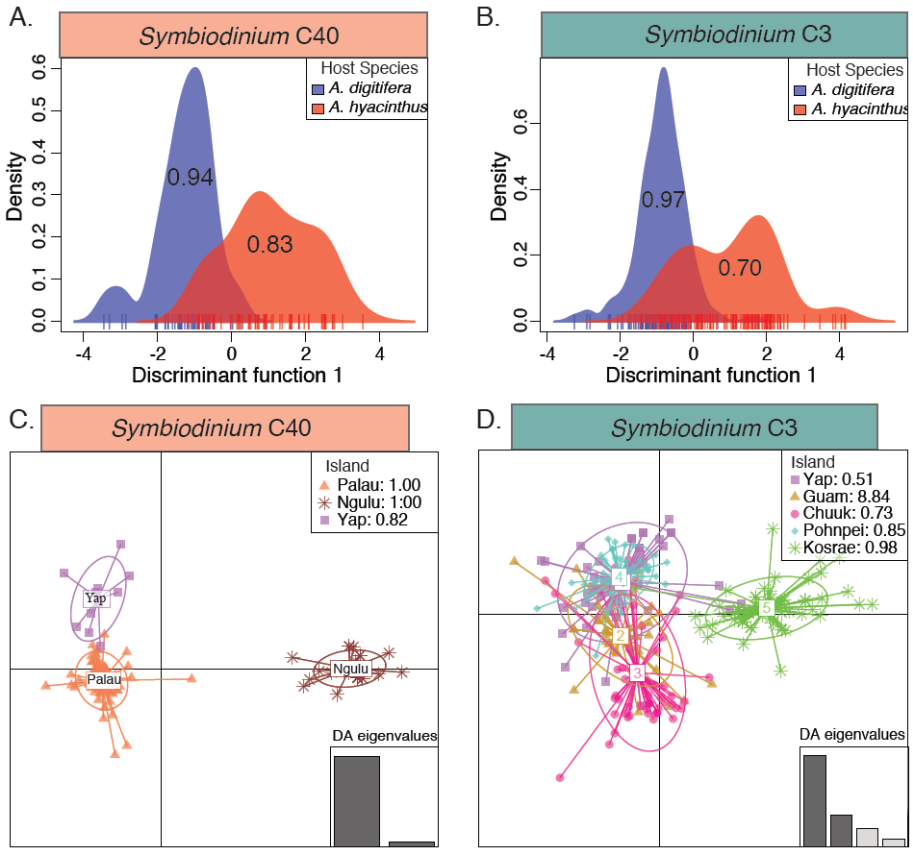


Figure 5: Discriminant analysis of principal components (DAPC) of MLG data for *Symbiodinium* C40 and C3 hosted by *Acropora hyacinthus* and *Acropora digitifera* at thirteen sites across seven islands in Micronesia. A. DAPC analysis on two discriminant functions demonstrating strong host species assignments 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 3.

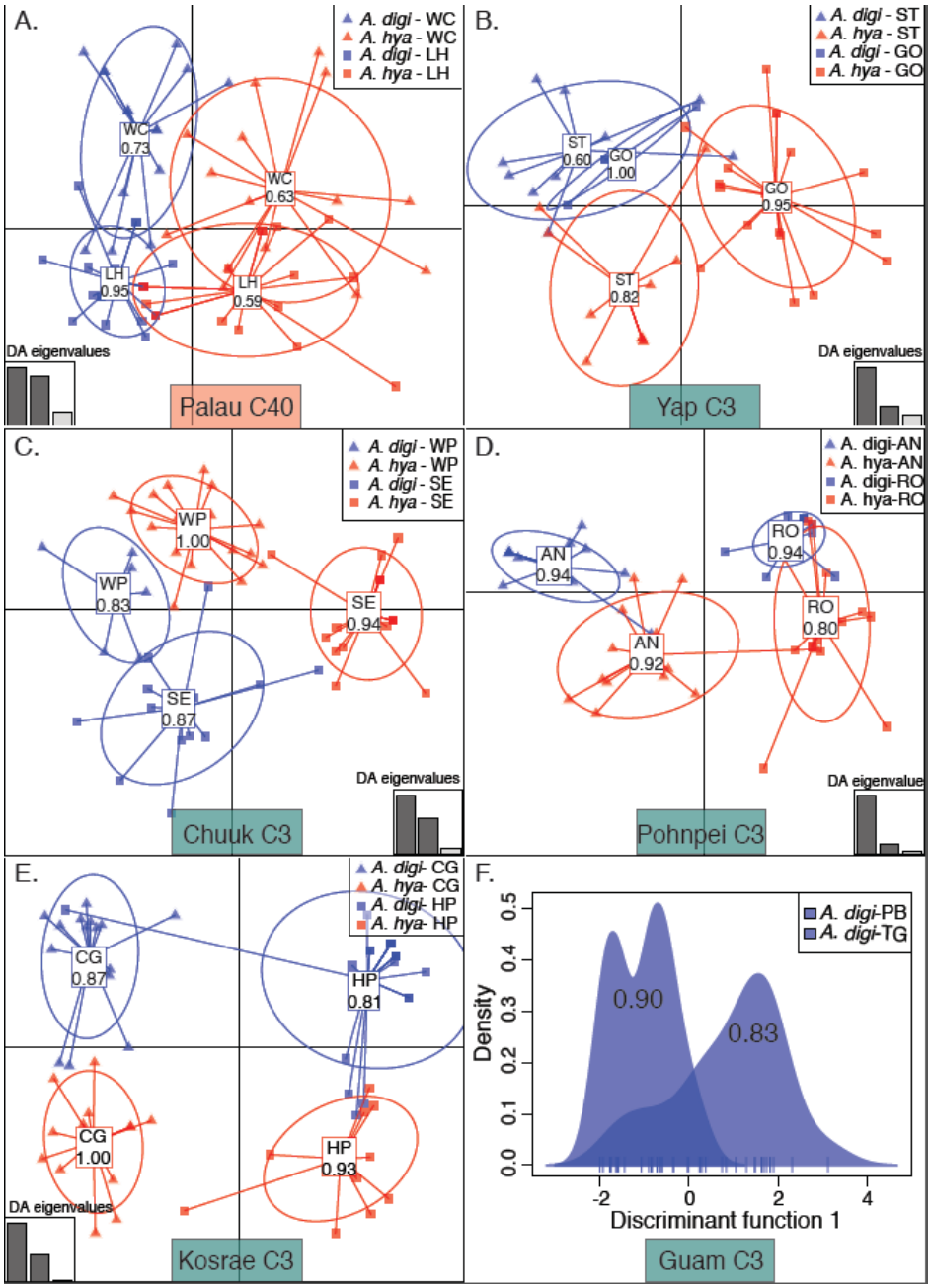


Figure 6: Discriminant analysis of principal components (DAPC) of MLG data for *Symbiodinium* C40 and C3 hosted by *Acropora hyacinthus* and *Acropora digitifera* at twelve sites across six islands (Ngulu not included) in Micronesia. DAPC analysis on two discriminant functions demonstrating host species 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 3.

245 **Discussion**

246 *Promiscuity of the coral host*

247 Clade C *Symbiodinium* are the dominant symbiont type found in Indo-Pacific reef-
248 building corals (43), presumably because this clade is capable of greater rate of carbon
249 fixation (44) and increased carbon translocation to hosts, which positively influences host
250 fitness (growth) when compared to other clades (44-46). Clade C *Symbiodinium* are
251 considered to be the most derived lineage within the genus *Symbiodinium* and exhibit
252 significantly higher within-clade diversity when compared to other, more basal, clades
253 (14, 47, 48). Across the Micronesian Pacific (Fig. 1), both coral hosts associated
254 exclusively with clade C *Symbiodinium*, which was represented by two distinct lineages–
255 C40 and C3, putatively corresponding to different species (Fig. 2). This observation
256 confirms the first prediction of the 'global host, local symbiont' hypothesis: coral hosts
257 show considerable flexibility in their symbiotic association across their range and within
258 their habitat.

259

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

261 Initially, we expected to find strong isolation by distance in *Symbiodinium*, since the
262 prevailing view of the *Symbiodinium* life cycle involves symbiotic existence in sedentary
263 hosts alternating with a free-living form that largely exists in the benthos where dispersal
264 by ocean currents must be limited (49-52). In contrast, we observed that genetic
265 differentiation between islands of *Symbiodinium* C3 across Micronesia did not exceed
266 $F_{ST} = 0.078$, which contrasts studies from other locations reporting symbiont F_{ST} as high
267 as 0.54 in clade A (53) and Φ_{ST} as high as 0.468 in clade C (54). This lack of physical
268 dispersal limitation is best demonstrated by comparing *Symbiodinium* C3 divergence to

269 divergence of their coral hosts, which has been shown to nearly perfectly follow
270 biophysical barriers (21). If *Symbiodinium* dispersal were limited by the same physical
271 barriers and to a greater extent, as our hypothesis posited, we would have observed
272 steeper increases in divergence with distance relative to the host; instead, no increase of
273 F_{ST} with distance was observed (Fig. 4A). Moreover, after correcting for mean
274 heterozygosity, genetic divergence (Jost's D) in *Symbiodinium* C3 was found to be
275 intermediate between *A. hyacinthus* and *A. digitifera* (Fig. 4B), once again demonstrating
276 that symbiont dispersal is less limited than their hosts. This finding contradicts the second
277 prediction of our 'global host, local symbiont' hypothesis and is contrary to our
278 expectations based on earlier F_{ST}-based reports of stronger *Symbiodinium* differentiation
279 compared to hosts (3, 25, 26, 54).
280
281 *Host specificity*
282 The majority of reef-building coral species associate with a specific strain (termed
283 "clade" or "subclade") of *Symbiodinium* broadly defined based on ribosomal and/or
284 chloroplast markers (3, 36, 38, 55). Previous *Symbiodinium* multilocus genotyping
285 studies revealed that each of these strains harbors greater diversity, both in terms of
286 genetic and functional diversity (25, 54, 56). Our data indicate that the local association
287 of hosts and symbionts of the same genotypic cluster is due to pervasive evolution of host
288 specificity in *Symbiodinium* (Fig. 5 & 6). As our study includes two coral species, we
289 also observe that this specificity is not perfect: at every location there were symbionts
290 that would have been assigned to another coral host based on their multilocus genotype
291 (Fig. 6). This suggests that host specialization in *Symbodinium* arises in the face of

292 considerable exchange between symbiont communities hosted by different coral species
293 at the same location. However, to which extent this between-host exchange contributes to
294 gene flow is unclear since it remains unknown if *Symbidinium* undergo sexual
295 reproduction within or outside the host.
296
297 *Environmental partitioning*
298 The most ecologically relevant postulate of the 'global host, local symbiont' hypothesis is
299 that locally available symbionts are also locally adapted, giving rise to a locally adapted
300 holobiont. Recurrent genetic divergence of symbionts between reef sites within the same
301 island (Fig. 6) despite the lack of physical dispersal limitation across much larger
302 distances (Fig. 4) suggests that symbiont genetic divergence is likely due to poor survival
303 of immigrants rather than to physical barriers to migration. Given that hosts are available
304 at every site, this leaves other ecological parameters of local reef environment as the most
305 likely barrier-forming force, preventing survival of immigrants adapted to a different
306 environment– a situation termed "phenotype-environment mismatch" (57). Thus,
307 although our study did not directly test for local adaptation of *Symbiodinium* as per
308 Kawecki and Ebert (58), genetic partitioning of symbionts among different reef
309 environments across small spatial scales suggests that their local adaptation does indeed
310 occur, thereby supporting our hypothesis. Notably, similar to the situation with host
311 specificity, there are several *Symbiodinium* genotypes that appear to be successful
312 migrants between reef sites (particularly clearly visible for Kosrae, Fig. 6E), indicating
313 that environmental partitioning is also incomplete and arises in the face of considerable
314 gene flow.

315 **Conclusions**

316 Across Micronesia, both coral host species associated with two divergent *Symbiodinium*
317 lineages (putatively corresponding to species), indicating high host promiscuity in
318 symbiont association. In contrast, within each *Symbiodinium* lineage, strong associations
319 with particular host species were observed, suggesting that host-specificity is an
320 important driver of *Symbiodinium* diversification. Unexpectedly, *Symbiodinium* genetic
321 divergence did not correlate with physical distance between islands, contrary to our initial
322 prediction that symbionts would exhibit a more pronounced isolation-by-distance pattern
323 than their hosts due to stronger barriers to dispersal. Instead, *Symbiodinium* genetic
324 structure was driven by the combined effects of local (within-island) environment and
325 host identity, suggesting that *Symbiodinium* assemblages across Micronesia are
326 comprised by broadly dispersing lineages dynamically adapting to specific coral hosts
327 and reef environments across their range. Notably, this pattern perfectly follows Baas
328 Becking's hypothesis for microbial communities: "Everything is everywhere, but the
329 environment selects" (59), assuming that host species themselves contribute to this
330 'environment'. Although this observation contradicts the original formulation of our
331 'global host, local symbiont' hypothesis, it supports the view that coral hosts could derive
332 fitness benefits from associating with locally available *Symbiodinium*. Finally, dramatic
333 differences in factors that structure genetic diversity in the coral host and their algal
334 symbionts render the 'hologenome' concept irrelevant to horizontally-transmitting coral-
335 algal symbioses.

336

337

338 **Materials and Methods**

339 ***Sampling Locations and Methodology:*** This study comprised a subset of samples
340 previously analyzed for coral host genetics in Davies *et al.* (21) (Table 5, Fig. 1).
341 Twenty-five individuals of each coral host species (*Acropora hyacinthus* and *A.*
342 *digitifera*) were examined at two reef sites within seven islands, with the exception of
343 Ngulu (the only species collected was *A. hyacinthus*) and Guam (no *A. hyacinthus* were
344 found and only *A. digitifera* were collected), for a total of thirteen sites.

345

346 Table 5. Reef Site Collections. GPS coordinates, main island group, number of *A. digitifera* and
347 *A. hyacinthus* hosts genotyped. The numbers in brackets are individuals hosting a single
348 *Symbiodinium* MLG, which were included in all analyses presented here. Site letter corresponds
349 to island insets in Figure 1.

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)**

350 * indicates that no individuals of this species were found
351 [1] indicates that individuals were not collected from this site but are likely present
352 [m] indicated sites where multiple *Symbiodinium* species were detected

353

354 ***Laboratory Procedures:*** Holobiont DNA was isolated following Davies *et al.* (60).
355 Microsatellite primers for this study consisted of six previously published clade C loci
356 (61, 62) and one novel locus mined using Msatcommander (63) from nucleotide EST data
357 for *Symbiodinium* sp. clade C3 in GenBank (64) (Table 6). Loci were multiplexed
358 according to annealing temperatures and fragment sizes. Each 20 µl polymerase chain

359 reaction (PCR) mixture contained 10 ng of DNA template, 0.1 µM of each forward
360 primer, 0.1 µM of each reverse primer, 0.2 mM dNTP, 1 µl 10X *ExTaq* buffer, 0.025 U
361 *ExTaq* Polymerase (Takara Biotechnology) and 0.0125 U *Pfu* Polymerase (Agilent
362 Technologies). Amplifications were performed using a DNA Engine Tetrad2 Thermal
363 Cycler (Bio-Rad, Hercules CA). Cycling began at 94°C for 5 min, followed by 35 cycles
364 of 94°C for 40 s, annealing temperature for 120 s, and 72°C for 60 s and a 10 minute
365 extension period at 72°C. Molecular weights were analyzed using the ABI 3130XL
366 capillary sequencer with an in-house ROX-labeled size standard. Data were binned by
367 repeat size and individuals failing to amplify at ≥3 loci were excluded from analyses.

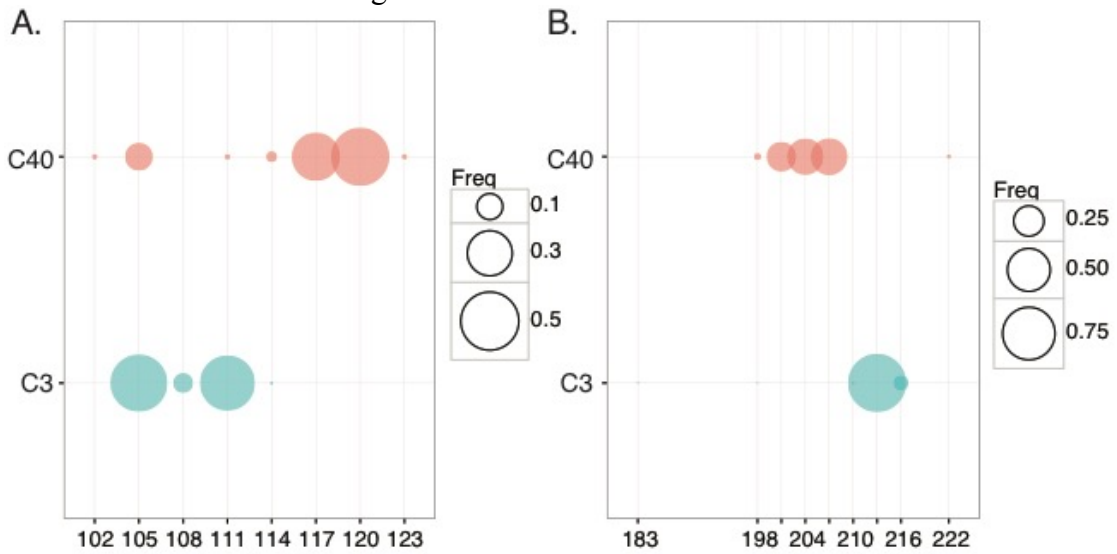
368

369 Table 6: Summary of six polymorphic microsatellite loci used to assess genetic variation
370 *Symbiodinium* clade C hosted by *A. hyacinthus* and *A. digitifera* and their corresponding
371 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

372 Fadp- labeled primers were indirectly labeled in each PCR reaction with an additional FAM labeled adapter
373 tag sequence: FAM: agcagcgaactcagtacaaca
374 Hadp- labeled primers were indirectly labeled in each PCR reaction with an additional FAM labeled
375 adapter tag sequence: HEX: tcgtcgcttgagtcatcgtta
376
377

Data Analysis: *Symbiodinium* in clade C microsatellite data were scored as diploid based on available information on the ploidy of these organisms (3, 62, 65) Since each individual host could potentially contain a population of genetically distinct *Symbiodinium* individuals, multi-locus genotypes (MLG) were only considered in this study if they contained two or fewer alleles across all loci, suggesting that all alelles originate from a single, clonally replicated, genome (65), which was the case for a total of 190 out of 277 for *A. digitifera* hosts and 178 out of 278 for *A. hyacinthus* hosts. We first applied a Bayesian approach implemented in STRUCTURE v2.3.3 (66). STRUCTURE uses a Monte Carlo Markov chain (MCMC) clustering algorithm to assign individuals with similar genotypes to populations. Mean and variance of log likelihood values of the number of populations K (1-10) were inferred by STRUCTURE with 10^6 iterations (burn in = 300,000 iterations) in ten replicate runs for each K. An admixture model was implemented with no location prior. Following the recommendations of (67), the ad hoc statistic ΔK was calculated based on the rate of change of the log-likelihood between consecutive K values, which is implemented in the program STRUCTURE Harvester (68). CLUMPP (69) and DISTRUCT (70) were used to produce graphics. We observed high percent assignment to two different clusters among sympatric individuals. Following the methodology of Wham and LaJeunesse (65), we investigated the relationship between these cluster assignments and allele identities. From this analysis we found a strong relationship between cluster assignments and allele identity, particularly at Sgr_21 and Spl_33 (Supplement Fig. S1A, B), and it became clear that two distinct *Symbiodinium* lineages were present. MLG data were then split into *Symbiodinium* lineages based on the probability of species assignment conditioned on their cluster assignment (65). Additional STRUCTURE runs were completed following descriptions above with these subsequent differences: 1) location prior was implemented and 2) instead of using the ΔK statistic, we presented the K that maximized the mean of the estimated probability of data while minimizing the standard deviation.



Supplementary Figure 1: 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.

410 Once data were partitioned by *Symbiodinium* lineages, genetic divergences among
411 islands and sites were investigated using F_{ST} (AMOVA, 9999 permutations in GENALEX
412 v6.5, (71)). Pairwise differentiations (F_{ST} and Jost's D) and pairwise island distances
413 were then correlated to test for isolation by distance (IBD) in *Symbiodinium* C3 (not
414 sufficient data for C40) and these IBD trends were compared to results from the host
415 species (72). Mean allelic diversities per island and numbers of private alleles per island
416 were also calculated in GENALEX v6.5.

417 To resolve differences between islands, between host species and between sites
418 and host species within each island, assignment of samples to genetic clusters using
419 discriminant analysis of principal components (DAPC) was performed in R (73) using the
420 ADEGENET package (74, 75). Here, *Symbiodinium* data were converted into principle
421 components and then a-scores were used to determine trade-offs between power of
422 discrimination and model over-fitting. Relationships were examined by DAPC, which
423 optimizes variation among clusters by minimizing variation within clusters, while
424 retaining the optimal number of principle components and maximum number of
425 discriminant functions. All information on DAPC model parameters and results are
426 contained in Table 3. Cluster assignment patterns were compared across islands, host
427 species, and among host species and sites within each island.

428

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

430 In order to confirm the assignments of individuals to different *Symbiodinium* lineages, we
431 selected a small subset of the full sample set and analyzed the non-coding region of the
432 circular plastid (psbA^{ncr}). We amplified the psbA^{ncr} locus with the primers 7.4-Forw and

433 7.8-Rev following the methods described by LaJeunesse and Thornhill (40). The
434 amplified product was directly sequenced and the resulting sequences were aligned with
435 ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) to clade C psbA^{ncr} sequences
436 from common *Symbiodinium* types found throughout the Indo-Pacific region
437 (Supplemental Table 1: JQ043587-JQ043676 from (40)). We reconstructed the
438 phylogeny of these samples using the default settings of Phylogeny.fr (76). The identities
439 of *Symbiodinium* types from our analysis were interpreted from their percent sequence
440 identity with these previously identified *Symbiodinium* types and their position on the
441 resulting phylogeny.
442

Acknowledgements

Thanks to field assistants Carly Kenkel, Tim Kiett, Irina Yakushenok and David Stump. Nida Zehra Khawaja Rahman was integral in the molecular work and James Derry helped with data management. We are grateful to Ulrich Mueller for numerous comments and suggestions. We also acknowledge all permit authorities. This study was supported by the grant from the Coral Reef Conservation program of the National Oceanic and Atmospheric Administration (administered through the Hawaiian Undersea Research Laboratory) and the National Science Foundation grant DEB-1054766 to M.V.M. DW was funded in part by Pennsylvania State University and grants from the National Science Foundation (OCE-0928764 and IOS-1258058).

455 **References**
456

457 1. Brucker RM & Bordenstein SR (2012) The roles of host evolutionary
458 relationships (genus: Nasonia) and development in structuring microbial
459 communities. *Evolution* 66(2):349-362.
460 2. Moran NA (2006) Symbiosis. *Curr Biol* 16(20):R866-871.
461 3. Thornhill DJ, Lewis AM, Wham DC, & LaJeunesse TC (2014) Host-specialist
462 lineages dominate the adaptive radiation of reef coral endosymbionts. *Evolution*
463 68(2):352-367.
464 4. Brucker RM & Bordenstein SR (2012) Speciation by symbiosis. *Trends Ecol Evol*
465 27(8):443-451.
466 5. Mereschkowsky K (1910) Theorie der zwei Plasmaarten als Grundlage der
467 Symbiogenesis, einer neuen Lehre von der Ent-stehung der Organismen. *Biol*
468 *Centralbl* 30:353-367.
469 6. Nakabachi A, Ishida K, Hongoh Y, Ohkuma M, & Miyagishima SY (2014) Aphid
470 gene of bacterial origin encodes a protein transported to an obligate
471 endosymbiont. *Curr Biol* 24(14):R640-641.
472 7. Shigenobu S & Wilson AC (2011) Genomic revelations of a mutualism: the pea
473 aphid and its obligate bacterial symbiont. *Cellular and molecular life sciences :*
474 *CMLS* 68(8):1297-1309.
475 8. Peksa O & Skaloud P (2011) Do photobionts influence the ecology of lichens? A
476 case study of environmental preferences in symbiotic green alga Asterochloris
477 (Trebouxiophyceae). *Mol Ecol* 20(18):3936-3948.
478 9. Fernandez-Mendoza F*, et al.* (2011) Population structure of mycobionts and
479 photobionts of the widespread lichen Cetraria aculeata. *Mol Ecol* 20(6):1208-
480 1232.
481 10. Henry LM*, et al.* (2013) Horizontally transmitted symbionts and host colonization
482 of ecological niches. *Curr Biol* 23(17):1713-1717.
483 11. Brucker RM & Bordenstein SR (2014) Response to Comment on "The
484 hologenomic basis of speciation: Gut bacteria cause hybrid lethality in the genus
485 Nasonia". *Science* 345(6200).
486 12. Zilber-Rosenberg I & Rosenberg E (2008) Role of microorganisms in the
487 evolution of animals and plants: the hologenome theory of evolution. *Fems*
488 *Microbiol Rev* 32(5):723-735.
489 13. Moran NA & Sloan DB (2015) The Hologenome Concept: Helpful or Hollow?
490 *PLoS biology* 13(12):e1002311.
491 14. Lesser MP, Stat M, & Gates RD (2013) The endosymbiotic dinoflagellates
492 (Symbiodinium sp.) of corals are parasites and mutualists. *Coral Reefs*:603-611.
493 15. Muscatine L (1990) The role of symbiotic algae in carbon and energy flux in reef
494 corals. *Ecosystems of the World* 25:75-87.
495 16. Barott KL, Venn AA, Perez SO, Tambutte S, & Tresguerres M (2015) Coral host
496 cells acidify symbiotic algal microenvironment to promote photosynthesis. *Proc*
497 *Natl Acad Sci U S A* 112(2):607-612.
498 17. Muscatine L & Cernichiari E (1969) Assimilation of photosynthetic products of
499 zooxanthellae by a reef coral. *The Biological Bulletin* 137(3):506-523.

18. Trench RK & Blank RJ (1987) Symbiodinium microadriaticum Fredenthal, S. Goreau sp. nov., S. Kawagut2 sp. nov., and S. pilosum sp. nov.: Gymnodinoid dinoflagellate symbionts of marine invertebrates. *J Phycol* 23(3):469-481.
19. Fadlallah YH (1983) Sexual reproduction, development and larval biology of scleractinian corals. A review. *Coral Reefs* 2:129-150.
20. Harrison PLW, C.C. (1990) *Reproduction, dispersal and recruitment of scleractinian corals* (elsevier Science Publications Amsterdam).
21. Davies SW, Treml EA, Kenkel CD, & Matz MV (2015) Exploring the role of Micronesian islands in the maintenance of coral genetic diversity in the Pacific Ocean. *Mol Ecol* 24(1):70-82.
22. Gorospe KD & Karl SA (2011) Small-Scale Spatial Analysis of In Situ Sea Temperature throughout a Single Coral Patch Reef. *Journal of Marine Biology* 2011:12.
23. Byler KA, Carmi-Veal M, Fine M, & Goulet TL (2013) Multiple symbiont acquisition strategies as an adaptive mechanism in the coral Stylophora pistillata. *Plos One* 8(3):e59596.
24. Rowan R & Knowlton N (1995) Intraspecific diversity and ecological zonation in coral-algal symbiosis. *Proc Natl Acad Sci U S A* 92(7):2850-2853.
25. Howells EJ*, et al.* (2012) Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nat Clim Change* 2:116-120.
26. Baums IB, Devlin-Durante MK, & LaJeunesse TC (2014) New insights into the dynamics between reef corals and their associated dinoflagellate endosymbionts from population genetic studies. *Mol Ecol* 23(17):4203-4215.
27. Abrego D, MJ VANO, & Willis BL (2009) Highly infectious symbiont dominates initial uptake in coral juveniles. *Mol Ecol* 18(16):3518-3531.
28. Gómez-Cabrera MdC, Ortiz JC, Loh WKW, Ward S, & Hoegh-Guldberg O (2008) Acquisition of symbiotic dinoflagellates (Symbiodinium) by juveniles of the coral Acropora longicyathus. *Coral Reefs* 27(1):219-226.
29. Little AF, van Oppen MJ, & Willis BL (2004) Flexibility in algal endosymbioses shapes growth in reef corals. *Science* 304(5676):1492-1494.
30. Abrego D, van Oppen MJ, & Willis B (2009) Onset of algal endosymbiont specificity varies among closely related species of Acropora corals during early ontogeny. *Mol Ecol* 18(16):3532-3543.
31. Ackerly DD, Schwilk DW, & Webb CO (2006) Niche evolution and adaptive radiation: testing the order of trait divergence. *Ecology* 87(7 Suppl):S50-61.
32. Coffroth MA, Santos SR, & Goulet TL (2001) Early ontogenetic expression of specificity in a cnidarian–algal symbiosis. *Mar Ecol Prog Ser* 222:85-96.
33. Finney JC*, et al.* (2010) The relative significance of host-habitat, depth, and geography on the ecology, endemism, and speciation of coral endosymbionts in the genus Symbiodinium. *Microbial ecology* 60(1):250-263.
34. Lajeunesse TC (2005) "Species" radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene-Pliocene transition. *Mol Biol Evol* 22(3):570-581.
35. LaJeunesse TC*, et al.* (2010) Host-symbiont recombination versus natural selection in the response of coral-dinoflagellate symbioses to environmental

545 disturbance. *Proceedings. Biological sciences / The Royal Society*
546 277(1696):2925-2934.
547 36. Rodriguez-Lanetty M, Krupp DA, & Weis VM (2004) Distinct ITS types of
548 Symbiodinium in Clade C correlate with cnidarian/dinoflagellate specificity
549 during onset of symbiosis. *Mar Ecol Prog Ser* 275(97-102).
550 37. Thornhill DJ, Xiang Y, Fitt WK, & Santos SR (2009) Reef endemism, host
551 specificity and temporal stability in populations of symbiotic dinoflagellates from
552 two ecologically dominant Caribbean corals. *Plos One* 4(7):e6262.
553 38. Weis VM, Reynolds WS, deBoer MD, & Krupp DA (2001) Host-symbiont
554 specificity during onset of symbiosis between the dinoflagellates Symbiodinium
555 spp. and planula larvae of the scleractinian coral Fungia scutaria. *Coral Reefs*
556 20(3):301-308.
557 39. Polato NR, Vera JC, & Baums IB (2011) Gene discovery in the threatened
558 elkhorn coral: 454 sequencing of the Acropora palmata transcriptome. *Plos One*
559 6(12):e28634.
560 40. LaJeunesse TC & Thornhill DJ (2011) Improved resolution of reef-coral
561 endosymbiont (Symbiodinium) species diversity, ecology, and evolution through
562 psbA non-coding region genotyping. *Plos One* 6(12):e29013.
563 41. Jakobsson M, Edge MD, & Rosenberg NA (2013) The Relationship Between F-
564 ST and the Frequency of the Most Frequent Allele. *Genetics* 193(2):515-528.
565 42. Jost L (2008) G(ST) and its relatives do not measure differentiation. *Mol Ecol*
566 17(18):4015-4026.
567 43. Quigley KM*, et al.* (2014) Deep-Sequencing Method for Quantifying Background
568 Abundances of Symbiodinium Types: Exploring the Rare Symbiodinium
569 Biosphere in Reef-Building Corals. *Plos One* 9(4).
570 44. Stat M, Morris E, & Gates RD (2008) Functional diversity in coral-dinoflagellate
571 symbiosis. *Proc Natl Acad Sci U S A* 105(27):9256-9261.
572 45. Jones A & Berkelmans R (2010) Potential costs of acclimatization to a warmer
573 climate: growth of a reef coral with heat tolerant vs. sensitive symbiont types.
574 *Plos One* 5(5):e10437.
575 46. Mieog JC*, et al.* (2009) The roles and interactions of symbiont, host and
576 environment in defining coral fitness. *Plos One* 4(7):e6364.
577 47. Pochon X & Gates RD (2010) A new Symbiodinium clade (Dinophyceae) from
578 soritid foraminifera in Hawai'i. *Molecular phylogenetics and evolution* 56(1):492-
579 497.
580 48. Pochon X, Montoya-Burgos JI, Stadelmann B, & Pawlowski J (2006) Molecular
581 phylogeny, evolutionary rates, and divergence timing of the symbiotic
582 dinoflagellate genus Symbiodinium. *Molecular phylogenetics and evolution*
583 38(1):20-30.
584 49. Fitt WK, Chang SS, & Trench RK (1981) Motility Patterns of Different Strains of
585 the Symbiotic Dinoflagellate Symbiodinium (=Gymnodinium) Microadriaticum
586 (Freudenthal) in Culture. *Bulletin of Marine Science* 31(2):436-443.
587 50. Fitt WK & Trench RK (1983) The Relation of Diel Patterns of Cell-Division to
588 Diel Patterns of Motility in the Symbiotic Dinoflagellate Symbiodinium-
589 Microadriaticum Freudenthal in Culture. *New Phytol* 94(3):421-432.

590 51. Yacobovitch T, Benayahu Y, & Weis VM (2004) Motility of zooxanthellae
591 isolated from the Red Sea soft coral Heteroxenia fuscescens (Cnidaria). *J Exp*
592 *Mar Biol Ecol* 298(1):35-48.
593 52. Littman RA, van Oppen MJH, & Willis BL (2008) Methods for sampling free-
594 living Symbiodinium (zooxanthellae) and their distribution and abundance at
595 Lizard Island (Great Barrier Reef). *J Exp Mar Biol Ecol* 364(1):48-53.
596 53. Andras JP, Kirk NL, & Harvell CD (2011) Range-wide population genetic
597 structure of Symbiodinium associated with the Caribbean Sea fan coral, Gorgonia
598 ventalina. *Mol Ecol* 20(12):2525-2542.
599 54. Howells EJ, van Oppen MJ, & Bay LK (2009) High genetic differentiation and
600 cross-shelf patterns of genetic diversity among Great Barrier Reef populations of
601 Symbiodinium. *Coral Reefs* 28(1):215-225.
602 55. Fabina NS, Putnam HM, Franklin EC, Stat M, & Gates RD (2013) Symbiotic
603 specificity, association patterns, and function determine community responses to
604 global changes: defining critical research areas for coral-Symbiodinium
605 symbioses. *Global Change Biol* 19(11):3306-3316.
606 56. Santos SR, Shearer TL, Hannes AR, & Coffroth MA (2004) Fine-scale diversity
607 and specificity in the most prevalent lineage of symbiotic dinoflagellates
608 (Symbiodinium, Dinophyceae) of the Caribbean. *Mol Ecol* 13(2):459-469.
609 57. Marshall DJ, Monro K, Bode M, Keough MJ, & Swearer S (2010) Phenotype-
610 environment mismatches reduce connectivity in the sea. *Ecol Lett* 13(1):128-140.
611 58. Kawecki TJ & Ebert D (2004) Conceptual issues in local adaptation. *Ecol Lett*
612 7(12):1225-1241.
613 59. Baas Becking LGM (1934) Geobiologie of inleiding tot de milieukunde. *Den*
614 *Haag [Netherlands]: W.P. Van Stockum & Zoon, N. V.*
615 60. Davies SW*, et al.* (2013) Novel polymorphic microsatellite markers for
616 population genetics of the endangered Caribbean star coral, Montastraea
617 faveolata. *Mar Biodivers* 43(2):167-172.
618 61. Bay LK, Howells EJ, & van Oppen MJ (2009) Isolation, characterisation and
619 cross amplification of thirteen microsatellite loci for coral endo-symbiotic
620 dinoflagellates (Symbiodinium clade C). *Conserv Genet Resour* 1(1):199-203.
621 62. Wham DC, Carmichael M, & LaJeunesse TC (2014) Microsatellite loci for
622 Symbiodinium goreaui and other Clade C Symbiodinium. *Conserv Genet Resour*
623 6(1):127-129.
624 63. Faircloth BC (2008) MSATCOMMANDER: detection of microsatellite repeat
625 arrays and automated, locus-specific primer design. *Molecular ecology resources*
626 8(1):92-94.
627 64. Leggat W, Hoegh-Guldberg O, Dove S, & Yellowlees D (2007) Analysis of an
628 EST library from the dinoflagellate (Symbiodinium sp.) symbiont of reef-building
629 corals. *J Phycol* 43(5):1010-1021.
630 65. Traylor-Knowles N*, et al.* (2011) Production of a reference transcriptome and
631 transcriptomic database (PocilloporaBase) for the cauliflower coral, Pocillopora
632 damicornis. *BMC genomics* 12:585.
633 66. Pritchard JK, Stephens M, & Donnelly P (2000) Inference of population structure
634 using multilocus genotype data. *Genetics* 155(2):945-959.

635 67. Evanno G, Regnaut S, & Goudet J (2005) Detecting the number of clusters of
636 individuals using the software STRUCTURE: a simulation study. *Mol Ecol*
637 14(8):2611-2620.
638 68. Earl DA & Vonholdt BM (2012) STRUCTURE HARVESTER: a website and
639 program for visualizing STRUCTURE output and implementing the Evanno
640 method. *Conserv Genet Resour* 4(2):359-361.
641 69. Jakobsson M & Rosenberg NA (2007) CLUMPP: a cluster matching and
642 permutation program for dealing with label switching and multimodality in
643 analysis of population structure. *Bioinformatics* 23(14):1801-1806.
644 70. Rosenberg NA (2004) DISTRUCT: a program for the graphical display of
645 population structure. *Mol Ecol Notes* 4(1):137-138.
646 71. Peakall R & Smouse PE (2006) GENALEX 6: genetic analysis in Excel.
647 Population genetic software for teaching and research. *Mol Ecol Notes* 6(1):288-
648 295.
649 72. Davies SW, Treml EA, Kenkel CD, & Matz MV (2015) Exploring the role of
650 Micronesian islands in the maintenance of coral genetic diversity in the Pacific
651 Ocean. *Mol Ecol* 24(1):70-82.
652 73. R Development Core Team (2015) R: A language and environment for statistical
653 computing. (R Foundation for Statistical Computing, Vienna, Austria).
654 74. Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic
655 markers. *Bioinformatics* 24(11):1403-1405.
656 75. Jombart T, Devillard S, & Balloux F (2010) Discriminant analysis of principal
657 components: a new method for the analysis of genetically structured populations.
658 *BMC genetics* 11:94.
659 76. Dereeper A*, et al.* (2008) Phylogeny.fr: robust phylogenetic analysis for the non-
660 specialist. *Nucleic Acids Res* 36(Web Server issue):W465-469.
661
662
663

664 Supplemental Table 1: Accession number and corresponding Clade C *Symbiodinium* lineage for
665 each unique sequence identifier from Figure 2.
666

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

667