Ecological factors rather than physical 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 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 considered 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.

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

To integrate these three evolutionary forces, we formulated a working hypothesis for this study, the 'global host, local symbiont' hypothesis. It posits that (i) coral hosts disperse widely and are able to establish symbiosis with diverse algal strains across locations; (ii) *Symbiodinium* algae are poor dispersers, 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. 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. 1). 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 (21). 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*.

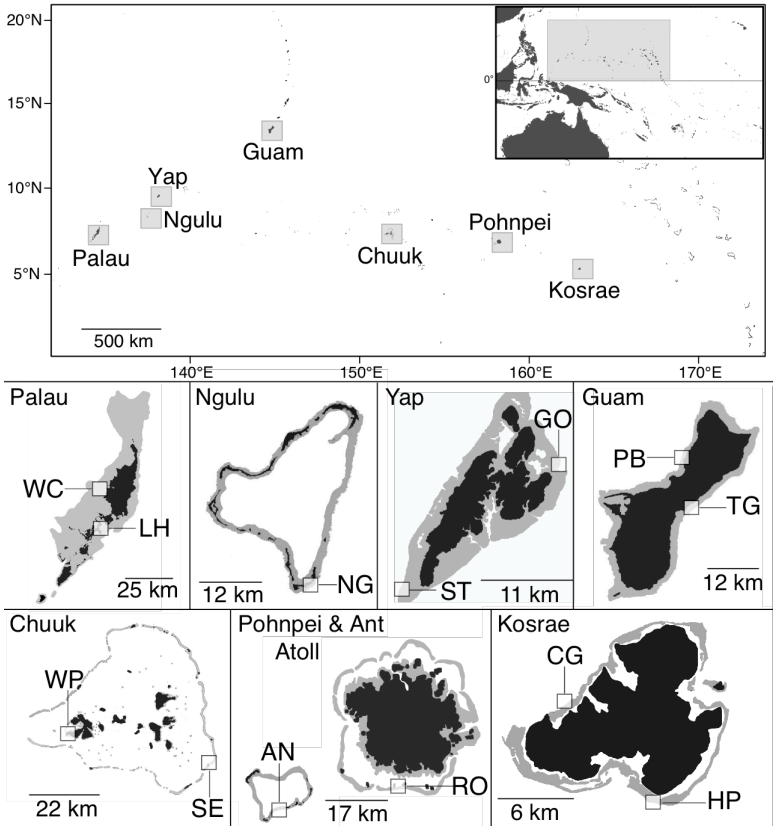


Figure 1: Geographic location of the Micronesian islands where *Acropora hyacinthus* and *A. digitifera* coral samples were collected. Top: Map of the Micronesian Pacific with an inset of the Pacific Ocean for reference. Islands where samples were collected and analyzed for *Symbiodnium* genetics are 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

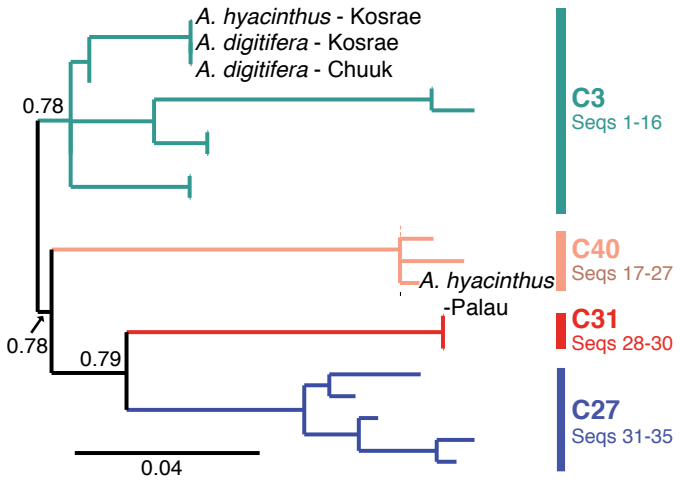


Figure 2. Phylogenetic analysis of psbA^{ncr} sequences from representative samples from this study (labeled branch tips) along with publically available psbA^{ncr} sequences from known *Symbiodinium* subclades identifies two lineages present (C3 and C40). Bootstrap support values are shown at the partitions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

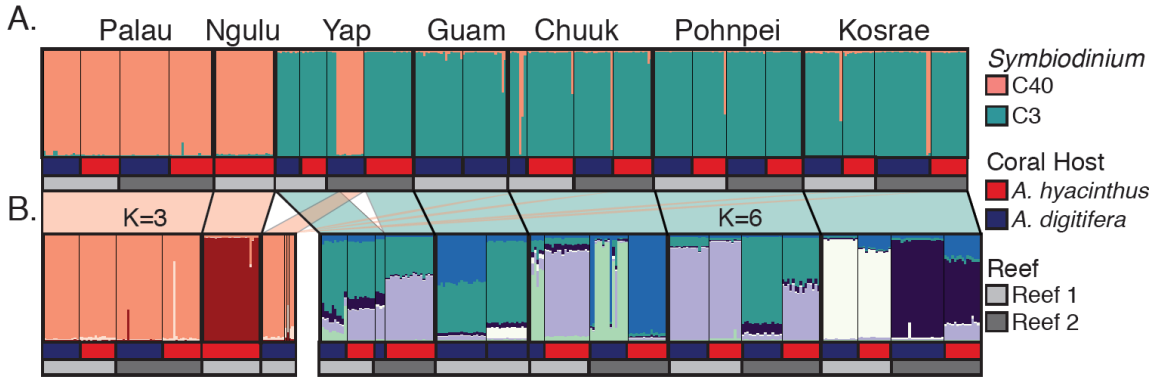


Figure 3: Analyses of microsatellite loci data for *Symbiodinium* hosted by *Acropora hyacinthus* and *Acropora digitifera* at thirteen sites across seven islands in Micronesia, using multilocus genotyping data. A. STRUCTURE population assignment for *Symbiodinium* from two *Acropora* host species across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

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

Table 2. 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

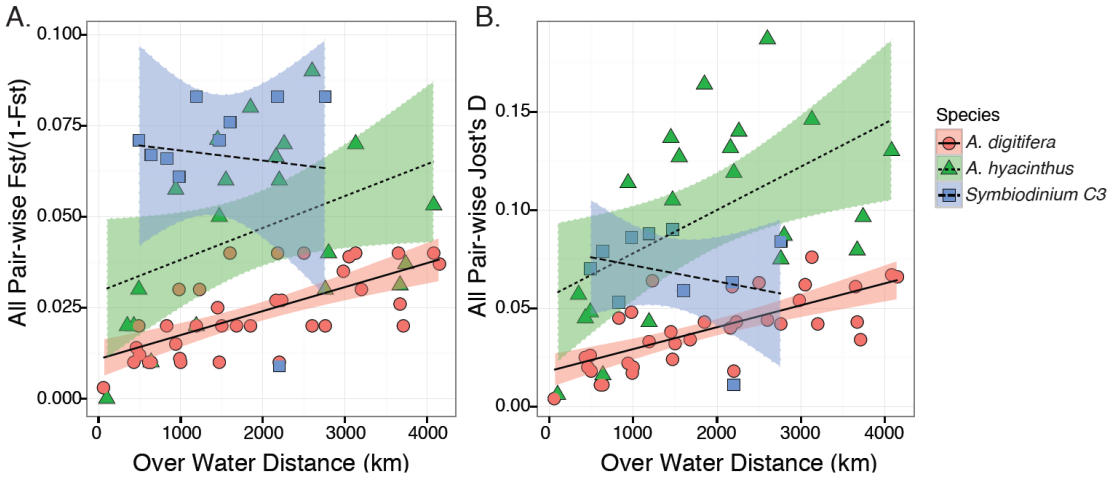


Figure 4: Comparison of two host species and *Symbiodinium* C3 differentiation. A. Pairwise genetic differentiation [(F_{ST} /(1-F_{ST})] of two species of *Acropora* coral and *Symbiodinium* C3 across linear distances (km) demonstrating significant isolation by distance for the two host species but 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

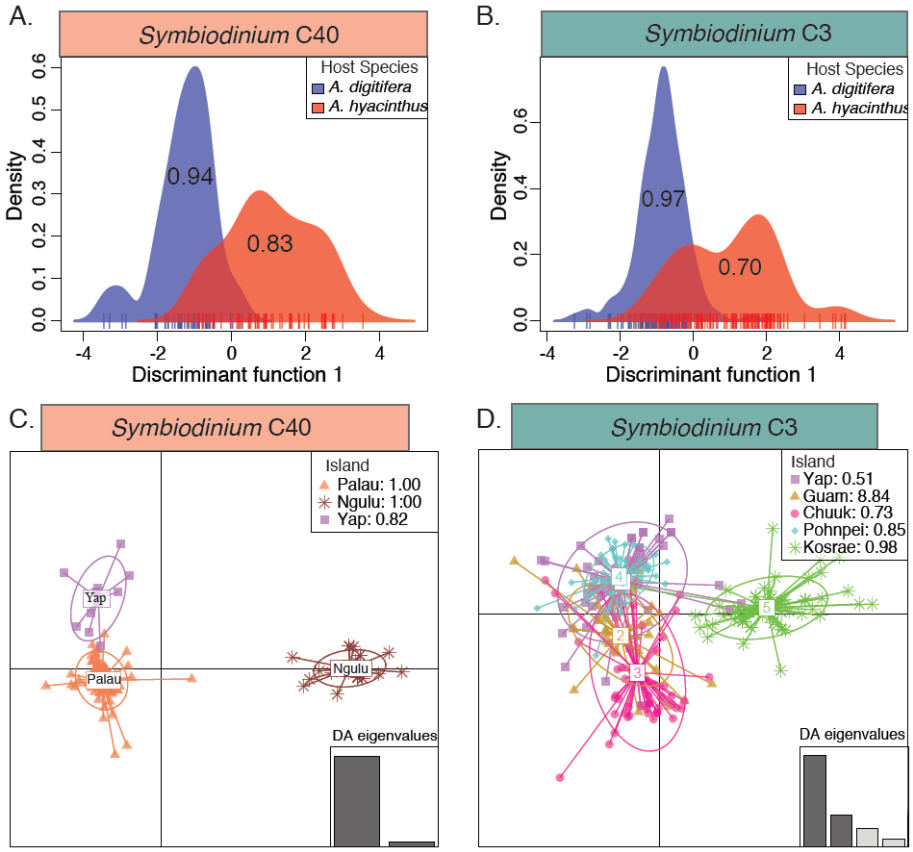


Figure 5: Discriminant analysis of principal components (DAPC) of MLG data for *Symbiodinium* C40 and C3 hosted by *Acropora hyacinthus* and *Acropora digitifera* at thirteen sites across seven islands in Micronesia. A. DAPC analysis on two discriminant functions demonstrating strong host species assignments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

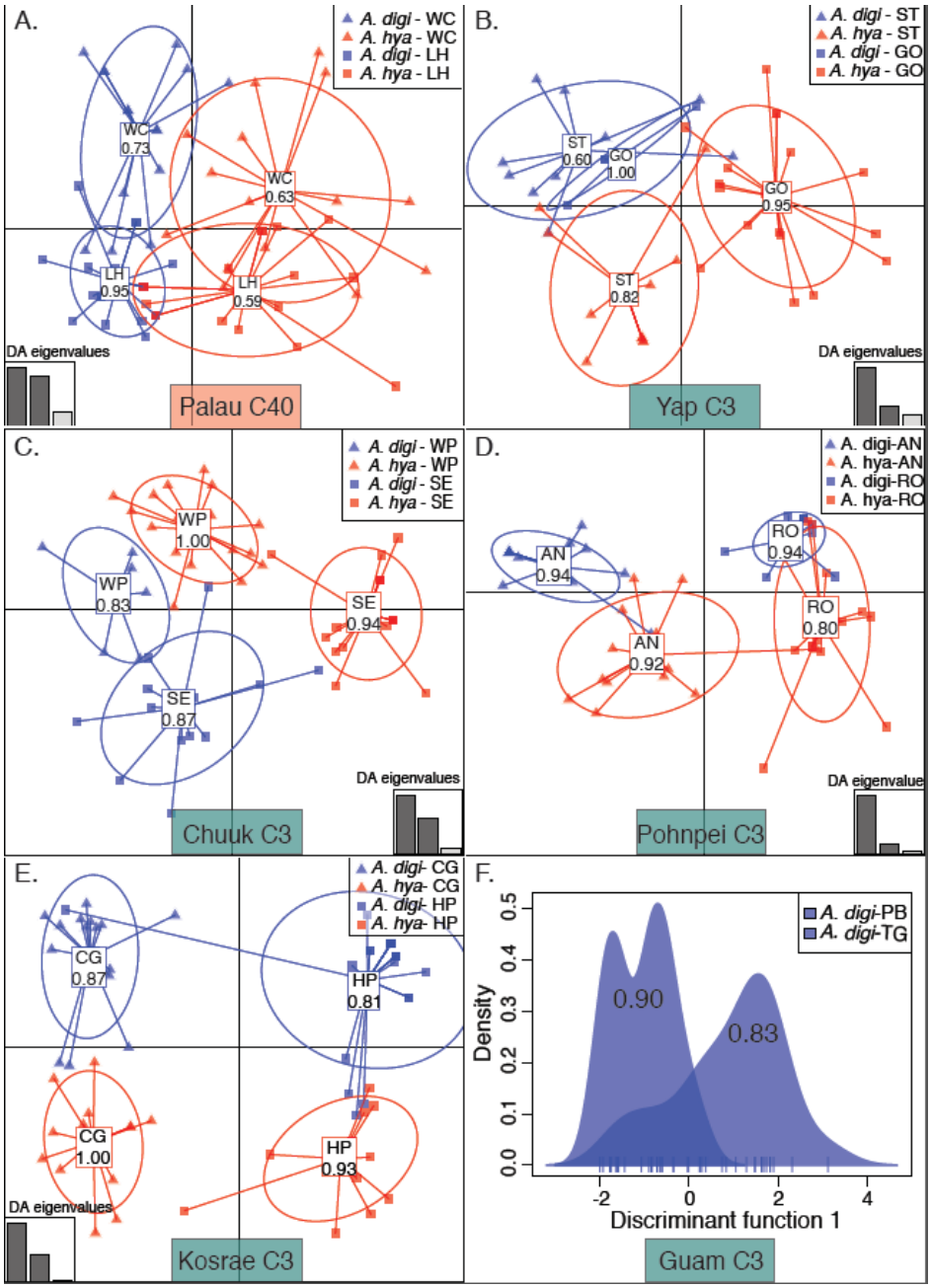


Figure 6: Discriminant analysis of principal components (DAPC) of MLG data for *Symbiodinium* C40 and C3 hosted by *Acropora hyacinthus* and *Acropora digitifera* at twelve sites across six islands (Ngulu not included) in Micronesia. DAPC analysis on two discriminant functions demonstrating host species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

246 **Discussion**

247 *Promiscuity of the coral host*

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

260

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

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

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

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

316 **Conclusions**

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

337

338

339 **Materials and Methods**

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

346

347 Table 5. Reef Site Collections. GPS coordinates, main island group, number of *A. digitifera* and
348 *A. hyacinthus* hosts genotyped. The numbers in brackets are individuals hosting a single
349 *Symbiodinium* MLG, which were included in all analyses presented here. Site letter corresponds
350 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)**

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

354

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

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

369

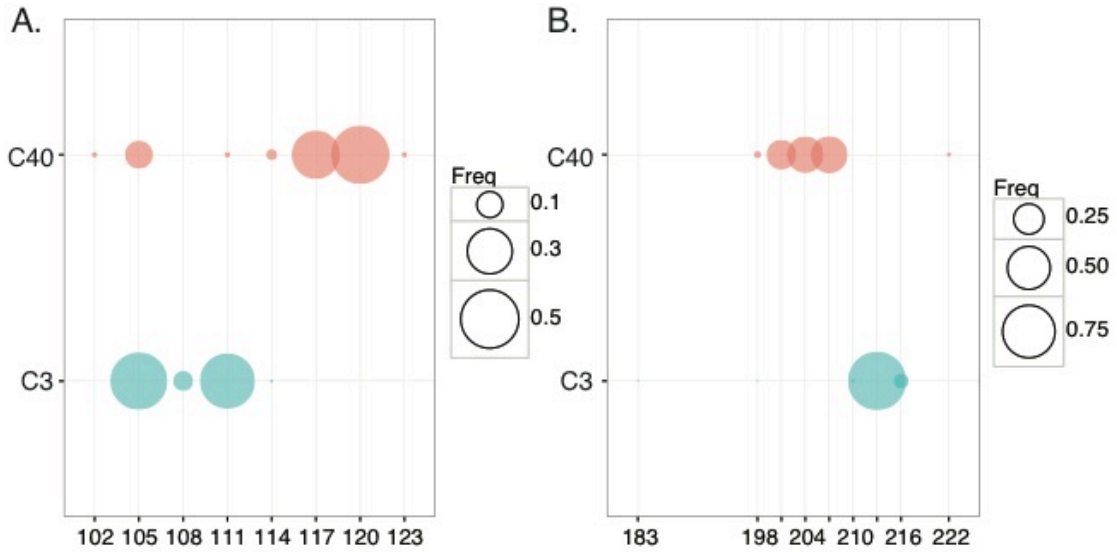
370 Table 6: Summary of six polymorphic microsatellite loci used to assess genetic variation
371 *Symbiodinium* clade C hosted by *A. hyacinthus* and *A. digitifera* and their corresponding
372 multiplexing groups.

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

373 Fadp- labeled primers were indirectly labeled in each PCR reaction with an additional FAM labeled adapter
374 tag sequence: FAM: agcagcgaactcagtacaaca
375 Hadp- labeled primers were indirectly labeled in each PCR reaction with an additional FAM labeled
376 adapter tag sequence: HEX: tcgtcgcttgagtcatcgtta
377
378

379 ***Data Analysis:*** *Symbiodinium* in clade C microsatellite data were scored as diploid based
380 on available information on the ploidy of these organisms (3, 62, 65) Since each
381 individual host could potentially contain a population of genetically distinct
382 *Symbiodinium* individuals, multi-locus genotypes (MLG) were only considered in this
383 study if they contained two or fewer alleles across all loci, suggesting that all alelles
384 originate from a single, clonally replicated, genome (65), which was the case for a total of
385 190 out of 277 for *A. digitifera* hosts and 178 out of 278 for *A. hyacinthus* hosts. We first
386 applied a Bayesian approach implemented in STRUCTURE v2.3.3 (66). STRUCTURE uses a
387 Monte Carlo Markov chain (MCMC) clustering algorithm to assign individuals with
388 similar genotypes to populations. Mean and variance of log likelihood values of the
389 number of populations K (1-10) were inferred by STRUCTURE with 10^6 iterations (burn in
390 = 300,000 iterations) in ten replicate runs for each K. An admixture model was
391 implemented with no location prior. Following the recommendations of (67), the ad hoc
392 statistic ΔK was calculated based on the rate of change of the log-likelihood between
393 consecutive K values, which is implemented in the program STRUCTURE Harvester (68).
394 CLUMPP (69) and DISTRUCT (70) were used to produce graphics. We observed high
395 percent assignment to two different clusters among sympatric individuals. Following the
396 methodology of Wham and LaJeunesse (65), we investigated the relationship between
397 these cluster assignments and allele identities. From this analysis we found a strong
398 relationship between cluster assignments and allele identity, particularly at Sgr_21 and
399 Spl_33 (Supplement Fig. S1A, B), and it became clear that two distinct *Symbiodinium*
400 lineages were present. MLG data were then split into *Symbiodinium* lineages based on the
401 probability of species assignment conditioned on their cluster assignment (65).

402 Additional STRUCTURE runs were completed following descriptions above with these
403 subsequent differences: 1) location prior was implemented and 2) instead of using the ΔK
404 statistic, we presented the K that maximized the mean of the estimated probability of data
405 while minimizing the standard deviation.



406

407 Supplementary Figure 1: Bubble plot of the SSR allele frequencies of C3 (red) and C40 (blue) at
408 the two loci that showed the largest discrimination power, Sgr_21 (A) and Spl_33 (B).
409 The size of each bubble is proportional to the allele frequency within each *Symbiodinium* type.
410

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

418 To resolve differences between islands, between host species and between sites
419 and host species within each island, assignment of samples to genetic clusters using

420 discriminant analysis of principal components (DAPC) was performed in R (73) using the
421 ADEGENET package (74, 75). Here, *Symbiodinium* data were converted into principle
422 components and then a-scores were used to determine trade-offs between power of
423 discrimination and model over-fitting. Relationships were examined by DAPC, which
424 optimizes variation among clusters by minimizing variation within clusters, while
425 retaining the optimal number of principle components and maximum number of
426 discriminant functions. All information on DAPC model parameters and results are
427 contained in Table 3. Cluster assignment patterns were compared across islands, host
428 species, and among host species and sites within each island.
429

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

431 In order to confirm the assignments of individuals to different *Symbiodinium* lineages, we
432 selected a small subset of the full sample set and analyzed the non-coding region of the
433 circular plastid (psbA^{ncr}). We amplified the psbA^{ncr} locus with the primers 7.4-Forw and
434 7.8-Rev following the methods described by LaJeunesse and Thornhill (40). The
435 amplified product was directly sequenced and the resulting sequences were aligned with
436 ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) to clade C psbA^{ncr} sequences
437 from common *Symbiodinium* types found throughout the Indo-Pacific region
438 (Supplemental Table 1: JQ043587-JQ043676 from (40)). We reconstructed the
439 phylogeny of these samples using the default settings of Phylogeny.fr (76). The identities
440 of *Symbiodinium* types from our analysis were interpreted from their percent sequence
441 identity with these previously identified *Symbiodinium* types and their position on the
442 resulting phylogeny.
443

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666
667
668

669 Supplemental Table 1: Accession number and corresponding Clade C *Symbiodinium* lineage for
670 each unique sequence identifier from Figure 2.
671

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

672