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Heritability of the *Symbiodinium* community in vertically- and horizontally-transmitting broadcast spawning corals

Kate M. Quigley[1,2*], Bette L. Willis[1,2], Line K. Bay[2,3]

[1]ARC Centre of Excellence for Coral Reef Studies, and College of Marine and Environmental Sciences, James Cook University, Townsville, QLD 4811, Australia

[2]AIMS@JCU, Australian Institute of Marine Science and James Cook University, Townsville, QLD 4811, Australia

[3]Australian Institute of Marine Science, PMB3, Townsville, Queensland 4810, Australia

*Corresponding author: KM Quigley

Address: College of Marine and Environmental Sciences, James Cook University, Townsville, QLD 4811, Australia

Email: katemarie.quigley@my.jcu.edu.au

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Abstract

The dinoflagellate-coral partnership influences host tolerance to thermal stress that causes bleaching. However, the comparative roles of host genetic versus environmental factors in determining the composition of this symbiosis are largely unknown. Here we quantify the heritability of *Symbiodinium* communities in two broadcast spawning corals with different symbiont transmission modes: *Acropora tenuis* has environmental acquisition, and *Montipora digitata* has maternal transmission. Using high throughput sequencing of the ITS-2 region to characterize communities in parental colonies, juveniles and eggs, we describe new *Symbiodinium* types in both coral species and previously unknown symbiont dynamics. After one month of natural uptake in the field, *Symbiodinium* communities associated with *A. tenuis* juveniles were dominated by A3, C1, D1, A-type CCMP828, and D1a in proportional abundances that were conserved across two years. In contrast, *M. digitata* eggs were predominantly characterized by C15, D1, and A3. On average, host genetic influences accounted for 29% of phenotypic variation found in *Symbiodinium* communities in *A. tenuis* and 62% in *M. digitata*. Our results reveal hitherto unknown flexibility in the acquisition of *Symbiodinium* communities and substantial heritability in both species provides ample material for selection to produce partnerships that are locally adapted to changing environmental conditions.

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Introduction

Coral bleaching, defined as either the loss of *Symbiodinium* cells from coral tissues or reduction in symbiont photosynthetic pigments, represents a threat to coral reefs world-wide as it increases in both frequency and magnitude [1–4]. If coral reefs are to persist under climate change, corals must either disperse to new unaffected habitats, acclimate through phenotypic plasticity, and/or adapt through evolutionary mechanisms [5]. However, the extent to which thermal tolerance can increase through changes to the host genome, *Symbiodinium* community hosted, or direct selection on the symbionts themselves is currently unclear.

Bleaching sensitivity is variable within and among species [6], but the mechanisms causing this variability remain relatively unknown. Although the coral hosts have a repertoire of molecular mechanisms that provide some capacity to respond to thermal stress (e.g. antioxidant pathways and fluorescent pigments) [7–9], the *Symbiodinium* community hosted by corals has long been recognized as the primary factor determining bleaching susceptibility [10,11]. Recent evidence shows that bleaching is primarily caused by the expulsion of *Symbiodinium* cells, rather than host-cell degradation or host-cell detachment [12], and further corroborates the hypothesis that coral holobiont stress responses are driven by attributes of the photosymbiont. The increased adaptive potential of *Symbiodinium*, because of their short generation times and large population sizes compared to corals [13], raises the possibility these endosymbiotic communities could influence host adaptation to changing climates through increased host niche expansion [14,15]. A major impediment to understanding the capacity of corals to adapt to a changing climate is the lack of knowledge about the extent to which *Symbiodinium* communities associated with corals are inherited and hence subject to selection.

There are nine recognized *Symbiodinium* clades [16] which encompass substantial sequence and functional variation at the intra-clade (type) level (reviewed in [17]. Traditional technologies used in the field have most often overlooked taxonomic resolution at the type level, leading to the majority of studies comparing *Symbiodinium* communities at the clade level and generally only for dominant symbionts. Deep sequencing technologies currently available can detect type level diversity even at low abundances [18] and are now being applied to understand adult coral-*Symbiodinium* diversity [19–21], but are lacking for the early life-history stages of corals. Therefore, there are gaps in our basic knowledge of the composition of *Symbiodinium* communities at lower, functionally relevant taxonomic levels, in particular, those at background abundances and in the eggs and juveniles of corals.

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Natural variation in the composition of coral-associated *Symbiodinium* communities exists among coral populations and species [17,22], with certain communities offering greater bleaching resistance compared to others [23,24]. It is not yet known what enhances or constrains the capacity of corals to harbour stress tolerant *Symbiodinium* types and whether changes to *Symbiodinium* communities in response to environmental stressors are stochastic or deterministic [25]. Given the importance of *Symbiodinium* communities for bleaching susceptibility and mortality of the coral holobiont [26,27], quantifying the proportional contributions of genetic and environmental factors to community formation, regulation and stress tolerance is important for understanding coral health. If the *Symbiodinium* community is heritable, changes to these communities may bring about adaptation of the holobiont as a whole. Under this scenario, *Symbiodinium* community shifts are equivalent to changes in host allele frequencies, thus opening up new avenues for natural and artificial selection, assisted evolution and microbiome engineering [25,28].

Symbiodinium communities associated with scleractinian corals are either acquired from the environment (horizontal transfer) or passed maternally from adults to eggs or larvae (vertical transfer). Approximately 85% of scleractinian coral species broadcast spawn eggs and sperm into the environment, and of these, ~80% acquire symbionts horizontally; the remaining ~20% acquire them vertically [29]. Vertically-transmitted symbiont communities are predominantly found in brooding corals with internal fertilization [29] and are theorized to be of lower diversity and higher fidelity [17]. Conversely, horizontal transmission has generally been assumed to result in weaker fidelity that can be increased through the development of strong genotype- associations between host and symbiont community [30]. Studies specifically quantifying the genetic component governing the *Symbiodinium* community established in offspring of both horizontal and vertical transmitters are needed to elucidate the potential for adaptation through symbiont community changes.

Heritability describes the genetic components of variability in a trait using analysis of co-variance among individuals with different relatedness [31]. The ratio of additive genetic variance to phenotypic variance (V_A/V_P) is defined as narrow-sense heritability (h^2) [32]. The degree of heritability of a trait ranges from 0 - 1, and describes the influence of parental genetics on the variability of that trait [32]. Therefore, the degree to which traits might change from one generation to the next can be predicted from measures of heritability, where the predicted change in offspring phenotype is proportional to h^2 (i.e., the breeder's equation) [33]. It is particularly important to determine the genetic contribution to understand the potential

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for adaptation and to predict the strength of response to selection (i.e, the 'evolvability' of a trait) [5,34,35].

To quantify the potential for selection of endosymbiotic *Symbiodinium* communities associated with broadcast spawning corals in response to changes in environmental conditions (i.e., climate change-induced), we characterized symbiont communities associated with adults, juveniles and eggs of the horizontal transmitter *Acropora tenuis* and the vertical transmitter *Montipora digitata* using high-throughput sequencing. Using a community diversity metric, we derive the narrow-sense heritability (h^2) of these communities and identify new and unique *Symbiodinium* types recovered from juveniles and eggs compared to their parental colonies. Finally, we describe previously unknown *Symbiodinium* community dynamics in the early life- history stages of these two common coral species.

Results

Symbiodinium communities associated with *Acropora tenuis*

After one month in the field, there were clear similarities at the clade level between *Symbiodinium* communities associated with the 2012 and 2013 families of *A. tenuis* juveniles, with 54 OTUs (17.1%) shared between the two years (Fig. 1, Supplementary Table S4). In both years, the majority of OTUs were recovered from three clades (A, C, and D) and the number of OTUs from each of these clades was similar between years (Supplementary Table S4). The greatest diversity of OTUs found in juveniles from both years belonged to C1, A3 and uncultured types, and a diversity of different OTUs within types A13, CCMP828, D1 and D1a were also present (Supplementary Fig. S1). The predominant patterns characterising *Symbiodinium* communities associated with the 2012 and 2013 families were the high abundance of *Symbiodinium* types A3, C1, D1, and A-type CCMP828, and the comparatively lower abundance of D1a (Fig. 2). However, substantial variation in *Symbiodinium* diversity and abundance existed between juveniles within the same family as well as between families of juveniles (supplementary results, Fig. 2). For example, juvenile families differed in their average OTU diversity and abundance as well as taxonomic composition (additional description in supplementary results, Fig. 2, Supplementary Table S5), where particular families contained juveniles of particularly high diversity (F14 and F18).

Juveniles from both years harboured more unique OTUs compared to adults (Juveniles vs. adults: 111 vs. 2 (2012), 151 vs. 2 (2013)), with far fewer OTUs shared between life stages (2012: 21, 2013: 28) (Fig. 1). Furthermore, the majority of OTUs in both years were at background abundances (Fig. 1). The majority of OTUs were also rare (112 -

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172 OTUs found in less than 25% of samples in 2012 and 2013), whilst 4 - 16 OTUs were common (25 -75% of samples), and 5 - 6 OTUs were core members (two A3 types, CCMP828, C1, D1, D1a) (Fig. 1).

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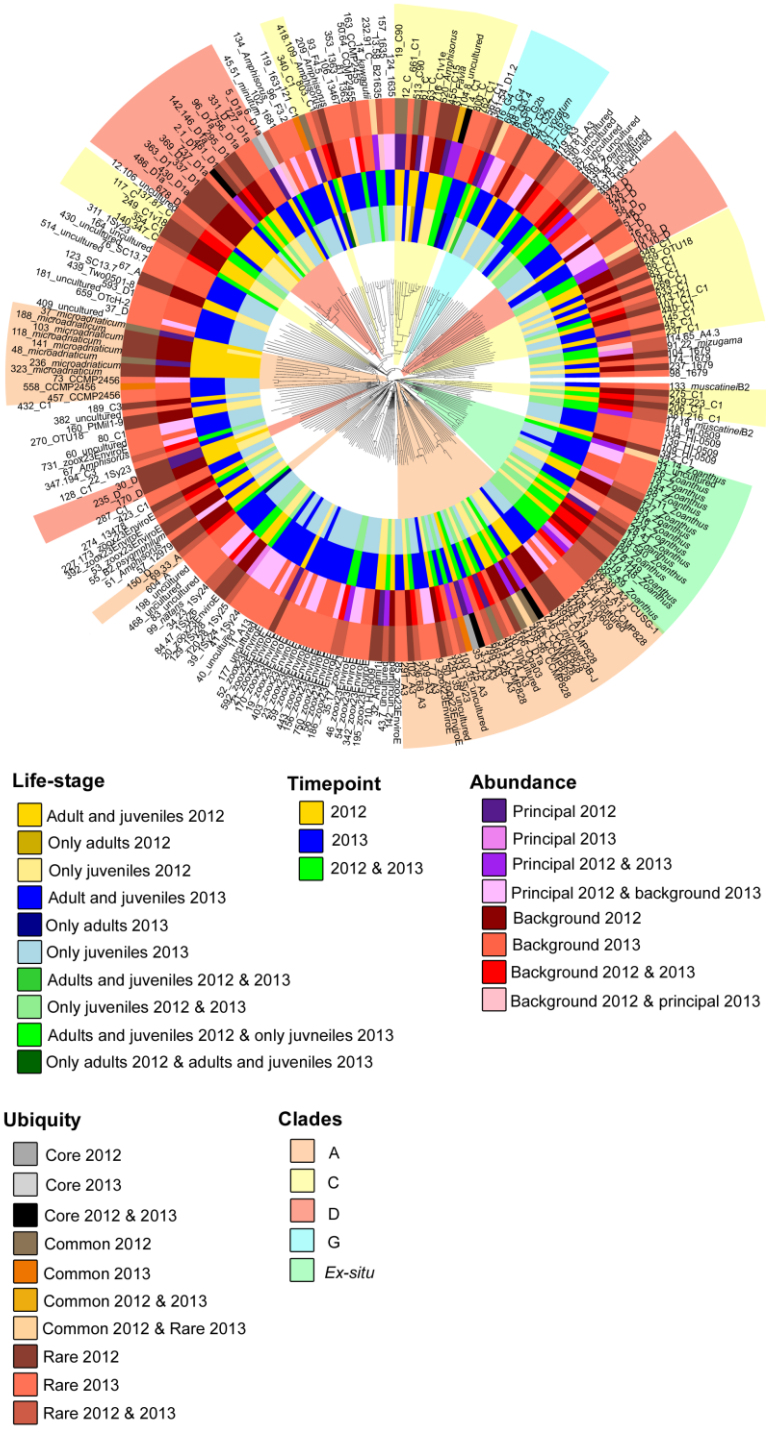

Figure 1. Fan dendrogram of 261 *Symbiodinium* ITS-2 OTUs retrieved from *Acropora tenuis* juveniles and adults in 2012 and 2013. The Neighbour-Joining dendrogram was constructed using raw APE alignments of only those OTUs that were retrieved from three or more samples (134/422 OTUs in 2012 and 181/568 OTUs in 2013). Concentric circles from innermost to the outermost position represent OTUs present: 1) life-stage, 2) year, 3) normalized abundance (principal: > 0.01%, background < 0.01%), 4) ubiquity (Core: >75% of samples, Common: 25-75%, Rare: < 25%). Semi-transparent backgrounds represent clade designations of individual OTUs.

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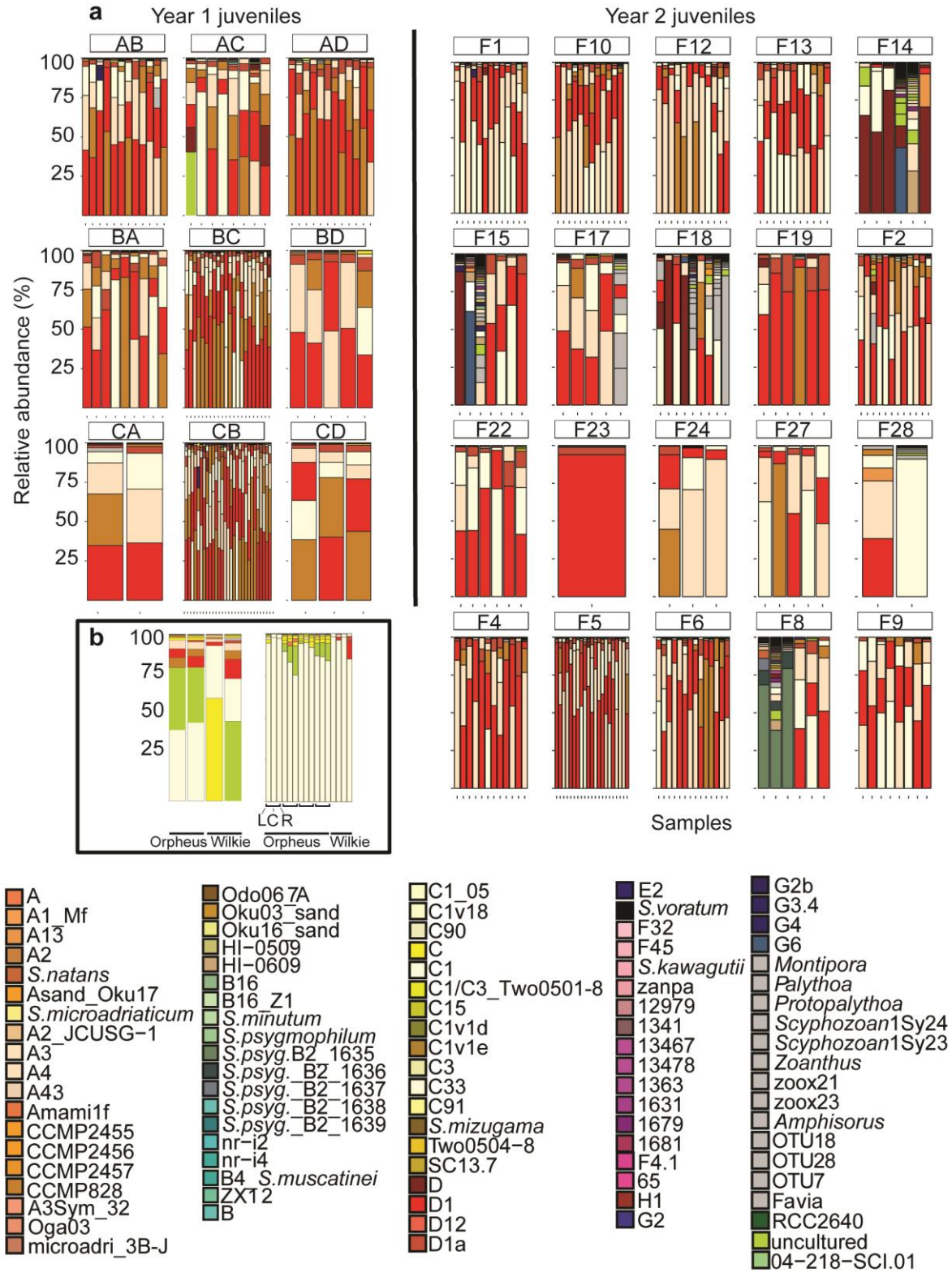

Figure 2. Barplots of variance-normalized abundances of *Symbiodinium* diversity associated with (a) juveniles and (b) adults of *Acropora tenuis* used in 2012 (Year 1) and 2013 (Year 2) crosses. Colours represent different *Symbiodinium* types. Origins of parent colonies are Orpheus and Wilkie reefs. *A. tenuis* adult colonies from Orpheus used for 2013 crosses included samples that were sequenced that represent the left side of the colony (L), center of the colony (C), and right side of the colony (R) to examine intra-colony *Symbiodinium* diversity.

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Symbiodinium* communities associated with *Montipora digitata

101 OTUs were found in *M. digitata* eggs and adults, with on average 7 (±0.9 SE) OTUs per egg and 5.3 (±0.9 SE) OTUs per adult. The highest diversities of OTUs were retrieved from clades A (73 OTUs) and C (18 OTUs), whereas D and *ex-situ* types each had three OTUs represented (Fig. 3). 99.1% of the total cleaned reads belonged to C15 (OTU1), with this type making up 98.8 % (±0.5 SE) and 99 % (±0.1 SE) of all reads retrieved from dams and eggs, respectively. The next most abundant OTUs were an *ex-situ Amphisorus* identified OTU (potentially another C15 type), D1, A3, and two other A types (A and CCMP828) (Fig. 4). Adults could generally be distinguished from eggs by the unique presence of A2, A3, HA3-5, C1_8, G3 (Fig. 3) and a greater proportional abundance of a type A symbiont (OTU4) in dams 29, 32, 7, 8 and 9 (Fig. 4). Of these five unique adult OTUs, none were found in more than two adult colonies. Eighty-two OTUs were found in eggs but not adults and 43 of these were found in three or more eggs, and a majority were uncultured types at background levels from the eggs of dam 29 (Fig. 3). Both inter- and intra- family variation in background *Symbiodinium* OTU composition and abundance were detected within eggs as well (further description in supplementary results, Supplementary Fig. S2, Supplementary Table S6).

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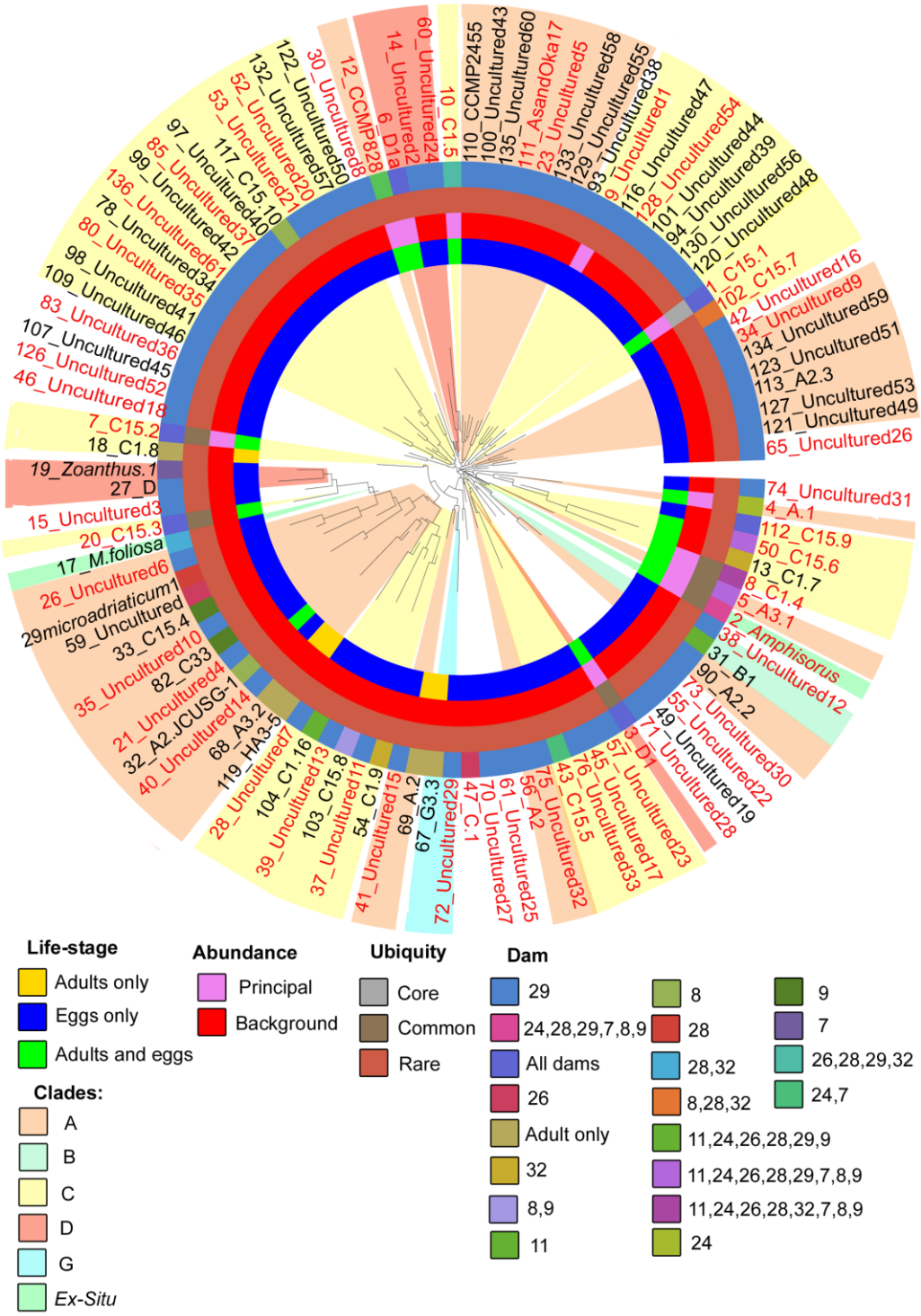

Figure 3. Fan dendrogram of 101 *Symbiodinium* ITS-2 OTUs retrieved from *Montipora digitata* eggs and adults. The Neighbour-Joining dendrogram was constructed using raw APE alignments. Concentric circles from innermost to the outermost position represent OTUs present: 1) life-stage, 2) normalized abundance (principal: > 0.01%, background < 0.01%), 3) ubiquity (Core: >75% of samples, Common: 25-75%, Rare: < 25%), and 4) dam identity. Semi-transparent backgrounds represent clade designations of individual OTUs. Red text indicates OTUs that were found in three or more eggs.

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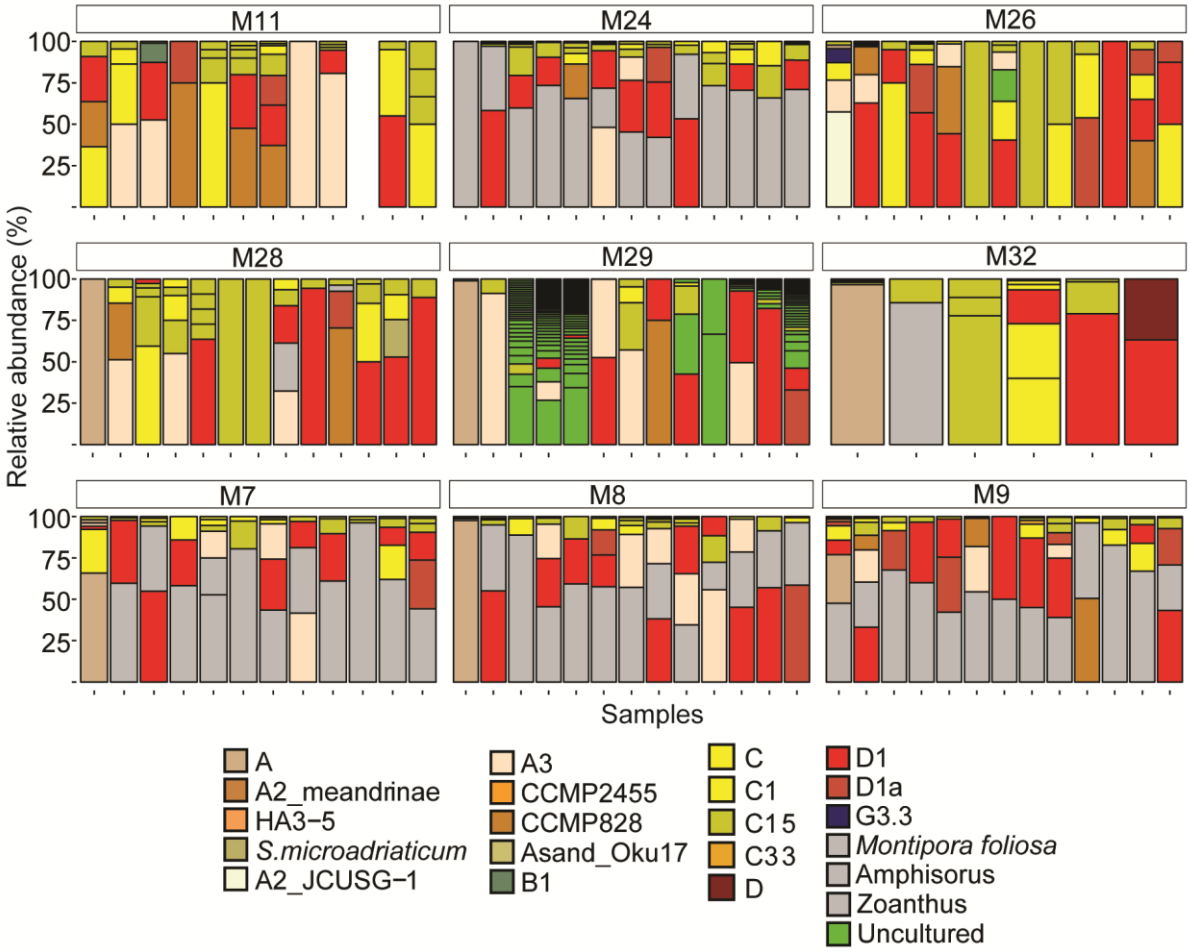

Figure 4. Barplot of variance-normalized abundances of the background *Symbiodinium* diversity associated with dams and eggs of *Montipora digitata*. Colours represent different *Symbiodinium* types. The dominant type, C15, was excluded for clarity. The first bar in each group is the spawning dam and the following bars represent her eggs. The tenth egg sample from dam 11 (M11) was made up of 100% C15, and was therefore not shown.

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208 **Narrow-sense heritability of *Symbiodinium* community in *A. tenuis* juveniles and *M.***
209 ***digitata* eggs**

210 Bayesian linear mixed models, and specifically, the animal model, were used to
211 estimate relatedness-based heritability as they are robust to unbalanced designs. Furthermore,
212 the animal model utilizes all levels of relatedness between individuals in a given dataset, and
213 not just parent-offspring comparisons, thus giving it greater power for data collected in field
214 studies [36]. The Bayesian narrow-sense heritability estimate (h^2) of the *Symbiodinium*
215 community in *A. tenuis* juveniles was 0.29, with a 95% Bayesian credibility interval for the
216 additive genetic component of 0.06-0.86. The mean heritability was 0.36 (±0.21 SD) (Table
217 1). The high density of estimates between 0.2 - 0.4 within the posterior distribution of h^2
218 suggests high statistical support around 0.29 despite the credibility interval being very large.
219 The maternal transfer of *Symbiodinium* in the broadcast spawning coral *M. digitata* had a
220 narrow-sense heritability estimate of 0.62 (0.27-0.86 95% Bayesian credibility interval), with
221 a mean heritability of 0.57 (±0.16 SD) (Table 1). We did not detect an effect of maternal
222 environment on similarities in *Symbiodinium* diversity among eggs or among juveniles.
223 Models that included maternal effects arising from eggs developing in a shared environment
224 (maternal environmental effects for both *A. tenuis* and *M. digitata*) were not significantly
225 better than those that did not include maternal effects (DIC no effects < DIC maternal
226 environmental effects included).

227 Mid-parent regression estimates for the 29 *A. tenuis* families from 2012 and 2013
228 indicated that trait-based h^2 of the *Symbiodinium* community was 0.3 (Supplementary Fig.
229 S3). Parent-offspring regression of the 99 *M. digitata* eggs genotyped from nine dams
230 resulted in a heritability estimate of 0.156 (slope= 0.078 x 2 as a single parent)
231 (Supplementary Fig. S4). Therefore, 30% and 16% of the measured variation in the
232 *Symbiodinium* community in *A. tenuis* and *M. digitata*, respectively, was due to genetic
233 differences between offspring.
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Table 1. Summary of the narrow-sense heritability estimates obtained for *A. tenuis* and *M. digitata* in this study using relatedness and trait-based heritability methods.

Symbiont acquisition mode	Heritability estimation method:	Relatedness-based (Bayesian)				Trait-based (regression)
		h^2 (mode)	95% Bayesian credibility interval	h^2 (mean)	± SD	h^2 (slope)
Environmental	*A. tenuis*	0.29	0.06-0.86	0.36	0.21	0.30
Maternal	*M. digitata*	0.62	0.27-0.86	0.57	0.16	0.16

Discussion

Substantial heritability of the *Symbiodinium* community in both vertically- and horizontally-transmitting corals highlights the important role of host genetics in governing the composition of symbiont communities within their tissues. Surprisingly, mean Bayesian heritability estimates for *Symbiodinium* communities associated with juveniles of *Acropora tenuis* were high (0.29) given expected low levels of fidelity for species with environmentally-acquired symbionts. Conversely, heritability estimates associated with eggs of *Montipora digitata* were low (0.62) given the high levels of fidelity expected for vertically-transmitted symbionts. Although our results differed from expectations of fidelity and heritability based on current transmission paradigms in corals, they are consistent with studies that have demonstrated the host genetic role in governing the composition of symbiotic bacterial communities in mammals, insects and other cnidarians [37–40], as well as the abundance of bacteria in insects [41] and humans [42]. Furthermore, these estimates are consistent with the characteristic hallmarks of host-controlled symbiont regulation. For example, *Symbiodinium* cells are enveloped in a host-derived symbiosome, with only a few (2-8) symbiont cells per host membrane [43]. This indicates that the coral host regulates *Symbiodinium* on an almost individual cell basis, facilitating overall population regulation [41] and potentially community composition within the holobiont. Thus, it is likely advantageous for the host's molecular architecture governing the *Symbiodinium* community to be passed from one generation to the next. Importantly, the partial genetic regulation in *Symbiodinium* communities found here suggests that there is potential for the symbioses to evolve and adapt and therefore potentially develop 'optimal' symbiont-host partnerships under changing environmental conditions.

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Our results provide the first in-depth picture of the complexity of the *Symbiodinium* community in *A. tenuis* juveniles during the initial month of uptake. No juveniles exclusively hosted a single clade or type, a result corroborated by lab and other field-based experiments [44–49]. Moreover, although the diversity measured here was much greater than those reported in these previous studies; we found strong temporal stability in OTU diversity and abundances between the two years. Therefore, the unexpectedly high fidelity of the symbiont community in conjunction with our heritability estimates suggests strong host genotype – symbiont community associations, a result also implicated in studies comparing symbiosis prevalence across phylogenetic relationships in *Hydra*, wasps, and primates [30]. Further work is needed to map the *Symbiodinium* diversity in juvenile corals as well as elucidate the molecular mechanisms regulating the establishment of this symbiosis.

The strong temporal stability in the relative proportions and numbers of OTUs at principal and background levels suggests that the genetic regulation governing the *Symbiodinium* community extends to OTUs found at very low abundance. Although their significance for juvenile physiology is currently unclear, *Symbiodinium* at background abundances in adults are central to coral health. For example, fine scale dynamics of *Symbiodinium* communities (i.e., changes in relative abundance and/or diversity of only a fraction of types) impact host bleaching susceptibility, recovery and physiology [27,50]. Although background types are likely to have a negligible effect compared to principal types like A3, C1, and D1 when corals are healthy, they may increase in importance when environmental conditions are sub-optimal. On the other hand, the presence of many low-abundance types may have negative fitness outcomes for coral juveniles if they reflect the inability of the coral host to maintain stable symbioses with beneficial types. This hypothesis is supported by our observation of a correlation between *Symbiodinium* diversity and juvenile mortality among particular coral families [51] and suggests that these high-mortality families had lost the ability to regulate community diversity, leading to a proliferation of potentially opportunistic types. This proliferation of initially low abundance types could represent dysbiosis of symbiotic communities, which is known to lead to disease in human populations [52,53] and could also impact coral health outcomes.

The heritable signal found for *Symbiodinium* communities associated with eggs of the vertically-transmitting coral *M. digitata* was strong (62%), but fidelity was less than expected given that eggs acquire *Symbiodinium* communities in the maternal environment. Despite *Symbiodinium* C15 dominating symbiont communities in both eggs and dams, maternal transfer lacked precision in one dam in particular (dam 29), whose eggs had highly variable

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Symbiodinium communities that included uncultured OTUs, similar to previous reports from this coral genus [54]. There are many precedents for inexact maternal transfer of symbiont communities, and studies on insects show that vertical transmission is rarely perfect [55] due to symbiont competition within hosts [56]. Such imprecision in maternal transfer is a derivative of fitness costs associated with the maintenance of superinfections (stable coexistence of multiple symbionts) and can be overcome if selection for coexistence is greater than the costs of its maintenance. Superinfections may provide a diversity of beneficial symbiont traits. For example, different symbionts provide different nutrients to the host insects [57]. For *M. digitata,* imprecision may represent a bet-hedging strategy to maximise the likelihood that some offspring will survive when eggs are dispersed and encounter environments that are different to their parents. This variation also highlights the potential flexibility of the *M. digitata-Symbiodinium* symbiosis, which may enable the host to vary its symbiotic partnerships in response to environmental change by benefitting from new host-symbiont combinations.

Surprisingly, much of the diversity found in *M. digitata* eggs was not present in parent colonies, a result previously observed in larvae of the brooding, vertically-transmitting coral *Seriatopora hystrix* (Quigley et al. *in-review*) and between *A. tenuis* juveniles and adults (this study). This suggests that eggs are acquiring symbionts from sources external to the maternal transmission process. Mixed systems involving both vertical and horizontal transmission are known (e.g. bacteria in clams; reviewed in [30] and have recently been demonstrated in brooding corals (Quigley et al. *in-review*). The cellular machinery needed for recognition of appropriate *Symbiodinium* types [43] would not be developed in the egg cytoplasm, where *Symbiodinium* are present pre-fertilization [58]. Therefore, eggs exposed to transient symbionts in the dam's gastrovascular cavity or by parasitic *Symbiodinium*-containing vectors (e.g. ciliates [59]; symbiont transmission by parasitic vectors: reviewed in [56]), may retain these communities until recognition systems of eggs, larvae or juveniles mature. Interestingly, one type (OTU111) found in three eggs from dam 29 were identified as a free-living A type recovered from Japanese marine sediments (EU106364, [60]), suggesting that these OTUs represent non-symbiotic, potentially opportunistic symbionts. Further work is needed to determine what potential ecological role these symbionts fulfil.

Maternal environmental effects, such as lipid contributions by dams, have well known effects on the early life stages of many marine organisms [61]. However, our Bayesian models were not significantly improved with the addition of dam identity, suggesting that significant heritability estimates are attributable to genetic effects and not due to maternal environmental effects [36] or cytoplasmic inheritance [62]. Whilst we can only speculate about the exact

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mechanisms that are being inherited by offspring, likely candidates include those involved in recognition and immunity pathways [43], with cell-surface proteins playing an important role in the selection of specific *Symbiodinium* strains by coral hosts [63–65]. For example, these may include Tachylectin-2-like lectins, which have been implicated in the acquisition of A3 and a D-type in *A. tenuis* [44,66,67]. Indeed, suppression or modification of the immune response has often been implicated in the formation of the *Symbiodinium*-cnidarian partnership [43,68,69]. Although this has not yet been demonstrated in corals, human studies have shown that immune system characteristics underpin heritable components of the genome [70] and at least 151 heritable immunity traits have been characterized, including 22 cell-surface proteins [71].

Similarly, the juvenile coral may be primed to take up specific *Symbiodinium* types through the transfer of genetic machinery that results in a bi-product(s) to ensure the juvenile is colonized by beneficial types and prevents colonization by unfavourable symbionts through competitive exclusion (e.g., maternal imprinting controlled by offspring loci [62]). Such bi-products may be akin to amino acids, which have been shown to regulate the abundances of *Symbiodinium* populations [72]. Sugars have also been found to influence bacterial communities in corals [73] and may have similar roles in regulating *Symbiodinium* communities. Trehalose, in particular, has been identified as an important chemical attractant between *Symbiodinium* and coral larvae and may help to regulate the early stages of symbiosis [74]. Human studies also provide examples of sugars (both maternal and offspring derived) that make infant intestines less habitable for harmful bacteria, setting up conditions for preferential colonization by favourable bacteria [75]. Bacterial diversity in cnidarian hosts can also be modulated through the production of antimicrobial peptides [37] and bacterial quorum sensing behaviour [76]. Although neither of these mechanisms have been explored in corals, similar host/symbiont bi-products may be influential in the regulation of *Symbiodinium* communities.

In conclusion, the results presented here provide new insights into the role of host genetics and inheritance in governing *Symbiodinium* communities in corals. This information is crucial for understanding factors governing coral health and fitness under stress conditions (i.e. bleaching risk), as well as the potential for host-symbiont partnerships to evolve. Variability in the symbiont community within and among families and evidence that that variation is heritable supports the likelihood that adaptive change is possible in this important symbiotic community. These results will also aid in the development of active reef restoration methods focused on the assisted evolution of hosts and symbionts as they take advantage of the link between genetic mechanisms and desired phenotypes, in which targeted traits with

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moderate to high heritability increase the efficacy of breeding schemes. Adaptive change through heritable variation of symbionts is therefore another mechanism that corals may use to contend with current and future stressors, such as climate change.

Materials and Methods

Experimental breeding design and sample collection

For crossing experiments, gravid colonies of the horizontally-transmitting broadcast-spawning coral *Acropora tenuis* were collected in 2012 and 2013 from the northern (Princess Charlotte Bay (PCB): 13°46'44.544"S, 143°38'26.0154"E) and central Great Barrier Reef (GBR) (Orpheus Island: 18°39'49.62"S, 146°29'47.26'E).

In 2012, nine families of larvae were produced by crossing gametes from four corals (OI: A-B, PCB: C-D) on 2 December following published methods [51]. The nine gamete crosses excluded self-crosses (Supplementary Table S1). Larvae were stocked at a density of 0.5 larvae per ml in one static culture vessels per family in a temperature-controlled room set at 27°C (ambient seawater temperature). Water was changed one day after fertilization and every two days thereafter with 1 µM filtered seawater at ambient temperature. To induce settlement, 25 settlement surfaces (colour-coded glass slides) were added to each larval culture vessel six days post-fertilization, along with chips of ground and autoclaved crustose coralline algae (CCA, *Porolithon onkodes* collected from SE Pelorus: 18°33'34.87"S, 146°30'4.87"E). The number of settled juveniles was quantified for each family, and then placed randomly within and among the three slide racks sealed with gutter guard mesh. The racks were affixed to star pickets above the sediments in Little Pioneer Bay (18°36'06.2"S, 146°29'19.1"E) 11 days post fertilization. Slide racks were collected 29 days later (11 January 2013), after which natural infection by *Symbiodinium* had occurred. Juveniles from each cross were sampled (n = 6 - 240 juveniles/family, depending on survival rates), fixed in 100% ethanol and stored at -20°C.

In 2013, 25 families were produced from gamete crosses among eight parental colonies: four from PCB and four from Orpheus Island (full details of colony collection, spawning, crossing and juvenile rearing in [51] (Supplementary Table S2). Larvae were raised in three replicate cultures per family. Settlement was induced by placing autoclaved chips of CCA onto settlement surfaces, which were either glass slides, calcium carbonate plugs or the bottom of the plastic culturing vessel. Settlement surfaces with attached juveniles were deployed randomly to the same location in Little Pioneer Bay as in 2012 19 days post

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fertilization and collected 26 days later. Samples of juveniles (n = 1 - 194 juveniles per family) were preserved and stored as in 2012.

Thirty-two gravid colonies of the vertically-transmitting broadcast spawner *Montipora digitata* were collected from Hazard Bay (S18°38.069', E146°29.781') and Pioneer Bay (S18°36.625', E146°29.430') at Orpheus Island on the 30th of March and 1st of April 2015. Colonies were placed in constant-flow, 0.5 µM filtered seawater in outdoor raceways at Orpheus Island Research Station. Egg-sperm bundles were collected from a total of nine different colonies on the 4th and 5th of April, separated with a 100 µm mesh and rinsed three times. Individual eggs and adult tissue samples were then placed in 100% ethanol and stored at -20°C until processing.

Sequencing of *Symbiodinium* ITS-2 in egg, juvenile and adult coral samples

The number of juveniles of *A. tenuis* sequenced from each of the 9 crosses in 2012 ranged from 2 - 29 individuals (average ± SE: 11.3 ± 3) (Supplementary Table S1) and a single sample from each parental colony was sequenced concurrently. In 2013, 1 - 21 *A. tenuis* juveniles (average ± SE: 8.6 ± 1) were sequenced from each of the 20 families (of the original 25) that survived field deployment (Supplementary Table S2). The adult samples sequenced included three samples per colony from Orpheus parents (from the edges and center of each colony) and one sample per colony for Wilkie parents. For *M. digitata,* 5 - 12 eggs per dam were sequenced, along with one sample per maternal colony.

DNA was extracted from juveniles of *A.tenuis* in 2012 and 2013 with a SDS method [51]. For *M. digitata,* single egg extractions used the same extraction buffers and bead beating steps as described in [51] , although without the subsequent washes and precipitation steps because of the small tissue volumes of single eggs [77]. Library preparation, sequencing and data analysis were performed separately for 2012 and 2013 samples of *A. tenuis* and *M. digitata,* as described in [51]. Briefly, the USEARCH pipeline (v. 7) [78] and custom-built database of all *Symbiodinium*-specific NCBI sequences were used to classify reads [79,80], with blast hits above an E-value threshold of 0.001 removed, as they likely represented non-specific amplification of other closely-related species within the Dinoflagellata phylum (Supplementary Table S3). Analysis of rarefaction curves suggested that differences in sequencing depth across samples did not affect diversity estimates (additional description in supplementary methods).

Data analysis and visualization

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Fan dendrograms were constructed using a raw alignment function and neighbour joining tree algorithm from the 'ape' package [81]. Sample metadata were mapped onto trees using the package 'diverstree' [82]. To aid in visualizing the phylogenetic relationships on the *A .tenuis* tree, only OTUs that were found within at least three samples were kept, reducing the total OTU count from 422 to 134 for 2012 samples and from 568 to 181 for 2013 samples, giving an overall total of 315 OTUs for *A. tenuis*. To determine the overlap in *Symbiodinium* OTUs from *A. tenuis* data between years that were clustered and mapped separately, the 315 OTUs were aligned in Clustal OMEGA [83]. OTUs that clustered and Blasted to the same accession number (54 of the 315) were deemed to be the same OTU, resulting in a total of 261 distinct OTUs. In total, 80 unique OTUs were found in 2012, 127 were found in 2013, and 54 were shared between years. OTUs with a relative normalized abundance of less than 0.01% were classified as "background", whilst those with abundances greater than 0.01% were considered "principal." OTUs were further classified by ubiquity across samples and "core" OTUs were found in >75% of samples, "common" were found in 25 -75% of samples and "rare" were found in < 25%. As far fewer OTUs were recovered from *M. digitata* samples, all 101 OTUs were visualized and classified by abundance and ubiquity as described above. Differential abundance testing was performed with 'DESeq2', with Benjamini-Hochberg p-adjusted values at 0.05 [84–86]. Networks and heatmaps were constructed using un-weighted Unifrac distances of the normalized *Symbiodinium* abundances in eggs only, where maximum distances were set at 0.4.

Heritability analyses

The *Symbiodinium* community associated with each adult, juvenile or egg of the two coral species was characterized as a continuous quantitative trait by converting community composition into a single diversity metric, as detailed in Quigley et al. *in-review*. Collapsing complex assemblage data into a single diversity value (local diversity measure) [87] was necessary to apply a univariate heritability statistic. The Leinster and Cobbold diversity metric incorporates variance-normalized OTU abundances from linear models using negative binomial distributions, OTU sequence diversity, and OTU rarity [87]. Incorporating both abundance and diversity of *Symbiodinium* types into heritability estimates is essential because changes in *Symbiodinium* community abundance dynamics can change the functional output of the symbiosis as a whole [27] and are important in determining coral resilience and bleaching susceptibility [26,88,89].

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Regression-based estimates of heritability: Phenotypic values of offspring can be regressed against parental midpoint (average) phenotypic values, with the slope being equal to the narrow-sense heritability of the trait of interest [32,33]. Parental midpoint values were calculated by taking the average of the dam and sire *Symbiodinium* diversities for each family and then regressed against diversity values for the offspring of each family. Precision of the heritability estimate increases when parents vary substantially in the trait of interest [32]. Coral colonies dominated by a single or mixed *Symbiodinium* communities (C, D, C/D communities) can be considered biological extremes and ample evidence describes their contrasting physiological impacts on coral hosts (i.e., growth, bleaching) when associated with D versus C communities in particular [27]. Therefore, parental colonies selected for breeding were dominated by C1 (families W5, 10) or had mixed communities of C1/D1 (W7), C1/D1/D1a (W11, PCB4, 6, 8, 9), or multiple A, C1 and D types (OI3, 4, 5, 6) (Fig. 2b).

Bayesian linear mixed model estimates of heritability: Heritability estimates were derived from estimates of additive genetic variance calculated from the 'animal model,' a type of quantitative genetic mixed effects model incorporating fixed and random effects, and relatedness coefficients amongst individuals [90]. The animal model was implemented using Bayesian statistics with the package 'MCMCglmm' [91]. The model incorporated the diversity metric calculated for each juvenile and the pedigree coefficient of relatedness as random effects. Bayesian heritability models were run with 1.5 x10^6 iterations, a thinning level of 800 (*A. tenuis*) or 250 (*M. digitata*), and a burn-in of 10% of the total iterations. A non-informative flat prior specification was used, following an inverse gamma distribution [36]. Assumptions of chain mixing, normality of posterior distributions and autocorrelation were met. The posterior heritability was calculated by dividing the model variance attributed to relatedness by the sum of additive and residual variance. The impact of environmental covariance (V_{EC}) was reduced by randomly placing families within the outplant area [32]. Maternal environmental effects were assessed and were not significant for either *A. tenuis* or *M. digitata*, based on Deviance Information Criteria (DIC) from Bayesian models [36]. The influence of different settlement surface for *A. tenuis* juveniles in 2013 was assessed using linear mixed models (fixed effect: substrate, random effect: family) in the 'nlme' package [92] using the first principal component extracted from PCoA plots incorporating weighted Unifrac distances of normalized *Symbiodinium* abundances for juveniles. Model assumptions of homogeneity of variance, normality, and linearity were met. Substrate type did not

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significantly explain *Symbiodinium* community differences among samples (LME: $F_{(4)}$= 1.05, p = 0.38).

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Author Contributions

K.M.Q., B.L.W., and L.K.B. designed and conducted the experiments, K.M.Q. analysed the data and wrote the manuscript, and all authors made comments on the manuscript.

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