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REPORT

Trans-generational specificity within a cnidarian–algal symbiosis

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Abstract Ocean warming and other anthropogenic stresses threaten the symbiosis between tropical reef cnidarians and their dinoflagellate endosymbionts (*Symbiodinium*). Offspring of many cnidarians acquire their algal symbionts from the environment, and such flexibility could allow corals to respond to environmental changes between generations. To investigate the effect of both habitat and host genotype on symbiont acquisition, we transplanted aposymbiotic offspring of the common Caribbean octocoral *Briareum asbestinum* to (1) an environmentally different habitat that lacked *B. asbestinum* and (2) an environmentally similar habitat where local adults harbored *Symbiodinium* phylotypes that differed from parental colonies. Symbiont acquisition and establishment of symbioses over time was followed using a within-clade DNA marker (23S chloroplast rDNA) and a within-phylotype marker (unique alleles at a single microsatellite locus). Early in the symbiosis, *B. asbestinum* juveniles harbored multiple symbiont phylotypes, regardless of source (parent or site). However, with time (~4 yr), offspring established symbioses with the symbiont phylotype dominant in the parental colonies, regardless of transplant location. Within-phylotype analyses of the symbionts revealed a similar pattern, with offspring acquiring the allelic variant common in symbionts in the parental population regardless of the environment in which the offspring was reared. These data suggest that in this host species, host–symbiont specificity is a genetically determined trait. If this level of specificity is widespread among other symbiotic cnidarians, many cnidarian–algal symbioses may not be able to respond to rapid, climate change-associated environmental changes by means of between-generation switching of symbionts.

Keywords *Symbiodinium* · Cnidarian · Host–symbiont specificity · *Briareum* · Symbiosis

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Introduction

Climate change is a strong selective force, and how organisms will respond to this pressure is largely uncertain. In the marine realm, increasing oceanic temperature is threatening many ecosystems. For example, endosymbiotic dinoflagellate algae in the genus *Symbiodinium* have important effects on the fitness of their cnidarian hosts and, thereby, the functionality of the entire coral reef ecosystem. Yet ocean warming can lead to the breakdown of this symbiosis (coral bleaching) and is threatening these important ecosystems.

Symbiodinium is a highly diverse genus, comprised of several divergent taxonomic lineages (clades) and many within-clade phylotypes or species (Coffroth and Santos 2005; Pochon and Gates 2010) that exhibit a range of ecophysiological traits. Furthermore, the pairing between symbiont and host taxa can vary depending on environmental conditions such as light and temperature (Rowan and Knowlton 1995; Baker 2003).

Indeed, it is well known that some scleractinians can harbor multiple *Symbiodinium* phylotypes simultaneously within a single host colony, successively with environmental changes and/or between conspecifics across reef habitats/environments (Rowan and Knowlton 1995; LaJeunesse 2002; Jones et al. 2008; Fay and Weber 2012). However, flexibility in the symbiosis and the role of environmental conditions in modulating the host–symbiont interaction vary among cnidarian hosts. Adults of many host species harbor the same dominant symbiont phylotype across their range (Goulet 2006) or across time (Goulet and Coffroth 2003; Thornhill et al. 2006). Other hosts harbor low levels of background phylotypes, which allows flexibility in changing environments (Mieog et al. 2007; Jones et al. 2008; LaJeunesse et al. 2009; Silverstein et al. 2012; Quigley et al. 2014; but see Lee et al. 2016). Even if some hosts show ecological flexibility in symbiont–host pairing, rapid changes in symbiont communities as a means to deal with environmental changes may not be possible for most corals (Lewis and Coffroth 2004; Coffroth et al. 2010).

Despite their importance to the host, the majority of these symbionts are horizontally transmitted, i.e., acquired by coral offspring from the environment, and not inherited from the maternal coral (Baird et al. 2009). Coral larvae often disperse from their natal reef so that the preponderance of horizontal transmission among coral–*Symbiodinium* symbioses is thought to confer an adaptive advantage to the host (Douglas 1998). Several studies have demonstrated the potential for acclimatization and/or adaptation in both host and symbiont, suggesting a potential for trans-generational switching to occur (Howells et al. 2012; Palumbi et al. 2014; Putnam and Gates 2015; van Oppen et al. 2015). Thus, switching symbionts *between* generations could provide the host with another mechanism for acclimatization or adaptation to a changing environment (Little et al. 2004; Baker 2003; Baird et al. 2007; Cumbo et al. 2013).

Larval and juvenile cnidarians (<1 yr) initially acquire a broad range of symbionts that often differ from the adult hosts' symbiont populations (Coffroth et al. 2001, 2006; Little et al. 2004; Abrego et al. 2009a; Poland et al. 2013). Yet selectivity does exist since not all available *Symbiodinium* infect a given juvenile host (Rodriguez-Lanetty et al. 2006; Voolstra et al. 2009; Poland et al. 2013). Longer-term studies (>1 yr) demonstrated that among *Acropora tenuis* recruits, homologous symbiont populations (relative to symbionts in parental hosts) were established by 3.5 yr, while over this same time period recruits of the sister species *A. millepora* had not established a symbiosis with the *Symbiodinium* phylotypes that dominated parental colonies (Abrego et al. 2009a). Thus, interspecific variation in timing of the establishment of specificity in coral–algal symbioses, along with local environmental conditions and symbiont availability, may play a role in determining the final symbiont assemblage (Abrego et al. 2009a; Thornhill et al. 2009; Poland et al. 2013) and could theoretically lead to selection for a locally adapted symbiont phylotype as the juvenile host ages. However, if specificity during the winnowing period is genetically determined, trans-generation switching of symbiont partners may not be an option for corals to adapt at the expected pace of climate change (IPCC 2013).

In this study, we further investigate the specificity and flexibility of symbiotic partnerships across generations using the octocoral *Briareum asbestinum*. Like scleractinians, *B. asbestinum* recruits are flexible in early symbiont acquisition and can accept at least 11 symbiont phylotypes, with single polyps capable of harboring at least six phylotypes simultaneously (Poland et al. 2013). In contrast, the adult colonies are found in symbiosis with only one or two symbiont phylotypes (Lewis and Coffroth 2004; Hannes et al. 2009). We tested the null hypothesis that adult *B. asbestinum–Symbiodinium* associations are determined by local habitat conditions, i.e., associations are similar to those of the local population where the juveniles are reared, and independent of the symbiont phylotypes detected in the source (parental) population. We performed two field-based transplantations of aposymbiotic larvae where (1) larvae were collected from a single source population and outplanted to a habitat that lacked *B. asbestinum* and (2) larvae were collected from two similar habitats where host populations were dominated by different symbiont phylotypes and establishment of in hospite *Symbiodinium* populations followed at a single site over extended (>1 yr) time scales.

Materials and methods

Study organism and larval collections

Briareum asbestinum is a common Caribbean octocoral that as an adult typically harbors *Symbiodinium* B178 with low frequencies of *Symbiodinium* B184 (nomenclature given as clade and fragment size based on variation in domain V of the chloroplast 23S rDNA; Santos et al. 2003a). These symbionts belong to the Pliocene (B19) (GenBank Accession No. EU449077) and the Pleistocene (B1) radiations (Parkinson 2014; Parkinson et al. 2015), respectively. Although clearly distinct lineages, these symbiont species have not been formally described so they are referred to herein as *Symbiodinium* B178 and B184 phylotypes. The B178 and B184 phylotypes within adult *Briareum* differ from other species that share the same cp23S fragment size (B178 or B184) and do not associate with *B. asbestinum* (Parkinson 2014). *Briareum asbestinum*

is a gonochoric surface brooder that releases aposymbiotic offspring during early summer which settle in a philopatric fashion (Brazeau and Lasker 1990). Planula larvae remain on the maternal colony for several days from which they can be collected using 50-mL syringes.

Transplant experiments

To determine whether symbiont acquisition among juveniles from the same source population varied depending on habitat (i.e., the effect of environment on symbiont acquisition in juveniles with a similar genetic background), symbiont acquisition was followed in two different habitats. Larvae were collected from ten maternal colonies at a site in the Middle Keys on the Florida Bay side of Long Key, FL, in 2002 (Florida Bay; Table 1) where *Symbiodinium* B178 was the only symbiont detected with cp23S rDNA primers in the maternal colonies, as well as 89% of all adults sampled ($n = 74$) at this site. Larvae were settled and reared as described below at (1) the parental site, a shallow (3 m), silt-covered, hard-bottom nearshore site in Florida Bay where octocorals and sediment-tolerant scleractinians were the dominant fauna, and (2) at a dense *Thalassia* sea grass bed with minimal coral cover, on the Atlantic Ocean side of Long Key at 5 m depth (Grassbed; Table 1). The two sites are located on either side of Long Key approximately 4 km apart and differ slightly in depth and turbidity. Although detailed environmental measurements were not taken, the ocean versus bay location, as well as the strikingly different communities, suggests that, in general, these represented different environments. More detailed descriptions of these sites are found in Poland et al. (2013).

To examine the effect of host genetic background versus local environment in the establishment of symbioses, larvae were collected in 2005 from maternal colonies at two sites with similar habitats—the Florida Bay site described above and a Content Keys site (Florida Bay, Lower Keys; Table 1) which was also a nearshore, silt-covered, hard-bottom site dominated by similar taxa found at the Florida Bay site. At the Florida Bay site, only B178 was detected in the majority of adults (89%) and all maternal colonies, while at Content Keys, only *Symbiodinium* B184 was detected in the maternal colonies and the majority (96%) of adult colonies sampled ($n = 98$). These larvae were reciprocally transplanted between the two sites to examine symbiont acquisition in similar habitats where adult colonies harbored symbiont phylotypes that differed between sites (Table 1). The Florida Bay B178 site was monitored for several years while only two months of data were available from the Content Keys B184 site, due to hurricane damage to this site.

Offspring larvae from each maternal colony were placed in separate, replicate 2-L containers with ten cleaned gorgonian axial branches as a settlement substrate. The top and bottom of each container was covered with 250-μm mesh to allow water exchange. Containers with larvae from a single colony were suspended 1 m off the bottom at the outplant site. After the larvae settled (approximately 5–10 d), the branches with newly metamorphosed polyps were removed from the container and floated approximately 1 m above the substrate (Poland et al. 2013) at the respective sites.

Sampling and molecular methods

Settlement varied among the branches for each maternal colony and was lower for those originating from local Florida Bay B178 colonies, resulting in lower sample sizes relative to Content Keys B184-source offspring. Due to the long-term nature of the study and the finite number of initial settled polyps, not all replicate settlement branches (per adult maternal colony) were sampled each time to ensure the possibility of future samples. Additionally, as polyps started asexual budding and forming colonies, colony overgrowth and/or merging decreased the number of colonies in addition to mortality, leading to variation in sample sizes over the course of these studies. However, at least 10–20 polyps per parental population were collected at each sampling time (though symbiont detection was not always successful for all samples).

Settled single-polyp juveniles and the resultant colonies were sampled or subsampled (respectively) from settlement branches and preserved in 95% ethanol or 20% salt-saturated DMSO (dimethyl sulfoxide), EDTA buffer (Seutin et al. 1991). DNA extraction followed the protocol

Table 1 Sites used during 2002 and 2005 transplant experiments of *Briareum asbestinum*

Site	Year	Latitude (N)	Longitude (W)	Depth (m)	*B. asbestinum* present?	Dominant *Symbiodinium*
Florida Bay	2002/2005	24°50.049′	80°48.548′	3	Yes	B178
Grassbed	2002	24°47.921′	80°48.739′	5	No	–
Content Keys	2005	24°47.695′	81°29.685′	4	Yes	B184

Dominant *Symbiodinium* cp23S types are given but do not preclude the presence of the other types

of Coffroth et al. (1992), and symbionts were characterized to phylotype using visual gel scoring of length heteroplasmy of partial chloroplast large subunit (23S) rDNA as described in Santos et al. (2003a). This method infers the presence/absence, not relative quantities, of symbiont phylotypes. Although this method can detect phylotypes at the level of 10–1000 cells per sample (Santos et al. 2003a), it is recognized that undetected symbiont phylotypes may be present in low abundance.

The B184 cp-23S rDNA phylotype was the dominant *Symbiodinium* within Content Keys adults, in developing juveniles at all sites regardless of parental symbiont phylotype (see "Results" section, Figs. 1, 2) and present in approximately 10% of Florida Bay adults (Fig. 2). Specificity within this phylotype was therefore further examined. To identify a marker that distinguished among symbionts within the B184 phylotype in these two populations, a series of microsatellite loci for Clade B *Symbiodinium* were screened for fixed allelic differences between B184 symbionts in adults from the Content Keys and Florida Bay sites following published protocols (Santos et al. 2003b; Andras et al. 2009; Pettay and LaJeunesse 2007).

Fixed allelic differences (one 4 bp GAAT-repeat indel) between B184 symbionts in adults from the Content Keys versus Florida Bay sites were detected at one locus (B7SYM34; Pettay and LaJeunesse 2007). This locus was used to screen juveniles to identify "Florida Bay" and "Content Keys" alleles (see "Results" section) within the B184 symbionts in *B. asbestinum* juveniles. PCR amplification conditions followed those of Pettay and LaJeunesse (2007), except the annealing temperature was lowered to 55 °C. Amplicons were visualized and scored using size standards on the LI-COR 4200 NEN Global IR2 DNA sequencing system. Adult *B. asbestinum* samples with B184 symbionts from 2003 and 2005 (Content Keys) and from 2005 and 2006 (Florida Bay) were screened to investigate any temporal allelic variation at this locus. In hospite symbionts are haploid (Santos and Coffroth 2003) and more than one allele detected per sample were scored as mixed symbiont populations. To characterize the source of allelic differences, samples representing each dominant B7SYM34 microsatellite allele in adult *B. asbestinum* were sequenced (GenBank accession numbers KM503098 and KM503099).

Statistical analyses

Briareum asbestinum recruits routinely harbor *Symbiodinium* phylotypes other than B178 and B184 (Figs. 1, 2) (Coffroth et al. 2006; Poland et al. 2013). These vary in occurrence over habitats and/or years during early ontogeny and have been further discussed in Poland et al. (2013).

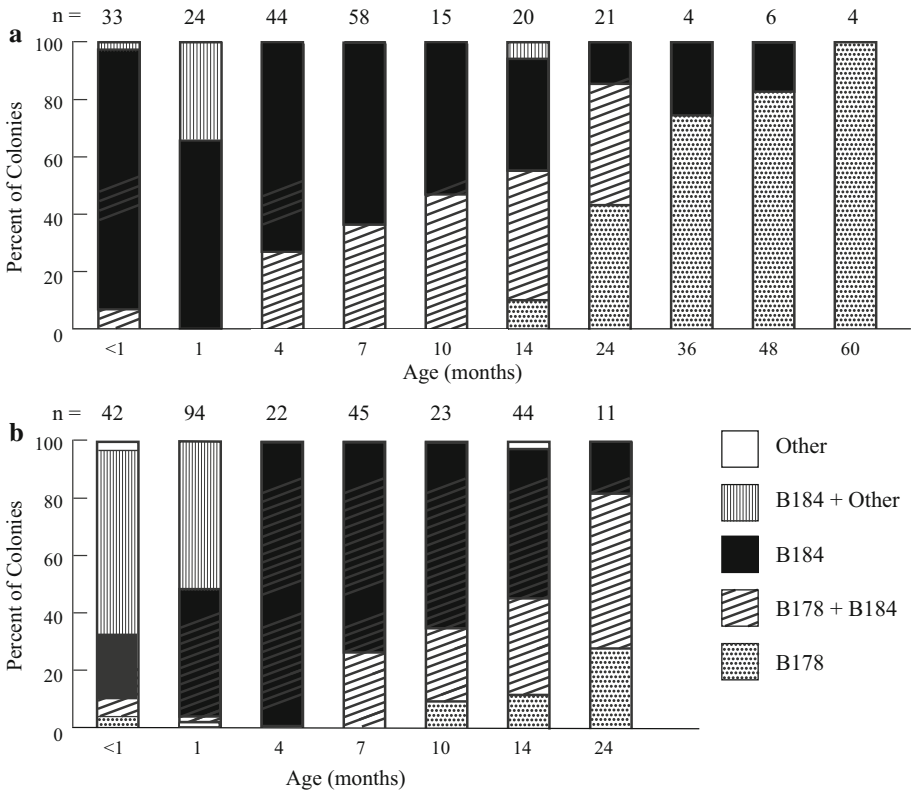

Fig. 1 *Symbiodinium* communities in 2002 *Briareum asbestinum* juveniles reared in one of two environments during **a** 2002–2006: Florida Bay (natal site), and **b** 2002–2004: Grassbeds (transplant site). Data show the percentage of colonies that harbored each symbiont type. Sample sizes are noted above columns. *Symbiodinium* types in the "other" category include symbionts belonging to clade A (phylotypes A194 and A198), clade B (phylotypes B211 and B224), and clade C (phylotype C180). Parents of these polyps hosted solely B178

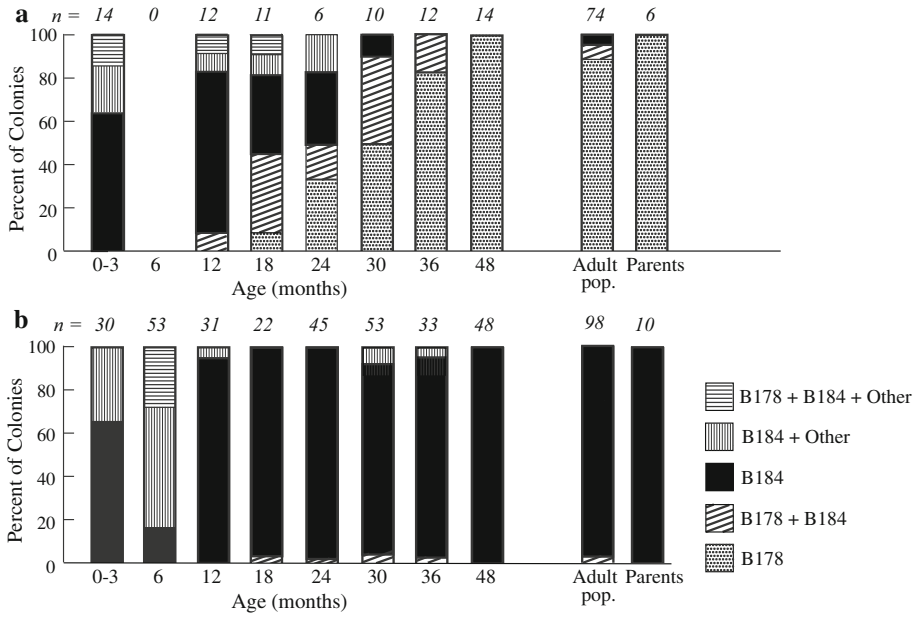

Fig. 2 *Symbiodinium* populations in *Briareum asbestinum* offspring collected in 2005 from **a** B178 colonies at Florida Bay and raised in the natal environment; and from **b** B184 colonies at Content Keys raised in the transplant site at Florida Bay. Data show the percentage of juvenile colonies that harbored each symbiont type from 2005–2009. Sample sizes (*n*) are shown above *each bar*. In each panel, "Parents" show the symbiont types within the maternal colonies and "Adult pop." shows the symbiont distributions from population surveys of adults at the Florida Bay (**a**) and the Content Keys (**b**) sites. *Symbiodinium* phylotypes in the "other" category include clade A phylotypes A194 and A198, clade B phylotypes B211 and B224 and, clade C phylotype C180

Beyond 12 months, however, B178 and B184 symbionts dominated the symbiosis and these were the targets for the present long-term study (Figs. 1, 2). For analyses, juveniles were categorized as harboring B178, B184 or B178 + B184, even if additional symbiont phylotypes within clades A, B, C and D were detected in colonies in any of the three categories. Those juveniles that contained *either* B178 or B184 alone or together with non-B178/B184 symbionts (the "+ other" categories in Figs. 1, 2) were accounted for in the two categories B178 or B184, respectively. Thus, the juveniles in B184 and the B184+ other categories were combined for analysis (Fig. 1). Similarly, those with B178 and B184 as well as those with B178 + B184 and other symbionts (B184 + B178 + other in Fig. 2) were grouped in the category B178 + B184. Juveniles with neither B184 nor B178 were grouped as "other" (Fig. 1). Hierarchical loglinear regression for differences between populations, time and symbiont type detected (B178, B184 or B178 + B184) was performed on the long-term data from the 2002 offspring raised at Florida Bay (natal offspring) and those raised at Grassbed transplant site.

In 2005, only offspring (sourced from B184-parent Content Keys and B178-parent Florida Bay sites) raised at Florida Bay (where local adults harbor mostly B178 symbionts; Table 1) were available for the entire time of the study (due to storm damage at Content Keys site). In most cases individual colonies were not identified so that succession of symbionts in individual offspring colonies could only be tracked in a few colonies that were repeatedly sampled at 18, 30, 36 and 48 months (Electronic Supplementary Material, ESM, Table S1). Given that the sample size was limited at each time point, results from individual colonies, branches and parental colonies were combined to increase statistical power. Categories were established as above and hierarchical loglinear regression for differences between host population, time and symbiont type (B178, B184 or B178 + B184) was used to compare symbiont composition between offspring from B184 colonies at Content Keys and offspring from B178 maternal colonies at Florida Bay.

Pairwise Chi-square tests with Bonferroni corrections were performed between populations first for the time period of 0–12 months where symbiont populations were more diverse, and then for the time period 18–30 months. Variation at the B7SYM34 microsatellite locus was also assessed through Chi-square tests, where allele frequencies were compared between parental populations and the two offspring populations in the common garden at Florida Bay at years 1 + 2 combined (for statistical power, as described above), and years 3 and 4. Data will be deposited with the Biological and Chemical Oceanography Data Management Office (http://www.bco-dmo.org/project/472478).

Results

Transplant experiments

Florida Bay hard-bottom versus ocean-side Grassbed habitat

During the first year, juvenile hosts at both sites acquired and maintained symbioses with several symbiont phylotypes where juvenile symbioses (up to 1 yr) were dominated by B184 symbionts, but other phylotypes occurred (Fig. 1). By 14 months 10–11% of all juveniles showed *Symbiodinium* B178 as their sole detectable symbiont partner at both the natal (Florida Bay) and transplant (Grassbed) sites (Fig. 1). The presence of B178 symbionts in juveniles continued to increase at both sites, but generally occurred in mixed assemblages with B184 for the first 2 yr (Fig. 1). *Symbiodinium* distributions in juveniles at the natal (Florida Bay) and transplant (Grassbed) sites did not differ significantly between sites between 10 and 24 months ($p > 0.05$ for all comparisons). After this time, only the juveniles reared at the natal Florida Bay site remained and in 2007, at five years of age, all remaining colonies associated with only B178 symbionts (Fig. 1a).

B178- and B184-source juveniles at Content Keys site

The high hurricane activity in the Florida Keys during 2005 (Hurricanes Katrina, Rita and Wilma) destroyed all offspring colonies transplanted to the Content Keys (B184) site after the two-month sampling, leaving only those B184-source offspring transplanted at the Florida Bay (B178) site for long-term studies. However, data from the two available months at Content Keys are informative. Offspring from parents with B184 symbionts (Content Keys offspring) showed only B184 ($n = 15$) or B184 + *Symbiodinium* types other than B178 ($n = 4$). Fourteen juveniles from Florida Bay B178 parents reared at the Content Keys site were sampled at two months and these had all acquired *Symbiodinium* B184. In addition, in two of these juveniles B178 symbionts were detected in combination with B184 and other algal types, demonstrating that *Symbiodinium* B178 was available at this site.

B178- and B184-source juveniles at Florida Bay site

At the Florida Bay site offspring were sampled over the 48-month study. *Symbiodinium* B178 was infrequent in all offspring for the first 12 months, occurring at most in approximately 14–16% of juveniles from Florida Bay (B178) adults (Fig. 2a). During this time 64–75% of B178-source offspring harbored only *Symbiodinium* B184, and in an additional 8–24% of juveniles, B184 was present with other (non-B178) symbiont types. The first occurrence of juveniles with only B178 was at 18 months, similar to what was seen in the 2002 cohort, and the proportion of B178-only colonies increased; by 48 months only B178 was detected in B178-source offspring (Fig. 2a).

Symbiodinium B184 also dominated the B184-source offspring reared at the Florida Bay site and there was no significant difference in the distribution in the symbiont communities between Florida Bay- and Content Keys-source offspring at 12 months ($\chi^2 = 0.682$, $df = 1$, $p = 0.682$; Fig. 2b). However, over the course of the entire study, *Symbiodinium* communities in B184-source (Content Keys) juveniles reared at the Florida Bay site differed significantly from the B178-source (Florida Bay) offspring (Likelihood ratio = 22.236, $df = 8$, $p < 0.01$). Interestingly, phylotype B178 was found in mixed communities (with B184 symbionts, among others) in 26.4% of 6-month-old Content Keys offspring, despite originating from B184 parental colonies, and was found at low frequencies with B184 across several sampling times from 18 to 36 months (Fig. 2b), demonstrating that B178 could infect these offspring. However, B178 was never the sole symbiont type in any of the Content Keys offspring. The continued dominance of B184 *Symbiodinium* in Content Keys (B184-source) juveniles stands in significant contrast to the increasing frequency of B178 over the same time frame in Florida Bay (B178-source) juveniles (18–30 months; $\chi^2 = 72.24$, $df = 2$, $p < 0.001$; Fig. 2b). At 48 months, all 14 remaining B178-source (Florida Bay) offspring colonies showed only B178 symbionts and all 48 B184-source (Content Keys) offspring reared at the Florida Bay site harbored only B184 symbionts (Fig. 2), matching the *Symbiodinium* populations of their respective source populations/maternal colonies.

Within-phylotype specificity

To identify a marker that would distinguish symbionts within the B184 phylotype, a total of 15 loci were screened; at most loci alleles were shared between sites. However, at one locus (B7SYM 34; Pettay and LaJeunesse 2007) fixed allelic differences were found between B184 harbored by *B. asbestinum* adults at the Florida Bay ($n = 16$) and Content Keys ($n = 49$) sites (Fig. 3).

The most frequent allele encountered in adult colonies at Florida Bay was at 263 bp (termed here the "Florida Bay allele"), while an allele at 267 bp was the most common allele in adult colonies at Content Keys (the "Content Keys allele"; Fig. 3). Several other alleles, observed at lower frequencies, were also unique to a site (Florida Bay: 259 bp; Content Keys: 271 and 275 bp; see 'others' in Fig. 3). Sequencing of the 263 bp and the 267 bp alleles

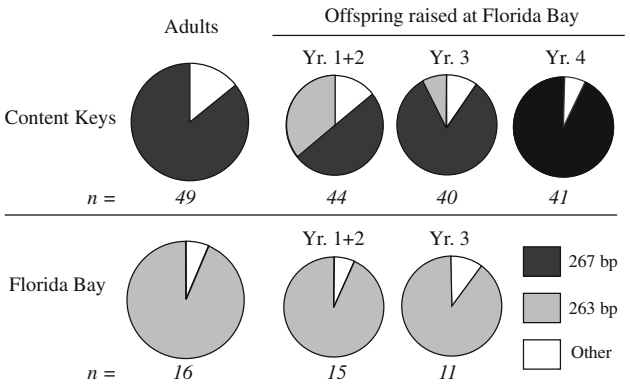

Fig. 3 Distribution of B7SYM34 alleles of B184 *Symbiodinium* (given by allele sizes in base pairs [bp]) in adult *Briareum asbestinum* in the parental populations Content Keys and Florida Bay and in offspring colonies from these two populations reared in the same habitat (Florida Bay). Alleles in the "other" category: 259 bp (Florida Bay); 271 and 275 bp (Content Keys)

from respective hosts confirmed eight and nine repeats of an identical four-nucleotide motif $(GAAT)_n$ with identical flanking regions (GenBank accession numbers KM503098 and KM503099), confirming that these are two distinct alleles at the same locus. Symbionts from hosts sampled in 2003 and 2006 were also screened to verify that these alleles were stable in the population. The sequences (number of repeats) for the two predominant symbiont alleles at each of the two sites were identical when compared between years, showing that the two alleles were stable over the timescale of this study.

The frequency of alleles at the B7SYM34 locus for the Florida Bay and Content Keys offspring raised together at Florida Bay differed significantly for the first two years, ($\chi^2 = 15.098$, $df = 2$, $p < 0.01$). Initially, some Content Keys offspring harbored symbionts with the Florida Bay allele (263 bp; Fig. 3), but the proportion of Content Keys offspring with the Florida Bay allele (263 bp) decreased with time, and by 4 yr old, this allele was no longer detected in any Content Keys offspring colonies ($n = 41$; Fig. 3). In Florida Bay offspring colonies, symbionts with the Florida Bay 263-bp allele were predominant throughout the 4-yr time period (Fig. 3).

Discussion

Final symbiont assemblage reflects parental symbiont populations

Over time, host–symbiont pairings in *B. asbestinum* juveniles most closely resembled those in the parental population with little obvious influence of the local habitat, suggesting a strong parental effect. Although the Grassbed and Florida Bay sites represented different habitats based on benthic cover, after 2 yr *Symbiodinium* B178 was the predominant symbiont within the recruits at both sites. Although it is possible that the B178 symbiont is favored in both habitats, the 2005 reciprocal transplant study further suggests a parental factor as symbiont assemblages in offspring reflected those of their parents. Despite the hypothesized adaptive value of horizontal uptake and the diverse symbiont populations in juvenile hosts, this study reveals fidelity in host–symbiont pairing at the level of symbiont phylotype and within-phylotype across generations.

The ontogenetic sequence of *B. asbestinum* is similar to that observed in the long-term study of *Acropora tenuis* juveniles where juveniles reared for 3.5 yr at their parental site acquired symbiont types that were dominant in the parental population (Abrego et al. 2009a). However, in contrast to our results, juveniles outplanted to a non-parental reef did not acquire the symbiont type found in adults at either the source or outplanted reefs (Abrego et al. 2009a).

An important factor in symbiont acquisition is symbiont availability. If the parental symbiont types are absent in the habitat where the offspring develops, recruits may establish symbioses with non-homologous symbiont types out of necessity. In *B. asbestinum*, however, symbiont availability was not a factor. The vast majority of juveniles, irrespective of source, initially harbored *Symbiodinium* B184 as the predominant partner and acquired a range of other symbiont types, *including* B178. Furthermore, a within-phylotype marker showed that juveniles initially acquired B184 alleles representative of symbionts in both adult populations, but by 4 yr all juveniles harbored the allelic variant unique to adults at the site of origin demonstrating specificity for the within-phylotype variant of the parental population (Fig. 3).

These data demonstrate that in *B. asbestinum*, host–symbiont specificity exists at a phylotype level (B184 vs. B178) and a within-*Symbiodinium* phylotype level (B184 microsatellite allelic variants). Population genetic studies have revealed substantial variation within *Symbiodinium* species (Santos et al. 2003b; Pettay and LaJeunesse 2007; Thornhill et al. 2009), and it is clear that there are functional differences between symbiont genotypes within symbiont species [e.g., symbiont responses to stress as in *Gorgonia ventalina* (Andras 2010), growth rates (Parkinson and Baums 2014), and infectivity (Schoenberg and Trench 1980)]. Functional differences most likely exist between the B184 and B178 phylotypes as well. For example, prior to an induced bleaching event, *B. asbestinum* colonies harbored primarily B178 (69%), but after bleaching, 79% of the colonies harbored B184, suggesting that B184 *Symbiodinium* may be more resistant to this stress or may recover more rapidly (Lewis and Coffroth 2004). This study, along with others, suggests the need for

continued ecological/physiological studies to examine the role of specific genotype-by-genotype pairing of host and symbiont using within-species level markers (Abrego et al. 2008; Baums et al. 2014; Parkinson and Baums 2014).

Succession of symbiont types in *B. asbestinum* juveniles

The winnowing of symbiont phylotypes during ontogeny to the dominant symbiont within the adult symbiosis is a slow process, taking up to 4 yr. The mechanisms behind this process are not clearly understood, and a range of possible processes has been suggested, e.g., delayed onset of allorecognition (either through maturation of host system or symbiont interference with the innate immune system) with subsequent elimination of suboptimal symbionts, post-infection sanctions based on symbiont type, competitive abilities of symbionts, or different microenvironments as the host grows (Fitt 1985; Coffroth et al. 2001; Little et al. 2004; Rodriguez-Lanetty et al. 2006; Abrego et al. 2009b; Dunn and Weis 2009; Voolstra et al. 2009; Puill-Stephan et al. 2012; Mohamed et al. 2016). Future research will be needed to fully understand the cellular recognition and communication pathways and host–symbiont and symbiont–symbiont interactions that give rise to the observed patterns of acquisition and maintenance of certain symbionts over others. However, mechanisms based on several ecological and physiological factors can be addressed by the present study.

The decreasing diversity and subsequent sorting of symbionts that accompanies the maturation of the symbiosis could be the result of a variety of processes, possibly occurring simultaneously. First, the ontogenetic shift in symbiont populations seen in Florida Bay, B178-source, *B. asbestinum* could arise if the host's needs vary over ontogeny with the benefits to the host varying among symbionts, as found with light tolerance (Gómez-Cabrera et al. 2008) or photosynthate relocation to the host (Cantin et al. 2009). Importantly, environmental factors may dictate the relative advantages of different symbiont partners to the host, as recently shown between C1 and D symbionts in *Acropora tenuis* (Baker et al. 2013) and *Pocillopora damicornis* (Cunning et al. 2015). However, increased benefits related to different ontogenic demands do not explain the continued dominance of *Symbiodinium* B184 in Content Key offspring while those offspring from Florida Bay became dominated by B178. All were reared in the same habitat where the physiological processes within the host are likely to be similar. Instead, we propose that the different ontogenetic symbiotic trajectories in *B. asbestinum* are due to genetically determined and parentally inherited traits. Interestingly, a subset of adult *B. asbestinum* at Florida Bay (B178 site) also host B184 (Fig. 2a), suggesting that the diverging ontogenies of the Florida Bay and Content Keys offspring are not due to adaptation to local conditions, but reflect host genetic background.

Second, differential host mortality during ontogeny could also explain the observed pattern of sorting. If initial symbiont uptake is relatively non-specific, over time Florida Bay juveniles harboring the heterologous B184 algal type might suffer higher mortality than juveniles with the homologous B178 *Symbiodinium* partner. Several lines of evidence from the current study, however, refute such symbiont-related host mortality for *B. asbestinum* juveniles: (1) B178 replaced B178 + B184 in juvenile colonies that were repeatedly subsampled over time (ESM Table S1), and (2) survivorship was not compromised in Content Keys, B184-source, offspring, which continued to associate almost exclusively with B184 from 12 months to adulthood (Fig. 2a).

Alternatively, B184 symbionts could be more successful colonizers. Opportunism has been proposed in the rapid colonization and dominance of Clade D *Symbiodinium* in *A. millepora* and *A. tenuis* (Abrego et al. 2009b) and may be a trait of B184. In a preliminary laboratory infection trial examining S*ymbiodinium* uptake by newly settled *B. asbestinum* recruits, B184 symbionts were dominant in juveniles that were offered approximately equal concentrations of B178 and B184 symbionts (ESM Laboratory infection trial), Fig. S1. This suggests that *Symbiodinium* B184 are highly successful host colonizers even if they do not necessarily confer a greater benefit to the host.

Finally, some symbionts may have evolved strategies with their host to initiate symbioses, and occur at high frequency in newly settled juveniles and then remain in low population densities at background levels during late ontogeny and adulthood (Abrego et al. 2009b; LaJeunesse et al. 2009; Silverstein et al. 2012). Several studies have demonstrated that *A. millepora* and *A. tenuis* juveniles harbor clade D while adults harbor *Symbiodinium* ITS types C1 and C2 (Little et al. 2004; Abrego et al. 2009b) and that clade D persists in adults of these species at low levels (Mieog et al. 2007). *Symbiodinium* B184 could, in this scenario, not be replaced by *Symbiodinium* B178, but be present at background levels within Florida Bay *B. asbestinum* colonies. After experimental bleaching, symbiont assemblages in some *B. asbestinum* colonies changed from B178 to B178 + B184, and it was suggested these B184 symbionts were present in low, undetectable levels in pre-bleached colonies (Lewis and Coffroth 2004; Poland 2010). It is therefore possible that B184 does not "disappear" from Florida Bay juveniles, but decreases to low background populations in adulthood. Indeed, approximately 10% of the adult *B. asbestinum* sampled at the Florida Bay site harbored both B178 and B184. The molecular tools employed in the present study do not have the resolution to resolve individual symbiont phylotypes in

mixed communities when present at low levels (see "Methods" section). Information on relative abundances of symbiont phylotypes in mixed populations in hospite (see "Methods" section) will require the development of phylotype-level qPCR techniques. However, the acquisition of specific B184 allelic variants and the subsequent preference for B178 symbionts that come to dominate Florida Bay juveniles suggests that this outcome may be influenced by genetically fixed traits.

Trans-generational switching of symbionts?

Specificity in most symbioses, including coral–algal symbioses, involves both host and symbiont genes that direct communication and recognition between partners (Schoenberg and Trench 1980; Markell and Wood-Charlson 2010; Mohamed et al. 2016). This cross-talk likely continues beyond the initial acquisition and may involve symbiont control of the host immune response (Voolstra et al. 2009; Puill-Stephan et al. 2012; Mohamed et al. 2016). Genetic control of specificity in other systems such as the legume–rhizobia symbiosis is well established with involvement of both host and symbiont genes (Yang et al. 2010; Wang et al. 2012).

Although there is much evidence of specificity in coral–algal symbioses, the level of host–symbiont specificity reported here is novel. Determining the genetic control of specificity that is operating in the *B. asbestinum–Symbiodinium* system requires further attention, and future studies should examine whether offspring in other *B. asbestinum* populations show the same fidelity to the parental symbiont type as seen in the two populations studied here. However, results from this study imply that, at least over one generation, and over the environmental conditions of this study, local environmental factors may be secondary to the genetic traits of the host source population.

Although background symbionts may become more prominent during thermal stress in some corals, they often revert to the original symbiont type once conditions return to normal (Thornhill et al. 2006; Jones et al. 2008; LaJeunesse et al. 2009, but see Palumbi et al. 2014). Host–symbiont specificity such as seen in this study and others (Parkinson and Baums 2014) may limit the holobiont's ability to respond to warming via symbiont switching (sensu Baker 2003). However, clearly, long-term changes in cnidarian–algal symbioses are possible (due to evolutionary processes of mutation and selection) as current biogeographic diversity in cnidarian–*Symbiodinium* symbioses is a result of host specialization and geographic isolation (LaJeunesse 2005; Thornhill et al. 2013), and recent studies have demonstrated that both host and symbionts have a capacity for acclimatization and/or adaptation over ecological timescales (Howells et al. 2012; Palumbi et al. 2014; van Oppen et al. 2015; Putnam and Gates 2015). These studies show that, in at least some symbiotic pairings, both host and symbiont have the capacity to adapt or acclimatize to changing conditions over ecologically relevant timescales leading to higher tolerance of environmental changes. If these findings are widespread, it is conceivable that the host specificity demonstrated here in *B. asbestinum* and elsewhere (Goulet 2006; Thornhill et al. 2009, 2013, 2014; LaJeunesse et al. 2010; Parkinson et al. 2015) would not limit the ability of the holobiont to tolerate elevated temperatures if at least one of the partners in the symbiosis can adapt or acclimatize to changing environmental conditions. However, the understanding of fine-scale physiological diversity of *Symbiodinium* at the species and within-species level is limited and clearly, in light of the results presented herein, there is a need for further research to determine the potential of this, and other cnidarian–algal symbioses, to adapt/acclimatize.

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