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ARTICLE

Inter-partner specificity limits the acquisition of thermotolerant symbionts in a model cnidarian-dinoflagellate symbiosis

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Abstract

The ability of corals and other cnidarians to survive climate change depends partly on the composition of their endosymbiont communities. The dinoflagellate family Symbiodiniaceae is genetically and physiologically diverse, and one proposed mechanism for cnidarians to acclimate to rising temperatures is to acquire more thermally tolerant symbionts. However, cnidarian-dinoflagellate associations vary in their degree of specificity, which may limit their capacity to alter symbiont communities. Here, we inoculated symbiont-free polyps of the sea anemone *Exaiptasia pallida* (commonly referred to as 'Aiptasia'), a model system for the cnidarian-dinoflagellate symbiosis, with simultaneous or sequential mixtures of thermally tolerant and thermally sensitive species of Symbiodiniaceae. We then monitored symbiont success (relative proportional abundance) at normal and elevated temperatures across two to four weeks. All anemones showed signs of bleaching at high temperature. During simultaneous inoculations, the native, thermally sensitive *Breviolum minutum* colonized polyps most successfully regardless of temperature when paired against the non-native but more thermally tolerant *Symbiodinium microadriaticum* or *Durusdinium trenchii*. Furthermore, anemones initially colonized with *B. minutum* and subsequently exposed to *S. microadriaticum* failed to acquire the new symbiont. These results highlight how partner specificity may place strong limitations on the ability of certain cnidarians to acquire more thermally tolerant symbionts, and hence their adaptive potential under climate change.

Introduction

The symbiosis between cnidarians (*e.g.* corals, anemones, and jellyfish) and photosymbionts (primarily dinoflagellates of the family Symbiodiniaceae) is widespread in the marine environment, and has particular ecological importance with respect to the growth and survival of coral reefs [1–3]. The family Symbiodiniaceae is genetically and physiologically diverse, with seven genera and numerous species currently described and many more awaiting formal description [4–6]. Symbiont community composition greatly affects host performance [7–10], and impacts the ability of the holobiont (the host and all of its symbionts) to thrive at different latitudes, depths, irradiances, and temperatures [11–14].

Despite the high diversity of Symbiodiniaceae, host-symbiont associations are nonrandom, exhibiting varying degrees of specificity. Some host species associate with multiple dominant or co-dominant symbiont types, while others associate with only one primary type, and nearly all contain background symbionts [15–17]. The degree of specificity depends on the host, the symbiont, and the environment [18–20]. The cellular mechanisms involved in determining specificity are still unclear, though cell surface recognition during phagocytosis of the symbiont by the host is known to be involved [21–24], as is the capacity of different symbiont types to circumvent the host's immune response [25]. Furthermore, the competitive interactions between different Symbiodiniaceae could play a role but have received little attention [26, 27].

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Understanding how host-symbiont specificity arises is not only important for understanding the evolution of particular partnerships, but also the potential to form new partnerships. This is especially important when considering the survival of scleractinian corals and other cnidarians as our climate changes, through the acquisition of new symbiont species or through adjustments in the relative numbers of current species [28–30]. Evidence for such transitions is abundant e.g., [31–38], but they typically occur under stressful conditions, such as those that cause coral bleaching —the loss of a majority of symbiont cells/pigments. Thus, under normal conditions, the greater colonization success of homologous symbionts (those that regularly associate with a particular host) appears to prevent persistent colonization by heterologous symbionts (those that typically do not associate with a given host), even if these 'foreign' symbionts manage to evade the host's immune system. Indeed, when hosts are rendered aposymbiotic (symbiont-free) under laboratory conditions they can typically form new associations when inoculated with a range of homologous or heterologous symbionts [10, 39–41]. Yet such a ready ability to form new, lasting partnerships does not appear to occur often in the wild [33, 42, 43].

The sea anemone Aiptasia (*Exaiptasia pallida*) is a widely adopted model system for the study of cnidarian-dinoflagellate symbioses [44, 45]. This anemone is distributed throughout the world's tropical seas, and in the Indo-Pacific associates only with *Breviolum minutum* (ITS2 type B1), though Atlantic populations can additionally associate with *Symbiodinium linucheae* (A4) and rarely with a member of the genus *Cladocopium* [46]. Consistent with this high degree of host-symbiont specificity, laboratory experiments show that *B. minutum* colonizes Indo-Pacific Aiptasia at a much faster rate than *S. microadriaticum* (A1) and *Durusdinium trenchii* (D1a), while *Effrenium voratum* (E1) and *Cladocopium* sp. (C3) fail to persist after just a few weeks [10]. Furthermore, *B. minutum* forms a more beneficial symbiosis than heterologous species, facilitating faster host growth and asexual reproduction consistent with greater rates of photosynthesis and carbon translocation [9, 10, 47]. It also appears to be a highly compatible symbiont, as Aiptasia is more immunotolerant of *B. minutum* than it is of heterologous *D. trenchii* [8]. The relationship between Aiptasia and *B. minutum* therefore provides an excellent model for elucidating the mechanisms that promote such a high degree of partner fidelity in the wild, despite the ability of this sea anemone to form a range of associations in a laboratory setting.

Here we measured the colonization success of *B. minutum*, the homologous partner of Aiptasia, relative to two thermally tolerant heterologous species: *S. microadriaticum* and *D. trenchii*. In a recent partial-rank aggregation analysis that included reports on the relative thermotolerance of Symbiodiniaceae both *in hospite* and *in vitro*, *B. minutum* was ranked the 38th most heat-tolerant type (out of 64), while *S. microadriaticum* and *D. trenchii* were ranked 26th and 8th, respectively [48]. We measured the relative abundance of these different symbiont species in a clonal Aiptasia line after: (1) inoculating symbiont-free polyps with each Symbiodiniaceae species in isolation, (2) inoculating symbiont-free polyps simultaneously with each pairwise combination of Symbiodiniaceae species; and (3) exposing polyps in established symbioses to alternate symbionts. We performed the experiments at 24, 32, and 34 °C to explore the effect of elevated temperature on symbiont dynamics. We hypothesized that, under elevated temperatures, the success of homologous *B. minutum* would decline, causing Aiptasia to transition to an alternate symbiont community composed of a greater proportion of heat-tolerant heterologous species. Ultimately, we aimed to assess the extent to which host-symbiont specificity might override the potential to establish novel symbioses with more thermally resistant partners even at elevated temperatures, and help to explain the strong partner fidelity seen in Aiptasia in the field.

Methods

Experimental organisms

All experiments were performed with a clonal culture of the symbiotic sea anemone *Exaiptasia pallida* (culture ID: NZ1; commonly referred to as 'Aiptasia') isolated from the Indo-Pacific region. Polyps were maintained in aquaria at 24 °C under a standard light regime of 80 µmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) on a 12:12 h (light:dark) photoperiod, and fed twice weekly with freshly hatched *Artemia* nauplii. To generate aposymbiotic anemones, polyps were menthol-bleached as described by Matthews et al. [49]. This treatment was repeated daily for 4 weeks or until no symbionts were present, as determined by the absence of chlorophyll fluorescence under confocal microscopy (IX81, Olympus New Zealand; 635 nm laser, 655–755 nm emission filter).

Cultured strains of the three focal Symbiodiniaceae species (*Breviolum minutum*, *Symbiodinium microadriaticum*, and *Durusdinium trenchii*) were chosen as inoculates based on their capacity to readily colonize aposymbiotic Aiptasia [10]; for more details about the cultures, see Table 1. The algae were sub-cultured from long-term (>5 years) laboratory stocks and grown in silica-free f/2 medium (Sigma-Aldrich, Auckland, New Zealand) under the same conditions as the anemones. All cultures were sampled for experimental use during the log-phase of growth.

Table 1 Symbiodiniaceae culture details

Culture ID	Original host source	Geographical location	Symbiodiniaceae species	ITS2 type	Relationship to the host
FlAp2	*Exaiptasia pallida*	Long Key, Florida	*Breviolum minutum*	B1	Homologous
CCMP2467	*Stylophora pistillata*	Gulf of Aqaba	*Symbiodinium microadriaticum*	A1	Heterologous
Ap2	Unknown anemone sp.	Okinawa	*Durusdinium trenchii*	D1a	Heterologous

Single species exposure

Aposymbiotic anemones ($n = 4$ polyps *per* symbiont culture, temperature, and time-point combination) were inoculated with one of three Symbiodiniaceae cultures: 100% *B. minutum*, 100% *S. microadriaticum*, or 100% *D. trenchii*. Polyps of similar size (2–3 mm oral disc diameter) were starved for a week and then transferred to 400-ml clear plastic jars (one anemone *per* jar) filled with 0.2 µm filtered seawater (FSW; IKA T-10, ThermoFisher Scientific) and allowed to acclimate for three days. Each algal culture was diluted in 25 ml FSW and mixed with one drop of *Artemia* nauplii, giving a final concentration of ~1×10^6 Symbiodiniaceae cells ml^{-1}. Using a glass pipette, 1 ml of inoculate was dispensed onto the oral disc of each polyp. Thereafter, anemones were fed once weekly, followed the next day by a water change. Inoculated anemones were maintained at 24, 32, or 34 °C under the standard light regime. Polyps were sacrificed at 1 and 2 weeks post-inoculation to determine the densities of different Symbiodiniaceae species. Each polyp was homogenized in 500 µl FSW and centrifuged (Sigma 3–16k) for 5 min at 400 RCF to separate the algal cells from the anemone tissues. A 100 µl sample was removed from the supernatant (host fraction) for protein determination *via* the Bradford assay [50] and the remaining supernatant was discarded. The algal pellet was then processed for DNA extraction and qPCR analysis (see below).

Simultaneous exposure

Aposymbiotic anemones ($n = 4$ polyps *per* symbiont mixture, temperature, and time point combination) were inoculated with one of three Symbiodiniaceae mixtures: 50% *B. minutum* + 50% *S. microadriaticum*, 50% *B. minutum* + 50% *D. trenchii*, or 50% *S. microadriaticum* + 50% *D. trenchii*. Inoculated anemones were maintained at 24, 32, or 34 °C under the standard light regime. Polyps were sacrificed as above at 1 and 2 weeks post-inoculation to determine the densities of different Symbiodiniaceae species *via* qPCR.

Delayed exposure

Aposymbiotic anemones ($n = 6$ polyps *per* symbiont culture, temperature, and time point combination) were first inoculated as above with either 100% *B. minutum* or 100% *S. microadriaticum*. The inoculated polyps were maintained at 24 °C until they became densely colonized (a minimum of 4 weeks). The anemones were then challenged *via* exposure to the alternate Symbiodiniaceae species (i.e. anemones colonized by *B. minutum* were re-inoculated with *S. microadriaticum* or *vice versa*), and maintained at 24, 32, or 34 °C under the standard light regime. Polyps were sacrificed as above at 1, 2, and 4 weeks post-inoculation to determine the densities of different Symbiodiniaceae species *via* qPCR.

Quantitative PCR

DNA extraction and qPCR amplification followed the protocol of Yamashita et al. [51] with modification. For full details, see the Supplementary Information. In brief, symbiont DNA was isolated following guanidinium extraction of the algal pellets and amplification with the Symbiodiniaceae-specific rDNA primers of Pawlowski et al. [52]. For a subset of samples, the rDNA was cloned into *E. coli* vectors, re-isolated, and sequenced ($n = 4$ sequences *per* Symbiodiniaceae species). Sequences were blasted against the NCBI database to confirm ITS2 type, and one sample from each type was chosen to generate standard curves for qPCR using the genus/clade-specific primers of Yamashita et al. [51]. All original sample extracts were then subjected to genus-specific qPCR, and the 28 S copy number *per* genus *per* cell was estimated by deduction from the regression lines and comparison with the standard curves. In this case, because there was only one species *per* genus in the experiment, genus identity resolved species identity.

To confirm cell enumeration by qPCR, one cultured sample of each of the three species was diluted, extracted, and amplified ($n = 6$ replicates *per* sample). For the combinations 50% *B. minutum* + 50% *S. microadriaticum* and 50% *B. minutum* + 50% *D. trenchii*, seven two-culture mixtures were generated with Symbiodiniaceae species ratios ranging from 1:99 to 99:1. Following qPCR, the predicted *versus* observed symbiont ratios were assessed for accuracy using least-squares linear regression. A subset of polyps from each treatment was tested periodically to confirm a lack of contamination by symbionts from other treatments (see Supplementary Information: Fig. S1; Tables S1 and S2).

Statistical analyses

To test the effects of temperature, time, and their interaction on symbiont density *per* species within each experimental treatment, the data were log-transformed to achieve normality and homoscedasticity then analyzed *via* two-way ANOVA and Tukey *post hoc* tests ($\alpha = 0.05$) in the R statistical environment [53]. Differences in the log-transformed symbiont density between species within each treatment were calculated *via* one-way ANOVA. The data were plotted with the package '*ggplot2*' [54]. All raw data and R code for the analyses are included in the Supplementary Information.

Results

Single species exposure

Colonization success (symbiont cell density) varied by symbiont species in the single species exposures. In independent host polyps, the homologous *B. minutum* (Fig. 1a) surpassed heterologous *S. microadriaticum* (Fig. 1b) and heterologous *D. trenchii* (Fig. 1c) regardless of treatment (one-way ANOVA, $p < 0.05$), whereas the density difference between *S. microadriaticum* and *D. trenchii* was rarely significant. The density of *B. minutum* varied with temperature and the time x temperature interaction, the density of *S. microadriaticum* varied by the interaction only, and the density of *D. trenchii* varied by temperature only (two-way ANOVA, $p < 0.05$). The density of the heterologous symbionts remained low throughout the experiment (average range: $0–3 \times 10^5$ cells *per* mg protein), but homologous *B. minutum* density was very high at 24 and 32 °C (average range: $1–6 \times 10^7$ cells *per* mg protein). However, *B. minutum* density dropped significantly at 34 °C (average range: $2–3 \times 10^5$ cells *per* mg protein; two-way ANOVA, $p < 0.05$), while still remaining higher than for the heterologous species in parallel treatments. A similar heat-induced bleaching pattern (though much smaller in magnitude) was observed for *D. trenchii*, but not for *S. microadriaticum*.

Simultaneous exposure

Colonization success varied by symbiont species when introduced in 50/50 mixtures simultaneously. When paired within the same host polyp, success of the homologous *B. minutum* surpassed that of the heterologous *S. microadriaticum* (Fig. 2a) or *D. trenchii* (Fig. 2b) regardless of treatment (one-way ANOVA, $p < 0.05$). In these two trials, the proportion of *B. minutum* was always higher than for the heterologous species, ranging from 93–100% (*versus S. microadriaticum*) or from 96–100% (*versus D. trenchii*). The density of the heterologous symbionts remained low throughout the experiments (never exceeding an average of 3×10^4 cells *per* mg protein in a given treatment), whereas homologous *B. minutum* density began low at 24 and 32 °C in week one, then increased dramatically by week two to levels comparable to the single species exposure. However, *B. minutum* density remained low at 34 °C. Thus, temperature and time either significantly or marginally influenced *B. minutum* density in both experiments, and the interaction was significant when *B. minutum* was paired with *S. microadriaticum* (two-way ANOVA, $p < 0.05$). Neither temperature nor time influenced *S. microadriaticum* density, and only temperature impacted *D. trenchii* density.

In the simultaneous exposure experiment between the two heterologous symbionts (Fig. 2c), densities of both species remained low regardless of treatment (never exceeding 2×10^4 cells *per* mg protein), and the only significant difference between species was detected at 24 °C, where *D. trenchii* was greater in abundance than *S. microadriaticum* (one-way ANOVA, $p < 0.05$). *Symbiodinium microadriaticum* colonization increased with temperature (from an average of 16% at 24 °C, to 47% at 32 °C, to 80% at 34 °C). By necessity, *D. trenchii* showed the opposite pattern: its proportion decreased as the temperature increased. However, temperature only significantly impacted *D. trenchii* density (two-way ANOVA, $p < 0.05$), and neither temperature, time, nor their interaction affected *S. microadriaticum* density. As in the other trials, *D. trenchii* density was lowest at 34 °C.

Delayed exposure

When anemones were challenged with heterologous *S. microadriaticum* after first forming a stable symbiosis with homologous *B. minutum*, *S. microadriaticum* did not manage to colonize the polyps in appreciable amounts, representing < 0.1% of the symbiont community at any combination of temperature and time point (Fig. 3a). Thus, *B. minutum* density always exceeded *S. microadriaticum* density (one-way ANOVA, $p < 0.05$). The density of *B. minutum* declined with time at 24 °C, though to a small degree (3×10^6 cells at week one to 6×10^5 cells at week four), while density remained relatively low at 32 and 34 °C regardless of time. Temperature, time, and their interaction all significantly impacted the density of both species (two-way ANOVA, $p < 0.05$).

In the alternate case, when anemones were challenged with homologous *B. minutum* after first forming a stable association with heterologous *S. microadriaticum*, *B. minutum* was able to partially colonize the anemone

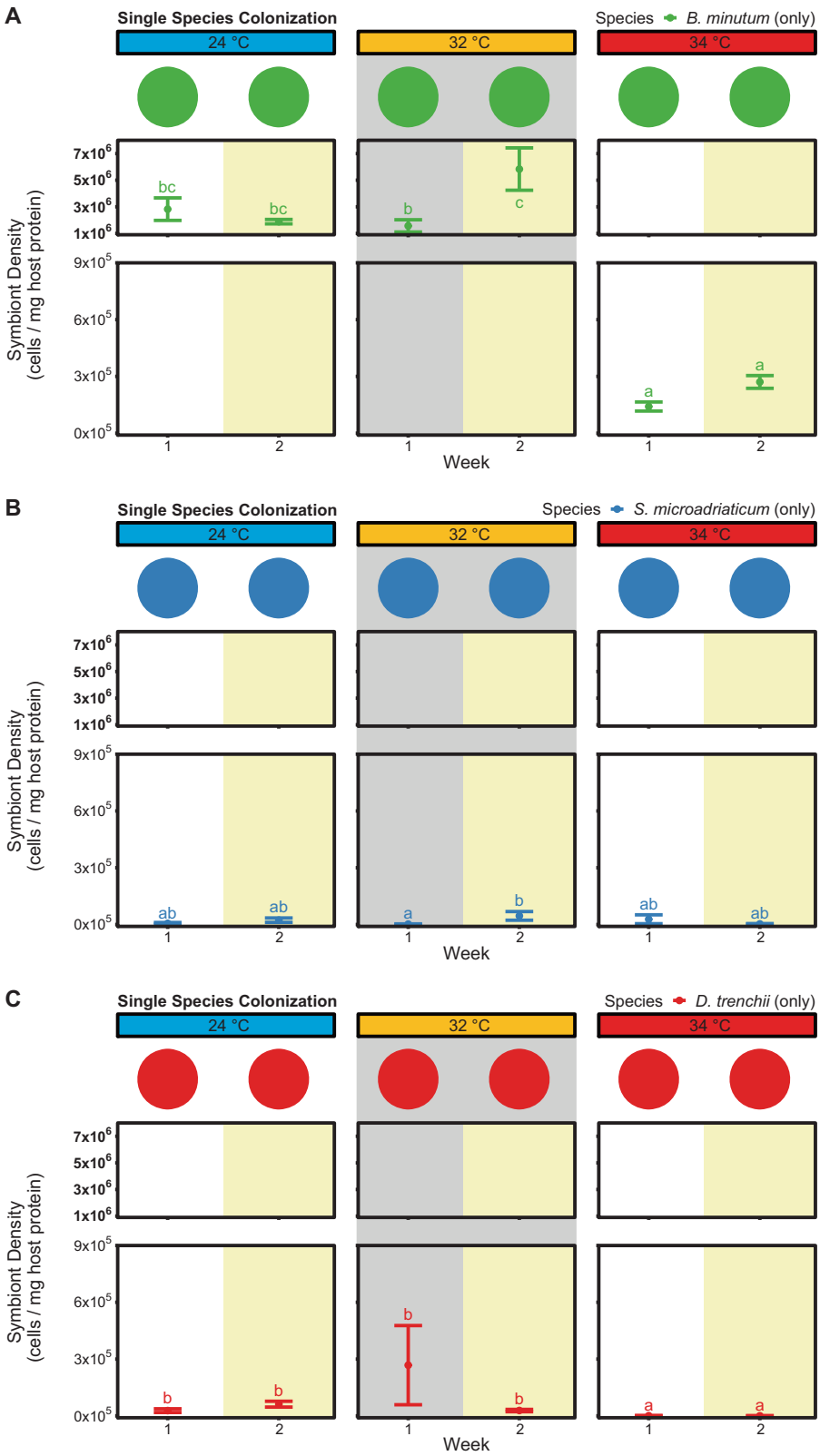

Fig. 1 Single species colonization experiments. Pure cultures of one Symbiodiniaceae species (**a** 100% *B. minutum*; **b** 100% *S. microadriaticum*; and **c** 100% *D. trenchii*) were introduced to aposymbiotic anemones ($n = 4$ polyps *per* treatment *per* time point). Symbiont densities (cells *per* mg host protein) were measured one and two weeks after inoculation under cool (24 °C), ambient (32 °C), and thermal stress conditions (34 °C) *via* genus-specific qPCR. Pie charts represent the average proportion of each species in the host's total symbiont community. Error bars represent the standard error of the mean. Letters correspond to distinct cell densities within a species across treatments (color coded; two-way ANOVA; $p < 0.05$). To visualize large cell density changes without obscuring small changes, results were depicted across two plots each: note the *y*-axis break and unique scales

(Fig. 3b). The average proportion of *B. minutum* across temperatures increased from 7% at week one to 36% at week four. Notably, the density of *S. microadriaticum* declined with time at 24 °C (2×10^5 cells at week one to 1×10^4 cells at week four), such that the change in proportion of symbiont types was accounted for mainly by a

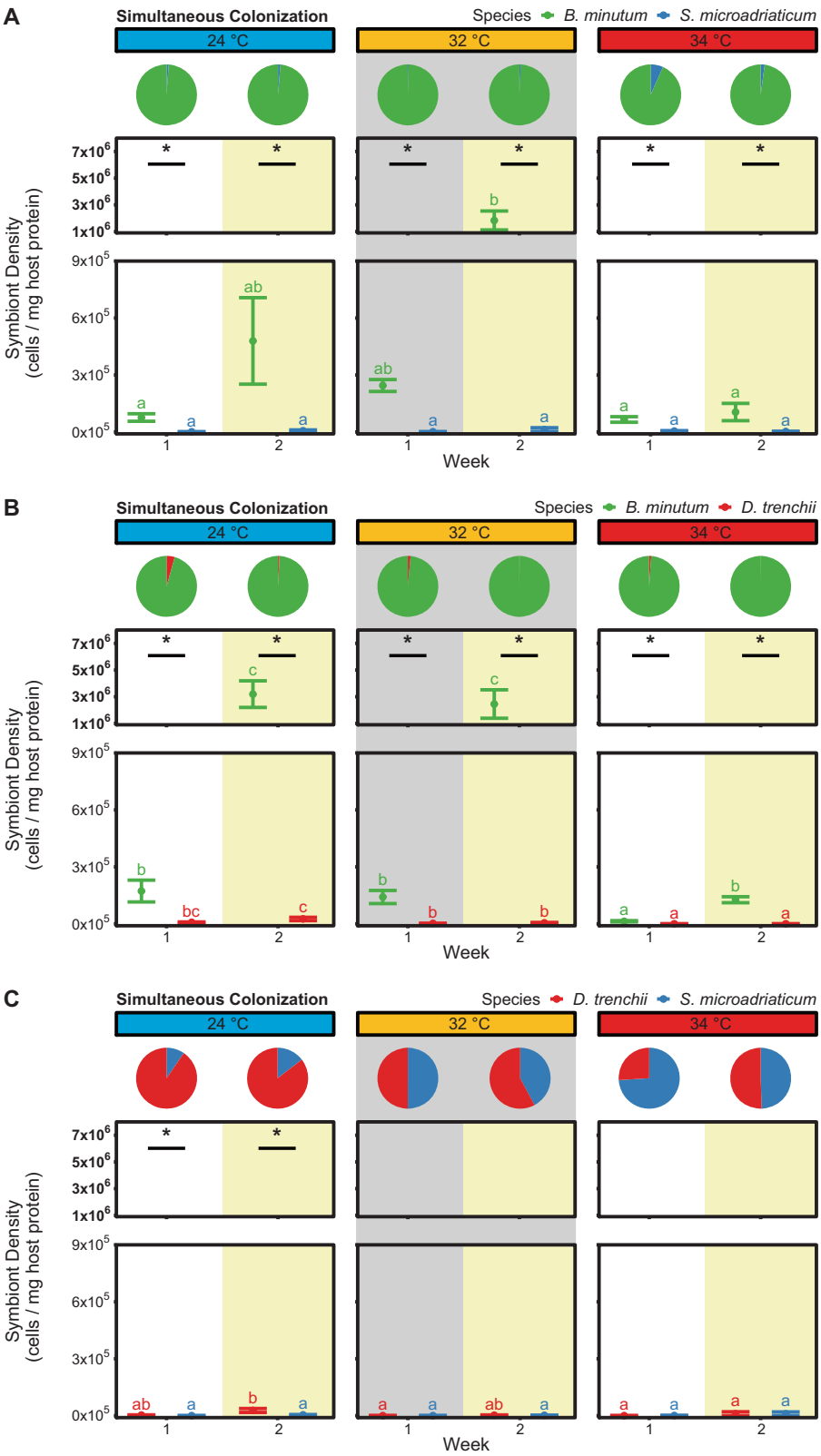

Fig. 2 Simultaneous colonization experiments. Simultaneous mixtures of two different Symbiodiniaceae species (**a** 50% *B. minutum* and 50% *S. microadriaticum*; **b** 50% *B. minutum* and 50% *D. trenchii*; and **c** 50% *S. microadriaticum* and 50% *D. trenchii*) were introduced to aposymbiotic anemones ($n = 4$ polyps *per* treatment *per* time point). Symbiont densities (cells *per* mg host protein) were measured one and two weeks after inoculation under cool (24 °C), ambient (32 °C), and thermal stress conditions (34 °C) *via* genus-specific qPCR. Pie charts represent the average proportion of each species in the host's total symbiont community. Error bars represent the standard error of the mean. Letters correspond to distinct cell densities within a species across treatments (color coded; two-way ANOVA; $p < 0.05$). Asterisks correspond to distinct cell densities across species within a treatment (one-way ANOVA; $p < 0.05$). To visualize large cell density changes without obscuring small changes, results were depicted across two plots each: note the *y*-axis break and unique scales

loss of the heterologous species rather than a gain in the homologous species. Density remained low for both species at 32 and 34 °C. The density of *S. microadriaticum* was significantly greater than the density of *B. minutum* during weeks one and two but not week four at 24 and 34 °C (one-way ANOVA, $p < 0.05$). In this

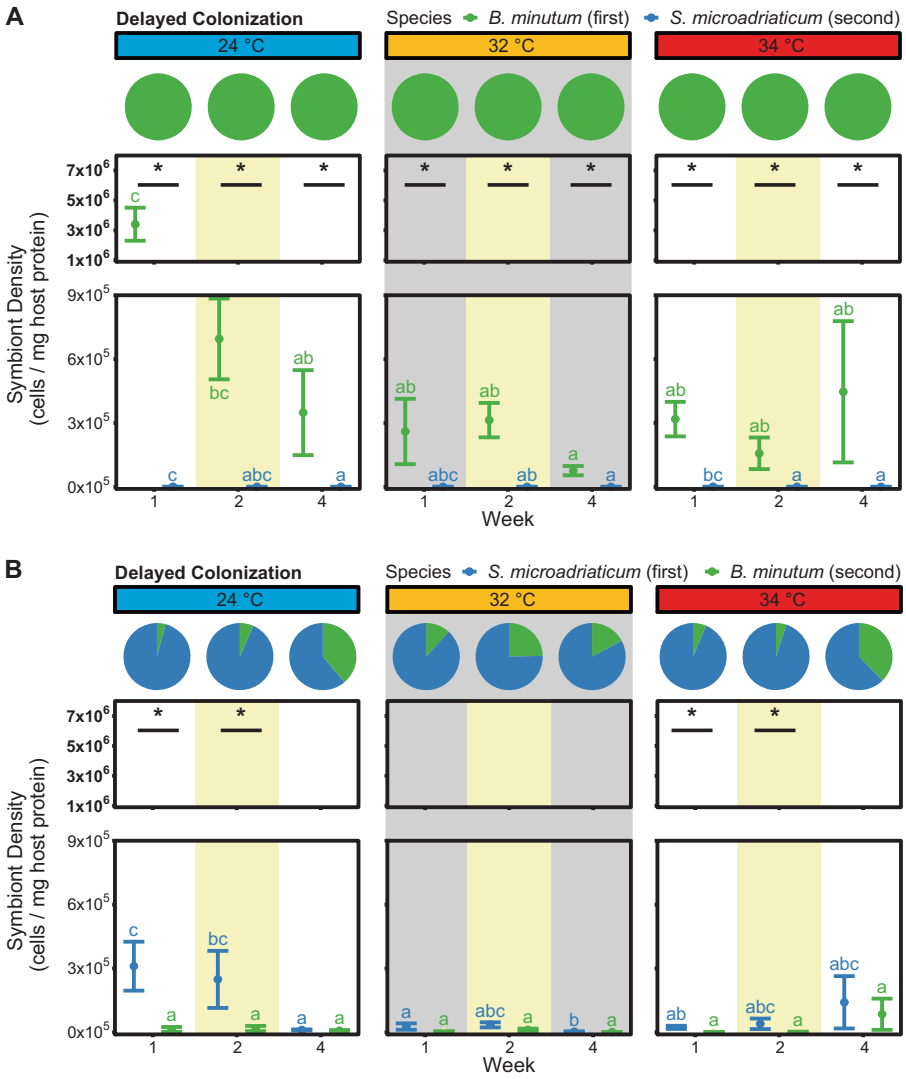

Fig. 3 Delayed colonization experiments. Delayed mixtures of two different Symbiodiniaceae species (**a** first 100% *B. minutum* until the symbiosis stabilized, followed by 100% *S. microadriaticum*; and **b** first 100% *S. microadriaticum* until the symbiosis stabilized, followed by 100% *B. minutum*) were introduced to aposymbiotic anemones (n = 6 polyps *per* treatment *per* time point). Symbiont densities (cells *per* mg host protein) were measured one, two, and four weeks after the secondary inoculation under cool (24 °C), ambient (32 °C), and thermal stress conditions (34 °C) *via* genus-specific qPCR. Pie charts represent the average proportion of each species in the host's total symbiont community. Error bars represent the standard error of the mean. Letters correspond to distinct cell densities within a species across treatments (color coded; two-way ANOVA; $p < 0.05$). Asterisks correspond to distinct cell densities across species within a treatment (one-way ANOVA; $p < 0.05$). To visualize large cell density changes without obscuring small changes, results were depicted across two plots each: note the *y*-axis break and unique scales

case, temperature, time, and their interaction affected *S. microadriaticum* density (two-way ANOVA, $p < 0.05$), but not *B. minutum* density.

Discussion

This study is the first to investigate the effects of thermal stress on multi-species symbiosis establishment dynamics in the Aiptasia—Symbiodiniaceae model system. Given the different thermal tolerances of the three symbiont species used (*D. trenchii* > *S. microadriaticum* > *B. minutum*) and previous evidence for symbiont transitions in other cnidarians under heat stress, we expected to see a shift away from a *B. minutum*-dominated community and towards an *S. microadriaticum*- and/or *D. trenchii*-dominated community in Aiptasia as temperatures rose. However, in all cases, *B. minutum* persisted as the dominant partner.

Single species exposure

As expected, when introduced as the only potential symbiont, the homologous *B. minutum* colonized aposymbiotic Aiptasia polyps far more readily than either *S. microadriaticum* or *D. trenchii*, achieving densities an order of magnitude greater than the two heterologous species at both 24 and 32 °C (Fig. 1). These results are similar to those of previous colonization studies performed under ambient conditions [10]. Marked declines in symbiont density were observed at 34 °C for *B. minutum* and *D. trenchii*, but even here *B. minutum* densities continued to exceed those of the other two species, despite their greater thermal tolerance under culture conditions. Therefore, while these heterologous symbionts have the potential to persist in Aiptasia at elevated temperatures, host-symbiont specificity likely limits their success relative to the native partner. Future work should examine whether these patterns are also observed during symbiont uptake when the

host is already under thermal stress, as opposed to exposure to thermal stress post-uptake as was performed here. These single-species data provided baseline colonization rates against which the simultaneous and delayed exposures could be compared.

Simultaneous exposure

The homologous *B. minutum* was dominant when introduced simultaneously with either heterologous species under ambient conditions, representing the greatest proportion of the symbiont community at the coolest temperature (24 °C; Fig. 2). Contrary to our expectations based upon its thermal sensitivity, however, *B. minutum* remained dominant at 32 and 34 °C, with the heterologous species rarely exceeding 2% of the total community, mirroring the single-species exposure results. Even though symbiont cell density was relatively low at the most extreme temperature, the homologous symbiont still dominated the more heat-tolerant alternatives, at least in terms of proportional abundance. A similar dominance by the native symbiont in Aiptasia at 'normal' temperature (25 °C) was reported by Belda-Baillie et al. [55], who showed that when anemones were inoculated with a mixture of 6 different Symbiodiniaceae isolates (genera *Symbiodinium*, *Breviolum* and *Cladocopium*) and maintained for 3 months, the homologous *B. minutum* was always dominant in the end, and remained so even when exposed again to this same symbiont mixture and maintained for another month.

Our results raise the question as to why the symbiont community did not shift even at elevated temperature. We offer two potential explanations, which are not mutually exclusive. First, the specificity between Aiptasia and *B. minutum* may supersede any potential for establishing primary associations with heterologous symbionts, regardless of the alternate symbiont's heat tolerance. This would mirror certain other high-fidelity associations among cnidarians [20, 28, 56]. Of course, certain hosts more predictably change their symbiont communities under thermal stresses imposed by transplantation or other experimental manipulation [35, 36, 57]. Thus, Aiptasia appears to be a poor model for highly flexible cnidarian-dinoflagellate associations, but a good model for the many marine symbioses that tend to be more specific. It is important to note that the terms "flexibility" and "specificity" can be overly simplistic, because many hosts capable of associating with multiple Symbiodiniaceae species do so with only a highly constrained subset, and nearly all hosts feature moonlighting background populations of diverse symbionts which may or may not become ecologically relevant under climate change scenarios [17, 43, 58].

Second, the interaction between *B. minutum* and its primary host Aiptasia may greatly improve the symbiont's performance *in hospite*, at least relative to heterologous species. Indeed, previous metabolomic and proteomic work on Aiptasia indicates that the host is impacted by thermal stress before symbiotic *B. minutum*, suggesting that the species is more thermally robust when residing in host tissue [59, 60]. This robustness may be facilitated by various host-buffering mechanisms to maintain a favorable internal cellular environment [61–63]. Notably, none of the published studies used in the consensus thermotolerance rankings of Swain et al. [48] examined the performance of *B. minutum* from Aiptasia *in hospite*, so its greater tolerance in its homologous host may have been overlooked in the past.

When compared to the single species exposure results, it is clear that the presence of a second potential symbiont hindered colonization by the homologous symbiont, as it took an extra week for *B. minutum* to reach control density when paired with *D. trenchii*. The same was true when it was paired with *S. microadriaticum* at 32 °C, but control densities were never reached at 24 °C. These data indicate that some sort of competitive interaction was at play, with the outcome being dependent upon the particular combination of symbionts. It remains unclear to what extent competition among symbionts (rather than, or in addition to, host control) may result in the observed specificity of particular cnidarian-dinoflagellate symbioses, but future studies should investigate the mechanisms that may underlie these patterns.

The simultaneous inoculation experiment with the two heterologous species (*S. microadriaticum* and *D. trenchii*) suggested that relative colonization success can change with environmental conditions, as the dominant partner shifted from *D. trenchii* to *S. microadriaticum* as temperature increased. This was a perplexing result because *D. trenchii* is typically one of the most tolerant and opportunistic species under extreme heat [33, 64, 65]. It should be noted, however, that these were artificial associations, and the absolute density of symbiont cells was very low at all temperatures. Hence, caution is warranted when interpreting these data.

Delayed exposure

The final set of experiments was designed to test the strength of the 'home field advantage.' That is, in an established symbiosis, can newly introduced low-abundance symbionts displace the dominant species? The answer is that it appears to depend on the degree of specificity of the host and symbiont for each other. When homologous *B. minutum* was first established as the dominant symbiont in Aiptasia, subsequent introduction of heterologous *S. microadriaticum* failed to cause any community shift (Fig. 3a). *S. microadriaticum* was relegated to background symbiont status, never exceeding 0.1% of the population. This was true even at the most extreme temperature (34 °C), where absolute cell density declined

dramatically relative to the ambient treatment. Thus, even bleaching was insufficient to dislodge the homologous species' numerical dominance, despite the alternate species' greater heat tolerance. A similar pattern has been observed previously in the jelly *Cassiopea xamachana*, where uptake of novel symbionts in the field was much less frequent when polyps had been experimentally inoculated beforehand with the native *S. microadriaticum* rather than with other symbiont types [7].

In contrast, when heterologous *S. microadriaticum* was introduced first (Fig. 3b), subsequent introduction of homologous *B. minutum* did drive a community shift (up to 39% *B. minutum* in one replicate after four weeks at ambient conditions). The change was similar at the extreme temperature, where again the cell density was quite low. Presumably, this shift to *B. minutum* would have continued had the experiment continued. Longer-term trials designed to determine if and when *B. minutum* takes over as the numerically dominant symbiont should be carried out in the future.

The results of both the simultaneous and delayed exposure experiments reinforce the idea that in this particular association, the homologous symbiont species maintains its dominance over more heat-tolerant heterologous symbionts even as temperatures rise and presumably improve the relative performance of the alternate species. Note that we did not measure the physiology or nutritional output of each symbiont species *in hospite*, and therefore we do not know if their relative performance actually changed. Nevertheless, *B. minutum*'s success owes itself to more than just a home field advantage—it appears able to supplant alternate symbionts whether it begins as the dominant species or a background symbiont. These population dynamics are consistent with the high fidelity of this symbiosis throughout the Indo-Pacific. Further work is now needed to better understand the cellular events that underlie these dynamics, and in particular the relative importance of symbiont expulsion, apoptosis, autophagy, and cell cycle control to the regulation of native *versus* non-native symbiont proliferation through the host's tissues [66]. Furthermore, we know little about how competitive interactions between individual symbiont cells or host-symbiont nutritional fluxes might contribute to the relative colonization success of symbionts in a mixed population. Of note though, recent work has demonstrated that, when colonized solely by *B. minutum* or *D. trenchii*, Aiptasia exhibits marked differences with respect to host metabolite profile, as well as various nutritional, stress moderation, and cell–cell signaling pathways [8, 9].

Ecological implications

The potential for symbiotic cnidarians to change their dinoflagellate partners as an acclimatory response to global climate change continues to intrigue researchers [34, 37, 67–70]. However, high host-symbiont specificity may constrain the potential for partner alteration in certain cnidarians, limiting their associations to one or few algal partners [56, 67, 71]. Our study indicates that the Aiptasia —*B. minutum* association is highly specific, at least among Indo-Pacific individuals. However, such partner fidelity might not be disadvantageous. The relationships between specialist cnidarians and symbionts may be the outcome of stringent coevolution [72], resulting in integrated and functionally optimal mutualisms that can persist under both stable and stressful conditions; indeed, to our knowledge there are no published reports of fully bleached Aiptasia in the field. On the other hand, the "symbiotic entrepreneurialism" of generalist hosts may open the door to competitive interactions between symbionts that ultimately impair holobiont function and destabilize the symbiosis [73].

For example, the high degree of host-symbiont specificity between Pacific scleractinian corals in the genus *Porites* and Symbiodiniaceae in the *Cladocopium* C15 lineage is believed to confer considerable ecological benefit when compared to more generalist scleractinian coral genera such as *Acropora* and *Pocillopora* [74–76]. Similarly, the widespread ecological success of Aiptasia across the Indo-Pacific region could be attributed, in part, to its high degree of partner fidelity. Moreover, even though this symbiosis exhibits bleaching at high temperature, the persistent dominance of *B. minutum* suggests that Aiptasia will retain its fidelity for this symbiont even as the climate warms. Whether *B. minutum* continues to offer the greatest ecological benefit to Aiptasia under these changing conditions warrants further study. Likewise, whether symbiont population dynamics in specialist hosts mirror those seen in the model Aiptasia system awaits further confirmation. Such knowledge will better inform predictions about the responses of symbiotic cnidarians, including reef-building corals, to climate change.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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