selection can occur between symbionts in adults in a manner similar to that observed in early ontogeny (*24*)

Corals have survived global changes since the first scleractinian coral-algal symbioses appeared during the Triassic, 225 million years ago (*25*). In recent decades, we have come to appreciate and better understand the fragile nature of coral reefs. The survival of individual colonies and populations should not be confused with the health of ecosystems. However, the ability of octocorals to reestablish symbiont populations from multiple sources provides a mechanism for resilience in the face of environmental change. The task ahead is to access the presence of similar processes in scleractinian corals and to determine if symbionts are available in hospite or exogenously that can allow corals to respond to future environmental changes.

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22. Although it is possible that symbionts containing the marker cp23S-genotypes were initially present as cryptic populations [detection threshold ~1000 cells (*21*)], in many cases the B178 and/or B184 cp23S-rDNA genotypes were detected after bleaching, whereas the marker cp23S-rDNA genotypes were not detected until the host colony was exposed to the isoclonal cultures. The symbionts with the marker genotypes subsequently appeared, replacing these initial genotypes. Furthermore, with one exception (see legend to Fig. 1), a given marker cp23S-rDNA genotype was only detected in colonies exposed to that culture.
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Materials and Methods

Table S1

References and Notes

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Flexibility in Algal Endosymbioses Shapes Growth in Reef Corals

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The relation between corals and their algal endosymbionts has been a key to the success of scleractinian (stony) corals as modern reef-builders, but little is known about early stages in the establishment of the symbiosis. Here, we show that initial uptake of zooxanthellae by juvenile corals during natural infection is nonspecific (a potentially adaptive trait); the association is flexible and characterized by a change in (dominant) zooxanthella strains over time; and growth rates of experimentally infected coral holobionts are partly contingent on the zooxanthella strain harbored, with clade C–infected juveniles growing two to three times as fast as those infected with clade D.

The recent discovery of the genetically diverse nature of the dinoflagellate genus *Symbiodinium* (zooxanthellae) that forms symbiotic associations with stony corals raises the possibility that physiological properties and tolerances of reef corals may vary according to the association established. The genus *Symbiodinium* consists of at least seven clades (A to G) based on sequence analysis of the internal transcribed spacer (ITS) region (*1*–*5*), as well as many genetic types within each clade, referred to as subclades or strains (e.g., C1, C2) (*4*–*6*). In most broadcast spawning corals, zooxanthellae are acquired from the environment in early ontogeny by horizontal transmission and become established in the endodermal cells of coral hosts as an endosymbiosis. This creates an opportunity for the host to establish an association with a variety of symbionts. Indeed, adults of some coral species form associations with more than one *Symbiodinium* strain according to the local environment (7, *8*) or microhabitats within a coral (*6*, *9*, *10*). Such polymorphic symbioses suggest that corals within a species may not be physiologically uniform (*11*) and that the taxonomic identity of the *Symbiodinium* partner(s) may be as significant as that of the host in determining the physiology of the holobiont (host-symbiont partnership). A recent review (*12*) highlights our limited understanding of the influence of symbiont type on physiological performance of the holobiont and the importance of understanding potential flexibility in *Symbiodinium* symbioses in an era of global coral reef deterioration.

Acropora tenuis and *A. millepora* are broadcast spawning corals with horizontal transmission of symbionts (*13*) that, as adults, express different specificities for *Symbiodinium* strains at Magnetic Island (an inshore reef in the central section of the Great Barrier Reef, Australia), where adult colonies of *A. millepora* contain a *Symbiodinium* D strain, whereas *A. tenuis* adults contain *Symbiodinium* strain C1 and occasionally strain C2 (*6*, *10*). The production of larvae free of zooxanthellae by both species provides the opportunity to observe natural patterns of zooxanthella infection and also to manipulate the strains offered for uptake in controlled experimental conditions to determine the impact of known strains on juvenile growth.

Larvae of *A. tenuis* were raised from spawned gametes (*14*) and settled onto tiles (*15*). Positions of juveniles on the tiles were mapped, and the tiles were then attached to the reef (Nelly Bay, Magnetic Island) in a zone where adult *A. tenuis* colonies were abundant (*15*). Thirty juvenile corals were sampled at about 1, 2, 4, and 9 months after settlement. Total DNA (both coral and algal) was extracted from the polyps, and the polymerase chain re-

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action (PCR) was used to amplify the rDNA *ITS1* region of the *Symbiodinium* genome (*6*). We identified zooxanthella genotypes by single-stranded conformation polymorphism (SSCP), using reference samples of known genotypes. Contrary to expectations, we found that the apparent specificity for strain C1 observed in adult populations of *A. tenuis* is not present in the early stages of infection. Two distinct *Symbiodinium* strains, D and C1, were acquired by juveniles in the first month. In the subsequent 4 months, the relative abundance of these two strains within the symbiosis changed, with a clear increase (from ~33 to >90%) in the number of juveniles harboring strain D, and a decrease in the number of juveniles harboring strain C1 (from ~50 to 0%) or a combination of the two strains (from ~17 to <6%) by 4.5 months (Fig. 1). The dominance of *Symbiodinium* D in early juveniles of *A. tenuis*, in contrast to the dominance of *Symbiodinium* C1 in adults of this species in Magnetic Island populations (*6*, *10*), suggests that there may be "active" selection by the host to maximize symbiont effectiveness that varies with differences in physiological requirements between juvenile and adult corals. For example, corals may have a higher demand for nutrients when they reach reproductive maturity, leading to a preference for one type to meet increased energy requirements. It is possible that *Symbiodinium* C1 persists in very low densities and is maintained as an undetectable "background" strain, because the SSCP method cannot detect a strain with a relative abundance below ~5% (*16*). Evidence supporting this interpretation is provided by zooxanthellae cultures, where cultured strains are often not the same as ones initially identified from the host used to establish the culture (*5*, *17*). Thus, the dynamics of coral-zooxanthellae associations may vary with the changing physiological needs of the host in response to life history stage requirements or ambient environmental conditions.

We also examined the impact of locally (i.e., relative to Magnetic Island populations) homologous and heterologous strains of zooxanthellae on growth of coral hosts as a surrogate measure of fitness. Larvae of *A. tenuis* and *A. millepora* were raised in sterile (0.5 μm filtered) seawater (*15*). Strain C1 and D zooxanthellae were isolated from adult corals of the two species, and each strain was added to half of the settled juveniles of each coral species ($N > 1000$) (*15*). As in the above study, the positions of juveniles on settlement tiles were mapped, and tiles were attached to the reef. Growth of juveniles was monitored for 6 months as the number of polyps per colony, a more sensitive measure than colony diameter in the earliest stages of coral growth. Juveniles of *A. tenuis* and *A. millepora* were each able to establish associations with both homologous and heterologous strains of *Symbiodinium*. At each sampling time, SSCP analysis verified that juveniles tested for each treatment contained only the strain initially offered (*15*). Some of the polyps were pale when placed in the field, which suggested that levels of experimental infection were low. Although juveniles gained normal pigmentation, none took up additional types from wild populations of zooxanthellae, which suggests that uptake of zooxanthella types is fixed at an early stage.

We found that juveniles of both species of *Acropora* grew fastest when associated with *Symbiodinium* C1 (*15*) (Fig. 2, A and B). For *A. tenuis*, the rate of polyp budding

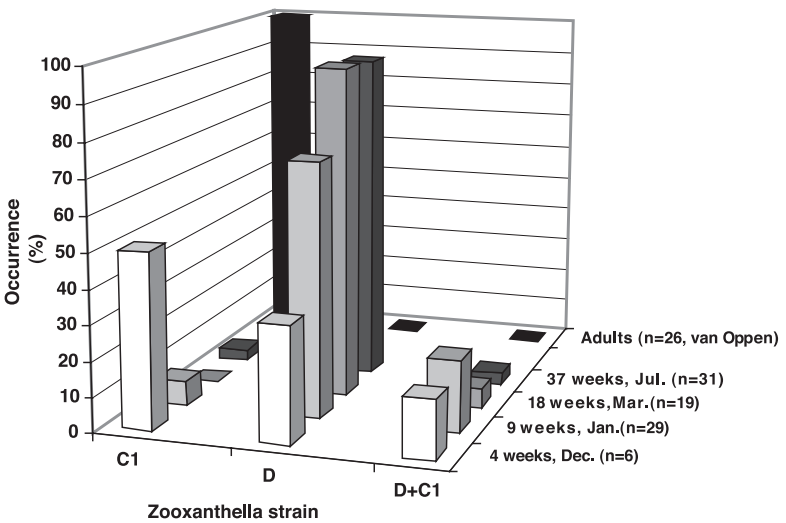

Fig. 1. Relative abundance of two *Symbiodinium* strains in juveniles (in the first 9 months after settlement) and adults (*16*) of *A. tenuis* at Magnetic Island. *n*, number of juveniles genotyped. The rDNA-*ITS1* region was successfully amplified for the majority of juveniles in the last three samples (96, 63, and 100% amplified, respectively), but only for 23% of samples collected at week 4, probably because of low densities of zooxanthellae in juveniles at this early stage.

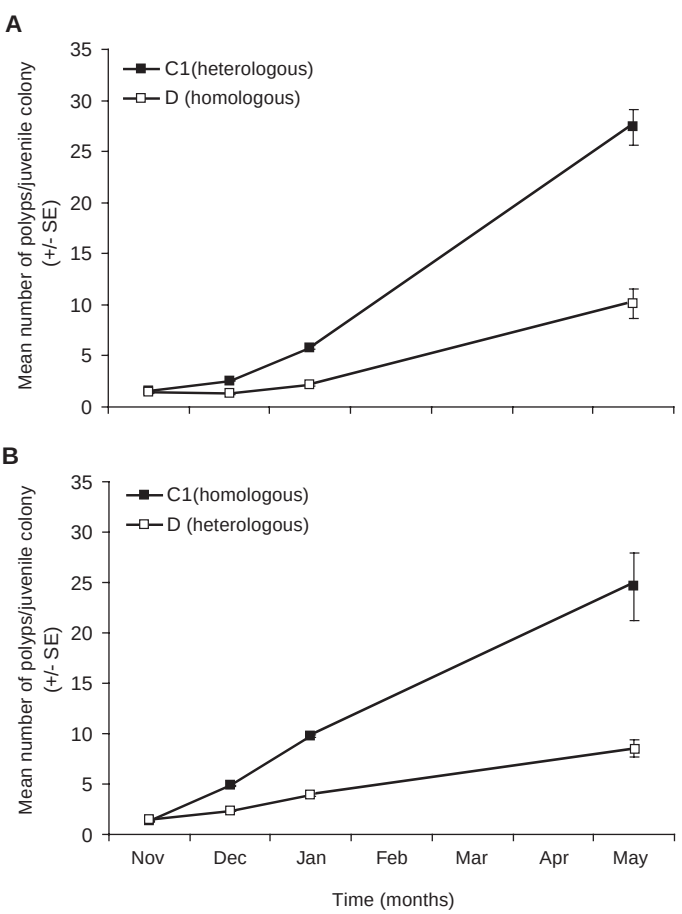

Fig. 2. Comparison of mean (±SEM) growth among coral juveniles infected with zooxanthellae strain C1 versus D over a 6-month period for (**A**) *A. millepora* (n = 2214, 1099, 810, and 203 for C1 juveniles; n = 736, 379, 150, and 13 for D juveniles in November, December, January, and May, respectively), and (**B**) *A. tenuis* (n = 2396, 1842, 1261, and 55 for C1 juveniles; n = 1740, 1473, 1026, and 45 for D juveniles in November, December, January, and May, respectively). Fused colonies were excluded. Where error bars are not visible, they are small and hidden by the symbols.

in the first 6 months was more than 2 times that in C1 juveniles (mean size = 25 ± 3.4 polyps per colony at 6 months) compared with D juveniles (9 ± 0.9 polyps per colony). Similarly, in *A. millepora,* growth of C1 juveniles (mean size = 27 ± 1.8 polyps per colony) during the same period was significantly greater than D juveniles (10 ± 1.5 polyps per colony). Faster growth of holobionts infected with *Symbiodinium* C may reflect a greater contribution of the symbiont to host nutrition through faster rates of population growth inside the host (*18*). For *A. tenuis*, the faster growth rates of C1 juveniles may explain why C1 adults are the most common at Magnetic Island (*10*). In contrast, the dominance of *Symbiodinium* D, known to be associated with greater thermal tolerance (*12*) in naturally infected, 6-month-old *A. tenuis*, may reflect distinct physiological needs of the juvenile holobiont, which recruits into populations at the beginning of summer.

The lack of detectable (by SSCP) acquisition of additional symbiont strains from field zooxanthella populations, when experimentally infected juveniles were reared on the reef, suggests that the temporal window for symbiont acquisition is relatively narrow. The increase in the proportion of *A. tenuis* juveniles with strain D through time may represent greater mortality of juveniles with clade C, or competitive exclusion of clade C within juvenile hosts, at least to undetectable levels, or alternatively, selective up-regulation of strain D by the host. In combination, our results are consistent with adjustments in the ratio of already coexisting symbiont populations or holobiont types, rather than uptake of additional symbiont types. Symbiont shuffling [sensu (*12*)] represents a mechanism for rapid acclimatization of the holobiont to environmental change, whereas the lack of specificity in initial uptake of zooxanthellae in early ontogeny demonstrated in our study provides a mechanism for establishing associations with multiple symbionts and, hence, may be adaptive. Use of more sensitive methods for detecting potential background strains would help determine the mechanism underlying changes in detectable symbiont genotypes in adult corals when moved from one light environment to another (*19–21*) and further our understanding of the "adaptive bleaching hypothesis" (ABH), which postulates that hosts may be repopulated by better-adapted algal endosymbionts after bleaching (*22*).

Our study demonstrates that coral-zooxanthella associations are both dynamic and flexible and that algal endosymbionts contribute significantly to physiological attributes of the coral holobiont. As yet, little is known about host factors that contribute to this symbiosis. Further studies are required for a better understanding of the implications of these new findings for the capacity of corals to cope with global climate change.

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Stomatal Development and Pattern Controlled by a MAPKK Kinase

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Stomata are epidermal structures that modulate gas exchange between a plant and its environment. During development, stomata are specified and positioned nonrandomly by the integration of asymmetric cell divisions and intercellular signaling. The *Arabidopsis* mitogen-activated protein kinase kinase kinase gene, *YODA*, acts as part of a molecular switch controlling cell identities in the epidermis. Null mutations in *YODA* lead to excess stomata, whereas constitutive activation of *YODA* eliminated stomata. Transcriptome analysis of seedlings with altered *YODA* activity was used to identify potential stomatal regulatory genes. A putative transcription factor from this set was shown to regulate the developmental behavior of stomatal precursors.

Pattern formation requires that the specification of individual cells be coordinated with mechanisms that create an ordered spatial arrangement of those cells. The *Arabidopsis* shoot epidermis consists of three major cell types: trichomes, pavement cells, and guard cells in a nonrandom arrangement. Guard cells flank a pore, the stoma, through which water vapor and carbon dioxide are exchanged between the plant and the environment. Mechanisms that control the abundance of these stomatal complexes, therefore, are important components of photosynthetic rate and water use efficiency. Guard cells are the terminal product of a lineage that arises postembryonically in the seedling epidermis. Asymmetric division of a protodermal cell generates a larger cell that is fated to become a pavement cell and a smaller cell, the meristemoid, that serves as a precursor to the guard cells. The meristemoid possesses stem-cell character and divides asymmetrically 1 to 3 times, then modifies its division potential and cell wall and differentiates into a guard mother cell (GMC) (*1*). The GMC makes a single symmetric division to form a pair of

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