Trends in
Microbiology

Review

The use of experimentally evolved coral photosymbionts for reef restoration

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The heat tolerance of corals is largely determined by their microbial photosymbionts (Symbiodiniaceae, colloquially known as zooxanthellae). Therefore, manipulating symbiont communities may enhance the ability of corals to survive summer heatwaves. Although heat-tolerant and -sensitive symbiont species occur in nature, even corals that harbour naturally tolerant symbionts have been observed to bleach during summer heatwaves. Experimental evolution (i.e., laboratory selection) of Symbiodiniaceae cultures under elevated temperatures has been successfully used to enhance their upper thermal tolerance, both *in vitro* and, in some instances, following their reintroduction into corals. In this review, we present the state of this intervention and its potential role within coral reef restoration, and discuss the next critical steps required to bridge the gap to implementation.

Highlights

The heat tolerance of corals is largely determined by their microbial photosymbionts, and manipulating these symbiont communities may enhance the ability of corals to survive summer heatwaves.

Experimental evolution of Symbiodiniaceae cultures under elevated temperatures has been successfully used to enhance the upper thermal tolerance of both symbiont cultures *in vitro* and corals following inoculation.

Novel culturing, upscaling, and biodiversity monitoring techniques are required to deliver experimentally evolved coral symbionts at scale, and success requires learning from, or directly utilising, adjacent industries (e.g., biotechnology for algae-based commodities).

This intervention has transitioned from stakeholder engagement to regulatory approval for risk assessments via small-scale field trials, which is an essential step on the path to implementation.

Novel approaches for protecting tropical coral reefs

Most countries with extended tropical coastlines derive considerable economic benefits from their coral reefs. These ecosystems support important industries, such as fisheries, tourism, and the development and production of pharmaceuticals. They also protect coastal infrastructure from erosion, are biodiversity hotspots, and hold substantial cultural and spiritual values, particularly for **Traditional Owners** (see Glossary). Unfortunately, these magnificent ecosystems face threats from various anthropogenic disturbances, primarily the escalating frequency and severity of climate change-driven summer heatwaves, which cause widespread coral bleaching and mortality (Figure 1). Coral bleaching is the loss of the Symbiodiniaceae symbionts from the coral tissues, resulting in paling of the coral and leaving it in a nutrient-starved state. The first global mass bleaching event in 1998 removed ~8% of all corals worldwide and 14% was lost in the decade between 2009 and 2018 [1]. Further declines of 70–99% in coral cover are predicted to occur within this century as a result of climate change [2]. To minimise additional climate warming and ensure the longevity of coral reefs for the benefit and enjoyment of future generations, it is imperative to rapidly reduce greenhouse gas emissions. However, even under best-case emission reductions scenarios, seawater temperatures are likely to exceed bleaching thresholds for decades [3]. Therefore, the use of both conventional management and active coral reef restoration is necessary to help avert the catastrophic loss of coral reefs.

Researchers around the world are developing a suite of novel coral reef restoration approaches. These include the use of early life stages of coral, such as: sexually produced larvae and recruits (instead of adult coral fragments), which are settled on devices that increase their survivorship and reduce the cost of deployment; the use of artificial structures that stabilise the reef substratum and increase survivorship of coral recruits; and interventions aimed at enhancing coral thermal

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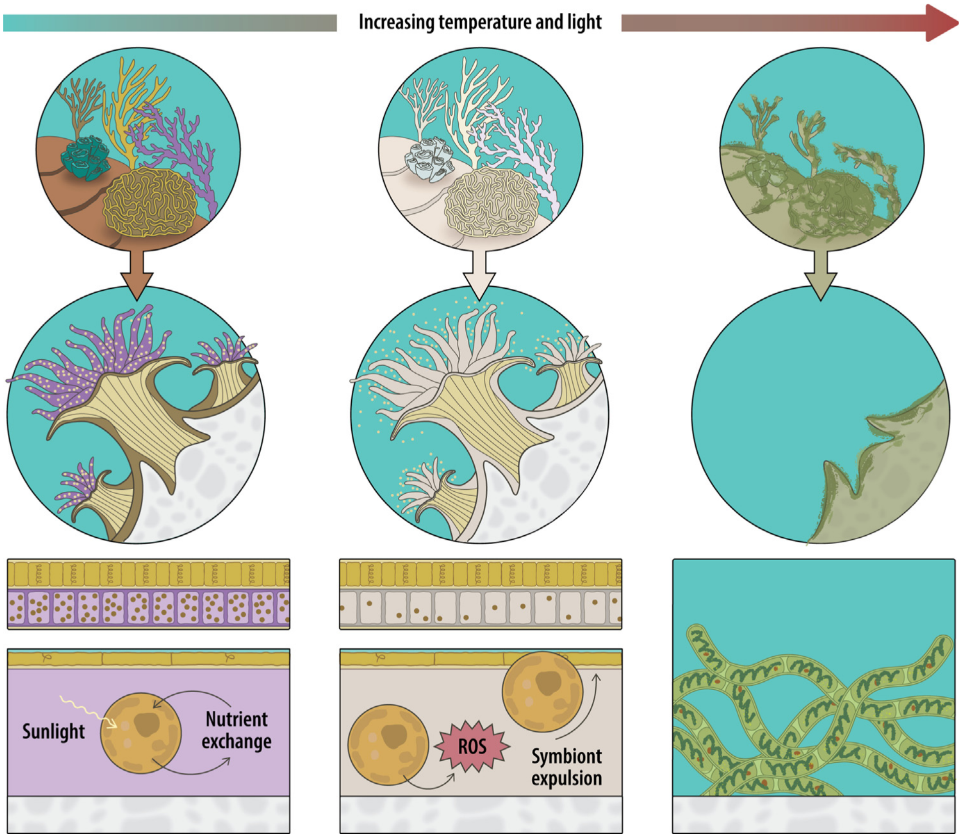


Trends in Microbiology

Figure 1. Coral–Symbiodiniaceae symbiosis and dysfunction under stress. Most stony corals (Scleractinia) form an obligate endosymbiosis with dinoflagellate microalgae in the family Symbiodiniaceae. Under typical conditions, the coral tissue provides a protective environment for the Symbiodiniaceae, which, in turn, produce oxygen through photosynthesis and convert the waste byproducts of coral cellular respiration into compounds essential for coral growth and further respiration. Anthropogenic climate change leading to elevated sea surface temperature (which acts synergistically with other stressors, such as light) can overwhelm the photosynthetic system of the Symbiodiniaceae and cause increased production and release of reactive oxygen species (ROS), which become a source of oxidative stress in the coral tissue. The coral stress response mechanism is to expel the Symbiodiniaceae to prevent damage to its tissues. In the absence of Symbiodiniaceae, the coral tissue is in a 'bleached' state, whereby metabolic processes slow and the coral begins to starve. If the elevated conditions persist and the symbionts fail to repopulate, the coral will die, which is followed by rapid degradation of the reef framework. However, many Symbiodiniaceae are not dependent on the coral host animal for their survival and have a free-living life phase. The survival of expelled symbionts during such conditions in surrounding reef habitats (e.g., sediments and water column) is unknown.

bleaching tolerance [4]. A promising method to elevate the upper thermal tolerance limit of corals involves ***ex hospite*** heat selection (i.e., **experimental evolution** or adaptive laboratory evolution; Figure 2) of their microalgal photosymbionts, which reside within the cells lining the coral gut. The upper thermal bleaching tolerance of corals is largely contingent on the specific Symbiodiniaceae community they harbour [5,6], with both heat-tolerant and -sensitive Symbiodiniaceae species found in nature. For instance, closely related taxa in the *Cladocopium* C15 lineage are associated with differential thermal-bleaching tolerance of their coral hosts [7,8]. Furthermore, differences in thermal tolerance between species in the *Durusdinium* and *Cladocopium* genera are well documented, where the former tend to have higher thermal tolerance, which is conferred on their coral hosts [9,10]. However, even corals that predominantly host *Durusdinium* have been observed to bleach during summer heatwaves [9]. Experimental

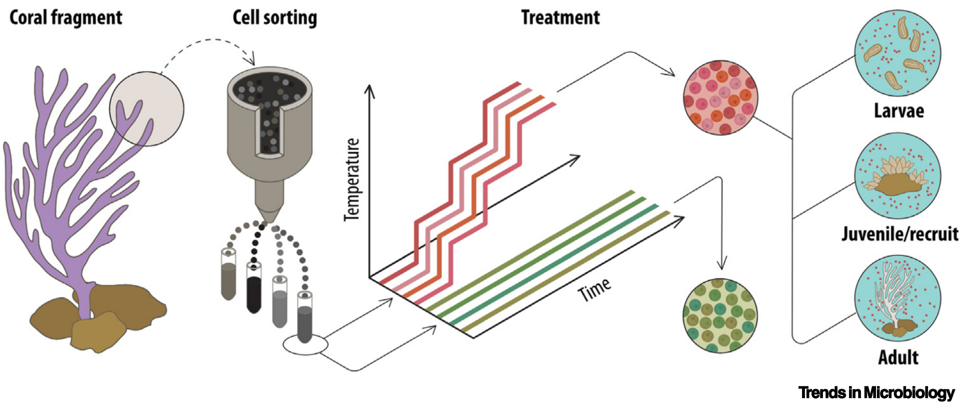


Figure 2. An experimental evolution framework for enhancing Symbiodiniaceae thermal tolerance. Experimental evolution of Symbiodiniaceae can start from a mixed community (multiple genotypes) isolated from a coral fragment or from a single cell (the latter is depicted here). The single cell is grown into a culture of multiple cells that will be genetically closely related [Symbiodiniaceae cultures primarily reproduce asexually, but a small proportion of meiotic cells has been observed in *Cladocopium proliferum* culture (E. Flynn, Master's thesis, University of Melbourne, 2022)]. Starting from a single cell rather than a mixed community permits in-depth understanding of genetic changes arising in response to selection. The culture may then be further divided into replicate subcultures, which are allocated among treatments and which produce 'wild-type' strains kept at ambient temperatures (e.g., 27°C) and heat-evolved strains subjected to temperatures ratcheted upwards (see Box 1) in small increments until reaching the temperature beyond which no growth occurs. After multiple generations under selection, heat-evolved strains are used to inoculate aposymbiotic larvae, juvenile, or bleached adult corals and the holobiont tolerance to heat stress is assessed.

evolution of Symbiodiniaceae offers a means to shift their thermal tolerance limit upward, increasing host resistance to bleaching [11–13].

Experimental evolution of Symbiodiniaceae

Experimental evolution is a common method to study **adaptive responses** to a small number of **selective agents**. It is often conducted with microbes that have short generation times, such as bacteria [14], yeast [15], and microalgae [16], although invertebrates [17] and vertebrates [18] have also been used. A second aim of experimental evolution can be to produce genotypes with improved traits that are relevant for commercial purposes, such as the generation of biofuels [19], or to develop genotypes better adapted to changing environmental conditions, such as Symbiodiniaceae with augmented thermal tolerance for use in coral reef restoration (Figure 2). Few research groups have carried out experimental evolution with Symbiodiniaceae cultures, and most efforts have been directed toward species isolated from Great Barrier Reef (GBR) corals. To the best of our knowledge, outside the GBR, only Symbiodiniaceae from the Mediterranean Sea and Florida Keys have been subjected to experimental evolution. Pierangelini *et al.* worked with three Symbiodiniaceae cultures in the genus *Symbiodinium*, which were isolated from the sea anemone *Anemonia viridis* in the Mediterranean Sea [20]. These cultures were transferred from 25°C to 32°C and maintained for 1 year, after which adaptive changes to elevated temperature were observed. However, the phenotypes were rapidly lost when the cultures were returned to 25°C; therefore, the authors concluded these phenotypes were due to acclimation (i.e., reversible physiological adjustments) rather than adaptation (i.e., fixed changes due to genetic mutation). Long-term thermal selection has also been conducted on *Breviolum antillogorgium*, the Symbiodiniaceae symbiont of the octocoral *Antillogorgia bipinnata* from the Florida Keys [21,22]. Replicate heterogenous symbiont cultures were exposed to 26°C or 30°C for 2 (256–284 generations) [21] and 5 (650–700 generations) years [22], and only cultures in which a single genotype remained were tested. Neither study yielded evidence for genetic adaptation to elevated temperature because the historical temperature (i.e., long-term selection temperature) was not a strong

Glossary

Adaptive response: genetic or epigenetic change in a population in response to a selective agent, resulting in a fitter phenotype and manifesting as enhanced resistance.

Aposymbiotic: state where a host organism is without its usual symbiotic partners.

Cophylogeny: where relationships between two or more groups of organisms, typically host species and their associated symbionts, result in congruent evolutionary history.

Coral holobiont: coral host animal and its associated microbiota.

Degree Heating Weeks: accumulation of temperature anomalies exceeding the monthly maximum mean sea surface temperature for a given region.

***Ex hospite*:** refers to outside the host organism, in this case, outside the coral host.

Experimental evolution: maintenance of replicate populations of an organism in different controlled environments for many generations, with the goal of studying adaptive response to selective forces, or enhancing certain traits of interest (e.g., thermotolerance).

Fitness: relative contribution of a genotype to succeeding generations through survival and/or reproduction.

Generalist species: species/symbionts with a broad host range.

Heat evolved: symbionts with enhanced thermal tolerance developed through experimental evolution under elevated temperature

***In hospite*:** within the host organism.

Local Symbiodiniaceae community: multiple symbiont species coexisting within a single colony.

Macroscale Symbiodiniaceae community: multiple symbiont species among conspecific corals at the site/reef/reef cluster scale.

Maternally transmitted: symbiont transmission occurring directly from coral parental colonies into oocytes before gamete release.

Mutagen: substance or agent that has the potential to induce genetic mutations (i.e., alterations in the DNA sequence of an organism).

Selective agent: any factor that acts for or against a trait, causing directional selection in a population/experimental treatment. Selective agents can be physical (i.e., light, temperature), predictor of contemporary temperature responses. In addition, distinct genotypes remained in the cultures exposed to 26°C and 30°C, respectively and, therefore, no true **wild-type** was available for comparison.

Research on GBR species represents the largest effort in the field of Symbiodiniaceae experimental evolution. Huertas *et al.* transferred two Symbiodiniaceae species from 22°C to 30°C and showed that they were able to grow at 30°C after 55–70 cell generations (100 days at exponential growth) of selection at this temperature, while the wild-type was unable to grow at 30°C [23]. The strains used for this work were isolated from the corals *Heliofungia actiformis* and *Pocillopora damicornis*, but their taxonomic affiliation was not assessed. The **generalist species**, *Cladocopium proliferum*, which is distributed throughout the Pacific [24], has also been subjected to laboratory selection under elevated temperatures. This work started with a heat-tolerant strain isolated from the common scleractinian coral *Acropora kenti* (formerly *Acropora tenuis*) [25] from an inshore reef in the central GBR [26]. Experimental evolution with elevated temperature as the selective agent was started in mid-2011, with ten replicate cultures (hereafter referred to as 'strains') of *C. proliferum*. When one strain was tested after 2.5 years (~80 generations), evidence for adaptation was detected *in vitro*, but adaptive responses of **coral holobionts** inoculated with this strain were minimal. When re-examined after ~4 years of experimental evolution (~120 generations), findings for this strain were similar, but three other strains (of the additional nine strains tested) were observed to enhance thermal bleaching tolerance in *A. kenti* larvae [11]. All ten strains showed enhanced tolerance *in vitro.* This rapid adaptive response (after 80–120 generations) for a microalga with a relatively large cell size and a large and complex genome [27,28] may be the result of the occurrence of occasional sexual reproduction [29–32]. Subsequent examination of a subset of the same **heat-evolved** strains inoculated into *A. kenti* juveniles [12] and experimentally bleached adults of a taxonomically divergent coral species, *Galaxea fascicularis* [33], confirmed the earlier findings and, in addition, demonstrated that enhanced thermal bleaching tolerance did not compromise coral growth (over 40 and 18 weeks, respectively) at ambient temperature. A trade-off against growth at ambient temperature is commonly reported in corals hosting natural, heat-tolerant symbionts [34,35]. As often seen in temperature adaption of other microalgae [16,36–38], ***in hospite*** differences between wild-type and heat-evolved strains were driven by differences in algal pigments [33], and rates of photosynthesis and carbon fixation [11]. Additional Symbiodiniaceae taxa from other genera (*Durusdinium*, *Fugacium*, *Gerakladium*, and *Symbiodinium*) have now also been heat evolved, with some showing *in vitro* adaptive responses after as few as 41–80 generations [39–41], and these are awaiting *in hospite* testing.

Reconciling results among studies and from culture to the holobiont

Limited commonalities can be found among these early studies on Symbiodiniaceae experimental evolution. This is unsurprising given the standing genetic [42] and functional [43] variation among the six genera subjected to selection and the biogeography of their hosts, the varying experimental designs (e.g., designation of wild-type strains) and thermal tolerance assays used, the suite of traits quantified, and the classes of cnidarian hosts (e.g., octocoral versus scleractinian coral) utilised for performance testing *in hospite*. As this field of research progresses, identifying the unresolved issues and embracing standardised techniques could pave the way for enhanced cross-comparability among future studies.

Many Symbiodiniaceae species exist *in hospite*, in reef water, and sediments [44], and some have been established in culture. Thermal tolerance may differ depending on these lifestyles and the experimental setting (e.g., *in hospite* versus *in vitro*), making it challenging to extrapolate findings from one environment to another. For example, Buerger *et al.* [11] and Chakravarti *et al.* [25] observed a lack of correlation between *in vitro* and *in hospite* phenotypes of heat-evolved strains,

chemical (i.e., **mutagen** or nutrients), or biological (i.e., pathogen).
Symbiont shuffling: in corals, refers to a change in the relative abundance of Symbiodiniaceae species that constitute the *in hospite* community.
Traditional Owners: in Australia, Traditional Owners are the descendants of the original inhabitants of a region before European settlement. Australian law acknowledges that Aboriginal and Torres Strait Islander individuals have rights and interests in the land and Sea Country across the length of the GBR based on their traditional laws and customs, recognising them as Traditional Owners.
Transgressive segregation: formation of extreme phenotypes, or transgressive phenotypes, in offspring due to novel combinations of parental alleles at different loci that underpin polygenic traits.
Wild-type: symbionts that have not undergone experimental evolution but have been maintained in a laboratory environment under historical conditions.

highlighting the role of host–symbiont interactions and the interdependencies of the symbiosis. Identifying *in vitro* traits predictive of holobiont performance (e.g., glutathione content [45]) may improve our ability to identify genotypes relevant for coral reef restoration. Additionally, mimicking the *in hospite* environment more closely in culture assays may further improve the transferability of results to the symbiotic lifestyle.

To date, most thermal tolerance assessments have been performed using reciprocal transplants of heat-evolved and wild-type strains to ambient and elevated temperatures that induce stress. However, reciprocal transplants to two environmental conditions provide a narrow lens of the impact of experimental evolution on the thermal tolerance of a strain and potential trade-offs. Thermal performance curves [46] may circumvent this limitation by capturing trait performance values across a wider range of conditions. Furthermore, such curves enable the parametrisation of trait performances, which can provide greater power to predict how evolved strains may perform once reintroduced to natural environments, and enable cross-study meta-analysis and modelling of effect sizes.

In most *in vitro* assessments of the thermal tolerance of heat-evolved Symbiodiniaceae, traits relating to growth (growth rates), photochemical efficiency (maximum quantum yield of photosystem II) and levels of oxidative stress (levels of intracellular/extracellular reactive oxygen species) have been measured. Additional metrics could be included to gain a more holistic characterisation of the likely contribution of a heat-evolved strain to coral holobiont thermal tolerance. For instance, nutrient uptake (nitrate, ammonium, and phosphate) and photosynthate translocation by heat-evolved strains are poorly understood but warrant further investigation because they influence coral holobiont performance [47]. Holobiont trait trade-offs associated with harbouring heat-evolved strains other than growth also need to be understood and monitored. For instance, a recent study found a correlation between the presence of symbionts in the genus *Durusdinium* and stony coral tissue loss disease in the Caribbean coral, *Orbicella faveolata* [48], suggesting that photosymbionts affect coral disease prevalence.

Going forward

New strategies for symbiont cultivation

Targeted isolation of naturally tolerant Symbiodiniaceae strains (e.g., genotypes adapted to the extremes of environmental gradients) could provide valuable starting material for experimental evolution (Box 1). Substantial literature on the thermal tolerances of Symbiodiniaceae species has accumulated via observations of patterns in coral bleaching and can form the basis for such targeted isolation [49,50]. However, Symbiodiniaceae isolation from corals is generally considered challenging, with varying levels of success, and progress toward a shared understanding of ‘best practice’ is hindered by under-reporting of failed culturing attempts [51]. Generating a collection of ecologically important Symbiodiniaceae cultures for experimental evolution may require innovating beyond standard autotrophic growth media (f/2 or IMK) as new understanding emerges of the varying strategies of Symbiodiniaceae resource acquisition (i.e., oligotrophic media, mixotrophic lifestyles [52], or symbiosis with prokaryotes [53]). Targeted isolations of generalist species (e.g., *C. proliferum* or *Durusdinium trenchii*) for experimental evolution will likely continue to be desirable due to the strong potential for return on investment (i.e., evolve one symbiont with applicability to many coral species). In addition to trophic considerations for cultivation, future strategies should also consider the life-histories of Symbiodiniaceae, including **maternally transmitted** symbionts with strong host **cophylogeny** [54]. To the best of our knowledge, maternally transmitted Symbiodiniaceae species are yet to be cultured, but are critical to coral reef function as the vital symbionts of some of the major reef builders (e.g., the *Cladocopium* symbionts of *Porites*). It may be essential to experimentally evolve such symbionts *in hospite*, for

Box 1. Impacts of different experimental evolution frameworks on the fitness landscape

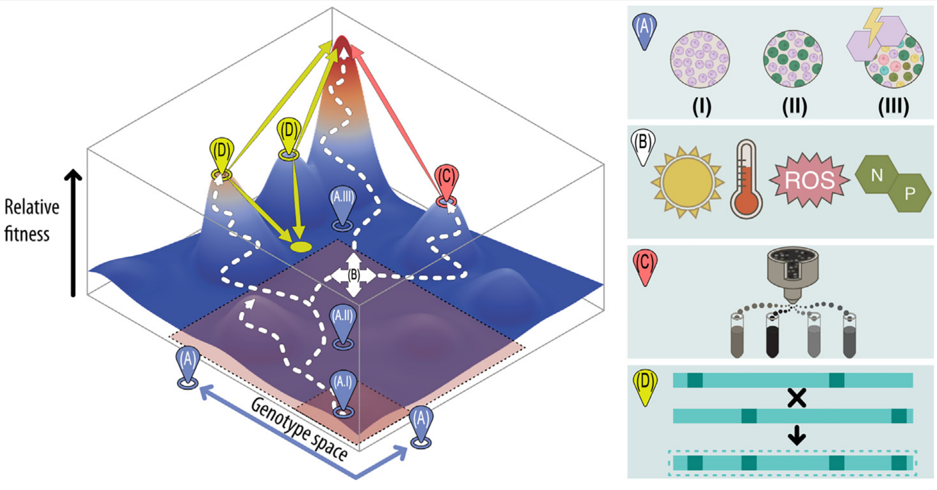
The **fitness** landscape (Figure I) is a concept used in evolutionary biology to represent the relationship between genotypes and their fitness levels in a population. In this landscape, each point represents a genotype, and the elevation of that point the respective fitness 'peak' [71]. Individuals with traits that allow them to succeed in a particular environment are favoured by selection. If these traits are heritable, the alleles that give rise to the trait increase in frequency, causing the population to evolve and adapt to the environment [72].

Experimental Evolution (EE) influences the fitness landscape by leveraging principles of Darwinian natural selection in laboratory environments. The standard framework for Symbiodiniaceae EE is the ratchet method, whereby cell cultures are subjected to stepwise changes in an environmental condition (e.g., temperature), maintaining a selective pressure (see Figure 2 in the main text). Temperature ratcheting has improved the thermal tolerance of Symbiodiniaceae [23,25,40]), phytoplankton [23], and bacteriophages [73], highlighting the strength of this technique.

Genetic variation (A) is the basis of evolutionary change; increasing the standing genetic variation, for example by generating a heterogeneous culture from a population with natural genetic variance (A.II), instead of a culture originated from a single isolated cell (A.I), or by introducing new alleles through *de novo* mutations (A.III), can speed up the adaptation of microorganisms to novel environments [74–77].

Alternative selective agents (B) may provide a shorter path to a desirable trait. For instance, light tolerance is often related to thermal tolerance due to shared physiological and adaptive responses in the photosynthetic machinery, such as coping with excessive reactive oxygen species [78]. In addition, selection for thermal resilience in microalgae often results in the reduction of cell size [45,79]. Selecting for small cell size and higher light tolerance (i.e., single versus multiple selective agents [80]) has not been attempted for Symbiodiniaceae. Incorporating fluctuations into the selection regime may promote different adaptive trajectories compared with selection under homogeneous conditions [37,41]. To add such complexity into EE frameworks, Symbiodiniaceae research must embrace technological advancements, such as automation and cell sorting (Figure IC), in prospecting for desirable traits [81–83].

The use of sexual reproduction (Figure ID) among different strains/species for EE awaits exploration. Recombination between genomes carrying adaptive alleles at different loci can produce phenotypes with increased fitness, although adverse outcomes are also possible due to negative epistasis [84]. For polygenic traits, such as thermal tolerance, it is likely that culture strains have evolved adaptive alleles at different loci. These alleles can be combined via sexual reproduction, potentially resulting in novel genotypes with better fitness (**transgressive segregation**).



Trends in Microbiology

Figure I. Using selective agents to explore the fitness landscape. The fitness landscape concept illustrates how genotypes relate to fitness levels, and how increasing genetic variation (A) through heterogeneous cultures or *de novo* mutations can accelerate adaptation. Exploring alternative selective agents (B), high-throughput phenotyping (C), and incorporating sexual reproduction (D) offer avenues for potentially discovering and enhancing desirable traits in organisms, such as Symbiodiniaceae. Abbreviation: ROS, reactive oxygen species.

example, using coral cell cultures [55], and use certain techniques (e.g., chemical bleaching) to integrate such symbionts into other corals. One limitation is that Symbiodiniaceae divide less frequently *in hospite*, but this may be overcome by using mutagenesis before *in hospite* selection. Further, exploratory co-culture of host-specialist symbionts (i.e., with naturally high thermal tolerance, such as *Cladocopium C15*) with diverse coral cell cultures from varying species may result in the evolution of host generalism [56].

Upscaling from a single cell to industrial-scale biomass

One strategy to utilising experimentally evolved coral symbionts for restoration at scale is to integrate them with the sexual production of coral (i.e., coral spawning), inoculating **aposymbiotic** recruits before field deployment. To our knowledge, research cultures are typically in the millilitre to litre volumes, yet thousands of litres would be required to inoculate millions of coral recruits reared from multiple reefs or regions (i.e., restoration at industrial scales). Upscaling from a single cell to biomass suitable for experimentation requires 6 to 9 months in addition to the 2 to 3 years [25] under selection required to shift trait values. Scaling these small experimental cultures to thousands of litres may be time and resource intensive, especially if a desirable trait (i.e., heat tolerance) is traded against culture growth rate at elevated temperatures. However, mass production of Symbiodiniaceae for restoration can look to the many solutions within industries that grow microalgae for the purpose of yielding valuable commodities (e.g., biofuels or nutraceuticals) to overcome bottlenecks. For example, it may be possible to fine-tune the culture conditions (e.g., media composition, lighting, or bacterial probiotics [57]) to match the stoichiometry of resource requirements as trait values change under experimental evolution. Symbiodiniaceae often exhibit benthic growth in culture (e.g., biofilm formation), which will likely result in nonlinearity of yields when attempting to grow biomass at greater densities (e.g., self-shading). Thin-film bioreactors or cascades (with high surface area:volume ratios) is one such solution [58] for the mass culture of Symbiodiniaceae. Experimental evolution aimed at restoration can further learn from, or directly utilise, such industries where strategies are used to prevent yield loss due to genetic/trait drift, which include cryopreservation and maintaining large volumes during subculturing to minimise the effect of repeated bottlenecking [59].

Monitoring biodiversity

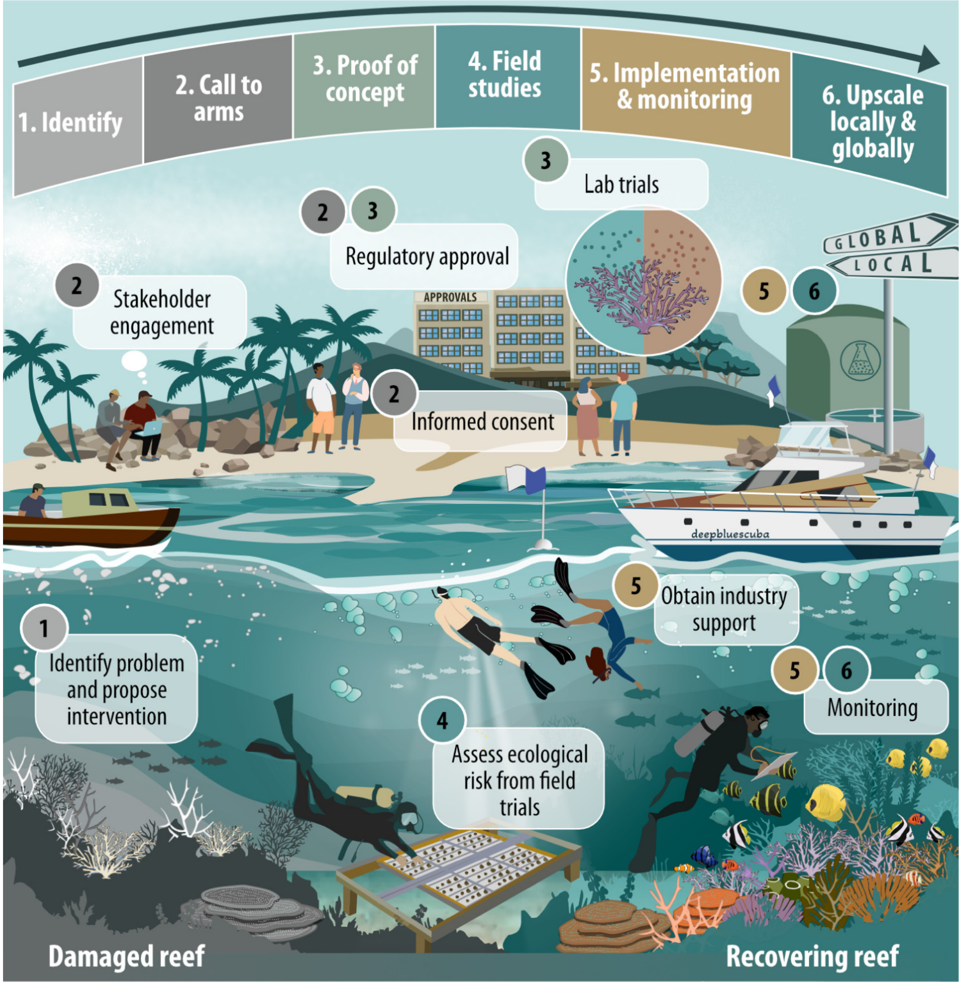
Reef restoration activities that alter the **local** (i.e., within a coral colony) and **macroscale** (i.e., among colonies or reefs) Symbiodiniaceae communities should be paired with significant upscaling in monitoring capability (e.g., genetic assays and high-throughput cell sorting). The most used approach for characterising Symbiodiniaceae community composition is metabarcoding of the Internal Transcribed Spacer 2 (ITS2) region [60]. This will likely be insufficient to identify and monitor heat-evolved strains because, after >10 years of subjecting *C. proliferum* to selection, the ITS2 intragenomic variants were unchanged [13]. Comparatively less research has been conducted on population genetics of Symbiodiniaceae species in the field, or among replicate cultures that are subjected to selection and maintained in isolation. Symbiodiniaceae population structure can emerge at small spatial scales [61], but there are also examples of single clones dominating coral colonies across hundreds of kilometres of reef [62]. Replicate cultures constitute a collection of allopatric populations and identifying diagnostic genetic signatures (e.g., microsatellites, single nucleotide polymorphisms, or genomic rearrangements) that emerge in these cultures against wild-type cultures or conspecific symbionts *in situ* will be essential for intervention monitoring. New genetic monitoring frameworks are needed to detect the future mixing of these populations when reunited within local or macroscale symbiont communities. For example, given the mounting evidence for sexual reproduction in the Symbiodiniaceae (Box 1), the potential for introgression of heat-evolved strains via intra- and interspecific hybridisation (i.e., the cross-breeding of distinct populations of the same species or different species) requires the development of techniques to detect and monitor hybrid offspring. This may be especially useful to examine whether hybrid phenotypes differ to those of both parents (i.e., hybrid vigour or outbreeding depression). In the absence of sexual reproduction, further potential for hybridisation has been observed in the form of Symbiodiniaceae protoplast fusion [63], although long-term viability and cell division following fusion have not yet been demonstrated.

Further knowledge gaps regarding the rearrangement of community structures include the possibility of reduced effect sizes (including the mitigation of trait trade-offs) when wild-type and/or homologous symbionts persist alongside heat-evolved strains in an individual coral [33]. While adult corals typically harbour a numerically dominant symbiont in addition to one or more low-abundance strains, juvenile corals appear to be predisposed toward harbouring more diverse assemblages [64]. The reasons for these ontogenetic changes are unknown, although it is possible that this supports the transition of recruits from cryptic habitats into the adult niche [65]. Perhaps symbiont-based interventions should inoculate coral juveniles with communities of multiple symbiont genera [66] experimentally evolved to coexist; this may maximise symbiont genetic and functional diversity and improve coral resilience. Thus, bridging these knowledge gaps and acquiring an enhanced capability to monitor coral symbionts in space and time is critically important.

Closing the gap to reef restoration

The development of a new biodiversity conservation/restoration intervention may entail the following steps (noting that the regulatory frameworks differ across countries): (1) identification of the problem and evaluation of initial proposals for intervention; (2) an open consultation with stakeholders, including the general public, to obtain the social licence to operate; free and prior informed consent for the work secured from relevant Traditional Owner groups; and permits obtained from regulators to collect biological material from the field or for working in the field; (3) demonstration of proof-of-concept in the lab, including via peer-reviewed scientific publications; (4) regulatory approval in place for small-scale field tests, including an ecological risk assessment for larger-scale deployment of the intervention based on results from the small field trial and the literature; (5) obtaining industry support for field testing and monitoring on a larger scale; and (6) implementation of the intervention at local and global scales (Figure 3). The use of coral inoculated with experimentally evolved photosymbionts is currently at Step 4, with data expected to be collected over the next year to permit progression to Step 5.

A critical decision about the efficacy of this intervention will revolve around determining whether field-deployed corals inoculated with heat-evolved symbionts exhibit greater tolerance to summer heatwaves compared with their native counterparts, because all observations so far have been made in the lab. The thermal tolerance phenotypes of the new symbioses are expected to vary across environments and, therefore, we define success of the intervention as follows. First, corals harbouring heat-evolved symbionts (alone or in combination with native symbionts) have thermal tolerance levels that are never lower compared with native corals and are higher at some reef habitats or sites. Second, experimentally evolved symbionts persist within the target corals through time. While heat-evolved symbionts remained in symbiosis with corals for the full duration of the longest lab experiment to date (2 years) [33], comparable data from field-deployed corals are not yet available. For a coral reef restoration intervention to be economically viable, benefits to the reef need to persist long term. Thus, the temporal stability of symbioses with heat-evolved strains, and whether heat-evolved symbionts can increase their relative abundance following retreat into the rare biosphere (i.e., **symbiont shuffling**) need to be examined in the field (see Outstanding questions). Third, experimentally evolved symbionts can be acquired by other corals from the surrounding environment. It is imperative to establish whether and how far



Trends in Microbiology

Figure 3. Pathway to implementation. Successful deployment of reef intervention measures, such as those described in this review, require multiple checkpoints, with experimental evolution of coral symbionts to enhance the thermal limits of corals currently at Step 4.

heat-evolved symbionts that are expelled from their coral host spread in the reef environment, and whether they can be acquired by other corals (healthy or bleached). Healthy corals are known to continuously expel symbionts to maintain stable symbiont cell densities, with many of these being viable [67,68]. If the new symbionts do spread, the benefits (i.e., upscaling of the intervention) and possible unintended consequences, such as heat-evolved symbionts outcompeting native symbionts (and a loss of genetic diversity of the environmental symbiont pool), or unexpected trait trade-offs, need to be understood.

Once the intervention has been implemented in one region, efforts can be made to upscale the approach locally and globally. Knowledge transfer and collaboration with relevant industries may be possible. For instance, the aquaculture industry may assist in growing heat-evolved strains at industrial scales for inoculation of millions of coral larvae that are reared for deployment on damaged reefs. Tourism operators may be able to provide support to implement this intervention on the reefs where they operate, and financial investment from other industries that rely on healthy coral reefs, such as commercial fisheries, may be sought. Knowledge around this intervention (and others) should also be transferred to other coral reef regions in the world, and there are now several grant schemes that are specifically designed for such a purpose (e.g., The Coral Research & Development Accelerator Platform).

Concluding remarks

The Caribbean summer of 2023 saw the most severe coral bleaching event to date, with temperature anomalies reaching >20 **Degree Heating Weeks**. Extreme levels of bleaching-related mortality occurred both in wild corals and corals that were nursery bred and subsequently outplanted to restore damaged reefs [69]. This emphasises the notion that reef restoration with coral stock that is not thermally enhanced may not be the best option because climate models predict that more extreme and frequent summer heatwaves will continue to be part of our immediate future. Therefore, the development of assisted evolution methods, such as the one discussed herein, to deployment-ready tools is a time-critical task. The Caribbean event has also taught scientists and restoration practitioners working in areas with less degraded coral reefs, such as the GBR, that we need to be prepared for the worst and explore all options. This includes an urgent need to invest in research and development for interventions that carry high levels of perceived risks (and high 'pay-off' if they are successful) and that have received less attention, such as the translocation of corals and symbionts from warmer reef regions (e.g., from the Coral Triangle or Red Sea to the GBR).

While we have provided evidence that heat-evolved symbionts can boost coral thermal bleaching tolerance, the effect size of this intervention alone may not suffice to protect corals from extreme heatwaves, such as the 2023 event mentioned above. It is more likely that multiple interventions will need to be combined in coral reef restoration efforts, such as assisted gene flow, managed breeding, or manipulation of coral-associated bacteria, in combination with the use of experimentally evolved Symbiodiniaceae [70]. Fortunately, many methods of enhancement can be relatively easily combined with novel coral reef restoration methods that use sexually produced coral stock [4]. All these interventions will be critical in maximising the likelihood that coral reefs persist into the future, but it is imperative that these occur in tandem with significant emissions reductions (e.g., of carbon dioxide) and science-based reef management.

Outstanding questions

Will symbioses between coral and experimentally evolved symbionts persist over time and continue to exhibit enhanced performance?

Are there trade-offs associated with heat-evolved symbionts (e.g., nutrient production/translocation, coral growth, reproductive output, or disease susceptibility)?

Should inoculation with communities of multiple heat-evolved symbiont species be considered, and would these provide larger enhancements to holobiont performance compared with single-symbiont inoculations?

What are the nutritional and physicochemical requirements of vertically transmitted symbionts? Solving this question will allow these symbionts to be cultured and subjected to experimental evolution.

How can the process of experimental evolution be sped up to reduce time to generate enhanced phenotypes/genotypes?

What are the barriers to upscaling symbiont cultures and which lessons or innovations can be leveraged from algae-based industries?

Can the uptake of heat-evolved symbionts by coral stock ear-marked for reef restoration be optimised?

Can uptake be improved by utilising symbiont behaviours (e.g., chemotaxis), easing the transition between culture and symbiotic lifestyles (e.g., nutrient gradients), and/or enabling host–symbiont recognition (e.g., cell surface modifications of the symbionts)?

Could genomic prediction be used to enable high-throughput screening of thermal tolerance traits that are independent of symbiotic state? If so, how do we account for differences observed *in vitro* versus *in hospite*?

How can the effect size of heat-evolved symbionts be quantified when they co-occur with homologous (or heterologous) symbionts in the coral host?

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Declaration of interests

None declared by authors.

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How can heat-evolved symbionts best be deployed on the reef (i.e., paired with sexually produced larvae or inoculated into adults; inoculated into reef sediments)?

To what extent do heat-evolved symbionts spread via horizontal transmission in the wild?

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