Evidence for de novo acquisition of microalgal symbionts by bleached adult corals

Hugo J. Scharfenstein, Wing Yan Chan, Patrick Buerger, Craig Humphrey and Madeleine J. H. van Oppen

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Early life stages of most coral species acquire microalgal endosymbionts (Symbiodiniaceae) from the environment, but whether exogenous symbiont uptake is possible in the adult life stage is unclear. Deep sequencing of the Symbiodiniaceae ITS2 genetic marker has revealed novel symbionts in adult corals following bleaching; however these strains may have already been present at densities below detection limits. To test whether acquisition of symbionts from the environment occurs, we subjected adult fragments of corals (six species in four families) to a chemical bleaching treatment (menthol and DCMU). The treatment reduced the native microalgal symbiont abundance to below 2% of their starting densities. The bleached corals were then inoculated with a cultured Cladocopium C1acro strain. Genotyping of the Symbiodiniaceae communities before bleaching and after reinoculation showed that fragments of all six coral species acquired the Cladocopium C1acro strain used for inoculation. Our results provide strong evidence for the uptake of Symbiodiniaceae from the environment by adult corals. We also demonstrate the feasibility of chemical bleaching followed by reinoculation to manipulate the Symbiodiniaceae communities of adult corals, providing an innovative approach to establish new symbioses between adult corals and heat-evolved microalgal symbionts, which could prove highly relevant to coral reef restoration efforts.

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Scleractinian corals prosper in oligotrophic waters by forming mutualistic relationships with microalgae (Symbiodiniaceae) that translocate photosynthate to their host [1]. Breakdown of the coral-Symbiodiniaceae symbiosis, i.e. coral bleaching, occurs in response to environmental stress and may result in widespread coral mortality [2]. Thermal stress is the primary cause of large-scale coral bleaching events, which have become more frequent and severe over recent years as climate change-driven marine heatwaves gain prevalence [3].

Bleaching tolerance of corals to elevated temperatures varies within and among species. This is partly determined by the physiological performances of their microalgal symbionts under thermal stress [4, 5]. For instance, thermotolerant Symbiodiniaceae species in the genus Durusdinium have been found to increase the bleaching threshold of the coral holobiont by 1–2 °C [6].

The Symbiodiniaceae comprise at least 15 genera and genus-level lineages which include many species [7]. Several microalgal symbionts may coexist within a coral host, with the Symbiodiniaceae communities of adult corals often being dominated by a single species [8]. Other members of the symbiont community are found in low abundances, forming a Symbiodiniaceae rare biosphere increasingly linked to coral bleaching resilience [9]. Changes in the relative abundance of Symbiodiniaceae species (shuffling) [10] and the acquisition of new symbionts from the environment (switching) [11] in adulthood are potential mechanisms for corals to adapt to increases in sea surface temperatures [4–6, 8–11]. Evidence of symbiont shuffling is widespread [4, 5, 10], yet reports of switching in adult corals remain limited to metabarcoding studies [12] and the Symbiodiniaceae rare biosphere [11], where the acquired symbionts may have been present below the detection limit before bleaching. Whilst environmental uptake of exogenous Symbiodiniaceae has been demonstrated in adult sea anemones [13] and octocorals [14], experimental evidence of switching in adult corals remains unconvincing with only a transient symbiosis being reported in adult colonies of Porites divaricata following bleaching [15].

Here we used chemical bleaching to remove >98% of the native symbiont cells from adult fragments of six coral species spanning four families (i.e. Diplastrea heliopora (Diplastreaeidae), Dipsastrea palida (Merulinidae), Echinopora lamellosa (Merulinidae), Platygryra daedalea (Merulinidae), Porites lobata (Poritidae) and Stylophora pistillata (Pocilloporidae)), which were then successfully reininfected with a cultured Symbiodiniaceae strain. Colonies were fragmented and chemically bleached (n = 16 per species) through exposure to menthol and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU; Supplementary methods section 1.3). Once bleached, the coral fragments underwent four different reinoculation treatments (n = 3 per species and treatment): (1) a negative control treatment (Ctl-) where corals were not reinoculated with any Symbiodiniaceae; (2) a positive control treatment (Ctl+) where corals were reinoculated with freshly isolated homologous Symbiodiniaceae (obtained through tissue blasting of conspecific coral fragments); (3) a reinoculation treatment (Ri) where corals were reinoculated with a cultured Cladocopium C1acro strain (SCF055-01.10) at 10^6
Fig. 1 Coral bleaching and repigmentation responses to chemical bleaching and reinoculation. A Symbiodiniacaeae cell densities in hospite before and after chemical bleaching (n = 4 per species) and after reinoculation (n = 6 per species, except for E. lamellosa: n = 2; P. daedalea: n = 5; S. pistillata: n = 5). Error bars represent 1 standard error. B Images of corals before and after chemical bleaching and nine weeks after reinoculation with a cultured Cladocopium C1 strain in the presence of sterilised sand (treatment RiS shown here). Both D. pallida and D. heliopora displayed thinner tissue after reinoculation resulting in their septa standing out. No tissue necrosis was recorded in these coral species, contrarily to E. lamellosa and P. lobata fragments. The Coral Watch Coral Health Chart depicts differences in pigmentation when a coral undergoes bleaching.
but not reinoculated with any Symbiodiniaceae. Ctl = reinoculated with freshly isolated homologous Symbiodiniaceae. Cladocopium species, the native Symbiodiniaceae were replaced with the symbionts from the environment. The chemical bleaching and reinoculation methodology may prove highly relevant for the study of coral-Symbiodiniaceae interactions and for the development of adult coral stock with enhanced thermal tolerance for reef restoration by inoculating them with heat-evolved Symbiodiniaceae 

The environmental acquisition of the Cladocopium C1acro strain by P. lobata and S. pistillata contributes to the growing body of evidence that vertically transmitting corals also possess the ability to acquire symbionts horizontally [17]. The presence of sand during reinoculation had a measurable effect on the composition of the Symbiodiniaceae community in P. daedalea only, which led to a mixed community with a Durasdinium phylotype that was initially detected in low abundance (<1%). Chemical bleaching is increasingly used in studies investigating cnidarian-symbiont interactions to obtain asymbiotic hosts for reinfection with cultured Symbiodiniaceae [13, 18]. Adult Stylophora pistillata and Isopora pallida corals have been found to lose 99% of their algal symbiont densities following menthol exposure [19]. Comparable levels of Symbiodiniaceae density reduction (98.3–99.8%) were observed for the six coral species used here. Unlike Exaiptasia diaphana [18], none of the coral species in this study were rendered completely asymbiotic from exposure to menthol and DCMU. Severe reduction of the native Symbiodiniaceae communities, rather than complete elimination, is thus sufficient for novel symbioses to establish, though the long-term stability (>9 weeks after reinoculation) of these symbioses needs to be investigated. The presence of sand during reinoculation had a noticeable effect on the composition of the Symbiodiniaceae community in P. daedalea only, which led to a mixed community with a Durasdinium phylotype that was initially detected in low abundance (<1%).
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AUTHOR CONTRIBUTIONS

All authors participated in the conception and design of the study. HS carried out the experiment and sample processing. HS, WC and PB performed the data analysis and figure drawing. HS, WC, PB and MvO provided critical biological interpretations of the data. HS wrote the paper. WC, PB, CH and MvO supervised this work and edited the paper.

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COMPETING INTERESTS

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ADDITIONAL INFORMATION

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Correspondence and requests for materials should be addressed to Hugo J. Scharfenstein.

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