Green fluorescence from cnidarian hosts attracts symbiotic algae

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Reef-building corals thrive in nutrient-poor marine environments because of an obligate symbiosis with photosynthetic dinoflagellates of the genus *Symbiodinium*. Symbiosis is established in most corals through the uptake of *Symbiodinium* from the environment. Corals are sessile for most of their life history, whereas free-living *Symbiodinium* are motile; hence, a mechanism to attract *Symbiodinium* would greatly increase the probability of encounter between host and symbiont. Here, we examined whether corals can attract free-living motile *Symbiodinium* by their green fluorescence, emitted by the excitation of endogenous GFP by purple-blue light. We found that *Symbiodinium* have positive and negative phototaxis toward weak green and strong purple-blue light, respectively. Under light conditions that cause corals to emit green fluorescence, (e.g., strong blue light), *Symbiodinium* were attracted toward live coral fragments. *Symbiodinium* were also attracted toward an artificial green fluorescence dye with similar excitation and emission spectra to coral-GFP. In the field, more *Symbiodinium* were found in traps painted with a green fluorescence dye than in controls. Our results revealed a biological signaling mechanism between the coral host and its potential symbionts.

phototaxis | GFP | symbiosis | fluorescence | coral

Reef-building corals form an obligate symbiotic relationship with dinoflagellates of the genus *Symbiodinium*. Some coral species acquire *Symbiodinium* via transmission from the parent to the oocyte. However, >70% of coral species acquire *Symbiodinium* from the environment after the propagules are released (1), and both larvae (2) and juveniles (3) have been shown to acquire symbionts in the field. Uptake of symbionts by adult corals is also thought to be possible, particularly after stress, such as bleaching (4, 5).

Free-living *Symbiodinium* can change morphology rapidly, transforming from a flagellated motile cell during the day to a spherical nonmotile form at night (6). In contrast, *Symbiodinium* within the host's cells are always nonmotile. In general, motility in free-living *Symbiodinium* peaks a few hours after exposure to light (6, 7). Given that corals are sessile for most of their life-history, with the exception of a comparatively brief larval stage, the coral hosts must rely on free-living *Symbiodinium* to come to them. *Symbiodinium* densities are very low on coral reefs (8–10), and therefore some mechanism of attraction would be of great benefit to the host. One hypothesis for the acquisition of *Symbiodinium* is via chemo-attraction by the host (11, 12). An alternative hypothesis, whereby symbionts are attracted to green fluorescence from endogenous GFPs in the host (13), has yet to be tested.

Many coral species are bright green on exposure to the UV/blue region of light. This green color is attributed to the fluorescence from endogenous GFP (14, 15). GFP was first isolated from the jellyfish *Aequorea victoria* (16), and its homolog genes are found in many cnidarians (15, 17), crustaceans (18), and chordates (19). The light absorption and fluorescence emission spectra differ among the homologs, of which there are four fluorescent (cyan, green, yellow, and red) and one nonfluorescent (purple-blue color) types (20, 21). The function of these fluorescent proteins has been the focus of much research. Fluorescence proteins have been proposed to change the light environment in the coral tissue, resulting in the enhancement of light suitable for photosynthesis by *Symbiodinium* (14) and protection of the photosynthetic machinery from damaging light wavelengths (22).

Previous research using white light dispersed by a prism at a single intensity demonstrated that *Symbiodinium* accumulated in the region of green light, leading to the hypothesis that free-living *Symbiodinium* swim toward green fluorescence emitted by corals (13). However, the emission of green fluorescence relies on light exposure (e.g., sunlight), and sunlight includes green light that might also attract free-living *Symbiodinium*. Thus, the hypothesis also assumes that *Symbiodinium* do not swim toward the light source. Indeed, it has yet to be proven that coral's green fluorescence attracts *Symbiodinium* either in the field or under controlled laboratory conditions. Here, we perform a series of experiments to test whether coral's green fluorescence can induce directional movement (i.e., phototaxis) in free-living motile *Symbiodinium*. Our results demonstrate that *Symbiodinium* have positive phototaxis mostly toward weak green light and negative phototaxis mostly toward strong purple-blue light, with the result that they swim toward corals, but not the light source, under

Significance

Reef-building corals cannot survive without symbiotic algae, *Symbiodinium*, on which they depend for most of their energy. Most coral species gain symbionts from the environment early in life, and possibly after bleaching (i.e., the loss of symbionts in response to stress). However, *Symbiodinium* density on coral reefs is very low. Although it has long been hypothesized that corals must be able to attract free-living *Symbiodinium*, such a mechanism has yet to be identified. Here, we use a series of experiments to demonstrate that corals attract *Symbiodinium* using their endogenous GFP-related green fluorescence, revealing a biological signaling mechanism that underlies the success of this symbioses that is the building block of coral reef ecosystems.

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conditions in which corals emit green fluorescence (e.g., strong blue-containing light).

Results

Characteristics of *Symbiodinium* Phototaxis. Previous research has established that *Symbiodinium* have phototaxis, the level of which varies throughout the day and among *Symbiodinium* phylotypes (6). To expand on this previous work, we first studied phototaxis using cultured *Symbiodinium* OTcH-1 (clade A) grown in a light (12 h)–dark (12 h) cycle. *Symbiodinium* cells were harvested every 2 h from between 2 and 12 h after exposure to light and were placed in a plastic container for monitoring phototaxis (Fig. 1*A*). The container was exposed to light at eight different light intensities from 0.28 to 40 μmol $m^{-2} \cdot s^{-1}$ from one side for 10 min. The culture was then equally partitioned into proximal (*P*) and distal (*D*) halves from the light source, and the phototaxis index was calculated as $[(P - D)/(P + D)]$. We used green light to measure the phototaxis index because previous work suggests that *Symbiodinium* have positive phototaxis toward green light (13). In our experimental conditions, phototaxis was seen in samples harvested after 2–8 h from the onset of light exposure, with the maximum levels occurring after 4 h (Fig. 1*B*). Phototaxis was always positive (i.e., *Symbiodinium* moved toward green light; peak at 525 nm). A similar maximum level of activity was evident under blue light (peak at 460 nm); however, low blue light caused positive phototaxis and high blue light caused negative phototaxis (*SI Appendix*, Fig. S1). Further experiments were therefore carried out using cells harvested between 2 and 5 h from the onset of light exposure.

To further understand the characteristics of *Symbiodinium* phototaxis, we tested the effect of the light spectrum (every 20 nm from 360 to 700 nm) on the phototaxis index, using the Okazaki large spectrograph that provides monochromatic light with a half-bandwidth of 10 nm. The phototaxis index was measured at eight different light intensities in each spectrum (Fig. 1*C*). *Symbiodinium* showed significant positive phototaxis toward purple (peak at 400 nm), blue (peak at 440 nm), green (peak at 510 nm), and red (peak at 680 nm) weak light, with the highest activity toward green light. Furthermore, *Symbiodinium* showed significant negative phototaxis against purple-blue (375–475 nm) strong light. Our experiments clearly demonstrate that *Symbiodinium* phototaxis can be both positive and negative, and that the direction of movement is influenced by both wavelength and intensity.

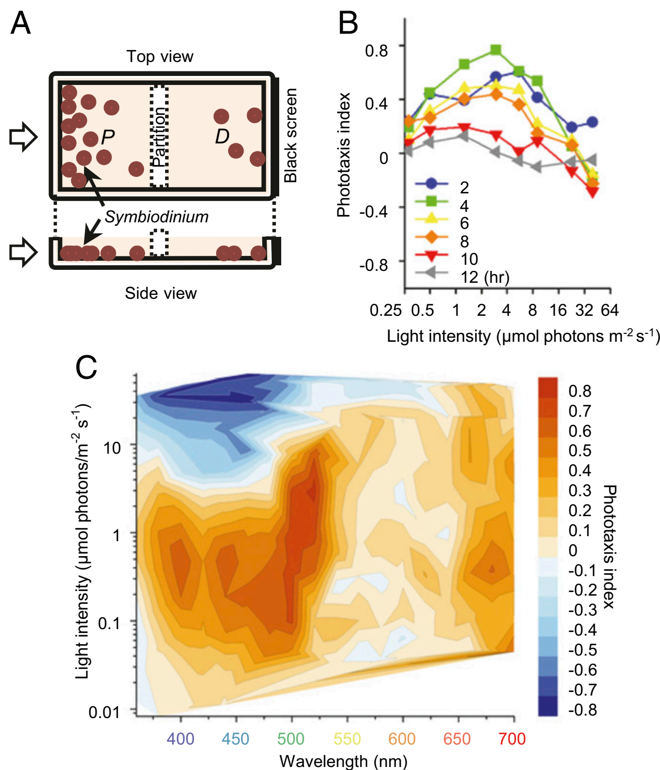

Fig. 1. Phototaxis in *Symbiodinium*. (*A*) An illustration of the experimental set-up used for evaluating the phototaxis index used in this study. The *Symbiodinium* OTcH-1 culture in the container was irradiated with light (*open arrows*) for 10 min, followed by equal partitioning of the container (*dashed square*) into moieties proximal and distal to the light. The cell densities of the proximal (*P*) and distal (*D*) moieties were then used to calculate a phototaxis index, using the equation described in the text. (*B*) The effect of time of day on phototaxis of *Symbiodinium* OTcH-1 toward a green LED. The *Symbiodinium* cells were harvested after the onset of light exposure, from 2 to 12 h, and were placed in a plastic container for monitoring phototaxis (n = 3 biological samples). (*C*) Contour map of the phototaxis action spectrum of *Symbiodinium* OTcH-1. The phototaxis index was determined from measurements taken under 18 different monochromatic light spectra at eight different light intensities (n = 3 biological samples).

Attraction of Motile *Symbiodinium* by Corals. Next, we examined whether living corals can attract motile *Symbiodinium*, using the coral *Echinophyllia aspera* (green morph; Fig. 2*A*), which fluoresces bright green under blue light. The maximum excitation and emission peaks for the green fluorescence of this coral were at 492 and 505 nm, respectively (Fig. 2*B*), consistent with characteristics of GFP (20). We placed two coral fragments (length × width × height = 8 mm × 8 mm × 2 mm) in each container. One was taken from live coral (live), and the other from dead (i.e., skeleton only) as a control. We then counted *Symbiodinium* cell numbers around each fragment after 10 min of light exposure (Fig. 2*C*). In our experiment, the coral fragments were coated with resin plastic to eliminate the possibility of chemotaxis. In conditions in which corals emit green fluorescence (i.e., blue light), *Symbiodinium* cells were found in significantly higher abundance near the coral fragment (Fig. 2*D* and Movie S1), and the cell concentration was 10 times greater than the initial density (Fig. 2*E*). In contrast, there was no attraction to the coral skeleton or to the live coral in light conditions that do not cause green fluorescence (i.e., under green or red light or in darkness; Fig. 2*E*).

Attraction of Motile *Symbiodinium* to a Green Fluorescent Dye. To examine whether the attraction of *Symbiodinium* to coral fragments is directly associated with green fluorescence, we monitored the attraction of *Symbiodinium* using an artificial object (a 2-mm high, 8-mm diameter, dome-shaped resin plastic disk) painted with a green fluorescent dye (GFD), and a nonpainted white disk as a control. The fluorescence emission spectrum of the GFD showed a maximum peak at 504 nm (Fig. 3*A*), similar to the maximum peak of the coral's GFP, at 505 nm (Fig. 2*B*). Under blue light, *Symbiodinium* cells gathered around the GFD-painted disk, but not around the nonpainted one (Fig. 3*B* and Movie S2). The accumulation of *Symbiodinium* around the disk did not happen in conditions in which GFD does not emit green fluorescence (i.e., under green or red or in darkness; Fig. 3*C*). These results demonstrated that GFD-associated green fluorescence attracts *Symbiodinium*, supporting our hypothesis that the accumulation of *Symbiodinium* around the coral fragment under blue light is related to GFP-associated green fluorescence. *Symbiodinium* also accumulated around the GFD under full-spectrum sunlight, but densities were much less than under blue light (Fig. 3*C*). Furthermore, *Symbiodinium* accumulation under full spectrum sunlight required much higher light intensity (60 μmol photons $m^{-2} \cdot s^{-1}$) than under blue light (3 μmol photons $m^{-2} \cdot s^{-1}$; Fig. 3*C*). The accumulation of *Symbiodinium* around the GFD-painted

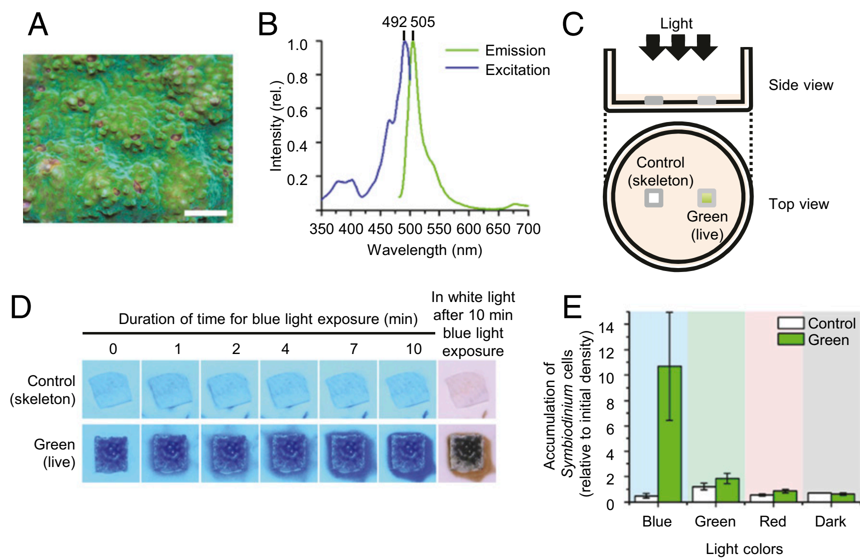

Fig. 2. Attraction of *Symbiodinium* cells to a green fluorescent coral. (*A*) Top surface of the plate-like coral, *E. aspera*, used in this study. Photograph was taken under natural light conditions. (Scale bar, 1 cm.) (*B*) Fluorescence spectrum (blue, excitation; green, emission) of the coral body. (*C*) Schematic illustration of the fluorescence attraction assay. (*D*) Representative pictures of the *Symbiodinium* accumulation around the green fluorescent coral fragment (square 8 mm on a side; *Lower*), but not around the coral skeleton control (*Upper*) during the exposure to blue light (20 μmol photons · $m^{-2} \cdot s^{-1}$) for 10 min. (*E*) Attraction of *Symbiodinium* cells around the coral fragments on exposure to different colors of light (20 μmol photons · $m^{-2} \cdot s^{-1}$) for 10 min. The values are relative to cell density of the culture before irradiation ($n = 3$ biological samples; bars, ±SE).

disk occurred under a wide range of fluorescence intensities, even when the GFD emitted only 25% of the *Echinophyllia aspera* fluorescence (Fig. 3*D*).

Attraction of *Symbiodinium* to a GFD on the Reef. On the basis of the results of the laboratory experiments, we next conducted field experiments to determine whether green fluorescence attracts *Symbiodinium* in natural light environments on the reef. We placed nine pairs of white traps, one painted with GFD and the other not painted (Fig. 4*A*), at depths of between 3 and 6 m on the fringing reef surrounding Sesoko Island, Okinawa, Japan, for 3 h (13:00–16:00). The seawater collected in the traps was then centrifuged, and the numbers of *Symbiodinium* cells were determined by real-time PCR analysis, using *Symbiodinium*-specific primers (23). The correlation between the cell number and the PCR signal (amount of DNA fragment amplified) was first tested using cultured *Symbiodinium* from clade C (*SI Appendix*, Fig. S2*A*), which is the most common *Symbiodinium* in

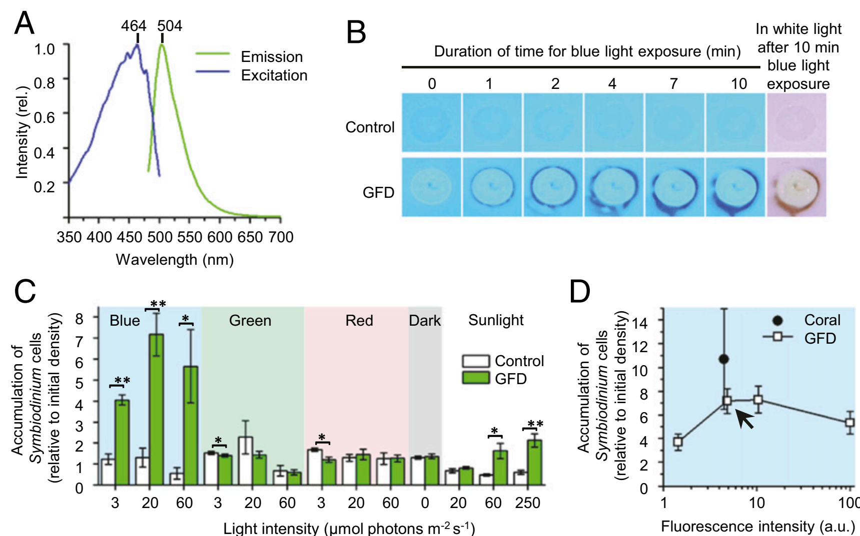

Fig. 3. Attraction of *Symbiodinium* cells to an artificial green fluorescent object. (*A*) The fluorescence spectrum (blue, excitation; green, emission) of the GFD used. (*B*) *Symbiodinium* accumulation around the GFD-painted disk (8 mm in diameter; *Lower*), but not around the control disk (*Upper*), during the exposure to blue light (20 μmol photons $m^{-2} \cdot s^{-1}$) for 10 min. (*C*) Attraction of *Symbiodinium* cells to the GFD-painted disk on exposure to different colors of light for 10 min. The values are relative to cell density of the culture before irradiation. $^{*}P < 0.05$; $^{**}P < 0.01$, Student's *t* test. (*D*) Effect of the intensity of green fluorescence of the coral and GFD on attraction of *Symbiodinium* cells on the exposure to blue-light (20 μmol photons $m^{-2} \cdot s^{-1}$) for 10 min. The arrow shows the GFD intensity used in experiments for *Symbiodinium* attraction (*A*–*C*). The value for the coral was the same as in Fig. 2*E*. (*C* and *D*) $n = 5$ biological samples for GFD-painted disk; $n = 3$ biological samples for the coral. Bars, ±SE.

Fig. 4. Attraction of *Symbiodinium* cells to green fluorescent traps in coral reefs. (*A*) Seawater traps with and without green fluorescent dye. (*B*) Box-and-whisker plot for the density of *Symbiodinium* in the green fluorescent trap relative to that in the non-GDF painted (white) trap. The box, line, and square represent the quartiles, median, and average, respectively. Data were taken at nine sampling sites.

corals around Okinawa (24). The *Symbiodinium* cell number was 2.5 times higher in the green fluorescent traps than in control traps (Fig. 4*B*), indicating that green fluorescence can cause accumulation of *Symbiodinium* on the reef.

Discussion

The attraction of motile *Symbiodinium* by coral fragments only occurred under light conditions in which corals emit green fluorescence (i.e., blue light; Fig. 2). In our experiments, coral fragments were coated with plastic resin to eliminate the possibility of chemotaxis. Therefore, the attraction of *Symbiodinium* by coral fragments was not caused by signal molecules released by corals. To support this hypothesis, we showed that a green fluorescent disk also attracted motile *Symbiodinium* in light conditions in which the disk emitted green fluorescence (i.e., blue light and full-spectrum sunlight; Fig. 3). The attraction of *Symbiodinium* occurred over a wide range of fluorescence intensities, even as low as 25% of the fluorescence emitted by *E. aspera*, the coral used in this study (Fig. 3*D*). This result suggests that the attraction of *Symbiodinium* by green fluorescence can also occur in other host organisms that emit less green fluorescence than *E. aspera*. We therefore conclude that green fluorescence emitted by cnidarians can attract motile *Symbiodinium*. In the field, GFD-painted traps collected more *Symbiodinium* cells than the controls (Fig. 4*B*). This result suggests that the attraction of *Symbiodinium* by green fluorescence can also occur on the reef.

Free-living *Symbiodinium* potentially sense at least two different phototactic signals from opposite directions; one is the light signal from the sun, and the other is the fluorescent signal from benthic corals. Thus, the attraction of *Symbiodinium* to corals occurs only if the positive phototaxis toward the fluorescent signal is dominant. The fact that the extent of *Symbiodinium* attraction by the green-fluorescent dye increased as light intensity increased suggests that this would be the case (Fig. 3*C*). The attraction of *Symbiodinium* by fluorescent dye required 3 μmol photons $m^{-2} \cdot s^{-1}$ under blue light (e.g., in deep water) and 60 μmol photons $m^{-2} \cdot s^{-1}$ under sunlight (e.g., in shallow water; Fig. 3*C*). It is therefore likely that the light intensity required for the attraction of *Symbiodinium* by green fluorescence differs with light spectra and depth. Because blue light penetrates seawater to a depth of ~50 m, the attraction of *Symbiodinium* by green fluorescence potentially occurs in both shallow and deep water.

Symbiodinium showed significant positive phototaxis toward weak purple (peak at 400 nm), blue (peak at 440 nm), green (peak at 510 nm), and red (peak at 680 nm) light, with the highest activity toward green light (Fig. 1*C*). *Symbiodinium* use chlorophyll *a* and *c*2, both of which have absorption peaks in the blue and red regions, and a unique carotenoid, peridinin, which has an absorption peak in the blue/green region (470–550 nm) (25), for photosynthesis (26). Thus, *Symbiodinium* showed positive phototaxis toward light wavelengths that match the absorption peaks of their photosynthetic antenna pigments, including chlorophylls and carotenoids. Furthermore, *Symbiodinium* showed negative phototaxis under purple/blue light (380–475 nm) at intensities greater than 3 μmol photons $m^{-2} \cdot s^{-1}$. Purple/blue light is known to damage the photosynthetic machinery (i.e., photosystem II) in *Symbiodinium* (27). It is therefore likely that *Symbiodinium* evolved phototaxis to locate a light environment suitable for photosynthesis, as has been demonstrated in other algae (28), rather than for finding host cnidarians.

Phototaxis is initiated by the absorption of light by photoreceptors, leading to the transduction of the captured light signal to the flagella. *Symbiodinium* have an optical system, known as an eyespot. However, the photoreceptor for phototaxis is unknown. So far, two types of photoreceptors have been identified in algae: rhodopsin (an opsin protein in combination with the chromophore retinal) in *Chlamydomonas reinhardtii* (29) and photoactivated adenylate cyclase in *Euglena gracilis* (30). In *Symbiodinium*, there are no photoactivated adenylate cyclase genes, but eight opsin genes with conserved retinal binding sites occur in the genome (*SI Appendix*, Fig. S3) (31), and at least some are expressed (32). Thus, rhodopsin is the most likely candidate for the phototactic photoreceptor of green light, and possibly other wavelengths.

Symbiodinium showed strong positive phototaxis to green light (485–525 nm) over a wide range of light intensities (Fig. 1*C*). The green wavelength region includes wavelengths in which other fluorescence proteins (i.e., cyan fluorescent proteins) have emission peaks (485–495 nm) (19). It is therefore conceivable that the florescence form cyan fluorescent proteins also attract motile *Symbiodinium*. There was no significant positive phototaxis to light wavelengths in which yellow fluorescent proteins (emission maxima between 525–570 nm) and red fluorescent proteins (emission maxima between 576–595 nm) have emission peaks (20, 21), suggesting that they are not involved in attracting *Symbiodinium*, at least for the strain used in our study. Many marine organisms associated with *Symbiodinium* have GFP (19), suggesting that the attraction of *Symbiodinium* using GFP might be common. However, many nonsymbiotic marine organisms also have GFP. Because phototaxis is a common phenomenon in motile organisms (see review ref. 28), fluorescent marine organisms might use GFP or other fluorescence proteins to attract motile plankton for predation (33).

Reef-building corals are obligate symbionts: they do not survive unless symbiosis is established very early in life. Therefore, the recruitment of free-living *Symbiodinium* from the environment is crucial for the ~70% of reef coral species that lack symbionts in the oocytes. Because infectivity is related to *Symbiodinium* density around the host (9, 10), the attraction of free-living *Symbiodinium* by green fluorescence helps corals recruit symbionts. However, not all *Symbiodinium* attracted necessarily end up in symbiosis because each host species can only associate with a limited number of *Symbiodinium* types (34–37). GFP-associated fluorescence is higher in larvae and juvenile coral polyps that lack symbionts compared with those that have symbionts, again suggesting a role for green fluorescence in attracting symbionts at this crucial life stage (38). The survival of corals affected by stress, such as the loss of symbionts after exposure to high temperature, might also rely on recruiting symbionts from the environment. This hypothesis is consistent with results showing

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that GFP-associated fluorescence is higher in bleached corals than in nonbleached ones (39). Furthermore, as bleaching sensitivity differs among symbiont *Symbiodinium* phylotypes, attracting a range of symbiont types might increase the chance of corals to survive bleaching (4). Our findings address a critical gap in our knowledge of the symbiosis between corals and algae, and suggest a role for GFP.

Materials and Methods

Cultures and Growth Conditions. *Symbiodinium* sp. OTcH-1 (clade A) were obtained from the National Institute of Technology and Evaluation (Chiba, Japan). *Symbiodinium* Mf1.05b (clade B1) were from Mary Alice Coffroth (State University of New York at Buffalo, Buffalo, NY). *Symbiodinium* Y103 (clade C) were originally maintained in Michio Hidaka's laboratory (University of the Ryukyus, Okinawa, Japan), and were gifted from Eiichi Shoguchi (Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan). OTcH-1, Mf1.05b, and Y103 were originally isolated from *Tridacna crocea*, *Orbicella faveolata*, and *Fragum* sp., respectively. *Symbiodinium* were cultured in 200 mL of artificial seawater (sea salts no. S-9883; Sigma-Aldrich) containing Daigo's IMK medium for marine microalgae (Wako) without shaking or air bubbling in a 500-mL Erlenmeyer flask with a silicone sponge closure at 25 °C under white fluorescent bulbs at 60 μmol photons · $m^{-2} \cdot s^{-1}$ with a light/dark cycle of 12 h/12 h. Cells in their midlogarithmic growth phase ($1–2 \times 10^5$ cells/mL) were used for the experiments. An automatic cell counter (TC20; Invitrogen) was used. Coral, *E. aspera* (green morph type), purchased from a coral supplier, was maintained in an aquarium tank with 1,100 L artificial seawater at 25 °C under white and blue LEDs at 100 μmol photons $m^{-2} \cdot s^{-1}$ with a light/dark cycle of 12 h/12 h. All the laboratory experiments were carried out at room temperature (24–25 °C).

Light Sources. For the action spectrum of phototaxis, the Okazaki Large Spectrograph (40) was used to provide 18 different monochromatic light spectra with a half-bandwidth of 10 nm. For the other experiments, LED panels (VBP-L444-W6666; Valore) were used to provide blue (460 nm), green (525 nm), and red (660 nm) light with a half-bandwidth of 16 nm (*SI Appendix*, Fig. S4). Spectra of the LED lights and sunlight used in the experiments are shown in *SI Appendix*, Fig. S4.

Evaluation of Phototaxis. *Symbiodinium* cells were placed in a plastic container (width × length × height = 39 × 68 × 15 mm) that was illuminated down the length of the container (Fig. 1*A*). The opposite side was colored black to prevent light reflection. After 10 min light exposure, the culture was equally partitioned into halves proximal (*P*) and distal (*D*) to the light source by a silicon spacer. The cell density in each half was determined by flow cytometry (Attune; Life Technologies). The phototaxis index was calculated as $[(P - D)/(P + D)]$, with values ranging from −1 to 1 (41). Positive and negative index values indicate positive and negative phototaxis, respectively.

Attraction of Symbiodinium by Coral Fragments or Artificial Objects. Both coral fragments (alive) and skeletons from similar fragments (control) were cut into 8-mm squares with 2-mm height with a cast cutter and coated with a plastic resin (Sunnote). The artificial fluorescent objects and the controls were made from resin plastic with a GFD (fluorescent-green acrylic lacquer, Atom support) or a white dye (nonfluorescent white acrylic lacquer, Atom support), respectively. Coral fragments and skeletons or GFD and white plastic objects were placed in round white plastic dishes (95 mm in diameter; Fig. 2*C*) with cultured *Symbiodinium* cells, and exposed to light for 10 min. Then, *Symbiodinium* cells around the coral fragments or plastic objects were separated by silicon rings (21 mm in diameter) and collected for measuring the cell density. A TC20 cell counter or an Attune flow cytometer were used for cell counting in coral or plastic experiments, respectively. Paired two-tailed Student's *t* tests were performed using MS Excel.

Evaluation of Fluorescence. Excitation and emission spectra of the green fluorescent coral fragments coated with the resin and green fluorescent resin objects were measured with a FluorMax-4 spectrophotometer (Horiba Jobin Yvon). Excitation spectra were detected at an emission wavelength of 520 nm, and emission spectra were detected at an excitation wavelength of 470 nm. The relative quantification of green fluorescence of the coral fragment and the GFD-painted object was performed with the fluorescence stereo microscope M205FA (Leica microsystems) with the optical filter set LEICA 10447408 (excitation, 450–490 nm; emission, 500–550 nm).

Attraction of Free-Living *Symbiodinium* on a Coral Reef to a Green Fluorescent Object. To examine whether a green fluorescent object attracted free-living *Symbiodinium* under natural seawater light conditions, we placed pairs of white traps, one painted with GFD and one without (Fig. 4*A*; 110 mm in diameter with 85 mm height), for 3 h at a depth of 3–6 m on the reef crest of Sesoko Island, Okinawa, Japan. The experiment was carried out on a sunny day between 1 and 4 PM on September 4, 2016. After 3 h on the reef, each trap was first sealed with a plastic lid and then transferred to the Sesoko Marine Station. To estimate the number of *Symbiodinium* cells in the traps, the seawater captured in each trap (400 mL) was centrifuged (1,600 × *g* for 2 min) with a Himac CT6E (Hitachi Koki), and pellets were stored at −30 °C. DNA was extracted from the pellets by using DNeasy plant-mini kit (Qiagen). Real-time PCR was performed with LightCycler (Roche) to quantify the concentration of small ribosomal subunit RNA gene fragments specific to *Symbiodinium* by quantification cycle (23). The primers used were ACGCAGTGCTCAGCTTCTGGAC and GATCCTTCCGCAGGTTCACCTAC. The concentrations of *Symbiodinium* cells in the seawater samples were estimated by using the *Symbiodinium* culture Y103 (clade C) as a standard (10^2, 10^3, and 10^4 cells/sample; *SI Appendix*, Fig. S2*A*). The PCR resulted in amplification of single ∼100-bp DNA fragments, as expected (*SI Appendix*, Fig. S2*B*). Similar PCR signal was seen in both clade B (Mf1.05b) and clad C (Y103) *Symbiodinium* strains, suggesting that the primers used target multiple *Symbiodinium* clades, as originally designed.

Phylogenetic Analysis. Using a bacteriorhodopsin protein sequence from *Halobacterium salinarum* strain ATCC 700922 (BACR_HALSA, UniProtID: P02945) as a query, similarity searches against the *Symbiodinium minutum* genome (marinegenomics.oist.jp/symb/viewer/info?project_id=21), the *Symbiodinium kawagutii* genome (web.malab.cn/symka_new/index.jsp), and the GenBank database (https://www.ncbi.nlm.nih.gov/) were performed to collect homologous sequences, followed by multiple sequence alignment and phylogenetic analysis, as described previously (42). RAxML (43) and IQ-TREE (44) were used to reconstruct phylogenetic trees using LG+G model with support values based on 1,000 bootstrap resamplings.

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