Skeletal structure and progression of growth anomalies in *Porites australiensis* in Okinawa, Japan

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ABSTRACT: Growth anomalies (GAs), one of the diseases recently reported for scleractinian corals, are characterized by an abnormal skeletal structure and reduced zooxanthella density. The pathological characteristics of GAs were studied in colonies of *Porites australiensis* on a reef in Kayo, Okinawa, Japan. Corallites in the GA region lost the skeletal architecture characteristic of *P. australiensis*, and polyp density had decreased in the GAs due to enlargement of both calices and the coenosteum. The gross productivity of isolated GA samples was lower than in healthy samples and decreased to almost 0 within 11 d after isolation. However, when GA samples were brought into contact with healthy-looking samples from the same colony, they fused and both the GA and healthy regions grew. Healthy samples fused with GA samples grew more slowly than those fused with healthy samples. For *in situ* GAs surrounded by healthy tissue, tissue death usually started at the center of the GA, probably due to a deficiency in the translocated energy supply from the surrounding tissue. The total area of the GA region and the dead area increased at a rate of $5.3 \pm 2.9 \text{ cm}^2 \text{ yr}^{-1}$. These results suggest that GA regions are maintained by energy supplies from surrounding healthy tissues and that GAs may have a negative impact on host corals.

KEY WORDS: Coral · Disease · Growth anomaly · *Porites australiensis*

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INTRODUCTION

In the last 2 decades, diseases of corals have increased in both frequency and the number of affected species. Coral diseases are considered serious factors affecting corals and reef ecosystems (Harvell et al. 1999, Porter & Tougas 2001, Sutherland et al. 2004). The causative agents, however, have not been identified, except for a few coral diseases (Rosenberg & Ben-Haim 2002, Sutherland et al. 2004).

Growth anomalies (GAs) of scleractinian corals have been called 'coral tumors,' 'neoplasia,' or 'skeletal anomalies' and are easily recognized as swollen, and in many cases bleached, tissue areas within a colony. Sutherland et al. (2004) noted that GAs affected 16

Caribbean and 24 Indo-Pacific scleractinian species, including dominant coral species such as those in the Acroporidae and Poritidae. Recently, GAs have been reported to occur worldwide (Yamashiro et al. 2000, Gateño et al. 2003, Kaczmarek 2006, Work et al. 2008, McClanahan et al. 2009, Stimson 2010).

Negative signs have been reported in GA-affected corals, including low fecundity (Yamashiro et al. 2000, Domart-Coulon et al. 2006, Irikawa et al. 2011), low lipid storage (Yamashiro et al. 2001), and soft tissue anomalies (Peters et al. 1986, Work & Rameyer 2005, Domart-Coulon et al. 2006, Work et al. 2008). Partial colony mortality due to GAs has also been documented (Cheney 1975, Bak 1983, Work et al. 2008, Stimson 2010, Irikawa et al. 2011). Patchy coral

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bleaching (McClanahan et al. 2009) and aging (Irikawa et al. 2011) have been suspected to be an endogenous causative agent of GAs, while infections by microorganisms such as algae and/or fungi (Loya et al. 1984, Kaczmarzsky & Richardson 2007), environmental factors such as UV radiation (Peters et al. 1986, Coles & Seapy 1998), high temperatures (Domart-Coulon et al. 2006, McClanahan et al. 2009, Stimson 2010), and/or high levels of photosynthetically active radiation (Stimson 2010) are suspected as exogenous factors. The causative factors of GAs, however, are still not well understood.

GAs have mainly been reported in *Acropora*, *Montipora*, and massive *Porites* corals in the Ryukyu archipelago (Yamashiro et al. 2000, 2001, Sato 2006, Yasuda et al. 2006, Irikawa et al. 2011). They have increased recently near Kerama Islands and Sekisei Lagoon in Okinawa (Sato 2006, Irikawa et al. 2011). In 2003, GA-affected massive *Porites* colonies were observed in Kayo, Okinawa, Japan (Yasuda et al. 2006). GA-affected *Porites* colonies are common in the Ryukyu archipelago, but they occur sporadically and are not abundant at individual sites (N. Yasuda pers. obs.). This is in contrast to reports of high densities of GA-affected massive *Porites* colonies on reefs in the Philippines (Kaczmarzsky 2006).

While the morphological and ecological aspects of GAs have been documented in the genus *Porites* (Hunter & Field 1997, Raymundo et al. 2005, Domart-Coulon et al. 2006, Kaczmarzsky 2006, Kaczmarzsky & Richardson 2007, McClanahan et al. 2009, Stimson 2010), the physiological aspects of GAs, such as production, have not been studied. Also, it is not known how GA regions grow and invade healthy areas in colonies despite the low density of zooxanthellae. The objective of the present study was to investigate the pathological and physiological characteristics of GAs in *Porites australiensis* colonies and to clarify the mechanisms of persistence and growth in GA regions and their impacts on adjacent healthy regions.

MATERIALS AND METHODS

Study site and sample collection

The study site was located on the back reef moat at Kayo (26° 33' N, 128° 08' E) on the east coast of Okinawa Island, Japan, where massive *Porites* colonies were abundant. Samples of GA and healthy-looking regions were collected from 2 colonies of *P. australiensis*. The 2 colonies, Colony 1 and 2, were 2.5 and 0.4 m in diameter, respectively, and were located

about 1 m apart from each other. The sampling site was the reef moat 100 m off the sandy shore at a depth of 2 to 3 m.

Morphological characteristics of GAs

Polyp density, algal density and skeletal structure

Samples for polyp density measurements were isolated from each of 10 GA regions and 8 healthy regions of 2 colonies (8 GA and 6 healthy-looking samples from Colony 1, and 2 GA and 2 healthy samples from Colony 2) using a chisel and a hammer. Photographs of randomly selected 3 to 6 squares (1 cm²) were taken for each sample using a digital camera. The number of polyps within each square was counted and mean polyp density for each sample was calculated.

Five GA samples (3 from Colony 1, and 2 from Colony 2) and 4 healthy samples (2 from Colony 1, and 2 from Colony 2) out of the samples used in the above measurement were used for zooxanthella density measurements. The coral samples were photographed and fixed in 10% formalin in filtered seawater, decalcified in 5% acetic acid, and washed with running tap water. Decalcified samples were homogenized, and the number of zooxanthellae was counted using a hemocytometer. Projected areas of coral samples were calculated from photographs of samples using Scion Image software. The mean areal density of zooxanthellae in GA and healthy regions was calculated for 2 *Porites australiensis* colonies.

To compare the skeletal structure of GA and healthy regions, 3 samples (2 from Colony 1, and 1 from Colony 2) were collected anew from each of GA and healthy regions. The samples were immersed in a 10% commercial hypochlorite solution to remove the soft tissue. The skeletal surface structure was observed under a scanning electron microscope (JSM-6060LV, JEOL). The number of the main structural elements of the corallite, such as the columella, the large palus, and the septa, as well as the number of denticles, was measured on 20 calices from Colony 1 and 11 calices from Colony 2 for each of the GA and healthy regions. The size of the calice, the length and width of the septa, the projected area of the columella and the tall palus were measured on scanning electron microscope (SEM) photographs of 31 calices (20 and 11 calices from Colony 1 and 2, respectively) using Adobe Photoshop 6.0 and Scion Image. The thickness of the theca/coenosteum, which was defined as minimum distance between neighboring calices, was measured on the same samples.

Histological observations of the spermary

Six samples, 3 from each of the GA and healthy regions, were collected from a male colony (Colony 1) of *Porites australiensis* on 2 July 2005, 3 wk before the expected date of spawning. The samples were fixed and decalcified with Bouin's fixative, dehydrated in a graded series of ethanol and benzene, and embedded in paraffin. Sections (7 μm thick) were cut and stained with Delafield's hematoxylin and eosin. The number of spermaries per polyp and the maximum diameter of the spermaries were measured on serial cross sections of the polyps. A total of 10 polyps were observed for each of the GA and healthy regions.

Growth of *in situ* and isolated GAs

Growth of *in situ* GAs

Underwater photographs of GAs were taken 2 to 4 times a year from 2003 to 2008, and once in 2009, using a digital camera (Canon, Camedia PowerShot A620) to monitor growth of *in situ* GAs. 17 GAs that developed in Colony 1 were monitored from 2003 to 2009. In 2007, we added twelve GAs, which were monitored from 2007 to 2009. The growth rates of GAs surrounded by healthy tissue were estimated from increases of the projected areas of the GAs on underwater photographs.

Growth of isolated GAs

Samples of approx. 0.5×1 cm were isolated from GA and healthy regions of the 2 *Porites australiensis* colonies. After a 3 d recovery period, 18 pairs of healthy and GA samples and 9 pairs of healthy samples were prepared. These pairs were placed in a tank at Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, for 25 wk. The tanks were supplied with non-filtered running seawater and illuminated by natural sunlight through glass ceiling and side windows. The position of each pair within the aquarium was changed randomly every 2 wk. There was no sign of stress on isolated healthy samples.

Healthy samples fused with paired partners regardless of whether the partner was a healthy or GA sample. Healthy and GA regions can be distinguished in fused pairs because of the white appearance of GA regions. Growth rates of healthy and GA

regions in fused pairs were measured by counting the number of polyps in each region at 0, 7, 8, 10, 17, and 25 wk after contact. The number of polyps was logarithmically transformed and plotted against time after contact. The slope of the regression line was calculated as a measure of the growth rate.

Productivity of isolated GA and healthy samples

Six cores (1.5 cm diameter) were collected from each of the healthy and GA regions from a *Porites australiensis* colony (Colony 1) using a cork borer in November 2006. The core samples were allowed to acclimate to the aquarium for 3 d, and then photosynthetic productivity was measured every fourth day for 12 d.

Coral samples were placed in a chamber filled with 250 ml of oxygen-saturated seawater. The seawater temperature was regulated at 25°C. The dissolved oxygen (DO) concentration was measured by an O_2 sensor (ASR fluorescence O_2 Analyzer Model FO-960). DO was measured for 15 min in darkness after a 5 min dark-adaptation period, and then for 15 min at saturated light intensity ($600 \mu\text{mol m}^{-2} \text{s}^{-1}$) provided by a metal-halide lamp (Dupla Electra) after a 5 min light-adaptation period. The measurements were repeated 3 times for each sample. The gross production rate was calculated by subtracting the rate of change in DO in darkness (respiration) from that under light (net production).

Statistical analyses

In this study, only 2 colonies with GAs were studied because GA-affected colonies of *Porites australiensis* were distributed sporadically and were not abundant at the study site. Morphological parameters were compared between the GA and healthy regions in each of the colonies using Student's *t*-test. The polyp densities, the number and size of spermaries, and productivity were compared between GA and healthy regions within the same colony (Colony 1). A Student's *t*-test was used when assumptions of normality (Shapiro-Wilks test) and homogeneity of variances (Barlett's test) were met. The data were logarithmically transformed when necessary. A Mann-Whitney *U*-test was used when the transformed data did not meet parametric requirements. In the growth rate measurement of isolated samples, the polyp numbers in the isolated GA and healthy samples were logarithmically trans-

Fig. 1. *Porites australiensis*. Scanning electron micrographs of (A,B) the corallite and (C,D) theca of (A,C) healthy and (B,D) growth anomaly (GA) regions. a: presence (A) or absence (B, dotted circle) of columella; b: palus; c: calice wall; d: denticle; e: dorsal directive; f: ventral directive; g: pair of lateral septa; h: triplet with free margins (dotted circle). Healthy and enlarged theca or coenosteum are shown inside the dotted lines in (C) and (D)

formed before regression analyses. The transformed data met parametric requirements. The slope of the regression line for each sample was used as a statistical unit and comparison of the growth rate among the 3 groups, healthy samples paired with healthy samples (H-H), healthy samples paired with GA samples (H-GA), and GA samples paired with healthy samples (GA-H), were tested using ANOVA and Bonferoni multiple comparison tests.

RESULTS

Polyp density and skeletal structure

Polyp density was significantly lower in GA regions (44.1 ± 11.6 polyps cm^{-2} , mean $\pm$ SD) than in healthy regions (89.2 ± 16.0 polyps cm^{-2}) (t -test, $p < 0.001$)

when 8 GA samples were compared with 6 healthy-looking regions within Colony 1. Colony 2 also showed similar polyp density values for 2 GA (51 polyps cm^{-2}) and 2 healthy (84.7 polyps cm^{-2}) regions.

Polyps in healthy regions of *Porites australiensis* had a corallite structure characteristic of the species, which was described by Veron (2000). The corallite had one large columella and 12 thick septa with denticles (Fig. 1A). The 12 septa consisted of 1 dorsal directive, 2 pairs of lateral septa on both sides, and 3 ventral directives. The dorsal directive and each pair of lateral septa had a tall palus, and the 3 ventral directives (triplet) usually had free margins with a short palus. Thus, 5 tall pali and 3 short pali were observed in the corallite. However, corallites in the GA region lost the skeletal architecture characteristic of *P. australiensis*. The size of the corallite, theca thickness, and the number and size of structural

Table 1. *Porites australiensis*. Comparison of corallite dimensions between the healthy and growth anomaly (GA) regions in 2 colonies (mean $\pm$ SD). Numbers in parentheses indicate the number of corallites measured except for the theca/coenosteum thickness where the number of measured sites is shown. Asterisks indicate significant differences between GA and healthy corallites. * Student's *t*-test, $p < 0.01$; *^u Mann-Whitney *U*-test, $p < 0.01$; nd: not determined because no columella was found in the corallites

Colony	Region	Calice size (mm ²)	Theca/coenosteum thickness (μ m)	Columella size ($\times 10^3 \mu$ m ²)	Tall palus size ($\times 10^3 \mu$ m ²)	Length of septa (μ m)	Width of septa (μ m)	Denticle
1	Healthy	1.13 $\pm$ 0.11 (20)	114.4 $\pm$ 39.3 (63)	6.4 $\pm$ 2.4 (20)	9.6 $\pm$ 1.9 (20)	339.2 $\pm$ 25.2 (20)	90.7 $\pm$ 14.6 (20)	12/corallite
	GA	1.36 $\pm$ 0.14 (20)*	326.8 $\pm$ 192.6 (66)*	5.2 $\pm$ 2.1 (13)	12.3 $\pm$ 3.1 (20)*	416.1 $\pm$ 33.9 (20)*	98.7 $\pm$ 25.6 (20)*	None/roundish ^a
2	Healthy	1.09 $\pm$ 0.11 (11)	124.1 $\pm$ 23.3 (44)	6.9 $\pm$ 1.5 (11)	10.3 $\pm$ 1.4 (11)	318.6 $\pm$ 23.3 (11)	81.1 $\pm$ 8.5 (11)	12/corallite
	GA	1.54 $\pm$ 0.16 (11)*	428.8 $\pm$ 213.6 (33)* ^u	nd	13.6 $\pm$ 2.2 (11)*	422.1 $\pm$ 23.8 (11)*	104.6 $\pm$ 9.1 (11)*	None/roundish ^a

^aDenticles were absent or, if present, became roundish instead of acicular

elements in the corallite were markedly different between corallites in GA regions and those in healthy regions in both Colony 1 and 2 (Table 1). Corallites in GA regions were larger than in healthy corallites (*t*-test, $p < 0.01$) and showed irregular skeletal structure (Fig. 1B). The theca was enlarged both in Colony 1 (*t*-test, $p < 0.01$) and Colony 2 (Mann-Whitney *U*-test, $p < 0.01$) and elevated when compared with corallites in healthy regions both in Colony 1 and 2 (Fig. 1C,D). The theca appeared as a coenosteum with a porous structure and well-developed denticles in both Colony 1 and 2. The columella had disappeared in 35% of the GA corallites in Colony 1 and 100% of those in Colony 2, while the size of the columella, if present, was not significantly different from those in healthy corallites in Colony 1 (*t*-test, $p > 0.01$). In 55% of the GA corallites in Colony 1 and in 65% of these in Colony 2, 1 or 2 out of 5 tall pali had disappeared, and the remaining pali were significantly larger than in healthy corallites in both Colony 1 and 2 (*t*-test, $p < 0.01$). In 20% of GA corallites in Colony 1 and 18% of these in Colony 2, 1 or 2 of 12 septa had disappeared, while the remaining septa were significantly longer and wider than in healthy corallites in both Colony 1 and 2 (Table 1; *t*-test, $p < 0.01$). Denticles on the septa were acicular in healthy corallites but roundish in GA corallites in both Colony 1 and 2.

Growth rates of *in situ* GAs

In total, 29 GAs on *Porites australiensis* Colony 1 were monitored; 12 GAs were monitored for 6 yr (2003–2009), and 17 GAs were monitored for 2 yr (2007–2009). In 10 of 12 GAs (83.3%) and in 9 of 17 GAs (52.9%), tissues in GA regions died partially or completely during the 6 and 2 yr observation periods, respectively. The remaining 10 GAs did not have dead areas within them during the observation periods. When GAs suffered partial or complete tissue death, tissues started dying at the center of the GA if the GA was surrounded by healthy tissue (Fig. 2A,B), but if the GA was located at the margin of a colony, tissue death started from the margin of the GA region (Fig. 2C).

The projected area of the GA region fluctuated with time because it was affected by both the death and growth of GA tissues. The growth rate of live GA regions, which was measured for 17 GAs that did not have dead areas, was $2.9 \pm 2.0 \text{ cm}^2 \text{ yr}^{-1}$ (mean $\pm$ SD). The area of GA-affected regions, that is, the sum of

Fig. 2. *Porites australiensis*. Growth anomaly (GA) regions on a colony monitored from 2003 to 2009. (A) GA region surrounded by healthy tissue (April 2003). (B) Same GA region observed 4 yr later (May 2007) with tissue dying from the central part outward. (C) GA region located at the margin of a colony (April 2009) with tissue death that started from the marginal part. Squares are 10×10 cm

live GA areas and dead areas, increased at a rate of $5.3 \pm 2.9 \text{ cm}^2 \text{ yr}^{-1}$ ($n = 8$). Increases in the projected areas of 7 GA-affected regions that were monitored for 6 yr are shown in Fig. 3.

GAs located at the margins of colonies also showed fluctuations in projected areas due to the death and growth of GA tissues. In all cases ($n = 12$), GAs invaded surrounding healthy regions after repeated death and growth in the colony. The complete replacement of GA regions by healthy regions was not observed during the observation period.

To investigate whether tissue death occurred when GAs grew beyond a certain size, we compared the size of GAs without dead areas to those with dead areas. The projected areas of 17 GAs that were located away from the colony margin and surrounded by healthy tissue were measured. The maximum area of the GAs that showed no tissue death during the observation period ranged from 1.7 to 34.1 cm^2 ($n = 10$). In 7 GAs, dead areas were not present during the first observation but were found during later observation periods. The maximum area of the 7 GAs measured before tissues started dying ranged from 5.6 to 58.5 cm^2 , while the projected area of these GA-affected regions (GA and dead area) was in the range of 6.1 to 68.1 cm^2 when the dead area was first observed. The additional 2 GAs had dead areas during the first observation were 73.6 and 95.2 cm^2 , and these area of GA-affected regions also increased on the coral surface with time (data not shown).

Growth rate of isolated GAs

When 2 healthy samples or healthy and GA samples that had been isolated from the same colony were brought into contact, they fused with each other (Fig. 4). The GA region grew around the margin of the healthy sample and appeared to surround the healthy region, while the healthy region grew in the opposite direction (Fig. 4D).

Fig. 3. *Porites australiensis*. Changes in the projected areas of 7 growth anomaly (GA)-affected regions in a colony in the field during a 6 yr (2003–2009) observation period. The projected area of the GA region, including dead areas, was plotted against the date of observation. The regression was significant and the slope of the regression line indicates the progression rate of the GA-affected area

Fig. 4. *Porites australiensis*. Photographs of sample pairs at the commencement of the contact experiment and 6 mo later. (A) A pair of healthy samples and (B) the same pair 6 mo later. (C) A GA sample paired with a healthy sample and (D) the same pair 6 mo later. Samples were from the same colony. Scale bar = 1 cm

The growth rates of GA and healthy samples were estimated from the slope of a regression line of the number of polyps in each sample against time after contact after logarithmic transformation of the data (Fig. 5). GAs that were in contact with healthy samples survived throughout the 25-wk observation period, while isolated GAs degenerated soon after isolation (see 'Algal density and productivity of isolated GAs'). The growth rates of healthy samples paired with GA samples were significantly lower than that of healthy samples paired with healthy samples (0.018 ± 0.005 vs. 0.025 ± 0.004 , mean $\pm$ SD, $n = 18$) and that of GA samples paired with healthy samples ($0.024 \pm 0.007 \log_{10}(\text{no. of polyps wk}^{-1})$, $n = 18$; Bonferroni multiple comparison test, $p < 0.05$).

Algal density and productivity of isolated GAs

The density of zooxanthellae in GA regions was about 1/5 of that in healthy regions in both Colony 1

($4.6 \pm 2.8 \times 10^5$ cells cm^{-2} [$n = 3$] vs. 24.1×10^5 cells cm^{-2} [$n = 2$], mean $\pm$ SD) and Colony 2 (4.7×10^5 cells cm^{-2} [$n = 2$] vs. 23.9×10^5 cells cm^{-2} [$n = 2$]).

The gross production of GA and healthy samples was measured at 4 d intervals for 12 d after a 3 d recovery period following the isolation procedure. The gross production rate of GA samples was significantly lower than that of healthy samples (Fig. 6) (t -test, $p < 0.05$). The gross production rate of GA samples had almost declined to zero by 11 d after isolation. Tissues in isolated GA samples gradually became thin and had retracted tentacles; by 11 d after isolation, tissues started to peel from the skeleton, leaving a small amount of tissue on the septa and around the mouth.

Reduced spermaries in the GAs

Spermaries were present in both healthy and GA regions 3 wk before spawning. However, the

Fig. 5. *Porites australiensis*. Growth of healthy and growth anomaly (GA) samples in the contact experiment. The log-transformed number of polyps in each sample was plotted against time (wk). The slope of the regression line indicates the growth rate of the sample. (A) Healthy samples in contact with healthy samples, (B) healthy samples in contact with GA samples, and (C) GA samples in contact with healthy samples. The number of samples (n) was 18 in each case

maximum diameter of spermaries in GA region polyps was significantly smaller than in healthy regions ($38.0 \pm 7.0 \mu\text{m}$ [$n = 10$ polyps] vs. $82.7 \pm 2.5 \mu\text{m}$ [$n = 10$ polyps], mean $\pm$ SD) (t -test, $p < 0.001$). The number of spermaries per polyp was also significantly smaller in GA regions than in healthy regions (2.6 ± 0.9 [$n = 10$] vs. 14.2 ± 2.0 [$n = 10$]) (t -test, $p < 0.001$).

Fig. 6. *Porites australiensis*. Changes in the gross production of healthy and growth anomaly (GA) samples after isolation from a colony. GA samples (◆) had significantly lower productivity than healthy samples (■) (t -test, * $p < 0.05$, ** $p < 0.01$). Mean $\pm$ SD ($n = 6$ each for healthy and GA samples)

DISCUSSION

Skeletal and tissue morphology of GAs

GAs of *Porites australiensis* were characterized by reduced polyp density, larger calices, deformed corallite structure, and an enlarged coenosteum. This is consistent with previous studies on GAs in the genus *Porites* (Hunter & Field 1997, Domart-Coulon et al. 2006, McClanahan et al. 2009). In GAs of *P. australiensis* the skeletal pattern appeared to change from cerioid (thecal walls shared by adjacent corallites) to plocoid (corallites separated by coenosteum) due to enlargement of the coenosteum. The deformed skeletons of GA regions in *P. australiensis* resulted from enlarged and porous coenosteum as well as enlarged and deformed corallites. Domart-Coulon et al. (2006) also suggested that larger corallites, enlarged coenosteum, and accelerated vertical skeletal extensions contribute to the formation of the hemispherical shape of GAs in *P. compressa*.

GAs of *Acropora* spp. are characterized by the development of a smooth and undulating skeletal structure associated with the loss of normal polyp structures. In some cases, a globular mass of skeleton without corallites covers healthy regions with a normal skeletal structure (Cheney 1975, Bak 1983, Peters et al. 1986, Coles & Seapy 1998, Work et al. 2008, Irikawa et al. 2011). In contrast, polyp structure was retained in GAs of *Porites* corals (Hunter & Field 1997, Domart-Coulon et al. 2006, Kaczmarek & Richardson 2007, McClanahan et al. 2009, the present study). The present study, however, showed that

the species-specific corallite structure of *P. australiensis* changed or disappeared in GAs. Columella and some pali and denticles disappeared in some corallites, while septa and pali became enlarged.

Nutrient supply from healthy regions to GAs

Isolated GAs of *Porites australiensis* showed low photosynthetic productivity and disintegrated within 1 to 2 wk. Polyps in GA regions did not show normal feeding behavior in response to *Artemia* spp. and *Brachionus* spp. (N. Yasuda pers. obs.). This suggests that GAs cannot feed on zooplankton as an alternate nutrient source. Isolated GAs likely could not survive alone because of nutrient deficiency due to decreased algal density and decreased capacity for zooplankton feeding. However, isolated GA samples survived and grew if they were fused with healthy samples. The areal growth rate of GA samples fused with healthy samples was estimated using the increase in the polyp number of the GA samples and the mean polyp density of the colonies and was found to be $4.7 \text{ cm}^2 \text{ yr}^{-1}$, which is comparable to the growth rate of *in situ* GAs ($2.9 \text{ cm}^2 \text{ yr}^{-1}$). These observations suggest that GAs are able to sustain their growth by using energy supplies from healthy partners. The growth rate of healthy samples paired with GAs was slower than those paired with healthy samples, indicating that healthy samples suffered energy losses when fused with GA samples.

Higher growth rates of GAs compared to healthy tissues have been reported for *Acropora formosa* (Cheney 1975), *A. palmata* (Peters et al. 1986), *Platygyra pini*, *P. sinensis* (Loya et al. 1984), and *Pavona clavus* (Gateño et al. 2003). GAs probably overgrow adjacent healthy regions using energy supplied from the adjacent tissue. Oren et al. (1997) detected translocations of nutrients toward regenerating areas in which energy demand was high in colonies of *Favia fava* and *Platygyra lamellina*. Such translocations of nutrients from healthy regions to GA regions have been assumed (Cheney 1975, Domart-Coulon et al. 2006) based on observations that GAs suppressed the growth of healthy regions in *Acropora* spp. (Cheney 1975, Bak 1983) and that storage lipids were depleted in GA tissues of *Montipora informis* (Yamashiro et al. 2001). Stimson (2010) reported that the growth rate of healthy *Porites compressa* branches decreased when they were in contact with GAs. The results of the present contact experiments together with the above observations strongly suggest that coral GAs grow by using energy supplied by adjacent healthy areas.

The GA regions of male colonies had small numbers of immature spermaries during the reproductive season. Domart-Coulon et al. (2006) also suggested that in GA tissues, energy resources are primarily allocated to tissue growth at the expense of reproduction. Other diseases have also been reported to reduce reproductive output in corals (Weil et al. 2009, Borger & Colley 2010) and soft corals (Petes et al. 2003). However, since bleaching also has a negative impact on reproduction of corals (Szmant & Gassman 1990, Ward et al. 2000), it remains to be studied whether the reduced reproduction in *Porites australiensis* GA tissue is due to its 'bleached' condition or changes in energy allocation, from reproduction to growth, in GA tissues.

Death and progression of GAs

In our field observations of GAs on a *Porites australiensis* colony, if GAs were surrounded by healthy tissue, tissue death first occurred in the central areas of the GAs. In GAs located at the margin of a colony, tissue died first in the margin of the GAs, where it did not face any healthy tissue. Thus, GAs started dying in an area distant from the border facing healthy tissue. These results suggest that GA tissues die from the depletion of nutrients that are translocated from adjacent healthy tissue.

Bak (1983) mentioned that in *Acropora palmata*, GAs in the maximum size range had dead areas in the central region. Work et al. (2008) also reported that large GAs tended to die and suggested that a size limit for GA survival may exist. We also found that large GAs tended to have dead areas, although we could not find clear size limits for the survival of intact GAs. The size distributions for GAs without dead areas and those with dead areas overlapped. Some small GAs (6.1 cm^2 , including dead area) had dead regions, while some GAs grew to 58.5 cm^2 without tissue death. These observations suggest that the depletion of translocated nutrients is not the only cause of tissue death in GAs.

Nutritional deficiencies might make GA tissues more susceptible to fungal or algal invasions. Le Campion-Alsumard et al. (1995) found endolithic algae and fungi in skeletons of *Porites lobata* and suggested that these microorganisms affected calcification and soft tissues in the coral. A correlation between algal and fungal penetration and coral disease has been suggested (Le Campion-Alsumard et al. 1995, Bentis et al. 2000, Sutherland et al. 2004). However, Work et al. (2008) observed necrosis of

intact GAs in the absence of associated organisms and argued that the necrosis of GAs in *Acropora* spp. is not necessarily dependent on invasive organisms such as algae or fungi. The possible role of invasive organisms in GA tissue death awaits further study.

Areas affected by GAs (GA region + dead area) grew at a rate of $5.3 \pm 2.9 \text{ cm}^2 \text{ yr}^{-1}$, showing that GAs had negative impacts on the colonies and could eventually kill them. GA growth can lead to reduced colony growth or partial colony death (Cheney 1975, Bak 1983, Work et al. 2008, Stimson 2010, Irikawa et al. 2011). The mechanism by which GAs invade surrounding healthy tissue is not fully understood. In the contact experiments, the GA regions grew to surround their healthy partners rather than overgrowing them. However, if no free space is available, GAs would likely overgrow healthy partners. Gateño et al. (2003) observed healthy tissues overgrown by GA tissues in *Porites clavus*. Peters et al. (1986) and Work et al. (2008) reported that progressive growth of GAs in *Acropora* spp. led to the death of surrounding normal tissues.

Several researchers have investigated whether GAs are transmissible (Peters et al. 1986, Gateño et al. 2003, Kaczmarzsky & Richardson 2007). Most studies have not detected positive transmissions, although transmissions of GAs by direct contact and waterborne substances were reported in *Porites* colonies (Kaczmarzsky & Richardson 2007). Work et al. (2008) and Irikawa et al. (2011) suggested the possibility of metastasis based on the clustered development of GAs on colonies of *Acropora* spp. In our contact experiments, we observed no new GAs on healthy samples during the 6-mo observation period.

CONCLUSIONS

GAs that developed on 2 colonies of *Porites australiensis* were characterized by reduced polyp density, abnormal skeletal architecture, reduced algal density, and decreased reproduction, as reported with other *Porites* corals. The reduction in polyp density was due to the enlargement of both the corallite and the coenosteum. Some intra-corallite structural elements became enlarged or lost. The skeleton appeared to change from cerioid to plocoid due to the enlarged, porous coenosteum, which had well-developed denticles. The gross production of isolated GA samples decreased almost to zero and the samples disintegrated within approximately 10 d after isolation. However, GA samples survived and grew if they fused with healthy partners. Healthy samples

that fused with GA samples grew more slowly than those that fused with other healthy samples. The average rate of increase for GA-affected areas (sum of GA regions and dead areas) was $5.3 \text{ cm}^2 \text{ yr}^{-1}$. The present results suggest that GA regions are maintained by energy supplied from surrounding healthy tissues and that GAs have a negative impact on host corals.

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