Proceedings of the 11th International Coral Reef Symposium, Ft. Lauderdale, Florida, 7-11 July 2008
Session number 25

Adaptation/Exaptation in the host coral *Favites complanata* (Ehrenberg, 1834; Scleractinia, Faviidae) to increased Seawater Temperatures

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Abstract. Recent evidence suggests that, as global climate changes and seawater temperatures rise, the primary cause of coral bleaching is zooxantheallar mortality indicated by cell apoptosis/necrosis. But are the coral hosts themselves sensitive to increasing temperatures, and, if so, to what degree? We exposed the coral *Favites complanata* (Ehrenberg, 1834) with their symbiotic zooxanthellae (*Symbiodinium* sp.) to experimental temperatures of 28 (control), 30, 32, and 34°C for 12h. We assessed coral and symbiont cells *in situ* for symptoms of apoptosis and necrosis using transmission electron microscopy (TEM). *F. complanata* host cells *in situ* exhibited little or no mortality from exposure to increased seawater temperatures, except at very high temperatures (34°C) for ≥12hrs. By contrast, we found high levels of apoptosis/necrosis in the zooxanthellae *in situ* under all experimentally elevated temperatures. These findings indicate that the host corals are adapted/exapted to seawater temperature increases. They also imply that the coral hosts do not suffer mortality from temperature stress but from loss of their symbionts – a process driving natural selection in *Symbiodinium* and most likely resulting in their rapid adaptation to a changing environment.

Key words: Adaptation, Apoptosis, Bleaching, Exaptation, Necrosis, Seawater temperature, *Symbiodinium*

Introduction

Concerns are growing as climate change and associated increased seawater temperatures continue to induce coral bleaching in the tropics and sub-tropics (Sheppard and Rioja-Jieto 2005; Baker et al. 2008). Coral bleaching, a process by which corals lose their algal symbionts called zooxanthellae (genus *Symbiodinium*), is a response to heat-stress where temperatures rise above the maximum tolerance of the zooxanthellate corals/holobionts (Hoegh-Guldberg 1999). Such variation in temperature may be an important selective factor in the evolution of the relationship between corals and their endosymbionts. Some investigators suggest that the loss of *Symbiodinium* cells may help corals adapt to changing environmental conditions by allowing them to "shuffle" (Buddemeier and Fautin 1993) their complement of zooxanthellae, benefiting the corals (the "adaptive bleaching hypothesis"; also see Kinzie et al. 2001). Others believe that *Symbiodinium* shuffling does not occur quickly enough (Hoegh-Guldberg et al. 2002), serving to prolong the eventual death of the host. The interim survivorship period may last for up to six weeks. Grottoli et al. (2006) have demonstrated that *Montipora capitata* can replenish its energy reserves and biomass in the absence of its symbionts by increasing its food intake five-fold after temperature-induced bleaching. Borell et al. (2008) found similar effects in *Stylophora pistillata* (Esper) and *Galaxea fascicularis* (Linnaeus).

Recent data have indicated that bleaching is a primary selective factor for zooxanthellae, causing very high levels of mortality in zooxanthellae *ex situ* (Strychar et al. 2004 a, b; 2005). This can be inferred from the 95-99% mortality rate of zooxanthellae expelled from scleractinian and alcyonacean corals exposed to temperatures of 32-34°C for 9-48 h (*ibid.*).

Survival of zooxanthellae exposed to high seawater temperatures may occur as a result of adaptation by ancestors that have survived prior exposure to these conditions. Survival may also be occurring due to exaptation to these temperatures. Exaptation is defined as a character that has evolved for another function, or no function at all, but which has been co-opted for a new use (Gould and Vrba 1982, cf Futuyma 1998; McLennan 2008; also see Strychar and Sammarco, 2009). Mettler and Gregg (1969) discuss exaptation, previously termed "pre-adaptation", as follows: "…populations can… generate many genotypes with varying degrees of adaptedness. Those [non-adapted] forms produced each generation…are weeded out by selection, but …are continuously produced and …considered to be 'stores on hand'…the progenitors of future generations in the event of a changed environment. They are 'pre-adapted' genotypes ready for new

situations which might be met by the population…."

The high mortality rates observed by Strychar et al. (2004a,b, 2005) were determined *via* assessing apoptosis and necrosis in the symbiont cells. Apoptosis is a genetically pre-programmed, physiological and biochemical event that leads to the removal of unwanted and/or abnormal cells (Strychar et al., 2004a). This helps to regulate abundant cell populations. Apoptosis is not a synonym for programmed cell death, which involve other mechanisms (e.g. paraptosis, pyroptosis, etc.; see reviews of Ameisen 2002; Fink and Cookson 2005). Necrosis, by contrast, is a passive, accidental, and unordered occurrence of cellular death (Strychar et al., 2004a), causing inflammation of host tissue. For example, increased production of apoptotic cells in mammalian hosts elicits removal of dead/dying cells *via* phagocytosis, preventing inflammation. Necrosis, on the other hand, causes uncontrolled inflammation (Fink and Cookson, 2005). In Cnidarians, increased concentrations of necrotic cells likely cause similar responses. In zooxanthellate corals, the occurrence of apoptosis or necrosis in the symbiont but not the host would indicate that the former is more sensitive to a perturbation than the latter.

The rate of coral mortality due to bleaching is believed dependent upon the severity (intensity and duration) of the temperature anomaly (Winter et al. 1998, 2006; Craig et al. 2001; but also see Podesta and Glynn 1997). We have shown that some host corals, specifically *Acropora hyacinthus* (Dana, 1846; Acroporidae) and *Porites solida* (Forskål, 1775; Poritidae) from the Great Barrier Reef, Australia have a much broader temperature tolerance than that of the zooxanthellae and exhibit adaptation/exaptation towards increased temperatures (Strychar and Sammarco, 2009). The zooxanthellae appear to be doing most of the adaptation within this co-evolved symbiotic relationship. Here we demonstrate that this phenomenon is not restricted to these two species, or families, but may be extended to *Favites complanata* (Ehrenberg, 1834; Faviidae), and that this adaptation / exaptation may be a common characteristic within the Scleractinia.

Material and Methods

Forty colonies of *Favites complanata* were collected at ~8.5m depth from Barren Reef (23°10'S, 151°55'E), Great Barrier Reef, Qld, Australia. Colonies were exposed to different experimental seawater temperatures, following a random blocks design (Zar 1998). Full details of the experimental design may be found in Strychar et al. (2004b) but will be summarized here. Filtered seawater was pumped through 10 and 1µm filters and deposited into a series of header tanks (total combined vol = 1000 l), mixed using submersible pumps. Water was then pumped to 8 two-liter plastic holding chambers at a constant flow rate of 20ml min^{-1} through 1µm Millipore® filters. Magnetic stir bars provided water movement within each chamber. Colonies were subjected to experimental temperatures of 28 (control), 30, 32, and 34°C for 12h. Ten replicate (n_i=10) coral fragments were used for each temperature. Data presented here are derived from experiments described in Strychar et al. (2004b); here, however, the focus has shifted to host and *Symbiodinium* cells *in situ*, as opposed to expelled cells. We acknowledge that the rates of temperature increase and/or time scales of exposure employed here are faster and/or shorter than may occur naturally in the reef environment. Hence, the coral and symbiont experimental responses may not be representative of a protracted natural bleaching event.

In these experiments, coral tissue (~5mm^2) was excised from a colony using a scalpel and fine dissecting forceps at the 3, 9, & 12h marks. A solution of sodium citrate (10%) and formic acid (20%) was used to decalcify skeletal tissue (Strychar and Sammarco, 2009). Any skeletal tissue remaining after 24h was gently crushed using a micro-tissue grinder, and the solution was centrifuged and washed 3x in phosphate buffered saline solution (PBS). Following a 3rd wash and removal of the supernatant, samples of host tissue, along with the symbiont cells, were embedded in 0.2ml of agar. Thus, both the symbiotic partners could be analyzed simultaneously. After solidification, samples were post-fixed in 1% osmium tetroxide in 0.1 M PBS for 24h at 48°C. They were then centrifuged (500x *g*) and the supernatant removed. At 30 min intervals, the samples were dehydrated in a graded series of acetone washes (30, 50, 70, 90, & 3x at 100%). The embedded samples were then suspended in a series of graded acetone–Spurr's resin to ensure infiltration of resin into the tissue. Samples were finally polymerized into a mould by incubation at 60°C for 3d.

An Ultracut T microtome was used to prepare thin sections of tissue, which were placed on 3mm copper grids coated with 1.2% (w/v) formvar in trichloro-methane. Aqueous uranyl acetate was then used to stain the thin sections for ~5 min, followed by staining with 1.5% (w/v) lead citrate for 5 min. The ultrastructural characteristics of each thin section were observed with a JEOL-1010 or Hitachi 7000 transmission electron microscope (TEM) and analyzed for ultrastructural symptoms of apoptosis, necrosis, and viability. Thin sections were examined during each sampling period; 100 host and 100 symbiont cells were randomly sampled per section per experimental temperature treatment at each time interval. Frequencies of each were calculated.

Cells exhibiting apoptosis were characterized by cell contraction, chromatin condensation, and cell fragmentation into membrane-bound fragments called apoptotic bodies (see Strychar et al., 2004a). Necrotic cells were identified by cellular and organelle swelling, eventually causing release of the cytosolic contents into the surrounding host tissue.

Results

Coral host and* Symbiodinium *cells – Main Effects

TEM successfully detected variation in host and *Symbiodinium* cell states under varying seawater temperatures. There was no significant difference in apoptosis and necrosis levels between the 28°C and 30°C treatments in either the *Favites complanata* host cells or the *Symbiodinium* cells ($p>0.05$, MANOVA). Apoptosis levels increased significantly, however, in host corals exposed to temperatures ≥32°C (main effects; $p<0.05$, MANOVA). Levels of necrosis in host cells did not change significantly under conditions of increased temperatures *in situ* ($p>0.05$). Endosymbiont cells exhibited significantly higher levels of apoptosis at temperatures >30°C ($p<0.05$). Necrosis was significantly more frequent in the symbionts at temperature treatments >30°C *vs.* 28°C (control). Symptoms of stress in host corals and their symbionts (i.e. apoptosis & necrosis) rose significantly >9h after initiation of the experiment (main effect – time; $p<0.05$, MANOVA).

Temporal Responses of* Favites complanata *host cells and symbiont cells* in-situ *at 28-34°C. At 28°C, both host and symbiont cells exhibited high percentages of viability *in situ* (Fig. 1). The number of viable symbiont cells, however, decreased markedly and significantly as experimental temperatures were increased from 28 to 34°C ($p<0.001$, Tukey HSD and Bonferroni; Fig. 1).

Fig. 2 illustrates how the frequencies of apoptotic cells in both host and symbionts were negligible at experimental treatments of 28-30°C through time. At 32°C and 34°C, however, the level of apoptosis increased significantly through time in the *Symbiodinium* cells ($p<0.001$, Tukey HSD and Bonferroni tests). The frequency of apoptosis in *Favia complanata* host cells *in situ* rose significantly only under the extreme experimental temperature of 34°C, when compared to the control ($p<0.05$).

The frequency of necrosis in the host cells *in situ* did not vary significantly between any of the experimental temperatures ($p>0.05$, Tukey HSD and Bonferroni tests; Fig. 3). By contrast, *Symbiodinium* cells exhibited significantly higher frequencies of necrosis at 34°C ($p<0.04$; Fig. 3).

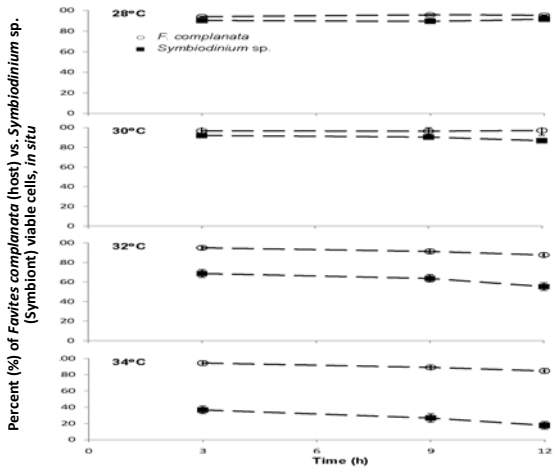

Figure 1: Effect of temperature on the relative frequencies of **viable** *Favites complanata* host and *Symbiodinium* cells *in-situ,* collected over 12h. Data are presented as the mean plus 95% confidence intervals from nine separate experiments; some confidence limits, too small to be seen. Each point = percentage of different cell types. The frequency of viable cells in *Symbiodinium* at 32°C and 34°C treatments were each significantly lower than all other treatments ($p<0.05$, Tukey HSD test). There was no significant difference between the 30°C and 32°C treatments in the host coral ($p>0.05$), no significant difference between the 28°C and 30°C treatments, and the 34°C treatment was significantly lower than the other treatments ($p<0.05$). There was an significant decrease in frequency of viable cells in both *Symbiodinium* and in the host cells between 3 and 12 hrs ($p<0.001$ & $p<0.05$, respectively).

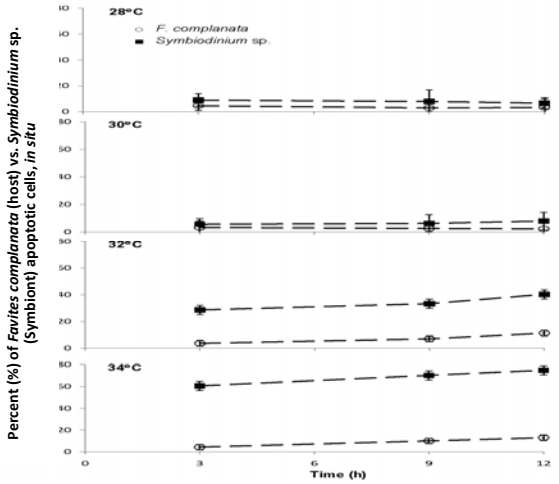

Figure 2: Effect of temperature on the relative frequencies of **apoptotic** *Favites complanata* host cells and *Symbiodinium* cells *in-situ,* collected over 12h. Data presented as the mean plus 95% confidence intervals; some confidence limits, too small to be seen. Each point = percentage of different cell types. The apoptotic cell frequencies in *Symbiodinium* at 32°C and 34°C were each significantly higher than all other treatments ($p<0.05$, Tukey HSD test). There was no significant difference between the 28°C and 30°C treatments ($p>0.05$). Apoptotic cell frequencies in the host cells were significantly higher at 34°C than at 28°C and 30°C ($p<0.05$). There was no significant difference between the 28°C and 30°C treatments ($p>0.05$). There was a significant increase in apoptotic *Symbiodinium* cells between 3 and 12hrs ($p<0.05$), but no difference in the host cells during this period ($p>0.05$).

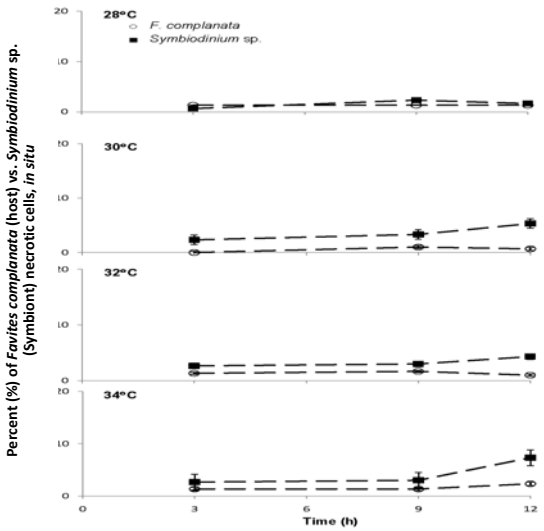

Figure 3: Effect of temperature on the percentage of **necrotic** *Favites complanata* host cells and *Symbiodinium* cells *in-situ,* collected over 12h. Data are presented as the mean plus 95% confidence intervals; some confidence limits, too small to be seen. Each point = percentage of different cell types. The frequency of necrotic cells in *Symbiodinium* at 34ºC was significantly higher than at 28ºC ($p<0.05$, Tukey HSD test). There was no significant difference in necrotic host cell frequencies between any temperature treatments ($p>0.05$). There was a significant increase in necrotic *Symbiodinium* cells between 3 and 12hrs ($p<0.05$), but no significant change in necrosis in host cells during that period ($p>0.05$).

Discussion

Our data indicate that the scleractinian coral *Favites complanata* is less heat-sensitive than its endo-symbionts. We hypothesize that increasing seawater temperatures, characteristic of global warming associated with climate change may be acting as a selective factor on the symbiotic zooxanthellae within the coral host, but not on the coral hosts themselves. This is because the coral hosts are already either adapted/exapted to high seawater temperatures. We have already desmonstrated adaptation/exaptation in the host corals *Acropora hyacinthus* and *Porites solida* - families Acroporidae and Poritidae, respectively. The data here show that this characteristic is restricted not only to these two species or families – but may also be extended to *Favites complanata* and its family – the Faviidae, suggesting that such temperature tolerances may be a widely distributed character in Scleractinain corals.

Like earlier studies, this suggests that it is the zooxanthellae that are responsible for doing most of the dying in this symbiotic relationship and the lion's share of the adaptation to rising seawater temperatures, certainly in the central Great Barrier Reef region. We propose that mortality is much more frequent and severe in the zooxanthellar populations during bleaching, and that the corals are suffering not from heat stress but from loss of their symbiotic partners. This is consistent with the results of Mise and Hidaka (2003) who studied effects of increased temperature on *Acropora nasuta*. They described rapid degradation of zooxanthellar cells under similar conditions, detailing symptoms characteristic of apoptosis and necrosis (also see Strychar et al., 2004a), and suggested that "degraded zooxanthellae are produced by host digestion". This implies that the host controls the disposal of the compromised portion of the zooxanthellar population *in situ*. Based on our data, we believe this may be the case.

Whether seawater temperatures rose above 32°C in the evolutionary past of the host animals, or whether the genes responsible for this tolerance originally applied to some other function, is not known. Their current function, however, is clear. *Symbiodinium* appears to still be adapting to current increases in SSTs, at its own rate. Can adaptation occur in both the host and the symbiont? We believe so. The literature suggests that it is quite possible for the algal symbionts to become adapted to these changes. For example, corals and their symbionts are known to thrive in environments in the Red Sea where SSTs routinely reach 34°C (Ateweberhan et al., 2005) and salinities reach up to 40.5ppt (Karako-Lampert et al., 2004).

If our higher temperatures were approached at a slower rate, this could have allowed time for some symbiont shuffling, lessening symptoms of bleaching. This could have decreased levels apoptosis/necrosis in the symbionts when higher temperatures were reached. The effects of slow temperature increases on symbiont shuffling, however, remains to be demonstrated, in the field or lab.

Here we have considered only *in situ* cells. Earlier (Strychar et al., 2004b), we demonstrated that *Symbiodinium* cell fractions expelled from the host exhibited apoptotic or necrotic characteristics with increased seawater temperature and time (up to 48h).This further supports the concept that the host cells are better adapted to elevated temperatures than their symbiotic zooxanthellae. We have also reported that we could only identify one clade of symbiont in *Favites complanata* - clade C (Strychar et al., 2005). Not all *F. complanata* have the same clade of symbionts, however. *Symbiodinium* clades A, B, C, D (Baker and Romanski 2007), and C3 (LaJeunesse et al., 2003) have all been identified from members of the Faviidae. It is possible that *F. complanata* could associate with multiple symbiont clades; thus, the response observed in our experimental colonies may not be representative of all *F. complanata* holobionts.

The adaptation/exaptation of a host coral to elevated seawater temperatures has now been demonstrated in three different hermatypic corals

from three separate families – the Faviidae, Acroporidae, and Poritidae – from the Great Barrier Reef. These corals and possibly families of corals are either already adapted to tolerate high temperatures from their ancestors having survived past high-temperature events in their evolutionary history, or they are exapted (Gould and Vrba, 1982; cf Futuyma, 1998; McLennan 2008) to these high temperatures *via* a pre-existing character associated with another physiological function. In the case of exaptation, it is not possible to discern from what character this tolerance may have been derived.

We thus hypothesize that this character is widespread among the Scleractinia of the Great Barrier Reef, and that death of the coral host in these organisms is due to their dependency upon the nutrients or other benefits that the symbionts provide. Coral hosts appear to be well adapted to temperature stress, while their endosymbiotic zooxanthellae either die or experience irreversible cell damage while still embedded in the coral host tissue, prior to expulsion from the coral. If the symbionts are not replaced or the loss of nutrients is not supplemented within some given time period, the host may die.

Adaptation and physiological tolerance to higher than normal temperatures while maintaining normal cell and tissue function is the key to survival in both the host corals and their symbiotic zooxanthellae. It would appear that it is the inherited adaptation or exaptation of pre-existing physiological traits from some ancestor that will permit survival of corals through its application to an environmental stress, the severity of which is increasing with time.

Acknowledgement
We thank the Great Barrier Reef Marine Park Authority for grants awarded to KBS. We also thank T. Piva and M. Coates for their contributions to earlier parts of this research. Finally, we thank J.S. Levinton his comments on an earlier version of the manuscript.

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