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JAMES COOK UNIVERSITY
AUSTRALIA

Coral symbionts in warming seas:

Population dynamics, adaptation and acclimatisation of *Symbiodinium*

Thesis submitted by

Emily Howells BSc. Hons

December 2011

for the degree of Doctor of Philosophy

in the School of Marine and Tropical Biology

James Cook University

Statement on the contribution of others

The research in this thesis was primarily designed, undertaken, analysed and written-up by Emily Howells. Contributions from collaborators are outlined at the beginning of each Chapter. Intellectual and editorial support was provided the supervisory team of Prof. Bette Willis (James Cook University), Dr. Madeleine van Oppen (the Australian Institute of Marine Science) and Dr. Line Bay (James Cook University and the Australian Institute of Marine Science).

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This thesis is dedicated to the inspirational women in my family

Victoria, Barbara, Dorothy and Peggy

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Chapter 3. Howells EJ, Jones AM, Bay LK, van Oppen MJH, Willis BL.
A change in *Symbiodinium* C2 microsatellite population composition harboured by a reef building coral following bleaching.
Marine Ecology Progress Series, in review

Chapter 4. Howells EJ, Beltran VH, Larsen NW, Bay LK, Willis BL, van Oppen MJH (2012).
Coral thermal tolerance is shaped by local adaptation of photosymbionts.
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Chapter 5. Howells EJ, Berkelmans R, van Oppen MJH, Willis BL, Bay LK (2011).
Historical thermal regimes define limits to coral acclimatisation.
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Appendix I. Bay LK, Howells EJ, van Oppen MJH (2009).
Isolation, characterisation and cross amplification of thirteen microsatellite loci for coral endosymbiotic dinoflagellates (*Symbiodinium* clade C).
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Appendix II. van Oppen MJH, Souter P, Howells EJ, Heyward A, Berkelmans R (2011).
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Abstract

Endosymbiotic photosymbionts, belonging to the dinoflagellate genus *Symbiodinium*, enable corals to succeed as dominant reef builders under ambient conditions, yet are also sensitive to anomalous changes in their thermal environment. Stressful temperatures cause photosynthetic damage to *Symbiodinium*, which can initiate lethal or sub-lethal coral bleaching. The capacity of *Symbiodinium* to resist and respond to stress (i.e. resilience) is crucial for the future persistence of corals. The aim of research presented in this thesis was to evaluate the influence of *Symbiodinium* population traits on the resilience of corals to warming seas. For within-type populations of *Symbiodinium*, spatial and temporal patterns of genetic diversity were investigated along with the potential for genetic adaptation and physiological acclimatisation to different thermal environments. Populations of the generalist *Symbiodinium* types C1, C2 and D (ITS1 rDNA) were used as a model system in the bleaching sensitive coral, *Acropora millepora*, on inshore reefs of the Great Barrier Reef (GBR).

Genetic diversity and connectivity were estimated for *Symbiodinium* C2 populations using eight polymorphic microsatellite DNA markers. At the central GBR, *Symbiodinium* C2 assemblages were genotyped in more than 400 colonies of *A. millepora* sampled at 7 sites (0.4 to 13 km apart) across a 12 year period. Within-host and within-reef assemblages were genetically diverse (up to 7 alleles per microsatellite locus per coral colony), with significant structure observed at all spatial and temporal scales investigated. Differentiation among sites accounted for 19-27% of the total genetic variation and was consistent with restricted hydrodynamic dispersal of *Symbiodinium* and the nature of local disturbance regimes. Differentiation among sampling years accounted for a lesser 7% of the total genetic variation and was associated with significant coral mortality during bleaching and cyclone events. Bleached corals hosted less diverse *Symbiodinium* C2 assemblages than healthy corals, indicating that genotypes may be lost during bleaching-induced reductions in *Symbiodinium* densities. Such population bottlenecks were investigated in more detail by following changes in *Symbiodinium* C2 assemblages before and after a severe bleaching episode at sites in the southern GBR. A 10 % decline in reef-wide genetic

diversity of *Symbiodinium* C2, including the loss of 16 alleles, was observed after the bleaching episode. The appearance of a few novel alleles after bleaching, combined with the high density of *Symbiodinium* on coral reefs, suggests that recovery of genetic diversity lost through bleaching is possible. However, if bleaching events become more frequent and severe, the genetic diversity of *Symbiodinium* populations could become eroded, especially as lost diversity is unlikely to be readily replenished by re-seeding from adjacent reefs.

Conversely, limited genetic connectivity between reefs may have positive implications for populations by promoting adaptation to local environmental conditions. Adaptation of *Symbiodinium* populations was examined by comparing the thermal tolerance of *Symbiodinium* type C1 from two central GBR reefs that differ in summer maximum temperatures by ~2°C. Following acclimation to a common thermal environment, *Symbiodinium* C1 populations displayed heat stress responses that correlated with their native thermal environment, both in symbiosis with a cohort of *A. millepora* juveniles and in cell cultures. In symbiosis, *Symbiodinium* C1 from the cooler reef underwent chronic photoinhibition at an elevated temperature of 32°C, causing severe bleaching and partial mortality of juvenile coral hosts. In contrast, *Symbiodinium* C1 from the warmer reef thrived at 32°C, with high rates of photochemical efficiency and rapid growth of juvenile coral hosts. A second heat stress experiment demonstrated that adaptive variation in the thermal tolerance of *Symbiodinium* C1 populations was maintained after more than 30 asexual generations in culture. Pigment profiles of *Symbiodinium* C1 showed that levels of photoprotective pigment (β-carotene relative to chlorophyll *a*) were more than twofold greater in the population native to the warmer reef indicating a functional basis for variation in thermal tolerance. These results demonstrate that *Symbiodinium* types can adapt to local thermal environments and that this adaptation shapes the fitness of coral hosts.

The contribution of physiological acclimatisation to adaptive variation in thermal tolerance was investigated during reciprocal transplantation of adult coral symbioses between the warm central and cool southern regions of the GBR. Throughout a year of transplantation, *A. millepora*-*Symbiodinium* D associations from the central GBR were exposed to gradually cooling temperatures and *A. millepora*-*Symbiodinium* C2 associations from the southern GBR were exposed to gradually warming temperatures.

In both locations, native corals remained healthy and transplanted corals were healthy over initial months when temperatures remained within native thermal regimes. However, during winter, *A. millepora-Symbiodinium* D associations transplanted to the southern GBR bleached and the majority suffered whole or partial mortality at temperatures <1°C below their native minimum. Similarly, during summer, *A. millepora-Symbiodinium* C2 associations transplanted to the central GBR bleached and suffered whole or partial mortality at temperatures 1-2°C above their native maximum. At the central GBR during summer bleaching, mortality was preceded by a change in the dominant *Symbiodinium* type from C2 to D within transplanted corals. The strong interaction between genotype and environment observed for bleaching and mortality (as well as for parameters of growth and reproduction) re-affirm the importance of genetic adaptation in defining the thermal limits of *A. millepora-Symbiodinium* partnerships. These results likely reflect differences in the thermal tolerance among *Symbiodinium* types and populations, however variation between coral host populations may also exist.

Findings presented in this thesis demonstrate that coral symbioses are adapted to their current thermal environments. Increases in thermal tolerance required for *A. millepora* symbioses to persist under warmer seas are dependent on continued genetic adaptation, as there is little potential for acclimatisation beyond current thermal regimes. Critical next steps to determine whether adaptation keeps pace with future warming include the application of functional genetic approaches to population genotyping and quantifying rates of adaptation for both *Symbiodinium* and coral hosts.

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Chapter 1. General Introduction

The relationship between the coral animal host and resident photosynthetic micro-organisms provides metabolic advantages that enable corals to succeed as diverse and dominant reef builders (Stanley 2006; Fig. 1.1). However, photosymbiotic corals are vulnerable to extinction under long-term shifts in their environment that occur as global climate changes. Photosymbiotic corals underwent disproportionate extinctions compared with non-photosymbiotic corals during geological periods of climate change (Stanley and van de Schootbrugge 2009; Barbeitos *et al.* 2010) and extant coral cover has recently declined by more than a third (Butchart *et al.* 2010) from rising sea temperatures acting in combination with other pressures (Hughes *et al.* 2003; Wilkinson 2004; Carpenter *et al.* 2008). The persistence of corals to further warming of 1.5 -3°C within the 21st century (Meehl *et al.* 2007) is dependent on the capacity of all partners in the symbiosis to respond together to changes in their environment. This thesis explores traits of photosymbiont populations which influence the resilience of their coral hosts to warming seas.

Fig. 1.1. Photosymbiosis in tropical reef corals

Left to right: Photosymbiont cells of the dinoflagellate genus *Symbiodinium* (scale = 10 µm, photo: E. Howells); coral polyps with brown pigmentation due to the presence of *Symbiodinium* cells at densities ~10^6 per cm^2 of coral tissue scale = 1 mm, photo: E. Howells); shallow tropical reef dominated by photosymbiotic corals (scale = 10 cm, photo: E. Howells); reefs built by photosymbiotic corals visible from space (scale = 100 km, photo: NASA).

1.1. *Symbiodinium* - ubiquitous driver of coral reef productivity

Obligate partnerships with photosymbiotic dinoflagellates in the genus *Symbiodinium* (also known as zooxanthellae) have evolved in numerous marine invertebrates and protists, including scleractinian (hard coral) and octocoral (soft coral and sea fan) hosts (Coffroth and Santos 2005). These symbioses facilitate high productivity through resource recycling. Nutrient-rich host habitats support high rates of *Symbiodinium* photosynthesis and almost all of the carbon fixed (up to 95%) is transferred to the coral host, providing energy necessary for daily metabolism as well as the surplus required for growth, reproduction and repair (Lewis and Smith 1971; Muscatine and Porter 1977; Falkowski *et al.* 1984). Many corals are almost entirely dependent on energy derived from photosymbiosis (autotrophy, Muscatine and Porter 1977; Falkowski *et al.* 1984), while others supplement their diet with varying degrees of particulate feeding (heterotrophy, Porter 1974; Bak *et al.* 1998; Anthony and Fabricius 2000). Host calcification is enhanced by *Symbiodinium* photosynthesis by an increase in internal pH and the availability of carbonate ions for precipitation (Al-Horani *et al.* 2003).

Corals acquire *Symbiodinium* either from the environment as larvae or juveniles (~85% of species) or via parental transmission to eggs, larvae or asexual propagules (Richmond 1997). Within the host environment, *Symbiodinium* cells are in a non-motile phase occupying vacuoles in the coral gastroderm at densities in the order of 10^6 cells per cm^2 of coral tissue (Drew 1972; Kawaguti and Nakayama 1973), which are maintained by cellular fission (Fig. 1.2), as opposed to continual uptake of ‘new’ *Symbiodinium* from the environment (see Goulet and Coffroth 2003a,b; Andras *et al.* 2011). Rates of asexual proliferation are determined by carbon fixation and usage (Stat *et al.* 2008; Cantin *et al.* 2009), available habitat (e.g. new or bleached tissue, Wilkerson et al. 1988, Fitt *et al* 1993, Jones *et al.* 1997) and supply of nutrients from the host (Cook *et al.* 1988; Titlyanov *et al.* 1996). Coral hosts further regulate their complement of *Symbiodinium* through cell digestion and extrusion (Muscatine and Pool 1979; Titlyanov *et al.* 1996). *Symbiodinium* asexual generation times (doubling rates) inside corals can be as short as a few days or as long as a few months (Wilkerson *et al.* 1988).

In addition to their very high densities in symbiosis, some *Symbiodinium* types maintain large free-living populations in benthic habitats (sediments and macroalgal beds, $>10^3$ cells per cm^3) and in the water column above reefs ($\sim 10^2$ cells per cm^3; Coffroth *et al.* 2006; Littman *et al.* 2008; Manning and Gates 2008; Porto *et al.* 2008). In free-living habitats, *Symbiodinium* cells are either non-motile cysts or in a motile flagellated phase (Fig 1.2). *Symbiodinium* cells enter the water column during diurnal periods of vertical migration of motile cells (suggested by observations of cultured and freshly isolated cells, Fitt *et al.* 1981; Fitt and Trench 1983; Trench and Blank 1987;Yacobovitch *et al.* 2004), during conditions that cause sediment re-suspension, and during release of intact cells from coral hosts (Stimson and Kinzie 1991; Ralph *et al.* 2001; Bhagooli and Hidaka 2004; Hill and Ralph 2007) or corallivore faeces (Muller-Parker 1984; Porto *et al.* 2008). In free-living habitats, growth rates of *Symbiodinium* are unknown, but asexual generation times in the order of days to weeks have been recorded for other species of free-living dinoflagellates (Wilkerson *et al.* 1988).

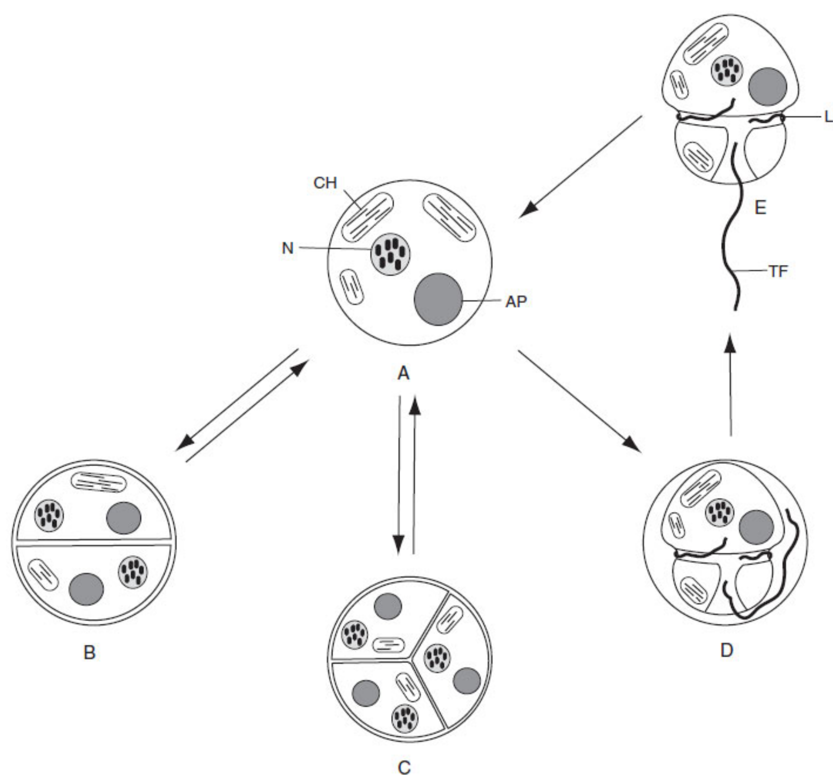


Fig. 1.2. **Life cycle of *Symbiodinium***

A. vegetative cyst; B. dividing vegetative cyst producing two daughter cells; C. dividing vegetative cyst producing three daughter cells; D. developing zoospore; E. zoospore.

CH: chloroplast; N: nucleus; AP: accumulation product; LF: longitudinal flagella; TF: transverse flagella. Figure from (Stat *et al.* 2006), adapted from (Freudenthal 1962) and (Schoenberg and Trench 1980b)

1.2. *Symbiodinium* photosynthetic dysfunction initiates the coral bleaching stress response

Coral-*Symbiodinium* associations are highly successful under ambient environments, yet they can be very sensitive to anomalous conditions, including changes in temperature, light, salinity (reviewed in Brown 1997) and water quality (Wooldridge and Done 2009). Current climate warming threatens coral-*Symbiodinium* associations, as summer temperature anomalies exceed the upper thermal limits of corals and their *Symbiodinium* partners with increasing frequency (Hoegh-Guldberg 1999). While the coral animal partner experiences direct thermal stress (Negri *et al.* 2007; Ainsworth *et al.* 2008; Rodriguez-Lanetty *et al.* 2009), the breakdown of photosymbiosis (coral bleaching) is initiated by photosynthetic damage in *Symbiodinium* cells (reviewed in Weis 2008; Figs.1.3 and 1.4). Coral bleaching is typically characterised by loss of *Symbiodinium* cells from host tissues, but can also include a reduction in photosynthetic pigments with or without cell removal. Most corals are unable to sustain metabolic requirements in the absence of *Symbiodinium* partners and suffer mortality when a functional photosymbiosis cannot be maintained or re-established. Large-scale episodes of thermal bleaching over the past few decades have caused or contributed to significant coral mortality on a global scale (Hoegh-Guldberg 1999; Wilkinson 2004) and where recovery has been inhibited, bleaching episodes have been linked to phase shifts from benthic ecosystems dominated by corals to algae (or other benthic biota, Bellwood *et al.* 2004).

Not all coral-*Symbiodinium* associations respond equally to thermal stress, and the temperatures that induce coral bleaching and mortality differ among reefs and regions (Hoegh-Guldberg 1999; Berkelmans 2002). For example, on the Great Barrier Reef, bleaching thresholds at inshore Magnetic Island are ~1.5°C higher than at offshore reefs at similar latitude, and up to ~2.0°C higher than inshore reefs at higher latitudes (Berkelmans 2002). Within a reef site, significant variation in bleaching sensitivity occurs among coral colonies belonging to different host species (Marshall and Baird 2000; Loya *et al.* 2001; McClanahan *et al.* 2004) or harbouring different *Symbiodinium* partners (Rowan *et al.* 1997; Baker *et al.* 2004; Jones *et al.* 2008; LaJeunesse *et al.*

2010; see section 1.3), as well as among colonies of the same host species harbouring the same *Symbiodinium* partner (Fig. 1.4 centre image; see section 1.4). Consequently, some coral-*Symbiodinium* associations may not be capable of withstanding future warming, while other individuals, populations and species may possess traits that enable them to enhance their thermal tolerance and buffer against disturbance. Such resilience-linked traits in coral-*Symbiodinium* associations are poorly understood especially for *Symbiodinium*, the more thermally sensitive partner in the symbiosis according to accumulating evidence (Strychar and Sammarco 2009; Yakovleva *et al.* 2009).

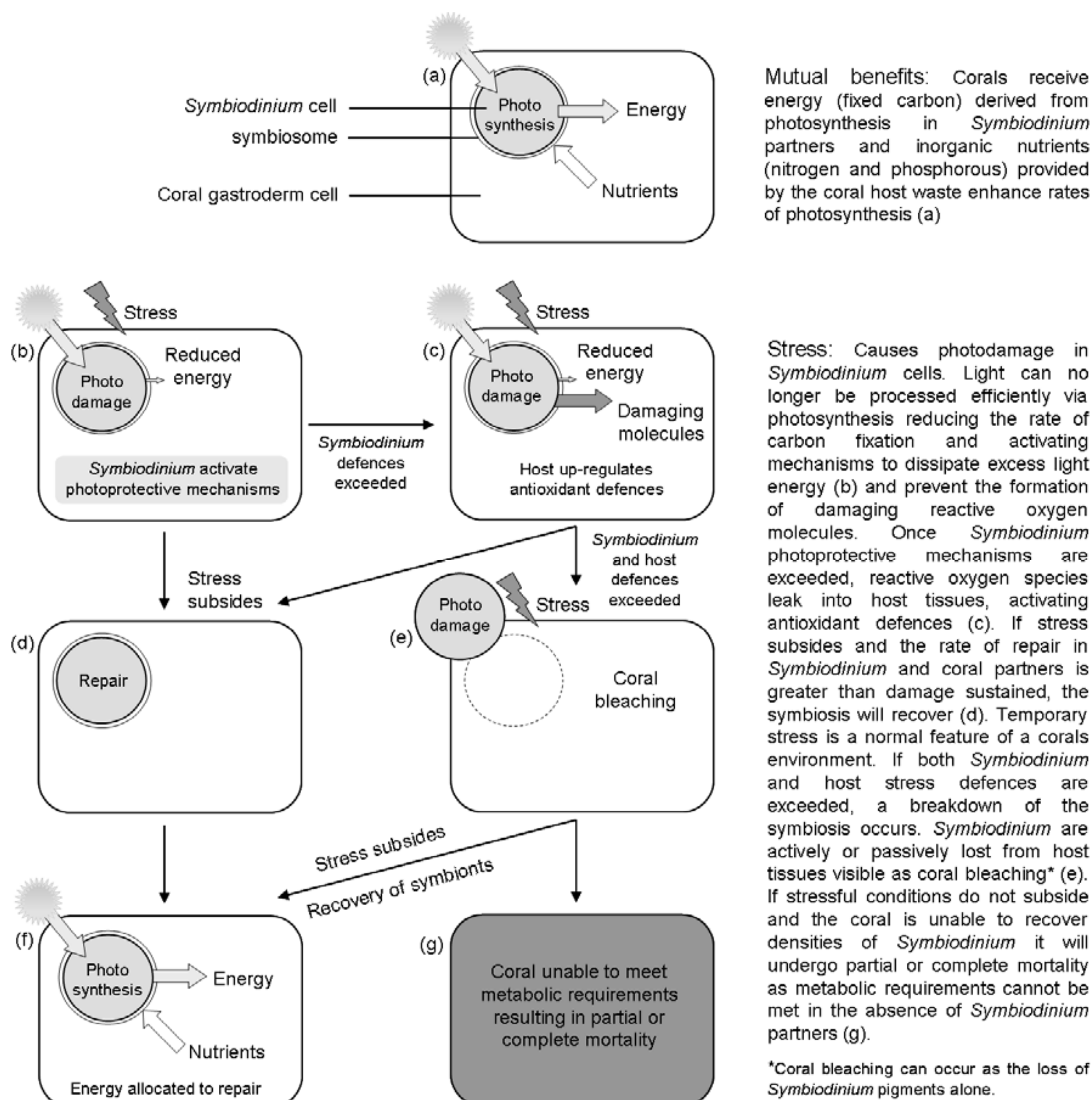


Fig. 1.3. Simplified model of the role of *Symbiodinium* in coral stress and the bleaching response

See (Weis 2008) for detailed mechanisms of coral bleaching.

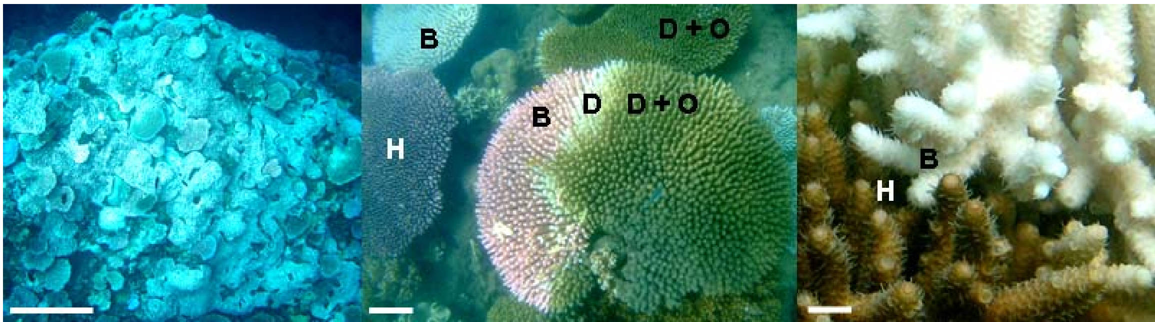


Fig.1.4. **Coral bleaching**

Left: Widespread coral bleaching observed during positive sea surface temperature anomaly in Okinawa, Japan, 2007 (scale = 1 m, photo: E. Howells).

Centre: Variation in bleaching response among colonies of *Acropora millepora* observed during flood plume event in the central Great Barrier Reef, Australia, 2009 (scale = 10 cm, photo: E. Howells). Healthy (H), bleached (B), recently dead (D), and dead overgrown with algae (D+O) areas are indicated. The forward colony is undergoing progressive mortality.

Right: Variation in bleaching response within a colony of *Acropora millepora* showing healthy (H) and bleached (B) coral tissue observed during thermal stress in a field experiment, central Great Barrier Reef, Australia in 2009 (scale = 1 cm, photo: E. Howells).

1.3. *Symbiodinium* type diversity – a source of resilience to thermal stress?

Knowledge of the diversity of *Symbiodinium* and their association with coral hosts has been rapidly advanced by a transition from morphological (e.g. Freudenthal 1962; Kinzie and Chee 1979; Schoenberg and Trench 1980b) to genetic (Rowan and Powers 1991,1992; Wilcox 1998) methods of identification and classification (Fig.1.5). The *Symbiodinium* genus encompasses levels of genetic diversity equivalent to order-level divergence in other groups of dinoflagellates (Rowan and Powers 1992) and is currently divided into nine lineages (clades A to I; clades A to D commonly associate with corals), which each encompass a number of widely distributed generalist and regionally endemic *Symbiodinium* types (LaJeunesse 2001; LaJeunesse 2002; LaJeunesse *et al.* 2004; LaJeunesse 2005). This exceptional diversity has been postulated to provide reservoirs of functionally distinct partners that coral hosts may exploit to form new

stress-tolerant associations (Buddemeier and Fautin 1993). Characterising functional variation among *Symbiodinium* clades and types and their influence on host stress tolerance has been a staple theme of coral reef research over the past few decades and there is now substantial evidence that the *Symbiodinium* type harboured exerts a strong influence on coral thermal tolerance and bleaching resistance (Rowan *et al.* 1997; Baker 2001; Baker *et al.* 2004; Rowan 2004; Berkelmans and van Oppen 2006; Abrego *et al.* 2008; Jones *et al.* 2008; Mieog *et al.* 2009; Csaszar *et al.* 2010; LaJeunesse *et al.* 2010).

Environmental acquisition of *Symbiodinium* in the majority of corals theoretically provides the opportunity to establish partnerships with *Symbiodinium* types best adapted to local environmental regimes during early life history (Douglas 1998). Some hosts are capable of establishing symbioses with multiple *Symbiodinium* types (Kinzie 1974; Kinzie and Chee 1979; Schoenberg and Trench 1980a; Schwarz *et al.* 1999; Coffroth *et al.* 2001; Weis *et al.* 2001; Little *et al.* 2004; Rodriguez-Lanetty *et al.* 2004; Gomez-Cabrera *et al.* 2008; Abrego *et al.* 2009a; Mieog *et al.* 2009), which influences their growth and thermal tolerance during early life stages (Kinzie and Chee 1979; Little *et al.* 2004; Abrego *et al.* 2008; Mieog *et al.* 2009). As symbioses mature, they tend to be dominated by a single *Symbiodinium* type (Coffroth *et al.* 2001; Gomez-Cabrera *et al.* 2008; Abrego *et al.* 2009b), although they may have additional types present in low background abundances (Mieog *et al.* 2007). In some instances, mature coral symbioses can undergo changes in the *Symbiodinium* type harboured during periods of thermal stress either by up-regulation of the abundance of more thermally tolerant background *Symbiodinium* type (“shuffling”, Chen *et al.* 2005; Mieog *et al.* 2007; Jones *et al.* 2008) or by uptake of *Symbiodinium* cells from the external environment (“switching”, Lewis and Coffroth 2004).

Enhanced thermal tolerance can come at the cost of reduced fixation and transfer of carbon to the coral host, limiting energy supply and growth (Little *et al.* 2004; Stat *et al.* 2008; Cantin *et al.* 2009; Mieog *et al.* 2009; Jones and Berkelmans 2010), which likely explains why novel symbionts typically do not persist in host tissues after stressful conditions have passed (Chen *et al.* 2005; Thornhill *et al.* 2006b; Jones *et al.* 2008). Consequently, it remains unclear whether the appearance of novel *Symbiodinium* types during coral stress represents active symbiont selection by the coral host to gain thermal

tolerance (Buddemeier and Fautin 1993) or passive exploitation of a stressed host by opportunistic types that out compete optimal *Symbiodinium* partners (Stat and Gates 2011). Furthermore, a number of coral species show no evidence of changing *Symbiodinium* partners under stressful conditions (Thornhill *et al.* 2006a; Thornhill *et al.* 2006b; Goulet *et al.* 2008a, Stat *et al.* 2009) and many species exhibit fidelity for particular *Symbiodinium* types across broad environmental ranges, including temperature gradients (LaJeunesse *et al.* 2004; Wicks *et al.* 2010; Cooper *et al.* 2011).

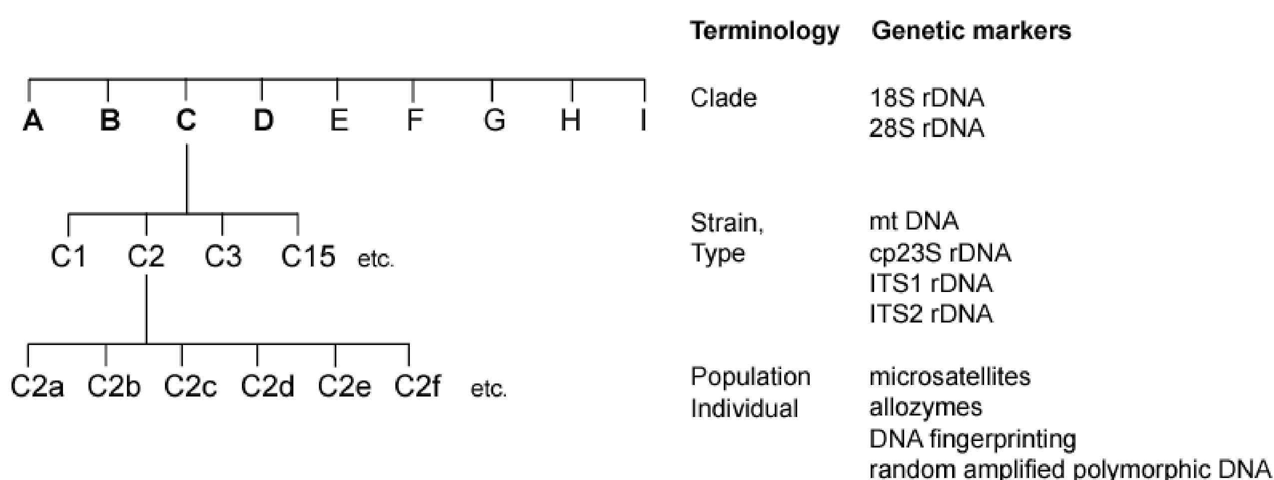


Fig. 1.5. Genetic markers used to identify and classify *Symbiodinium*

Symbiodinium is currently divided into nine lineages (clades A to I), based on nuclear 18S and 28S ribosomal DNA sequences (Rowan and Powers 1991,1992; Pochon and Gates 2010), four of which commonly occur in corals (clades A to D, highlighted in bold). Each clade contains many types or putative species based on variation at internal transcribed spacer regions of ribosomal DNA (ITS1 rDNA, (van Oppen *et al.* 2001); ITS2 rDNA, (LaJeunesse 2001; LaJeunesse 2002; LaJeunesse 2005)), chloroplast DNA regions (Santos *et al.* 2002; Moore *et al.* 2003) and mitochondrial DNA (Takabayashi *et al.* 2004). Within types, *Symbiodinium* maintain diverse populations at the scale of individual coral colonies, reefs and regions based on the polymorphism of higher resolution genetic markers including DNA fingerprints (Goulet and Coffroth 2003b,a), random amplified polymorphic DNA (Baillie *et al.* 2000), allozymes (Baillie *et al.* 1998; Belda-Baillie *et al.* 1999), microsatellite flanking (Santos *et al.* 2004) and repeat (Santos *et al.* 2003; Magalon *et al.* 2006; Bay *et al.* 2009; Howells *et al.* 2009; Kirk *et al.* 2009; Pettay and Lajeunesse 2009; Thornhill *et al.* 2009; Andras *et al.* 2011; Wham *et al.* 2011) regions. Figure modified after (Coffroth and Santos 2005).

1.4. Resilience traits of *Symbiodinium* populations

Surprisingly little research has been undertaken to investigate the resilience of *Symbiodinium* populations under warming scenarios, which is defined in this thesis as their capacity to respond to environmental change and retain their ecological function as mutualistic symbionts. As coral species have evolved associations with single or a small sub-set of *Symbiodinium* types that occur in a given reef environment, I argue that the future persistence of reef corals will be influenced largely by the resilience of existing mutualistic *Symbiodinium* types, rather than their capacity to associate with novel *Symbiodinium* types. Resilience is dependent on population traits that enhance thermal tolerance and buffer against disturbances such as coral bleaching (Table 1.1).

Symbiodinium populations may enhance thermal tolerance by acclimatisation involving adjustments in cell biochemistry. *Symbiodinium* cells acclimatise to seasonal temperatures via variation in the production of photosynthetic and photoprotective pigments (Fitt *et al.* 2000), photoprotective mycosporine-like amino acids (Linan-Cabello *et al.* 2010) and heat shock proteins (Downs *et al.* 2002). However, it is unclear to what extent acclimatisation can continue under gradual rises in temperature that exceed present thermal regimes. Acclimatisation potential is ultimately constrained by genetic make-up and any further gains in thermal tolerance require genetic adaptation. This occurs via selection of genotypes linked to optimal physiological performance under a given set of environmental conditions. Adaptive tolerance to specific environments including extreme temperatures has been documented in aquatic microalgae (Costas *et al.* 2001; Costas *et al.* 2007; Costas *et al.* 2008; Lopez-Rodas *et al.* 2008a; Lopez-Rodas *et al.* 2008b; Lopez-Rodas *et al.* 2009a; Lopez-Rodas *et al.* 2009b; Marva *et al.* 2010), but neither historical adaptation nor future adaptive potential have been investigated for *Symbiodinium*.

Adaptation potential is expected to be maximised if genetic diversity (the substrate for natural selection) is maintained within populations, yet there are only a few estimates of population genetic diversity for *Symbiodinium*. Some *Symbiodinium* types maintain monoclonal or low diversity populations within hosts and reefs (Goulet and Coffroth

2003a; Santos *et al.* 2003; Kirk *et al.* 2009; Thornhill *et al.* 2009; Andras *et al.* 2011), while populations of other types are highly diverse (Magalon *et al.* 2006; Howells *et al.* 2009). Adaptation is further shaped by the extent to which individual genotypes disperse and recruit among reefs (connectivity). Low connectivity can facilitate adaptation to local environmental conditions as the adapted population is not diluted by recruitment from external sources carrying maladapted alleles (Sanford and Kelly 2011). However, high connectivity may spread beneficial genetic variants, such as those linked to thermal tolerance, among reefs and is important in the re-seeding of reefs following disturbance, such as episodes of bleaching (van Oppen and Gates 2006). There have been only a few investigations of connectivity among *Symbiodinium* reef populations, and these mostly indicate limited connectivity across scales of 10s of km or less (Santos *et al.* 2003; Howells *et al.* 2009; Kirk *et al.* 2009; Thornhill *et al.* 2009; Andras *et al.* 2011; but see Magalon *et al.* 2006). However, additional research on different oceanic regions, host species and *Symbiodinium* types is required to corroborate scales of *Symbiodinium* dispersal and recruitment.

Table 1.1. Resilience traits of *Symbiodinium* populations

Shaded table cells indicate research areas to be addressed in this thesis

Trait	Benefit	Trade-off	Knowledge state	Knowledge gap
Abundance within hosts and free-living habitats	Buffers against population size reductions and losses of genetic diversity	Nil	Limited quantification of cell densities in water column and sediments[1]	Unknown abundance of specific types in free living habitats
Genetic diversity within hosts and free-living habitats	Required for adaptation via natural selection	Nil	Few studies with limited coverage of different *Symbiodinium* types, host species, and geographic regions[2]	No information for types hosted by scleractinian corals on the Great Barrier Reef
Genetic connectivity among reefs	Re-seeds populations following size reductions and spreads beneficial genetic mutations	Counteracts local adaptation	Few studies with limited coverage of different *Symbiodinium* types, host species, and geographic regions[3]	No information for types hosted by scleractinian corals on the Great Barrier Reef
Physiological acclimatisation within cells	Capacity to adjust tolerance to broad range of environmental conditions	Potentially energetically costly; extent of response constrained by genotype	Responses to seasonal, changes in temperature[4]	Limited information on the capacity for gradual acclimatisation to temperatures exceeding present thermal regimes
Genetic adaptation via selection within hosts and free-living habitats	Specialisation to particular set of environmental conditions	Susceptible to changes in environment that exceed rate of adaptation	No information	No evidence of historical adaptation and no quantification of rates of adaptation

[1] Littman *et al.* 2008
[2] Goulet and Coffroth 2003b,a; Santos *et al.* 2003; Magalon *et al.* 2006; Howells *et al.* 2009; Kirk *et al.* 2009; Thornhill *et al.* 2009; Andras *et al.* 2011
[3] Santos *et al.* 2003; Magalon *et al.* 2006; Howells *et al.* 2009; Kirk *et al.* 2009; Thornhill *et al.* 2009; Andras *et al.* 2011
[4] Fitt *et al.* 2000; Downs *et al.* 2002; Linan-Cabello *et al.* 2010

1.5. Thesis outline

My aims in this thesis were to evaluate the resilience of *Symbiodinium* populations to warming seas and investigate the influence of symbiont resilience on coral thermal tolerance. *Symbiodinium* partnerships in the scleractinian coral *Acropora millepora* (Acroporidae; Fig. 1.6) were selected as a model system because of their common occurrence at sites across the Great Barrier Reef (GBR), existing knowledge of their *Symbiodinium* type associations, and the availability of historical samples for comparative analysis. *A. millepora* typically occupies the reef flat and crest habitats, is often locally abundant, yet is sensitive to bleaching and associated mortality (Baird and Marshall 2002). Across the length of the GBR, *A. millepora* most commonly associates with *Symbiodinium* type C2 (ITS1 rDNA, equivalent to ITS2 type C3), although, at some offshore sites, a distinct C2 type occurs (C2*, Cooper *et al.* 2011). At some inshore sites, types C1 and D are locally dominant, especially during episodes of thermal stress, or are present within some colonies in low background abundance (Mieog *et al.* 2007; Jones *et al.* 2008; Cooper *et al.* 2011). *Symbiodinium* C1, C2 and D are generalist types which occur in a number of coral hosts on the GBR (van Oppen *et al.* 2001; LaJeunesse *et al.* 2004).

Fig. 1.6.
Acropora millepora

Halfway Island,
southern Great Barrier Reef
(scale = 10 cm, photo: James Tan)

Research presented in this thesis investigated resilience traits in populations belonging to the common *Symbiodinium* types C1, C2 and D, as outlined below for Chapters 2 to 6.

Chapter 2 describes the influence of spatial isolation and disturbance regimes in shaping the genetic diversity and structure of *Symbiodinium* populations harboured by *A. millepora*. The capacity of *Symbiodinium* C2 to disperse and recruit across small spatial scales was evaluated in reference to regional oceanography, and the influence of disturbance events was established by comparison of samples collected between major disturbances and during coral bleaching. Results provide the first insight into the population genetics information of a *Symbiodinium* type hosted by scleractinian corals on the GBR and advance knowledge of *Symbiodinium* diversity and connectivity.

Chapter 3 investigates whether coral bleaching episodes have unseen impacts on the genetic diversity and composition of *Symbiodinium* C2 populations harboured by *A. millepora*. *Symbiodinium* C2 in tagged coral colonies was genotyped before a rapid loss of population numbers during sub-lethal bleaching and after a gradual re-population of host tissues. The results identify losses of population genetic diversity following bleaching and provide insight into mechanisms of repopulation of *Symbiodinium* within coral tissues during bleaching recovery.

Chapter 4 documents adaptation of *Symbiodinium* populations to local thermal environments. Heat stress responses were quantified in populations of *Symbiodinium* C1 native to different thermal environments on the GBR following acclimatisation within host fragments and in culture. Furthermore, the influence of *Symbiodinium* local adaptation on the coral host was tested by inoculation of juvenile *A. millepora* corals with *Symbiodinium* C1 native to different thermal environments. Results provide information on functional variation in thermal tolerance between populations belonging to the same *Symbiodinium* type and its influence on the fitness traits of coral symbioses.

Chapter 5 describes the capacity of coral-*Symbiodinium* partnerships to acclimatise to gradual changes in temperature. Reciprocal transplantation of adult colony halves of *A. millepora* between different thermal regions of the GBR investigated acclimatisation of *Symbiodinium* C2 partnerships to warming temperatures and acclimatisation of *Symbiodinium* D partnerships to cooling temperatures. Native and transplanted corals were monitored for more than one year, providing information on the relationship between acclimatisation potential and long-term thermal history.

Chapter 6 discusses the traits of *Symbiodinium* populations that positively and negatively influence their resilience to warming seas. The findings of preceding Chapters are synthesised to evaluate the role of *Symbiodinium* responses in the persistence of *A. millepora* symbioses under warming seas. The applicability of findings to coral-*Symbiodinium* associations with different life history traits and bleaching sensitivities are also discussed. Approaches for future research to refine predictions of the long term persistence of corals are highlighted.

Chapter 2. Spatial isolation and disturbance shape the genetic structure of *Symbiodinium* populations

This Chapter is inserted without abstract as currently *in review* at the journal *Molecular Ecology*:

Howells E, Willis B, Bay L, van Oppen M.
Spatial isolation and disturbance shape the genetic structure of *Symbiodinium* populations.

E. Howells collected and genotyed samples, analysed the data and wrote the manuscript. B. Willis and M. van Oppen collected historical samples and contributed to manuscript writing. L. Bay contributed to manuscript writing.

2.1. Introduction

Dinoflagellate photosymbionts of corals, once considered a single pandemic species (*Symbiodinium microadriaticum*, Taylor 1974) are now recognised to encompass numerous genetically and functionally distinct clades and types that play a fundamental role in defining the physiological tolerances of corals (Baker 2003; Coffroth and Santos 2005). The majority of coral species (~85%) acquire their *Symbiodinium* partners from the environment during early life history (Richmond 1997) and demonstrate fidelity for one, or a few, particular types as the symbiosis matures (Coffroth *et al.* 2001; Abrego *et al.* 2009b; Cooper *et al.* 2011). The environmental availability (see Coffroth *et al.* 2006; Manning and Gates 2008; Adams *et al.* 2009) of optimal *Symbiodinium* types influences the success of these host-*Symbiodinium* partnerships, yet the factors that influence the population dynamics of *Symbiodinium* types at local reefs are poorly understood.

Genetic markers are an essential tool in the identification of *Symbiodinium* clades and types (Rowan and Powers 1992; LaJeunesse 2001; van Oppen *et al.* 2001; Sampayo *et al.* 2009), and are equally important in determining population processes, as the morphological similarity and small size of *Symbiodinium* (6-12 µm, Fitt 1985) render visual surveys virtually impossible. Early investigations of population genetic variation in *Symbiodinium* demonstrated diverse populations of *Symbiodinium* hosted by juvenile (Belda-Baillie *et al.* 1999) and adult (Baillie *et al.* 1998; Baillie *et al.* 2000) giant clams using random amplified polymorphic DNA and allozymes, as well as inter-colony diversity and temporal stability of *Symbiodinium* associations in octocorals (Goulet and Coffroth 2003a,b) using DNA fingerprinting. Recent development of *Symbiodinium* clade-specific microsatellite markers (Santos *et al.* 2003; Magalon *et al.* 2004; Pettay and Lajeunesse 2007; Andras *et al.* 2009; Howells *et al.* 2009; Pettay and Lajeunesse 2009; Pinzon *et al.* 2011; Wham *et al.* 2011; simple sequence repeats typically located in regions of the genome not under selection, Lowe *et al.* 2004) have improved understanding of *Symbiodinium* population genetic diversity and connectivity, traits important for population resilience (van Oppen and Gates 2006).

The most well-described variation in the population genetics of *Symbiodinium* is for B1 types in the Caribbean. High genetic differentiation of *Symbiodinium* B1 populations has been observed at spatial scales of ~1 to10's of km in both scleractinian (*Montastraea faveolata* and *M. annularis*, Thornhill *et al.* 2009) and octocoral (*Pseudopterogorgia elisabethae*, Santos *et al.* 2003; and *Gorgonia ventalina* Kirk *et al.* 2009; Andras *et al.* 2011) hosts. Low connectivity between reefs and patterns of high genetic structure consistent with limited dispersal of *Symbiodinium* via prevailing ocean currents (Santos *et al.* 2003; Howells *et al.* 2009; Kirk *et al.* 2009; Andras *et al.* 2011) are congruent with the life history traits of *Symbiodinium*. Active dispersal among reefs does not occur in *Symbiodinium* (Fitt *et al.* 1981; Fitt and Trench 1983; Yacobovitch *et al.* 2004), and passive dispersal is likely restricted by both an endosymbiotic lifestyle (~10^6 cells per cm^2 in coral hosts, Drew 1972; Kawaguti and Nakayama 1973) and the predominance of free-living *Symbiodinium* in benthic habitats (>10^3 cells per cm^3, Littman *et al.* 2008) compared with the water column (~10^2 cells per cm^3, Littman *et al.* 2008). Two investigations of *Symbiodinium* C1 types in the Pacific Ocean showed higher variation within coral hosts compared with Caribbean *Symbiodinium* B1 associations, but differed in levels of population differentiation. For *Symbiodinium* C1:3a hosted by the octocoral *Sinularia flexibilis* on the Great Barrier Reef (Howells *et al.* 2009), population genetic differentiation at a spatial scale of 10s of km suggested that mechanisms of connectivity were similar with those described for *Symbiodinium* B1 populations (Santos *et al.* 2003; Kirk *et al.* 2009; Andras *et al.* 2011). Conversely, for *Symbiodinium* C1 in the scleractinian coral *Pocillopora meandrina* in the South Pacific (Magalon *et al.* 2006), populations were undifferentiated at distances up to ~250 km, indicating significantly greater connectivity among *Symbiodinium* reef populations, potentially because of enhanced *Symbiodinium* dispersal via coral larvae (*P. meandrina* transmit symbionts maternally). However, this hypothesis remains to be tested.

Within reef populations, episodes of disturbance potentially further influence the genetic structure of *Symbiodinium* populations. Bleaching and mortality of corals during anomalous weather conditions (reviewed in Brown 1997) and the destruction and fragmentation of corals during cyclones and storms (Harmelin-Vivien and Laboute 1986; Massel and Done 1993; Fabricius *et al.* 2008) may drive changes in

Symbiodinium populations through the mortality, displacement and redistribution of individual genotypes. Monitoring of *Symbiodinium* population genotypes across bleaching episodes has shown relative stability of *Symbiodinium* B1 genotypes in *Montastraea* spp. corals in the Caribbean (Thornhill *et al.* 2009). In contrast, changes in allele frequencies, including a loss of diversity in *Symbiodinium* type C2 in *Acropora millepora* colonies following bleaching on the Great Barrier Reef, indicate a strong role of such disturbances in shaping the genetic structure of *Symbiodinium* populations (Howells *et al.* in review, Chapter 3). These studies focussed on changes in *Symbiodinium* genotypes within individual coral hosts during sub-lethal disturbances. Potential changes in the genetic structure of *Symbiodinium* populations following significant mortality and recruitment events at the population level of the coral host have not yet been investigated.

My aim was to expand existing population genetic information for *Symbiodinium* by investigating the combined influences of spatial isolation and disturbance on the genetic structure of a generalist type of *Symbiodinium*, hosted by a wide range of scleractinian corals on the Great Barrier Reef (van Oppen *et al.* 2001; LaJeunesse *et al.* 2004). I genotyped type C2 *Symbiodinium,* as characterised by ITS1 rDNA (equivalent to ITS2 type C3) hosted by colonies of the scleractinian coral *Acropora millepora* using eight polymorphic microsatellite markers. To enable spatial and temporal comparisons at sites influenced by different disturbance regimes, I analysed coral samples from bleached and non-bleached corals at multiple sites in the Palm Islands, as well as historical sample collections. I describe patterns of genetic structure among sites, years and coral bleaching status, and find evidence that both oceanographic processes and disturbance events shape *Symbiodinium* populations.

2.2. Methods

2.2.1. Sample collection

Within-host assemblages of *Symbiodinium* C2 were genotyped from 401 individual colonies of *Acropora millepora* from the Palm Island group in the central Great Barrier Reef (GBR; Fig. 2.1). Samples were collected from a total of 7 sites separated by 0.4 to 13 km on either the windward or leeward sides of islands. Coral populations at windward sites are shaped by cyclone impacts, whereas those at leeward sites are more susceptible to lowered salinity and decreased water quality associated with flood plumes (Devlin *et al.* 2001; King *et al.* 2002; Schaffelke *et al.* 2009). During 2009, coral communities on the leeward side of islands underwent a natural bleaching episode induced by flood plume exposure. Accordingly, samples collected from *A. millepora* colonies were visually recorded as non-bleached (normal pigmentation) or bleached (moderately pale to white colouration; Fig. 2.2) at sites P2, O2, O3 and O4, and from an additional windward site (F1) where no bleaching was observed (Table 2.1). *Symbiodinium* assemblages in additional samples *of A. millepora* from historical collections from sites F1, P1 and O1 in 1997 and 2004 were also genotyped. *Symbiodinium* samples for the collective set of corals from a specific site and time point are referred to as a population.

2.2.2. Genotyping

Mixed host (*A. millepora*) and symbiont (*Symbiodinium*) DNA was extracted from ethanol-preserved samples using a modified version of the protocol outlined in Wilson *et al.* (2002). To reveal the *Symbiodinium* type(s) hosted by *A. millepora* at each reef site, the internal transcribed spacer 1 (ITS1) region of nuclear ribosomal DNA was amplified using polymerase chain reaction (PCR) amplification (van Oppen *et al.* 2001) and genotyped using single stranded conformation polymorphism (van Oppen *et al.* 2005b). In almost all (399 of 401) samples, *Symbiodinium* C2 was the only type detected, thus these samples were well-suited for microsatellite analysis using clade C-specific primers. Although two samples contained background levels of clade D, this was unlikely to affect

population genotyping because the microsatellites used in this study do not amplify clade D types (Bay *et al.* 2009).

To evaluate genetic variation of *Symbiodinium* C2 at the population level, samples were genotyped with 8 polymorphic microsatellite loci: *SymC_3-02*, *SymC_3-04* (Howells *et al.* 2009); *C1.01*, *C1.02*, *C1.05*, *C1.07*, *C1.15*, *C2.08* (Bay *et al.* 2009, Appendix I). PCR was used to amplify microsatellite loci and purified products were genotyped by capillary electrophoresis (*MegaBACE 1000*) at the Genetic Analysis Facility at James Cook University as described in (Bay *et al.* 2009). Fluorescent peaks at each locus were scored using *MegaBACE Fragment Profiler 1.2* (*Amersham Biosciences*).

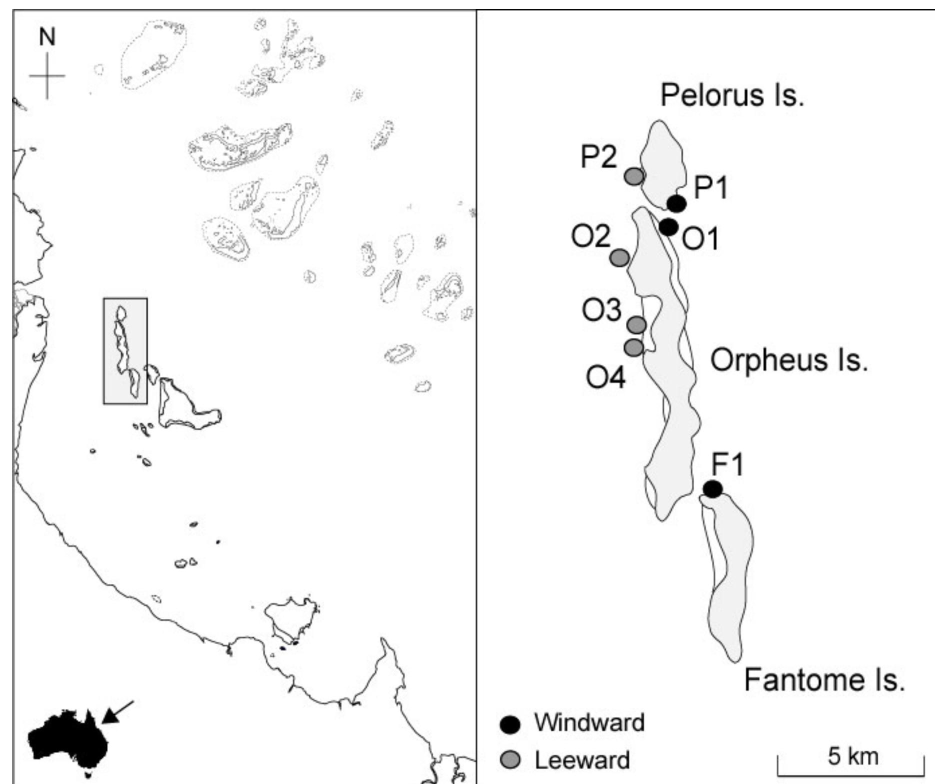


Fig. 2.1. Location of sampling sites (F1 to P2) of *Symbiodinium* C2 populations at the Palm Islands, Great Barrier Reef, Australia.

Sample sizes, collection years and bleaching levels of *A. millepora* hosts are provided in Table 1.

Table 2.1. **Sample sizes of normally pigmented and bleached colonies of *Acropora millepora* hosting *Symbiodinium* C2 populations analysed from each of the sites and collection years in this study.**

Year	Site	Normal	Bleached
1997	P1	9	-
	O1	17	-
2004	P1	50	-
	O1	50	-
	F1	50	-
2009	F1	19	-
	P2	24	34
	O2	24	29
	O3	14	38
	O4	19	24

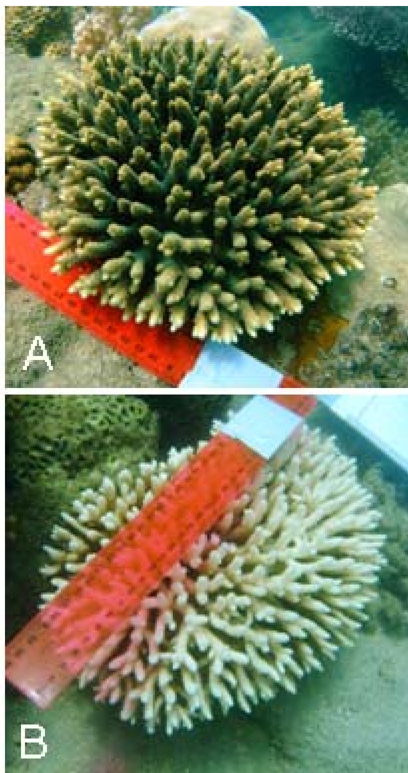


Fig. 2.2.

Visual appearance of *Acropora millepora* – *Symbiodinium* C2 symbioses from site O3 in the Palms Islands, Great Barrier Reef in 2009:

(A) normal pigmentation, and
(B) and bleached pigmentation.

2.2.3. Data analysis

Symbiodinium C2 microsatellite data were treated as a haploid binary data set based on the presence or absence of alleles. Observed peaks were scored as alleles if they were within the expected size range, in phase with the locus repeat motif, and at a minimum intensity of 10% of the most intense peak within a sample. Because DNA extracts were derived from thousands of *Symbiodinium* cells, each sample could potentially contain multiple alleles at each locus, representing genetically distinct haploid individuals. Although the single-copy nature of *Symbiodinium* microsatellite loci has not been unequivocally established, we treated microsatellite loci as single-copy because single alleles were recovered from a small number of coral samples at all loci, as well as in monoclonal cultures at *C1.01*, *C1.02*, *C1.07*.

Genetic diversity among sites, years and normally pigmented versus bleached corals was measured both at the colony and population level and was compared using analysis of variance (ANOVA), with Fisher's LSD post hoc test used to identify pairwise differences (performed in *Statistica 10*, *StatSoft*). Colony-level diversity was calculated as the number of alleles hosted per locus and population-level diversity was calculated using Shannon's index, H ($H = \sum p_i \log(p_i)$, where p_i is the proportion of the ith allele within the population) and expressed as a proportion of the total diversity per locus. To eliminate sample size (n) bias in calculations of H, equal sample sizes from each population were generated by random reductions in samples size to $n = 14$ (site P1 in 1997 was excluded from statistical comparison as $n < 14$).

Partitioning of *Symbiodinium* C2 genetic variation among sites and years was investigated with hierarchical analysis of molecular variance (AMOVA), with sites nested within years (run for 9,999 permutations in *GenAlEx 6*, Peakall and Smouse 2006). Only samples from non-bleached colonies were included for 2009 comparisons. The influence of bleaching on *Symbiodinium* C2 genetic structure was evaluated in a separate hierarchical AMOVA, in which samples from normally pigmented and bleached *A. millepora* colonies were nested within sites. The relationship of geographic distance (km) to the spatial genetic structure of *Symbiodinium* C2 (Φ_{ST}) was

investigated using Mantel correlations (run for 9,999 permutations in *GenAlEx 6*). Temporal genetic distances were not included in comparisons.

Patterns of *Symbiodinium* C2 genetic structure within and among sites, years and normally pigmented versus bleached corals were further investigated with hierarchical and model-based cluster analysis. Relationships among sites were viewed with hierarchical clustering, which grouped the Euclidean distances of allele frequencies using unweighted pair group means and the reliability of clusters was assessed with bootstrap probabilities and approximately unbiased *P* values calculated by multiscale bootstrap sampling (10^5 bootstrap replications on 10 scaled sample sizes, performed in *R*, using the package *pvclust*, Shimodaira 2004; Suzuki and Shimodaira 2006).

Model-based assignment of samples to genetic clusters using discriminant analysis of principal components (DAPC performed in *R*, using the package *adegenet*, Jombart 2008; Jombart *et al.* 2010) revealed genetic structure within and among populations, as well as the genetic relationships among assigned clusters. In the analysis, *Symbiodinium* C2 allelic data were converted into ~100 principal components and then clustered using a *k*-means algorithm. The optimal number of clusters (*k*) that resolved the most variation was assessed using Bayesian information criterion (BIC) and was determined to be $k = 6$ (where values of BIC plateaued). Samples were assigned to clusters and the relationships among these were examined by DAPC which optimises variation among clusters by minimising variation within clusters. The first 50 principal components and 5 discriminant functions were retained for DAPC, corresponding to 93% of the variation in the data set. Cluster assignment patterns were compared among and within sites, years and bleaching levels.

2.3. Results

A total of 108 alleles at 8 microsatellite loci were sampled from *Symbiodinium* C2 populations at 7 sites in the Palm Islands (Table AIII.1, Appendix III) and the number of alleles hosted by individual colonies of *Acropora millepora* ranged from 1 to 7 per locus. Analysis of the genetic structure and diversity of the *Symbiodinium* C2

populations demonstrated significant variation among sites and sampling years, as well as between bleached and non-bleached corals.

2.3.1. *Symbiodinium* C2 structure and diversity among sites

Variation among sites was the major factor structuring *Symbiodinium* C2 populations, and accounted for 19% and 27% of the genetic variation in AMOVA comparisons among sampling years (Table 2.2a) and levels of bleaching, respectively (Table 2.3a). *Symbiodinium* populations were significantly differentiated from one another at spatial scales ranging from 13 km to as little as 0.4 km between sites O3 and O4, both of which were located within a bay at Orpheus Island (Table 2.2b). The only populations that were not differentiated from one another were those at the neighbouring sites P1 and O1 (1.3 km apart) in 1997, and P2 and O2 (2.5 km apart) in 2009. *Symbiodinium* population genetic differentiation was moderately, but not significantly, related to distance within leeward (R^2 = 0.43) and windward (R^2 = 0.49) groups of sites (Fig. 2.3). Over small distances, populations at leeward sites showed higher genetic differentiation than windward sites and consequently there was no relationship to geographic distance when leeward and windward sites were considered together (R^2 = 0.06).

Hierarchical and model-based clustering corroborated patterns of genetic structure related to distance and exposure. The sites geographically closest to one another (P1 and O1, P2 and O2, O3 and O4) clustered together in the hierarchical analysis (Fig. 2.4), and sites O3 and O4 were the most distantly related to all other sites. Assignment of individual samples to the 6 model clusters obtained using DAPC was consistent with the hierarchical analysis (Fig. 2.5). Population samples from sites O3 and O4 were assigned to *k5,* which was the most distantly related of the genetic clusters. At the remaining leeward sites 4-6 km away, populations at P2 and O2 were assigned to *k3, 4* or *6*. In contrast, at windward sites (P1, O1, F1), the majority of samples were assigned to either of the closely related clusters *k1*, *2* or *3*.

Sites were differentiated from one another on the basis of alleles unique to individual sites or groups of sites, as well as differences in the frequencies of alleles that were

common to all sites (see Table AIII.1, Appendix III). In particular, there were a number of alleles that were rare or absent at O3 and O4, in addition to alleles that were unique to these two sites, which in combination, strongly differentiated these sites from the remaining sites (Table 2.2b). Within-colony genetic diversity was ~3 alleles per locus at all sites, except for O3 and O4 where diversity was significantly lower at ~2 alleles per locus (ANOVA, Table 2.4; Fig. 2.6a). Population-wide genetic diversity was similar among sites, except for at O4 where diversity was 16 to 38% lower than at other sites (Fig. 2.6b).

2.3.2. *Symbiodinium* C2 structure and diversity among years

Genetic structure among years accounted for a lesser, but significant, proportion (7%) of *Symbiodinium* C2 genetic variation (AMOVA, Table 2.2a). *Symbiodinium* C2 populations underwent a significant genetic change at P1 and O1 between 1997 and 2004, and at F1 between 2004 and 2009 (Table 2.2b). These changes were also evident in the hierarchical and model clustering. Populations at windward sites were more closely related to each other within years than they were to the same site between years (Fig. 2.4). In the modelled clusters, 44% and 41% of the samples at P1 and O1 were assigned to *k3* in 1997, whereas only 1 sample at each site was assigned to *k3* in 2004 (Fig. 2.5b). Similarly, no samples were assigned to *k2* at site O1 in 1997 yet 90% of samples were assigned to *k2* in 2004. At site F1, assignment to *k3* was common in both 2004 and 2009, but there was a decline through time in assignment to both *k1* (14% to 0%) and *k2* (34% to 5%).

Genetic differentiation among years was based on a few alleles that were either unique to or absent from a particular sampling year (Table AIII.1, Appendix III). Marginal changes in genetic diversity within coral colonies occurred between years. At sites P1 and O1, diversity was 6-10% higher in 1997 than in 2004, and at site F1, diversity was 8% higher in 2009 than in 2004 (Fig. 2.6a). However this did not affect population-wide genetic diversity, which was unchanged between years (Fig. 2.6b).

2.3.3. Diversity and structure of *Symbiodinium* C2 during coral bleaching

Bleaching of host *A. millepora* colonies contributed to only 3% of *Symbiodinium* C2 genetic variation (AMOVA, Table 2.3a), but significant changes in genetic diversity were observed between non-bleached and bleached corals.

At sites P2 and O2, subpopulations of *Symbiodinium* C2 in non-bleached and bleached corals were significantly differentiated (Table 2.3b). Notably, *Symbiodinium* C2 assemblages in bleached corals at these two sites were more closely related than they were to *Symbiodinium* C2 in healthy corals at the same site. (Fig. 2.4). The extent of differentiation (which was weaker than that among sites greater than 1.5 km apart) was due to a small (~8%) loss of genetic diversity within bleached corals compared to healthy corals (Fig. 2.6a, Table 2.4b). This loss of diversity was illustrated in the DAPC analysis, and was a consequence of an increasing proportion of samples assigned to *k6*, a model cluster characterised by low genetic diversity, in combination with a decline in the proportion of samples assigned to *k3*, a cluster characterised by high genetic diversity (Fig. 2.5b). This reduction in diversity at the level of individual coral colonies did not correspond to any changes in genetic diversity at the site level (Fig. 2.6b).

At sites O3 and O4, no significant differentiation or changes in diversity were observed among *Symbiodinium* C2 in non-bleached and bleached corals, however these sites were characterised by lower genetic diversity than was found at all other sites.

Table 2.2a. Analysis of molecular variation partitioning of microsatellite genetic variation among sampling years (1997, 2004 and 2009) and sites for *Symbiodinium* C2 populations hosted by *Acropora millepora* at the Palm Islands, Great Barrier Reef.

Note that for 2009 samples, only non-bleached colonies were included in the analysis.

Source of variation	df	SS	MS	Est. Var.	% Var.	Φ	P
Φ_{RT} : Among years	2	145.4	72.7	0.476	7%	0.071	0.001
Φ_{PR} : Among sites within years	7	274.2	39.2	1.254	19%	0.203	0.001
Φ_{PT} : Within sites	266	1312.4	4.9	4.934	74%	0.260	0.001

Table 2.2b. Analysis of molecular variation pairwise estimates of genetic differentiation among sampling years and sites for *Symbiodinium* C2 populations hosted by *Acropora millepora* at the Palm Islands, Great Barrier Reef.

Φ values (above the diagonal) indicate the magnitude of genetic differentiation between populations, and P values (below the diagonal) in bold face indicate significance after sequential Bonferroni correction at $\alpha = 0.05$.

		1997		2004			2009				
		P1	O1	P1	O1	F1	F1	P2	O2	O3	O4
1997	P1		0.000	0.160	0.238	0.203	0.304	0.260	0.231	0.412	0.438
	O1	0.445		0.114	0.191	0.201	0.320	0.280	0.241	0.389	0.389
2004	P1	**0.001**	**0.001**		0.105	0.159	0.350	0.267	0.233	0.403	0.364
	O1	**0.001**	**0.001**	**0.001**		0.102	0.308	0.225	0.186	0.348	0.307
	F1	**0.001**	**0.001**	**0.001**	**0.001**		0.209	0.142	0.089	0.361	0.329
2009	F1	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**		0.151	0.209	0.465	0.472
	P2	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**		0.044	0.402	0.387
	O2	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	0.004		0.436	0.415
	O3	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**		0.134
	O4	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	

Table 2.3a. Analysis of molecular variation partitioning of microsatellite genetic variation among sites and normal pigmented versus bleached *Acropora millepora* hosts for *Symbiodinium* C2 populations in 2009 at the Palm Islands, Great Barrier Reef.

Source of variation	df	SS	MS	Est. Var.	% Var.	Φ	P
Φ_{RT} : Among sites	3	344.9	115.0	2.028	27%	0.274	0.001
Φ_{PR} : Among bleaching within sites	4	40.8	10.2	0.210	3%	0.039	0.002
Φ_{PT} : Within bleaching	198	1021.4	5.2	5.159	70%	0.303	0.001

Table 2.3b. Analysis of molecular variation pairwise estimates of genetic differentiation among sites and normally pigmented versus bleached *Acropora millepora* hosts for *Symbiodinium* C2 populations at the Palm Islands, Great Barrier Reef.

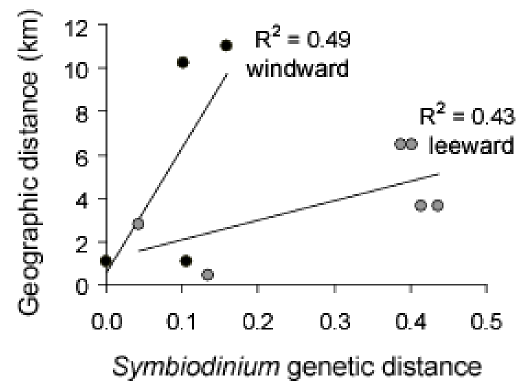
Φ values (above the diagonal) indicate the magnitude of genetic differentiation between populations, and P values (below the diagonal) in bold face indicate significance at $\alpha = 0.05$ following sequential Bonferroni correction. N = normal pigmentation, B = bleached.

		P2		O2		O3		O4	
		N	B	N	B	N	B	N	B
P2	N		0.051	0.044	0.022	0.402	0.407	0.387	0.321
	B	**0.002**		0.160	0.066	0.343	0.367	0.335	0.269
O2	N	0.003	**0.001**		0.058	0.436	0.426	0.415	0.357
	B	0.030	**0.001**	**0.001**		0.435	0.431	0.413	0.340
O3	N	**0.001**	**0.001**	**0.001**	**0.001**		0.000	0.134	0.128
	B	**0.001**	**0.001**	**0.001**	**0.001**	0.401		0.070	0.076
O4	N	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**		0.029
	B	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	0.053	

Fig. 2.3.

Relationship between *Symbiodinium* C2 genetic distance (Φ_{ST} values) and geographic distance (km) at 3 windward (black circles) and 4 leeward (grey circles) in the Palm Islands, Great Barrier Reef.

$R^2 = 0.06$ for all sites (windward and leeward combined). Relationships were non-significant ($P > 0.05$).



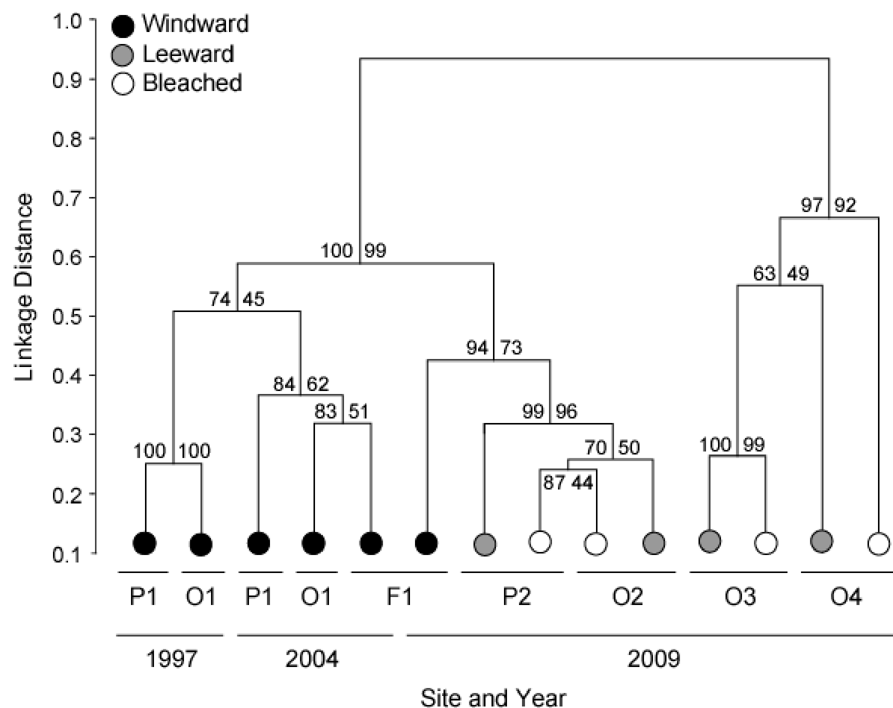


Fig. 2.4. **Genetic structure of *Symbiodinium* C2 populations hosted by *Acropora millepora* among sites, years and pigmentation categories at 7 sites in the Palm Islands, Great Barrier Reef.**

Topology was inferred using hierarchical cluster analysis of Euclidean distances of allele frequencies grouped using unweighted pair group means. Bootstrap values are on right side of cluster nodes and approximately unbiased probability values are on left side of nodes.

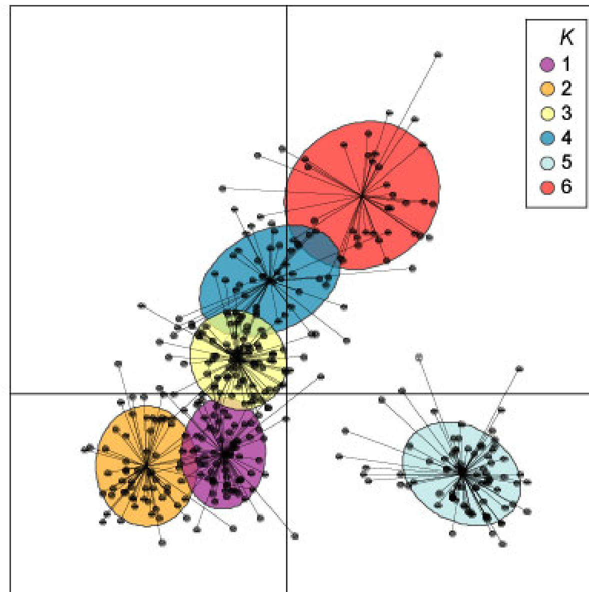


Fig. 2.5a. **Scatterplot of the discriminant analysis of principal components (DAPC) of microsatellite data for *Symbiodinium* C2 hosted by *Acropora millepora* at 7 sites in the Palm Islands, Great Barrier Reef.**

Individual samples (represented by dots) were assigned to six genetic clusters (*k1* to *k6*) detected within the data set. The analysis accounted for 93% of the genetic variation in the data set of which 43% and 35% is explained in first (x-axis) and second (y-axis) principal components.

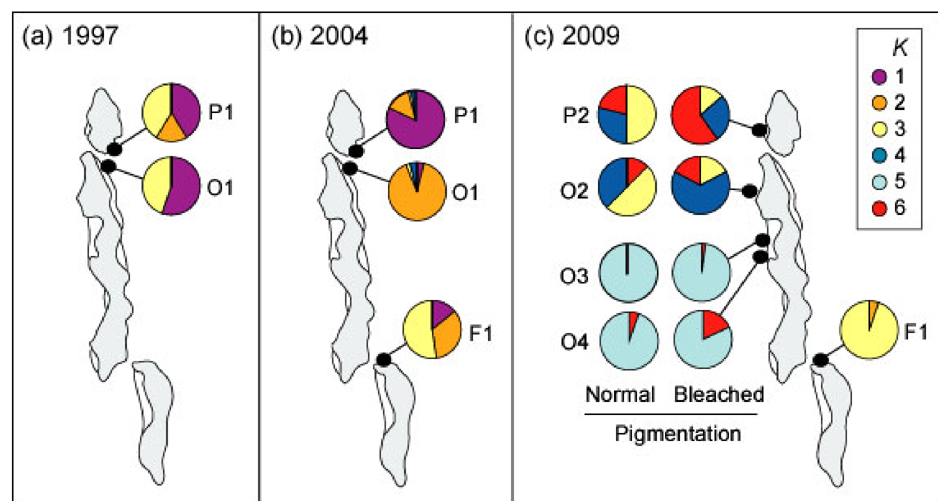


Fig. 2.5b. **Assignment of samples of *Symbiodinium* C2 to DAPC genetic clusters (*k1* to *k6*) among sites, years and normally pigmented versus bleached *Acropora millepora* hosts at the Palm Islands, Great Barrier Reef.**

a. 1997 samples, **b.** 2004 samples, **c.** 2009 samples in normally pigmented and bleached hosts.

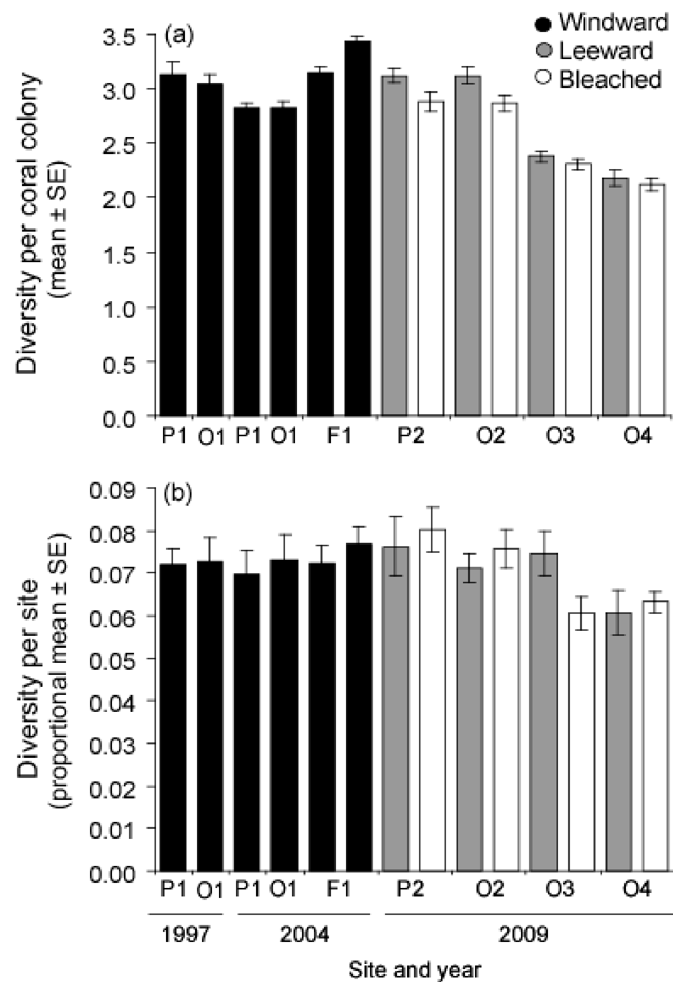


Fig. 2.6. **Genetic diversity of *Symbiodinium* C2 among sites, years and normally pigmented versus bleached *Acropora millepora* hosts at the Palm Islands, Great Barrier Reef.**

a. Number of alleles per microsatellite locus within individual colonies. **b.** Proportion of microsatellite locus diversity (Shannon's index, H) within each population. For calculations of H, even sample sizes were obtained by random reductions at n, except at site P1 where sample size was below n = 14.

Table 2.4a. Analysis of variation for comparisons of genetic diversity within individual colonies (allelic richness) and populations (Shannon's index, *H*) among sites, years and normally pigmented versus bleached *Acropora millepora* hosts for *Symbiodinium* C2 populations at the Palm Islands, Great Barrier Reef.

Source of variation	df	SS	MS	*F*	*P*
Individual colonies	13	51.22	3.94	32.85	0.001
Site populations	12	0.004	0.0003	1.61	0.102

Table 2.4b. Analysis of variation Fishers LSD post-hoc *P* values for comparisons of genetic diversity among sites, years and normally pigmented versus bleached *Acropora millepora* hosts for *Symbiodinium* C2 populations at the Palm Islands, Great Barrier Reef.

P values above the diagonal are for genetic diversity within individual colonies (allelic richness) and *P* values below the diagonal are for genetic diversity within populations (Shannon's index, *H*). *P* values in bold face indicate significance at $\alpha = 0.05$. N = normally pigmented, B = bleached. n/a = not included in analysis due to small sample size.

			1997		**2004**			**2009**								
			P1	**O1**	**P1**	**O1**	**F1**		**P2**		**O2**		**O3**		**O4**	
			N	**N**	**N**	**N**	**N**	**N**	**N**	**B**	**N**	**B**	**N**	**B**	**N**	**B**
1997	**P1**	**N**		0.508	**0.012**	**0.014**	0.910	**0.035**	0.907	**0.046**	0.907	**0.040**	**0.001**	**0.001**	**0.001**	**0.001**
	O1	**N**	n/a		**0.023**	**0.027**	0.264	**0.001**	0.473	0.109	0.473	0.093	**0.001**	**0.001**	**0.001**	**0.001**
2004	**P1**	**N**	n/a	0.686		0.919	**0.001**	**0.001**	**0.001**	0.461	**0.001**	0.584	**0.001**	**0.001**	**0.001**	**0.001**
	O1	**N**	n/a	0.957	0.647		**0.001**	**0.001**	**0.001**	0.518	**0.001**	0.645	**0.001**	**0.001**	**0.001**	**0.001**
	F1	**N**	n/a	0.972	0.713	0.929		**0.003**	0.727	**0.001**	0.727	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
2009	**F1**	**N**	n/a	0.535	0.306	0.571	0.512		**0.004**	**0.001**	**0.004**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
	P2	**N**	n/a	0.603	0.356	0.641	0.579	0.919		**0.008**	1.000	**0.008**	**0.001**	**0.001**	**0.001**	**0.001**
		B	n/a	0.275	0.136	0.299	0.260	0.636	0.566		**0.008**	0.886	**0.001**	**0.001**	**0.001**	**0.001**
	O2	**N**	n/a	0.851	0.829	0.808	0.879	0.419	0.479	0.201		**0.008**	**0.001**	**0.001**	**0.001**	**0.001**
		B	n/a	0.642	0.385	0.681	0.616	0.876	0.957	0.529	0.513		**0.001**	**0.001**	**0.001**	**0.001**
	O3	**N**	n/a	0.778	0.494	0.820	0.751	0.734	0.811	0.417	0.639	0.854		0.500	0.108	**0.027**
		B	n/a	0.087	0.189	0.078	0.094	**0.021**	**0.027**	**0.006**	0.127	**0.031**	**0.047**		0.205	**0.041**
	O4	**N**	n/a	0.091	0.196	0.082	0.098	**0.022**	**0.028**	**0.006**	0.133	**0.032**	**0.050**	0.983		0.564
		B	n/a	0.179	0.345	0.162	0.190	0.051	0.064	**0.016**	0.247	0.072	0.105	0.710	0.726	

2.4. Discussion

Over a period of 12 years, populations of the host coral *Acropora millepora* within the Palm Islands, Great Barrier Reef (GBR) were dominated by a single *Symbiodinium* type. Yet within these *Symbiodinium* type C2 associations, significant genetic structure was detected among sites and sampling years, as well as among non-bleached and bleached coral hosts, demonstrating the roles that spatial isolation and disturbance play in shaping the genetic composition of *Symbiodinium* populations within reefs.

2.4.1. Spatial genetic structure: Influence of oceanographic features

Spatial isolation was the major factor determining population genetic structure of *Symbiodinium* C2 at seven sites within the Palm Islands. This conclusion is consistent with previous reports of high spatial genetic structure over scales from 10s of metres to 10s kilometres in populations of *Symbiodinium* types that are acquired by corals from the external environment, including populations of a *Symbiodinium* C1 type hosted by a soft coral on the GBR (Howells *et al.* 2009) and populations belonging to B1 types hosted by scleractinian corals (Thornhill *et al.* 2009) and octocorals (Santos *et al.* 2003; Kirk *et al.* 2009; Andras *et al.* 2011) on Caribbean reefs. These results are consistent with limited dispersal and recruitment of *Symbiodinium* between reefs, although differences in disturbance regimes at windward and leeward sites are also likely to have contributed to the high spatial structure detected at these sites.

Regional water circulation in the central GBR lagoon is driven alongshore (south-easterly and north-easterly) by the East Australia current and wind-driven currents (Williams *et al.* 1984; Wolanski and Pickard 1985). The Palm Islands are situated parallel to these currents, forming an effective barrier to dispersal between windward and leeward sites. Although there is a channel between Orpheus and Pelorus Islands, its shallow bathymetry (<20m, Durkan 2006) restricts flow volume (C. Steinberg, pers. comm.). Such regional circulation patterns would explain the closer genetic relatedness of populations at neighbouring sites (P1 and O1, P2 and O2, O3 and O4) within the

windward and leeward sides of the Palm Islands, as well as the moderate (although not significant) relationship between genetic differentiation and distance between sites. Lower spatial genetic connectivity among leeward sites compared to windward sites may be related to currents on the leeward side of the Palms Islands being predominantly driven by inconsistent winds, in contrast to stronger influence of the East Australia current and higher wave energy on the windward sides of the Islands (Bannister 2007), which would provide steady hydrodynamic transport among sites (C. Steinberg, pers. comm.).

2.4.2. Spatial genetic structure: Influence of disturbance regimes

The Palm Islands have been shaped by hundreds of years of tropical cyclones and river flood plumes, as documented in recent monitoring (Thompson and Dolman ; Done *et al.* 2007; Sweatman *et al.* 2008; Schaffelke *et al.* 2009), modelling (King *et al.* 2002) and historical investigations (Isdale *et al.* 1998; Hayne and Chappell 2001; Nott and Hayne 2001; McCulloch *et al.* 2003; Roff 2010). Such events are likely to have further contributed to genetic differentiation found between windward and leeward *Symbiodinium* populations. Windward sites have been influenced by cyclonic waves, with 30 tropical cyclones or storms passing within 100 km of the Palm Islands during the past century (1910-2011, Austalian Bureau of Meteorology; Roff 2010). I hypothesise that cyclones affect *Symbiodinium* populations through physical damage and mortality of coral hosts, leading to death of *Symbiodinium* populations *in hospite*. Conversely, it is also possible that reworking of substrates during high wind events may act to redistribute viable free-living *Symbiodinium* cells. Although leeward sites are typically sheltered from cyclones, they are exposed to floodwaters more frequently, and are exposed to poorer water quality for a longer duration than windward sites (King *et al.* 2002). Floodwaters from mainland rivers regularly extend to the Palm Islands, exposing coral communities once every few years to salinities below 30 ppt and to elevated loads of sediments, nutrients and pollutants originating from land use practices in river catchments that have been farmed or grazed since the late 19th century (Devlin *et al.* 2001; McCulloch *et al.* 2003; Lewis *et al.* 2009). Bleaching associated with flood plumes was recorded at leeward sites in 2009 and while I did not find earlier records of flood plume-associated bleaching specific to the study sites, coral bleaching and

mortality have been recorded at other inshore regions of the GBR during previous flood plume events (VanWoesik *et al.* 1995). Such events potentially result in the mortality of *Symbiodinium* cells, given that photosynthesis is inhibited by flood plume parameters, including low salinity (Muthiga and Szmant 1987; Moberg *et al.* 1997; Ferrier-Pages *et al.* 1999; Kerswell and Jones 2003), high sedimentation (reviewed in Fabricius 2005), reduced light (reviewed in Fabricius 2005), and herbicide exposure (reviewed in Jones 2005). On top of these persistent disturbance regimes, windward and leeward sides of the islands were both impacted by thermal bleaching in 1998 (Baird and Marshall 2002), as discussed in the next section.

The dissimilarity of O3 and O4 *Symbiodinium* populations within Pioneer Bay at Orpheus Island from the remainder of sites and their lower genetic diversity may reflect the enclosed nature of this bay, which could reduce oceanographic connectivity with sites outside of the bay. Alternatively, historical extractive use of *A. millepora* at sites O3 and O4, which are situated in the same bay as Orpheus Island Research Station, may have altered their population genetic structure. Genetic differentiation of *Symbiodinium* populations at O3 and O4, which are separated by only 0.4 km, may be attributed to the designation of site O4 as a rehabilitation area for corals in recent years, while site O3 has remained subject to extractive use.

2.4.3. Temporal genetic structure: Disturbance frequency and genetic drift

Observed genetic differences in *Symbiodinium* C2 populations between years at windward sites are likely to reflect disturbances in the Palm Islands between sampling years. These results are in contrast to previous temporal studies of *Symbiodinium* populations, which found few or no temporal changes in clade B genotypes in Caribbean corals (Thornhill *et al.* 2009) and sea fans (Goulet and Coffroth 2003b) over months to years of monitoring. However, these studies did not encompass disturbance events that resulted in significant morality and recruitment in coral communities. Interestingly, a strong relationship between age of sea fans and the *Symbiodinium* B1 genotype harboured suggests that dominant *Symbiodinium* genotypes change over time in free-living environments (Andras *et al.* 2011), potentially due to genetic drift and or changing environmental selection pressures within spatially isolated populations.

Consequently, corals that recruit to reefs in different temporal periods might be expected to establish symbioses with genetically distinct complements of *Symbiodinium*.

In this study, differentiation between the *Symbiodinium* C2 populations at Pelorus and Orpheus Islands (sites P1 and O1) between 1997 and 2004 was consistent with genetic restructuring of the host *A. millepora* population between these years, as described in a separate study (Souter *et al.* 2010). The Palm Islands were severely impacted by thermal bleaching in 1998, when all surveyed colonies of *A. millepora* in the vicinity of sites P1 and O1 bleached, with a mortality rate of 32% (Baird and Marshall 2002). Additional disturbance events following the 1998 bleaching, including Cyclone Tessi in 2000 and minor thermal bleaching causing reductions in coral cover at nearby reefs in 2002 (Schaffelke *et al.* 2009), may have further impacted populations or hindered their recovery.

Since sampling in 2004, corals on the windward sides of the Palm Islands were further impacted by tropical cyclone Larry in 2006, which substantially reduced the cover of *A. millepora* (A. Paley, pers. comm.). At Fantome Island (site F1) in 2009, this history of recent disturbance was evident in the low abundance and small population size structure of *A. millepora*. Size-based estimates of the age of sampled colonies of *A. millepora* (D. Abrego, pers. comm.) suggest that only 26% of colonies were present in the population during the previous sampling period in 2004 (longest diameter ≥20cm). Of the remaining colonies, 37% were likely to have recruited to the site before cyclone Larry in 2006 (5 yrs, longest diameter 14-19cm), and the remaining 37% most likely recruited afterwards (2.5yrs, longest diameter ≤11cm).

Following this study, *Acropora* populations at windward sites in the Palm Islands were further impacted by tropical cyclone Yasi in 2011 (A. Paley and Y. Sato, pers. comm.), highlighting the high frequency of disturbances shaping coral-*Symbiodinium* partnerships in the Palm Islands.

2.4.4. Flood plume-induced coral bleaching: Impacts on genetic diversity

Exposure to flood plumes in 2009 caused sub-lethal bleaching in ~60% of *A. millepora* colonies at leeward sites in the Palm Islands (whole colony mortality was 6% at the time of sampling), whereas corals at windward sites were unaffected. The 8% lower genetic diversity within bleached compared with non-bleached hosts at two sites (P2 and O2) is consistent with loss of the least abundant genotypes (Nei *et al.* 1975) as a consequence of reductions in the within-host density of *Symbiodinium* following bleaching (Howells *et al.* in review, Chapter 3). However, because remaining genotypes differed among individual colony hosts, there was no apparent reduction in genetic diversity at the reef-wide scale. Similarly, there has been no decline in genetic diversity among years, despite frequent disturbance events in the Palm Islands. This is in contrast to an observed decline of 10% of the reef-wide genetic diversity of *Symbiodinium* C2 hosted by *A. millepora* following a bleaching episode in the southern GBR (Howells *et al.*, in review). I hypothesise that variation in alleles hosted among individual colonies in the Palm Islands and large population sizes may have been sufficient to buffer any losses of *Symbiodinium* C2 genetic diversity at the reef-wide scale. However, it is not known how bleaching and other disturbances influence the genetic diversity of free-living *Symbiodinium* populations.

2.4.5. Conclusion

Disturbance events interact with patterns of spatial isolation to determine genetic structure within and among reef populations of *Symbiodinium* C2. Limited spatial connectivity of populations and lower genetic diversity in bleached corals suggests that populations of *Symbiodinium* within reef sites may be vulnerable to losses of genetic diversity. While I did not observe losses of genetic diversity at sites during bleaching or among years, the increasing frequency of bleaching events, cyclones and flooding predicted under climate change (Nicholls *et al.* 2007) may begin to erode standing genetic diversity in populations, further threatening the resilience of these nearshore host-*Symbiodinium* partnerships.

Chapter 3. Coral bleaching changes the genetic composition of *Symbiodinium* populations

This Chapter is inserted without abstract as currently *in review* at the journal *Marine Ecology Progress Series*:

Howells E, Jones A, Bay L, van Oppen M, Willis B (2011)
A change in *Symbiodinium* C2 microsatellite population composition harboured by a reef building coral following bleaching

EH genotyed samples, analysed the data and wrote the manuscript. AJ collected samples and provided data on *Symbiodinium* type, coral bleaching and mortality. LB, MvO and BW contributed to manuscript writing.

3.1. Introduction

Coral bleaching, the breakdown of the obligate symbiotic relationship between coral animals and dinoflagellates in the genus *Symbiodinium*, is a stress response induced by anomalous environmental conditions, including changes in light, temperature, and water quality (reviewed in Brown 1997). Coral bleaching episodes have increased in frequency and severity over the past few decades as a consequence of increasing sea temperatures and declining water quality (Hoegh-Guldberg 1999; Wooldridge and Done 2009), which has resulted in widespread coral mortality and subsequent declines in global coral cover (Wilkinson 2004; Wilkinson 2008). However, bleaching can also be a sub-lethal response, and in such cases, significant proportions of bleached corals recover and survive (Fitt *et al.* 1993; Baird and Marshall 2002; Jones 2008). Sub-lethal bleaching can drive changes in the composition and diversity of *Symbiodinium* types present in corals, which may determine their capacity to withstand future disturbances (Buddemeier and Fautin 1993; Stat and Gates 2011). Numerous studies have demonstrated that the *Symbiodinium* community composition can change within corals during and following bleaching events (Baker 2001; Toller *et al.* 2001; Berkelmans and van Oppen 2006; Jones *et al.* 2008), but it is not known whether genetic changes also occur within populations of the dominant *Symbiodinium* type typically harboured.

Symbiodinium occupy their coral hosts at densities in the order of 10^6 cells per cm^2 under ambient conditions (Drew 1972; Fagoonee *et al.* 1999), but during bleaching episodes, numbers decline rapidly over days to weeks as symbiont cells are lost from the host tissues (Weis 2008). Such acute reductions in numbers constitute “population bottlenecks” that can cause loss of genetic diversity (Nei *et al.* 1975) and leave recovering populations less equipped to respond to selective pressures (Frankham 2005). *Symbiodinium* populations within corals have been observed to recover from declines as great as 90% following bleaching (Fitt *et al.* 1993; Fitt *et al.* 2000; Jones 2008), yet it is not known whether these bottlenecks impact on the genetic diversity at the population level (i.e. within *Symbiodinium* types).

During bleaching and recovery, the identity of *Symbiodinium* types within coral colonies can remain stable (Thornhill *et al.* 2006b; Stat *et al.* 2009) or undergo a shift to new dominant *Symbiodinium* types (Baker 2001; Toller *et al.* 2001; Berkelmans and van Oppen 2006; Jones *et al.* 2008). While shifting *Symbiodinium* types has been linked to preferential survival of the coral host (Berkelmans and van Oppen 2006; Jones *et al.* 2008), the *Symbiodinium* assemblage often reverts back to the original pre-bleaching *Symbiodinium* type over time (Thornhill *et al.* 2006b). However, it is not known whether bleaching also results in changes in the genetic structure of *Symbiodinium* at the population level, either for assemblages of genotypes within coral colonies or at the reef-wide scale. While little change was observed in the population-level composition of *Symbiodinium* type B1 in *Montastraea* spp. colonies across a bleaching episode in the Caribbean (Thornhill *et al.* 2009), data are lacking for different *Symbiodinium* lineages, host species and oceanographic regions.

This study describes the population genetic diversity and structure of an Indo-Pacific generalist *Symbiodinium* type across a bleaching episode on the Great Barrier Reef. I used microsatellite genotyping to follow variation within *Symbiodinium* type C2 (ITS 1 rDNA) hosted by tagged colonies of the scleractinian coral, *Acropora millepora*, before and after a natural bleaching episode. I describe changes in the genetic composition of *Symbiodinium* type C2 within individual coral colonies and at a reef-wide scale, including a loss of genetic diversity and the appearance of novel genotypes.

3.2. Methods

3.2.1. Sample collection

Single branches were collected from 33 tagged colonies of *Acropora millepora* before and after a severe thermal bleaching episode at Miall Island (23°09'S, 150°54'E) in the southern Great Barrier Reef (GBR). Samples were collected from individually tagged colonies on the reef flat in June 2005 (8 months before bleaching) and August 2006 (6 months after bleaching) and are a subset of samples described in Jones *et al.* (2008).

Colonies from Miall Island included in this study all underwent bleaching, and survived the bleaching episode with only partial mortality (Table AIII.2, Appendix III).

3.2.2. *Symbiodinium* type identity

Colonies of *A. millepora* analysed in this study recovered from the 2005 bleaching episode with predominantly *Symbiodinium* C2 or mixtures of *Symbiodinium* C2 and D. Before bleaching, *Symbiodinium* assemblages in these colonies were dominated (>95%) by *Symbiodinium* C2, as assessed using single stranded conformation polymorphism of ITS1 rDNA (Jones *et al.* 2008). After bleaching, the majority of colonies (79%) recovered with predominantly *Symbiodinium* C2, but 21% of colonies had a mixture of *Symbiodinium* D and C2 (Table AIII.2, Appendix III). Microsatellites used for population genotyping in this study can amplify multiple types within *Symbiodinium* clade C (Bay *et al.* 2009), therefore colonies that hosted C types other than C2 were excluded. However, they do not amplify *Symbiodinium* clade D types (Bay *et al.* 2009).

3.2.3. Microsatellite genotyping

To evaluate genetic variation of *Symbiodinium* C2 at the population-level, samples were genotyped with 8 polymorphic microsatellite loci as described in Chapter 2 using DNA extracted in Jones *et al.* (Jones *et al.* 2008).

3.2.4. Data analysis

Symbiodinium C2 microsatellite data were analysed as described in Chapter 2. Genetic diversity of *Symbiodinium* populations at Miall Island before and after bleaching was estimated by allelic richness at the within-colony level and by allelic richness and Shannon's index (*H*) at the site level (i.e. among all 33 coral colonies). The proportions of the total diversity at microsatellite loci before and after bleaching were compared using analysis of variance (ANOVA; performed in *Statistica 10*, *StatSoft*). Genetic differentiation of *Symbiodinium* C2 before and after the bleaching episode was evaluated with analysis of molecular variance (AMOVA), run for 9,999 permutations

(performed in *GenAlEx 6*, Peakall and Smouse 2006). Patterns of genetic structure within and between sampling times were further investigated using discriminant analysis of principal components (DAPC; performed in *R*, using the package *adegenet* (Jombart 2008, see Chapter 2 for description of the analysis). For the *Symbiodinium* C2 data in this Chapter, 40 principal components were used to find clusters in the data set and the first 20 of these, which comprised 85% of the genetic variation, were retained for DAPC. Values of BIC plateaued at k =3, indicating that 3 clusters best described the data variation. Assignments to these clusters in samples of *Symbiodinium* C2 from *A. millepora* colonies were compared before and after bleaching.

3.3. Results

The allelic composition of *Symbiodinium* C2 assemblages in each tagged colony of *Acropora millepora* changed over the period from 6 months before to 8 months after the bleaching episode at Miall Island. In total, 79 alleles across all loci were recorded before bleaching among the 33 coral colonies. Sixteen of these (20%) were low frequency alleles that were not observed after bleaching (see Table AIII.3, Appendix III). Nine new alleles, which were not observed prior to the bleaching event, were present at Miall Island after bleaching. This resulted in an 8-10% loss of genetic diversity within the *Symbiodinium* C2 population hosted by the *A. millepora* colonies sampled after the bleaching episode (Shannon's index 8%, ANOVA, $F_{(1, 65)} = 10.34$, $P = 0.006$; allelic richness 10%, $F_{(1, 65)} = 6.21$,$P = 0.025$; Fig. 3.1a). Individual *A. millepora* colonies hosted 1 to 7 alleles per microsatellite locus and many alleles were retained within individual colonies across the bleaching episode. However a small number of alleles were either lost and/or gained within *A. millepora* colonies, resulting in no change in within-colony allelic richness (Fig. 3.1b).

Differences in the genetic structure of *Symbiodinium* C2 populations before and after bleaching accounted for 14% of the total genetic variation among all samples (AMOVA, $\Phi_{ST\,(1, 65)} = 0.137$, $P = 0.001$), but the majority of genetic variation was explained by differences in *Symbiodinium* C2 assemblages among individual coral colonies. Assignment of individual samples to the three DAPC model clusters (*k1* to *k3*, Fig. 3.2) clearly illustrated that *Symbiodinium* C2 assemblages within individual colonies changed after the bleaching episode, with the majority of coral colonies (67%)

being assigned to different clusters before and after bleaching (Fig. 3.3). All 3 clusters were represented among *Symbiodinium* C2 samples prior to bleaching, with *k2* and *k3* being most similar genetically and more distantly related to *k1* (Fig. 3.2, note that the majority of genetic variation is represented on the x-axis). While *k1* was the most common cluster among samples before bleaching, no samples were assigned to this cluster after bleaching, when almost all samples (91%) were assigned to *k3*.

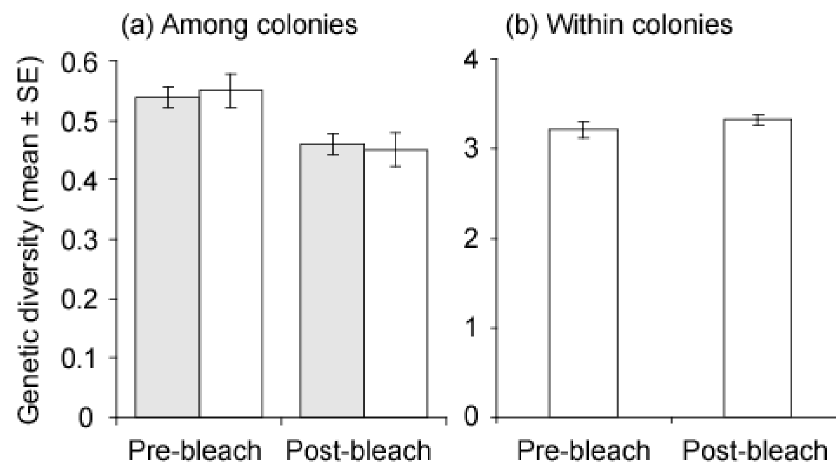


Fig. 3.1. Genetic diversity of *Symbiodinium* C2 from tagged colonies of *Acropora millepora* sampled before and after a bleaching episode at Miall Island on the Great Barrier Reef.

a. Shannon's diversity index (shaded columns) and allelic richness (unshaded columns) among coral colonies, which are expressed as a proportion of the total diversity across 8 microsatellite loci. **b.** Allelic richness within coral colonies across 8 microsatellite loci.

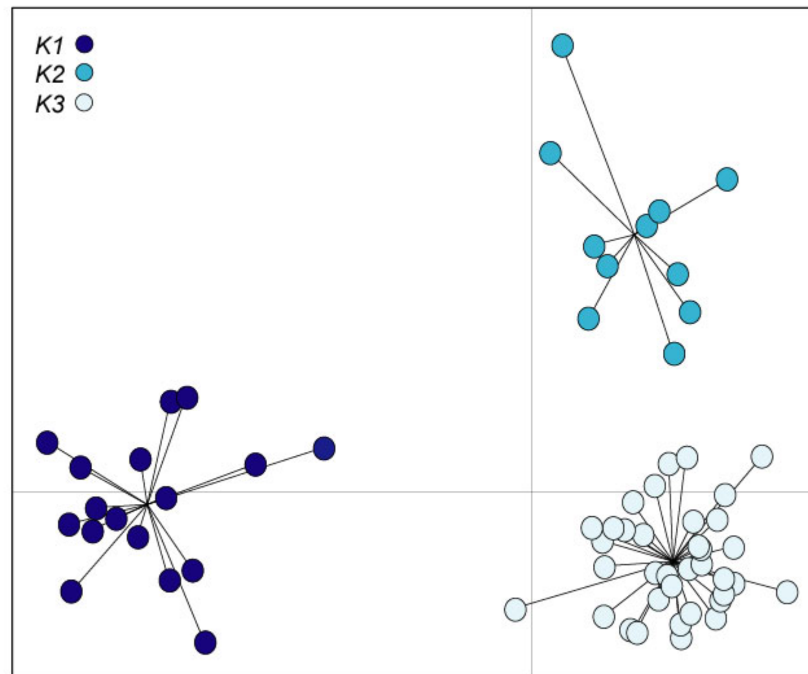


Fig. 3.2. **Scatterplot of the discriminant analysis of principal components of *Symbiodinium* C2 microsatellite data from tagged colonies of *Acropora millepora* sampled across a bleaching episode at Miall Island on the Great Barrier Reef.**

Symbiodinium populations in individual coral colonies (represented by circles) were assigned to three genetic clusters (*k1* to *k3*). The amount of genetic variation explained in the first (x-axis) and second (y-axis) principal component is 71% and 14%, respectively (85% total).

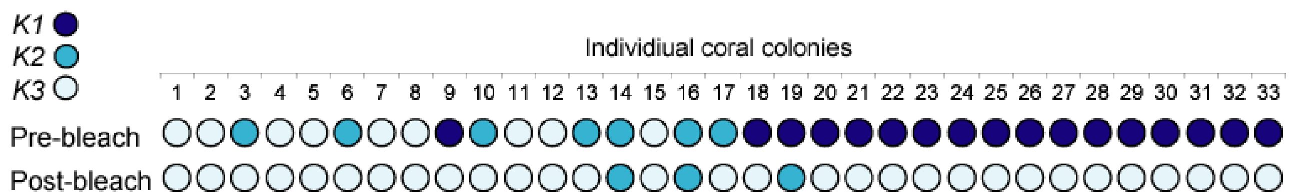


Fig. 3.3. **Assignment of *Symbiodinium* C2 populations within 33 colonies of *Acropora millepora* to genetic clusters (*k1* to *k3*) before and after thermal bleaching on Miall Island on the Great Barrier Reef.**

3.4. Discussion

This study demonstrates that bleaching events drive significant changes in the genetic diversity and structure of *Symbiodinium* populations in symbiosis with reef corals. *Symbiodinium* C2 populations hosted by the coral *Acropora millepora* underwent a 10% decline in genetic diversity 6 months after a bleaching event at Miall Island. The loss of 16 alleles (20%) across the 33 coral colonies is consistent with the expected loss of rarer alleles following a population bottleneck (Nei *et al.* 1975). Declines in genetic diversity in populations of other marine species have been observed following bottlenecks caused by anomalous sea temperatures (Faugeron *et al.* 2004; De Oliveira *et al.* 2009) and over harvesting (Kretzmann *et al.* 1997; Hutchinson *et al.* 2003; Harkonen *et al.* 2005). Bleaching rapidly reduces *Symbiodinium* population numbers, both within individual coral hosts and potentially on reef-wide scales, as *Symbiodinium* cells are either degraded when expelled (Franklin *et al.* 2004; but see Ralph *et al.* 2001), or become degraded in the hours to days after expulsion (Hill and Ralph 2007). The temporary displacement of normally dominant *Symbiodinium* types, such as C2 by type D in some of the *A. millepora* colonies analysed in this study (Jones *et al.* 2008), could further amplify bottleneck effects by inhibiting the capacity of the *Symbiodinium* type typically hosted by the coral to repopulate host tissues.

Interestingly, losses of genetic diversity in this study were also accompanied by the appearance of novel *Symbiodinium* C2 genotypes within and among coral colonies following bleaching. Seven out of nine novel alleles were detected in more than one coral colony, suggesting that the recovery of *Symbiodinium* densities may involve acquisition of *Symbiodinium* from the external environment, leading to the establishment of new genotypes in coral colonies. However, there is limited evidence supporting environmental acquisition of *Symbiodinium* following bleaching (Lewis and Coffroth 2004) and its role in enabling corals to gain new symbiont genotypes remains elusive. Symbiont shuffling, which is viewed as the most plausible explanation for changes in dominant *Symbiodinium* types that sometimes occur within corals during bleaching (Mieog *et al.* 2007; Jones *et al.* 2008), may also occur among genotypes belonging to a single *Symbiodinium* type. In this study, genotypes perceived as lost

could have persisted within coral colonies at levels below detection after the bleaching; similarly, those gained could have been present at low levels before the bleaching. Such changes would imply that specific microsatellite genotypes are linked to phenotypic differences; however, it is not known whether the microsatellite loci in this study are linked to DNA regions under selection. Sexual recombination inside the host is an alternative explanation for the appearance of new genotypes, but is considered unlikely as *Symbiodinium* are believed to be haploid inside the host (Santos and Coffroth 2003) and sexual reproduction has not been observed. New genetic variants in *Symbiodinium* are likely to be created primarily through somatic mutations (Correa and Baker 2011; van Oppen *et al.* 2011b), and the appearance of new alleles following bleaching potentially reflects somatic mutations at the microsatellite loci examined and may be important in the recovery of reef-wide genetic diversity.

My findings of genetic changes in *Symbiodinium* C2 populations across a bleaching event are in contrast with no or limited changes in the *Symbiodinium* clade B microsatellite genotypes (Kirk *et al.* 2005; Thornhill *et al.* 2009) and DNA fingerprints (Goulet and Coffroth 2003b) within Caribbean corals. In the absence of bleaching, no changes in *Symbiodinium* clade B fingerprints were detected within individual colonies of the octocoral *Plexaura kuna* over 10 years of monitoring (Goulet and Coffroth 2003b), while *Symbiodinium* type B1 (ITS2 rDNA) genotypes within 40% of colonies of *Montastraea* spp. changed between a low number of genotypes (2-3) sampled at a given reef at some point during almost three years of monitoring (Thornhill *et al.* 2009). Compared with changes in *Symbiodinium* C2 in 100% of *A. millepora* colonies in this study, genetic differences in *Symbiodinium* B1 were observed in only 20% of *Montastraea* spp. colonies during a natural bleaching episode (Thornhill *et al.* 2009), and in no colonies of the octocoral, *Gorgonia ventalina* during experimental heat stress (Kirk *et al.* 2005). *Symbiodinium* B1 types from the Caribbean are characterised by low genetic diversity within individual coral colonies (Santos *et al.* 2003; Kirk *et al.* 2009; Thornhill *et al.* 2009; Andras *et al.* 2011) and in some instances, within individual reefs (Santos *et al.* 2003; Thornhill *et al.* 2009) compared with clade C types of *Symbiodinium* from the Pacific (Magalon *et al.* 2006; Howells *et al.* 2009). Consequently, there may be less scope for population changes to occur within individual corals when *Symbiodinium* genetic diversity is low.

It has been hypothesised that coral bleaching selects for stress tolerant *Symbiodinium* populations within coral hosts and on reefs (Buddemeier and Fautin 1993), but this study demonstrates that bleaching episodes can also impact genetic diversity by acting as drivers of population bottlenecks. On a positive note, the appearance of novel alleles within colonies 6 months after bleaching, combined with the high density of *Symbiodinium* on coral reefs (within hosts and in free-living habitats), suggests that recovery of genetic diversity lost through bleaching is possible. However, if bleaching events continue to become more frequent and severe, as expected under climate change scenarios (Hoegh-Guldberg 1999), the genetic diversity of *Symbiodinium* populations could become eroded, leaving populations less equipped to adapt to warming conditions and other environmental changes (Frankham 2005). Understanding the long-term impacts of bleaching and other disturbances on the genetic diversity of *Symbiodinium* and coral populations requires greater temporal genetic monitoring of populations, including the analysis of historical samples.

Chapter 4. Coral thermal tolerance is shaped by local adaptation of *Symbiodinium* populations

This Chapter is inserted with re-formatting as published in the journal *Nature Climate Change*:

Coral thermal tolerance is shaped by local adaptation of photosymbionts. *Nature Climate Change* 2: 116-120

EH collected samples, conducted experiments, analysed the data and wrote the manuscript. VB initiated and maintained *Symbiodinium* cultures and contributed to manuscript writing. NL provided experimental assistance and contributed to manuscript writing. LB, MvO and BW contributed to manuscript writing.

4.1. Introduction

Coral thermal tolerance is strongly influenced by the identity of obligate photosymbionts, which encompass numerous types belonging to the dinoflagellate genus *Symbiodinium* (Coffroth and Santos 2005). Most coral species establish partnerships with local populations of *Symbiodinium* acquired from the reef on which they settle (Richmond 1997), yet many coral species exhibit fidelity for particular types of *Symbiodinium* across broad environmental gradients, including temperature (LaJeunesse *et al.* 2004; Goulet *et al.* 2008b; Cooper *et al.* 2011). Oceanographic distributions of many *Symbiodinium* types span temperature ranges as great as 15°C (LaJeunesse *et al.* 2004; Wicks *et al.* 2010; Cooper *et al.* 2011), but *Symbiodinium* types often persist as genetically isolated populations at the scale of individual reefs (Santos *et al.* 2003; Howells *et al.* 2009; Howells *et al.* in review, Chapter 2). Accordingly, it follows that populations of *Symbiodinium* from different reefs could exhibit functional heterogeneity as a consequence of adaptation to local environmental conditions. In this Chapter, I describe differences in thermal tolerance between populations of a generalist *Symbiodinium* type (C1) with a global distribution (LaJeunesse 2005). I show that variation in thermal tolerance is consistent with adaptation of *Symbiodinium* populations to local thermal regimes and contributes significantly to the fitness of the coral host under heat stress conditions.

4.2. Methods

4.2.1. *Symbiodinium* sources

Populations of *Symbiodinium* type C1 were sourced from fragments of the coral *Acropora tenuis* collected at ~3m depth from two inshore reefs in the Great Barrier Reef Marine Park that are similar in water quality (Schaffelke *et al.* 2011) aside from differences in temperature (Fig. 4.1). South Molle Island (20°16’33”S, 148°49’36”E), the cooler of the two reefs, has an average summer maximum of 28.2°C (10 year daily average), and temperatures rarely exceed 30°C. Temperature regimes at Magnetic Island (19°10’6”S, 146°50’60”E) are approximately 2°C warmer, with a long-term summer maximum of 30.1°C and maxima occasionally exceeding 32°C. *Symbiodinium* type was identified by the nucleotide sequence of the ITS1 and ITS2 nuclear ribosomal DNA regions, which were identical for *Symbiodinium* C1 from both locations (*GenBank* accession numbers JN129483-JN129484, http://www.ncbi.nlm.nih.gov/genbank/).

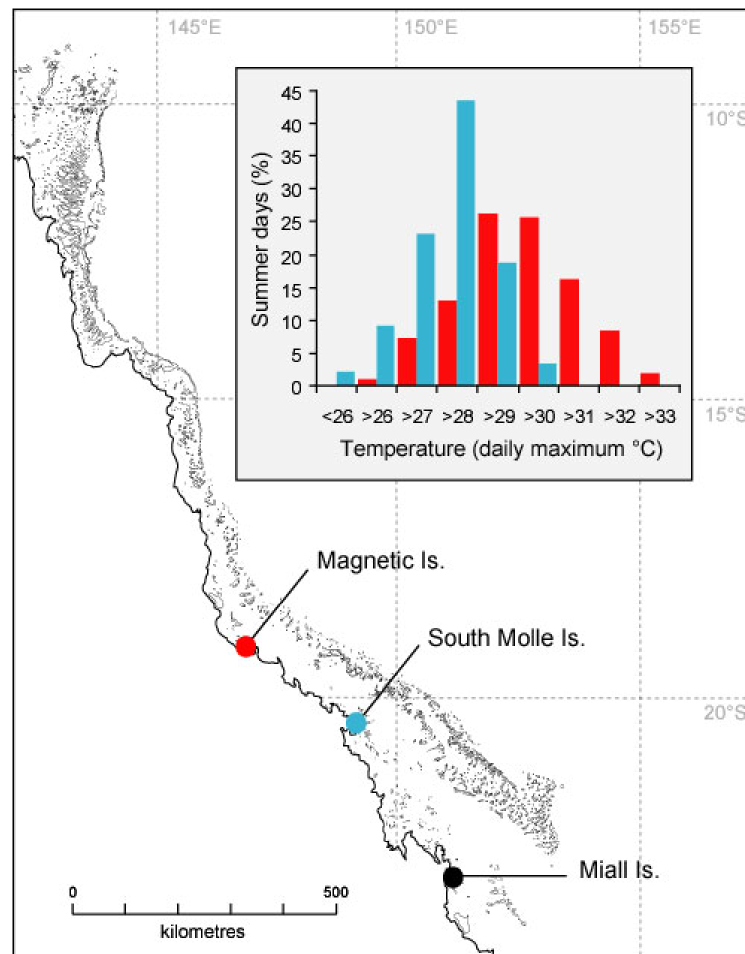


Fig. 4.1. **Location and summer temperature trends of *Symbiodinium* C1 populations (red, blue) and location of the *Acropora millepora* coral population (black) in the Great Barrier Reef, Australia.**

Temperature data sourced from data loggers deployed on reef flat locations managed by Ray Berkelmans at the Australian Institute of Marine Science. Summer trends were calculated from daily values from December to February, 1996 to 2009. Logger distances from sampling sites ranged from 0 to 3km.

4.2.2. Symbiosis experiment

A. tenuis fragments from both locations were acclimated to 27° C in shaded aquaria (maximum of ~200 µmol.quanta.$m^{-2}.s^{-1}$) for three weeks before *Symbiodinium* were extracted to inoculate coral juveniles. To control for genetic and environmental differences between coral hosts, juvenile corals were reared from a single larval cohort from eight parental colonies of *A. millepora* from a third reef location (Miall Island , 23°9'14"S, 150°54'13"E; Fig. 4.1). *A. millepora* is a natural host of *Symbiodinium* C1 (Cooper *et al.* 2011) and previous studies have shown that healthy symbioses develop between *A. millepora* juveniles and *Symbiodinium* C1 isolated from *A. tenuis* (Mieog *et al.* 2007; Cantin *et al.* 2009). Symbioses were established with both *Symbiodinium* populations, although juveniles inoculated with *Symbiodinium* from Magnetic Island showed a higher density of *Symbiodinium* cells than juveniles inoculated with *Symbiodinium* from South Molle Island (see Fig. 4.2b).

Two weeks after establishment commenced, juvenile corals in symbiosis with each of the two *Symbiodinium* populations (n = >200 juveniles for each *Symbiodinium* population) were divided between an ambient (27°C) and an elevated temperature treatment (32°C) with two replicate aquaria per temperature and *Symbiodinium* population combination. Temperatures were ramped from 27°C at 0.05°C.hr^{-1} to reach the 32°C target temperature. Light was provided at an intensity of 130 µmol.quanta.$m^{-2}.s^{-1}$ on a 12 hour light-dark cycle using 17,000K/420nm fluorescent lights.

The physiological responses of *Symbiodinium* and their coral hosts were monitored for 12 days. Maximum quantum yields of photosystem II (F_v/F_m) were measured every second day (immediately before lights were turned on) using chlorophyll *a* fluorescence measurements with a pulse amplitude modulated fluorometer (*Imaging-PAM*; *Walz*). *Symbiodinium* cell densities within juvenile corals were counted at 6 day intervals using replicate haemocytometer counts on decalcified and homogenised samples. Coral size and mortality was measured at 4-10 day intervals using photo-microscope mapping of individual juveniles. *Symbiodinium* C1 genotypes were verified among samples from source coral fragments, freshly isolated *Symbiodinium* cell preparations and juvenile

corals at the start and end of the experiment. Samples were genotyped at the ITS1 and ITS2 regions of rDNA using polymerase chain reaction and either single stranded conformation polymorphism (ITS1 rDNA, van Oppen *et al.* 2001; van Oppen *et al.* 2005b) or denaturing gradient gel electrophoresis (ITS2 rDNA, LaJeunesse and Trench 2000; LaJeunesse 2002), as well as direct sequencing.

4.2.3. Culture experiment

Symbiodinium C1 extracted from coral hosts for the above experiment was used to establish culture populations of *Symbiodinium* C1 from Magnetic and South Molle Islands. After one year of growth in a controlled environment at 25°C (>30 asexual generations), a second heat stress experiment was undertaken. Cultured *Symbiodinium* populations were distributed into three replicate 96-well plates at a density of 4 x 10^5 cells per well in a total volume of 250 µl. Temperatures in incubators were ramped from 24°C at 0.5°C per hour to reach target temperatures of 27°C, 30°C and 32°C. Light was provided at an intensity of 30 µmol.quanta.m^{-2}.s^{-1} as above. Values of F_v/F_m were measured every second day (as in 4.2.2) and proportions of dead and alive cells were measured at 4-10 day intervals using a fluorescent dead-cell stain (*SYTOX® Green*, *Invitrogen*) and replicate haemocytometer counts. *Symbiodinium* pigment concentrations were determined by high pressure liquid chromatography of methanol-extracted samples alongside pigment standards using a modified protocol of van Heukelem and Thomas (2001). *Symbiodinium* C1 genotypes were verified during the culturing period and at the start of the experiment as in 4.2.2.

4.2.4. Data analysis

The effect of *Symbiodinium* C1 population, temperature treatment and sampling day on the physiological responses measured in both experiments were evaluated using analysis of variance (ANOVA; performed in *Statistica 10*, *StatSoft*) with either Tukeys or Newman-Keuls post-hot tests. A repeated measures ANOVA was used to analyse F_v/F_m data in the culture experiment as the same wells in experimental plates were measured at each time interval. For all other response variables, replicates were treated as independent. F_v/F_m data values (for both experiments) and *Symbiodinium* cell

mortality data (culture experiment) were divided by pre-heating treatment means (day -1). Additionally, *Symbiodinium* cell mortality data were square-root transformed to meet the ANOVA assumptions of normality and homogeneity of variance.

4.3. Results

4.3.1. Symbiosis experiment

Symbiodinium C1 populations from South Molle Island and Magnetic Island exhibited divergent responses to temperature when in symbiosis with juvenile corals. Chronic photodamage was observed in the cooler South Molle Island population following exposure to elevated temperature, with F_v/F_m values being 15% lower at 32°C than at 27°C after 11 days of heating (Fig. 4.2a; ANOVA, population x temperature x day, P = 0.001, Appendix III, Table AIII.4). This was accompanied by severe bleaching of the coral hosts at 32°C, indicated by a loss of 80% of *Symbiodinium* cells from host tissues (Fig. 4.2b; ANOVA, population x temperature x day, P = 0.040, Appendix III, Table AIII.5). In contrast, the warmer Magnetic Island population not only exhibited no sign of stress at elevated temperature, but also displayed optimal photochemical performance at 32°C, with maximum quantum yields of PSII being 16% higher at 32°C than at 27°C after 11 days of heating.

Growth of the coral juveniles was strongly related to the physiological responses of the endosymbiotic *Symbiodinium* they hosted. Corals hosting *Symbiodinium* from the cooler South Molle Island were 13% smaller at 32°C than at 27°C because of significant partial mortality resulting from bleaching stress (Fig. 4.2c; ANOVA, population x temperature x day, P = 0.001, Appendix III, Table AIII.6). In contrast, corals hosting *Symbiodinium* from the warmer Magnetic Island grew 15% faster at 32°C than at 27°C, presumably because of increased photosynthate supply.

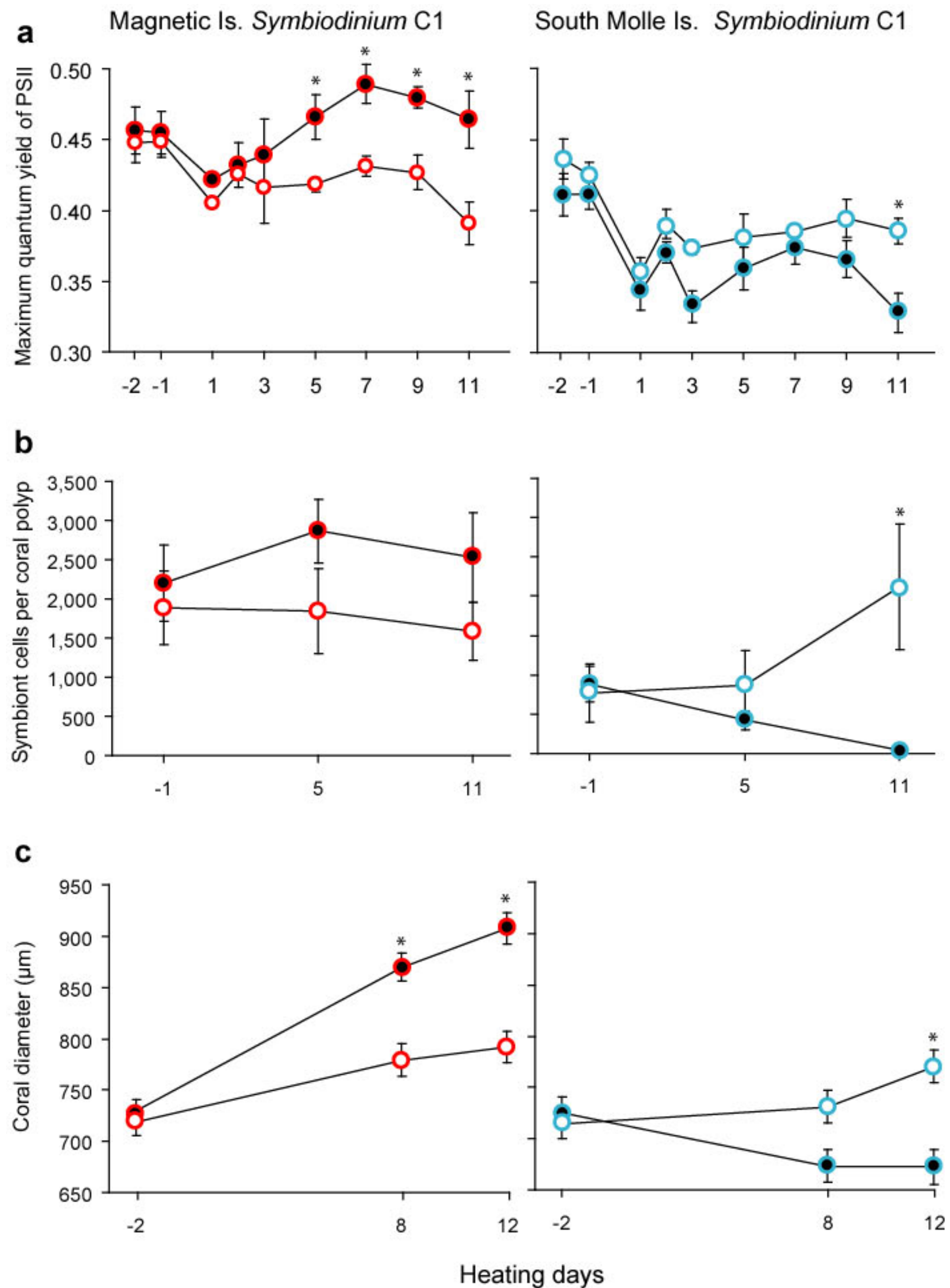


Fig. 4.2. **Responses of *Symbiodinium-Acropora millepora* symbioses to ambient and elevated temperature**

Circles represent temperature treatments of 27°C (white) and 32°C (black) at days relative to the start of heating (pre-heating temperature was at 27°C). **a.** Maximum quantum yield of photosystem II (mean ± SE) **b.** Cell density of *Symbiodinium* within corals (mean ± SE). **c.** Coral diameter (mean ± SE). Significant differences ($\alpha = 0.05$) among temperature treatments determined by ANOVA and post-hoc analyses (a: Newman-Keuls; b,c: Tukeys) are indicated with asterisks

4.3.2. Culture experiment

Adaptive variation in the thermal tolerance of *Symbiodinium* C1 populations in culture was retained following a year of growth in a controlled environment at 25°C. After more than 30 asexual generations in culture, the *Symbiodinium* population from the cooler reef exhibited a significantly greater stress response when exposed to 32°C than was observed in the population from the warmer reef, although the latter was also negatively affected by elevated temperature. Conservative estimates of *Symbiodinium* cell death showed a 33% decline in survivorship for the cooler reef population after 24 days exposure to 32°C, compared to only a 5% decline in the warmer reef population (Fig. 4.3a; ANOVA, temperature x day, $P = 0.019$; temperature x population, $P = 0.009$; population x day $P = 0.018$, Appendix III, Table AIII.7). *Symbiodinium* cell death was preceded by chronic photodamage, which was 57% greater in the cooler reef population exposed to 32°C, as indicated by the extent of reduction in the maximum quantum yield of PSII (Fig. 4.3b; ANOVA, temperature x population, $P = 0.001$, Appendix III, Table AIII.8). Continued elevations in the pressure over photosystem II at 32°C in the cooler reef population from day 9 of heating suggests that this population was unable to repair photodamage as fast as the warmer reef population, and a lack of change in pressure in the warmer reef population until day 23 suggests a greater capacity to engage photorepair mechanisms (Appendix III, Fig. AIII.1). This differential thermal tolerance may be an attribute of functional pigment profile, as a greater investment in pigments with photoprotective function was observed in the *Symbiodinium* population native to the warmer environment (Table 4.1). Despite a year of growth in identical conditions, the ratio of β-carotene to chlorophyll *a* was 61% higher in the warmer Magnetic Island culture than in the cooler South Molle Island cultures (ANOVA, Appendix III, Table AIII.8). In addition to light harvesting, β-carotene can prevent the formation of damaging reactive oxygen species by quenching ^3Chl and $^1O_2$ (Siefermann-Harms 1987).

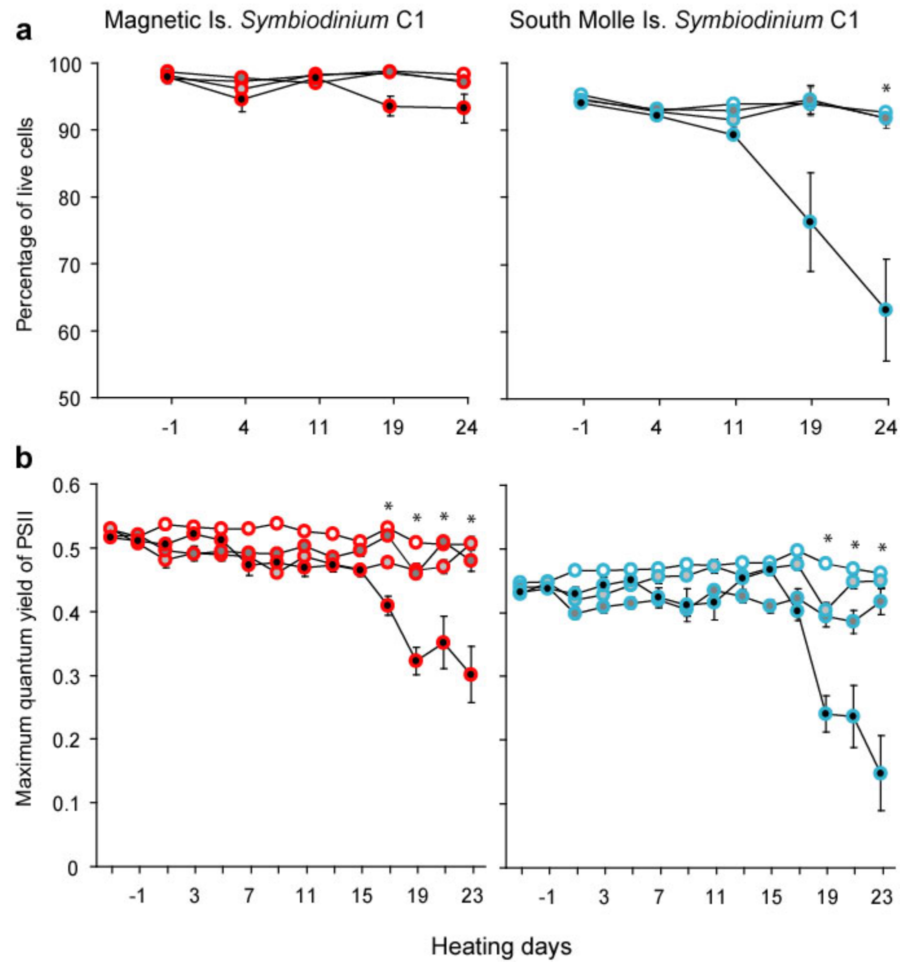


Fig. 4.3. ***Symbiodinium*** **C1 population responses to ambient and elevated temperatures following 1 year of growth in cell cultures**

Circles represent temperature treatments of 24°C (white), 27°C (light grey), 30°C (dark grey) and 32°C (black) at days relative to the start of heating (pre-heating temperature was 24°C). **a.** Percentage of living cells (mean ± SE). **b.** Maximum quantum yield of photosystem II (mean ± SE). Significant differences ($\alpha = 0.05$) between 32°C and all other temperature treatments on heating days determined with ANOVA and Tukeys post-hoc tests are indicated with asterisks.

Table 4.1. Properties of *Symbiodinium* C1 populations after 1 year in cell culture

Values are means ± SE per cell and ratios are given in moles. Chl *a* = chlorophyll *a*, Dd+Dt = diadinoxanthin + diatoxanthin, *β*-car = *β*-carotene. Significant differences ($\alpha = 0.05$) between populations assessed with ANOVA and Tukeys post-hoc tests are indicated with asterisks. Concentrations of accessory photosynthetic pigments are provided in Appendix III (Table AIII.10).

Population	**Diameter (μm)**	**Chl *a* (pg)**	**Dd+Dt : Chl *a***	***β*-car : Chl *a***
Magnetic Is.	10.37 ± 0.19 *	1.70 ± 0.26 *	0.26 ± 0.02	0.029 ± 0.001 *
South Molle Is.	9.41 ± 0.14 *	1.05 ± 0.18 *	0.29 ± 0.02	0.018 ± 0.001 *

4.4. Discussion

These results provide the first evidence that adaptive differences in heat sensitivity and heat tolerance exist, not only among divergent clades and types of *Symbiodinium* (Baker *et al.* 2004; Rowan 2004; Jones *et al.* 2008), but also between populations belonging to a single *Symbiodinium* type. Local adaptation of *Symbiodinium* populations has likely been a key mechanism enabling coral symbioses to optimise fitness under spatial variation in thermal regimes characteristic of coral reefs. Importantly, I show that the capacity to associate with divergent clades and types of *Symbiodinium*, a trait which appears to be absent in many corals (Goulet and Coffroth 2003b; Thornhill *et al.* 2006b; Goulet *et al.* 2008c; Thornhill *et al.* 2009; Stat *et al.* 2009), is not a prerequisite for corals to associate with functionally distinct symbionts. In contrast to previous comparisons of the thermal tolerance of *Symbiodinium* clades, gains in thermal tolerance in this study did not come at a cost of reduced coral growth (Jones and Berkelmans 2010, but see Abrego *et al.* 2008).

The capacity to rapidly adapt to changes in environmental conditions has been demonstrated in aquatic asexual organisms, including single-celled eukaryotic algae (*Chlorella vulgaris* Meyer *et al.* 2006; *Dictyosphaerium chlorelloides*, Costas *et al.* 2008). In experimental cultures, selection of resistant genetic variants arose from spontaneous somatic mutations in the order of 10^{-7} to 10^{-5} resistant variants per cell division. If rates of somatic mutation in *Symbiodinium* are similar to other species of

single-celled eukaryotic algae, significant adaptive potential exists in wild populations, given their rapid asexual generation times (days to weeks within coral hosts, Wilkerson *et al.* 1988) and large population sizes within corals (~10^6 cells.cm^{-2}, Drew 1972) and free-living habitats (Littman *et al.* 2008). Furthermore, episodes of bleaching induced by elevated sea temperature (Buddemeier and Fautin 1993; Correa and Baker 2011) and symbiotic lifestyle (Voolstra *et al.* 2009) may provide strong selective pressure favouring the persistence and proliferation of heat resistant genetic variants. Quantification of rates of adaptation in *Symbiodinium* and corals is necessary to improve predictions of the persistence versus extinction of these symbiotic partnerships under a rapidly changing climate.

Chapter 5. Historical thermal regimes define limits to the acclimatisation of coral-*Symbiodinium* partnerships

This Chapter is inserted without abstract as submitted to the journal *Ecology*:

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EH collected and analysed the data, with the exception of growth measurements collected by LB and RB. EH wrote the manuscript with contributions from all other authors. LB, RB and MvO designed the study.

5.1. Introduction

Coral reefs are experiencing changes in biodiversity and ecosystem function due to rising sea temperatures acting in synergy with additional environmental pressures (e.g. ocean acidification, pollution and overharvesting; (Hoegh-Guldberg 1999; Bellwood *et al.* 2004; De'ath *et al.* 2009) which is threatening their capacity to persist. Temperature anomalies have caused or contributed to significant losses of global coral cover over the past few decades (Wilkinson 2004; Carpenter *et al.* 2008; Wilkinson 2008), and reefs are expected to experience further warming of 1.5 -3°C within the 21st century (Meeehl *et al.* 2007). The extent to which corals can acclimatise or adapt to changes in their thermal environment remains uncertain, yet accurate projections of reef futures (Pandolfi *et al.* 2011) and effective conservation strategies (Mumby *et al.* 2011) are dependent on information on these resilience-linked processes. Addressing this uncertainty requires knowledge of the contribution of physiological acclimatisation versus genetic adaptation in defining the present thermal limits of coral animals and their endosymbiotic dinoflagellates (*Symbiodinium* spp.) and the potential for these limits to be extended in response to current and future environmental changes (Coles and Brown 2003).

Thermal tolerance in reef building corals is primarily governed by the obligate relationship between the coral animal and its photosymbiotic *Symbiodinium* partner (Rowan 2004; Abrego *et al.* 2008; Jones *et al.* 2008). Anomalous increases and decreases in temperature are highly stressful for *Symbiodinium* (Iglesias-Prieto *et al.* 1992; Saxby *et al.* 2003, although coral animal stress responses have also been described, Ainsworth *et al.* 2008; Rodriguez-Lanetty *et al.* 2009). Impaired rates of photosynthesis and damage to photosynthetic structures result in reactive oxygen species accumulating in *Symbiodinium* cells and potentially leaking into host tissues (Iglesias-Prieto *et al.* 1992; Fitt and Warner 1995; Jones *et al.* 1998; Warner *et al.* 1999). When the capacity of photoprotective and antioxidant mechanisms that prevent and remove these damaging molecules is exceeded, oxidative stress (reviewed in (Lesser 2006) promotes disassociation of coral and *Symbiodinium* partners, visible as coral bleaching (reviewed in Weis 2008). Temperatures that induce bleaching and

mortality are only a couple of degrees above local ambient summer temperatures and vary among reefs and regions with different thermal regimes (Hoegh-Guldberg 1999; Berkelmans 2002). However, the extent to which spatial variation in coral thermal tolerance is due to processes of acclimatisation or adaptation of *Symbiodinium* to local environmental conditions is unknown.

In *Symbiodinium,* temperature regulates processes of light acclimatisation, which is a balance between photosynthetic and photoprotective light absorption (see Falkowski and Dubinsky 1981; Iglesias-Prieto and Trench 1994; Shick *et al.* 1995). In symbiosis, *Symbiodinium* acclimatise to seasonal temperatures via variation in the production of photosynthetic and photoprotective pigments (Fitt *et al.* 2000), photoprotective mycosporine-like amino acids (Linan-Cabello *et al.* 2010), heat shock proteins (Downs *et al.* 2002), changes in cell densities (Fagoonee *et al.* 1999; Fitt *et al.* 2000; Ulstrup *et al.* 2008), and the relative abundance of functionally distinct *Symbiodinium* types in some host species (Chen *et al.* 2005; Berkelmans and van Oppen 2006; Jones *et al.* 2008; Suwa *et al.* 2008). In the coral animal, thermal acclimatisation occurs via increased production of heat shock proteins (Sharp *et al.* 1997; Brown *et al.* 2002c; Downs *et al.* 2002; Robbart *et al.* 2004; Fitt *et al.* 2009), antioxidant enzymes (Brown *et al.* 2002c; Downs *et al.* 2002; Yakovleva *et al.* 2004; Linan-Cabello *et al.* 2010), and potentially also fluorescent proteins with photoprotective and antioxidant properties (Salih *et al.* 2000; Bou-Abdallah *et al.* 2006 but see Dove 2004), as well as biochemical mechanisms not yet characterised. Behavioural responses, such as increased heterotrophic feeding (Grottoli *et al.* 2006; Borell and Bischof 2008) and tissue retraction (Brown *et al.* 2002a), can further facilitate acclimatisation to stressful environmental changes. Coral thermal responses have been monitored extensively over rapid timescales (i.e. hours to days, e.g., Iglesias-Prieto *et al.* 1992; Brown *et al.* 2002c; Bhagooli and Hidaka 2003; Saxby *et al.* 2003; Dove 2004; Yakovleva *et al.* 2004; Desalvo *et al.* 2008; Fitt *et al.* 2009), or over longer periods (i.e. months) but within seasonal temperature ranges (e.g. Fitt *et al.* 2000; Downs *et al.* 2002; Warner *et al.* 2002; Chen *et al.* 2005; Ulstrup *et al.* 2008; Linan-Cabello *et al.* 2010). The extent of acclimatisation in coral-*Symbiodinium* partnerships to gradual changes in temperature beyond current thermal regimes remains unclear (but see Berkelmans and van Oppen 2006).

Acclimatisation is ultimately limited by the genetic make-up of both partners in coral-*Symbiodinium* partnerships, as shown by taxon-specific responses of both *Symbiodinium* and the coral animal in acclimatisation studies (Iglesias-Prieto and Trench 1994; Robbart *et al.* 2004; Tchernov *et al.* 2004; Robison and Warner 2006). Once the capacity for acclimatisation has been exceeded, any further gains in thermal tolerance require adaptation through the selection of tolerant genetic variants. Differential thermal tolerance limits of corals that correlate with their native thermal environment have been observed during heat stress experiments following short periods of acclimatisation, indicating local adaptation (Coles and Jokiel 1977; Ulstrup *et al.* 2006). However, the amount of functional genetic variation in thermal tolerance available within coral species and *Symbiodinium* types for selection to act upon is largely unknown (but see Meyer *et al.* 2009a; Csaszar *et al.* 2010; van Oppen *et al.* 2011b), although spatial limits to gene flow are expected to have enhanced adaptive divergence of populations (van Oppen and Gates 2006; Sanford and Kelly 2011). Few studies have demonstrated physiological signatures attributable to local thermal adaptation in coral and *Symbiodinium* partners, due to the difficulty of controlling for acclimatisation effects in adult corals sourced from different environments (but see (Polato *et al.* 2010; Howells *et al.* 2011, Chapter 4). Consequently, understanding whether genetic effects constrain the acclimatisation potential to circumstances involving gradual long-term changes in temperature requires further investigation.

The objective of this study was to determine whether coral-*Symbiodinium* partnerships can acclimatise to gradual changes in temperature beyond their historical thermal ranges. The common bleaching sensitive coral, *Acropora millepora*, was reciprocally transplanted between the central and southern GBR for up to 14 months to investigate the capacity of heat tolerant coral-*Symbiodinium* D partnerships to acclimatise to gradually cooling temperatures (~0.35°C per week) and heat sensitive coral-*Symbiodinium* C2 partnerships to acclimatise to gradually warming temperatures (~0.6°C per week). Successful transplantation of corals during months, when temperatures were within native thermal regimes, followed by bleaching and mortality when temperatures exceeded upper and lower native temperatures demonstrates that coral thermal tolerance limits are determined by genetic adaptation of one, or both, symbiotic partners.

5.2. Methods

Colonies of the coral *Acropora millepora* were reciprocally transplanted between inshore fringing reefs at Magnetic and Miall Islands, which are located in the central and southern Great Barrier Reef regions, respectively (Fig. 5.1). *A. millepora* colonies native to Magnetic Island (n = 10) and Miall Island (n = 20) were split into two fragments, tagged and fixed to wire racks at approximately 4m depth adjacent to fringing reef at the native and transplant sites. *A. millepora* fragments were transplanted at the beginning of autumn and monitored at 1 to 4 month intervals over 14 months (April 2008 to May 2009). Previous genotyping and physiological studies have shown differences in *Symbiodinium* types hosted by *A. millepora* populations at the two locations; *A. millepora* forms heat-tolerant partnerships with *Symbiodinium* ITS1 rDNA type D at Magnetic Island, but heat-sensitive partnerships with *Symbiodinium* type C2 are typical at Miall Island (Berkelmans and van Oppen 2006; Jones *et al.* 2008).

5.2.1. Environmental monitoring

Long-term sea temperatures differ by 0.9°C (winter) to 2.7°C (summer) between the two sites. Daily averages range from 19.1 to 32.5°C at Magnetic Island (mean 26.2°C), and from 18.0 to 29.8°C at Miall Island (mean 24.0°C; http://data.aims.gov.au/aimsrtds, records from 1996 to 2010). Additional environmental parameters, including light, turbidity, chlorophyll and nutrient levels, are highly variable at both locations due to wind-driven sediment resuspension and seasonal river floods (Schaffelke *et al.* 2011). Data collected at comparable locations near the study sites (3 km from Magnetic Island site, 10 km from Miall island site) indicate higher turbidity and nitrogen levels at Magnetic Island (Schaffelke *et al.* 2011). Limited irradiance data collected over 6 weeks from the wire racks at each location indicates an average midday irradiance of 425 μmol.quanta.$m^{-2}.s^{-1}$ at the central GBR site and 608 μmol.quanta.$m^{-2}.s^{-1}$ at the southern GBR site. For the duration of the study, temperature and turbidity were recorded by continuous data loggers at 5m depth located near the wire racks (Temperature: reef flat loggers, http://data.aims.gov.au/aimsrtds, 1km from Magnetic Island site, 8km from Miall island site; Turbidity: *WETLabs Eco FLNTUSB* sensors,

3km from Magnetic Island site, 10km from Miall island site). Regional water quality data collected at intervals during the study are published in Schaffelke *et al.* (2009).

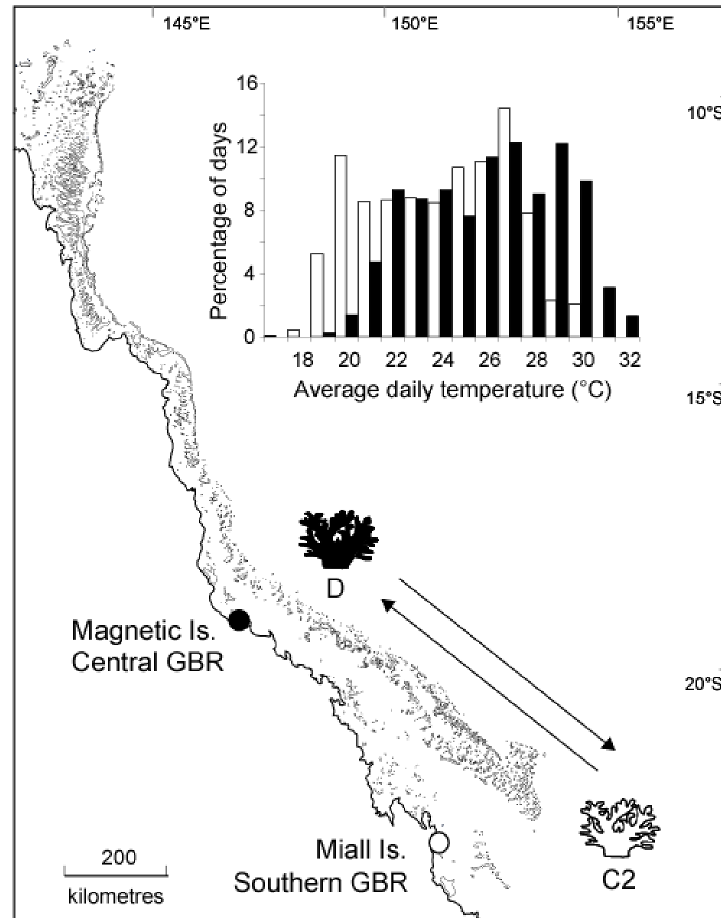

Fig. 5.1. Long-term sea temperatures at sites of reciprocal coral transplantation between the central (Magnetic Island) and southern (Miall Island) Great Barrier Reef (GBR) regions, between which corals were reciprocally transplanted.

Transplanted coral fragments represented were partnerships between *Acropora millepora* and *Symbiodinium* type D native to Magnetic Island, and *A. millepora* and *Symbiodinium* type C2 native to at Miall Island. Daily average temperature data from 1996 to 2010 sourced from reef flat data loggers (deployed 0 to 8km distance from transplant sites; http://data.aims.gov.au/aimsrtds).

5.2.2. Biological monitoring

Variation in the condition of coral fragments between native and transplant sites was assessed by visual surveys of bleaching and tissue mortality. To monitor any changes in *Symbiodinium* type within symbiont communities, a branch was collected from the centre of each fragment during monitoring surveys. Total DNA was extracted from snap-frozen samples (protocol modified from Wilson *et al.* 2002) and the ITS1 rDNA region of *Symbiodinium* was genotyped using polymerase chain reaction and single stranded conformation polymorphism (van Oppen *et al.* 2001; van Oppen *et al.* 2005b). Throughout coral spawning periods in October and November, transplanted *A. millepora* fragments were briefly removed from the field following each full moon to prevent the introduction of non-native coral gametes into the local environment. Reproductive timing was determined from a combination of spawning observations and histological slides of samples (decalcified tissue) collected in October, November and December. Eight of the fragments transplanted to the central GBR were not returned to the field after the spawning period, as they still contained gametes. Genotypic and environmental effects on coral colonies were analysed with Fishers exact tests (Bonferroni sequential adjusted P at $\alpha = 0.05$) on counts of bleaching, partial mortality, whole mortality and change in dominant *Symbiodinium* genotype during the course of the study.

During December at the central GBR site, photosynthetic stress in coral fragments was assessed by *in situ* measurements of the effective (F_v/F_m', midday) and maximum (F_v/F_m, dark-adapted) quantum yields of photosystem II using chlorophyll *a* fluorescence (*Diving PAM*, *Walz*). For each fragment, three replicate measurements were taken of colony branches ~1 cm below axial polyps. The growth rate of coral fragments over the first 7 months of transplantation was calculated at each site from buoyant weights (Jokiel *et al.* 1978) measured initially in April and then in October (central GBR) or November (southern GBR). Photochemical yields and growth rate data were compared among coral fragments with analysis of variance (ANOVA) and Tukeys or Fishers least significant difference post-hoc tests (*Statistica 10*, *StatSoft*). Growth data were $\log_{10}(x+1)$ transformed to meet the ANOVA assumption of homogeneity of variance.

5.3. Results

The responses of *Acropora millepora – Symbiodinium* partnerships during reciprocal transplantation between the central and southern GBR showed a strong interaction between the genotypes of the native partnership and the local environment for all parameters investigated, including bleaching, growth, reproduction and mortality. Coral bleaching, mortality and differences in *Symbiodinium* type occurred when transplanted coral fragments were exposed to temperatures outside of their long-term thermal history. Variation in growth rates and reproductive timing occurred between native and transplant corals, regardless of whether or not they showed visual signs of stress.

5.3.1. Coral condition

At the southern GBR site, transplanted colonies (native to the central GBR) remained visually healthy as sea temperatures declined by ~0.35°C per week from April to August, with the exception of mild bleaching in one fragment. During August through early September, transplanted fragments were exposed to daily average sea temperatures less than 19°C, which were below the lowest temperature recorded over 15 years at their native site (Fig. 5.2a). Bleaching was first observed in transplanted colonies in September at the end of this cold period (A. Jones, pers. comm.). By the time of the next survey in October, all coral fragments still alive had bleached and 50% (n = 10) had suffered recent partial or whole colony mortality (Figs. 5.3, 5.4). Bleaching was sustained through November and as a result, 40% of fragments died, 50% suffered partial mortality and only one fragment recovered from bleaching without undergoing any tissue mortality. Of the native fragments, only 10% bleached in September, but only mildly and with no associated mortality.

At the central GBR site, transplanted colonies remained visually healthy as seawater temperatures initially cooled from April to August and subsequently increased by ~0.6°C per week from August to November. However, from the end of November, daily average sea temperatures were above 30°C, and exceeded values recorded in 15 years of data collection at the native southern site (Fig. 2a). In the first week of December, all

transplanted fragments bleached, and by early January, 50% (*n* = 12) of the transplanted coral fragments were dead, and the remainder had little surviving tissue (Figs. 5.3, 5.4). In contrast, the native corals appeared unaffected, retaining their normal pigmentation. During February and March, the site was exposed to a flood plume for ~2 months (Devlin *et al.* 2010), which caused mortality of the majority of the native fragments (70%, *n* = 10), as well as extensive mortality in the wider *A. millepora* population (>90 % of colonies). The interaction between the genetic combination of the host-*Symbiodinium* partnership and site conditions was significant in accounting for whether corals bleached or underwent partial or whole mortality (Fig. 5.5, Fishers exact tests, $P < 0.05$, Appendix III, Table AIII.10).

Turbidity was three times higher at the central GBR site than at the southern GBR site (Fig. 5.2b). However, in the months immediately before and during the bleaching of transplants at both the southern and central GBR sites, turbidity levels did not exceed ranges typically experienced in their native habitat. Turbidity reached exceptionally high levels (>20 NTU, Nephelometric Turbidity Units) during the flood plume event in mid-January at the central GBR site.

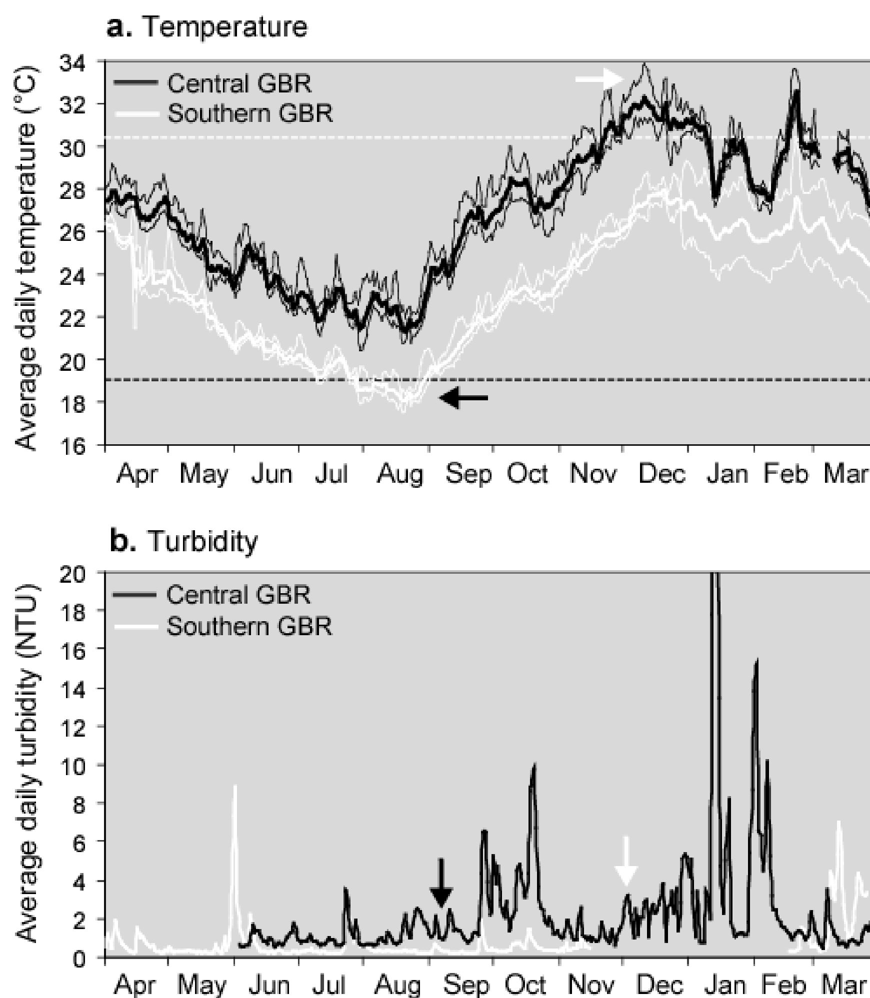

Fig. 5.2. **Daily temperature (a) and turbidity (b) data during reciprocal transplantation of fragments of the coral *Acropora millepora* between the central (Magnetic Island) and southern (Miall Island) Great Barrier Reef (GBR).**

Temperature data are daily means (bold line) and ranges (fine line) from April 2008 to March 2009, sourced from reef flat data loggers (1 to 8km at study from sites; http://data.aims.gov.au/aimsrtds). Dashed lines indicate the upper historical temperature range at the central GBR site (white line) and the lower historical range at the southern GBR site (black line), as per Fig. 1. Turbidity data are daily means from *WETLabs Eco FLNTUSB* sensors, located 3km from Magnetic Island site, 10km from Miall island site (see (Schaffelke *et al.* 2011)). Arrows indicate the time when bleaching was first observed in corals transplanted from the central to the southern GBR (black arrows) and in corals transplanted from the southern GBR to the central GBR (white arrows).

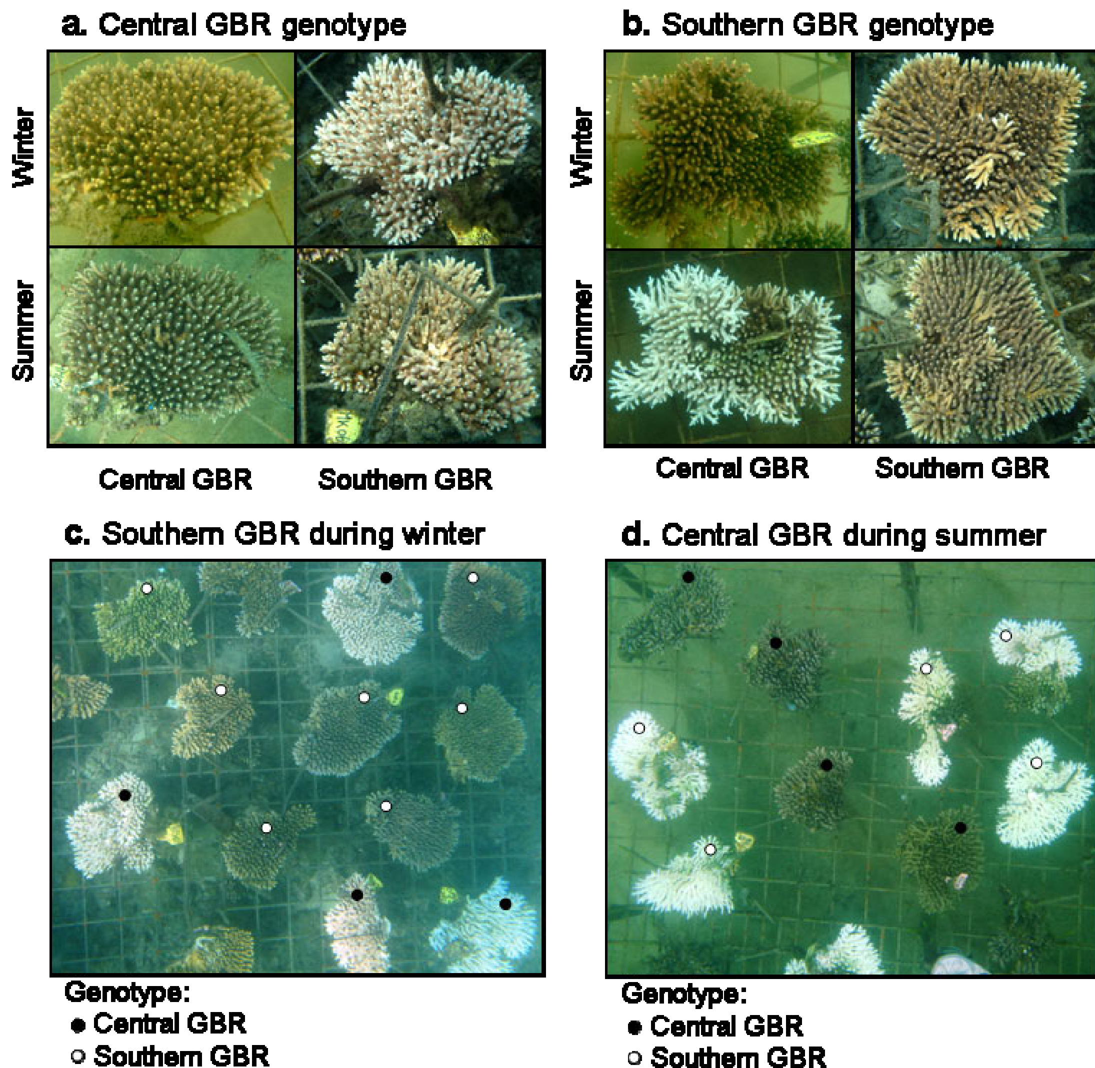

Fig. 5.3. Genotypic and environmental variation in the bleaching response of fragments of the coral *Acropora millepora* at native and transplant sites in the central (Magnetic Island) and southern (Miall Island) Great Barrier Reef regions.

a. Fragments of the same colony from the central GBR in October and December 2008, when located at the native (top to bottom, left panels) and transplant (top to bottom, right panels) sites. **b.** Fragments of the same colony from the southern GBR in October and December 2008, when located at the native (top to bottom, right panels) and transplant (top to bottom, left panels) sites. **c.** Native (white circles) and transplant (black circles) coral fragments at the southern GBR site in October 2008 (7 months after transplantation). **d.** Native (black circles) and transplant (white circles) coral fragments at the central GBR site in December 2008 (9 months after transplantation).

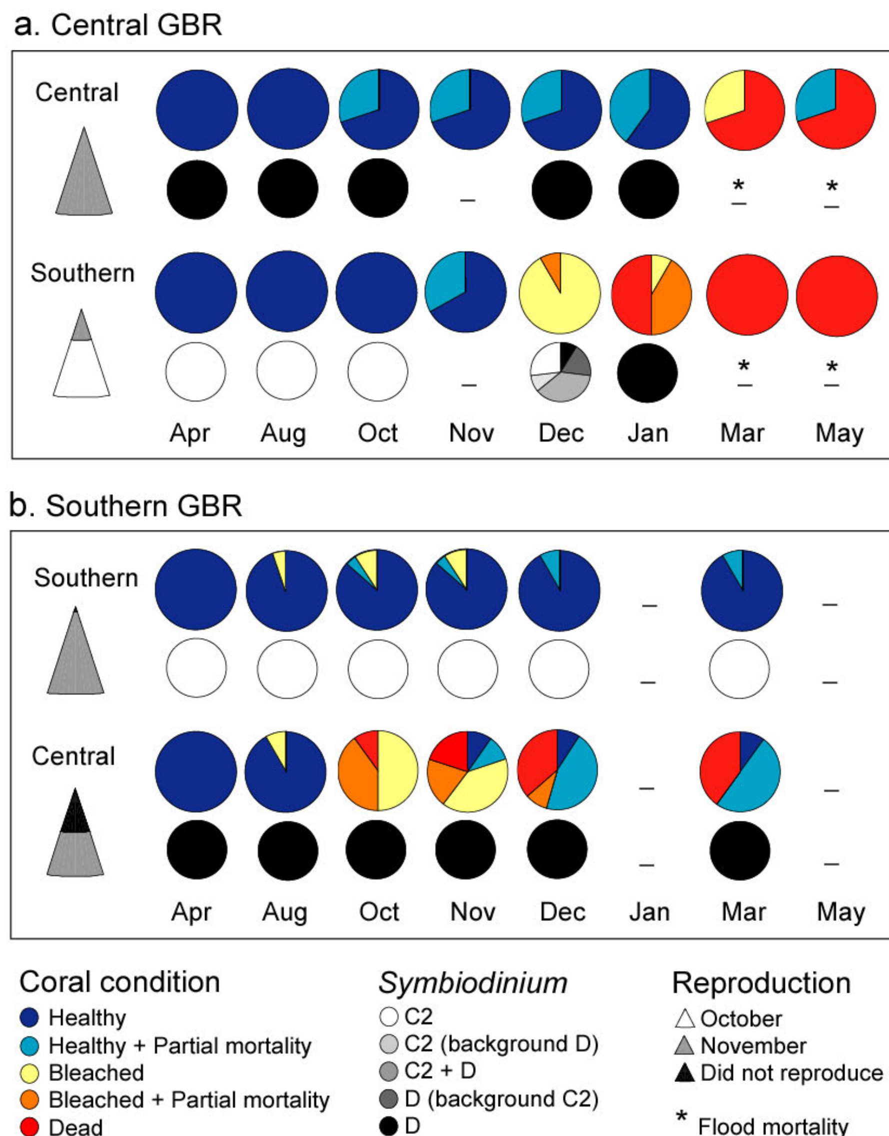

Fig. 5.4. **Genotypic and environmental variation in native and transplanted fragments of the coral *Acropora millepora* at the central (a. Magnetic Island) and southern (b. Miall Island) Great Barrier Reef (GBR) sites.**

Coral condition (large pie charts), *Symbiodinium* types hosted (small pie charts) and reproduction (pyramid area charts) data collected from April 2008 to May 2009. Sample sizes: 10 for corals retained at the central GBR site; 20 for southern GBR corals transplanted to the central GBR site (12 from November onwards), 20 for corals retained at the southern GBR site, 10 for central GBR corals transplanted to the southern GBR. The central GBR was affected by flooding in February to March 2009 causing significant coral mortality.

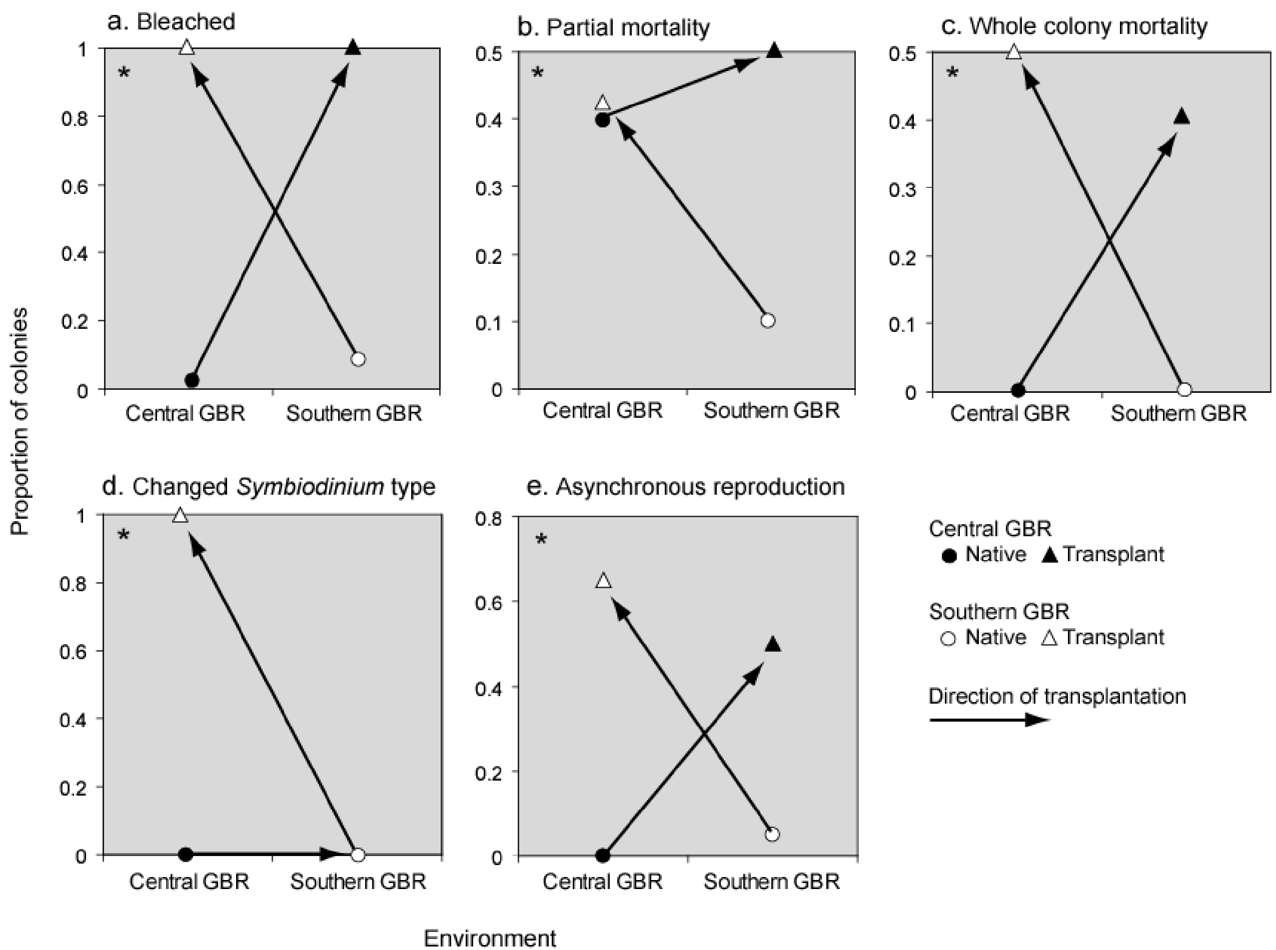

Fig. 5.5. **Summary of genotype and environment interactions for the responses of *Acropora millepora* coral fragments at native and transplant sites in the central (Magnetic Island) and southern (Miall Island) Great Barrier Reef (GBR) regions.**

Interaction plots represent the total proportion of coral fragments which (**a**) bleached, (**b**) underwent partial tissue mortality, (**c**) underwent partial whole colony mortality, (**d**) changed *Symbiodinium* type, and (**e**) reproduced out of synchrony with their respective native populations, which spawned in November at both sites. Observations are totals from the period April 2008 and January 2009 (March 2009 observations including mortality from flood plume exposure at the central GBR have not been included). Significant interactions (Fishers exact tests, Bonferroni sequential adjusted P at α =0.05, Table AIII.11) are indicated with an asterisk.

5.3.2. *Symbiodinium* type

The dominant *Symbiodinium* type remained constant in coral fragments throughout the study, except following bleaching of transplanted corals at the central GBR site (Fig. 5.5; Fishers exact test, $P < 0.05$, Appendix III, Table AIII.11). Coral fragments from the southern GBR site maintained associations with *Symbiodinium* type C2 at both the native and transplant sites, but only until transplants bleached during early summer at the latter central GBR site (Fig. 5.4). During bleaching, *Symbiodinium* D became increasingly prevalent and was detected as a dominant or background type in 75% of fragments in December ($n = 12$), and was the sole *Symbiodinium* type detected in transplanted fragments one month later in January. Interestingly, this change in *Symbiodinium* composition was not accompanied by a gain in symbiont photosynthetic efficiency. Maximum and effective quantum yields of photosystem II in bleached transplanted corals were significantly reduced, and were 60-70% lower than those measured for normally pigmented native coral fragments (Fig. 5.6; ANOVA, genotype x environment, $P = 0.001$, Appendix III, Table AIII.12). However yields were equally low in bleached fragments regardless of whether they had dominant associations with *Symbiodinium* type D, C2 or a mix of C2 and D.

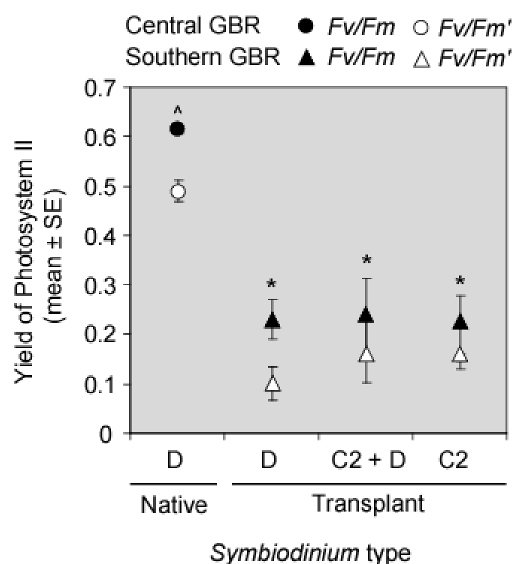

Fig. 5.6. Variation in maximum (F_v/F_m) and effective (F_v/F_m') quantum yields of photosystem II among *Symbiodinium* types hosted by *Acropora millepora,* for native and transplanted coral fragments located at Magnetic Island in the central Great Barrier Reef region during December 2008.

Symbols above standard error (SE) bars indicate homogenous groups with ANOVA and Tukeys post-hoc test ($\alpha = 0.001$). Sample sizes were: native D type = 10, transplant D type = 3, C2 + D = 5, C2 = 3. Note that native coral fragments were normally pigmented and transplant fragments were visually bleached.

5.3.3. Coral growth

Coral growth was measured over the first 7 months of the experiment and was substantially slower at transplant sites for both central and southern GBR genotypes, regardless of whether or not coral fragments exhibited visual signs of stress (Fig. 5.7; ANOVA, genotype x environment, $P = 0.002$, Appendix III, Table AIII.13). Central GBR genotypes, which underwent bleaching at the transplant location during the 2 months prior to the final growth measurement, grew 74% slower at the southern GBR site than in their native habitat. Similarly, southern GBR genotypes, which did not experience any bleaching over the months when growth was measured, grew 59% slower at the central GBR site than in their native habitat. Growth of colony fragments

in their native location did not differ significantly between the central and southern GBR, but was slightly higher and less variable in southern GBR genotypes.

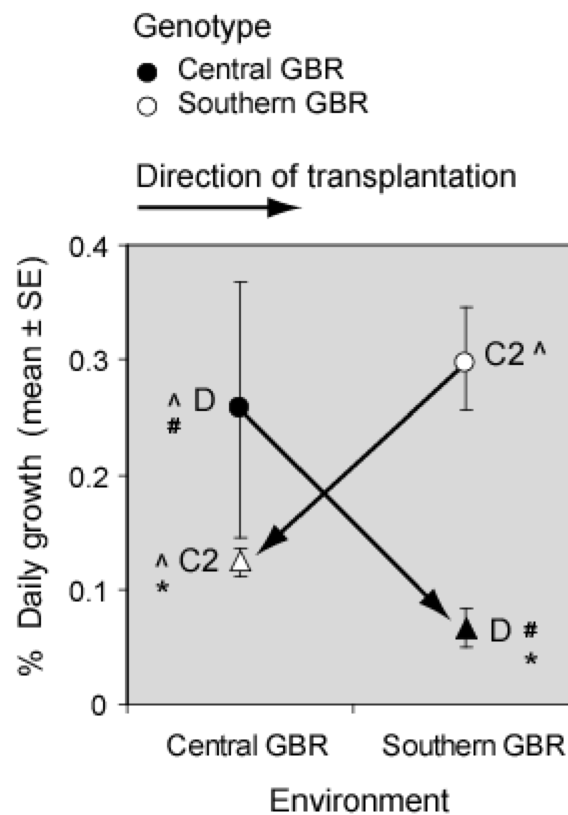

Fig. 5.7. Growth rates of *Acropora millepora* fragments at native and transplant sites in the central (Magnetic Island) and southern (Miall Island) regions of the Great Barrier Reef (GBR), measured from April to October/November 2008.

The *Symbiodinium* type hosted by fragments is indicated next to symbols. Symbols adjacent to standard error (SE) bars indicate homogenous groups identified with ANOVA and Fishers LSD post-hoc test ($\alpha = 0.05$).

5.3.4. Coral reproduction

Coral colonies retained at their native site spawned in November, at both the central (100%, n =10 colonies) and southern (95%, n = 20 colonies) GBR sites, which was consistent with the timing of spawning in the wider *A. millepora* population at both sites in 2008 (Figs. 5.4 and 5.5; Fishers exact test, $P < 0.05$, Appendix III, Table AIII.10).

However, there was a split spawning (*sensu* Willis *et al.* 1985) in 2008 at the central GBR site, and some proportion of the wider native Magnetic Island population also spawned in October. Corals transplanted from the central to southern GBR were recovering from bleaching and partial mortality during the breeding season and, although immature eggs and spermaries were present in 75% of colonies (n = 8) in October, only 50% went on to spawn in November (no colonies contained gametes in December). Corals transplanted from the southern to central GBR were visibly healthy up to and including the breeding season, and 65% (n = 20 colonies) spawned in October, one month earlier than their colony halves at the native southern GBR site. The remaining 35% of colonies contained gametes after the October spawning, and were assumed to spawn in November (but were not returned to the field after the October spawning period). Temperatures at the central GBR site during the October breeding season were slightly warmer than those at the native southern GBR site during November breeding season.

5.4. Discussion

This reciprocal transplant study demonstrated that coral-*Symbiodinium* symbioses have a poor capacity to acclimatise to changes in temperature that exceed the upper or lower limits of their local thermal regimes. Following 8 months of exposure to a warmer transplant environment, *Acropora millepora-Symbiodinium* type C2 partnerships from the southern GBR suffered bleaching and mortality when summer temperatures rose 1-2°C above the historical maximum recorded at their native site (Figs. 5.2-5.5). Similarly, after 5 months of exposure to a cooler transplant environment, *A. millepora-Symbiodinium* type D partnerships from the central GBR underwent bleaching and mortality when winter temperatures fell 0.5-1°C below the historical minimum at their native site (Fig. 5.2-5.5). In both cases, the timing of bleaching and subsequent mortality was consistent with a temperature-driven response (Fig. 2a), although turbidity (Fig. 2b) and other environmental parameters presumably varied between locations due to the field setting of the study. These results indicate that thermal tolerance limits of the coral *A. millepora* are determined primarily by genetic adaptation to local thermal regimes, either of the *Symbiodinium* partner, the host coral partner, or both partners combined.

5.4.1. Limits to acclimatisation: Too hot and too cold

Conditions inducing warm-water bleaching and mortality of *A. millepora* transplants from the southern GBR were congruent with previously defined bleaching (~28.5-29.5°C) and mortality (~30°C) thresholds for corals in the southern GBR (Berkelmans 2002, 2009). This affirms little or no gain in thermal tolerance of the symbiosis following transplantation and gradual exposure to the warmer summer temperature regime at the central GBR site. The shift from *Symbiodinium* C2 to D in summer in these transplanted corals was similar to the bleaching-related shift found in an earlier study involving transplantation of *A. millepora-Symbiodinium* C2 partnerships from the southern to central GBR in 2002-2003 (Berkelmans and van Oppen 2006), however, levels of bleaching and mortality reported were lower than those found in the present 2008-2009 study. During the 2002/2003 summer, severe bleaching occurred in 50% of transplanted corals and resulted in 36% whole colony mortality. However, during the 2008/2009 summer, severe bleaching occurred in 100% of southern transplants at the warmer central GBR site, which was accompanied by 50% whole colony and 42% partial mortality (Fig. 5.4). More than half of the transplanted fragments in 2002/2003 recovered, whereas transplants in this study were either entirely dead or almost dead prior to a flood plume mortality event that also impacted the local native *A. millepora* population. Variation in bleaching and mortality responses between these two transplant studies is likely attributable to differences in temperature profiles between years. In particular, temperatures exceeded the 30°C mortality threshold for southern GBR corals for 52 consecutive days during the 2008/2009 summer (November to March daily average temperature, total days = 65), compared with only 5 consecutive days above 30°C during the 2002/2003 summer (total days = 10). Thus, while changing *Symbiodinium* partners can enable transplanted corals to withstand bleaching when excursions above their native threshold are small or short (Berkelmans and van Oppen 2006), this mechanism was not sufficient to enable corals to respond to prolonged exposure to temperatures above this threshold.

At the central GBR site, cold-water bleaching of *A. millepora* and the wider coral community has not been recorded previously, presumably as seasonal temperatures do not drop below their lower thermal limits. However following transplantation of these *A. millepora – Symbiodinium* D associations to the southern GBR, a 74% reduction in growth was observed and all colonies bleached at temperatures less than 1°C degree below their historical minimum, resulting in 50% partial and 40% whole colony mortality. In contrast, native corals were relatively unaffected by winter temperatures at the southern site in 2008/2009 (10% of colonies bleached), although natural episodes of cold-water bleaching in southern GBR populations have been reported in other years (Hoegh-Guldberg *et al.* 2005). Similar limits to cold water acclimatisation have been observed during year-long transplantation of single colonies of *A. cervicornis* in the Caribbean (Shinn 1966), and two-month aquarium acclimation of *Montipora verrucosa* fragments in Hawaii (Coles and Jokiel 1978), where reduced growth, bleaching and mortality were observed when corals were exposed to temperatures below their native minimum. These findings demonstrate that species-specific differences in lower thermal limits (Coles and Fadlallah 1991; Kemp *et al.* 2011) also extend to population-specific limits of the coral host or variation in the tolerances of their *Symbiodinium* partners.

5.4.2. Adaptive variation in thermal tolerance among *Symbiodinium* types and populations

Coral bleaching susceptibility and associated mortality is often strongly dependent on the *Symbiodinium* type harboured (Rowan *et al.* 1997; Jones *et al.* 2008; LaJeunesse *et al.* 2010). For *A. millepora-Symbiodinium* symbioses, variation among *Symbiodinium* types (rather than host populations) predominantly determines coral growth, fecundity, survival and thermal tolerance (Berkelmans and van Oppen 2006; Mieog *et al.* 2009; Jones and Berkelmans 2011). Heat sensitivity of *Symbiodinium* C2 and heat tolerance of *Symbiodinium* D when associated with *A. millepora* have both been documented in studies of natural (Jones *et al.* 2008) and experimental (Berkelmans and van Oppen 2006; Mieog *et al.* 2009) heat stress, and it is likely that these thermal characteristics of the two *Symbiodinium* types contributed to differential thermal tolerance of *A. millepora* partnerships from the central and southern GBR in this study (but see discussion of potential host effects, below).

Unlike in previous bleaching studies of *A. millepora* (Berkelmans and van Oppen 2006; Jones *et al.* 2008), no apparent gain in photosynthetic efficiency was detected in transplanted corals following shifts from *Symbiodinium* C2 to D, as determined by subsequently exposing them to temperatures above their native bleaching thresholds (Fig. 5.6). If type D symbionts in southern corals transplanted to the central site originated from background levels present within coral tissues (Mieog *et al.* 2007), the lack of enhanced heat tolerance in the symbiosis may reflect regional differences in the heat tolerance of *Symbiodinium* type D. *Symbiodinium* D naturally occurs at both the central and southern GBR sites, and given the low likelihood of gene exchange over the > 600 km distance separating the two locations (e.g. Howells *et al.* 2009; Howells *et al*. in review, Chapter 2), it is likely that the *Symbiodinium* D populations are functionally distinct (see Howells *et al.* 2011, Chapter 4). Alternatively, the observation that *Symbiodinium* D did not appear in coral fragments in this study until bleaching was underway (see Fig. 5.4) may reflect invasion of an opportunistic, local cohort of *Symbiodinium* D, which did not provide benefits for the symbiosis under warmer conditions (Stat and Gates 2011). Interestingly, heat sensitive *Symbiodinium* C2 has only once been detected in cnidarian hosts at the warm central GBR location of Magnetic Island (Ulstrup and Van Oppen 2003, see Abrego *et al.* 2009a), despite being common elsewhere on the GBR (Cooper *et al.* 2011).

Variation in cold temperature tolerance among *Symbiodinium* types has been the subject of surprisingly few investigations (but see Suwa *et al.* 2008; Thornhill *et al.* 2008; LaJeunesse *et al.* 2010). Cold sensitivity has not been documented previously for *Symbiodinium* D, although variation in lower thermal limits has been observed among *Symbiodinium* types in culture (Thornhill *et al.* 2008) and in the field (LaJeunesse *et al.* 2010). Results of cold-water bleaching surveys indicated broad stress tolerance in a *Symbiodinium* D type hosted by *Pocillopora* spp. colonies, however this D type (ITS2 type D1) is genetically distinct from the *Symbiodinium* D type hosted by *A. millepora* on the GBR (ITS2 type D1a, GenBank Accession EU024793 in van Oppen *et al.* 2001). Assuming that cold-water bleaching of *A. millepora* transplants in this study was influenced by *Symbiodinium* type, transplant results highlight that clade D *Symbiodinium* types often described as stress tolerant are not always well adapted to withstand a broad spectrum of environmental conditions. This could be a consequence

of local adaptation in central versus southern GBR *Symbiodinium* D populations. However, it may also be an adaptive trait at the type level and could explain, in addition to reduced productivity (Cantin *et al.* 2009), why the abundance of *Symbiodinium* D dominates some corals during warm conditions but does not persist when cooler conditions return (Thornhill *et al.* 2006b; Jones *et al.* 2008).

The apparent inability of *Symbiodinium* to respond to changes in their thermal environment not experienced in the last 15 years is likely due to the evolution of regional differences in pigment ratios, lipid composition, and the size of photosynthetic units among populations of the same type (Howells *et al.* 2011), as well as variation between *Symbiodinium* types (Iglesias-Prieto and Trench 1994; Tchernov *et al.* 2004; Hennige *et al.* 2009), which constrain the extent of physiological acclimatisation. For example, *Symbiodinium* types differ in the lipid composition of their thylakoid membranes, which determines membrane stability and susceptibility to oxidative damage (Tchernov *et al.* 2004).

5.4.3. Adaptive variation in thermal tolerance among *Acropora millepora* populations

There is increasing evidence that coral populations have the potential to adapt to their local thermal environments. Population genetic studies demonstrate restricted gene flow among reefs and regions for a number of coral species (reviewed in van Oppen and Gates 2006), which could facilitate regional divergence of thermal tolerance (reviewed in Sanford and Kelly 2011). *A. millepora* populations in the central and southern GBR have been shown to be genetically distinct at both allozyme (Smith-Keune and van Oppen 2006) and microsatellite loci (van Oppen *et al.* 2011a), with regular dispersal between the inshore reefs in the two regions inhibited by an oceanographic front (van Oppen *et al.* 2011a). In a study of *Montastraea faveolata* in the Caribbean, where populations were found not to be genetically differentiated, evidence of local adaptation was observed in aposymbiotic larval cohorts from different thermal regions, based on differences in patterns of gene expression and developmental malformation under elevated temperature (Polato *et al.* 2010). Different thermal responses among individual larval crosses of *A. millepora* from the same population indicate variation in thermal

tolerance that could potentially facilitate population adaptation (Meyer *et al.* 2009a; Meyer *et al.* 2011). Furthermore, population influences on coral growth and survivorship in adult corals have been observed in other long-term (>6 month) transplantation studies (Raymundo 2001; Smith *et al.* 2007).

5.4.4. Influence of other environmental variables and reproductive evidence of acclimatisation

In addition to temperature, corals are sensitive to anomalous changes in other environmental parameters. Because of the field nature of the study, levels of light, suspended sediments and nutrients fluctuated throughout the year in both locations (see Fig. 5.2b; Schaffelke *et al.* 2011) and may have adversely affected experimental colonies, especially those that were transplanted. This may have contributed to the slower growth rates of transplanted corals measured over the first 7 months of transplantation, which were less than half those of native corals at both locations (Fig. 5.7). Slower growth of coral transplants at the southern GBR site can be at least partially attributed to cold water bleaching, however slower growth of transplants at the central GBR site was measured 6 weeks before warm water bleaching occurred. Corals transplanted to non-native sites were likely receiving less nutrition, potentially because *Symbiodinium* partners were photoacclimated to their native environments (Dustan 1979; Falkowski and Dubinsky 1981; Iglesias-Prieto and Trench 1994) or because coral hosts were less able to utilise different heterotrophic food sources available at transplant sites (Anthony 2000). Alternatively, energy acquired by coral transplants may have been directed towards processes other than growth, for example towards mechanisms enabling them to acclimatise to their new environment, or faster gamete development stimulated by the warmer temperature regime. In the initial months following transplantation, the pigmentation of corals transplanted to the central GBR darkened, whereas corals transplanted to the southern GBR lightened. Such changes in pigmentation are consistent with acclimatisation to more turbid conditions at the central GBR site and less turbid conditions at the southern GBR site. However, this interpretation remains speculative, as signatures of light acclimatisation, such as changes in *Symbiodinium* density and cellular chlorophyll concentration (Falkowski and Dubinsky 1981), were not measured. A change in reproductive timing in corals

transplanted from the southern to central GBR, from a November spawning to a split October (65%) – November (35%) spawning, was similar to the split spawning (*sensu* (Willis *et al.* 1985) found for the wider *A. millepora* population, which spawned in both months at the central GBR site. This change in reproductive timing provides tentative evidence that the transplants were at least partially acclimatised to the central GBR location. However, none of the native *A. millepora* colonies attached to the wire study rack spawned until November. Similarly, colonies of *Echinopora lamellosa* transplanted to a cooler location in Northern Taiwan shifted their reproductive timing to that of the local population, while colonies of *E. lamellosa* transplanted to a warmer location in Southern Taiwan reproduced earlier than the native population (Fan and Dai 1999). In both of these studies, temperature is expected to have strongly influenced the timing of gamete development and release, affirming environmental rather than genetic drivers of reproductive timing in corals (reviewed in Harrison 2011).

5.4.5. Implications for coral reef conservation and reef futures

Knowledge of the degree to which corals can undergo physiological acclimatisation or genetic adaptation in response to changes in their thermal environment is crucial to the success of coral reef conservation strategies. By demonstrating that corals do not acclimatise effectively to temperature changes beyond their historical thermal regimes, my findings highlight the importance of conserving local coral populations, as efforts to restore lost coral cover through transplantation efforts are likely to have a low success rate when environmental regimes at donor and recipient reefs are mismatched (see for example Job *et al.* 2006; Guest *et al.* 2007; Fisk *et al.* 2009). Overall, there are only a few published examples of long-term survivorship and growth of corals transplanted from one reef to another (Dizon and Yap 2006) or from a nursery to reef habitat (Omori 2011). Proposed introductions of corals from warmer regions to cooler regions, designed to enhance the upper thermal limits of populations (Hoegh-Guldberg *et al.* 2008), may not be feasible as corals also vary in their lower thermal limits.

A. millepora corals are bleaching-sensitive symbioses, and their inability to acclimatise to temperatures outside of their historical thermal regimes is probably representative of other bleaching-sensitive acroporid corals (Marshall and Baird 2000; Loya *et al.* 2001).

Bleaching tolerant coral species have higher rates of protein turnover than acroporid corals, indicating a greater potential for thermal acclimatisation and hence survivorship (Gates and Edmunds 1999). However this may not necessarily result in long-term displacement of bleaching-sensitive species from reefs, as tolerant coral species tend to grow slowly, whereas sensitive species tend to have high rates of recruitment and grow rapidly (Pandolfi *et al.* 2011; van Woesik *et al.* 2011). Under continued ocean warming, the persistence of bleaching-sensitive corals is likely to be strongly influenced by rates of beneficial adaptation in *Symbiodinium* (see van Oppen *et al.* 2011b) and the extent to which anthropogenic environmental pressures are mitigated.

Chapter 6. General discussion

The survivorship and reef-building capacity of corals is dependent on functional associations with *Symbiodinium* photosymbionts (Chapter 1). Coral physiology is strongly influenced by *Symbiodinium* tolerances, and the extent to which *Symbiodinium* are resilient to warming seas will shape the capacity of corals to persist during climate change. In this Chapter, the population traits of *Symbiodinium* types that positively and negatively affect resilience to warming seas are discussed. The major findings of my thesis are synthesised and the importance of *Symbiodinium* population responses to the persistence of coral symbioses is evaluated. The relevance of the thesis outcomes for reef management is highlighted, along with approaches for future research directions.

Fig. 6.1. *Symbiodinium* cells visible within the tentacles of a coral polyp

Symbiodinium type C1 within a polyp of an adult colony of *Acropora tenuis* (scale = 1 mm; photo: Jean-Baptise Raina)

6.1. Importance of, and evidence for, variation in thermal tolerance among populations of *Symbiodinium* types

Variation in the thermal tolerance of *Symbiodinium* partners can enable corals to resist thermal stress (Rowan *et al.* 2004, Berkelmans and van Oppen 2006, Ulstrup *et al.* 2006, Abrego *et al.* 2008, Jones *et al.* 2008), suggesting that *Symbiodinium* partners will play an important role in the persistence of reef corals under climate warming. A number of factors indicate that variation in thermal tolerance exists, not just among *Symbiodinium* clades and types, but also among (and potentially within) populations belonging to the same *Symbiodinium* type. Research presented in this thesis contributes substantially to establishing that genetic and functional variation exists within and among *Symbiodinium* populations (summarised in Table 6.1). First, distributions of generalist *Symbiodinium* types span broad environmental ranges, including temperature gradients. For example, the *Symbiodinium* types C1, C2 and D (ITS1 rDNA terminology) investigated in this thesis occur along the ~2,000 km length of the Great Barrier Reef (GBR) at sites ranging in annual average temperatures from below 24°C (southern GBR, monthly range ~18-27°C) to above 28°C (far northern GBR, monthly range ~24-32°C; http://data.aims.gov.au/aimsrtds; LaJeunesse et al. 2004; van Oppen et al. 2005a; Cooper et al. 2011), as well as on cooler temperate reefs off the East Australian coastline (Wicks et al. 2010). To succeed across this wide range of thermal environments, populations of *Symbiodinium* types from different reefs and regions have either adapted to local differences in temperature, or have the plasticity to acclimatise to a wide range of temperatures. Second, genetic diversity within *Symbiodinium* types is not homogenously distributed among populations located at different reefs and regions. In this thesis, populations of *Symbiodinium* C2, the most common type of *Symbiodinium* hosted by acroporid corals on the GBR (van Oppen *et al.* 2001; LaJeunesse *et al.* 2004; van Oppen *et al.* 2005a; Cooper *et al.* 2011), showed genetic separation at distances as small as ~1 km (Chapter 2). Small spatial scales of genetic structure have also been reported for populations of a *Symbiodinium* C1 type on the GBR (Howells *et al.* 2009), and also for populations of *Symbiodinium* B1 types in the Caribbean, although the latter exhibited stronger differentiation due to the occurrence of unique genotypes at

Table 6.1. Summary of the outcomes of this thesis and their relevance to biology, management and future research directions

Outcome	Biological implications	Relevance to management	Future directions
Symbiodinium maintain genetically diverse populations within coral hosts and reefs that are genetically isolated from one another at very small spatial scales (<10 km; Chapter 2).	Diverse populations are required for adaptation via natural selection. Limited connectivity may facilitate adaptation of populations to local environmental conditions but may also render populations vulnerable to disturbances (see below).	*Symbiodinium* dispersal distances are smaller than those of their coral hosts. Source and sink exchanges between reefs identified for corals (and fish) are not necessarily going to be the same for *Symbiodinium* populations, which will be more dependent on local scale protection (see below).	Determine the distribution of functional genetic diversity in *Symbiodinium* types that underlies variation in thermal tolerance.
Coral bleaching events reduce genetic diversity and change the genetic composition of *Symbiodinium* within corals and reef populations (Chapters 2 and 3).	Continued bleaching events may erode the diversity of populations. Losses of genetic diversity reduce capacity to respond to environmental pressures, as there is less diversity for natural selection to act upon.	Actions to reduce multiple stressors on coral reefs (e.g. thermal stress combined with poor water quality) may reduce the frequency and severity of bleaching and will be beneficial for populations of both coral and *Symbiodinium* partners	Temporal monitoring of individual colonies and reef populations to track changes in diversity and composition. Application of functional genetic markers (see above) may provide insight into selective processes.
Symbiodinium populations are adapted to their local thermal environment (Chapter 4).	Enables corals to optimise thermal tolerance as they acquire *Symbiodinium* best adapted to local conditions at the reef to which they recruit. Future increases in thermal tolerance may occur if *Symbiodinium* can continue to adapt with climate change-related rises in sea temperature.	Conservation of local populations is important, including those that are already adapted to living at high temperatures. See above.	Quantify rates of adaptation to determine whether they can match projected rises in sea temperatures.
Apparent limited acclimatisation of *Symbiodinium* populations to changes in temperature that exceed historical thermal regimes (Chapter 5).	Local adaptation of *Symbiodinium* types is expected to place genetic constraints on the capacity for acclimatisation.	Coral transplantation is not likely to be successful if the conditions at the recipient site exceed the historical thermal regime of the donor population. This applies to the restoration of damaged reefs and introductions of "warm adapted" corals to increase the thermal tolerance of recipient populations.	Investigate acclimatisation potential in coral symbioses with a range of life history traits, including bleaching sensitive and bleaching tolerant symbioses Distinguish adaptive variation attributed to *Symbiodinium* and host partners that constrain the capacity for the symbioses to acclimatise.

individual reefs (Santos *et al.* 2003; Kirk *et al.* 2009; Thornhill *et al.* 2009; Andras *et al.* 2011). Third, within reefs and individual hosts, *Symbiodinium* populations can be composed of many genotypes (Chapters 2 and 3; Magalon *et al.* 2006; Howells *et al.* 2009; but see Santos *et al.* 2003; Thornhill and Santos 2009). This genetic diversity, in combination with their high replication rates (Wilkerson *et al.* 1988), large population sizes (Drew 1972; Kawaguti and Nakayama 1973; Littman *et al.* 2008) and the likelihood of high mutation rates (van Oppen et al. 2011b) suggest that *Symbiodinium* populations may be well equipped to adapt to local thermal regimes. The presence of genetically diverse populations comprised of a large number of rapidly replicating and mutating cells indicates that there are functional differences among individuals upon which natural selection can act (see Correa and Baker 2011; van Oppen *et al.* 2011b). Furthermore, genetic isolation among reefs indicates that selection processes are unlikely to be neutralised by recruitment from external populations potentially carrying maladapted alleles (see Sanford and Kelly 2011).

Despite substantial theoretical evidence, experimental demonstration of population-level adaptation in *Symbiodinium* has been lacking, as the majority of research on functional variation in *Symbiodinium* focuses on differences between clades and types (Rowan et al. 1997, Baker et al. 2001, Rowan et al. 2004, Iglesias-Prieto et al. 2004, Berkelmans and van Oppen et al. 2006, Abrego et al. 2008, Mieog et al. 2009). In this thesis, I provide the first experimental evidence that populations belonging to a single *Symbiodinium* type can adapt to different thermal environments (Chapter 4). I show that *Symbiodinium* C1 from a warm reef can thrive at elevated temperatures (32°C) typically experienced in summer at their native reef, while *Symbiodinium* C1 from a cooler reef suffer photoinhibition and mortality under the same conditions, which exceed summer temperatures at their native reef. Importantly, when juvenile *A. millepora* corals native to an even cooler reef were associated with the warm-adapted *Symbiodinium* C1, they grew rapidly and resisted coral bleaching. Consequently, if *Symbiodinium* populations are able to further adapt to increases in temperature at the pace at which oceans warm, they may assist corals to increase their thermal tolerance and persist into the future.

The evolution of fixed differences in biochemical traits among *Symbiodinium* types (Iglesias-Prieto and Trench 1994; Tchernov *et al.* 2004; Hennige *et al.* 2009) and populations (Howell *et al.* 2011, Chapter 4) may constrain phenotypic plasticity. For the *Symbiodinium* C1 populations investigated in Chapter 4, I documented signicant differences in cell size and pigment composition between populations from the warm and the cool reef after a year of growth in identical culture conditions. Particularly, two-fold higher levels of β-carotene (relative to chlorophyll *a*) in *Symbiodinium* C1 from the warm reef are likely to provide resistance to heat stress by preventing the formation of damaging reactive oxygen species (Siefermann-Harms 1987). Genotypic constraints to thermal acclimatisation in *Symbiodinium* types and populations are likely to have contributed to the failure of *A. millepora – Symbiodinium* partnerships to adjust to temperatures beyond historical thermal regimes, as described in Chapter 5. Reciprocal transplantation of corals to different thermal regimes demonstrated that *A. millepora* - *Symbiodinium* associations are able to gradually acclimatise to cooler and warmer conditions, but only within their historical thermal regimes. Bleaching and mortality were observed in coral partnerships with *Symbiodinium* D when temperatures dropped <1°C below their native 15 year minimum, and in partnerships with *Symbiodinium* C2 when temperatures rose 1-2°C above their native 15 year maximum. While the role of host effects requires further investigation, these findings indicate that gains in the thermal tolerance required to match rising sea temperatures in bleaching sensitive corals will be more dependent on genetic adaptation, rather than physiological acclimatisation, of *Symbiodinium* partners.

6.2. Can *Symbiodinium* populations adapt to future rises in temperature?

While rates of adaptation in *Symbiodinium* populations are not known, the capacity to rapidly adapt to changes in environmental conditions, including temperature (Bennett *et al.* 1990), salinity (Costas *et al.* 2008), acidity (Amaral-Zettler *et al.* 2002; Costas *et al.* 2008), predation (Meyer *et al.* 2006) and toxin exposure (Costas *et al.* 2001; Lopez-Rodas *et al.* 2008a; Lopez-Rodas *et al.* 2008b; Sanchez-Fortun *et al.* 2009; Marva *et al.* 2010), has been demonstrated in aquatic asexual organisms, including single-celled eukaryotic algae. In laboratory experiments, adaptation via selection of resistant genetic variants was observed in algae in response to environmental treatments, rather than mechanisms of physiological acclimatisation (Costas *et al.* 2001; Amaral-Zettler *et al.*

2002; Costas *et al.* 2008; Lopez-Rodas *et al.* 2008a; Lopez-Rodas *et al.* 2008b; Sanchez-Fortun *et al.* 2009; Marva *et al.* 2010). Resistant genetic variants arose from spontaneous somatic mutations arising during cell divisions in the order of 10^{-7} to 10^{-5} resistant variants per cell division. If similar rates of mutation exist in *Symbiodinium*, significant adaptive potential exists in populations, given their fast asexual generation times (Wilkerson *et al.* 1988) and high numbers within individual coral colonies (~10^6 cells.cm^{-2}, Drew 1972) and reef communities (including free-living habitats, Littman *et al.* 2008).

Based on the mutation rates above, van Oppen *et al.* (2011b, Appendix II) estimate that at any given time, 10^3 to 10^5 beneficially mutant cells arise within a *Symbiodinium* assemblage in an average sized coral colony (30 cm diameter). With new beneficial mutations appearing in the *Symbiodinium* assemblage with each asexual generation, rapid adaptation could potentially occur under continual selection pressure. Following this line of argument, it is possible that selection occurred in the *Symbiodinium* culture populations investigated in this thesis (Chapter 4). An observed decline in the upper thermal tolerance of *Symbiodinium* C1 from Magnetic Island after one year of growth (>30 generations) in culture at 24°C may reflect adaptation to a cooler environment. However, this observation could also reflect acclimatisation effects, thus rates of beneficial mutations in *Symbiodinium* remain to be quantified.

The strength of selection under ambient, naturally-fluctuating environmental conditions is difficult to estimate on coral reefs. However, as average temperatures are predicted to increase by 1.5-3°C over the coming decades (Meehl *et al.* 2007), selection for heat-resistant genetic variants is expected to occur. Bleaching episodes induced by elevated sea temperature may further provide rapid and strong selection for heat resistant variants within populations (Buddemeier and Fautin 1993; Correa and Baker 2011). In some instances, corals recover from a loss of up to 90% of their *Symbiodinium* assemblages during bleaching, with symbiont repopulation occurring from a low number of remnant *Symbiodinium* cells or by uptake from the external environment (Chapter 2; see also Lewis and Coffroth 2004; Mieog *et al.* 2007). *Symbiodinium* cells involved in repopulation may be individuals that resisted bleaching because they were occupying microhabitat refuges (e.g. shaded branches or the reef benthos) or they may constitute genotypes that were more tolerant to the bleaching stressor (e.g. elevated temperature).

Interestingly, symbiosis has been shown to accelerate rates of adaptation in endosymbiont populations. In bacteria and fungi, a greater number of mutations occur in vertically transmitted endosymbiotic lineages than in close free-living relatives Moran 1996; Woolfit and Bromham 2003). This is attributed to the small size of endosymbiont populations undergoing a bottleneck at each host generation, which promotes non-synonymous mutations (i.e. those that result in amino-acid substitutions) to drift toward fixation. These endosymbiotic systems are analogous to the ~15% of coral species that transmit *Symbiodinium* maternally (Richmond 1997), for which there is evidence of co-evolution of host and symbiont populations (Frade *et al.* 2008; Bongaerts *et al.* 2010). Even in the majority of coral species that acquire endosymbionts from the environment at each new host generation, *Symbiodinium* experience bottlenecks during sub-lethal bleaching. In such cases, *Symbiodinium* are lost and may be subsequently recovered from background levels (Chapter 3), and thus may undergo accelerated adaptation.

Adapted genotypes are likely to replicate and spread within the coral host, and indeed, this phenomenon has been observed during recovery from bleaching, when novel *Symbiodinium* type(s) become more abundant than the usual type (e.g. Berkelmans and van Oppen 2006; Jones 2008). Such changes are also possible within a *Symbiodinium* type, given the high number of genetic variants (observed at microsatellite alleles, Chapters 2 and 3, Howells *et al.* 2009) and new variants theoretically arising from spontaneous mutation (van Oppen *et al.* 2011b), upon which selection could act. Interestingly, microsatellite genotyping of *Symbiodinium* in *A. millepora* colonies before and after a thermal bleaching episode at Miall Island (southern GBR) showed that bleaching resulted in changes in the genetic composition of *Symbiodinium* C2 within individual coral colonies and on a reef-wide scale (Chapter 3). Additionally, during a flood plume bleaching episode at the Palm Islands (central GBR), differences were observed in the genetic composition of *Symbiodinium* C2 in bleached and unbleached coral sub-populations (Chapter 2). It is unclear whether these changes reflect functional changes in the symbiosis, as it is unknown whether the microsatellites used in this study (Appendix I) are linked to DNA regions under selection. Reductions in genetic diversity were also observed during and following these bleaching episodes, and erosion of diversity may result if bleaching episodes become more frequent or severe (e.g. Hoegh-Guldberg 1999). If this occurs, the genetic variation that natural

selection can act upon may be significantly reduced before positive adaptations can occur (Frankham 2005).

6.3. Influence of *Symbiodinium* population responses on the persistence of corals under warming seas

Symbiodinium are considered to be the more thermally sensitive partner in coral-dinoflagellate symbioses due to their photoinhibition and cellular damage at anomalous temperatures (Weis 2008; Strychar and Sammarco 2009) and the substantially reduced stress responses of corals in the absence of *Symbiodinium* partners (Yakovleva *et al.* 2009). Thus, any gain in the thermal tolerance of *Symbiodinium* populations is expected to have a positive outcome for coral hosts. A number of studies have demonstrated increased thermal tolerance in corals when thermally tolerant types of *Symbiodinium* are incorporated into the symbiosis (Baker 2001; Rowan 2004; Chen *et al.* 2005; Berkelmans and van Oppen 2006; Abrego *et al.* 2008; Jones *et al.* 2008; Mieog *et al.* 2009). However these associations are often temporary in natural environments (Chen *et al.* 2005; Thornhill *et al.* 2006b; Jones *et al.* 2008) and can result in reduced growth, as some tolerant symbiont types supply less energy to their host (Little *et al.* 2004; Cantin *et al.* 2009; see Stat *et al.* 2008; Stat and Gates 2011 for discussion of opportunistic versus mutualistic traits of *Symbiodinium* partners). Furthermore, not all corals appear to be able to form associations with multiple *Symbiodinium* types (Thornhill *et al.* 2006a; Thornhill *et al.* 2006b; Goulet *et al.* 2008a; Stat *et al.* 2009). In this thesis, corals that established symbioses with a heat-adapted population of a generalist *Symbiodinium* type not only resisted bleaching and mortality at high temperature, but maintained rapid growth rates at a developmental stage where increased size confers a survival advantage (Jackson 1986; Chapter 4). If *Symbiodinium* can continue to adapt in reef populations, corals that acquire their symbionts from the environment may be able to form more thermally tolerant symbioses at each new host generation (see Douglas 1998), with the potential for additional adaptation within the established symbioses (van Oppen *et al.* 2011b).

Ultimately, thermal tolerance in photosymbiotic corals is governed not only by *Symbiodinium* partners, but also by interactions among responses of the coral animal and *Symbiodinium* (and potentially also other associated microbes, Rosenberg *et al.* 2007). Thus, the likelihood of particular coral-*Symbiodinium* partnerships persisting under future warming will be influenced by the degree to which coral animals can provide photoprotection to their *Symbiodinium* partners, upregulate mechanisms to protect themselves from oxidative damage and supplement their diet with heterotrophic nutrition (reviewed in Baird *et al.* 2009). Symbiotic associations in the bleaching-sensitive coral *A. millepora* were the focus of this thesis and did not show any potential to acclimatise to temperatures that exceeded their historical thermal regimes. Corals that are known to have a greater bleaching resistance (see Marshall and Baird 2000; Loya *et al.* 2001 for examples) may show greater potential for host-driven acclimatisation (Gates and Edmunds 1999; Baird *et al.* 2009); however, this hypothesis requires further investigation (but see Brown *et al.* 2002b; Brown *et al.* 2002c; Grottoli *et al.* 2006).

Coral animals themselves also experience a degree of stress at high temperatures (Ainsworth *et al.* 2008; Rodriguez-Lanetty *et al.* 2009; Yakovleva *et al.* 2009), indicating that they too will need to increase their acclimatisation capacities or undergo genetic adaptation to warming conditions. Host responses may be possible on faster timescales than previously realised (see Stockwell *et al.* 2003) because of their modular make-up (Monro and Poore 2004; van Oppen *et al.* 2011b) and co-evolutionary interactions with *Symbiodinium* (see Frade *et al.* 2008; Bongaerts *et al.* 2010 for examples in corals where *Symbiodinium* is vertically transmitted). There is evidence that corals have recently increased their thermal thresholds on the GBR over a timescale of less than a decade (Berkelmans 2009). This may have occurred through acclimatisation of the symbiosis or selection of more tolerant coral and *Symbiodinium* genotypes. While short-term associations with more thermally tolerant *Symbiodinium* types can occur during bleaching events on the GBR (e.g. Jones *et al.* 2008), there has been no observed long-term shift towards such types. This may reflect sampling effort, or alternatively, responses of existing *Symbiodinium* partners or their coral hosts may have contributed to increases in the temperature thresholds at which corals bleach.

6.4. Implications of major thesis outcomes and future research directions

In addition to contributing biological knowledge on the resilience of *Symbiodinium* to warming seas, the outcomes of this thesis provide useful information that can be applied to reef management, especially for the GBR (summarised in Table 6.1). Effective management of *Symbiodinium* populations at local scales will be important because their limited spatial connectivity makes them vulnerable to disturbances. Furthermore, conservation efforts involving the transplantation of adult coral colonies or fragments (Hoegh-Guldberg *et al.* 2008; Edwards 2010) are not likely to be successful if thermal regimes at donor and recipient reefs are mismatched. The potential of locally adapted *Symbiodinium* populations to respond to future warming will be maximised if exposure to additive stressors, such as poor water quality, are minimised (see Wooldridge 2009; Wooldridge and Done 2009; Negri and Hoogenboom 2011). On the GBR, nutrient loading exacerbates thermal stress experienced by corals on inshore reefs during summer months, and thermal tolerance at these reefs would likely be improved by ~2-2.5°C (Wooldridge 2009) through changes to agricultural practices in river catchments. Such local management steps provide relatively rapid benefits compared to reductions in greenhouse gas emissions, given that temperatures will continue to rise for the remainder of the century even if greenhouse gas emissions are stabilised (Bernstein *et al.* 2007). Nonetheless, global emissions reductions are crucial in determining whether anthropogenic rises in sea temperature can be held to ~2°C by the end of the century (Bernstein *et al.* 2007; Allison *et al.* 2009).

Continued investigation of the contributions of *Symbiodinium* populations to the fitness traits of corals will advance understanding of the evolution of thermal tolerance in coral symbioses and the potential for future adaptation to warming seas. Applying functional genetic approaches to genotyping of *Symbiodinium* populations, such as analysis of single nucleotide polymorphisms (see Kruglyak and Nickerson 2001; Meyer *et al.* 2009b), may enable identification of the genes linked to thermal tolerance and the distribution of variation at these genes among coral colonies, reefs and regions. Rates of adaptation to specific and synergistic environmental stressors could be quantified by running selection experiments on *Symbiodinium* cultures (see Huertas *et al.* 2011) and

would provide valuable information on whether rates of adaptation in *Symbiodinium* are likely to keep pace with projected rates of warming. Overall, future work should aim to combine information on the singular and interactive responses of *Symbiodinium* and coral partners, including symbioses in coral species that vary in their present thermal thresholds (see Marshall and Baird 2000; Loya *et al.* 2001).

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Appendix I. Development of population genetic markers for *Symbiodinium* clade C

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Bay LK*, Howells EJ*, van Oppen MJH (2009) Isolation, characterisation and cross amplification of thirteen microsatellite loci for coral endosymbiotic dinoflagellates(*Symbiodinium* clade C). *Conservation Genetics Resources* 1: 199-203

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Data were collected by E. Howells using microsatellite primers developed by L. Bay. Analsyses were undertaken by E. Howells and L.Bay. The manuscript was writted by L. Bay with contributions from E. Howells and M. van Oppen

AI.1. Introduction

Coral reefs are under increasing threat from a diverse range of disturbances primarily associated with human activities (Hughes *et al.* 2003). Reef corals often respond to disturbances by bleaching, a process where the mutualistic symbiosis between scleractinian corals and dinoflagellate algae (*Symbiodinium microadriaticum*) breaks down. As a consequence, corals lose their photosymbionts and/or photosynthetic pigments, which constitute their major food source, and turn characteristically pale or white (Baker 2003). If bleaching conditions persist for extended periods of time, this can result in widespread coral mortality. Because the ecological and evolutionary success of coral reefs is largely supported by this symbiosis, a detailed understanding of the species and population structure of *Symbiodinium* communities in natural and disturbed coral habitats is urgently required.

Due to lack of significant morphological differentiation, the identity of *Symbiodinium* spp. and types is mostly determined based on ribosomal (rDNA) and chloroplast DNA (cpDNA) sequence variation. Eight genetically distinct clades are currently recognized (Clade A to H: Coffroth and Santos 2005). Extensive genetic diversity (based on sequence variation in rDNA ITS1 and 2) exists within clades, and types can display distinctive geographic and host species associations (Apprill and Gates 2007). Furthermore, physiological performance can vary greatly among types of *Symbiodinium*; it can affect rates of photosynthate translocation (Cantin *et al.* 2009), coral growth (Little *et al.* 2004) and bleaching susceptibility (e.g. Berkelmans and van Oppen 2006). Indeed, coral bleaching may change the relative abundance of Symbiodinium clades and types within populations (Jones *et al.* 2008) and potentially also the genetic diversity of types (Howells *et al.* 2009).

Despite their ecological and evolutionary importance, the species status and population structure of *Symbiodinium* types, their distribution and abundance within and among coral hosts and population dynamics following disturbances, such as bleaching, are still not well understood. DNA sequence analysis of rDNA and cpDNA markers does not provide the resolution required to resolve population genetic parameters and hence, microsatellite markers are increasingly being developed (e.g. Pettay and LaJeunesse

2007). Significant genetic structure can be detected among reefs separated by tens (Santos *et al.* 2003; Howells *et al.* 2009) to thousands (Magalon *et al.* 2004) of kilometers. So far, microsatellite analyses have revealed substantial type specificity (Pettay and LaJeunesse 2007), which has required the development of markers for each of the numerous *Symbiodinium* types hosted by corals. To facilitate further population genetic analyses microsatellite markers were developed for *Symbiodinium* clade C, which is the most common group of *Symbiodinium* hosted by corals on the Great Barrier Reef (van Oppen *et al.* 2001; LaJeunesse *et al.* 2004).

AI.2. Methods

A partial genomic library was constructed using a magnetic bead enrichment protocol (Glenn and Schable 2005). Fresh *Symbiodinium* type C1 and C2 extracts were obtained from *Acropora tenuis* (C1) and *A. millepora* (C2) corals from Orpheus Island (18°36’35S, 146°29’16E) in the central Great Barrier Reef, Australia, because cultures of these local types were not available. *Symbiodinium* cell extracts were obtained by submerging coral branches in fresh water for 10 min, returning to filtered sea-water and then collecting expelled *Symbiodinium* cells after three to five hours. *Symbiodinium* cell extracts were purified by repeated seawater washes and centrifugation at 600 *g* but some coral cells remained. Genomic DNA was extracted using a CTAB extraction protocol (Sambrook and Russell 2001). *Symbiodinium* cell extracts were confirmed to only host a single type of *Symbiodinium* using polymerase chain reaction (PCR) and single stranded conformation polymorphism (SSCP) analysis of the rDNA ITS1 region (van Oppen *et al.* 2001), where PCR products of cell extracts were run alongside known reference types. Two to four µg DNA from each type was digested using 10 U *Rsa*1 restriction enzyme (*New England Biolab*s) and double stranded SNX linkers were ligated to the DNA fragments in the presence of 10 U *Xmn*1 (to prevent linkers dimerisation) using 800 U T4 DNA Ligase (*New England Biolabs*). Five µL linker-ligated DNA and ten µL PCR amplified linker-ligated DNA was hybridised to 1 µM each of biotinylated microsatellite probe ($(AC)_{13}$, $(AG)_{13}$, $(AAC)_6$, $(ATC)_8$, $(AATG)_6$, $(ACAG)_6$), keeping the di-, tri- and tetramers separate. Five hundred µg streptavidin-coated magnetic beads (*Dynal*) were used to capture microsatellite-containing DNA fragments, which were subsequently PCR amplified and purified using *QIAGEN* PCR clean-up columns. Seventy-five ng purified PCR product was ligated into 100 ng pGEM T-easy vector and

384 clones for type C1 and 196 clones for type C2 enrichments were sequenced at the Australian Genome Research Facility, Brisbane, Australia. Thirty-one specific primers for types C1 and C2 were designed using Primer3 (Rozen and Skaletsky 2000) and tested for amplification success and *Symbiodinium* specificity (indicated by no amplification in *Symbiodinium*-free DNA extracted from sperm of *Acropora millepora* and *A. tenuis*). Seven loci reliably produced amplicons in the expected size range with no amplification of coral sperm samples. Levels of polymorphism of these loci were tested in *Symbiodinium* C2 populations isolated from 30 coral hosts (*A. millepora* southern GBR, 23°08'06S, 153°89'04E). SSCP analysis indicated the presence of a single C2 type in these branches. PCR was carried out in 10 µL reactions containing 2.5 mM MgCl, 0.4 mM of each primer (forward primer fluoro-labelled with either TET or FAM), 0.2 mM dNTPs, 0.3 U Taq Polymerase and ~5 ng DNA using the cycling conditions: 95°C for 3 min, 30 cycles of 95°C, T_a (Table 2.1.1) and 72°C for 30 s each, followed by an extension step at 72°C for 10 min. PCR products were purified using Sephadex (G-25) resin and genotyped on a *Megabase* capillary sequencer at the Genetic Analysis Facility at James Cook University. Microsatellite peak profiles were analysed using *Fragment Profiler 1.2* (*Amersham Biosciences*). Peaks were conservatively scored that had a minimum intensity of 5% of the most intense peak, were in phase with the locus repeat motif, and were identified in at least two coral host samples. The seven novel primer pairs and all (six) previously published clade C *Symbiodinium* primers were cross-tested on five different clade C types and one D clade type using published locus specific amplification conditions (Magalon *et al.* 2004; Howells *et al.* 2009), genotyped and scored as above.

Symbiodinium cells are probably haploid (Santos and Coffroth 2003) and may reach densities of millions of cells per cm^2 of coral gastrodermal tissue (Drew 1972). Extracts from coral branches represent a community of *Symbiodinium* cells that may contain many genotypes from one dominant type as well as additional background types (Mieog *et al.* 2007). Because of these biological features, microsatellite profiles of *Symbiodinium* isolated from coral branches resemble a dominant binary matrix preventing the construction of multilocus genotypes and, therefore, examinations of Hardy–Weinberg conformation and linkage disequilibrium. Here, it is assumed that identified peaks represent alleles in the population and report the mean number of

alleles per locus and genetic diversity (based on Shannon's index H [$H = -\sum p_i \ln(p_i)$], where p_i is the proportion of the ith allele within the population).

AI.3. Results and Discussion

The seven novel microsatellite primer pairs consistently amplified between 1 and 8 alleles per coral host branch with the mean number of alleles ranging from 1.9 to 4.0 in the *Symbiodinium* C2 type used. Genetic diversities were generally high (0.71–2.76) but lower in locus *C2.08* (Table 2.1.1). The novel microsatellite loci consistently amplified between 1 and 10 alleles in four other C types indicating their general utility within this *Symbiodinium* clade. Geographic variation in the amplification success between *S. hystrix* populations from Western Australia and the GBR indicate some spatial specificity in cross amplification success. Three of six previously published clade C microsatellite loci amplified 1–6 alleles in two or more novel C types tested. One locus amplified three alleles in a single type but did not amplify in three other novel types (Table 2.2.2). None of the clade C primers amplified clade D *Symbiodinium* (ITS1 GenBank accession: EU024793 data not presented) confirming the clade specificity of these primers. The primers presented here will therefore be useful in elucidating the population genetic structure of *Symbiodinium* clade C populations in corals.

Table AI.1. Characteristics of the seven clade C *Symbiodinium* microsatellite loci including primer sequence, motif sequence, allele size range, annealing temperature (T_a), mean number of alleles per host (range of alleles per host), genetic diversity (Shannon's Index) and *GenBank* accession number.

Locus	Primer sequence (5' – 3')	Motif sequence	Exp. Amplicon size (bp)	T_a (°C)	Mean no. alleles / host (range)	Genetic diversity	GenBank accession no.
C1.01	F: CAAGAGGTGAGGTGATGTCG R: TATCTTTCATCCGCCTGAGC	Trimer 1	241 (109-232)	50	3.4 (1-7)	2.76	GQ254813
C1.02	F: TTGTAAGCCTGGGAACTTGG R: CAAAATAGGACACCCGCACT	Trimer 2	182 (121-136)	50	2.3(1-3)	0.71	GQ254814
C1.05	F: TTTCGTTGTTGGACATTGTTATG R: GGACTGAAAGGTGCTTGAGG	Tetramer 1	311 (235-287)	55	3.0 (2-4)	0.92	GQ254815
C1.07	F: GGAGTCATGACACACAAACCA R: AACCTGCCAGACATGTCACC	Trimer 3	144 (110-240)	50	4.0 (3-8)	2.07	GQ254816
C1.15	F: GGTTTTACGGCTATTGATTAAAAA R: CGCCATATGAACATACACGAA	Trimer 4	250 (130-253)	50	3.4 (2 - 4)	1.85	GQ254812
C2.08	F: ATCACGGATCCACAGAGACC R: AAGTCGGCGCTATCCTACC	Trimer 5	202(197-215)	55	4.0 (4)	0.27	GQ254810
C2.12	F: ACTGACGAGCTCCTGACGAT R: GCACCGTCATCATCAAAATG	Trimer 6	221(112-326)	50	1.9 (1-5)	1.89	GQ254811

Trimer 1 = $(CAT)_7GAT(CAT)_5CAAGATCAAGAA(CAAGAT)_2$
Trimer 2 = $(GAT)_4(TAT)_5(GAT)_2TCT(GAT)_8(GAC)_4$
Trimer 3 = $(CAA)_5(CAG)_2(CAA)_2CAG(CAA)_4CAG(CAA)_2$
Trimer 4 = $GATGAC(GATGGT)_6(GTT)_2(GAT)_6N_4(GAT)_2N_8GAT(TGAGGA)_3GATAGA(TGGTGGA)_4(TGA)$
Trimer 5 = $(GAT)GGT(GAT)_4(G(GAT)_2)3N_4(GAT)G(GAT)_5(GAA)_2$
Trimer 6 = $(GAT)10N_3(GAT)_{17}N_2(GAT)_3GAA(GAT)_5GACGATGGT(GAT)_5N_2(GAT)_6(CAT(GAT)_3)_2$
Tetramer 1 = $(AGAT)_{12}N_{12}(AGAT)_9(AGAC)_3AGAT(AGAC)_3(AGAT)_3AGAC(AGAT)_2N_3(TAGG)_3$

Table AI.2. Amplification success of cross species testing of all published *Symbiodinium* clade C microsatellite primers including host coral *Symbiodinium* type (host species, location) and ITS1 *GenBank* accession number, Amplification (Amp) = Number of tests/number of successful amplifications, Range = Amplicon range (bp), N_a = number of alleles

Locus	*Symbiodinium* strain (host/location)														
	C2* *Acropora millepora*, GBR AY643495			C1* *Acropora tenuis*, GBR AF380551			C1:3a# *Sinularia flexibilis*, GBR AY758439			C^ *Seriatopora hystrix*, WA			C^* *Seriatopora hystrix*, GBR		
	Amp.	Range	N_a	Amp.	Range	N_a	Amp.	Range	N_a	Amp.	Range.	N_a	Amp.	Range	N_a
SymC_3-01 [1]	0/6			0/6			As per[1]			0/6			0/4		
SymC_3-02 [1]	0/6	141- 144	2	0/6			As per[1]			0/6			4/4	102–169	6
SymC_3-03 [1]	0/6	123-127	2	0/6			As per[1]			0/6			4/4	151–160	3
SymC_3-04 [1]	0/6			6/6	128	1	As per[1]			0/6			4/4	104–182	5
C1.01 [2]	As per Table 2.1.1			6/6	117–297	11	6/6	113–305	15	2/6	116	1	4/4	116–293	7
C1.02 [2]	As per Table 2.1.1			6/6	122–206	5	6/6	119–326	6	3/6	104	1	4/4	104–326	4
C1.05 [2]	As per Table 2.1.1			5/6	106–315	6	6/6	122–311	5	2/6	102	1	4/4	130–274	5
C1.07 [2]	As per Table 2.1.1			6/6	110–314	4	6/6	110–257	6	0/6			4/4	271	1
C1.15 [2]	As per Table 2.1.1			5/6	257–284	3	6/6	125–272	6	0/6			4/4	263–269	2
C2.08 [2]	As per Table 2.1.1			6/6	177–218	6	6/6	186–228	4	0/6			4/4	202–222	4
C2.12 [2]	As per Table 2.1.1			6/6	127–229	10	6/6	109–256	13	0/6			3/4	101–253	5
PV1 [3]	6/6	189–295	2	6/6	189–295	5	0/6			0/6			0/4		
PV4 [3]	0/6			0/6			0/6			0/6			0/4		

Microsatellite sources: [1](Howells *et al.* 2009), [2] This study, [3] (Magalon *et al.* 2004)
* sensu van Oppen *et al.* (2001), # sensu LaJeunesse *et al.* (2004)
^ and ^* ITS1 strains have not been sequenced

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Appendix II. Adaptive potential through somatic mutation in coral and *Symbiodinium* populations

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AII.1. Introduction

The speed at which our climate is changing is likely unprecedented in the past 50 million years (Jansen *et al.* 2007). Of particular concern for reef corals are increases in seawater temperature and lowering of seawater pH (acidification). Corals are sensitive to even small temperature elevations beyond the norm, which can lead to a break-down of the obligate symbiosis between the coral animal and its algal endosymbionts (unicellular dinoflagellates of the genus *Symbiodinium*, also known as zooxanthellae). This results in a paling of the coral tissues due to loss of *Symbiodinium* cells and/or their pigments and is therefore referred to as coral bleaching. During unusually warm summers, bleaching events often extend across many reefs and reef systems. Such mass bleaching events affect many coral species severely and can cause considerable mortality. Ocean acidification, through dissolution of elevated atmospheric CO_2 into the seawater, is likely to affect calcification, reducing skeletal extension rates (i.e., growth rates) and skeletal density, as well as the corals' productivity (Anthony *et al.* 2011). These deleterious effects may further compound each other, as corals show increased sensitivity to temperature under high CO_2 levels (Anthony *et al.* 2008). On a world-wide scale, contemporary coral reefs have suffered large declines in coral cover (Gardner *et al.* 2003; Bruno and Selig 2007, but see Sweatman *et al.* 2011 for data on some regional patterns) and ~15% are seriously threatened with complete loss within the next 10-20 years (Wilkinson 2008). These observations indicate that unless corals can adapt, further loss of coral cover and diversity is expected to occur over the next decades.

It is generally believed that the rate of adaptation of the coral animal is too slow to keep up with the predicted pace of warming and ocean acidification (Hoegh-Guldberg 1999; Hoegh-Guldberg *et al.* 2007) due to the long generation times of corals (but see Maynard *et al.* 2008a; Baird *et al.* 2009 for alternative opinions). Some coral species indeed have long sexual generation times of >30 years, but many common and abundant corals, such as species of *Acropora* and *Pocillopora*, mature early (from ~4 yr of age, Babcock 1991; Omori 2010). This "slow adaptation" hypothesis in corals considers adaptation solely as the result of selection on standing genetic variation from one sexual

generation to the next. Corals possess attributes that may warrant more complex models of adaptation via selection on cells with different genotypes arising through somatic mutation (i.e., mosaicism) and their evolutionary role through both asexual and sexual reproduction. Furthermore, the physiological tolerances of corals, including thermal tolerance, are strongly influenced by their *Symbiodinium* partners (Rowan 2004; Ulstrup *et al.* 2006; Abrego *et al.* 2008; Mieog *et al.* 2009), which are therefore likely to be subject to strong selective pressures under climate change. *In hospite* populations of *Symbiodinium* are likely haploid (Blank 1987; Santos and Coffroth 2003) and maintained asexually, while sexual reproduction has never been observed (Santos *et al.* 2003; Correa and Baker 2009). Non-neutral somatic mutations in *Symbiodinium* arising *in hospite* are subject to selection and if the mutation is beneficial, the new mutant can theoretically displace most if not all of the original genotype(s) within the coral colony (Fig. AII.1), as has been shown in several free-living microalgae when exposed to extreme toxicity (Baos *et al.* 2002; Costas *et al.* 2007). Somatic mutations and selective sweeps in non-eukaryotic microbes associated with corals may also play a role in their adaptation to climate change. These organisms are not specifically addressed here, although what is discussed applies to them also (Correa and Baker 2011). The role of somatic mutations in adaptation of the coral animal was first considered by Fautin (1997) and Buddemeier *et al.* (1995) and those in the associated *Symbiodinium* by Correa and Baker (Correa and Baker 2011). The ideas of these authors are expanded upon here, and it is argued that evolution within mitotic cell lineages of both the coral host and its associated *Symbiodinium*, also known as cell-lineage selection, may play a role in the adaptation of corals to climate change. This warrants testing via robust scientific experimentation.

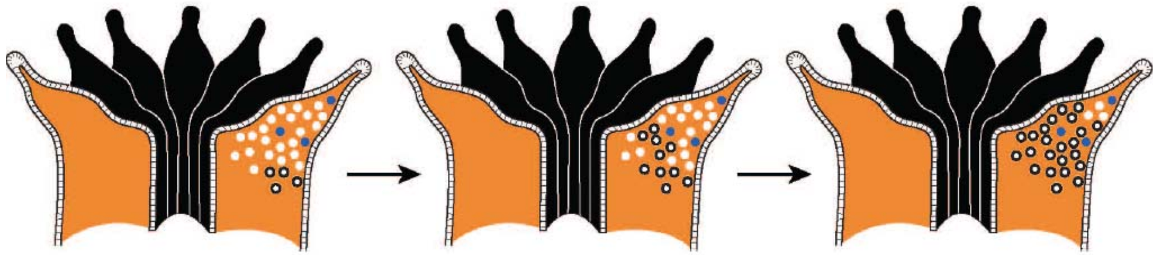

Fig. AII.1. **Cartoon of the spread of a beneficial mutation in a *Symbiodinium* community *in hospite*.**

The blue and white circles represent two wild-type *Symbiodinium* types, the white circles with a black border represent mutant *Symbiodinium* cells which have evolved from the white type. The mutant cells are under positive selection and increase their relative abundance over time (from left to right).

AII.2. Somatic mutations

Somatic mutation rates are consistently greater than germ line mutation rates, both on a per cell division basis as well as on a per generation basis (Lynch 2010). For example, tissue assays of mouse and rat show that somatic cells accumulate two- to six-fold more mutations than germ cells in the testes by the time of sexual maturity, and in humans the average mutation rate in four somatic cell types is 17 times higher than in the germ line (Lynch 2010). In organisms that reproduce sexually and where the differentiation between somatic and germ line cells (i.e., germ line cell sequestration) occurs early in ontogeny, a clear evolutionary difference exist between a somatic and a germ line mutation, with the former mostly not being heritable (unless the mutation arises during the first few cell divisions prior to germ line cell sequestration) and hence having no impact on the fitness of future generations. In contrast, corals and many other modular marine invertebrates, as well as many plants, lack segregation of germ line and somatic cells (Buss 1983). In these organisms**,** germ line cell differentiation occurs continuously from somatic stem cells, resulting in a higher probability of somatic mutations becoming incorporated in coral gametes than in organisms with germ-soma segregation. This means that somatic mutations can provide a substrate for selection and form the basis of adaptation. In humans, for instance, the number of cell divisions from the fertilised egg to formation of the female gamete is only ~24 (Crow 1993). Sperm

produced in adolescents at the age of 13 has undergone ~36 cell divisions, followed by ~23 cell divisions per year (Crow 1993). Yet in corals, innumerable cell divisions occur before gametes are formed, both over the life time of a coral colony or between episodes of sexual reproduction (typically a year in broad-cast spawning corals). In essence, mutant cell lineages that arise though somatic mutations are legitimate units of selection, either where a somatic cell lineage undergoes a large number of divisions before sequestering the germ line or through asexual reproduction by fragmentation, budding or other means (see below; Rinkevich and Matranga 2009). Thus, in corals, not only gametic mutations, but also somatic mutations can eventually be represented in gametes and hence can be passed on to the next generation.

AII.3. Evolution through cell lineage selection in organisms other than corals

The importance of somatic mutations in evolution and adaptation is well recognised for some organisms and life forms, such as prokaryotes and viruses (reviewed in Lushai *et al.* 2003), but is often ignored in eukaryotic taxa (but for examples see Fagerstrom *et al.* 1998; Pineda-Krch and Fagerstrom 1999; Monro and Poore 2004,2009). However, asexuality is found in over half of all eukaryotic phyla (Buss 1983; Fagerstrom *et al.* 1998); many higher plants, algae and animals are exclusively or largely clonal, including a range of species of aphids, cladocerans, freshwater snails, bryozoans and corals (reviewed in Gill *et al.* 1995). Some asexual animal species are believed to be ancient, including darwinulid ostracods (100 Myr; Butlin *et al.* 1998), the brine shrimp *Artemia salina* (30 Myr; Perez *et al.* 1994), bdelloid rotifers (at least 35-40 Myr; Welch and Meselson 2000; Welch and Meselson 2003), the weevil, *Aramigus tessellates* (2 Myr; Normark 1996) and salamanders of the genus *Ambystoma* (~4–5 Myr; Hedges *et al.* 1992). These organisms have evolved through somatic mutation and selection in the absence of sex (Welch and Meselson 2000). In support of this notion, variance in many anatomical traits, karyotype, genetic markers and fecundity have been observed in natural populations of asexual aphid species (reviewed in Loxdale and Lushai 2003). Furthermore, cell lineage selection was observed in asexual (apomictic) peach potato aphids, which led to an approximately 30-fold increase in the level of esterase production (which confers resistance to pesticides) after only 14 asexual generations under laboratory selection (Bunting and Vanemden 1980). Intra-clonal heritable genetic

variation is also present in the red seaweed species *Asparagopsis armata* and *Delisea pulchra* (Monro and Poore 2004), and significant responses to cell lineage selection have been documented in the former (Monro and Poore 2009). Moreover, somatic mutations have been documented in long-lived trees (Wittlieb *et al.* 2006; Ally *et al.* 2008), and many plants with long generation times are well able to keep up the arms race with their short-lived pests (Gill *et al.* 1995), further supporting that evolution can be fast in organisms with infrequent sexual reproduction. Microalgae, including dinoflagellates, undergo spontaneous genetic mutations in culture at a detectable rate, some of which are under positive selection. For example, genetic adaptation to increased nutrients and temperature was demonstrated in the asexually growing dinoflagellate *Prorocentrum triestinum* (Flores-Moya *et al.* 2008). The chlorophycean, *Scenedesmus intermedius*, showed the occurrence of beneficial spontaneous mutations at the rate of 2.12×10^{-5} mutants per mitotic cell division when grown under stress from acid wastes rich in heavy metals/metalloids (Baos *et al.* 2002). The chlorophycean *Dictyosphaerium chlorelloides* produced mutants resistant to various environmental stressors, including low pH, TNT, and heavy metals, at a rate of $\sim 1 \times 10^{-5}$ to $\sim 1 \times 10^{-7}$per cell per generation (Garcia-Villada *et al.* 2002; Costas *et al.* 2007; Lopez-Rodas *et al.* 2008; Lopez-Rodas *et al.* 2009). The studies listed are only a small subset of the many known examples of detectable somatic mutations and the evolution through selection on somatic mutations, but demonstrate that evolution occurs and can be fast in the absence of sex.

AII.4. Asexuality in corals and their associated *Symbiodinium*

Asexual reproduction is a key attribute of colonial corals that may facilitate the proliferation of localised mutation within colonies and subsequently propagation as new independent colonies. A number of different modes of asexual reproduction are recognised in reef building corals (reviewed in Wells 1956; Cairns 1988; Borneman 2006; Harrison 2011). Growth within colonies typically requires production of the modular polyp units via extratentacular budding (polyps forming from tissues adjacent to or in between existing polyps), or intratentacular budding (polyps forming by internal division of existing polyps). Subsets of these polyps can further proliferate into distinct clonal colonies via fragmentation (portions of the colony breaking off or becoming physically separated from the rest of the colony). In branching corals, where new

branches originate as single apical polyps buds off from radial polyps, fragmentation can be the dominant mode of colony propagation (Sammarco 1982). This mode of asexual reproduction has also been documented in massive species (Highsmith 1982). Other less well known forms of asexual reproduction in reef building corals include transverse division (polyps forming within the tentacular ring, also known as "strobulation", exhibited in many milleporinids; reviewed in Cairns 1988), asexual planulae (planulae genetically identical to parent polyps, exhibited by *Pocillopora damicornis*, several species of *Tubastrea* and *Oulastrea crispata*; reviewed in Harrison 2011), polyp extrusions (extensions of tissue which flow out from corallites and attach to the substrate, exhibited by at least 20 species in 9 families, including Acroporidae, Favidae, Pocilloporidae and Agariciidae; reviewed in Borneman 2006), polyp bailout (expulsion or detachment and relocation of individual polyps from the skeleton, often due to stress, exhibited by several pocilloporids, *Acropora* sp., *Euphillia* sp., *Favia favus* and *Oculia Patagonia*; Sammarco 1982; KramarskyWinter *et al.* 1997; Borneman 2006), and polyp balls (growth of round protrusions of polyps and skeleton on the surface or margins of some *Goniopora* and faviid species; Rosen and Taylor 1969; Borneman 2006). Almost all these modes of asexual reproduction result from a single polyp, making the propagation of a somatic mutation in one polyp into a whole new colony a distinct possibility (Fig. AII.2: C, D). The diversity of sexual and asexual reproductive modes displayed by corals may provide them with adaptive mechanisms similar to those in some plants, where mosaicism can play a significant role in adaptation (O'Connell and Ritland 2004). In some species, such as the massive *Porites*, individual colonies can live and reproduce for centuries, growing indeterminately through asexual budding, and consequently producing large colonies of several million polyps. In such long lived species, there is a high probability that somatic mutations will be preserved and accumulated.

Knowledge on new polyp formation in cnidarians is primarily derived from pioneering work conducted on the model coelenterate *Hydra*. In the early twentieth century, Tannreuther (1909) described in detail how new buds commence with an increase in volume and division of cells in the wall of the parent *Hydra*. Once these cells have increased in volume once or twice, the ectoderm bulges out. Cells in this bulge become differentiated into two regions: the central part which becomes the distal end of the new polyp where cells become dormant and the region on either side in which the cells

divide rapidly and contribute almost entirely to the growth of the bud (Fig. AII.2: A). Since only one or two cells on either side of the distal end of the bud are involved in growth, any mutation within these cells has a high probability of making up the majority of the new polyp. More recently, work conducted in the Thomas Bosch laboratory has shown that in addition to the involvement of cells at the site formation of the new bud, endodermal cell motility can be involved in bud formation (Bosch 2009; Venera-Ponton *et al.* 2010). Transgenic cells expressing a green fluorescent protein were observed to migrate to distant sites within the polyp (including the site of formation of the new bud), and oriented cell division of the transgenic cells occurred during the migration process (Wittlieb *et al.* 2006) Thus, not only mutant cells at the site of polyp formation but also mutant cells arisen elsewhere in the body may be incorporated in the newly budded polyp (Kenyon 1997; Fig. AII.2: B).

Corals acquire *Symbiodinium* either from the environment as larvae or primary polyps (~85% of species; Richmond 1997) or via parental transmission to eggs, larvae or asexual propagules. *Symbiodinium* cells occupy vacuoles within the coral gastroderm, maintaining large population sizes in the order of 10^6 cells per cm^2 of coral tissue, which vary in healthy coral symbioses from 0.5 to 9 x 10^6 cells per cm^2 among species (Drew 1972; Kawaguti and Nakayama 1973) and in response to environmental parameters (Dustan 1979; Falkowski and Dubinsky 1981; McCloskey and Muscatine 1984; Stimson 1997; Fagoonee *et al.* 1999). *Symbiodinium* also forms symbioses with other invertebrate and protist hosts (reviewed in Coffroth and Santos 2005) and occurs free-living in various habitats including reef sediments (>10^3 cells per cm^3), seawater (~10^2 cells per cm^3; Littman *et al.* 2008) and the surface of benthic macroalgae (Porto *et al.* 2008). Populations are maintained by binary fission and it is not known whether sexual reproduction exists within the *Symbiodinium* life cycle (LaJeunesse 2001). *Symbiodinium* cells in the process of dividing account for 0.4 to 12% of the *in hospite* population (Muscatine *et al.* 1984; Hoegh-Guldberg *et al.* 1987; Wilkerson *et al.* 1988) corresponding to generation times of 3 to 74 days, with ~7 days being common in most corals (Wilkerson *et al.* 1988). *Symbiodinium* growth is inversely related to density, with the fastest rates observed in new host tissue (e.g. the tips of coral branches; Wilkerson *et al.* 1988) and in the re-population of bleached tissue (Fitt *et al.* 1993; Jones and Yellowlees 1997). Growth is regulated by the rate of carbon fixation and

utilization (Stat *et al.* 2008; Cantin *et al.* 2009) and the amount of nutrients provided by the host (Cook *et al.* 1988; Titlyanov *et al.* 1996), with further regulation of population sizes *in hospite* by host digestion and extrusion of *Symbiodinium* cells (Muscatine and Pool 1979; Titlyanov *et al.* 1996). In free-living habitats, growth rates of *Symbiodinium* are not known, but generation times in the order of days to weeks have been recorded for other species of free-living dinoflagellates (Wilkerson *et al.* 1988). In culture, *Symbiodinium* are capable of rapid growth with typical generation times of less than 3 days (Fitt and Trench 1983; Kinzie *et al.* 2001; Orive 2001; Taguchi and Kinzie 2001), although some strains grow more slowly (Purkis *et al.* 2011). The asexual mode of reproduction and large *in hospite* population sizes suggest that somatic mutations are likely to be an important source of novel genetic variation for *Symbiodinium*. The fitness effects of mutation are generally more rapidly expressed in haploid organisms, such as *Symbiodinium*, as the impact of the mutation is not confounded by the wild-type allele of the homologous chromosome as is the case in diploid organisms (i.e. heterozygous state).

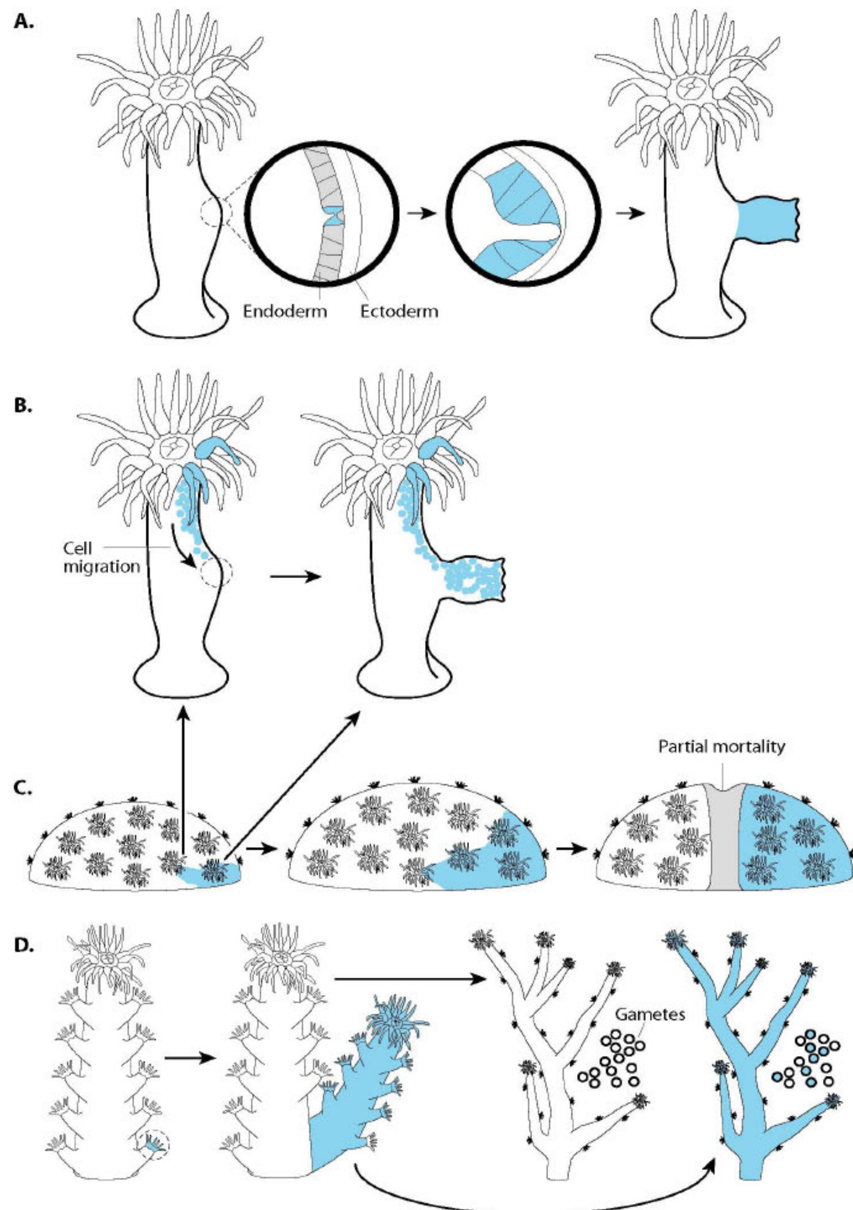

Fig. AII.2. Cartoon showing several ways in which mutant cells (shown in blue) can spread in corals

A) Coral polyp with a mutation arisen in a cell of the endodermis (=gastrodermis) at the site of new polyp formation. As the budding process progresses, the mutated cell divides to form a mutant cell lineage which constitutes the endoderm of the newly budded polyp. B) Mutant cells arisen in the upper part of the coral polyp migrate to some of the polyp's tentacles as well as into the budding polyp. As the budding process progresses, most of the endodermal cells of the newly budded polyp are of the mutant genotype. C) Massive coral colony in which a mutation has arisen and spreads as the colony grows. At some point in time, partial colony mortality divides the colony into two physically separated units that are of different genotypes (wild-type and mutant). D) Branching coral in which a mutated branch brakes off and re-establishes itself. The wild-type 'mother' colony produces wild-type gametes only, while the mutant colony produces both wild-type and mutant gametes as corals are diploid (Kenyon 1997) and only half of the gametes in a heterozygous individual will carry the mutation following meiosis.

AII.5. Are somatic mutations fuel for adaptation of corals?

The evidence summarized above from a wide range of partially or fully asexual organisms, indicates that evolution through somatic mutations and cell lineage selection in corals and associated *Symbiodinium* is likely to be an important mechanism for adaptation to climate change. Figures AII.3: A-D show field corals displaying intra-colony phenotypic variation. It is hypothesised that such variation may in some instances reflect mosaicism within the host and/or its associated *Symbiodinium* population, but this remains to be tested. Based on measurements from a coral population of *Acropora millepora* in the southern GBR, it is estimated that ~100 million somatic mutations can arise within a single coral colony with a diameter of 30 cm (Table AII.1). This estimate is based solely on the number of cells that constitute a colony of that size, and ignores cell renewal because it is not known whether cell renewal takes place in corals. In *Hydra*, cells are continuously being renewed and each individual cell has a relatively short life span (Bosch 2009). A 30 cm *A. millepora* colony contains approximately half a million polyps, each of which spawns on average 5 eggs (Orive 2001) and a vast number of sperm cells, hence, there is considerable potential for somatic mutations to be passed on to the next sexual generation (Fig. AII.2: D). Further, somatic mutations may be passed on to asexually produced larvae or to other polyps and colony fragments (Fig.AII.2: A-D). The vast majority of spontaneous mutations, however, are likely to be selectively neutral, i.e., have no impact on the phenotype or fitness, and the persistence of cells carrying a non-neutral mutation will depend the strength of selection for that mutation (Otto and Hastings 1998). Nevertheless, estimates of beneficial mutations under extreme experimental stress in unicellular algae are astonishingly high and only 100-10,000 fold smaller than the estimates for the combined neutral and non-neutral somatic mutations in coral (10^{-5}-10^{-7} per cell generation, Table AII.1). Climate change is predicted to result in an increased frequency of extreme weather events (Jones 2008), which act as strong selective forces on coral and *Symbiodinium* populations. Mosaicism within the coral host tissues or *Symbiodinium* population may result in different levels of environmental tolerance across a single coral colony and it is well documented that partial rather than whole colony mortality is often observed after severe bleaching events (Jones 2008).

Coral bleaching events reduce *in hospite Symbiodinium* densities to in some cases as low as 10 % of the pre-bleaching population in coral colonies that survive the stress event (e.g. Hoegh Guldberg *et al.* 1987; Jones and Yellowlees 1997; Jones 2008). Remaining *Symbiodinium* cells may represent individuals that resisted bleaching because they were occupying microhabitat refuges (e.g., shaded branches) or they may represent genotypes that were better adapted to the bleaching stressor, e.g. elevated temperature. On repopulating the coral's tissues during recovery, the adapted genotype is likely to spread and increase in numbers. Such shifts have been documented for different *Symbiodinium* types (some even belonging to different clades) under natural (Jones *et al.* 2008) and experimental (Ulstrup *et al.* 2006) field conditions following severe bleaching, but may have gone undetected if mutant cells better adapted to heat stress were present within a *Symbiodinium* type. In addition to extreme events, climate change is expected to cause a gradual and persistent increase in mean seawater temperatures (Allison *et al.* 2009) and this is a continuous selective force which will push coral and *Symbiodinium* populations towards increased thermal tolerance if these have the ability to evolve in that direction. Field observations of reduced impact of thermal stress events on coral communities in recent years compared with earlier thermal stress events of the same or lesser severity, suggest that adaptation may already have occurred on some reefs through selective mortality of the less tolerant (host and/or *Symbiodinium*) genotypes (Maynard *et al.* 2008b; Middlebrook *et al.* 2008; Berkelmans 2009). Whether this involved selection on recently evolved (i.e., mutant) genotypes is, however, unknown.

At the population level, the maintenance of genetic diversity is a balance between random genetic drift, migration and natural selection, which either increase or decrease the prevalence of any particular mutation within a population or species (Hartl and Clark 1997). For functional genetic divergence to occur between populations (i.e., local adaptation), the intensity of selection on a certain adaptive trait or beneficial mutation needs to be greater than the combined effects of random genetic drift and migration (Beaumont and Balding 2004). The emerging picture on levels of connectivity of both coral hosts and *Symbiodinium* is that gene flow is relatively restricted for most species of host and symbiont, i.e., that most populations are largely self-seeding (Hughes *et al.* 1992; Frankham 1995; Kirk *et al.* 2009), at least over the time scales relevant to adaptation to climate change impacts. This will facilitate local adaptation as 'dilution'

through input of less adapted alleles from outside the population is likely to be low. Drift becomes more important with decreasing N_e, and low frequency alleles (such as new mutant alleles) have a high probability of being lost in populations with small N_e. Effective population sizes (N_e) of corals are likely significantly smaller than their census sizes (Wallace 1985; Loya and Sakai 2008), because (1) populations are known to greatly fluctuate in size due to a range of perturbations, including crown-of-thorn outbreaks, storms and cyclones, extreme wet seasons and bleaching events (Sweatman *et al.* 2011), (2) fecundity in corals is highly variable (Hoegh Guldberg *et al.* 1987), (3) asexual reproduction is common in corals (Harrison 2011), and (4) in gonochoric species, sex-ratios may be biased (e.g., Hoegh Guldberg *et al.* 1987). No estimates of N_e exist for common species of coral, and the role of drift on the likelihood of a mutant genotype to establish itself within a population is therefore virtually impossible to assess. This is further complicated by the occurrence of asexual reproduction in most coral species. Other factors that affect the likelihood of a new mutation spreading through a population have been elucidated through a recent model of co-evolutionary interactions between the coral host and associated *Symbiodinium* (Day *et al.* 2008). This model predicts that bleaching resistance alleles spread more quickly through the population when bleaching results from death of the coral-*Symbiodinium* partnership rather than expulsion of *Symbiodinium* without death, because the death of the partnership is more efficient at removing the disfavoured allele from the coral host and *Symbiodinium* populations (Day *et al.* 2008) This finding would extend to partial mortality of tissues with less adapted genotypes within a mosaic colony. The way in which the genomes of the two symbiotic partners interact and the trade-offs between fitness traits (such as thermal tolerance and growth rate) were also found to have a considerable effect on the speed of the spread of resistance alleles. Further, in species that have to acquire *Symbiodinium* from the environment each generation (~85% of all species (Richmond 1997), the spread of *Symbiodinium* alleles will be affected by the force and direction of selection *in* and *ex hospite*, which need not be the same, and the extent of gene flow between the *in hospite* and free-living *Symbiodinium* populations.

Table AII.1. Estimates of the number of somatic mutations arising in coral and *Symbiodinium* cells.

Estimates are for a median size *Acropora millepora* colony based on the number of cells within the colony (i.e., not taking into account cell renewal), using data from a small fringing reef in the southern Great Barrier Reef (Miall Island). Note that estimate of all spontaneous somatic mutations are presented for the coral host, but an estimate of only beneficial and phenotypically observable somatic mutations for *Symbiodinium*. The reason for this is that the latter is based on estimates for free-living unicellular algae, for which mutation rates have been estimated through laboratory selection experiments under various simulated environmental stress conditions, and mutants were phenotypically identified.

Attribute	**Estimate of variable**	**Reference**
Median colony diameter (cm)	30	E. Puill-Stephan, pers. comm.
No. branches/30 cm colony	400	E. Puill-Stephan, pers. comm.
Surface area/branch (cm^2)	13	R. Berkelmans, unpubl.
Surface area/30 cm colony (cm^2)	400 x 13 = 5200	
No. *Symbiodinium* cells/cm^2	1.5×10^6	R. Berkelmans, unpubl. and (Purkis *et al.* 2011)
No. *Symbiodinium* cells/30 cm colony	$1.5 \times 10^6 \times 5200 = 7.8 \times 10^9$	
Ratio of *Symbiodinium*/host cells	0.15	(Purkis *et al.* 2011)
No. coral host cells/30 cm colony	$7.8 \times 10^9/0.15 = 5.2 \times 10^{10}$	
Coral host somatic mutation rate per cell generation and genome (assuming ~2×10^4 genes in corals)	2×10^{-3}	(Orive 2001; Technau *et al.* 2005)
No. somatic mutations arising in coral cells in 30 cm colony	$10.4 \times 10^7 \cong 10^8$	
Symbiodinium beneficial and phenotypically observable somatic mutation rate per cell generation	10^{-5} - 10^{-7}	(Baos *et al.* 2002; Garcia-Villada *et al.* 2002; Costas *et al.* 2007; Lopez-Rodas *et al.* 2008; Lopez-Rodas *et al.* 2009)
No. beneficial mutant *Symbiodinium* cells in 30 cm colony	$7.8 \times 10^2 - 7.8 \times 10^4 \cong 10^3 - 10^5$	

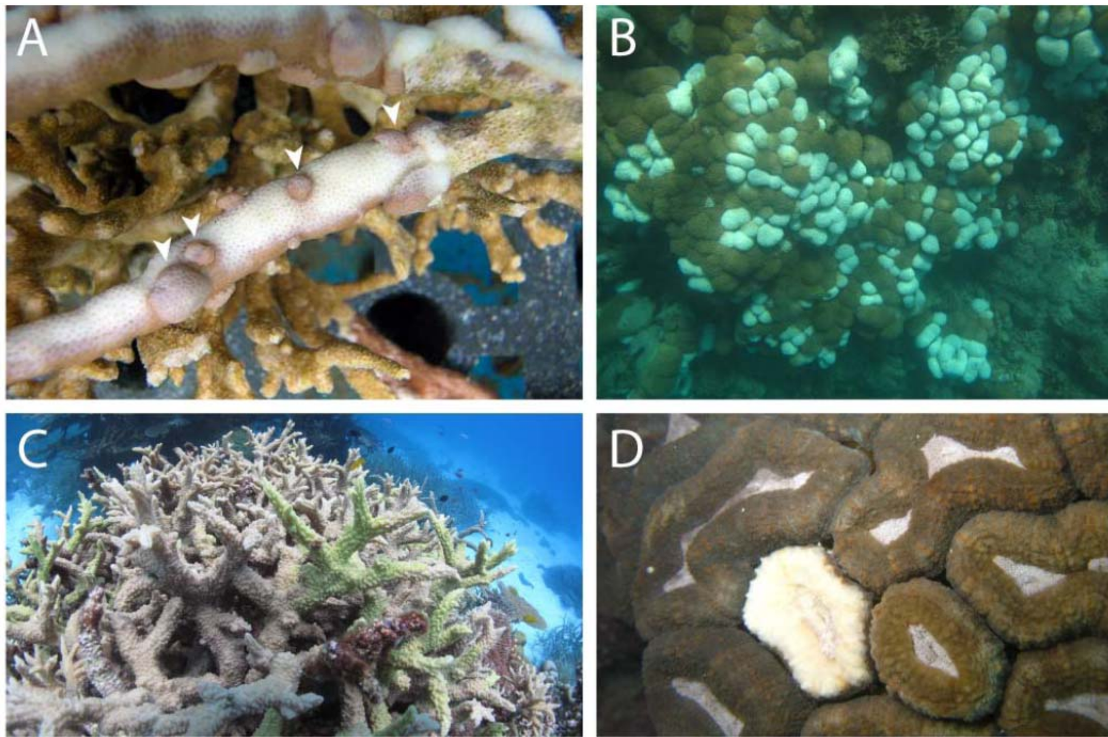

Fig. AII.3. Field examples of intra-colony phenotypic variation in reef corals.

A: “Tumour”-like structure on a *Montipora* branch that has not bleached while the main branch has (photo: Andrew Heyward). B: Healthy colony of *Lobophyllia hemprichii*, showing one severely bleached polyp (photo: Emily Howells). C: Differential expression of coral host pigments across a *Hydnophora rigida* colony (photo: Ray Berkelmans). D: Patchy bleaching across a *Goniopora* sp. Colony (photo: Emily Howells).

AII.6. Conclusions

Corals possess a set of attributes that theoretically provides them with considerable adaptive potential through somatic mutations and selection thereon. Many species show extensive asexual reproduction, indeterminate growth, asexual regeneration following partial colony mortality, and longevity, resulting in many mitotic cell divisions and hence, opportunities for somatic mutations to arise within an individual. All coral species lack germ cell lines. Instead, germ cells arise from somatic cells, and therefore, many cell divisions take place prior to the formation of gametes. The model cnidarian, *Hydra*, is believed to integrate foreign genetic material relatively easily into its genome (Steele *et al.* 2004; Habetha and Bosch 2005), and has been shown to readily accept and incorporate mutant cells into its body (Wittlieb *et al.* 2006). If these traits are also

present in corals, they will facilitate cell lineage selection. All reef-building corals form an intimate symbiosis with *Symbiodinium*, which to a large extent determines the coral's physiological tolerances. *Symbiodinium* also have attributes that promote adaptation, such as large population sizes and rapid turn-over rates. Baskett *et al.*'s (2009) model of symbiont evolutionary dynamics predicts that some level of adaptation within the following decades is possible in the presence of either genetic or community-level variation in *Symbiodinium* thermal tolerance, however, these model outcomes have not yet been experimentally validated. It is proposed that future work should focus on examining the response of *Symbiodinium* and possibly coral tissues to selection in the laboratory (e.g., Huertas *et al.* 2010). Recent laboratory selection experiments on 12 unicellular algal species (11 phytoplankton species and *Symbiodinium* sp.) have shown that adaptation to increased temperatures is possible within 8–150 generations, with the extent of the increase in temperature tolerance and the rate at which adaptation occurred varying among species (Huertas *et al.* 2011). Whole genome sequencing of cells/tissues prior to and following the *in vitro* selection experiment may reveal the mutations responsible for the adaptive response.

AII.7. References

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Appendix III. Supplementary data

AIII.1. Supplementary data for Chapter 2

Table AIII. 1. Microsatellite allele frequencies for *Symbiodinium* C2 populations hosted by *Acropora millepora* at the Palm Islands, Great Barrier Reef.

Alleles at each locus (*SymC_3-02*, *SymC_3-04*, *C1.01*, *C1.02*, *C1.05*, *C1.07*, *C1.15*, *C2.08*) are indicated by the number of base-pairs.

Continued on following pages

	1997		2004			2009								
	P1	O1	P1	O1	F1	F1	P2		O2		O3		O4	
	N	N	N	N	N	N	N	B	N	B	N	B	N	B
C 3-02														
132	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-
135	-	-	0.01	-	-	-	-	-	-	-	0.07	-	-	-
138	0.05	0.03	-	-	-	0.05	-	-	-	0.02	-	-	-	-
141	0.47	0.49	0.48	0.49	0.50	0.49	0.49	0.46	0.51	0.50	0.45	0.49	0.50	0.50
144	0.47	0.49	0.49	0.49	0.50	0.46	0.51	0.53	0.49	0.48	0.41	0.47	0.47	0.50
147	-	-	-	-	-	-	-	-	-	-	0.07	0.04	0.03	-
157	-	-	0.01	0.01	-	-	-	-	-	-	-	-	-	-
C 3-04														
123	0.44	0.45	0.46	0.45	0.52	0.47	0.39	0.42	0.43	0.38	0.55	0.51	0.51	0.47
126	0.50	0.42	0.38	0.41	0.34	0.42	0.43	0.44	0.45	0.47	0.27	0.35	0.43	0.42
132	-	-	0.02	0.02	-	-	0.06	-	0.02	0.02	-	0.02	-	-
216	-	-	0.01	0.01	0.02	0.03	-	-	-	-	-	-	-	-
219	-	-	0.02	0.03	0.01	-	0.06	0.05	0.02	-	0.05	0.03	0.03	0.05
222	-	-	-	-	-	-	-	-	-	-	0.05	0.05	-	0.02
225	0.06	0.12	0.10	0.07	0.10	0.08	0.06	0.09	0.09	0.13	0.09	0.05	0.03	0.05
C1.01														
118	-	-	0.06	0.05	-	0.01	-	0.02	0.04	-	-	-	-	-
121	-	-	0.18	0.16	0.22	0.13	0.23	0.24	0.17	0.24	-	-	-	-
127	-	-	-	0.01	0.01	0.03	0.01	0.02	0.01	0.02	-	-	-	-
133	-	-	-	0.01	0.01	-	0.01	-	-	-	-	-	-	-
142	-	-	-	-	0.01	-	-	0.01	-	-	0.07	0.14	0.09	0.10
151	-	-	-	0.01	0.02	-	0.02	0.01	0.01	0.01	-	-	-	-
160	-	-	-	-	-	-	-	0.01	-	-	-	-	-	0.03
163	-	-	-	-	-	-	-	-	-	-	-	0.02	-	0.03
166	-	-	-	-	0.01	-	-	0.03	-	-	-	-	-	0.03

169	-	-	-	-	-	-	-	-	-	-	-	-	-	-
172	-	-	0.02	0.02	0.01	0.03	-	0.02	-	-	-	-	-	-
175	-	-	-	-	-	-	-	-	-	-	-	-	0.05	-
178	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03
181	-	-	0.01	-	-	-	-	0.01	-	-	-	-	-	-
187	-	-	-	-	-	-	-	-	0.01	0.02	-	-	-	-
202	-	-	-	0.01	-	-	-	-	0.01	-	-	-	-	-
214	-	-	-	-	-	-	-	-	0.01	-	-	-	0.05	-
217	-	-	0.06	0.06	0.13	0.17	0.20	0.24	0.22	0.25	-	-	-	-
220	-	-	-	-	-	-	-	-	-	-	-	-	-	-
223	-	-	0.07	0.06	0.05	0.07	0.06	0.11	0.02	0.07	-	-	-	-
226	-	-	-	-	-	-	0.06	0.05	0.01	0.01	-	-	-	0.06
229	0.35	0.44	0.28	0.27	0.25	0.26	0.18	0.09	0.23	0.17	-	-	-	-
232	0.18	0.11	0.02	0.03	0.01	0.01	0.01	-	-	0.01	0.03	-	-	0.10
235	0.29	0.33	0.25	0.06	0.02	0.03	-	-	0.01	0.01	-	-	-	-
238	0.12	0.11	0.05	0.25	0.20	0.26	0.20	0.13	0.24	0.19	0.47	0.56	0.77	0.55
241	0.06	-	-	0.01	0.05	-	0.01	-	0.01	0.01	0.37	0.29	0.05	0.06
244	-	-	-	-	-	-	-	0.02	-	-	0.07	-	-	-
C1.02														
115	-	-	0.00	0.02	0.05	0.05	0.06	0.09	0.04	0.08	0.01	0.03	0.01	0.03
118	0.07	0.05	-	-	-	-	-	-	-	-	-	-	-	-
121	0.14	0.13	0.04	0.02	0.11	0.14	0.14	0.14	0.14	0.13	0.10	0.10	0.07	0.06
124	-	-	-	-	0.00	0.01	-	0.01	-	-	-	-	-	-
127	0.16	0.18	0.24	0.25	0.18	0.14	0.15	0.13	0.16	0.14	0.21	0.20	0.21	0.09
130	0.14	0.18	0.22	0.25	0.18	0.14	0.14	0.12	0.16	0.14	0.19	0.19	0.21	0.63
133	0.04	0.01	0.05	-	-	-	-	-	-	-	0.01	0.01	-	-
136	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-
145	-	-	-	-	-	-	-	0.01	-	0.01	-	0.01	-	-
148	-	-	-	-	0.01	0.01	-	0.01	-	0.01	-	-	-	0.01
157	-	-	-	-	-	-	-	0.01	0.01	0.01	-	0.01	-	-
160	0.09	0.05	-	0.01	0.08	0.13	0.12	0.12	0.11	0.13	0.03	0.07	0.03	0.03
163	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-
166	0.04	0.02	-	-	0.01	0.10	0.04	0.01	0.03	0.02	-	0.01	-	-
C1.05														
267	-	-	-	0.01	0.02	-	-	-	-	-	-	0.01	-	-
271	-	-	-	-	0.01	-	-	0.02	0.01	0.01	0.01	0.01	0.02	0.00
275	0.16	0.18	0.23	0.23	0.17	0.14	0.14	0.13	0.16	0.15	0.21	0.19	0.21	0.07
279	-	-	-	-	0.01	0.01	0.03	0.06	0.01	0.02	0.01	0.01	0.01	-
283	0.16	0.18	0.22	0.22	0.18	0.14	0.15	0.12	0.16	0.15	0.19	0.19	0.21	0.07
287	-	-	-	-	0.00	-	0.04	0.03	-	0.01	-	0.01	-	0.01
C1.07														
131	0.16	0.17	0.16	0.18	0.16	0.16	0.20	0.22	0.19	0.29	-	0.01	0.02	-
134	-	0.01	0.01	0.01	-	-	-	0.01	0.03	0.05	-	-	-	-

140	-	-	0.02	-	-	-	-	0.01	-	-	-	-	-	-
143	0.14	0.15	0.12	0.13	0.10	0.15	0.17	0.21	0.17	0.20	-	-	-	0.02
149	-	-	0.00	-	-	-	0.02	0.04	0.01	-	-	-	-	-
152	-	-	-	0.01	-	-	-	0.01	-	-	-	-	-	-
158	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-
179	0.11	0.06	0.04	0.04	0.04	0.02	0.13	0.08	0.10	0.14	-	-	-	-
188	-	-	0.00	0.02	0.05	0.04	0.06	0.08	0.01	-	-	0.01	0.02	0.05
191	-	-	-	-	0.01	0.01	0.01	0.07	-	-	-	-	-	-
218	-	-	0.01	-	0.00	-	-	0.01	0.01	-	-	-	0.02	0.02
221	0.18	0.15	0.15	0.20	0.21	0.20	0.11	0.05	0.13	0.05	0.28	0.30	0.28	0.25
224	-	0.05	0.03	-	0.01	-	-	0.01	0.02	-	0.02	0.03	0.05	0.02
227	-	-	0.00	-	-	-	-	-	-	-	0.02	0.02	-	-
230	0.20	0.20	0.22	0.23	0.21	0.21	0.18	0.14	0.20	0.19	0.30	0.31	0.30	0.34
233	-	-	0.00	0.01	-	-	-	-	-	-	0.07	0.02	0.03	0.02
236	-	-	0.01	-	-	-	-	-	-	-	-	0.01	-	-
239	0.20	0.20	0.20	0.19	0.21	0.21	0.12	0.04	0.14	0.08	0.30	0.29	0.30	0.29
C1.15														
123	-	-	-	-	-	0.02	-	0.02	0.03	-	-	-	-	-
126	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-
129	-	0.03	0.05	0.18	0.02	-	-	-	-	-	-	-	-	-
132	0.03	0.07	0.08	0.08	0.10	0.06	-	-	-	0.02	-	-	-	-
135	-	0.03	0.03	0.07	0.04	0.02	0.02	0.02	-	0.02	-	-	-	-
138	0.06	0.03	0.06	0.10	0.10	0.09	-	0.02	0.02	-	-	-	-	-
141	0.10	0.08	0.06	0.04	0.03	-	-	-	0.05	0.05	-	-	-	-
144	-	-	0.01	0.04	0.02	-	-	-	-	-	-	-	-	-
147	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-
150	-	-	0.01	-	-	-	-	-	-	0.02	-	-	-	-
156	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-
210	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-
219	-	-	-	-	0.01	-	-	0.02	-	-	0.04	-	-	-
222	-	-	-	-	0.02	-	-	-	0.03	-	-	0.02	0.03	0.02
225	0.16	0.17	0.03	0.01	0.03	0.06	-	0.02	0.03	-	0.07	0.07	0.03	0.02
228	0.29	0.22	0.31	0.23	0.29	0.36	0.47	0.47	0.39	0.46	0.33	0.37	0.46	0.49
234	-	-	-	-	-	-	-	-	-	-	0.26	0.21	-	0.02
237	-	-	-	-	0.01	-	-	-	-	-	0.04	0.01	-	-
240	-	0.02	0.02	-	0.01	-	-	-	0.02	-	0.04	0.01	0.03	-
243	-	-	-	-	0.01	-	0.02	-	0.03	0.02	0.07	0.07	0.08	-
246	0.29	0.20	0.29	0.23	0.31	0.38	0.47	0.43	0.39	0.43	0.15	0.23	0.38	0.44
249	0.06	0.12	0.05	0.01	-	-	-	-	-	-	-	0.01	-	-
C2.08														
197	-	-	-	-	0.00	0.17	0.10	0.13	-	0.03	0.03	0.04	-	-
200	-	-	-	0.00	0.01	-	-	0.01	-	-	0.03	0.01	0.02	-
203	0.27	0.27	0.25	0.25	0.24	0.19	0.22	0.24	0.25	0.26	0.41	0.43	0.40	0.44

209	-	-	-	0.01	0.02	0.01	-	-	-	0.01	-	-	-	-
212	-	-	-	-	-	0.18	0.07	0.10	-	0.02	-	0.01	-	-
215	0.24	0.24	0.25	0.24	0.24	0.18	0.21	0.19	0.25	0.25	0.09	0.04	0.12	0.08
218	0.27	0.27	0.25	0.25	0.24	0.16	0.22	0.20	0.25	0.25	0.41	0.44	0.43	0.42
221	0.21	0.22	0.25	0.24	0.24	0.13	0.18	0.13	0.24	0.18	0.03	0.04	0.02	0.06
224	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-

AIII.2. Supplementary data for Chapter 3

Table AIII.2.

***Symbiodinium* ITS1 type(s) hosted by tagged colonies of *Acropora millepora* at Miall Island and experienced mortality following a bleaching episode at Miall Island on the Great Barrier Reef.**

Background *Symbiodinium* types were present in samples are indicated in parentheses. Data are from: Jones AM, Berkelmans R, van Oppen MJH, Mieog JC, Sinclair W (2008) A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. Proceedings of the Royal Society B-Biological Sciences 275: 1359-1365.

	***Symbiodinium* ITS1 type**		
Colony #	**Before bleaching**	**After bleaching**	**Coral mortality**
1	C2	C2 (D)	Partial
2	C2	D (C2)	Partial
3	C2	D (C2)	Partial
4	C2	C2 (D)	Partial
5	C2	D (C2)	Partial
6	C2	C2 (D)	Partial
7	C2	C2 (D)	Partial
8	C2	C2 (D)	None
9	C2	C2	Partial
10	C2	C2 (D)	Partial
11	C2	C2	Partial
12	C2	C2	Partial
13	C2	C2	Partial
14	C2	D (C2)	Partial
15	C2	C2	> 90%
16	C2	C2 (D)	Partial
17	C2	C2	Partial
18	C2	D (C2)	Partial
19	C2	C2	Partial
20	C2	C2	> 90%
21	C2	C2	Partial
22	C2	C2	Partial
23	C2	C2	Partial
24	C2	C2 (D)	None
25	C2	D (C2)	Partial
26	C2	C2	None
27	C2	C2	> 90%
28	C2	C2	None
29	C2	C2	None
30	C2	C2	Partial
31	C2	C2 (D)	None
32	C2	C2 (D)	Partial
33	C2	D (C2)	Partial

Table AIII. 3. **Microsatellite allele frequencies for *Symbiodinium* C2 harboured by *Acropora millepora* colonies before and after bleaching at Miall Island on the Great Barrier Reef.**

Alleles at each locus (*SymC_3-02*, *SymC_3-04*, *C1.01*, *C1.02*, *C1.05*, *C1.07*, *C1.15*, *C2.08*) are identified by their length (in base pairs).

C_3-02	132	135	138	141	144														
Before	0.42	0.03	0.03	0.47	0.05														
After	0.48	-	-	0.52	-														
C_3-04	124	127	133	145	148	220	226												
Before	0.42	0.41	0.05	0.02	0.03	0.05	0.03												
After	0.46	0.44	-	-	0.02	0.05	0.03												
C1.01	118	121	127	151	154	187	217	220	223	229	232	238	241	256	268				
Before	-	0.08	0.04	0.01	0.01	-	0.15	0.27	0.09	0.26	0.03	0.03	0.01	-	0.01				
After	0.11	0.04	-	0.02	-	0.07	0.09	0.32	0.01	0.31	0.02	-	-	0.01					
C1.02	115	121	127	130	133	136	148	160	163	166									
Before	0.11	0.23	0.17	0.18	0.01	0.01	0.03	0.18	0.01	0.06									
After	0.08	0.17	0.18	0.17	-	0.08	0.02	0.17	0.01	0.11									
C1.05	275	279	283	287															
Before	0.17	0.30	0.17	0.36															
After	0.02	0.48	0.02	0.49															
C1.07	130	133	144	148	154	157	160	178	187	193	205	214	217	220	223	229	235	238	241
Before	0.21	0.05	0.15	0.01	0.01	0.01	0.01	0.06	0.03	0.02	0.01	-	0.12	0.01	-	0.14	-	0.02	0.14
After	0.17	0.04	0.13	0.01	0.01	0.02	0.01	0.03	0.01	0.01	0.01	0.01	0.17	-	0.01	0.17	0.01	0.01	0.17
C1.15	129	135	141	219	225	228	231	234	240	243	246	252							
Before	0.01	-	0.05	0.02	0.01	0.31	-	0.14	0.03	-	0.28	0.15							
After	-	0.02	0.11	-	0.01	0.22	0.02	0.27	0.01	0.02	0.08	0.26							
C2.08	197	203	209	212	215	218	221												
Before	0.20	0.19	0.02	0.18	0.24	0.11	0.05												
After	0.25	0.21	0.02	0.24	0.27	-	-												

AIII.3. Supplementary data for Chapter 4

Table AIII. 4a. **Analysis of variance for comparisons of the maximum quantum yield of photosystem II for *Symbiodinium* C1 within coral juveniles.**

Comparisons were among different *Symbiodinium* C1 populations (Magnetic Island and South Molle Island), temperature treatments (27°C and 32°C), and sampling days (n = > 20 juveniles per comparison). P values in bold face indicate significance at $\alpha = 0.05$.

Source of variation	df	SS	MS	*F*	*P*
Population	1	3.756	3.756	452.3	**0.001**
Temperature	1	0.214	0.214	25.8	**0.001**
Day	6	1.146	0.191	23	**0.001**
Population x Temperature	1	0.576	0.576	69.4	**0.001**
Population x Day	6	0.324	0.054	6.5	**0.001**
Temperature x Day	6	0.426	0.071	8.5	**0.001**
Population x Temperature x Day	6	0.573	0.096	11.5	**0.001**

Table AIII. 4b. **Analysis of variance Newman-Keuls post-hoc *P* values for the maximum quantum yield of photosystem II for *Symbiodinium* C1 within coral juveniles.**

Comparisons were among *Symbiodinium* C1 from different populations (Magnetic Island, MI and South Molle Island, SM), temperature treatments (27°C and 32°C), and sampling days (1 to 11). P values in bold face indicate significance at $\alpha = 0.05$.

Following page

		Pop.	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI
		Temp.	27	27	27	27	27	27	27	32	32	32	32	32	32	32
		Day	1	2	3	5	7	9	11	1	2	3	5	7	9	11
MI	27	1														
MI	27	2	0.287													
MI	27	3	0.507	0.652												
MI	27	5	0.626	0.800	0.891											
MI	27	7	0.120	0.933	0.833	0.741										
MI	27	9	0.117	0.847	0.750	0.691	0.962									
MI	27	11	0.728	0.066	0.178	0.317	**0.015**	**0.016**								
MI	32	1	0.801	0.546	0.736	0.722	0.357	0.335	0.632							
MI	32	2	0.557	0.792	0.843	0.795	0.814	0.752	0.231	0.733						
MI	32	3	0.230	0.848	0.797	0.806	0.913	0.721	**0.044**	0.501	0.835					
MI	32	5	**0.001**	**0.025**	**0.008**	**0.003**	**0.018**	**0.042**	**0.001**	**0.001**	**0.006**	**0.028**				
MI	32	7	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.039**			
MI	32	9	**0.001**	**0.001**	**0.001**	**0.001**	**0.002**	**0.002**	**0.001**	**0.001**	**0.001**	**0.002**	0.217	0.229		
MI	32	11	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.000**	**0.001**	**0.001**	

		Pop.	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM
		Temp.	27	27	27	27	27	27	27	32	32	32	32	32	32	32
		Day	1	2	3	5	7	9	11	1	2	3	5	7	9	11
SM	27	1														
SM	27	2	0.081													
SM	27	3	0.476	0.324												
SM	27	5	0.366	0.721	0.689											
SM	27	7	**0.034**	0.905	0.198	0.682										
SM	27	9	0.072	0.966	0.284	0.574	0.966									
SM	27	11	0.382	0.712	0.652	0.827	0.622	0.612								
SM	32	1	0.320	**0.004**	0.202	0.056	**0.002**	**0.003**	0.072							
SM	32	2	0.388	0.616	0.745	0.913	0.652	0.371	0.942	0.055						
SM	32	3	0.102	**0.001**	**0.023**	**0.001**	**0.001**	**0.001**	**0.002**	0.402	**0.002**					
SM	32	5	0.272	0.774	0.408	0.960	0.658	0.705	0.957	0.055	0.981	**0.002**				
SM	32	7	**0.002**	0.663	**0.021**	0.245	0.627	0.734	0.181	**0.001**	0.252	**0.001**	0.188			
SM	32	9	**0.002**	0.671	**0.011**	0.188	0.756	0.713	0.128	**0.001**	0.206	**0.001**	0.128	0.960		
SM	32	11	0.149	**0.001**	**0.049**	**0.004**	**0.001**	**0.001**	**0.007**	0.384	**0.004**	0.676	**0.006**	**0.001**	**0.001**	

		Pop.	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM
		Temp.	27	27	27	27	27	27	27	32	32	32	32	32	32	32
		Day	1	2	3	5	7	9	11	1	2	3	5	7	9	11
MI	27	1	**0.006**	0.775	0.060	0.413	0.596	0.857	0.339	**0.001**	0.407	**0.001**	0.358	0.696	0.911	**0.001**
MI	27	2	**0.001**	**0.043**	**0.001**	**0.001**	0.103	**0.044**	**0.002**	**0.001**	**0.002**	**0.001**	**0.002**	0.466	0.466	**0.001**
MI	27	3	**0.001**	0.131	**0.001**	**0.007**	0.246	0.137	**0.004**	**0.001**	**0.010**	**0.001**	**0.003**	0.684	0.628	**0.001**
MI	27	5	**0.001**	0.263	**0.002**	**0.028**	0.382	0.284	**0.015**	**0.001**	**0.033**	**0.001**	**0.015**	0.743	0.459	**0.001**
MI	27	7	**0.001**	**0.008**	**0.001**	**0.001**	**0.029**	**0.008**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	0.259	0.324	**0.001**
MI	27	9	**0.001**	**0.009**	**0.001**	**0.001**	**0.029**	**0.009**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	0.247	0.297	**0.001**
MI	27	11	0.057	0.845	0.270	0.726	0.819	0.969	0.689	**0.002**	0.669	**0.001**	0.736	0.658	0.714	**0.001**
MI	32	1	**0.002**	0.606	**0.010**	0.172	0.650	0.662	0.119	**0.001**	0.184	**0.001**	0.120	0.808	0.977	**0.001**
MI	32	2	**0.001**	0.180	**0.001**	**0.013**	0.300	0.191	**0.007**	**0.001**	**0.017**	**0.001**	**0.006**	0.709	0.577	**0.001**
MI	32	3	**0.001**	**0.027**	**0.001**	**0.002**	0.073	**0.027**	**0.001**	**0.001**	**0.002**	**0.001**	**0.001**	0.407	0.440	**0.001**
MI	32	5	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
MI	32	7	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
MI	32	9	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
MI	32	11	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**

Table AIII. 5a. **Analysis of variance for comparisons of *Symbiodinium* cell density within coral juveniles.**

Comparisons were among *Symbiodinium* C1 from different populations (Magnetic Island and South Molle Island), temperature treatments (27°C and 32°C), and sampling days (*n* = > 4 juveniles per comparison). *P* values in bold face indicate significance at $\alpha = 0.05$.

Source of variation	**df**	**SS**	**MS**	***F***	***P***
Population	1	1.29E+07	1.29E+07	11.1	**0.002**
Temperature	1	4.21E+05	4.21E+05	0.36	0.551
Day	1	1.52E+05	1.52E+05	0.13	0.72
Population x Temperature	1	7.59E+06	7.59E+06	6.51	**0.015**
Population x Day	1	1.23E+05	1.23E+05	0.11	0.747
Temperature x Day	1	1.55E+06	1.55E+06	1.32	0.257
Population x Temperature x Day	1	5.26E+06	5.26E+06	4.51	**0.040**

Table AIII. 5b. **Analysis of variance Tukeys post-hoc *P* values for *Symbiodinium* cell density within coral juveniles.**

Comparisons were among *Symbiodinium* C1 from different populations (Magnetic Island, MI and South Molle Island, SM), temperature treatments (27°C and 32°C), and sampling days (-1 and 11). *P* values in bold face indicate significance at $\alpha = 0.05$.

		Pop.	MI	MI	MI	MI	SM	SM	SM	SM
		Temp.	27	27	32	32	27	27	32	32
Pop.	**Temp.**	**Day**	-1	11	-1	11	-1	11	-1	11
MI	27	-1								
MI	27	11	1.000							
MI	32	-1	1.000	0.979						
MI	32	11	0.967	0.795	1.000					
SM	27	-1	0.703	0.930	0.446	0.154				
SM	27	11	1.000	0.991	1.000	0.998	0.522			
SM	32	-1	0.783	0.959	0.539	0.220	1.000	0.614		
SM	32	11	0.091	0.241	**0.041**	**0.007**	0.901	0.054	0.901	

Table AIII. 6a. **Analysis of variance for comparisons of the diameter of coral juveniles.**

Comparisons were among juveniles hosting *Symbiodinium* C1 from different populations (Magnetic Island and South Molle Island), temperature treatments (27°C and 32°C), and sampling days (n = > 100 juveniles per comparison). P values in bold face indicate significance at $\alpha = 0.05$.

Source of variation	**df**	**SS**	**MS**	***F***	***P***
Day	2	0.774	0.387	18.52	**0.001**
Population	1	1.955	1.955	93.61	**0.001**
Temperature	1	0.038	0.038	1.83	0.176
Day x Population	2	0.936	0.468	22.41	**0.001**
Day x Temperature	2	0.002	0.001	0.06	0.942
Population x Temperature	1	0.998	0.998	47.78	**0.001**
Day x Population x Temperature	2	0.559	0.279	13.37	**0.001**

Table AIII. 6b. **Analysis of variance Tukeys post-hoc *P* values for comparisons of the diameter of coral juveniles.**

Comparisons were among juveniles hosting *Symbiodinium* C1 from different populations (Magnetic Island, MI and South Molle Island, SM), temperature treatments (27°C and 32°C), and sampling days (-2, 8 and 12). P values in bold face indicate significance at $\alpha = 0.05$.

		Day	- 2	- 2	- 2	- 2	8	8	8	8	12	12	12	12
		Pop.	MI	MI	SM	SM	MI	MI	SM	SM	MI	MI	SM	SM
		Temp.	27	32	27	32	27	32	27	32	27	32	27	32
- 2	MI	27												
- 2	MI	32	0.998											
- 2	SM	27	1.000	1.000										
- 2	SM	32	1.000	1.000	1.000									
8	MI	27	0.360	0.939	0.612	0.941								
8	MI	32	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**							
8	SM	27	1.000	1.000	1.000	1.000	0.948	**0.000**						
12	SM	32	0.565	0.099	0.581	0.338	**0.001**	**0.000**	0.283					
12	MI	27	**0.041**	0.391	0.129	0.477	0.998	**0.040**	0.486	**0.000**				
12	MI	32	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.998	**0.000**	**0.000**	**0.001**			
12	SM	27	0.314	0.866	0.515	0.869	1.000	**0.022**	0.880	**0.002**	1.000	**0.001**		
12	SM	32	0.646	0.149	0.652	0.407	**0.003**	**0.000**	0.351	1.000	**0.000**	**0.000**	**0.004**	

Table AIII. 7a. **Analysis of variance for comparisons of the proportion of dead cells in *Symbiodinium* C1 cultures.**

Comparisons were among different *Symbiodinium* C1 populations (Magnetic Island and South Molle Island) and temperature treatments (24, 27, 30, and 32°C), and sampling days (n = 6). P values in bold face indicate significance at $\alpha = 0.05$.

Source of variation	df	SS	MS	*F*	*P*
Population	1	2.233	2.233	14.21	**0.001**
Temperature	3	6.155	2.052	13.05	**0.001**
Day	3	1.297	0.432	2.75	**0.045**
Population x Temperature	3	1.623	0.541	3.44	**0.019**
Population x Day	3	1.646	0.549	3.49	**0.018**
Temperature x Day	9	3.658	0.406	2.59	**0.009**
Population x Temperature x Day	9	1.696	0.188	1.2	0.301

Table AIII. 7b. **Analysis of variance Tukeys post-hoc *P* values of the proportion of dead cells in *Symbiodinium* C1 cultures.**

Comparisons were among different *Symbiodinium* C1 from different populations (Magnetic Island and South Molle Island) and temperature treatments (24, 27, 30, and 32°C). P values in bold face indicate significance at $\alpha = 0.05$.

Continued on following page

		Pop.	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI
		Temp.	24	24	24	24	27	27	27	27	30	30	30	30	32	32	32	32
		Day	4	11	19	24	4	11	19	24	4	11	19	24	4	11	19	24
MI	24	4																
MI	24	11	1.000															
MI	24	19	0.989	1.000														
MI	24	24	1.000	1.000	1.000													
MI	27	4	1.000	1.000	0.622	1.000												
MI	27	11	1.000	1.000	1.000	1.000	0.996											
MI	27	19	1.000	1.000	1.000	1.000	0.992	1.000										
MI	27	24	1.000	1.000	0.996	1.000	1.000	1.000	1.000									
MI	30	4	1.000	1.000	0.699	0.999	1.000	0.996	0.993	1.000								
MI	30	11	0.999	0.857	0.138	0.843	1.000	0.707	0.654	0.996	1.000							
MI	30	19	1.000	1.000	1.000	1.000	0.992	1.000	1.000	1.000	0.992	0.698						
MI	30	24	1.000	1.000	0.994	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000					
MI	32	4	0.998	0.843	0.129	0.828	1.000	0.687	0.634	0.995	1.000	1.000	0.680	1.000				
MI	32	11	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.971	1.000	1.000	0.967			
MI	32	19	1.000	0.934	0.221	0.925	1.000	0.829	0.787	0.999	1.000	1.000	0.813	1.000	1.000	0.990		
MI	32	24	1.000	0.996	0.526	0.995	1.000	0.979	0.968	1.000	1.000	1.000	0.968	1.000	1.000	1.000	1.000	

		Pop.	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM
		Temp.	24	24	24	24	27	27	27	27	30	30	30	30	32	32	32	32
		Day	4	11	19	24	4	11	19	24	4	11	19	24	4	11	19	24
SM	24	4																
SM	24	11	1.000															
SM	24	19	1.000	1.000														
SM	24	24	1.000	1.000	1.000													
SM	27	4	1.000	1.000	1.000	1.000												
SM	27	11	1.000	1.000	1.000	1.000	1.000											
SM	27	19	1.000	1.000	1.000	1.000	1.000	1.000										
SM	27	24	1.000	1.000	1.000	1.000	1.000	1.000	1.000									
SM	30	4	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000								
SM	30	11	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000							
SM	30	19	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000						
SM	30	24	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000					
SM	32	4	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000				
SM	32	11	1.000	1.000	1.000	1.000	1.000	1.000	0.999	1.000	1.000	1.000	0.996	1.000	1.000			
SM	32	19	0.464	0.205	0.195	0.536	0.261	0.515	**0.027**	0.420	0.220	0.226	**0.029**	0.445	0.678	0.971		
SM	32	24	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.002**	**0.024**	0.993	

		Pop.	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI	MI
		Temp.	24	24	24	24	27	27	27	27	30	30	30	30	32	32	32
		Day	4	11	19	24	4	11	19	24	4	11	19	24	4	11	19
SM	24	4	1.000	0.999	0.568	0.999	1.000	0.993	0.987	1.000	1.000	1.000	0.988	1.000	1.000	1.000	1.000
SM	24	11	1.000	1.000	0.872	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
SM	24	19	1.000	1.000	0.883	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
SM	24	24	1.000	0.998	0.487	0.998	1.000	0.985	0.975	1.000	1.000	1.000	0.978	1.000	1.000	1.000	1.000
SM	27	4	1.000	1.000	0.808	1.000	1.000	1.000	0.999	1.000	1.000	1.000	0.999	1.000	1.000	1.000	1.000
SM	27	11	1.000	0.999	0.511	0.998	1.000	0.988	0.979	1.000	1.000	1.000	0.982	1.000	1.000	1.000	1.000
SM	27	19	1.000	1.000	0.999	1.000	1.000	1.000	1.000	1.000	1.000	0.988	1.000	1.000	0.986	1.000	0.997
SM	27	24	1.000	1.000	0.620	1.000	1.000	0.996	0.992	1.000	1.000	1.000	0.992	1.000	1.000	1.000	1.000
SM	30	4	1.000	1.000	0.995	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
SM	30	11	1.000	1.000	0.995	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
SM	30	19	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.967	1.000	1.000	0.962	1.000	0.989
SM	30	24	1.000	1.000	0.945	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
SM	32	4	1.000	1.000	0.798	1.000	1.000	0.999	0.998	1.000	1.000	1.000	0.997	1.000	1.000	1.000	1.000
SM	32	11	1.000	0.968	0.300	0.962	1.000	0.897	0.865	1.000	1.000	1.000	0.879	1.000	1.000	0.996	1.000
SM	32	19	0.066	**0.004**	**0.001**	**0.004**	0.418	**0.002**	**0.001**	**0.043**	0.771	0.996	**0.003**	0.230	0.997	**0.031**	0.987
SM	32	24	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.004**	0.063	**0.001**	**0.001**	0.068	**0.001**	**0.036**

Table AIII. 8a. **Analysis of variance for comparisons of repeated measures across experimental days for the maximum quantum yield of photosystem II in *Symbiodinium* C1 cultures.**

Comparisons were among different *Symbiodinium* C1 from different populations (Magnetic Island and South Molle Island) and temperature treatments (24, 27, 30, and 32°C). *P* values in bold face indicate significance at $\alpha = 0.05$.

Source of variation	Effect df	Error df	*F*	*P*
Population	12	117	16.9	**0.000**
Temperature	36	346	25.6	**0.000**
Population x Temperature	36	346	3.3	**0.000**

Table AIII. 8b. **Analysis of variance Tukeys post-hoc *P* values (by day) for comparisons of the maximum quantum yield of photosystem II in *Symbiodinium* C1 cultures.**

Comparisons were among different *Symbiodinium* C1 from different populations (Magnetic Island and South Molle Island) and temperature treatments (24, 27, 30, and 32°C). *P* values in bold face indicate significance at $\alpha = 0.05$.

Continued on following pages

Day 1		MI	MI	MI	MI	SM	SM	SM	SM
Pop.	**Temp.**	24	27	30	32	24	27	30	32
MI	24								
MI	27	**0.005**							
MI	30	**0.047**	0.997						
MI	32	0.998	0.597	0.873					
SM	24	1.000	**0.001**	**0.016**	0.988				
SM	27	**0.001**	0.752	0.281	0.064	**0.001**			
SM	30	**0.014**	1.000	1.000	0.717	**0.004**	0.607		
SM	32	0.985	0.775	0.959	1.000	0.944	0.133	0.866	

Day 3		MI	MI	MI	MI	SM	SM	SM	SM
Pop.	**Temp.**	24	27	30	32	24	27	30	32
MI	24								
MI	27	0.148							
MI	30	**0.024**	0.999						
MI	32	1.000	0.537	0.280					
SM	24	1.000	**0.049**	**0.006**	1.000				
SM	27	**0.001**	0.815	0.984	0.062	**0.001**			
SM	30	0.197	1.000	0.998	0.586	0.071	0.781		
SM	32	0.994	0.204	0.075	1.000	1.000	**0.010**	0.238	

Day 5		MI	MI	MI	MI	SM	SM	SM	SM
Pop.	**Temp.**	24	27	30	32	24	27	30	32
MI	24								
MI	27	0.094							
MI	30	0.051	1.000						
MI	32	1.000	0.429	0.346					
SM	24	0.980	**0.006**	**0.002**	1.000				
SM	27	**0.004**	0.987	0.996	0.120	**0.001**			
SM	30	0.992	0.594	0.461	0.994	0.684	0.110		
SM	32	0.897	**0.039**	**0.025**	0.991	0.997	**0.004**	0.613	

Day 7		MI	MI	MI	MI	SM	SM	SM	SM
Pop.	**Temp.**	24	27	30	32	24	27	30	32
MI	24								
MI	27	0.082							
MI	30	0.070	1.000						
MI	32	1.000	0.830	0.820					
SM	24	0.965	**0.003**	**0.002**	0.957				
SM	27	0.077	1.000	1.000	0.831	**0.003**			
SM	30	0.999	**0.031**	**0.026**	0.994	1.000	**0.028**		
SM	32	1.000	0.330	0.316	0.998	1.000	0.329	1.000	

Day 9		MI	MI	MI	MI	SM	SM	SM	SM
Pop.	**Temp.**	24	27	30	32	24	27	30	32
MI	24								
MI	27	**0.001**							
MI	30	**0.001**	0.523						
MI	32	1.000	**0.005**	0.250					
SM	24	0.974	**0.001**	**0.001**	0.976				
SM	27	**0.001**	1.000	0.833	**0.017**	**0.001**			
SM	30	1.000	**0.001**	**0.002**	1.000	0.982	**0.001**		
SM	32	0.676	**0.001**	**0.001**	0.728	0.963	**0.001**	0.707	

Day 11		MI	MI	MI	MI	SM	SM	SM	SM
Pop.	**Temp.**	24	27	30	32	24	27	30	32
MI	24								
MI	27	0.240							
MI	30	0.712	0.996						
MI	32	0.992	0.994	1.000					
SM	24	0.512	**0.001**	**0.010**	0.439				
SM	27	0.932	0.940	1.000	1.000	**0.047**			
SM	30	0.200	**0.001**	**0.002**	0.221	0.998	**0.011**		
SM	32	0.432	**0.008**	**0.039**	0.299	0.992	0.092	1.000	

Day 13		MI	MI	MI	MI	SM	SM	SM	SM
Pop.	**Temp.**	24	27	30	32	24	27	30	32
MI	24								
MI	27	**0.011**							
MI	30	**0.014**	1.000						
MI	32	0.293	1.000	1.000					
SM	24	**0.033**	**0.001**	**0.001**	**0.001**				
SM	27	0.469	0.836	0.881	0.982	**0.001**			
SM	30	0.773	**0.001**	**0.001**	**0.025**	0.863	**0.017**		
SM	32	0.281	**0.001**	**0.001**	**0.006**	1.000	**0.008**	0.928	

Day 15		MI	MI	MI	MI	SM	SM	SM	SM
Pop.	**Temp.**	24	27	30	32	24	27	30	32
MI	24								
MI	27	0.107							
MI	30	0.972	0.701						
MI	32	0.167	0.997	0.580					
SM	24	**0.005**	**0.001**	**0.001**	**0.001**				
SM	27	0.311	1.000	0.933	0.967	**0.001**			
SM	30	**0.008**	**0.001**	**0.001**	**0.001**	1.000	**0.001**		
SM	32	0.822	**0.026**	0.410	**0.030**	0.987	0.069	0.981	

Day 17		MI	MI	MI	MI	SM	SM	SM	SM
Pop.	**Temp.**	24	27	30	32	24	27	30	32
MI	24								
MI	27	0.115							
MI	30	0.997	0.513						
MI	32	**0.001**	**0.003**	**0.001**					
SM	24	**0.032**	**0.001**	**0.005**	**0.001**				
SM	27	0.261	1.000	0.755	**0.001**	**0.001**			
SM	30	0.504	**0.001**	0.180	**0.001**	0.975	**0.001**		
SM	32	**0.025**	0.863	0.110	0.505	**0.001**	0.714	**0.001**	

Day 19		MI	MI	MI	MI	SM	SM	SM	SM
Pop.	**Temp.**	24	27	30	32	24	27	30	32
MI	24								
MI	27	0.588							
MI	30	**0.046**	0.953						
MI	32	**0.001**	**0.001**	**0.001**					
SM	24	0.086	**0.001**	**0.001**	**0.001**				
SM	27	0.064	0.973	1.000	**0.001**	**0.001**			
SM	30	0.391	1.000	0.993	**0.001**	**0.001**	0.997		
SM	32	**0.001**	**0.001**	**0.001**	0.791	**0.001**	**0.001**	**0.001**	

Day 21		MI	MI	MI	MI	SM	SM	SM	SM
Pop.	**Temp.**	24	27	30	32	24	27	30	32
MI	24								
MI	27	0.978							
MI	30	1.000	0.920						
MI	32	**0.001**	**0.001**	**0.001**					
SM	24	0.422	0.066	0.703	**0.001**				
SM	27	0.097	0.685	0.058	**0.006**	**0.001**			
SM	30	0.942	0.470	0.992	**0.001**	0.994	**0.005**		
SM	32	**0.001**	**0.001**	**0.001**	0.320	**0.001**	**0.001**	**0.001**	

Day 23		MI	MI	MI	MI	SM	SM	SM	SM
Pop.	**Temp.**	24	27	30	32	24	27	30	32
MI	24								
MI	27	0.991							
MI	30	0.966	0.624						
MI	32	**0.001**	**0.001**	**0.001**					
SM	24	0.894	1.000	0.296	**0.001**				
SM	27	0.998	0.844	1.000	**0.001**	0.542			
SM	30	0.997	1.000	0.724	**0.001**	0.999	0.906		
SM	32	**0.001**	**0.001**	**0.001**	**0.032**	**0.001**	**0.001**	**0.001**	

Table AIII. 9. **Analysis of variance for comparisons of the properties of *Symbiodinium* C1 populations.**

Comparisons were among *Symbiodinium* C1 from Magnetic Island and South Molle Island after one year of growth in cell cultures at 25°C. *P* values in bold face indicate significance at $\alpha = 0.05$.

Source of variation	**df_{effect}**	**df_{error}**	**SS**	**MS**	***F***	***P***
Chlorophyll *a* (pg per cell)	1	5	0.363	0.073	11.001	**0.021**
β-carotene / chlorophyll *a*	1	5	0.000	0.000	22.910	**0.005**
Diatoxanthin + Diadinoxanthin / chlorophyll *a*	1	5	0.002	0.000	0.121	0.742
Cell diameter	1	77	18.000	18.000	16.530	**0.001**

Table AIII. 10. ***Symbiodinium* C1 pigment content following 1 year of growth in cell cultures.**

Values are mean ± SE pigment (ρg) per cell.

Population	Chlorophyll *a*	Chlorophyll *c*	Peridinin	Diadinoxanthin	Diatoxanthin	*β*-carotene
Magnetic Is.	1.70 ± 0.26	0.46 ± 0.06	2.10 ± 0.29	0.26 ± 0.04	0.034 ± 0.014	0.029 ± 0.004
South Molle Is.	1.05 ± 0.18	0.32 ± 0.05	1.51 ± 0.24	0.17 ± 0.03	0.025 ± 0.004	0.011 ± 0.001

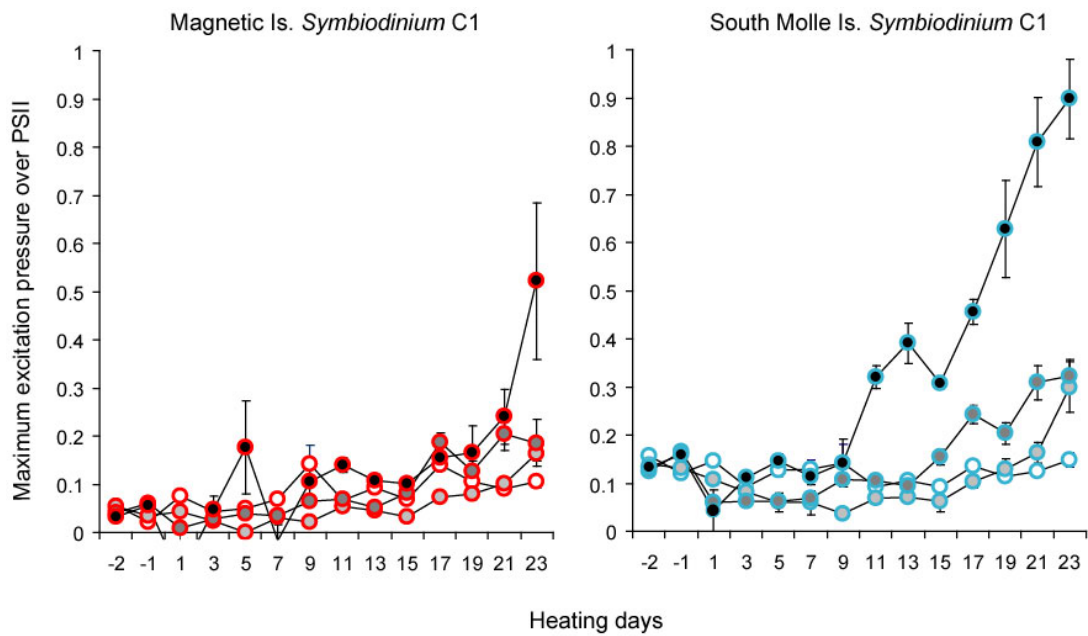

Fig. AIII. 1 **Maximum excitation pressure over photosystem II (mean ± SE) in *Symbiodinium* C1 populations following 1 year of growth in cell cultures at days relative to the start of heating (pre-heating temperature was 24°C).**

Circles represent temperature treatments of 24°C (white), 27°C (light grey), 30°C (dark grey) and 32°C (black).

AIII.4. Supplementary data for Chapter 5

Table AIII. 11. Pairwise comparisons (Fishers exact tests) of coral groups that bleached, underwent partial tissue mortality, underwent whole colony mortality, changed *Symbiodinium* type, and reproduced in different months.

Observations are totals from the period April 2008 and January 2009 (March 2009 observations including mortality from flood plume exposure at the central GBR site have not been included). Significant interactions after Bonferroni sequential correction of P at α =0.05 are indicated with an asterisk.

Following page

Categories	Native /	Transplant	Native /	Transplant	d.f.	*P*
Bleached						
No / Yes	Central	Central	Central	Southern	3	0.0001 *
	Central	Central	Southern	Southern	3	0.5400
	Central	Central	Southern	Central	3	0.0001 *
	Central	Southern	Southern	Southern	3	0.0001 *
	Central	Southern	Southern	Central	3	1.0000
	Southern	Southern	Southern	Central	3	0.0001 *
Partial mortality						
No / Yes	Central	Central	Central	Southern	3	0.1451
	Central	Central	Southern	Southern	3	0.1413
	Central	Central	Southern	Central	3	0.1451
	Central	Southern	Southern	Southern	3	0.0018 *
	Central	Southern	Southern	Central	3	1.0000
	Southern	Southern	Southern	Central	3	0.0018 *
Whole mortality						
No /Yes	Central	Central	Central	Southern	3	0.0152 *
	Central	Central	Southern	Southern	3	1.0000
	Central	Central	Southern	Central	3	0.0047 *
	Central	Southern	Southern	Southern	3	0.0006 *
	Central	Southern	Southern	Central	3	1.0000
	Southern	Southern	Southern	Central	3	0.0001 *
Changed Sym type						
No / Change to type D	Central	Central	Central	Southern	3	1.0000
	Central	Central	Southern	Southern	3	1.0000
	Central	Central	Southern	Central	3	0.0001 *
	Central	Southern	Southern	Southern	3	1.0000
	Central	Southern	Southern	Central	3	0.0001 *
	Southern	Southern	Southern	Central	3	0.0001 *
Spawning timing						
Oct / Nov / None	Central	Central	Central	Southern	4	0.0229 *
	Central	Central	Southern	Southern	1	1.0000
	Central	Central	Southern	Central	10	0.0011 *
	Central	Southern	Southern	Southern	5	0.0172 *
	Central	Southern	Southern	Central	34	0.0002 *
	Southern	Southern	Southern	Central	27	0.0001 *

Table AIII. 12a. **Analysis of variance for comparison of the maximum and effective quantum yield of photosystem II for *Symbiodinium* types D, D + C2 or C2 hosted by *Acropora millepora* colonies during summer bleaching at the central Great Barrier Reef.**

Comparisons were among coral partnerships native to the central and southern GBR. *P* values in bold face indicate significance at $\alpha = 0.05$.

Source of Variation	**Effect df**	**Error df**	***F***	***P***
Symbiodinium type	2	16	22.57	**0.001**
Error	6	32	335.8	**0.001**

Table AIII. 12b. **Analysis of variance Tukeys post-hoc *P* values for comparisons of the maximum and effective quantum yield of photosystem II for *Symbiodinium* types D, D + C2 or C2 hosted by *Acropora millepora* colonies during summer bleaching at the central Great Barrier Reef.**

Comparisons were among coral partnerships native to the central and southern GBR. *P* values in bold face indicate significance at $\alpha = 0.05$.

	Native site	Central	Southern	Southern	Southern
Native site	***Sym.* type**	D	D	C2 + D	C2
Central	D				
Southern	D	**0.001**			
Southern	C2 + D	**0.001**	0.989		
Southern	C2	**0.001**	0.987	0.999	

Table AIII. 13a. **Analysis of variance for the growth rate of *Acropora millepora* partnerships at native and transplant sites in the central and southern regions of the Great Barrier Reef.**

P values in bold face indicate significance at $\alpha = 0.05$.

Source of Variation	**df**	**SS**	**MS**	***F***	***P***
Genotype	1	0.007	0.007	2.04	0.159
Environment	1	0.001	0.001	0.20	0.977
Genotype x Environment	1	0.046	0.046	12.73	0.001

Table AIII. 13b. **Analysis of variance Fishers LSD post-hoc *P* values for the growth rate of *Acropora millepora* partnerships at native and transplant sites in the central and southern regions of the Great Barrier Reef.**

P values in bold face indicate significance at $\alpha = 0.05$.

		Southern	Southern	Central	Central
		Central	Southern	Central	Southern
Southern	Central				
Southern	Southern	**0.003**			
Central	Central	0.136	0.308		
Central	Southern	0.327	**0.001**	**0.034**	