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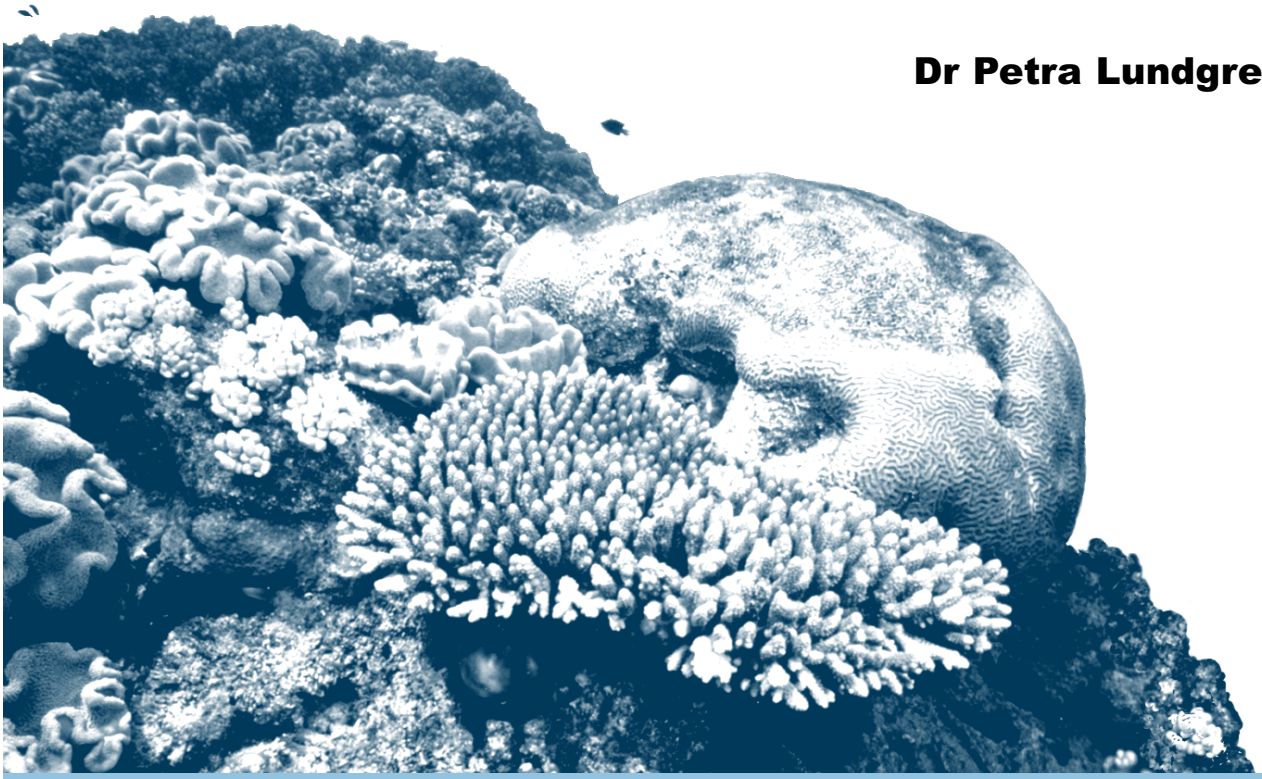
RESEARCH PUBLICATION NO. 107

Genetics and genetic tools in coral reef management

A synthesis of current research and its application in the management of coral reefs



Dr Petra Lundgren

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Table of Contents

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SUMMARY

This report forms a synthesis of current knowledge and future applications of genetics in coral reef management. It is divided up into three parts, (1) the coral host, (2) the Symbiodinium, and (3) other coral associated microbes. Within each of these parts current knowledge is summarised and examples are provided to illustrate how this knowledge may be applicable to the management of the Great Barrier Reef. The synthesis ends with a summary of how it is all linked together and possible future developments, uses and risks associated with the use of genetics in coral reef management. Words in dark blue with a dotted underline are part of the glossary, which can be found at the end of the document.

INTRODUCTION/BACKGROUND

Corals form the key structural component of coral reefs, where their expansive lime stone structures harbour one of the world's most biologically diverse ecosystems. The energy required to deposit the calcium carbonate skeleton of corals is to a large extent derived from a range of intra- and extra-cellular symbionts. These include photosynthetic dinoflagellates of the genus *Symbiodinium*, and a range of bacteria, archaea and viruses. Together the coral animal and symbionts make up the coral holobiont. Through its photosynthesis, the *Symbiodinium* provide the coral with nutrients that allow the coral to grow and survive in nutrient poor environments. Bacteria have been found to contribute to the nitrogen fixation capacity, chemical signalling pathways and defence mechanisms of the coral. The roles of other bacteria, viruses and archaea are less well understood, but as with all living organisms, they form an integral part of the general health status of the coral holobiont, and thus the health and productivity of the coral reef ecosystem.

Coral reefs around the world are under threat from a range of human stressors including destructive fishing, pollution and land run off. However, the biggest current threat to coral reefs on a global scale are increased sea surface temperatures and reduced ocean pH that are caused by climate change[1]. When corals are exposed to sea surface temperatures beyond their maximum thermal tolerance the symbiosis between the coral host and the *Symbiodinium* breaks down. The *Symbiodinium* are lost from the host tissue, causing a paling of the coral referred to as coral bleaching. Depending on the extent and duration of the bleaching event, the coral will either be re-populated with *Symbiodinium* and recover, or die. Corals that survive a bleaching event exhibit a reduction in growth and reproductive output[2] and an increased susceptibility to disease[3]. However, it has been shown that coral reefs that recover from a bleaching event tend to show an increased tolerance to elevated temperatures, either through acclimatisation[4] or possibly by rapid selection for and increased prevalence of more tolerant colonies on the affected reefs (adaptation).

Contrary to the touted concept that corals are only found in nutrient poor tropical waters and within a narrow thermal range, coral reefs are in fact known to exist in a range of environments. Sub-tropical reefs, such as the ones found around Lord Howe Island off the coast of New South Wales in Australia experience temperature ranges from 18 - 25°C and reach their thermal maximum at about 28°C[5], while the same species of coral are known to exist in extremely warm environments such as the Persian Gulf where maximum temperatures often exceed 35°C, with a bleaching threshold of >38°C[4] (Figure 1). Furthermore, many coral reefs thrive in highly turbid waters, including many of the inshore reefs on the Great Barrier Reef. Clearly, corals have the capacity to adapt to and exist in a range of temperatures and water qualities; the question that remains to be answered is if they can adapt rapidly enough locally to keep up with climate change and other anthropogenic disturbances.

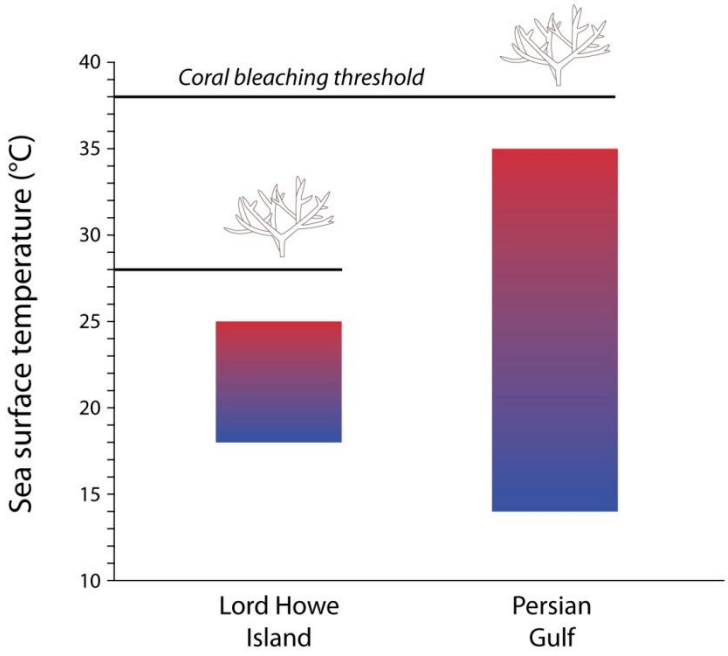

Figure 1 *Seriatopora hystrix* is one example of a coral species that can be found across a wide geographical range. This species is clearly capable of adapting to a range of thermal environments, and it's upper (and lower) thermal tolerance varies accordingly.

Due to the imminent threat of climate change to coral reefs around the world, many management and conservation efforts are focused on monitoring responses of coral reef ecosystems to climate change related stressors (thermal, disease, and acidification). One clear outcome of these monitoring efforts is the insight into the large geographical[6] and species level variations[7,8] in stress tolerance and recovery potential following severe disturbances. This variation highlights the potential for coral reefs to adapt to a changing climate and/or ambient environmental conditions. On an ecosystem scale, adaption can occur either through a shift in species composition, latitudinal expansion or through active selection for more tolerant individuals within a species.

For example, if two similar colonies of the same species, on the same patch of reef show a clear difference in stress tolerance it may be due to a difference at the molecular level, signalling the advantage of one genotype over another during periods of stress (Figure 2). Understanding the underlying molecular variation in stress tolerance and its role in adaptation and resilience will improve our ability to manage, restore and predict the health of coral reefs into the future.

Figure 2 Four colonies of *Acropora millepora* on the same patch of reef showing different stress responses. The colony at the front and the small colony at the very back display higher stress tolerance than the two pale (bleached) colonies in the middle (photo L. Bay).

GENETICS AND CORALS

DNA forms the blueprint of all living organisms. It is the code that underlies everything that an organism is and defines the boundaries to what it can become. It is the basis for evolutionary adaptation and, to a large extent it determines how capable an organism is at acclimatising within its lifetime. The difference between adaptation and acclimatisation lies in the DNA. Adaptation is a change in the DNA which results in a phenotypic trait difference that is passed onto future generations. Acclimatisation is a single organism's capacity to change its phenotype during its lifetime to accommodate environmental changes (Figure 3). How much an organism can acclimatise is ultimately governed by its genetic code.

The genetic code of each individual organism is unique. Hence DNA patterns can be used to pinpoint the identity of any sampled individual, from defining its species to determining its source population and parentage. A range of genetic markers exist that allow us to determine what any individual is and where it originates from (provided there is enough genetic data available for the species). In many model organisms, including humans, the fruit fly (*Drosophila melongaster)* and the flower *Arabidopsis thaliana*, the entire genome has been sequenced and characterised in a large number of individuals. This allows precise predictions to be made regarding an individual's phenotype and family history.

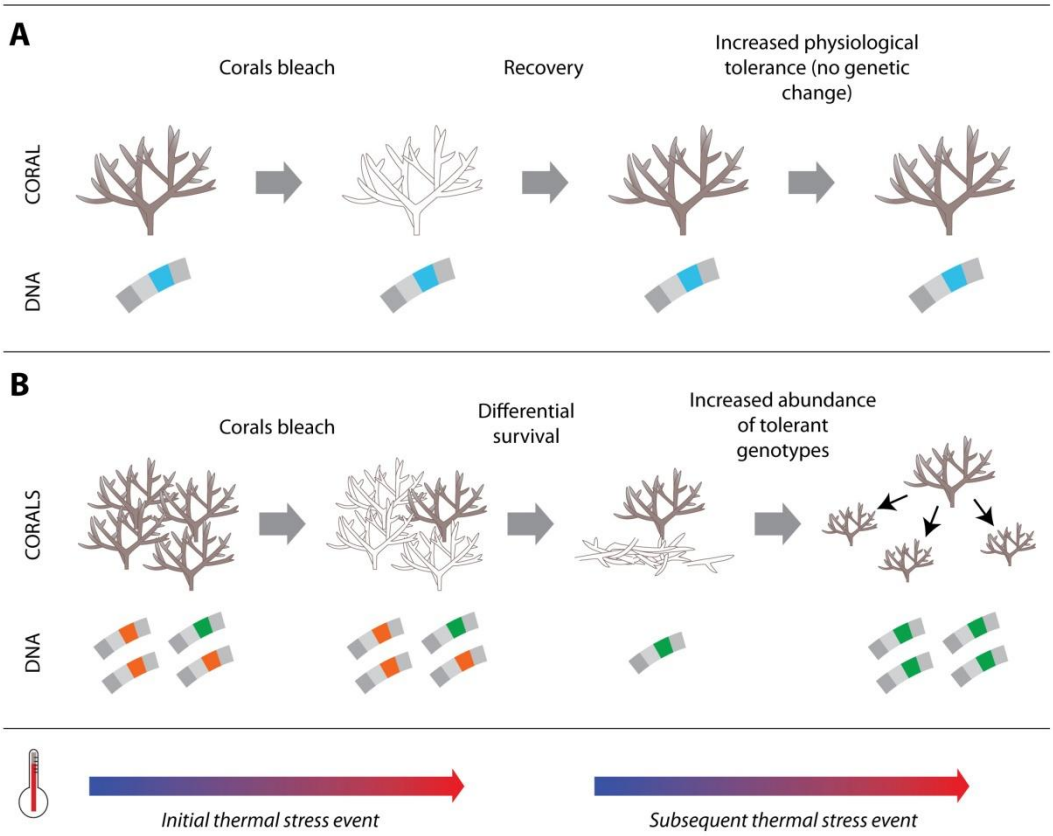

Figure 3 (A) Acclimatisation occurs at the level of the individual. It is the capacity for an individual to get used to increased levels of stress. It is not passed onto the next generation. (B) Adaptation is a change in the DNA that causes a change in the phenotype (for example increased thermal stress tolerance). These changes are passed onto subsequent generations.

Access to the entire genome in multiple individuals that differ in their phenotypes provides scientists with the possibility to develop a range of diagnostic markers. For example in humans, there are genetic markers that can be used to predict an individual's potential to develop certain diseases including Alzheimer's, certain forms of cancer and diabetes. The medical industry is using this information to design genotype specific drugs and diagnostic tools. Thanks to this research into the human genome, and to the development of accessible and affordable high throughput genotyping protocols, you can now send your own blood or tissue sample to an increasing number of commercial genotype facilities, and find out your precise family history and place(s) of ancestry.

While genetic methods provide a powerful tool to gain insights into the molecular mechanisms of stress response, resilience and adaptation, they often rely on expensive, time consuming and spatially restricted sample collection and processing. To date, the coral genome and its complex variations, remains mostly a mystery. Thus, the application of genetics to coral reef management is currently limited to individual or population scale studies or as a means to provide data for increased accuracy in the development of predictive models. However, the potential to develop accurate predictive genetic tools is rapidly approaching reality. Firstly the cost of genotyping and the development of novel genetic tools are decreasing drastically as DNA sequencing methods are increasingly streamlined. Secondly, the genetic understanding of corals is

improving rapidly, and now includes two published coral genomes, *Acropora millepora* (http://www.coralcoe.org.au/news_stories/coralgenome.html) and *Acropora digitifera*[9] as well as large databases of expressed genes that are publically available. All of these factors allow more accurate, cheaper and more spatially applicable markers to be developed.

It is often argued that evolutionary adaptation is a slow process, and that corals are unlikely to adapt at a rate that keeps pace with current climate change predictions [10]. However, thanks to the complex life history characters of corals and their associated microbes, adaptation through selection on novel mutations or standing genetic variation, may be highly relevant[11]. Corals reproduce both sexually and asexually[12], and their gametes are formed continuously from somatic cells. This fact, coupled with the adaptive potential of the asexually reproducing *Symbiodinium*, provide a source for relevant, adaptive selection on millions of cell level mutations during the life span of a coral.

THE CORAL HOST

Genetic connectivity

The most common application of genetic studies in coral reef management to date is the use of population genetic data in the design of Marine Protected Areas (MPAs). The current and rapid degradation of coral reefs around the world has sparked increasing efforts to optimise MPA design to protect these biologically and economically important ecosystems under a changing climate. The optimal design of MPAs requires knowledge about dispersal distance of coral larvae (and other coral reef associated organisms), source and sink reefs and degree of self seeding.[13] In addition to providing information regarding the optimal design of MPAs, this type of data can be used to assess the ability of reefs to recover from severe disturbances by calculating the probability of re-seeding from healthy reefs or remnant local populations.[14] To date, about 50 studies have been published in the peer reviewed literature on the subject of genetic connectivity in tropical scleractinian corals (ISI web of science search June 2011). The early discrepancy in population genetic data on corals stems primarily from the difficulty in developing reliable, neutral genetic markers for this type of study.[15] However, since the first coral microsatellite markers were developed and published in 2004,[16,17,18] almost 650 microsatellite sequences have been published from 26 species of reef coral (source: National Centre of Biotechnology Information NCBI http://www.ncbi.nlm.nih.gov). Population genetic studies remain highly relevant and we are only scratching the surface of the information this type of data can provide.

The Great Barrier Reef spans 2300 km along the coast of Queensland and encompasses almost 3000 reefs (10% of the worlds coral reef area) http://www.gbrmpa.gov.au/outlook-for-the-reef/great-barrier-reef-outlook-report. However, connectivity studies from the Great Barrier Reef are currently restricted to few species and few locations.[19,20,21,22,23,24] There are currently two studies that span across several sections of the Reef. The first is a stud of the brooding coral *Seriatopora hystrix* , which spans across 22 populations at 14 reefs[24] (Figure 4) and the second one is the broad cast spawner *Acropora millepora*, which spans from Wallace reef at the

very north of the Reef down to the Swains reefs at the south eastern point[25] . It is important to recognise that one or two species and a smattering of populations will not give an accurate picture of the Reef wide connectivity, thus each additional species and additional population will add to an increasing understanding of the connections between the reefs in the Great Barrier Reef ecosystem.

Population genetic studies are not limited to one dimensional spatial information, temporal population genetic data provides information regarding changes in community structure, for example before and after a major disturbance[22] and studies along depth gradients are providing important data about the potential importance of deep water populations for the recovery of degraded, shallow reefs.[23]

An important factor relating to genetic connectivity and its relevance to management is the differentiation between evolutionary and current levels of gene flow. Traditional statistical tools (Wright's F_{ST} [26]) used to infer population genetic patterns simply measured how genetically different populations were from each other. However, they could not separate what patterns were caused by historical levels of gene flow between populations from those that were occurring now. From a management perspective, it is the current level of dispersal between reefs that is important.

A range of statistical tools are now available to allow measurements of current migration using a range of assignment methods (GeneClass, STRUCTURE etc) and a majority of the more recent studies include these analyses (Figure 4). By employing these statistics it is possible to determine if an individual originates from the population where it was sampled, but it is more difficult to determine exactly where an individual originates from unless you have sufficient numbers of samples from all possible source locations. Hence, some care should be taken when interpreting this type of data regarding the source populations.

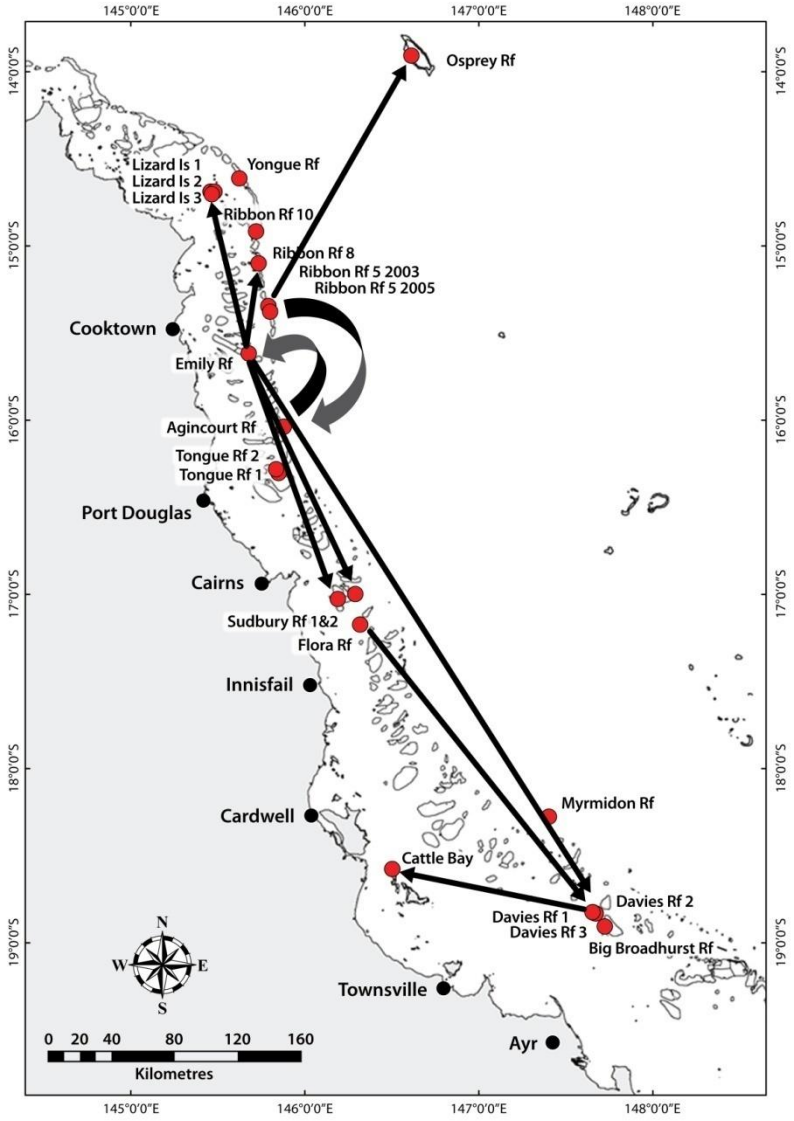

Figure 4 Results of genetic assignment of migrant individuals of *Seriatopora hystrix* on the Great Barrier Reef. In this study, 4% (44/1100) of the colonies were found to originate from sites other than where they were sampled. Fourteen of these had genotypes that could be matched to one of the other sampling locations that were included in the study (figure from van Oppen et al 2008).

Inferring dispersal and genetic connectivity is done using so called neutral genetic markers. These genetic markers do not code for a change in the physiological appearance or behaviour of the organism and hence are not affected by selection. In fact, the most commonly used markers (microsatellites) are found in a part of the genome that does not get translated into proteins at all. Neutral genetic markers enable predictions relating to dispersal, population of origin and parentage analysis. They will tell us how likely it is that larvae disperse and successfully settle and mature at some given distance from their natal reef or habitat. Genetic connectivity studies have thus provided insights into a range of management options (size of and distance between MPAs), models and predictions (larval dispersal, genetic diversity). Many of these studies have also made it clear how difficult it is to predict dispersal and recovery potential of degraded reefs due to a range of factors including (but not limited to) post

settlement survival (local adaptation), temporal variability (a year class of larvae ending up in a different location than previous years due to different wind and water movements between years) and complex, small scale hydrodynamic patterns.

Genetic diversity

The preservation of genetic diversity remains an important and overarching conservation goal for all ecosystems and species.[27] The loss of genetic diversity means loss of adaptive capacity, population viability and fitness[28] (Figure 5). Within the scope of this report, the focus will be on a couple of fundamental factors relating to genetic diversity and its relevance for management, namely taxonomy and adaptive diversity.

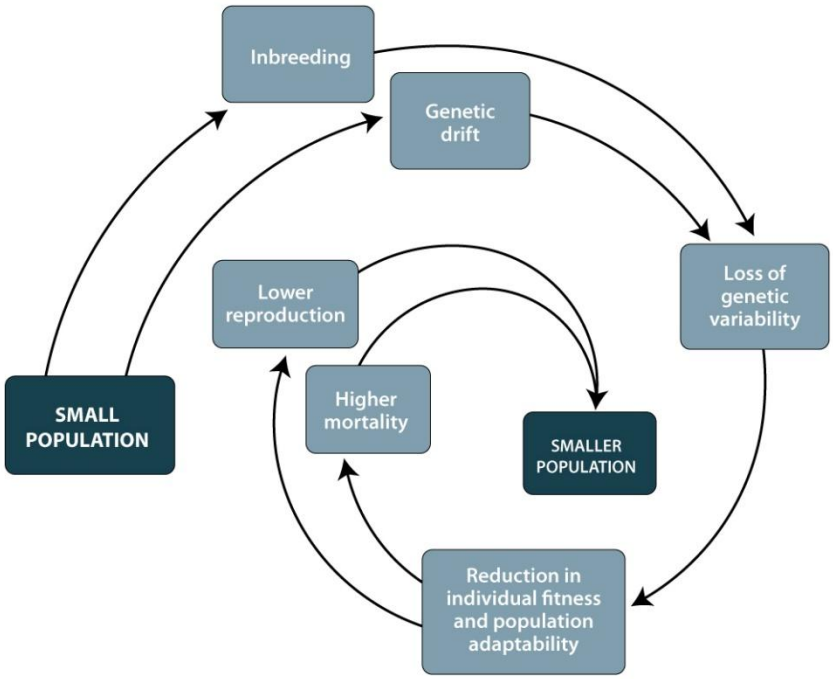

Figure 5 The *Extinction vortex*. An illustration of the connections between reduced population size, genetic diversity, adaptive capacity and further loss of population size. (Image adapted from Blomqvist, D., Pauliny, A., Larsson, M. and Flodin, L. (2003) Trapped in the extinction vortex? Strong genetic effects in a declining vertebrate population. *Evolutionary Biology*, 10(33))

Taxonomy

To effectively manage an ecosystem and to understand its various levels of function, it is important to know what is out there and at what levels diversity exists. Correct taxonomical knowledge is important because it allows accurate predictions regarding diversity, geographic uniqueness and conservation status of a species. You can't protect a species unless you know it exists. Conversely, managers may find themselves utilising resources to protect something that isn't as rare or threatened as predicted, as was the case of *Sideastrea glynni*. Genetic studies revealed that this species is a recent introduction to the Pacific coast of Panama from the Caribbean and hence not as rare or unique as previously thought.[29]

Coral (and *Symbiodinium*, which is addressed below) taxonomy is highly complex due to a range of factors including hybridisation,[30] morphologically indistinguishable species[16,31,32,33] and the resulting un-resolved species boundaries. For example, like many other species of coral, Pocilloporid corals are capable of morphological change in response to environmental cues and are thus difficult to tell apart in the field. Current species within this[34] genus are defined on morphology alone where many of the descriptive characters are found across several species.[35] A range of recent studies have indicated that this family may consist of several more species than previously thought[33,36,37]. It is currently argued that the species *Pocillopora damicornis* actually consists of at least three genetically distinct groups (lineages) on the reef, that are reproductively isolated despite their overlapping morphological characteristics (Schimdt-Roach in review) (Figure 6). The implications here are that each separate species is less genetically diverse than previously thought but that diversity at the species level is higher. It has also been found that these putative new species associate with different types and clades of *Symbiodinium*, making predictions about disturbance based on *Symbiodinium* types wrong if you assume they are all the same species and associated with the same clade in an undisturbed state.

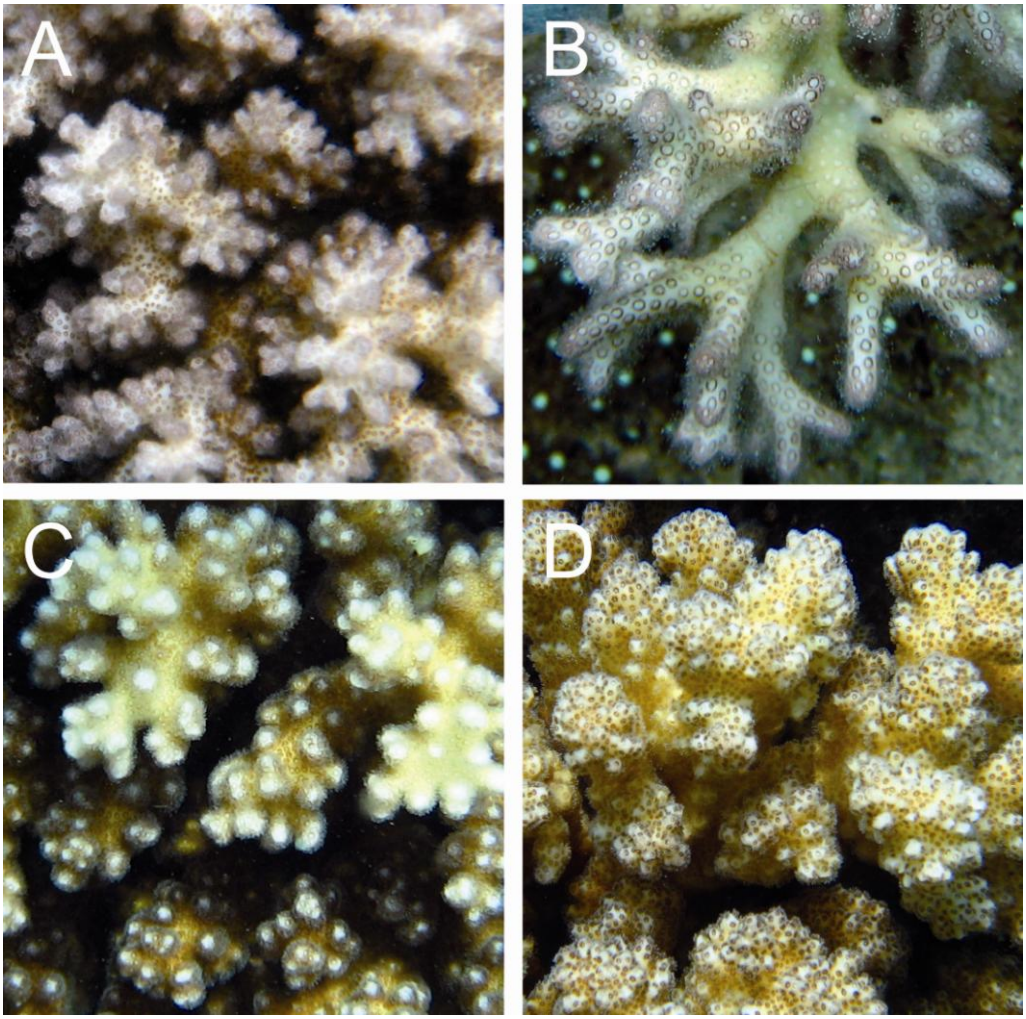

Figure 6 Several morphotypes of *Pocillopora damicornis* (A-C). Genetic data indicates that A and B are the same species but distinct from C. Plate D is *Pocillopora verrucosa* (photo: S. Schmidt-Roach).

Adaptive diversity

Genetic diversity is crucial for the survival of a species. It forms the basis of variation and population viability (Figure 5). The most obvious benefit of genetic diversity is its role in adaptation. Selection towards increased tolerance to environmental stress can only occur if there is a range of genotypes to "select" from. However, genetic diversity is not equally distributed across the entire genome, and diversity at a few neutral genetic loci does not necessarily translate to high genetic diversity at relevant functional markers. Adaptive diversity and the inherent capacity of corals to adapt to a rapidly changing climate is increasingly relevant and requires a different genetic approach to that of genetic connectivity or speciation, namely that of functional genomics, which is focused on the part of the genome that translates into function.

Functional genomics and adaptation

The future of coral reefs critically depends on the ability of corals to respond to the rapid environmental change with evolutionary adaptation.[1,38] Although substantial effort is being devoted to understanding physiological mechanisms of coral stress tolerance and acclimatisation,[14,39] virtually nothing is known about the mechanisms enabling genetic adaptation to climatic conditions over the course of generations.

Many genes that are involved in the maintenance of health during stress are conserved across species, allowing scientist to identify a range of target proteins (see glossary for link between gene and protein) for their studies of coral stress responses.[14] Stress marker development in corals is a relatively novel field. The first studies of gene expression (of the Heat shock protein Hsp 70) in corals emerged around the turn of the century[40]. A range of studies looking at stress related gene expression followed suit and the outcome can be summarised as highly variable; temporally, spatially and even within a single colony.[14,41,42,43] Furthermore genomic and transcriptomic studies remain, at large, too costly and logistically challenging to apply as a regular management tool on large spatial scales. The management application of these methods is also limited because measuring changes in expression as an indicator of stress can only provide information about current (and possibly past) stress levels and the response of a coral or population to an ongoing stress event. However, it will not allow predictions on how a population or colony will respond to a stress that has not yet eventuated. That type of prediction can only be made if molecular markers are developed that correlate the fixed DNA sequence to a predicted stress response. The capacity to express the right amount of the correct protein when the stressor arrives is important. The ability to identify the coral colonies that will do just that, even before it happens would provide managers with a valuable molecular tool for resilience mapping and restoration efforts.

Variation in tolerance to environmental stressors (including variation in the expression of stress response proteins) may be the result of the presence of a certain allele (i.e., gene variant) in individuals and populations. If carriers of allele "A" have higher reproductive success (fitness) than those that carry its alterative form "a" in a particular environment, allele A will be under positive selection and its frequency will increase in future generations. The less advantageous variant will eventually disappear from the population (unless it is linked to an alternative advantage and selected for under a different scenario). Genetic markers that are under selection can be identified by

comparing the relative frequency distributions of their alleles between populations to that of neutral genetic markers. If they are significantly more or less different than the neutral markers, there is reason to believe that they are influenced by selection. Another approach is to compare frequencies of alleles between populations spanning a range of (relevant) environments. If the allele frequencies of a gene correlate with environmental parameters, it is likely that this gene is influenced by environmental selection. For example, a correlation between the prevalence of allele “A” and increasing temperature would indicate that carriers of “A” have a higher thermal tolerance.

The latter approach has been developed at AIMS over the past three years, and correlations between the prevalence of a certain gene variant and thermal and turbidity gradients on the GBR have been found for both *Acropora millepora* and *Pocillopora damicornis (type A & B)* (Lundgren et al in review) (Figure 7).

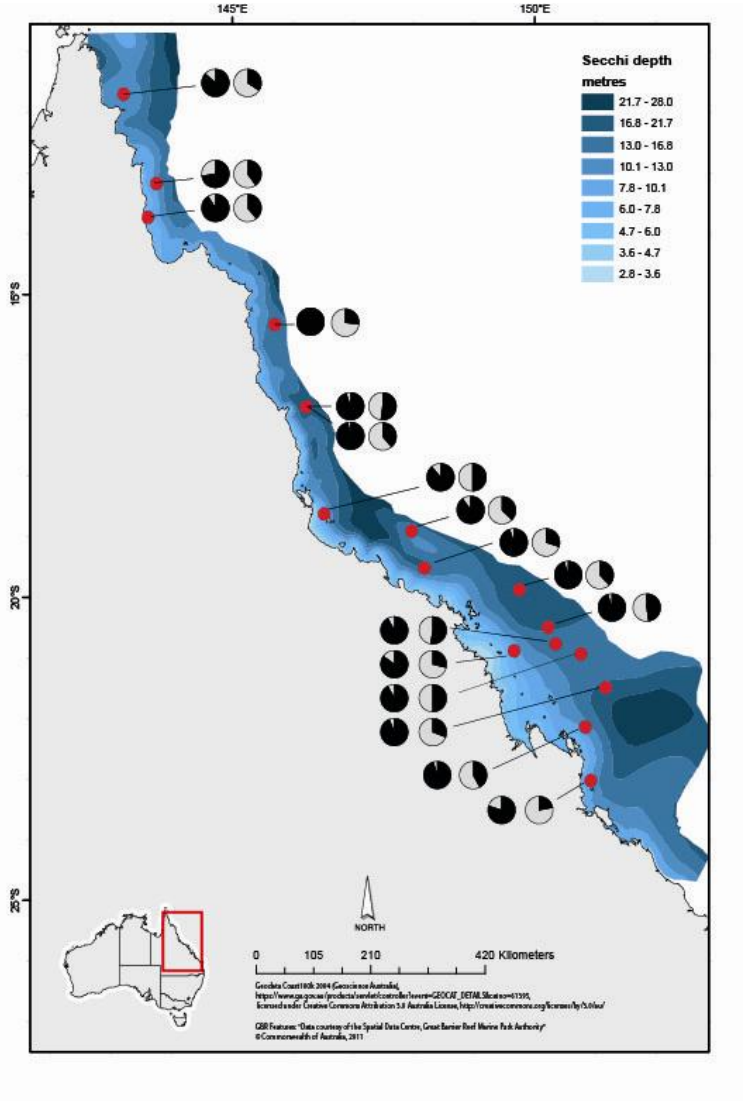

Figure 7 Genotype – environment associations. Pie charts depict the relative frequency of gene variants of genes encoding for the proteins Thioredoxin and Ligand of Numb X2, across 17 populations of the coral *Acropora millepora* on the Great Barrier Reef. The relative frequency of the each allele at these two genetic loci was found to be significantly correlated turbidity (Lundgren et al in review).

Experimental validation of these markers is needed to verify that they do correlate to differences in physiological stress response, both in controlled aquaria and on the reef. Once validated, such markers provide a powerful, predictive genetic tool, which can be incorporated in the development of models relating to adaptive capacity and for targeted breeding of adapted genotypes for conservation, preservation, restoration and translocation purposes.

In forestry and agriculture, the concept of “micropropagation” is commonly used in conservation and restoration efforts. It is defined as the planting and spreading of mature, elite genotypes; threatened or endangered species; or genotypes with known disease or pest resistance[44]. Similar methods are likely to be applicable to corals.

Restoration genetics

The time frame of corals’ capacity to adapt to climate change remains debated[10,45,46,47,48,49,50] but it is generally agreed that it involves a complex interaction between the coral host and its symbiotic microorganisms. Regardless of this debate, coral reefs are deteriorating, and corals remain unlikely to adapt to more acute changes such as those caused by destructive fishing methods, oil spills and ship groundings, hence active restoration of degraded reefs remains an important management consideration. Reef restoration projects are diverse and increasingly commonplace and recent advances in creating stocks from coral larvae allows farming of large numbers of genetically diverse colonies at low cost and effort.[51] Other proposed methods range from simply re-attaching fragmented corals[52] to suggestions of large scale interventions such as active translocation/assisted colonization of coral colonies from warmer latitudes to “boost” warming low latitude reefs.[53]

There are several genetic considerations to active restoration of degraded reefs,[54] including breaking up locally adapted gene complexes by introducing new genotypes, reduced fitness due to genetic incompatibilities between genetically distinct populations and loss of genetic diversity if the restored population originates from a very limited number of surviving individuals. One avenue to overcome many of these “risks” is to define genetically similar zones on the Great Barrier Reef within which restoration efforts such as translocations and enhanced breeding efforts could be done with minimum risk of disrupting the natural genetic profile of the degraded reef.

Restoration genetics is not only about managing the risks of restoration efforts, but also to ensure that the efforts that are put in place are successful. Genetics and genetic tools can aid managers in determining appropriate sources of propagules, improve spatial arrangements of transplants and track the success of restoration efforts through tracking the performance and dispersal of restored reefs into the future.[54]

Restoration efforts are not a realistic Reef wide management option; rather they need to be targeted to key sites. Such sites may include important tourism reefs or sites that are identified as important from a resilience point of view and thus warrant additional protection and possibly active intervention. In other parts of the world such "key" reefs have been selected for being areas of relatively low physical stress,[55] reefs that show signs of adaptation through increased thermal tolerance following previous bleaching events[47] or for being source reefs for coral larvae (connectivity studies). Intervention

strategies could include trials with selectively bred coral genotypes, or *in situ* acclimatized colonies.

THE SYMBIODINIUM

Genetic diversity

One of the biggest challenges in regards to genetics and genetic tools for *Symbiodinium* is their high level of genetic diversity and complex genome structure.[14,56] They are currently divided into nine distinct clades (A-I), with clade C and D being most commonly associated with corals on the GBR. Each clade is further subdivided into sub-clades (also referred to as types), with clade C comprising over 100 sub-clades, whereas clade D is divided into 10. The level of genetic diversity seems to be matched by their physiological diversity, including that of light and temperature stress tolerance and, not surprisingly, thermal tolerance in corals has been shown to be correlated to what type of *Symbiodinium* symbiont it harbours.

Thermal tolerance and clades

Symbiodinium associations vary geographically, temporally and between coral species [56]. Some coral species have been shown to harbour multiple clades, whose relative abundance inside the coral host varies through time.[57,58,59] A classic example of acclimatisation is symbiont shuffling, which refers to corals capacity to change the relative abundances of their associated S*ymbiodinium* type, and as a consequence increase their thermal tolerance.[59,60,61]

It is well documented that clade D symbionts are more common, or increase in prevalence in corals that experience thermal[4,60] and sedimentation[62,63] stress. However, the precise physiological response they trigger in the coral host, why and how that response is mediated and how the coral acquires the symbionts remains poorly understood.[56]

The current state of knowledge about clade C vs. clade D on the GBR can be summarised by:

- Clade D is less common than clade C
- Not all corals that harbour clade D show increased thermal tolerance
    - the response of the coral holobiont is potentially species specific, with some species (*Acropora tenuis)* inferring no physiological benefits when infected with clade D compared to being infected with type C1.[34]
- There are population level differences in thermal tolerance within sub-clades of type C, (Howells et al accepted manuscript)
- Associating with clade D results in reduced growth rates[64,65]
- Clade D is mostly found in circumstances where the coral host is stressed (and may thus simply be an opportunist invasion)
- In most cases, corals revert to clade C once the stress event has passed (usually within 2-3 years)[66]

- The bacterial flora of juvenile *A. tenuis* hosting clade D is dominated by *Vibrio* species indicative of increased susceptibility to disease invasion.[67]

Nonetheless, monitoring the prevalence of clade D remains a useful management tool, as it may indicate increased stress and thus provide an indicator of negative changes in coral health. However, treating an increased prevalence of clade D in a coral population as a sign of adaptation may not be accurate and caution should be made in assumptions that it provides a long term solution for corals to adapt to increasing sea surface temperatures (SSTs).

Additional studies are needed into the physiological tradeoffs that clade D infers and some of the key questions that remain unanswered are:

- What physiological changes correlate with a "shuffle" from clade C to D?
    - A further reduction in growth following severe stress event (beyond that resulting for the stress itself) and increased susceptibility to disease may mean there is no benefit to associate with clade D.
- The mechanism by which corals associate with clade D.
    - Do they change in relative frequency within the host or are they taken up from the water column?
- The capacity of clade C to adapt (or already be better adapted to) increasing temperatures.

Many types of microalgae have the capacity to respond and genetically adapt to extreme stress (toxicity) within a time frame of weeks.[68] In the case of *Symbiodinium*, preliminary studies of the heritability across a limited number of traits have shown that they are most likely source of rapid adaptation in the coral holobiont.[69] Furthermore, population level studies of *Symbiodinium* type C1 show significant differences in thermal tolerance between populations (E Howells un-published material). Hence, studying the adaptive response of the *Symbiodinium* and how that may translate to adaptation of the coral holobiont remains a priority if we are to predict the possible future states of the Great Barrier Reef.

BACTERIA, VIRUSES AND ARCHAEA

Health and disease

Bacteria, viruses and archaea are known to play an important role in all living organisms. They are most commonly thought of as carriers of disease (pathogens). However, it is well known that they fill a vital and mutualistic role in organism health and evolution. Corals have been shown to harbour large, diverse and highly specific populations of bacteria,[70] which confer benefits such as nitrogen fixation, decomposition of toxins and infection prevention. During periods of stress, certain microorganisms cause coral bleaching and disease. The mucus layer of corals harbour 100 – 1000 fold higher densities of bacteria than the surrounding water column and bacteria are found throughout the coral, including the coral skeleton and on the surface

of the *Symbiodinium*.[71] It has been found that the coral associated bacteria is highly specific even within species, and that each species of coral harbours a similar bacterial biota, regardless of geographic location.[70,72] This close association between the coral and its bacterial biota indicates they have co-evolved as a symbiosis.[71]

Stressed corals exhibit a change in their bacterial flora, from the more beneficial bacteria towards an increasing abundance of pathogens, dominated by a range of *Vibrio* bacteria.[73] The exact number of coral diseases is not known, but estimates range from 18 to 29 and the causative agent for six of these have thus far been isolated and described.[74] From a management perspective, the relevance of this research lies in the capacity to predict and prevent disease outbreaks; hence research into the development of easy, cheap and portable diagnostic and antibiotic tools (Figure 9) would be beneficial. Consequently, the development of improved sensory technology has been identified as a research priority by scientists at the Australian Institute of Marine Science (D Bourne pers comm.), where recent developments along this path are underway, including the development of a laboratory based assay to detect very low numbers of *Vibrio coraliiyticus* bacteria in sea water and coral before the signs of white syndrome are evident.[75]

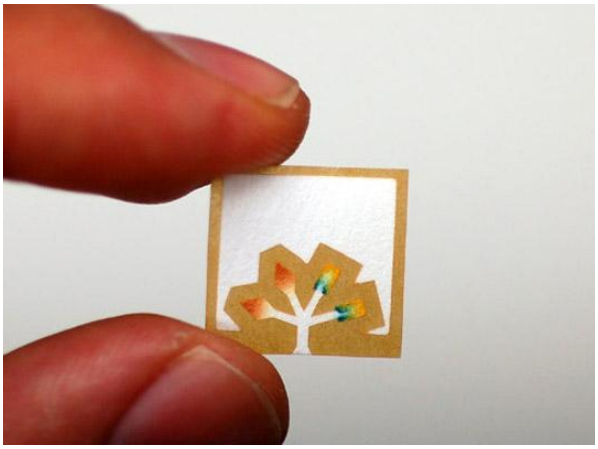

Figure 8 This small piece of paper is a diagnostic tool for the detection of diseases in humans, including malaria, HIV, hepatitis and tuberculosis. It works using only a single drop of blood. Similar tools should be feasible to develop to detect the coral pathogens from swabs of coral mucus.

As with bacteria, an enormous diversity of virus like particles (VPLs) has been detected throughout the coral. Until recently they were primarily thought to play a role as pathogens and their numbers have been shown to increase in abundance around coral colonies following coral bleaching and disease.[76] However, their potential role in host evolution and as a mutualist with the host and its associated symbionts should not be ignored.[77]

Very little is known about the role of Archaea and viruses in corals. Archaea communities do not differ from that of the surrounding water column and hence are not thought to be specifically associated with their coral host.

Cues for settlement and metamorphosis

Further to their role within the coral host, microbial communities are likely to play a key role in recruitment of corals and other reef associated invertebrates.[78,79] In fact, recent

studies have identified a specific chemical, which is produced by bacteria that induce metamorphosis in corals.[80] Chemicals that induce coral larvae to settle have been used in experiments to identify thermal thresholds for coral recruitment and examine the interactive effects of climate change and water quality[81,82] (Figure 9). From this research, it is evident that an improved understanding of the microbial community and its associated chemistry[83] may further enhance the potential to re-seed reefs damaged by climate change or other human impacts.[84] Thus, manipulating the microbiology of reefs may offer a further management option for reef rehabilitation.

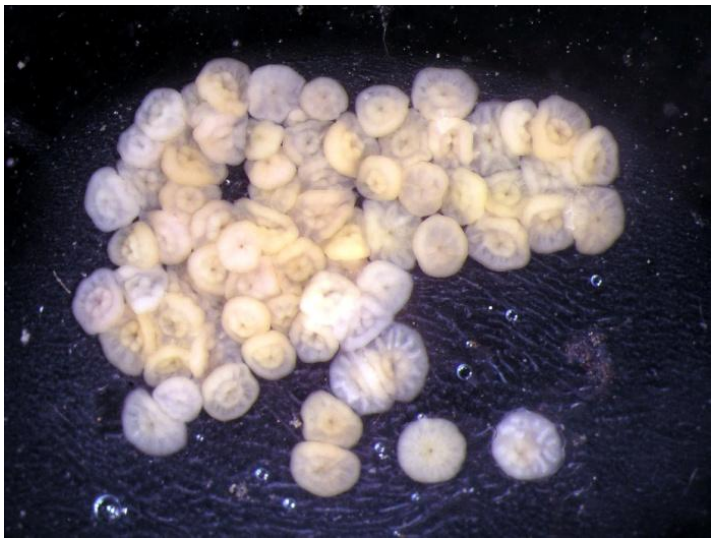

Figure 9 Coral larvae settling and metamorphosing on artificial biofilm (Photo A. Negri)

CONCLUSIONS

Despite their different roles, none of the above processes are independent from each other. In addition to the actual dispersal from one reef to the other, or from one patch of reef to the next, genetic connectivity relies on successful settlement, growth and reproduction of the migrant coral larvae. Settlement relies on the correct chemical cues to be present, while subsequent growth, survival and reproductive success are linked to how well adapted the newly settled coral is to the prevailing environment. In the event of a stressor, a cascade of responses is triggered, where each partner of the coral holobiont plays a crucial role in the ensuing health of the coral colony. It has even been found that the responses of coral associated bacterial communities to heat stress differ with *Symbiodinium* type on the same coral host[85]

Raising corals in captivity for deployment onto damaged reefs is a key strategy for reef rehabilitation.[86] However, the successful rearing of corals and their subsequent deployment requires knowledge regarding the viability of the reared colonies at the damaged reef. This includes factors such as their relative environmental stress tolerance, their species and population specific range of symbionts (*Symbiodinium* and bacteria) as well as their capacity to form viable offspring with possible remnant, native populations.

Possible future applications

Table 1 summarises much of what is presented in this report, including present and possible future applications. The risk and controversy associated to these applications vary. For example, the concept of a Great Barrier Reef wide, multi-species connectivity map which allows the identification of “genetic zones” can be regarded as very low risk. The only caution is that genetic zones are likely to vary between species, so it should never be assumed that it applies to a species which is not part of the map. However, it can be used as an indicator and should be based on a large range of variable species to improve its accuracy.

On the other end of the scale of risks is the idea of generating genetically modified corals to restore coral reefs in areas where they may no longer exist within the realms of natural adaptation. There is an ongoing debate surrounding genetically modified organisms (GMOs) and their possible benefits or dangers and the application of genetic modification as a strategy for conservation remains mostly un-tested. The concept of introducing foreign genes into organisms remains controversial, despite its common application in disease and pathogen resistance in agriculture and its use to increase harvest and growth periods. However, the introduction of “foreign” DNA, including that of large scale re-locations and transplantations between genetically distinct populations, should not be proposed as a possible fix for climate change threatened coral reefs without serious and rigorous risk analysis and mitigation. A less controversial approach may be selective breeding to enhance desirable traits within a restored or enhanced population.

It is well know that the impacts of artificial translocations of species and population don’t always turn out as predicted. Some classic examples include the introduction of cane toads to deal with the cane beetle and instances where the accidental release of aquacultured shellfish and fish has introduced pathogens to the environment resulting in the extinction of native species. Many of these considerations are the same as those associated with GMOs; namely the introduction of foreign genes and the possible negative consequences of that. A foreign genotype may be resistant to (and thus carrier of) a pathogen that poses a threat to the native populations (as per example of aquacultured species above). Hybridisation between genetically distinct populations may weaken or break up locally beneficial genetic adaptation or simply out-compete all native individuals and reduce the genetic diversity of the population. However, if handled with caution using clear guidelines and when rigorous scientific testing is carried out beforehand, they may contribute to enhance corals and thus coral reefs capacity to keep up with the adaptive arms race that climate change is challenging them with today.

GLOSSARY

Acclimatisation: an organisms capacity to “get used to” change. It is a change in physiology that is not passed onto future generations.

Adaptation: Unlike acclimatisation, adaptation is an evolutionary relevant change and happens on the timescale of generations rather than at the timescale of an individual’s life span. It is a change in the DNA (a mutation) that corresponds to a change in physiology, which improves health and fitness of the individuals that carry the alternate DNA sequence (**allele**).

Allele: One variant of a genetic marker. Most genetic markers (**locus**, plural **loci**) have more than one “version”. Organisms with two copies of each chromosome (diploid organisms) have two copies of each locus. An individual that carry two identical copies is referred to as a homozygote. If they are different copies (two different alleles) then the individual is heterozygote.

Archaea: A prokaryote (lacking distinct cell nucleus) microorganism, similar to bacteria, but thought to be more basal (an earlier life form).

Clade: A taxonomic unit given to organisms within a genus where strict species boundaries are not defined.

Conserved (genes): Has stayed the same for a long time. The use of the term conserved in genetics means that the DNA sequence that is “conserved” looks the same across several species, even very remotely related species. Heat shock proteins, which have a vital role in the stress response of almost all living organisms, are a good example of “highly conserved genes”. The DNA sequence that codes for these proteins looks almost identical regardless of organism.

Coral husbandry: Coral farming involving active selection of corals exhibiting desirable traits (thermal tolerance, disease resistance etc).

Cryo-preservation: Preserve biological specimens, including gametes by storing them at extremely low temperatures (which shuts down all protein activity).

Dinoflagellate: A family of unicellular algae. *Symbiodinium* are a genus within this family.

Expression of a protein (gene expression): The translation of a DNA sequence into the synthesis of a protein. A gene is the genetic code for a protein. A string of three nucleotides (AAG for example) corresponds to an amino acid and a string of amino acids make up a protein. To “express” a protein means that the particular gene is being translated in the cell, thus allowing the cell to synthesis the protein.

Functional genetic markers: Genetic markers that correlate to an actual function in the organism. Variation at functional markers correlates to a change in the physiology of the organism.

Gene: A gene is a DNA sequence that codes for and translates to a specific protein.

Gene banks: Storage of gametes to preserve current genetic diversity for future use in restoration or genetic enhancement programs.

Gene flow: the “flow” of DNA (genes) between individuals and populations through the transfer of gametes (between individuals), larvae, juveniles or adults (between populations).

Genetic drift: A random change in the frequency of alleles in a population from one generation to the next. Rare alleles are often lost from small populations simply by chance alone unless they are under very strong positive selection, hence the loss of genetic diversity (rare alleles) from small populations and its role in the extinction vortex.

Genetic markers (same as molecular markers): These are small parts of the DNA of an organism that are copied and amplified and scored to determine a genotype. If you want to study concepts such as gene flow, dispersal of larvae, genetic connectivity between populations, family history or parentage, then you look at parts of the genome that is NOT under selection, i.e. **neutral genetic markers**. If on the other hand you want to study selection and adaptation, you chose to look at the parts of the genome that ARE under selection, i.e. they code for a **function** that is expressed in the individual (such as size, colour, stress tolerance).

Genome: The entire DNA sequence of each chromosome of an organism. Including both the translated and un-translated parts.

Genotype: The DNA sequence pattern of an organism. It is often referred to in relation to a specific set of genotyped loci. For example, a unique microsatellite genotype infers that an individual is not equal to any other genotyped individual at the studied microsatellite loci.

Heritability: How likely it is that a trait is “inherited”, that is passed on from one generation to the next. In an evolutionary/adaptation sense it simply means a change in physiology that is correlated to a change in the DNA and hence can be passed down from parent to offspring.

Holobiont: The combined term for the coral animal and all its associated microbes (*Symbiodinium*, bacteria, viruses and archaea).

Hybridisation: The creation of hybrids, which are a mix of two species. Mules are hybrids between horses and donkeys.

Metamorphosis: In this context the change that occurs when the coral larvae transforms into an adult polyp. More generally defined as a change in shape.

Microsatellites are short, tandem repeats of DNA that form in non-translated (non functional) parts of the genome and they differ between individuals in the number of repeats, hence can be distinguished by their relative size to each other. If the repeat is AGG, then each “allele” differs by three base pairs depending on how many repeats of AGG that particular individual has at that microsatellite loci.

Morphologically plastic: Something that is capable of changing its shape (morphology).

Mutualism: Where two organisms live together and both depend on the other for survival.

Neutral genetic markers distribute randomly between individuals if sexual reproduction is random (each individual within a population is equally likely to reproduce with any other individual). Some of the most commonly used neutral genetic markers are microsatellites.

Parentage: The family origin of an individual. The parents. The source of the gametes that made the individual.

Phenotype: The physiological being of an organism, its size, shape, colour, smell, speed. The actual function and form of the organism.

Resilience: The capacity for an organism, population or ecosystem to return to its "pre-stressed" state after a disturbance.

Somatic: Cells that are not specifically programmed to form gametes in sexually reproducing organisms (those that are programmed to turn into gametes are referred to as germ or germ line cells).

Source and sink: This is a term commonly used in population genetic studies that relate to the design of protected areas or the management of wild populations. Through inferences of directions and magnitudes of **gene flow**, it is possible to determine what populations contribute gametes, juveniles or adults to others (source) and what populations are primarily on the receiving end of the equation (sink).

***Symbiodinium*:** A genus of dinoflagellate that from a mutualistic, intra-cellular symbiosis with the coral animal. Through its photosynthesis the *Symbiodinium* provides the coral with up to 90 per cent of its energy requirements. In return the coral host provides protection and a continuous supply of carbon dioxide from respiration, which is a key component of the photosynthetic cycle. They are commonly referred to as **zooxanthellae**, however, zooxanthellae is a broader term that simply means "small yellow cell that is found inside an animal host" and hence incorporates all algal endosymbionts not only *Symbiodinium*. In this report I have chosen to use the correct and more specific term of *Symbiodinium*.

Symbionts: A close and often long term interaction between organisms of different species where both species benefit from the interaction. In corals the symbiosis is between the coral animal and a range of micro organisms that live on (extra) or inside (intra) it's cells.

Trait: One specific part of an organism's phenotype. A colour is a "trait".

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Table 1. Summary of key processes, current knowledge, research gaps and present and future applications.

Aspect	Current knowledge	Research gaps	Direct application and relevance for managers	Possible future applications	Scale
Genetic connectivity	Stochastic and highly variable at different spatial scales. Some patterns emerging – Swains "different" some Keppel reefs more isolated.	Additional species. Temporal scales. Cohort tracking. Hierarchal scale interpretations.	Identify reefs that are more or less isolated. Identify source and sink reefs Conservation efforts to protect genetically unique reefs and reefs that are important from a re-seeding point of view.	Great Barrier Reef wide, multi-species connectivity maps. Identify and map genetic areas/zones of genetically similar and naturally connected populations to allow the conservation of relevant local adaptation for active restoration of degraded reefs.	Great Barrier Reef wide
Genetic diversity	Molecular species boundaries uncertain. Genetic diversity not necessarily lost following a major disturbance.	Revision of taxonomy of some families. Develop strategies to preserve genetic diversity in the event of a major disturbance. Better estimates of effective population sizes.	Accurate predictions of biodiversity. Accurate definitions of vulnerability status of single species. Accurate predictions of minimum numbers of source colonies required for a reef to recover or be restored after a major disturbance	Controlled increases of genetic diversity by introduction of new genetic material from outside populations.	Great Barrier Reef wide

Functional genomics and adaptation and Restoration genetics	Expression of stress response genes is highly variable. A number of genetic markers have been found to be significantly correlated to environmental gradients (temperature and secchi depth).	Validate markers that can be used to identify colonies and populations that have higher stress tolerance (temperature, salinity, turbidity). Study physiological response of combined stressors and correlate to genotype. Incorporation of prevalence and spatial extent of adapted genotypes into resilience models. Gene manipulation, transgenic trials to increase environmental stress tolerance.		Breeding more resistant genotypes for future restoration efforts through coral husbandry programs. Set up gene banks of known genotypes (include knowledge on genetic diversity to maintain adaptive capacity). Apply improved resilience models to new management plans and zoning efforts. Transgenic corals, targeted breeding or GMO corals manipulated for increased environmental stress tolerance.	Targeted – local Modelling, mapping – Great Barrier Reef wide

Symbiodinium	Genetically diverse Clade D not necessarily best bet for thermal tolerance. Micro algae (other than *Symbiodinium*) are capable of rapid adaptation	Identify relevant taxonomic units. Investigate clade and sub-clade differences. Enhancement of thermal tolerance in laboratory reared strains of *Symbiodinium* through artificial selection experiments. Measurements of rates of beneficial mutation rates in response to changed conditions. Investigate adaptive potential of beneficial types and clades of *Symbiodinium*.	Monitor for increases in clade D association as indicator of ongoing stress.	Identify adapted populations and colonies through known genotypic changes in *Symbiodinium.* Utilise strains of laboratory reared *Symbiodinium* that have enhanced thermal tolerance in coral husbandry or restoration programs. Manipulate *Symbiodinium* uptake, association and population type at threatened and degraded reefs as part of restoration effort.	Great Barrier Reef wide Small scale following acute disturbance to determine “stress”.

Bacteria, archaea and viruses	Assay developed to determine presence of *Vbirio coraliiyticus* bacteria in sea water and coral also before onset of white band disease. Anti bodies against *Vibrio coraliiyticus* identified and successfully produced in laboratory cultures. Chemical cues from microbial bio films are involved in coral larvae metamorphosis.	Increased understanding of the role of the bacteria and virus community in coral health, development and evolution and. Development of accurate, feasible and cost efficient sensory technology.	Determine prevalence of *Vbirio coraliiyticus* on stressed or degraded reefs (and healthy reefs). Chemical cues can be manufactured for improved settlement of aquarium reared larvae.	Protect reefs that show increased prevalence of disease pathogens (reduce human impact, shade). Quarantine measures to avoid spread of pathogens. Development and use of antibiotics on a reef wide scale to curb disease.	Current, small scale. With better sensory technology, Great Barrier Reef wide.