Exploring Coral Reefs Using the Tools of Molecular Genetics

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Abstract

The tools of molecular genetics have been transformed over the last decades and have in turn transformed our understanding of coral reefs. Initially limited to information on single genes, we are now capable of analyzing entire genomes. These developments make it possible to do many things that were either impossible or extremely difficult before: identify cryptic species, microbes, larvae and gut contents; determine relationships among populations and species; characterize reproductive and dispersal patterns; infer mechanisms of speciation; and soon perhaps embark on genetic engineering. Notably, many aspects of coral reef conservation can and will increasingly benefit from insights derived from the application of molecular genetic tools.

Keywords

Identification • Bioinformatics • Barcoding • Phylogenetics • Speciation

6.1 Introduction

As in other fields of biology, the tools of molecular genetics have transformed our understanding of coral reefs, giving us information that is difficult or impossible to access with the naked eye, or even high-powered microscopes. Though molecular genetics is no substitute for a deep understanding of the natural history of coral reefs, there is also no doubting the transformative advances that these tools have made possible. Indeed, molecular genetic methods have become so commonplace that this chapter can only touch on a few of the many contributions that have been made in this field. Using "DNA" and "coral reef" or "coral reefs" as coupled search terms yields over 600 papers published from 2000 to 2014 on the Web of Science.

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Underpinning this progress in understanding have been enormous technical advances, permitting ever more powerful analytic tools to be employed. Those who have witnessed the history over decades of the sequential adoption by coral reef scientists of new genetic tools and approaches (e.g., protein electrophoresis, the polymerase chain reaction, restriction fragment length polymorphism, Sanger sequencing, microsatellites, microarrays, and high-throughput sequencing technologies; see **Glossary**) know that what today seems doable but challenging will be routine in the not-too-distant future. A high-throughput DNA sequencer has even been brought on a ship to analyze samples from some of the most remote reefs of the central Pacific (Lim et al. 2014). In fact, the difficulties and costs associated with collecting molecular data are declining so precipitously that we now face an entirely new challenge, namely how to analyze the massive amounts of data that can be obtained. The biggest obstacles to be overcome are increasingly in the realm of bioinformatics.

With this history of rapid methodological turnover in mind, our chapter will concentrate on important discoveries based on molecular methods, rather than focusing in detail

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on specific techniques. The discoveries to be reviewed fall into seven fields: (1) species identification, (2) ecological relationships, (3) population structure, (4) evolutionary relationships, (5) speciation, (6) sexual systems, and (7) physiology and development. The chapter will conclude with a discussion of how molecular genetics may contribute to the conservation of coral reef organisms in the context of global change.

6.2 Species Identification

Many coral reef species are notoriously difficult to identify because of the problem of cryptic or sibling species, that is species that are difficult to distinguish using conventional morphological traits (Knowlton and Jackson 1994). Factors contributing to this problem include (1) paucity of diagnostic characters, (2) traditional preservation methods that eliminate informative characters such as color pattern, and (3) the fact that in many marine species, mate recognition is mediated by chemical cues rather than traits that are easily recognized by scientists.

The existence of complexes of cryptic species is typically confirmed using molecular genetic approaches (Knowlton 2000), although subtle morphological differences can often be detected in retrospect (e.g., Jackson and Cheetham 1990). Cryptic species complexes discovered using these methods are now so numerous as to defy summary, but they have notably included dominant members of coral reef ecosystems, such as the Caribbean coral *Orbicella* (formerly *Montastraea*) *annularis* (Fukami et al. 2004), the Indo-west Pacific coral *Stylophora pistillata* (Keshavmurthy et al. 2013) (Fig. 6.1), the Indo-Pacific crown-of-thorns seastar *Acanthaster planci* (Vogler et al. 2008), and the snapping shrimp symbiont of corals *Alpheus lottini* (Williams et al. 2003).

In some cases widespread "species" are now recognized as large numbers of geographically circumscribed taxa (e.g., the gastropod *Astralium* in the archipelagoes of the Indo-West Pacific, Meyer et al. 2005); in other cases large numbers of cryptic species are found to be sympatric (e.g., the brown alga *Lobophora* in New Caledonia, Vieira et al. 2014). The criteria for distinguishing cryptic species in sympatry are relatively straightforward. For nuclear genes, fixed differences between groups with no heterozygote individuals is strongly suggestive of the absence of interbreeding. For mitochondrial loci, the strict association of a diagnostic mitochondrial difference with another independent difference (molecular or morphological) is similarly informative. In cases of allopatry, there is more room for argument, as there is no universally accepted genetic definition of a species. However, groups defined by multiple fixed independent differences (e.g., Meyer et al. 2005) or by large genetic differences at a single locus (e.g., Vogler et al. 2008) are often recognized as distinct species.

The facility with which species can be identified by molecular traits has led to the widespread adoption of DNA "barcoding" of standard genetic markers [e.g. for animals, a portion of the mitochondrial cytochrome oxidase I (COI) gene]. The result has been the growth of barcode data in genetic databases [e.g., GenBank, Barcode of Life Data Systems (BOLD)], thereby facilitating the identification of specimens and comparisons across studies. However, the ability of a single mitochondrial gene to reliably define species depends on there being a consistent gap between the amount of variation that typically exists within species versus between species. Studies of cowries, a highly diverse and taxonomically well-known group often associated with coral reefs, suggest that error rates (recognizing taxa that are not distinct species or failing to recognize taxa that are) are not trivial (Meyer and Paulay 2005).

Moreover, some animals, including unfortunately corals, exhibit very little genetic variation in the mitochondrial genome, limiting the utility of COI-based barcoding for these groups (Shearer and Coffroth 2008; Huang et al. 2008). In some cases other genes have been surveyed and show promise [e.g., in octocorals, McFadden et al. (2011)]. Nevertheless, a mixture of genetic and other traits is often needed to distinguish closely related species in these problematic groups, and many unresolved species complexes remain (Prada et al. 2014a). The development of high-throughput sequencing, which allows the routine sequencing of numerous stretches of DNA simultaneously, may make a focus on particular barcoding genes obsolete.

Despite these limitations, DNA barcoding has found a wide variety of applications. For example, even in species that are readily distinguished as adults, barcoding is far more reliable for identifying egg and larval stages (Hubert et al. 2015), although identification efficiency depends on having a good database against which to match sequences, which is lacking for many groups and locations (Puillandre et al. 2009; Leray and Knowlton 2015). DNA barcoding also has the potential to dramatically increase our understanding of coral reef diversity as a whole by expanding analyses beyond the relatively small number of groups that are conspicuous and well known taxonomically [such as those surveyed by Roberts et al. (2002)]. For example, Plaisance et al. (2011) (Fig. 6.2) found 168 barcode-defined species (i.e. Operational Taxonomic Units or OTUs) of brachyuran crabs in just 6.3 m^2 of coral reef samples, a number representing almost 80 % of the diversity of this group for all European seas. These methods are also now being used for coral reef fisheries assessment. In the US, mislabeling of red snapper, a depleted reef fish, was found to be so extensive that 77 % of fish so labeled were likely other species, in some cases from distant locations (Marko et al. 2004).

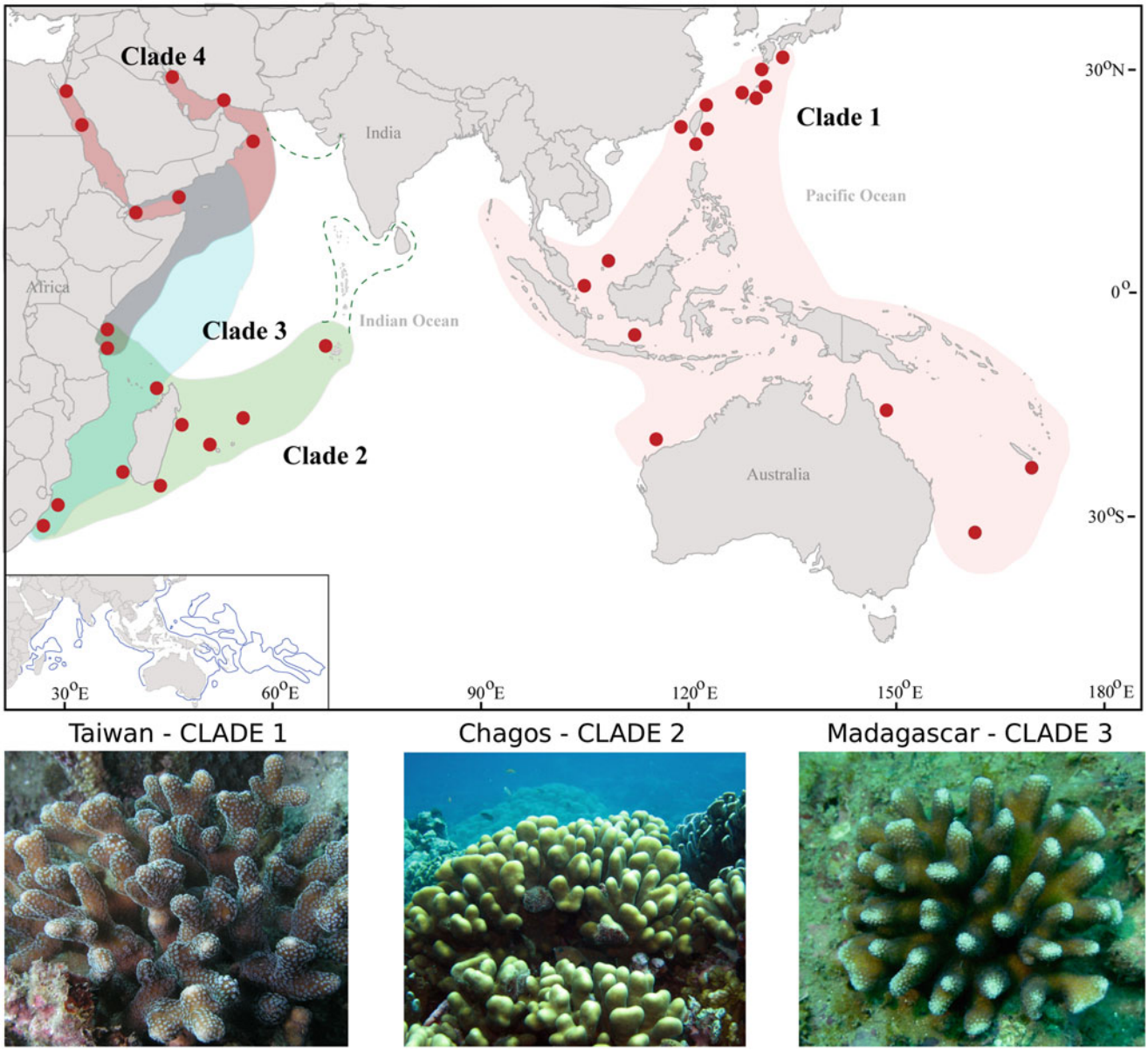

Fig. 6.1 DNA analysis has helped recognize that many species of fish and invertebrates that were once thought to have widespread distributions are in fact made up of multiple distinct genetic lineages. For example, Keshavmurthy et al. (2013, *figure above* modified from Figs. 1 and 2) used DNA barcoding to identify four distinct genetic clades in the widely distributed coral species *Stylophora pistillata*. The degree of differentiation suggests that they diverged from one another 51.5–29.6 million years ago

In Belize, 5–7 % of all fillets tested from markets and restaurants were found to be illegally harvested parrotfish, and 32–51 % of the fillets tested were misidentified (Cox et al. 2013).

Finally, traditional DNA barcoding efforts require identifying organisms one specimen at a time. The relatively recent application of high-throughput sequencing approaches promises to transform this field, because tens of thousands of sequences can be obtained from analyses of a single sample. Censusing diversity by barcoding DNA from samples of the environment containing many organisms is termed metabarcoding. This approach has been widely used to assess microbial diversity based on the 16S rRNA gene (the standard barcode for Bacteria) and has revealed staggering numbers of taxa, including on coral reefs. For example, it was estimated that a single 115 m transect would yield from 135,326 to 468,600 bacterial taxa, with algae harboring an even more diverse community of Bacteria than corals (Barrott et al. 2011). High-throughput sequencing has also been used to detect rare *Symbiodinium* within coral tissues (Quigley et al. 2014). Ironically, metabarcoding of multicellular eukaryotes has been more

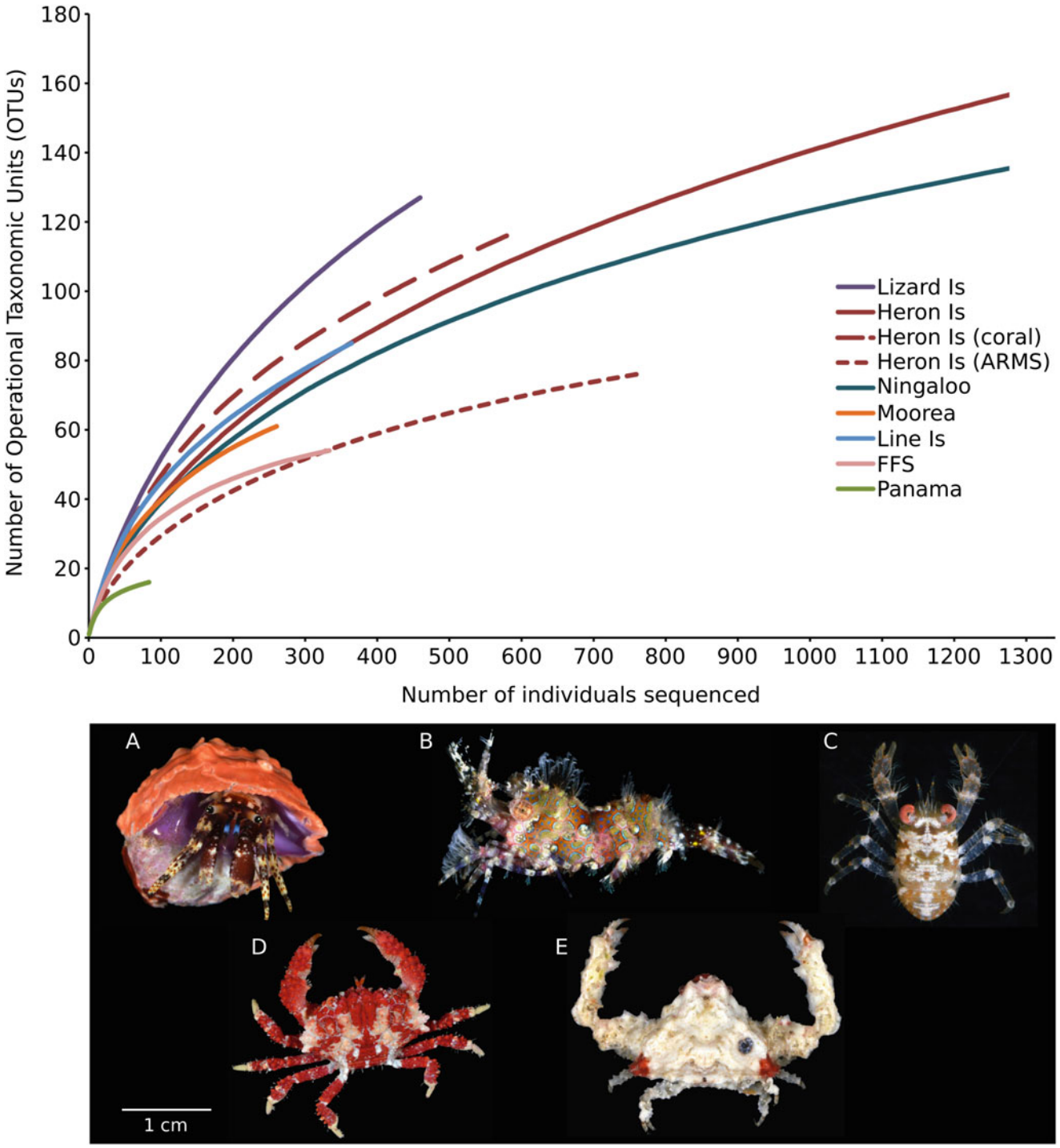

Fig. 6.2 Fish and corals have traditionally been used as surrogates to assess patterns of diversity on coral reefs because they are taxonomically well documented. However, short DNA sequences (*barcodes*) can now supplement traditional morphological approaches when identification can otherwise be time consuming, such as in decapods (*A*: *Calcinus* sp., *B*: *Saron* sp., *C*: *Galathea* cf. *ahyongi*, *D*: *Epiactaea* sp., *E*: *Daldorfia* sp.; all collected in the Red Sea; photo credit: Matthieu Leray). Plaisance et al. (2011, *top panel* modified from Fig. 4) used standardized sampling of dead coral heads and settlement structures (ARMS) with DNA barcoding to describe a gradient of decapod diversity across seven sites; even with large samples diversity had not reached a plateau in most locations

technically challenging, although applications for these organisms are also increasing (Leray et al. 2013; Leray and Knowlton 2015).

6.3 Ecological Relationships

Knowing what species are found where underpins most ecological analyses. For this reason, the identification of species using molecular tools, as described above, has transformed understanding of coral reef ecology in a number of areas where traditional methods for identification are time-consuming or inefficient. Here we review three: microbial symbiotic associations between corals and dinoflagellates, coral disease, and the dietary niches of reef fish.

If one had to choose a single area of coral reef science that has been most transformed by molecular genetics, it would be our understanding of the ecology of the symbiosis of corals and other coral reef organisms with dinoflagellates belonging to the genus *Symbiodinium* (Chap. 5). Indeed, a large proportion of the knowledge reviewed in Chap. 5 is due to the molecular methods that allow one to identify these single celled organisms, many of which cannot be readily discriminated morphologically. It is even possible to extract and sequence *Symbiodinium* DNA from museum specimens of octocorals collected over 150 years ago (Baker et al. 2013).

Previous research spanning decades on host-symbiont associations was upended by the realization that what was once thought to be a single species, *S. microadriaticum*, was in fact many. The initial discoveries were supported by protein electrophoresis and indicated that different hosts harbored genetically distinct symbionts; later work using DNA–based approaches revealed enormous diversity distributed among multiple subgenera of *Symbiodinium* (reviewed by Baker 2003). Multiple surveys have revealed some generalist and many specialist taxa with respect to host, and a wide variety of habitat preferences with respect to light and temperature [e.g. as in a recent survey of the northern Great Barrier Reef, Tonk et al. (2014)]. Discoveries of even more ecological specialization continue as additional cryptic diversity of both hosts and symbionts is uncovered (Prada et al. 2014b). The discovery of novel symbionts in unusually warm waters is of particular interest in the context of global warming [for a recent example see Hume et al. (2015)].

Symbiodinium are, of course, not the only microbial associates of corals and coral reefs. Like dinoflagellates, the Bacteria, Archaea, Fungi and protists found on and in the waters associated with coral reefs can only be easily studied with molecular genetic tools. These organisms have been the subject of especially intensive research of late, in part because of the increasing importance of coral disease on many coral reefs (Chap. 8). Analyses of changes in the microbial communities in corals in response to stress have revealed that manipulations of temperature, pH, nutrients, and dissolved organic carbon each result in distinct changes in the microbial communities (Thurber et al. 2009). Similarly, water samples taken from reefs characterized by different levels of overfishing have distinctive microbial communities, with more potential pathogens in the waters of the most anthropogenically impacted reefs (Dinsdale et al. 2008). DNA-based diagnostic tools for coral diseases have considerable and still largely untapped potential (Pollock et al. 2011).

Turning to the larger organisms on reefs, who eats whom is central to ecological understanding. Because observations of predation events are scarce even for conspicuous species, gut contents have been the traditional source of dietary information for fishes, the only group that has been intensively studied in this regard. However, there have been very few comprehensive analyses due to the diversity of potential prey combined with the difficulty of identifying semi-digested material (Bascompte et al. 2005). Molecular analysis of gut contents eliminates some of these challenges; a barcoding analysis of the gut contents of Mexican lionfish, for example, detected 34 species of prey from 157 individuals (Valdez-Moreno et al. 2012). Metabarcoding approaches (see above) provide even more information because they can characterize DNA from tissue slurries. The power of this approach is exemplified by a recent analysis of 16 reef fish guts (Leray et al. 2013) (Fig. 6.3), which revealed the presence of 334 prey taxa belonging to 14 phyla, 52.5 % of which could be identified to species by matches to genetic databases. Broad-scale application of this approach is likely to reveal diets that are more diverse than has been realized to date.

6.4 Population Structure

Long before molecular genetic data were available, scientists understood that marine species have far greater potential for long-distance dispersal than do terrestrial species. Dispersal typically takes places during the larval stage, but long distances can also be traversed by adults in some species. However, only with molecular genetic data has it been possible to evaluate clearly the extent to which that potential is realized.

Coral reef species vary considerably in this regard. In some cases, genetic data indicate surprisingly little genetic differentiation across large distances, for example across the 5000 km separating the reefs of the central and eastern Pacific (Lessios and Robertson 2006). In other cases, there is far more genetic structure dividing populations separated

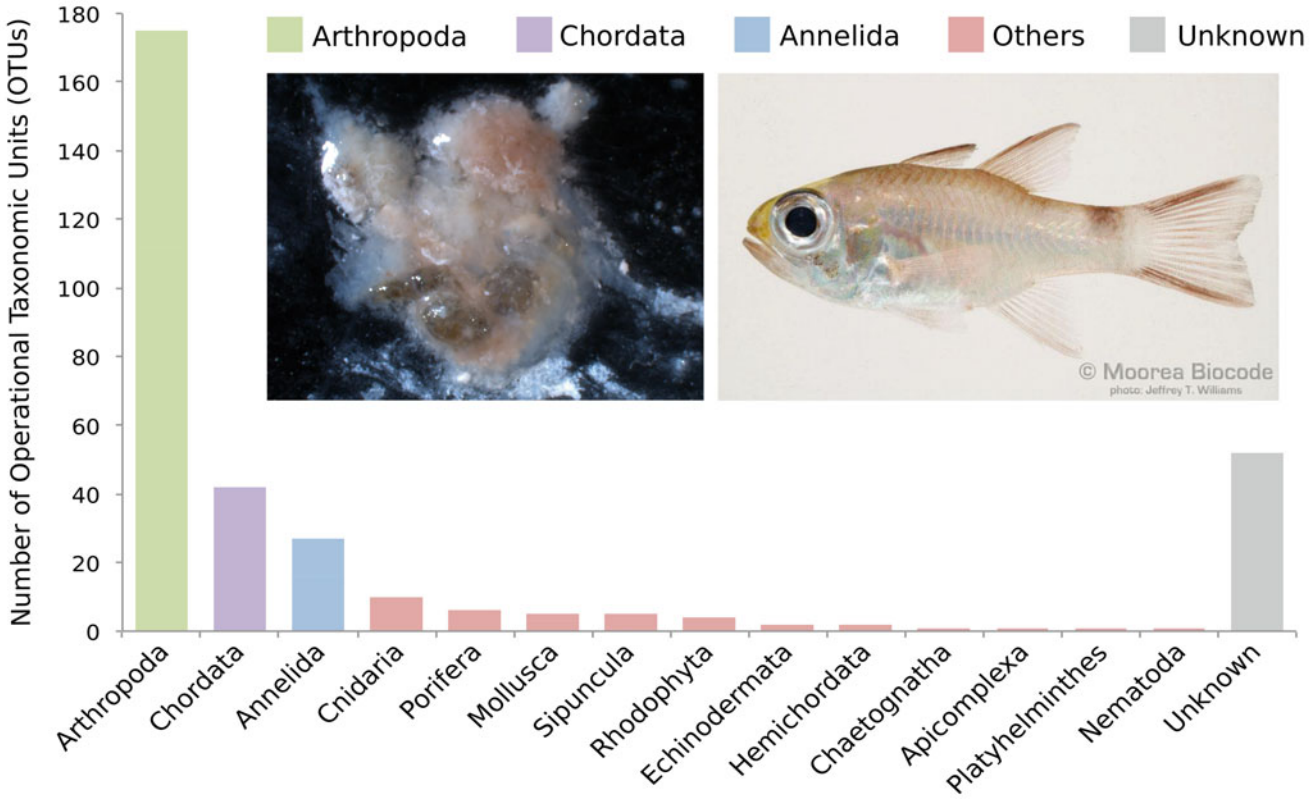

Fig. 6.3 Consumer-resource interactions are difficult to document on coral reefs and have often relied on morphological identification of prey remains in guts. However, genetic analyses have proven very powerful because they allow the detection of prey-specific DNA fragments from unidentifiable prey items (tissue homogenate, *left image*, photo credit: Matthieu Leray) even after several hours of digestion. For example, Leray et al. (2013, *bar graph* modified from Fig. 4a) used high-throughput sequencing to unveil the wide diversity of prey in the gut contents of *Nectamia savayensis* (*right image*, photo credit: Jeffrey T Williams), *Myripristis berndti* and *Sargocentron microstoma*

by short distances than might be expected (Barber et al. 2000; Rocha et al. 2005). Moreover, a recent study showing little difference in genetic diversity between closely related narrowly endemic versus widespread species (Delrieu-Trottin et al. 2014) suggests that genetic connectivity between widely separated populations is less than might be expected based simply on genetic differentiation.

Patterns of genetic connectivity have been of particular interest in the context of knowing the extent to which populations are self-sustaining. Some of the best data come from detailed analyses of anemonefishes, where it is possible to extensively sample adults and juveniles because of the discrete and conspicuous nature of the anemone host habitat. Results based on genetic parentage analysis vary widely depending on the setting, with more isolated sites exhibiting, as expected, more self-recruitment (Saenz-Agudelo et al. 2011). Moreover, even high levels of self-recruitment in contemporary time do not preclude long-distance genetic exchange over evolutionary time. One anemonefish species restricted to three offshore reefs in subtropical Australia had 68–84 % self-recruitment based on microsatellite analyses, whereas past connectivity over evolutionary time explained the shared mitochondrial lineages across locations (van der Meer et al. 2012). In the three-spot dascyllus, not only is some recruitment localized, but a pair of likely full sibs (based on their genetic similarity) were observed to settle together on a single night after spending nearly a month in the plankton (Bernardi et al. 2012).

The extent of routine connectivity between locations has important implications for the design of marine protected areas and other management decisions (Rocha et al. 2007). For example, Almany et al. (2013) (Fig. 6.4) documented that larvae of the squaretail coral grouper, a commercially important fish, often settle within 30 kms of their parents, suggesting that local communities could benefit directly by protecting spawning populations.

Patterns of genetic connectivity are also important for understanding sources of population explosions such as occur in the coral-eating seastar *Acanthaster planci*. One explanation for the temporal patterning of outbreaks is that an initial outbreak would seed secondary outbreaks via larval dispersal. In the central Pacific, unlike the Great Barrier Reef, the genetic distinctiveness of populations from different archipelagoes does not support a seeding hypothesis,

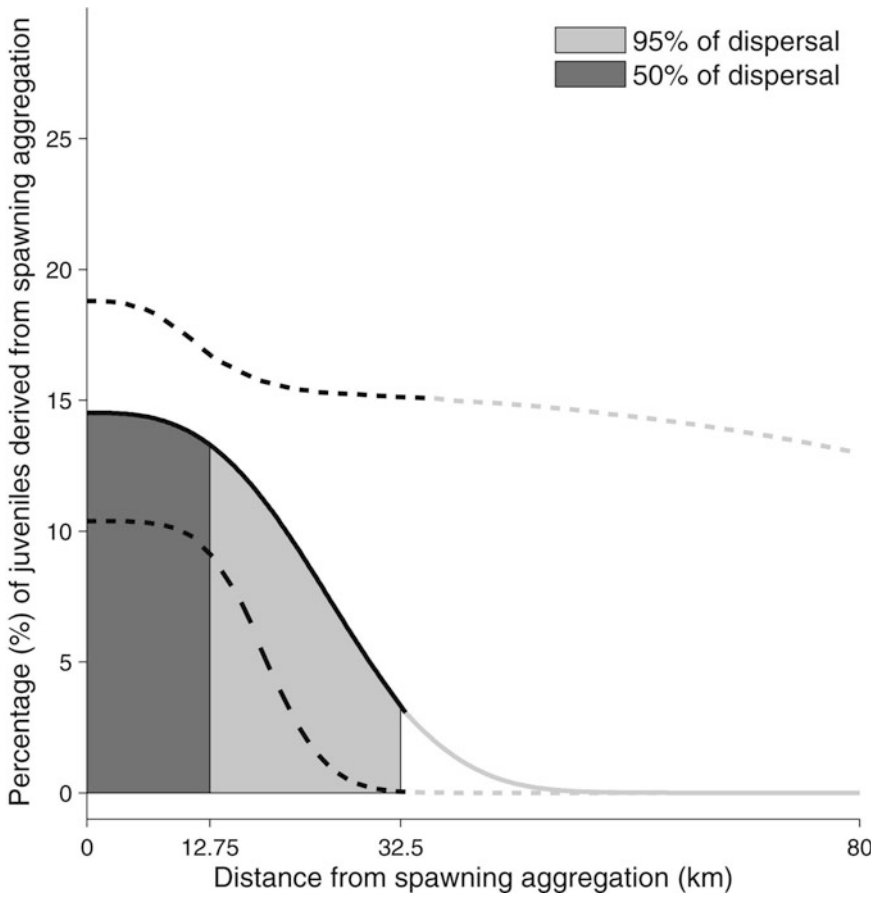

Fig. 6.4 Measuring the benefit of management actions for fisheries requires direct estimates of larval export for the replenishment of adjacent populations. Parentage analysis using microsatellites has been used as a tool to estimate distances of larval dispersal by assigning juveniles to their parents. Using this approach, Almany et al. (2013, panel above from Fig. 2) showed that 50 % of larvae produced by a managed spawning aggregation of squaretail coral grouper (*Plectropomus arelolatus*) settled within 14 kms of the aggregation, suggesting that management can provide fisheries benefits over small spatial scales

suggesting that clustering of outbreaks is instead due to shared environmental conditions (Timmers et al. 2012).

Finally, molecular genetic data allow one to estimate the demographic history of a species based on DNA sequence variation in extant populations. One of the most interesting case studies involves the Caribbean sea urchin *Diadema antillarum*, because of the potential for a genetic bottleneck following the loss of more than 97 % of its numbers due to a disease in the 1980s and because of the possibility that overfishing might have caused it to be more abundant immediately prior to the disease than it was in the more distant past. Surprisingly, genetic analyses supported neither of these scenarios, suggesting that the population of *D. antillarum* expanded more than 100,000 years ago with no loss of genetic variability following the recent population crash (Lessios et al. 2001).

6.5 Evolutionary Relationships

Our understanding of the evolutionary relationships among species has been transformed thanks to the application of molecular genetic methods in many groups, with coral reef organisms being no exception. These studies initially focused on mitochondrial genes, but phylogenetic studies now routinely involve multiple mitochondrial and nuclear genes.

Nowhere has this approach been more revolutionary in its findings than in the corals themselves, and hypotheses about relationships based on traditional morphological characters have been in many cases overturned following the application of molecular methods. One of the first major upheavals came with the establishment that corals could be divided into the so-called robust and complex corals, two highly divergent lineages that represent groupings quite distinctive from traditional arrangements of suborders (Romano and Palumbi 1996). Subsequent molecular analyses confirmed these results and showed that traditional families were also often unsupported (Fukami et al. 2008; Kitahara et al. 2010) (Fig. 6.5). Indeed, molecular genetic analyses have shown that some Atlantic genera conventionally assigned to different families are more closely related to each other than they are to "congeneric" taxa from the Pacific, suggesting a much deeper divide between Atlantic and Pacific reef groups than usually assumed. Considerable work on various sections of the scleractinian tree (e.g. Huang et al. 2014) will be required to sort out the many taxonomic messes that remain at a variety of taxonomic levels.

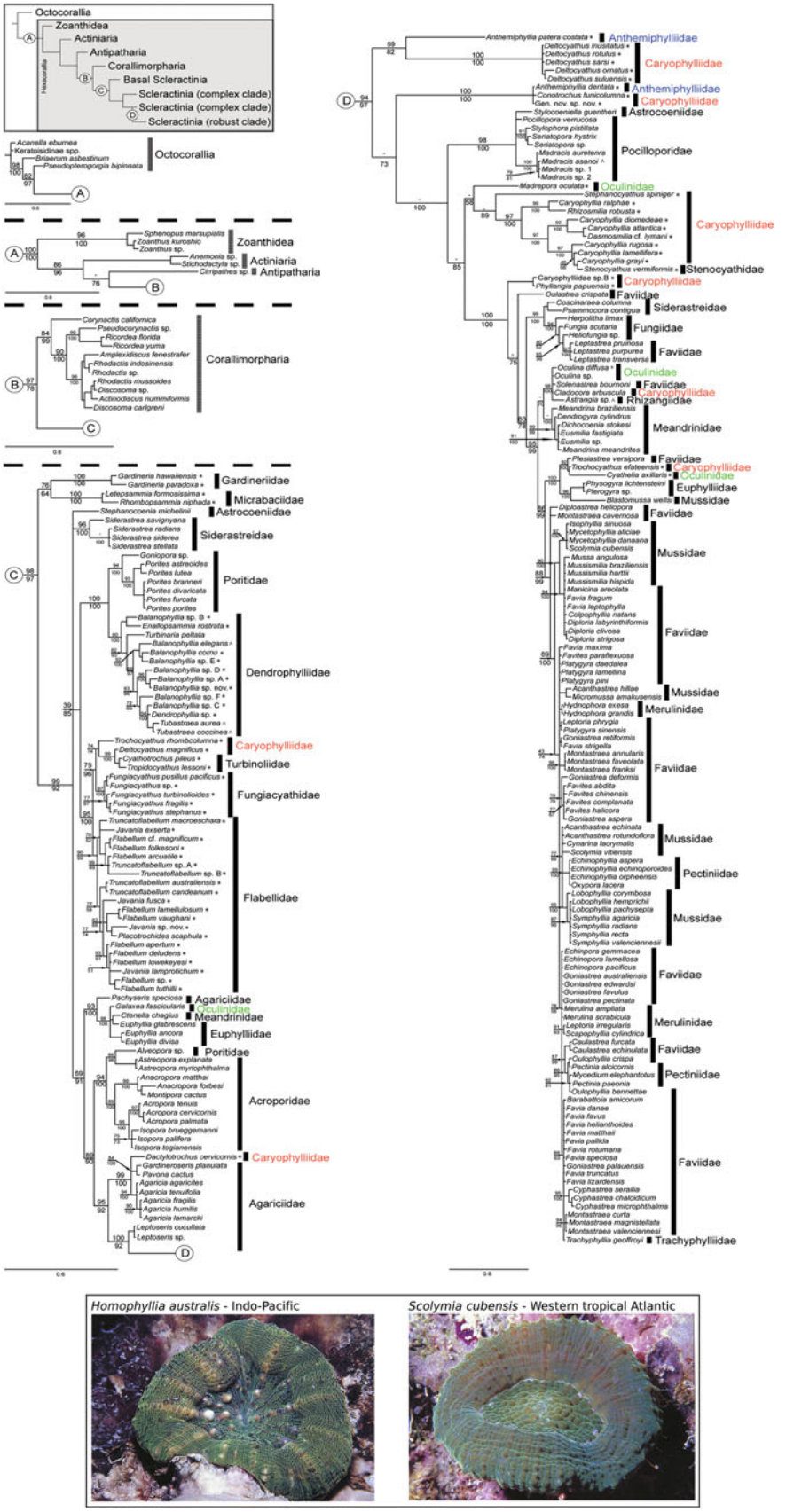

Fig. 6.5 Molecular analyses have had a profound impact on our understanding of evolutionary relationships of many coral reef organisms, including corals. Kitahara et al. (2010, *tree above* from Fig. 1), in a recent comprehensive phylogenetic analysis of scleractinian corals, showed that deep-sea azooxanthellate species are basal to the group and most recognized shallow-water zooxanthellate families are polyphyletic. In some cases, Atlantic and Pacific species once thought to belong to the same genus, are now placed in different families, such as *Homophyllia* (formerly *Scolymia*) *australis* from the Pacific (Lobophylliidae, *left image*) and *Scolymia cubensis* from the Atlantic (Mussidae, *right image*) (Photos courtesy of JEN Veron)

Taxonomic revisions based on molecular analyses are not limited to corals. Other groups of important coral reef organisms with major reevaluations of relationships based on molecular data include the damselfish (Quenouille et al. 2004), wrasses (Westneat and Alfaro 2005), cowries (Meyer 2003) and cone snails (Puillandre et al. 2014). Where considerable natural history is available to overlay on the phylogeny, it is possible to reconstruct the evolution of other features such as diet. In cone snails, for example, molecular phylogenies reveal that polychaete feeding is the ancestral state, and the ability to feed on mollusks and fishes most likely evolved only once in each case.

These kinds of genetic analyses can also give us insights into the geography of diversification (phylogeography). Coral reefs have played a prominent role in such studies because of their importance in the context of the latitudinal species gradient and attempts to understand why marine diversity is concentrated today in the western tropical Pacific (the so-called Coral Triangle). In evaluating the strengths of competing hypotheses (e.g. the Coral Triangle as a center of origin, center of accumulation, or center of overlap), a recent review (Bowen et al. 2013) concluded that phylogeographic analyses of a wide variety of organisms provide support for all three.

6.6 Speciation

A related controversy concerns the relative importance of allopatric and non-allopatric speciation in the sea (reviewed in Bowen et al. 2013). Impenetrable barriers are uncommon in the ocean, one exception being the Isthmus of Panama, which blocks connections for tropical organisms between the western Atlantic and the eastern Pacific. This situation is a model system for understanding allopatric speciation in the sea (reviewed by Lessios 2008) because closure was relatively recent (~3 million years ago) and because the geology of the area has been extensively studied. Analyses of the extent of molecular divergence between sister taxa on the two sides of the Isthmus showed that the impact of the Isthmus in breaking genetic connections began substantially before final closure, a finding with large implications for the calibration of molecular clocks for various genes and taxa (Knowlton and Weigt 1998).

Increasingly, however, speciation in the absence of complete physical barriers has been documented (Bowen et al. 2013). Cases for coral reef organisms involve speciation across strong ecological gradients (Rocha et al 2005; Duran and Rützler 2006), as a function of host shifts (Duffy 1996; Munday et al. 2004), and as mediated by assortative mating (Puebla et al. 2012) (Fig. 6.6). In each of these cases, genetic analyses have been used to confirm the absence or reduction in gene flow that underpins the speciation event. Speciation in the absence of geographic barriers can happen quite rapidly. For example, two sister species of tropical seastars, one a broadcast spawner and the other a self-fertilizing brooder, are thought to have diverged only 6000 years ago (Puritz et al. 2012).

Molecular methods are also critical to understanding the frequency with which barriers between species break down. Hybridization has been widely assumed in corals and undoubtedly occurs. Indeed, *Acropora prolifera* in the Caribbean is known to be an F1 hybrid of *A. palmata* and *A. cervicornis* (Vollmer and Palumbi 2002), two species that have weak barriers to crossing (Fogarty et al. 2012). In this and other cases, molecular data are crucial for determining the extent to which gene flow occurs across species boundaries and for determining the parentage of larvae resulting from experiments in which eggs are offered a choice between sperm of two species. In several other well studied cases, however, gene flow has turned out to be more restricted than initially assumed, and it is often difficult to distinguish between hybridization and the maintenance of ancestral polymorphisms when individuals carry the genetic signature of two closely related species (Palumbi et al. 2012).

Finally, the mechanistic processes underlying evolutionary diversification can also be analyzed using molecular genetics. For marine organisms, these have been most thoroughly explored to understand gamete recognition in species where fertilization occurs externally (Lessios 2011). Because many marine organisms release eggs and sperm into the water, genes that influence the interactions of sperm and eggs are tightly connected with the process of speciation itself. Sea urchins and abalones have been the best studied, and some of these sea urchins are prominent members of coral reef communities (e.g. *Echinometra*). In sea urchins, gametic compatibility is strongly influenced by the bindin protein and its receptor. The evolution of bindin has been extensively studied, but the receptor is enormous (4595 amino acids) and much less well known. Nevertheless, analyses of bindin and the extremely low incidence of genetically confirmed hybrids in nature suggest that bindin is one of several factors that contribute to interspecific boundaries, and that its evolution may have more to do with intraspecific sexual selection and avoidance of polyspermy (the fertilization of one egg by many sperm) than with prevention of interspecific hybridization (Lessios 2007).

6.7 Sexual Systems

Molecular analyses are also useful for studying mating biology within species, including the identification of partners; indeed when gametes are released into the water column in

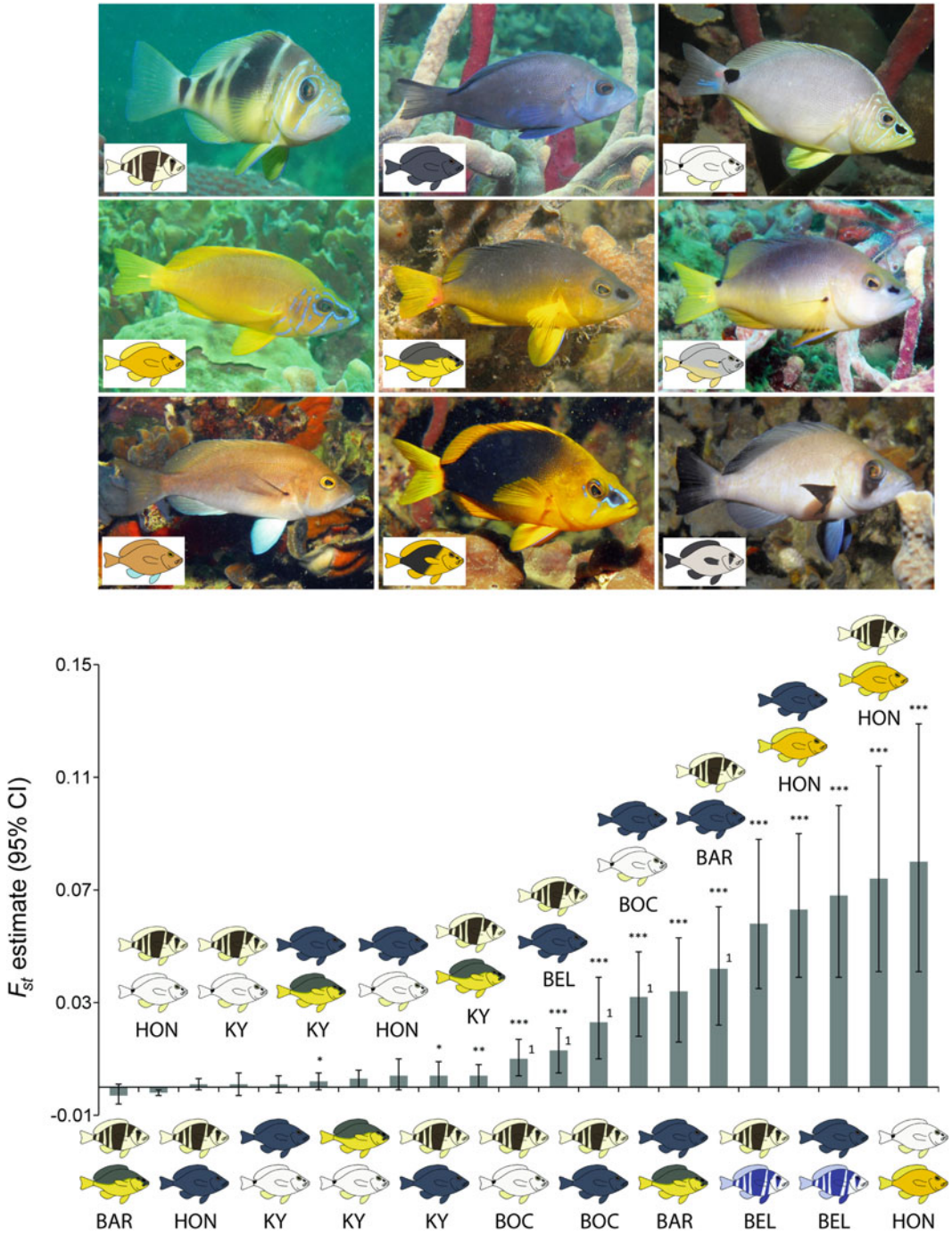

Fig. 6.6 Evolutionary mechanisms underpinning speciation on coral reefs can be readily studied with the tools of molecular genetics. Using behavioral observation, population genetic analysis and individual-based simulation, Puebla et al. (2012, above modified from Figs. 1 and 5) demonstrated the role of sexual selection in driving the evolution of assortative mating in hamlets (*Hypoplectrus* species). Low levels of genetic differentiation (F_{st}) at neutral genetic markers between sympatric color-morphs that occasionally hybridize showed that speciation is occurring in the presence of gene flow

the absence of clear pairing behavior, genetic analyses are the only way to analyze mating success. This approach has been used to determine paternity in spawning fish (Wooninck et al. 2000) as well as in some invertebrates. In one species of stony coral, molecular analyses showed that larvae are often the product of nearby individuals releasing their gametes nearly simultaneously (Levitan et al. 2011). Similarly, in a study of the soft coral *Pseudopterogorgia elisabethae*, most planula larvae appeared to have been sired within the 20 × 20 m of the study area, although closer

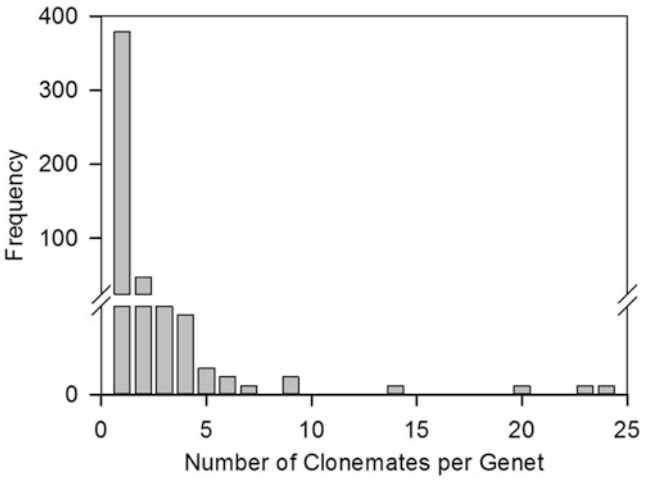

Fig. 6.7 Corals are able to reproduce sexually and asexually but the prevalence of asexual propagation has been difficult to evaluate. Foster et al. (2013, *panel above* from Fig. 2) used microsatellite markers to genotype individual colonies of *Orbicella annularis* and show the contribution of clonal reproduction at various sites across the species' range. They showed that sites varied widely with respect to the number of clonemates per genet (each genet consists of a colony or collection of colonies derived from a single fertilized egg), with asexual reproduction being more common in locations with greater hurricane frequency

or larger males surprisingly did not appear to have an advantage (Lasker et al. 2008). Planula larvae that disperse short distances can lead to aggregations of close relatives, and genetic signatures of inbreeding have been detected in some of these species (Richards and van Oppen 2012; Carlon and Lippé 2011).

Although sexual reproduction is the dominant form of reproduction in corals and many other organisms, asexual reproduction is also sometimes common. Fragmentation produces large numbers of clonemates that can be readily recognized with genetic methods (Foster et al. 2013) (Fig. 6.7). Documenting the geography of clonal reproduction is important because in many mass-spawning corals, self-fertilization is not possible, with implications for reproductive success. A less common form of asexual reproduction is the production of asexual larvae. The best studied case among corals is that of *Pocillopora damicornis*; a recent analysis using microsatellite data suggested that over 90 % of the larvae released were produced parthenogenetically (Combosch and Vollmer 2013).

Of course, asexual reproduction does not prevent the occurrence of mutations. Analogous to the forms of asexual reproduction described above is the clonal reproduction of units within colonies (polyps in corals, zooids in bryozoans, etc.), and growth in general is by its nature a form of asexual reproduction even in non-colonial forms. Recently, interest has increased in determining the prevalence of mutations occurring within individuals due to somatic mutations (mosaicism), a phenomenon that can only be routinely detected with genetic methods. One study using microsatellites found that 10 of 14 colonies of the coral *Acropora hyacinthus* exhibited genetic mosaicism with the potential to transfer this variation to the next generation via eggs (Schweinsberg et al. 2014).

6.8 Physiology and Development

The preceding sections have reviewed the profound contributions of molecular genetics to our understanding of ecology and evolution. At the level of individual organisms, genes control the structure and production of proteins, so it goes without saying that these methods have enormous power to elucidate physiological and developmental processes as well. Until recently, our ability to explore these aspects of coral biology depended on identifying genes known to be associated with certain functions in other metazoans and then using that information to search for related genes in corals. For example, Levy et al. (2007) were able to identify genes related to the ability to sense blue light that could be involved in the synchronization of spawning.

The landscape of what is possible has changed dramatically, however, now that gene expression, transcriptomes (sequences of transcribed genes) and entire genomes can be readily studied in non-model organisms, in particular corals. For example, comparisons of healthy versus diseased *Acropora cervicornis* and *Orbicella faveolata* show distinct patterns of gene expression as a function of coral health (Closek et al. 2014; Libro et al. 2013) (Fig. 6.8). A comparison of gene expression in the bases and branch tips of the closely related *A. cervicornis* and *A. palmata* revealed marked differences in gene expression that may underpin their morphological differences (Hemond et al. 2014). An array of genomic and proteomic data has revealed a suite of 36 proteins likely involved in the formation of coral skeletons (Drake et al. 2013). Gene expression responses associated with algal competition, high temperature, excess nutrients, and ocean acidification are providing a window on the complex physiologic responses of corals to stressors associated with locally driven reef degradation and rising CO_2 emissions (Rosic et al. 2014; Shearer et al. 2014; Moya et al. 2015).

Shinzato et al. (2014) provide a recent snapshot of genomic resources for corals and *Symbiodinium*. For corals, their list contains one published scleractinian genome (*Acropora digitifera*) and ten published scleractinian transcriptomes [four of which were for *Acropora*, two for *Porites*, and one each for *Favia*, *Orbicella* (listed as *Montastraea*), *Stylophora*, and *Pocillopora*]. The *A. digitifera* genome was about 420 Mbp in size and represented over 23,000 coding genes, about 93 % of which could be related to

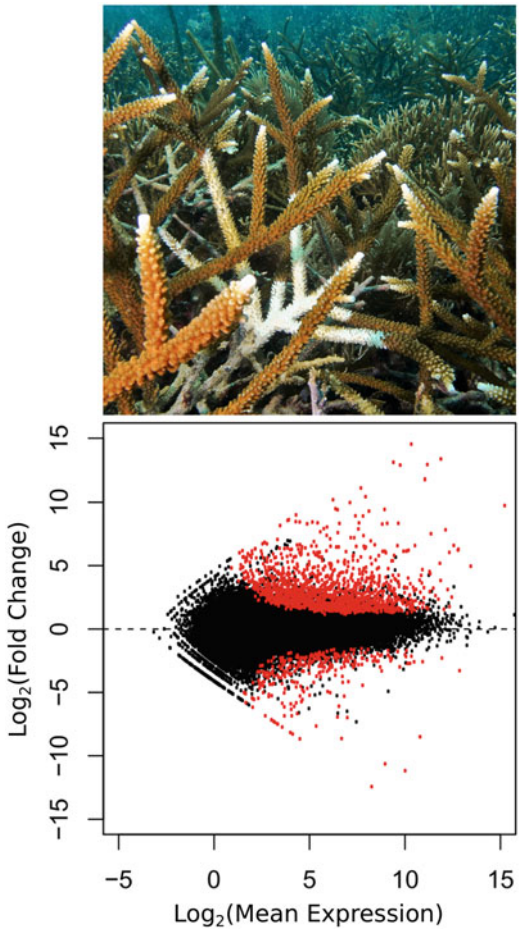

Fig. 6.8 High-throughput RNA sequencing is increasingly used as a tool to understand the physiological response of corals to stressors. Libro et al. (2013, above from Figs. 1 and 2) investigated the immune response of *Acropora cervicornis* to White-Band Disease (*upper photo*) by comparing RNA-seq profiles of healthy and infected tissues. Their approach identified a series up- and down-regulated genes that represent 4 % of the coral transcriptome (plotted in *red* below)

genes in other metazoans. Among these are genes linked to processes associated with calcification, *Hox* developmental genes, genes associated with innate immunity (some of which may be involved in the establishment and maintenance of symbiosis), genes involved in protection against UV damage, fluorescent protein genes, and photoreceptor and circadian clock genes. For *Symbiodinium*, one genome (~1500 Mbp) and 13 transcriptomes were cited in the review (the latter including representatives of the four clades commonly found with scleractinian corals). Our understanding of these data is more limited because of the scarcity of comparative data on dinoflagellate genomes compared to metazoans, as well as the large and peculiar features of the dinoflagellate genome itself. For both corals and *Symbiodinium*, the genomic information landscape is changing so rapidly that any review is almost immediately out of date.

Finally, microbes, being single celled, sit at the intersection of physiology and ecology. Because of their importance in biogeochemical cycling, one way to explore the metabolism of a reef is through analyzing microbial genes. Kelly et al. (2014) using a metagenomic approach found for an array of islands spanning a variety of biological, oceanographic and geographic characteristics, that the type of organisms living on the bottom (especially the amount of living coral) was the best predictor of the taxonomic composition of the microbes, whereas distance from the equator was the strongest predictor of metabolic characteristics of the microbial community.

6.9 Concluding Remarks: Molecular Genetics and the Future of Coral Reefs

Part of what drives science is the desire for technical breakthroughs, and molecular genetics is arguably the scientific field most transformed by such achievements in recent decades. Yet, despite their seductive appeal, it is important to remember that these powerful methods are tools. As with any tool, what really matters are the problems that need solving and the questions that need answering. For this reason, the insights provided by molecular genetics are most compelling when placed in a broader context. In that light, one of the most important frontiers is increasingly the linkage between insights on the functioning of coral reefs provided by molecular genetics and the challenges posed to reefs by the Anthropocene.

Many of the topics reviewed above have clear conservation implications. The dispersal capabilities of fish have important ramifications for the design of marine protected areas (Almany et al. 2013). The identification of stress resistant genotypes could be useful in facilitating adaptation to climate change (Chap. 7, Lundgren et al. 2013; Hume et al. 2015). The characterization of pathogens can form the underpinnings for the management of disease (Pollock et al. 2011). Evolutionary trees can be used to predict the implications of extinctions for the preservation of biodiversity (Huang and Roy 2015). Genetics can play critical roles in the analysis of diet and populations of invasive species (Betancur-R. et al. 2011; Valdez-Moreno et al. 2012) and in tracking the sources of population explosions of native species such as *Acanthaster* (Timmers et al. 2012). Rapid biomonitoring of diversity, including as a response to management, is made possible by metabarcoding (Leray and Knowlton 2015). DNA forensics allows one to assess the extent to which fisheries regulations are being followed (Cox et al. 2013). Indeed, the list is nearly endless.

Could genetic engineering be next? Van Oppen et al. (2015) have considered just that in their thoughtful essay on using assisted evolution to help save coral reefs.

Though they stop well short of the creation of Frankenstein corals, their list of topics to explore includes exposing *Symbiodinium* cultures to mutagens with the goal of producing strains better able to cope with rising temperatures and declining pH.

In sum, the future of molecular genetics in reef studies is bright indeed. One can only hope that these powerful methods will also serve to brighten the future of the reefs themselves.

Glossary[1]

Barcode is a standard short stretch of DNA that is unique to each species and therefore used to delineate species or identify unknown specimens. In animals, the most common barcode is a 658 bp segment of the mitochondrial cytochrome c oxidase subunit I gene.

Barcode of Life Data Systems (BOLD) is an online platform for the storage, analysis and publication of DNA barcode records.

Coalescent theory uses a mathematical model to reconstruct the genealogy of genes back to their common ancestor.

DNA microarray analyses are most commonly used to measure the expression levels of a large number of target genes simultaneously. Microarray chips contain thousands of microscopic spots where specific DNA probes are inserted. The relative abundance of target genes is quantified via fluorescence when target DNA hybridizes to probes.

Environmental DNA is the sum total of DNA in an environmental sample. It comprises not only the DNA from intact organisms in the sample, but also other sources of cellular or extracellular DNA released by an organism into the environment (e.g. in mucus, gametes, feces).

Expressed Sequence Tags (ESTs) are portions of complementary DNA (cDNA) that are constructed from messenger RNA. These fragments of expressed coding genes are used for gene discovery, mapping, gene prediction, gene expression and polymorphism analysis.

GenBank is a publicly available collection of DNA sequences hosted by the National Institute of Health (NIH).

Genome is the entirety of the genetic information contained in an organism.

Genomic library is the collection of DNA fragments representing the genome of an organism stored as short fragments within many individual bacteria or yeast cells. Such collections facilitated early efforts at genome sequencing.

Genomics is the quantitative analysis of the genome.

High-throughput sequencing technologies (i.e. pyrosequencing, semiconductor sequencing) produce millions of sequences concurrently within a few hours. These technologies have drastically lowered the cost of studies that require large amounts of sequence data.

Metabarcoding uses DNA-based species identification and high-throughput sequencing as a cost- and time-effective way to infer the species composition of environmental samples (e.g. plankton, sediments).

Metagenomics is the study of the genetic material collected from the environment. It provides a profile of diversity, including many small organisms that cannot be cultured, and a detailed characterization of the metabolic genes present in an environmental sample.

MicroRNA (miRNA) are short non-coding RNA molecules (approximately 22 nucleotides in length) found in the genomes of plants, animals and some viruses that play a key role in the regulation of gene expression.

Microsatellites are short repeating DNA sequences (two to ten base pairs in length) found across the genome of a species. Because they evolve rapidly, they are especially useful for population studies and individual fingerprinting.

Molecular clock is a mathematical approach that uses the fossil record and rates of DNA sequence evolution to estimate the time since two species or a group of species diverged.

Molecular cloning is a technique that uses a host organism (easy-to-grow bacteria) to replicate a single DNA molecule into multiple identical copies.

Nuclear DNA/organelle DNA/ribosomal DNA are different types of DNA found in the nucleus and organelles (such as the mitochondria, mtDNA) of eukaryotic organisms. Ribosomal DNA (rDNA) refers to the genes that code the RNA that makes up the ribosomes.

Operational Taxonomic Units (OTUs) are low-level taxa often equivalent to species that are defined genetically rather than being identified to species using traditional morphological methods.

Phylogeography is the study of historical processes (i.e. vicariance, population expansion) that explain the present day distribution of populations or species using mitochondrial/nuclear gene genealogies.

Polymerase Chain Reaction (PCR) is used to replicate a single copy of a DNA fragment into millions of copies of the same DNA fragment within a few hours, allowing the DNA to be sequenced.

Primers are strands of nucleic acids used as a starting point for DNA replication during the polymerase chain reaction.

Protein electrophoresis is a laboratory technique used to separate individual proteins from complex mixtures using

[1] Some terms and concepts useful for understanding molecular genetic studies.

differences in size and electric charge. This was one of the first molecular genetic tools to be used in coral reef studies.

Proteomics is the study of the composition, structure and function of the whole set of proteins produced by the coding genes of an organism.

Restriction-site Associated DNA (or RAD) sequencing is a method used to sample thousands of random parts of the genome of many individuals simultaneously using high-throughput sequencing. Because it analyzes a small fraction of the entire genome, it allows affordable study of many markers across the genome for population genetic studies in non-model species.

Restriction Fragment Length Polymorphism (RFLP) analysis is a DNA profiling technique that uses restriction enzymes to cut stretches of DNA at specific genetic sequences within a gene, followed by analysis of variation in the lengths of the fragments.

Sanger Capillary Sequencing is an automated DNA sequencing technology developed in 1977 by Fred Sanger. It uses a laser to read the position and identity of dye-labeled nucleotides on DNA fragments previously amplified via PCR.

Shotgun Sequencing is a method used to read the sequence of very long stretches of DNA (i.e. genomes). The process involves shearing the long DNA stretch into smaller fragments ($<$1000 bp) that can be sequenced individually and later reassembled bioinformatically using overlapping regions.

Single Nucleotide Polymorphism (SNP) is a genetic variant at one position in a DNA sequence shared by multiple individuals in a population. The frequency of different SNP alleles can be analyzed with respect to such factors as environment or geographic locale.

Transcriptomics is the study of sets of genes expressed in the genome of a given organism under specific conditions.

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