10 *Acropora*—The Most-Studied Coral Genus

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10.1 HISTORY AND TAXONOMIC STATUS OF THE GENUS

Corals belong to the phylum Cnidaria, the class Anthozoa (along with the sea anemones) and the Order Scleractinia (the stony corals). Within this order, there are two major clades, the Complexa and Robusta (Romano & Palumbi 1996). These clades, which were originally separated on the basis of 16S sRNA sequences and named on the basis of their skeletal characteristics, have been confirmed by more recent sequencing approaches that have resulted in the phylogenetic reclassification of corals at all taxonomic levels (Kitahara et al. 2016; Ying et al. 2018). The family Acroporidae, to which the genus *Acropora* belongs, falls within the complex clade and is the most speciose family of corals (Madin et al. 2016; Renema et al. 2016), as well as being responsible for much of the three-dimensional structure of modern reefs. Members of this family are commonly known as staghorn or elkhorn corals.

As summarized in Table 10.1, based on the number of mentions in Google Scholar, the genus *Acropora* is by far the most-studied genus of corals, and this has meant that we have had to be very selective in what to include in this chapter. For this, we apologize to the many authors whose excellent work we have failed to cite.

Our goal has been to provide the information required for an understanding of the basic biology of members of the genus *Acropora* and then to focus on some of the most recent findings and debates. Within the genus, the Caribbean species *Acropora palmata* (#1) and *Acropora cervicornis* (#3)

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TABLE 10.1
Most-Studied Corals Based on Number of Mentions in Google Scholar 2020

Widely studied coral genera	
Acropora	78,500
Pocillopora	19,000
Orbicella (Montastraea)	14,990
Stylophora	12,200
Widely studied species within the genus *Acropora*	
Acropora palmata	10,800
Acropora millepora	10,300
Acropora cervicornis	8,310
Acropora digitifera	3,230
Acropora tenuis	3,190

rank highly on the scale of mentions. The five most-studied *Acropora* species, as listed in Table 10.1, are pictured in Figure 10.1a–e, while Figure 10.1f–l shows the diverse morphology of other members of the genus.

In spite of the popularity of the Caribbean species, much of the *Acropora* research of this century has focused on Indo-Pacific species, partly due to the rise of large research centers in Australia (e.g. the ARC Centre of Excellence for Coral Reef Studies, James Cook University and the Australian Institute of Marine Science, all in the Townsville area, and the University of Queensland in Brisbane), as well as the Okinawa Institute of Science and Technology in Japan. There are additional major foci of coral research at King Abdullah University of Science and Technology (KAUST) in Saudi Arabia and in Israel, although with somewhat less emphasis on *Acropora* research, perhaps reflecting the composition of the fauna.

There has been a long-standing debate over what the type specimen of the genus *Acropora* should be. The situation was summarized in 1999 by Stephen Cairns (quoted in Wallace 1999) as follows: "The largest and most important genus of hermatypic Scleractinia does not have a recognisable type species". After an extensive historical review of names, Wallace designated a neotype for *Acropora muricata* (originally described as *Millepora muricata* by Linnaeus 1758) (Wallace 1999, p. iv). The description by Linnaeus was based on a drawing of a specimen from Ambon, Indonesia, by G.E. Rumphius, and therefore did not include a type specimen, necessitating Wallace to designate a neotype. The first use of the name *Acropora* for the genus was by Oken (1815), although most nominal *Acropora* species were described as *Madrepora* until Verrill (1901) formalized the genus *Acropora* within the newly designated family Acroporidae.

The genus *Acropora* currently contains approximately 408 nominal species (Hoeksema & Cairns 2020). However, many of these nominal species were synonymized in taxonomic works based on skeletal morphology in the late 20th century, while the status of others remains unresolved (Veron & Wallace 1984; Wallace 1999). Based largely on morphological features, Wallace (1999) recognized only 114 species, leaving almost three-quarters of nominal species either synonymized or unresolved. This was followed in 2012 by a revised monograph recognizing 122 species (Wallace et al. 2012). However, this monograph was completed just as molecular phylogeny was emerging, changing many of our views on relationships throughout the animal kingdom, including among corals, where environmental factors can have a major effect on micromorphology and few taxonomically informative morphological features have been identified. The switch from a taxonomy based exclusively on morphology to one utilizing an integrated approach combining morphology with sequence data has resulted in frequently changing views of relationships within the Scleractinia. Although molecular phylogenetics has largely stabilized genus- and family-level relationships (Kitahara et al. 2016), there is still considerable uncertainty at the species level in many groups, especially in the hyperdiverse family Acroporidae. Fortunately, newly developed molecular techniques such as targeted capture of conserved loci may allow resolution of species-level relationships (Cowman et al. 2020) and, combined with comparison to type material, should allow the testing of species boundaries and identification of informative characters for delineating species. This work suggests that the diversity of the genus *Acropora* is far higher than currently appreciated and that many species are not widespread across the Indo-Pacific, but restricted to specific biogeographic regions. So, while much of the material on structure and biology in Wallace's 1999 book is still valid and useful, the taxonomy is mostly in the process of revision.

Acropora taxonomy, as traditionally practiced, was based on qualitative morphological differences which were not easily recognized by the non-specialist, a situation which is problematic in a genus with environmentally induced morphological variability. This problem is exacerbated by the issue of potential hybridization among species in the genus, as was first brought to widespread attention by J.E.N. Veron in his book *Corals in Space and Time* (1995). This book popularized the idea of reticulate evolution in corals and called into question the definition of a species. For the species, Veron suggested substituting a grouping called a syngameon, which is an interconnected group of potentially interbreeding populations. Hybridization, to the extent it exists, will make it difficult to define a species, but molecular phylogenetics is also calling into question many of the morphological characters formerly used to define species. Indeed, several studies have highlighted extensive "cryptic" species complexes within morphological species (e.g. Richards et al. 2016; Sheets et al. 2018), and at least some of the characters used to define morphological species and species groups are invalid (Cowman et al. 2020). The existence of "cryptic" species is also supported by other lines of evidence. For example, the putatively widespread species *Acropora tenuis* was chosen for detailed study of spawning patterns by Gilmour et al. (2016) specifically because it was thought to be easily recognizable in the field. However,

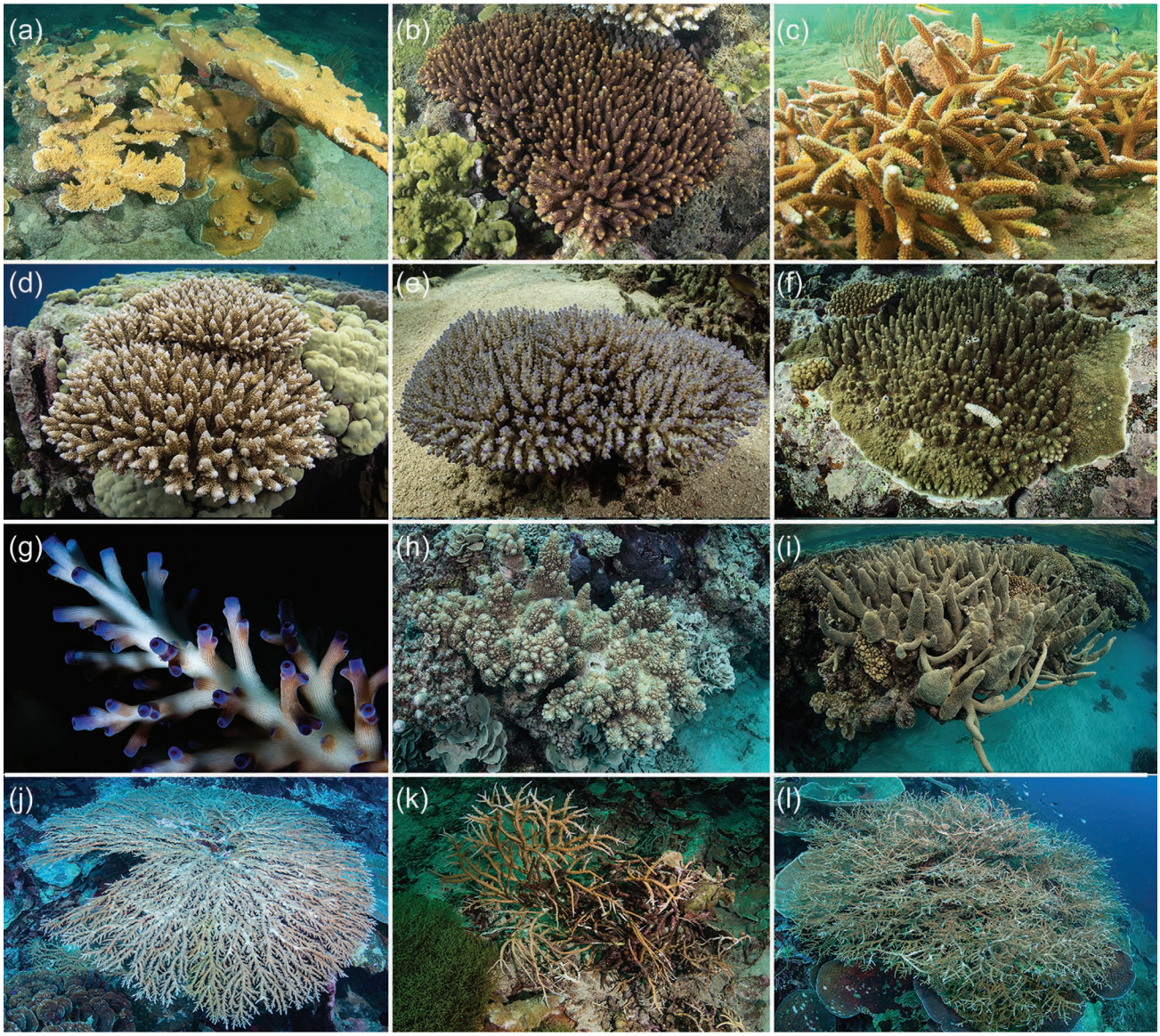

FIGURE 10.1 Diverse morphologies within the genus *Acropora*. (a–e) The five most-studied species: (a) *A. palmata* (Florida), (b) *A. millepora* (Magnetic Island, central Great Barrier Reef), (c) *A. cervicornis* (Florida), (d) *A.* cf *digitifera* (Kimbe Bay, New Britain, Papua New Guinea), (e) *A. tenuis* (Fiji), (f) *A.* aff. *palmerae* (Tonga), (g) *A. echinata* (Mantis Reef, northern Great Barrier Reef), (h) *A.* aff. *listeri* (Ha'apai, Tonga), (i) *A.* cf. *pacifica* (Ha'apai, Tonga), (j) *A. pichoni* (Kimbe Bay, Papua New Guinea), (k) *Acropora cf. rongelapensis* (Pohnpei, Micronesia), (l) *A. walindii* (Kimbe Bay, Papua New Guinea). Species identifications based on comparisons to type material of all nominal species using open nomenclature outlined in Cowman et al. (2020). (Photos [a,c] courtesy Peter Leahy; [b, d–l] Tom Bridge. Copyright is retained by the photographers.)

in spite of morphological similarity, the population was divided into two genetically distinct groups, as judged by microsatellites and time of spawning. In this chapter, we have retained the names used by the authors of the papers cited while noting that these identifications may be subject to future revision.

In spite of these difficulties, taxonomy is fundamental to the study of coral biology, especially for the field biologist, and no one has proposed a practical way to do without the concept of a species. Several efforts are underway to try to improve identification while maintaining the species concept. In one approach, Kitchen et al. (2019) used shallow genome sequencing to sample multiple populations of the two Caribbean acroporids, *A. palmata* and *A. cervicornis*, to establish the degree of intraspecific genomic variability and to find single nucleotide variants that allowed the two species to be distinguished. They also set up computational tools and stored workflows on the Galaxy server, to which others can add data from other *Acropora* species as these become available. A second approach uses targeted sequence capture of conserved genomic elements found in all corals to produce phylogenies that are stronger than those based on one or a few genomic loci and at a lower cost than whole genome sequencing (Cowman et al. 2020). These

robust phylogenies can then be combined with other lines of evidence (e.g. morphological, ecological or geographic data) to support the delineation of species. As in other coral taxa examined using such approaches (e.g. Benzoni et al. 2010; Budd et al. 2012; Huang et al. 2014), there is evidence that morphological characters for delineating species and therefore useful for field research do exist, although they are sometimes incongruent with traditional taxonomic classification.

This integrated approach combining phylogenomics with other lines of evidence, such as spawning times and geographical partitioning, forms the basis for re-examining the taxonomy of the group. The strong evidence for extensive "cryptic" speciation within putatively widespread *Acropora* species (e.g. Richards et al. 2016) necessitates comparison of operational taxonomic units (OTUs) to the type material of all 408 nominal species, not just those accepted in recent revisions, given that many of these "cryptic" species likely represent nominal species that have been synonymized based on morphological characters.

Possible approaches to dealing with the identification problem for future workers include collection of field photos and voucher specimens, use of single nucleotide polymorphisms (which unfortunately can only be done post-hoc back in the lab) and a better understanding of phylogenetically informative morphological features which can be used to identify species in the field.

Staghorn corals are the most important contributors to the three-dimensional structure of modern reefs and are therefore vital for maintaining the biodiversity of these systems (Renema et al. 2016). Much of their success has been due to their mutualistic association with photosynthetic endosymbionts belonging to the family Symbiodinaceae, on which they depend for much of the energy needed for growth. They are therefore most common at shallow depths with good light penetration in tropical and sub-tropical regions, although some species have become specialized to mesophotic coral ecosystems. Originally all of the photosynthetic endosymbionts were treated as a single species, but they are now known to form a diverse group and are placed in different genera. They confer different physiological properties on the colonies that contain them, one of which is resistance to bleaching. The relationship between the coral and its symbionts is a very active area of research, as will be discussed in later sections.

10.2 GEOGRAPHICAL OCCURRENCE—PAST AND PRESENT

The geographical occurrence and paleontology of staghorn corals have recently been summarized by Renema et al. (2016). The earliest described *Acropora* is from the Paleocene, with 10 species known by the end of the Oligocene, 37 in the Miocene, 60 in the Pleistocene and up to 408 nominal species at present (Wallace & Rosen 2006; Santodomingo et al. 2015). However, it should be noted that because these identifications were based on morphology, they are probably conservative, because recent molecular phylogenies have suggested different relationships and will probably increase the number of species (Cowman et al. 2020). In addition, the fragile skeletons of many *Acropora* species are not well suited to fossilization, making their identification in fossil assemblages extremely difficult, particularly at the species level. In spite of their long history, staghorn corals were not dominant reef builders until approximately 1.8 million years ago at the start of a period of high amplitude sea level fluctuations which favored *Acropora* due to high growth rates and the ability to propagate by fragmentation as well as sexually (Renema et al. 2016).

The diversity of staghorn corals belonging to the genus *Acropora* is greater now than at any time in the past. As shown in Figure 10.2, they are currently found in the tropics and subtropics in all three of the world's major oceans between 30°N and 30°S, with their peak distribution in the Central Indo-Pacific. Within this range, they are found in diverse habitats, including reef flats, reef crests and slopes and down to the mesophotic zone (reviewed in Wallace 1999; Muir et al. 2015).

It appears that all species presently described as belonging to the genus *Acropora* reproduce by releasing their buoyant gametes into the water column where fertilization occurs, a process known as "broadcast spawning". Older literature (e.g. Kojis 1986a, 1986b) describes brooding in *Acropora palifera*, but all brooding species are now included in the sister genus *Isopora* (Wallace et al. 2007). In several parts of the world, most notably in northeastern Australia and in the waters around Okinawa, multiple species of *Acropora* spawn together on just a few nights of the year, in a phenomenon known as mass spawning. The term "mass spawning" is controversial (see Baird et al. 2009), but we are using it to refer to spawning on the same night by multiple species in a limited area. Once the egg has been fertilized, the resulting larva can survive for weeks or months on its stored lipid, perhaps supplemented by captured organic matter (Ball et al. 2002a). The longest documented survival time for an *Acropora* larva that we know of is 209 days (Graham et al. 2008), although in the field, much of a larval population is likely to have died long before that. This longevity is facilitated by a rapid decline in larval metabolism (Graham et al. 2013) during which larvae could theoretically be carried hundreds of kilometers by currents before settling to found colonies which could then colonize a new area by a combination of fragmentation and further mass spawning.

Although the Quaternary has seen a peak in *Acropora* abundance and diversity, populations started to shrink in the 20th century due to myriad anthropogenically induced threats to coral health. The greatest of these threats is global warming. Most corals live near their upper thermal limits, so a temperature rise of as little as 3°C for more than a few days causes them to lose the photosynthetic endosymbionts, members of the dinoflagellate family Symbiodinaceae, on which they depend for much of their energy, in a phenomenon known as coral bleaching. If bleaching is prolonged,

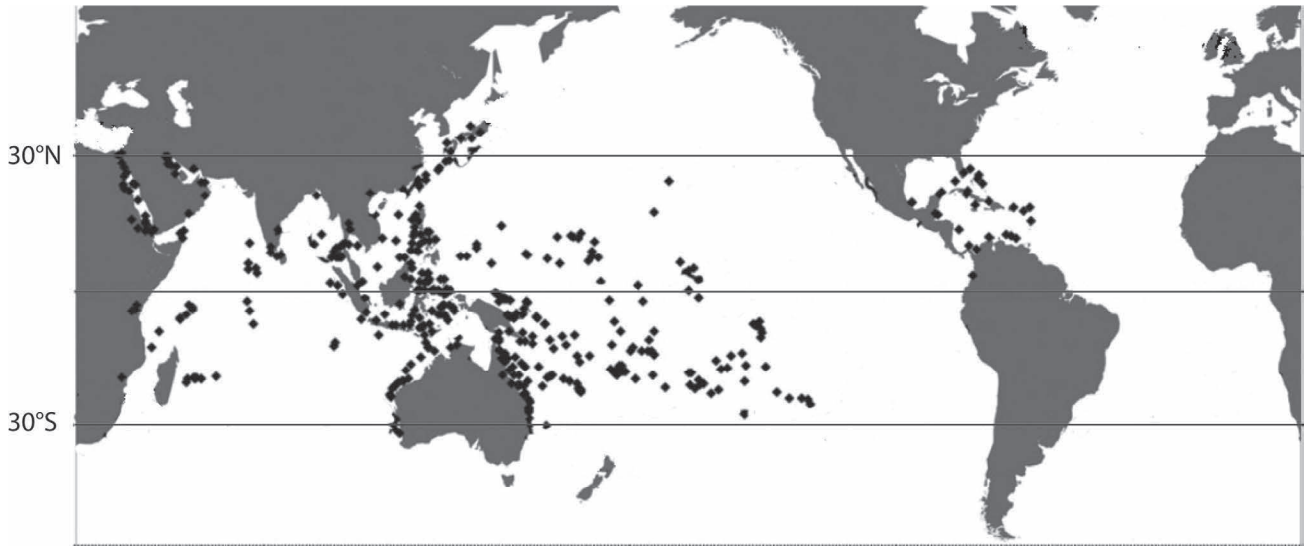

FIGURE 10.2 The worldwide distribution of *Acropora* species is essentially between 30°N and 30°S. (Modified from Wallace and Rosen 2006.)

the corals die, and members of the genus *Acropora* are particularly susceptible to bleaching. Episodes of bleaching are becoming increasingly widespread and frequent and have considerably reduced *Acropora* populations worldwide (Hughes et al. 2017, 2018). In addition to global warming, a second threat arising from rising atmospheric CO_2 levels is ocean acidification. Although a less immediate threat than bleaching, ocean acidification slows the rate of calcification and weakens coral skeletons and may therefore prove significant in the longer term. Other anthropogenic threats include severe weather events, reduced water quality, predator outbreaks (e.g. Crown of Thorns on the Great Barrier Reef), incidental damage due to fishing and diving, the aquarium trade and so on. All of these threats will result in changes to the distribution of individual species and may result in the extinction of some within this century.

10.3 LIFE CYCLE

There is a vast literature on various aspects of reproduction in *Acropora* to which we can't hope to do justice. Among the major reviews of coral reproduction which include information on *Acropora* are those of Harrison and Wallace (1990), Baird et al. (2009) and Harrison (2011), as well as a chapter specifically on reproduction in *Acropora* (Morita & Kitanobo 2020). In addition, the other references cited in this chapter contain many further references. Here we focus our discussion on the life cycle of *A. millepora*, as that is the species with which we are most familiar, but to the best of our knowledge, the life cycles of all members of the genus are very similar.

The month and day of spawning are determined mainly by seawater temperatures in the weeks before potential spawning dates and by phases of the moon, which in turn determine the tides (Keith et al. 2016). The importance of a rapid increase in temperature as a cue for spawning is evident on the central Great Barrier Reef (GBR) where corals on inshore reefs, where the water warms first, frequently spawn one month ahead of offshore reefs, although separated from the latter by only tens of kilometers. Thus, on the central GBR, inshore reefs usually spawn three to five days after the full moon in October or November, with offshore reefs a month later. The night of spawning is not totally synchronous within a population, as spawning may extend over a few nights, although peak spawning is usually restricted to a single night. Not only is there a peak night, but there is usually a peak time of the night at which each species characteristically spawns. For instance, at Magnetic Island, *A. tenuis* usually spawns approximately two hours before *A. millepora* (personal observation). For broadcast spawning corals, onset of darkness is typically the final cue determining the hour of spawning (Babcock et al. 1986). Fukami et al. (2003) describe a similar temporal separation of spawning times in sympatric acroporids in Okinawa.

In some years on the GBR, there is a split spawning, with part of the population spawning in one month and the remainder a month later. A recent modeling study using seven years of data from the GBR has combined data on the time and place of *Acropora* spawning with oceanographic data and has found that split spawning increases the robustness of coral larval supply and inter-reef connectivity due to temporal changes in the currents (Hock et al. 2019).

While the spectacular synchronous multispecies mass spawnings on the Great Barrier Reef have attracted considerable popular and scientific attention, synchrony is by no means universal, even there. In fact, in eastern Australia, synchrony is greatest at mid-latitudes and is reduced to both north and south, and populations in the north often have two spawnings per year.

A major study of *Acropora* spawning patterns was undertaken at Scott Reef (14°S) off northwestern Australia (Gilmour et al. 2016), where 13 species of *Acropora* were followed over three years (n = 1,855 colonies). Of these, seven species spawned in both autumn and spring, five only in autumn and one only in spring. However, the vast majority of individuals spawned only once a year in the same season. The most-studied species, *A. tenuis*, was divided into two genetically distinct but morphologically indistinguishable groups, one spawning in autumn and the other in spring.

On the night of spawning, egg–sperm bundles, which have been developing on the mesenteries of the individual polyps of the colony, are released from their mouths. The egg-sperm bundles contain a number of eggs, surrounding a mass of sperm. They are buoyant due to the high lipid content of the eggs, which is mainly in the form of wax esters (Harii et al. 2007). Once these bundles are released, they float to the surface, breaking up as they go and releasing the sperm. However, how synchronization between colonies is achieved is unknown. One possibility is a so-far-undescribed chemical cue, and there appears to be nothing in the literature to indicate that this has been investigated. In a mass spawning event, the eggs and sperm from one colony will join millions of others coming from diverse individuals and species, although the neighbors will often be predominantly of the same species, thus facilitating fertilization. It seems likely, just on consideration of gamete density, that the majority of fertilizations will occur within the first hour or two of gamete release, although Willis et al. (1997) report that gamete viability does not fall for six to eight hours after release. Cross-fertilization between closely related species is minimized in several ways. First, temporal separation of spawning times is important, as most eggs are apparently fertilized within a relatively short period after release. Second, according to Morita et al. (2006) *Acropora* sperm are not motile when spawned and only become so in the vicinity of conspecific eggs, first swimming in circles and then in a straight line as they get nearer to the egg. However, apparent hybridization between recognizably different morphospecies does occur, reaffirming questions about the nature of "species" in *Acropora*. Several generalizations emerged from the extensive hybridization experiments reported by Willis et al. (1997). First, self-fertilization of eggs from a colony by sperm from that same colony was rare, indicating that sperm can distinguish eggs from their own colony from those from other conspecific colonies. Second, morphologically similar "species" were more likely to hybridize than those which were dissimilar. Third, fertilization success was bimodal in *Acropora millepora*, and on closer inspection, it was found that low fertilization success was associated with differing morphologies of the parent colonies, suggesting the existence of two distinct populations (or of two separate species), one thick branched and the other thin branched. This is a particularly interesting case if the two morphs were both sympatric and spawning at similar times. Apparent cases of hybridization were recorded in more than one-third of 42 species pairs tested, but these results must be considered in light of more recent understanding of species boundaries. Hybrids survived just as well as non-hybrids. The paper of Willis et al. (1997) considers the many implications of their hybridization experiments and concludes, "The complexity in coral mating systems revealed by our experimental crosses suggest that a number of alternative speciation processes, as well as reticulate evolutionary pathways, may have contributed to shaping modern coral species". The take-home lesson for present-day workers is the need to carefully document their experimental material in every way possible, including photos, exact locality data and, if possible, molecular data to support the accurate delineation of species.

Moving on from these complications, the life cycle itself (Figure 10.3) seems to be basically similar for all of the species that have been studied. Once the egg has been fertilized, it continues to float for at least an hour before starting

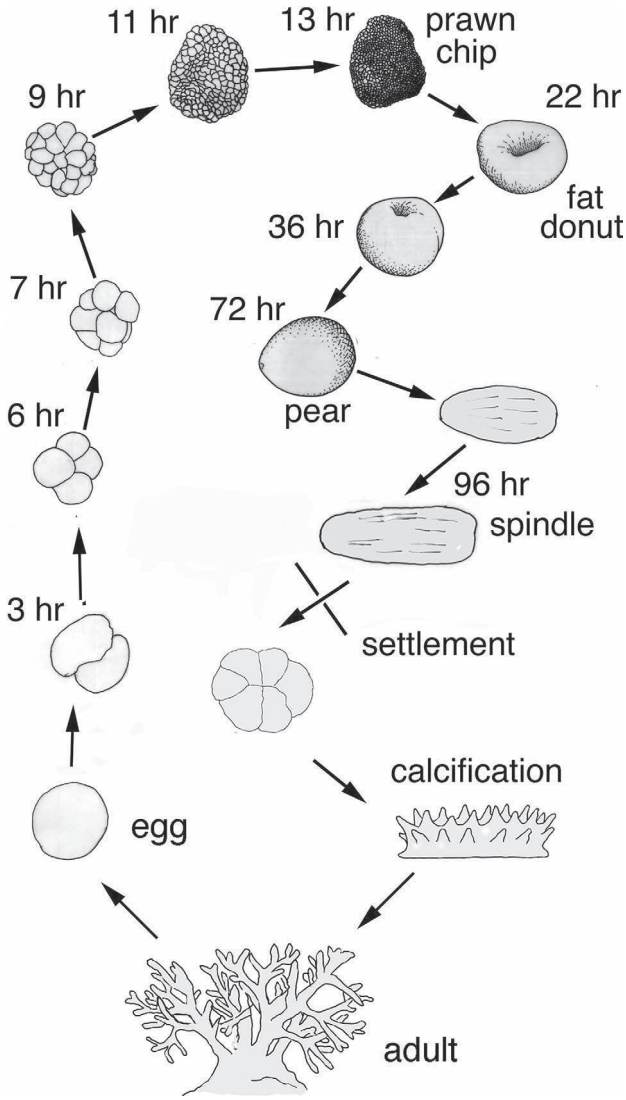

FIGURE 10.3 Life cycle of *A. millepora* in diagrammatic form. (Modified and reproduced with permission of UPV/EHU Press from Ball et al. 2002b. Coral development: from classical embryology to molecular control. *Int. J. Dev. Biol.* 46: 671–678.)

to divide. Then, once cell division has started, it progresses fairly steadily in a temperature-dependent fashion, initially resulting in a ball of cells, known as a morula (Figure 10.4h, i). This then flattens into a stage known colloquially as a prawn chip, due to its resemblance to a prawn cracker (Figure 10.4j–m). As cell division continues, this structure bends and thickens, taking on the appearance of a fat donut, with a depression in one side (Figure 10.4o). Tissue then sinks into this hole, the blastopore, which gradually closes as cells move in from the sides until a closed sphere is formed (Figure 10.4p). At about this stage, cilia appear and the sphere begins to elongate, taking on a pear shape with an oral pore at its apex (Figure 10.4q). The process of elongation continues until, at an age of four to five days, the planula larva has achieved the shape of a ciliated spindle, swimming independently through the water column. Up to this point, the population has remained relatively synchronous in its morphological development. Once elongation to a spindle has occurred, there is relatively little overt morphological change until just before settlement, although differentiation is continuing at the cellular level with an accompanying increase in the number of genes expressed. Somewhere between four and seven days in culture, the developmental synchrony breaks down, and a portion of the population shows a dramatic change in behavior, changing from horizontal swimming to corkscrew swimming into the bottom, apparently testing the substratum. By seven days post-fertilization >50% of the population studied by Strader et al. (2018) had settled and metamorphosed. The delay in settlement by part of a population occurs even in members of a single cross (Meyer et al. 2011; Strader et al. 2018), and its basis is not understood. An interesting correlate of this difference is that those larvae with higher levels of expression of red fluorescent protein are less responsive to settlement cues (Kenkel et al. 2011) and have "gene expression signatures of cell cycle arrest and decreased transcription accompanied by elevated ribosome production and heightened defenses against oxidative stress" (Strader et al. 2016). This pattern of gene expression is consistent with elevated thermal tolerance and greater dispersal potential.

For details of the settlement process, see the section on unresolved problems, but as far as the life cycle is concerned, at the time of settlement, the planula larva samples the substratum with unknown receptors on or toward its aboral end. Once it detects a favorable chemical signal, it flattens onto the substratum, and the oral end spreads to form a primary polyp. The morphology of larvae at this stage is remarkably labile, as they can appear to start to settle but then resume swimming in a matter of seconds. However, shortly after settlement, they attach themselves to the substratum and within a day or so have begun to calcify, first forming a basal plate and then starting to erect septa in a six-part symmetry corresponding to the mesenteries which divide the developing polyp into chambers. Growth is at first two-dimensional along the substratum, with additional polyps appearing in the developing tissue mass beside the first. Then the colony becomes dome shaped as polyps are added over the next few months, and finally vertical branches are sent up from the dome-shaped structure (Abrego et al. 2009). In *A. tenuis*, reproduction begins at colony diameters >10 cm, with the percentage of colonies reproducing steadily rising from there; once colony diameter is >21 cm, all are reproductively mature (Abrego et al. 2009).

10.4 EMBRYOGENESIS

The important stages in *Acropora* development were outlined in the previous section and are similar in all of the *Acropora* species studied. These include *A. hyacinthus*, *A. nasuta*, *A. florida* and *A. secale* (Hayashibara et al. 1997); *A. millepora* (Hayward et al. 2002, 2004, 2015; Okubo et al. 2016); *A. intermedia, A. solitaryensis*, *A. hyacinthus*, *A. digitifera* and *A. tenuis* (Okubo & Motokawa 2007); *A. digitifera* (Harii et al. 2009); and *A. digitifera* and *A. tenuis* (Yasuoka et al. 2016), and the embryology of several of these species has been studied in considerable detail.

As in the life cycle, we will start with release of an egg–sperm bundle by the adult coral. This consists of 4–17 eggs surrounding a tightly packed core of sperm (Hayashibara et al. 1997; Okubo & Motokawa 2007). The eggs are at first compressed into ellipsoidal shapes but round up to form a sphere (Figure 10.4a) within an hour of release. Sperm consist of an anterior head and a collar surrounding the base of a flagellum (Figure 10.5a). Ultrastructural features of the sperm are described by Harrison and Wallace (1990) and Wallace (1999). The speed at which cell division occurs varies with the temperature, but following the timetable in Figure 10.3, by three hours, the two-cell stage has been reached. The first cleavage division is equal and holoblastic and occurs by progressive furrow formation; the cleavage furrow initiates on one side of the fertilized egg and moves across to the opposite side, resulting in the formation of two equal blastomeres (Figure 10.4b–d). At this stage, the blastomeres may be parallel (Figure 10.4c) or at right angles to each other (Figure 10.4d). At the four-cell stage, the blastomeres lie in a single plane (Figure 10.4e), but as cell division continues, they form a cube (Figure 10.4f, g). With further cell division, the cube of cells becomes more rounded (Figure 10.4h). Anti-tubulin staining at this stage reveals no clear pattern in the orientation of dividing cells (Figure 10.5b). Next a depression appears in one side of the mass of dividing cells (Figure 10.4i); then the cells spread and flatten, eventually forming a bilayer (Figure 10.4j–m, 10.5c, d). At this stage, lipid is distributed evenly within the cells (Figure 10.5c, d), and DAPI staining reveals extra-nuclear bodies (Figure 10.5e, arrowheads) for which we have no explanation, unless they are mitochondria. As development continues, this bilayer thickens and rounds up, probably by a combination of cell movement and cell division (Figure 10.5f), although the relative contribution of these two processes has not been established (Figure 10.4n, o). We have described this process as gastrulation, as cells expressing

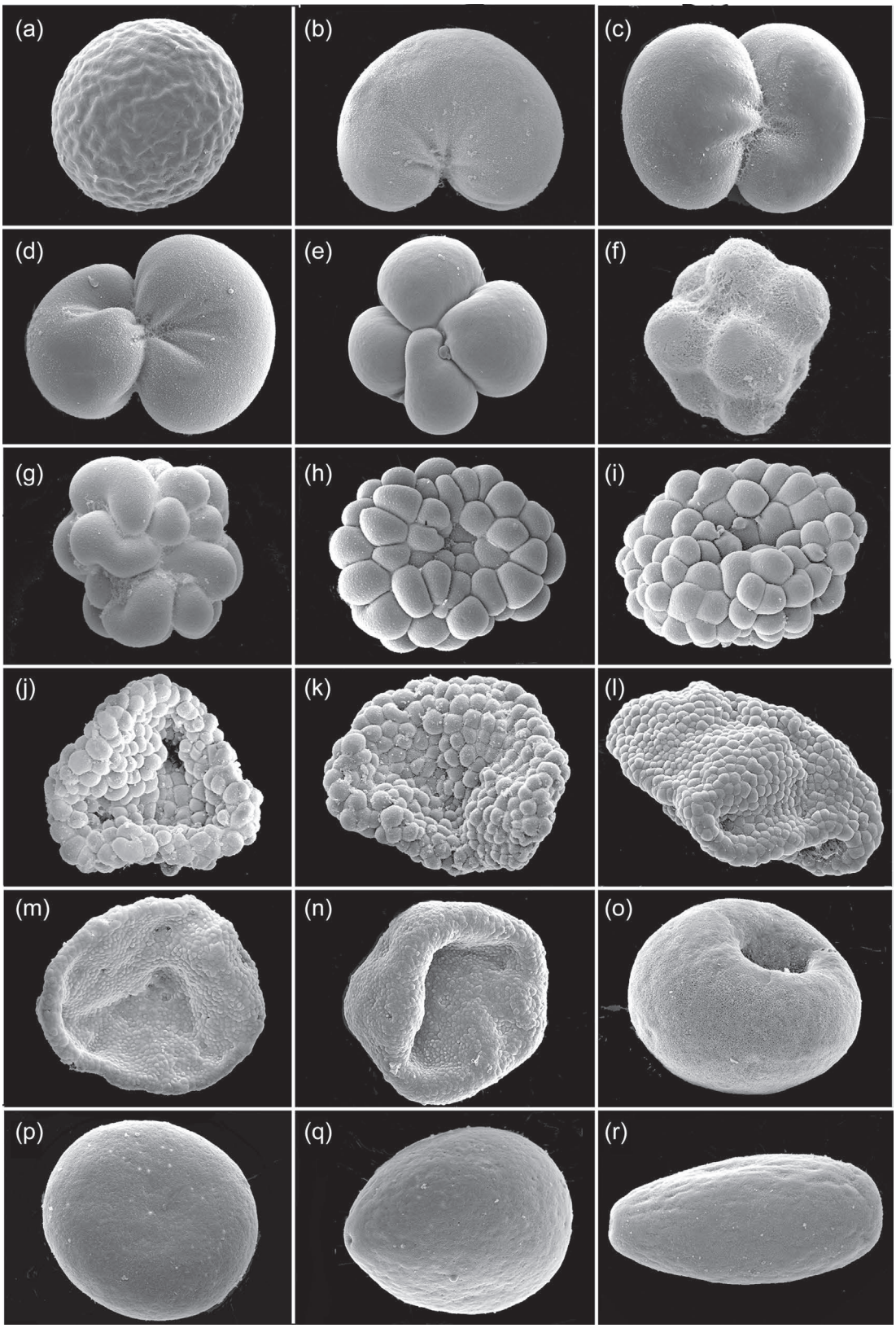

FIGURE 10.4 Scanning electron micrographs of critical point dried embryos corresponding to many of the stages shown in Figure 10.3 (life cycle). (a) Egg; (b) first cleavage division; (c) two-cell stage, blastomeres parallel; (d) two-cell stage, blastomeres at right angles; (e) four-cell stage; (f) eight-cell stage, divisions becoming asynchronous; (g) approximately 20 cells; (h,i) morula stage; (j–m) prawn chip stage, consisting of a steadily increasing number of cells; (n) the transition from prawn chip to gastrula; (o) gastrulation—cells are moving inward as the blastopore closes; (p) the blastopore has closed, and the embryo is spherical; (q) cilia have formed, and the sphere is elongating to form a pear; (r) the planula stage—this is the basic morphology until settlement, although the planula can change shape rapidly and dynamically.

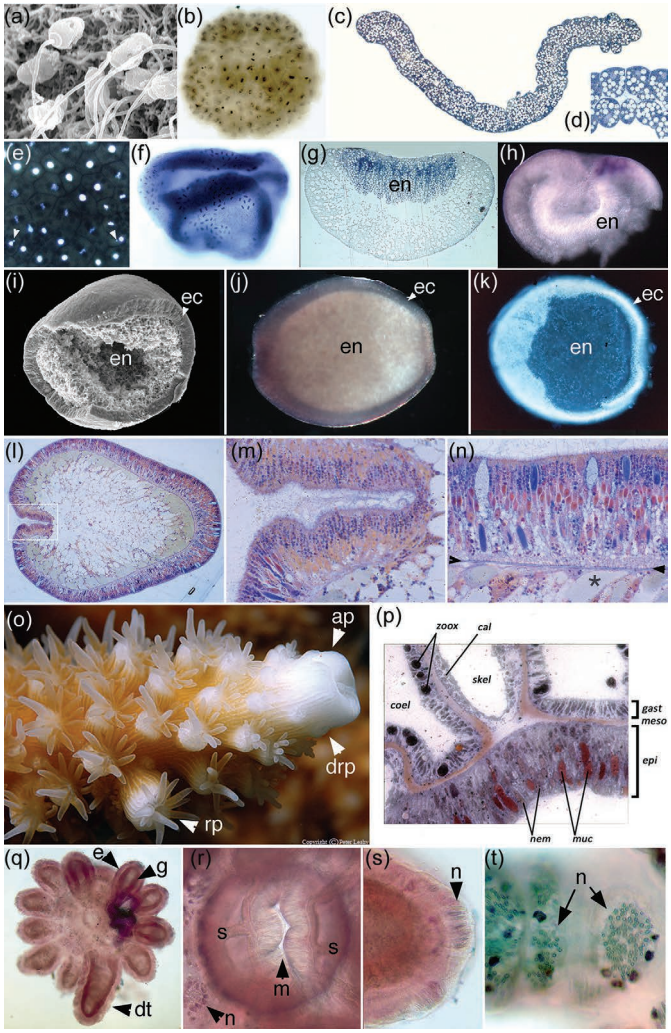

FIGURE 10.5 Aspects of *Acropora* development and anatomy visualized using different technologies. (a) Scanning electron micrograph of critical point dried *Acropora* sperm on the surface of an egg. (b) Anti-tubulin staining of mitotic spindles reveals no clearly ordered pattern of cell division at the morula stage. (c) Transverse section of a prawn chip stained with methylene blue and fuchsin, showing that it consists of a bilayer of cells containing evenly distributed droplets of lipid. (d) Higher magnification view of a portion of (c). (e) DAPI-stained whole mount of a prawn chip with mysterious extranuclear bodies (arrowheads). (f) Late prawn chip stained with anti-tubulin to reveal the patterns of cell division. (g) Section of an in situ hybridization of a bowl-shaped embryo. Tissue expressing the *snail* gene is moving inward to form the endoderm (en). (h) Section of a BMP2/4 in situ preparation reveals a well-developed endoderm at this stage. (i–k) Three embryos at the pear/planula stage examined using different technologies: (i) critical point drying reveals a clear demarcation between ectoderm (ec) and endoderm (en). Solvents used in preparation have removed lipid from the endoderm, giving it a frothy appearance. The central cavity is an artifact of the way in which the embryo fractured. (j) Light micrograph of an unstained embryo showing the highly reflective endodermal lipid (en) contrasting with the much less reflective ectoderm (ec). (k) DAPI staining of an embryo of similar age reveals the contrasting density of cells in the ectoderm (ec), as compared to the endoderm (en). This is consistent with the trichrome stained section shown in (l), in which the large, lipid-filled cells with small nuclei are apparent. (m) Blow-up of the boxed portion of the embryo shown in (l). The uniform nature and appearance of cells in this region contrast with the diversity of cell types apparent elsewhere in the ectoderm and are consistent with a possible function in extracellular digestion. (n) Trichrome staining reveals the diversity of cell types in the body wall away from the oral pore. Clearly apparent are dark-blue-staining cnidocytes (containing nematocysts) and gland cells (large empty-appearing cells). Arrowheads mark the mesoglea, beneath which lie lipid-filled cells (*), as well as smaller cells of unknown function. (o) Branch tip of *A. cervicornis*, showing the arrangement of the two types of polyps. At the tip of the branch is the large axial polyp (ap) which lacks zooxanthellae; behind it are small developing radial polyps (drp), and further proximally lie full-sized radial polyps (rp). (p) Polyp cross-section of *A. longicyathus* showing tissue layers. The coelenteron is lined with gastrodermis containing photosynthetic dinoflagellates (zoox). The calicoblastic epithelium (cal) lines areas occupied by the skeleton (skel) prior to decalcification for sectioning. The epithelium of the body wall contains mucocytes (muc) and nematocytes (nem) and is separated from gastrodermis by the acellular mesoglea (meso). (q) A radial polyp showing the longer directive tentacle (dt). The ectoderm (e), gastrodermis (g) and hollow nature of the tentacles are clearly visible. (r) The muscular mouth (m), showing the arrangement of the septa (s) and the abundant nematocysts (n) located on the oral disc. (s, t) Nematocysts (n) are abundant at the tips of the tentacles (s), particularly on their oral sides (t). (Photo in [o] courtesy Peter Leahy; photo in [p] courtesy Daniel Bucher and Peter Harrison from Bucher and Harrison 2018.)

the gene *snail* move inward through the pore (Figure 10.5g) to form a second tissue layer (Figure 10.5h). As development continues, the pore closes, forming a sphere (Figure 10.4p). Shortly thereafter, the sphere starts to elongate, becoming pear shaped (Figure 10.4q, 10.5i–k), and cilia form. As this elongation occurs, an oral pore (the future mouth of the polyp) opens at or near the site of the blastopore (Okubo & Motokawa 2007). Then, over the next 24–36 hours, cell division continues, new cell types differentiate and the pear elongates into a spindle-shaped planula larva (Figure 10.4r, 10.5l–n), a stage in which it may remain for days or weeks before settlement. Hayashibara et al. (2000) studied the development of cnidae in *Acropora nasuta* and found two types in planulae, a microbasic b-mastigophore nematocyst and a spirocyst. The appearance of cnidae in the planula at three to four days coincided with the start of settlement, and their abundance peaked at eight days, coinciding with maximum settlement. Interestingly, the number of spirocysts then fell in planulae which had failed to settle after eight days, possibly because they were used up in failed attempts to do so. These same two types of cnidae were present in the primary polyp, along with two additional types, the microbasic p-mastigophore and the holotrichous isorhiza.

10.5 ANATOMY

Before turning to anatomical details, a note on terminology relating to tissue layers is needed. The terms "endoderm" and "gastroderm/gastrodermis" are used interchangeably in the literature, as are "ectoderm" and "epithelium". Technically, the former term in each pair refers to embryonic tissue layers, while the latter is used for adult tissues, but this convention is often ignored.

There is no detailed account of what happens immediately after settlement for any one species, but by combining descriptions from several species, it is possible to put together a description that probably is correct in its general outlines for all species. The early steps in the process described in the following are shown in Figure 10.6a.

According to Goreau and Hayes (1977), working on *Porites*, the first step, once the planula larva has chosen a place to settle, is the laying down of a pad of a mucoid substance. Then, within a few hours or days of settlement, depending on species and conditions, the nature of the aboral ectoderm adjacent to the substratum undergoes a morphological change from a columnar epithelium consisting of multiple cell types to a flattened squamous epithelium consisting of a single cell type—the calicoblast cell. This process has been most studied in the genus *Pocillopora* (Vandermeulen 1975; LeTissier 1988; Clode & Marshall 2004), but those observations are consistent with what is known for *Acropora*. Hirose et al. (2008) has a series of photos showing the development of the living primary polyp, while a corresponding sequence of the early stages of skeleton formation in *A. millepora* is shown in Figure 8 of Wallace (1999). According to this sequence, by the third day after settlement, a disc-shaped basal plate has been laid down on which are 12 equally spaced protosepta radiating from the central area occupied by the polyp, like spokes of a wheel (Figure 10.6a4). By the fifth day, the inner ends of the septa have grown laterally and joined to form a circle known as a synapticular ring. The places where these lateral outgrowths meet are called nodes. By the seventh day, the nodes send projections centrally, and a second synapticular ring has formed concentric to and outside of the first (Figure 10.6a5). Further upward and outward growth occurs by addition of more synapticular rings. It is actually outgrowths from the nodes, rather than further development of the protosepta, that will form the adult septa (Piromvaragorn, cited in Wallace 1999). Once the tissue of the primary polyp has spread laterally across the substratum, secondary polyps start to appear by its side. As polyps are added, the colony becomes dome shaped. Then, once a colony consists of 15–20 polyps, some of these start to elongate, founding branches (Abrego et al. 2009).

Adult colonies of all species consist of numerous branches. The colony is organized so that the living tissue lies over of the skeleton that it is secreting (Figure 10.6d). The tissue throughout the colony is organized into two layers, an outer epidermis (or ectoderm) and an inner gastrodermis (Figure 10.5p, 10.6d). The nature of these two layers varies depending on where they are on the colony. At the tip of each branch, there is an axial polyp (Figure 10.5o, ap), while below it, on the sides of the branch, developing radial polyps are budded off (Figure 10.5o, drp) as the colony grows steadily larger. The axial polyp is the largest and fastest-growing polyp. It lacks zooxanthellae and contrasts in color with the radial polyps and the tissue covering the lower part of the branch, which contain zooxanthellae as well as often being pigmented.

Branches of *A. cervicornis* have been recorded to extend by as much as 300 um/day under favorable conditions (Gladfelter 1982). The axial and radial polyps are interconnected by a gastrovascular system of canals (Figure 10.6d–h) filled with fluid and lined with ciliated gastrodermal cells. It has been suggested that this allows sharing of photosynthate produced by zooxanthellate parts of the colony with the rapidly growing axial polyp, which lacks zooxanthellae of its own (Pearse & Muscatine 1971). Bucher and Harrison (2018) have hypothesized that the axial polyp may suppress others from forming as long as the photosynthate supply is limiting. Using time-lapse photography at six-hour intervals, Barnes and Crossland (1980) established that the peak period of daily branch extension was 1200–0600 and did not correspond to the peak period of accretion (0600–1200) as measured using ^{45}Ca. Gladfelter (1982) hypothesized that these observations could be explained by the rapidly growing axial polyp laying down a relatively flimsy framework during the first period, which is then filled in by continuing calcification behind the tip in the second. This is consistent with the observation that permeability and porosity of the

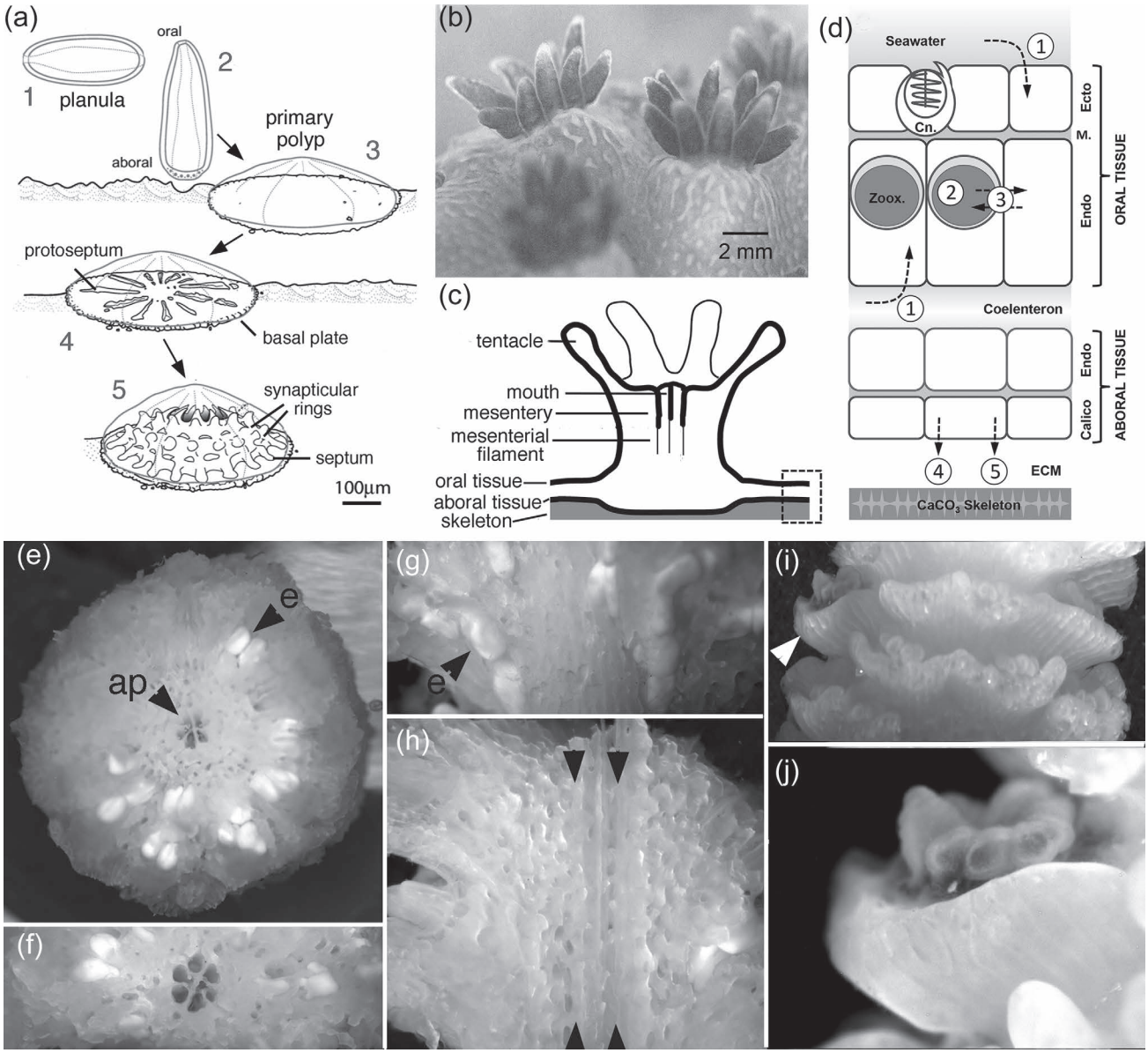

FIGURE 10.6 Anatomy. (a) Settlement, metamorphosis and the initiation of calcification. (a1) Initially, the planula larva swims horizontally well away from the bottom. (a2) When ready to settle, the planula initiates searching behavior, swimming into the bottom in a corkscrew fashion and apparently testing the substratum. (a3) Once a site is selected, the planula flattens in the oral/aboral axis and expands laterally, and a mucoid pad is laid down. (a4) Next calcification begins, first with the deposition of a calcified basal plate and then with the erection of radial protosepta on it. (a5) The protosepta are replaced by septa, which expand laterally at their inner ends to form a synapticular ring. Then more rings are added as the polyp grows. (b–d) Anatomy and function of the adult. (b) Expanded polyps of *A. digitifera*. (c) Diagrammatic view of a polyp with the parts labeled. (d) Histological organization of an area of calcifying tissue showing the relation of the tissue layers and the main metabolic pathways: (1) nutrient uptake, (2) photosynthesis, (3) nutrient exchange, (4) ion secretion, (5) organic matrix secretion. Cn, cnidocyte; M, mesoglea; ECM, extracellular matrix. (e–j) The skeleton. (e) Transverse section of a branch of *A. millepora* showing the central canal leading from the axial polyp (ap) and egg–sperm bundles (e) in canals leading from the radial polyps. (f) Blow-up of the central portion of (e). (g) Branch broken in the long axis showing the arrangement of the egg–sperm bundles in the canals leading to the radial polyps. (h) Another branch broken axially in the plane of the central canal (arrowheads). (i) Lateral view of a branch, showing the organization of the radial polyps. (j) Blow-up of the corallite arrowed in (i) showing a radial polyp with its long directive tentacle. ([a] Modified from Reyes-Bermudez et al. 2009; [b–d] modified from Bertucci et al. 2015.)

skeleton decrease with increasing distance from the branch tip (Gladfelter 1982).

The coral skeleton consists of calcium carbonate ($CaCO_3$) in the form of aragonite in an organic matrix consisting mostly of proteins, polysaccharides and lipids. The composition of the *A. millepora* (Ramos-Silva et al. 2013) and *A. digitifera* (Takeuchi et al. 2016) organic matrices has been determined, and progress has been made toward understanding basic mechanisms of calcification in other species (reviewed in Drake et al. 2019). However, how the

characteristic morphology of individual species is produced is still not understood.

As the colony grows, new branches are founded by appearance of a new axial polyp somewhere along an existing branch or by conversion of a radial polyp into an axial polyp (Wallace 1999). The tentacles of the polyps are mostly in multiples of six (hence the classification of *Acropora* in the Hexacorallia), with 12 tentacles being the most frequent (Figure 10.5o, q; Figure 10.6b, c, j). The radial polyps are retractile and can withdraw into the skeleton surrounding them when disturbed. The parts of a radial polyp are shown schematically in Figure 10.6c and in greater detail in Figure 10.5q–t. One tentacle (known as the directive tentacle) is consistently longer than all of the rest and is typically unpigmented, in contrast to the others (Figure 10.5o, q;10.6i, j). The organization of a radial polyp is clearly apparent in Figure 10.5q. Each tentacle is hollow and consists of an outer layer of ectoderm surrounding an inner layer of gastroderm, which in turn surrounds a hollow cavity, connecting to the central cavity, or coelenteron, of the columnar polyp. The mouth is at the center of a flattened area known as the oral disc and is closed by a muscular sphincter (Figure 10.5r). The central cavity is partially partitioned by mesenteries from which hang mesenterial filaments, containing nematocysts which help to subdue struggling prey. Nematocysts are also abundant at the tips of the tentacles (Figure 10.5s) and particularly on their oral sides (Figure 10.5t). The ectoderm consists of diverse cell types, including cnidocytes (which produce several types of nematocyst) as well as gland cells and neurons. Gastrodermal cells are ciliated, have a digestive function and frequently contain photosynthetic dinoflagellates belonging to the family Symbiodinaceae (LaJeunesse et al. 2018).

10.6. GENOMICS

Prior to 2011, only limited transcriptomic and genomic data were available for corals (reviewed in Miller et al. 2011), but in that year, the first coral whole genome assembly was published (Shinzato et al. 2011). Fittingly, the species sequenced was *A. digitifera*—a common species that dominates reefs in many parts of Okinawa and on which Japanese biologists regularly carry out research. Comparison of the *A. digitifera* genome with that of the sea anemone *Nematostella vectensis* (the first cnidarian whole genome sequence assembly) revealed a number of differences. For example, it was suggested that the requirement for a sophisticated symbiont recognition system might underlie the observed enrichment of predicted immune receptors in the *A. digitifera* genome relative to *N. vectensis* (Shinzato et al. 2011). Another surprise was the discovery in *A. digitifera* of a suite of genes that together may enable biosynthesis of mycosporine-like amino acids, "natural sunscreen" which was previously assumed to be produced by the algal symbionts rather than the coral animal. A third key finding arising from analyses of the *A. digitifera* genome was that this coral lacked cystathionine ß-synthase (Cbs), one of the enzymes required for biosynthesis of cysteine. All *Acropora* species examined to date lack Cbs, although a Cbs homolog is present in a wide range of other corals (Shinzato et al. 2011).

The early availability of significant bodies of molecular data for *A. millepora* (e.g. Kortschak et al. 2003; Meyer et al. 2009; Moya et al. 2012) led to widespread use of this coral for experimental purposes, making this species an obvious target for whole genome sequencing. In 2019, the first genome assembly for *A. millepora* became available (Ying et al. 2019); as with the *A. digitifera* assembly, the first *A. millepora* genome was based on short-read data, but a long-read–based assembly became available shortly thereafter (Fuller et al. 2020). There has recently been a rapid increase in the number of genome assemblies available for *Acropora* species, largely carried out at the Okinawa Institute for Science and Technology (OIST)—the institution responsible for the first coral genome assembly. Mao et al. (2018) generated short-read assemblies for four additional species of *Acropora* (*A. gemmifera*, *A. echinata*, *A. subglabra* and *A. tenuis*), and Shinzato et al. (2020) analyzed the genomes of an additional 11 *Acropora* species and those of the confamilial taxa *Montipora cactus*, *M. efflorescens* and *Astreopora myriophthalma*.

Although genomes were not actually assembled, extensive genomic sequence data are also available for the Caribbean species *A. palmata* and *A. cervicornis* (Kitchen et al. 2019).

10.6.1 What Have We Learned from All of Those Genomes?

Despite early speculation on the possibility of a whole genome duplication having facilitated the evolutionary success of *Acropora* (Mao & Satoh 2019), it is now clear that such a duplication is unlikely to have occurred (Shinzato et al. 2020). Rather, many independent gene duplication events occurred in the *Acropora* lineage (Hislop et al. 2005; Shinzato et al. 2020).

The genomes of *Acropora* species vary surprisingly little. Based on short-read assemblies, Shinzato et al. (2020) estimated gene numbers across the genus to be around 22–24,000. However, gene predictions from the two long-read assemblies are significantly higher—28,000 for *A. millepora* (Fuller et al. 2020) and around 30,000 for *A. tenuis* (Cooke et al. 2020). Within the genus *Acropora*, some gene families have been dramatically expanded, interesting examples of which are those encoding the atypical two-domain caspase-X, small cysteine-rich proteins (SCRiPs) and dimethylsulfoniopropionate (DMSP)-lyases (Shinzato et al. 2020). The caspase-X proteins have both active and inactive caspase domains, the latter being likely to normally hold the protein in an inactive state in a manner resembling the interaction of caspase-8 and c-FLIP (Moya et al. 2016). SCRiPs have been implicated in a wide range of functions, including skeletogenesis (Sunagawa et al. 2009; Hayward et al. 2011) and stress responses (DeSalvo et al. 2008; Meyer et al. 2011; Moya et al. 2012), as toxins

(Jouiaei et al. 2015) and possibly also in symbiont acquisition (Mohamed et al. 2020a). *Acropora* spp. are known to produce large amounts of DMSP, which is cleaved by DMSP-lyase to dimethyl sulfate (DMS) and acrylate. As DMS is volatile and can seed cloud formation, a role in local climate moderation has been proposed (Vallina & Simó 2007). Although roles for SCRiPs and caspase-X proteins in stress responses and for DMSP-lyases in mitigating solar radiation have been interpreted as adaptations within the *Acropora* lineage to deal with environmental stressors (Shinzato et al. 2020), *Acropora* species remain among the most sensitive of reef-building corals to thermal stress, and at this stage, it is unclear whether these gene family expansions are related to that.

With the exceptions of the Fuller et al. (2020) assembly for *A. millepora* and the Cooke et al. (2020) assembly for *A. tenuis*, all of these other genomes have been based on short-read data. So, while they have provided some high-quality gene prediction datasets, they do not provide comprehensive coverage. Comparison between the Cooke et al. (2020) *A. tenuis* and the Fuller et al. (2020) *A. millepora* assemblies shows a remarkable level of macrosynteny (Cooke et al. 2020). Given that these species are highly diverged within the genus (Cowman et al. 2020), it is likely that the overall genome architecture varies little within *Acropora*—note that data from Shinzato et al. (2020) are consistent with this view.

10.6.2 How Does the *Acropora* Genome Compare with Those of Other Coral Genera?

With the caveat that, at the time of writing, data are not available for a representative range of reef-building corals, based on the long-read assemblies, at around ~480 Mb (Fuller et al. 2020; Cooke et al. 2020), the estimated size of the *Acropor*a genome appears to be fairly typical of corals. Although estimates of both genome size and gene number for some members of the Robusta are much larger (Ying et al. 2018), these were based on short-read assemblies, and it is as yet unclear whether the larger genomes are consequences of higher content of repetitive elements and transposons—as in the case of several bilaterian lineages—or higher gene content. Until higher-quality genome assemblies are available for a phylogenetically representative range of corals, general evolutionary patterns will remain unclear.

10.6.3 Why Has *Acropora* Been Such an Evolutionary Success Story?

Throughout the Indo-Pacific, *Acropora* is the dominant reef-building coral and is one of the most speciose coral genera. As speculated on by Shinzato et al. (2020) and others, its evolutionary success may be due to acquisition and amplification of gene families that have enabled rapid adaptation to changing conditions. However, *Acropora* is almost always associated with one particular genus of Symbiodiniaceae, *Cladocopium*, and we speculate that this partnership may have facilitated the observed rise to dominance of this genus. Comparative transcriptomics has demonstrated the over-representation of (for example) ABC-transporters in *Cladocopium goreaui* compared to *Breviolum minutum* and *Fugacium kawagutii*—other Symbiodiniaceae associated with corals—and among the transporters known so far only in *Cladocopium*, there are components of transport systems for both cysteine and histidine (Mohamed et al. 2020b). The significance of cysteine in the case of *Acropora* was discussed previously; although members of the Robusta are capable of histidine biosynthesis, along with other Complexa and bilaterians, *Acropora* species cannot synthesize it. Hence the association between *Acropora* as host and *Cladocopium* as symbiont may be a particularly good "fit" and have contributed to the rise of the genus during the Neogene and Quaternary.

10.7 FUNCTIONAL APPROACHES: TOOLS FOR MOLECULAR AND CELLULAR ANALYSES

For many reasons, the functional approaches that have proven so fruitful in other organisms such as *Drosophila* and *Caenorhabditis* have been difficult or impossible to implement in *Acropora*. First, there is ease and cost of culture. While adult corals have been kept in aquaria for years, albeit in varying degrees of health, it is only in the past year that there has been a report in the literature of successful production of a second generation of *Acropora* in captivity (Craggs et al. 2020), and this required a sophisticated and expensive aquarium system. Second, there is the problem of generation time; it is probably at least three years before a second generation of *Acropora* would produce sufficient embryos for experimental purposes. Third, there is genome size. Compared to the best-understood "model" organism, *Drosophila melanogaster* (genome size ~140 Mb; 15,700 genes), at 400–500 Mb and with ~28–30,000 genes, the genomes of *A. millepora* and *A. tenuis*, the two *Acropora* species for which we have the best data, are relatively large. In addition, *Drosophila* has only 8 chromosomes (four pairs), while *A. millepora* has 28 (Kenyon 1997; Flot et al. 2006), as does *A. digitifera* (Supp Fig 1 in Shinzato et al. 2011). Twenty-eight chromosomes is most common in the genus, as Kenyon (1997) found this number in 16 species, but this is by no means universal, as 6 other species had 24, 30 (2 species), 42, 48 and 54.

Studies on *Acropora* also require several additional considerations that may not be relevant to other organisms. One is the taxonomic problem dealt with in Section 10.1. Molecular markers may be required in the future to be sure that one is really dealing with the same species in different parts of the world. A final difficulty is that a coral is in fact a holobiont, usually consisting of the coral itself, one or more species of photosynthetic microalgae and numerous other micro-organisms. In nature, this assemblage will vary

somewhat from coral to coral and locality to locality and may have considerable effects on the health and physiology of the individual coral and therefore on experimental repeatability.

Genetic and cell biological manipulations have been done on other cnidarians, most notably on *Hydra* and *Nematostella*, in both of which gene knockdown experiments have been successful. However, culturing these species is much less demanding than for corals. Of greater relevance to studies on corals have been experiments on the sea anemone *Exaiptasia* (often under the name *Aiptasia*), which is relatively easy to culture and which shares with corals the presence of photosynthetic endosymbionts. There has been an attempt by the *Exaiptasia* community to standardize strains of anemone and endosymbionts in order to achieve a greater level of experimental consistency across the community (e.g. Cziesielski et al. 2018), but this will be difficult in the case of *Acropora*.

In spite of the challenges noted previously, there have been some successful attempts at experimental manipulation in corals. For example, lithium chloride and 1-azakenpaullone (AZ) have been used to inhibit GSK3 and activate the wnt pathway in *A. digitifera* (Yasuoka et al. 2016), resulting in the expansion of *brachyury* expression throughout the embryonic ectoderm in a dose-dependent manner. In contrast, wnt/ßcatenin signaling inhibitors (pyrvinium pamoate, IWR1 or iCRT14) reduced *Adi_bra* expression in a dose-dependent fashion, leading to the conclusion that it is positively regulated by wnt/ßcatenin signalling. In a following experiment, FITC-labeled anti-sense morpholinos were designed to bind to and inhibit *Adi_bra* RNAs, resulting in loss of function of the *brachyury* gene and a lack of pharynx formation in the morphants, although gastrulation still occurred. The authors then went on to compare bra-morphants, control morphants and uninjected embryos using RNA seq in order to identify genes downstream from *Adi_bra*.

Although morpholinos gave results which could be interpreted in the case described previously, in most studies in other organisms, they have now been replaced by CRISPR/Cas9 gene editing technology, which can result in permanent heritable genetic changes. This was first applied to corals by Cleves et al. (2018), who targeted the *A. millepora* genes encoding fibroblast growth factor 1a (FGF1a), green fluorescent protein (GFP) and red fluorescent protein (RFP) in an attempt to prove that CRISPR/Cas9 could be applied to corals. FGF1a is a single copy gene chosen for its probable role "in sensing the environment and/or in modulating gene expression during larval settlement and metamorphosis". The GFP and RFP are multicopy but were chosen for ease of assay and for their probable ecological importance as well as the ability to target multiple copies due to their sequence similarity. Sequencing of 11 mutant larvae revealed both wild type and multiple different mutant alleles of target genes, indicating that the injected sgRNA-Cas9 remained active for several cell cycles after injection and that the target gene was never knocked out biallelically (i.e. on both copies of the chromosome). While this study was a great technical success, the authors are careful to point out some of its limitations and provide recommendations for further studies using this technique. They point out that "As there is little immediate prospect of raising mutagenized animals to adulthood and generating homozygous individuals by genetic crosses, obtaining animals that have sustained early biallelic mutations will be critical to the analysis of phenotypes of interest". A further consideration, in order to avoid equivocal results, is the need to choose a single copy gene with a clear assay for whether gene knockout has been achieved.

The examples discussed previously were both carried out by injecting eggs, and it should be stressed that such experiments require a high degree of organization on the part of the experimenters because eggs from mass-spawning acroporids are only available for a few nights once or twice per year. A promising new gene knockdown technology has recently been developed using electroporation of short hairpin RNA that has been successfully used on *Nematostella* (Karabulut et al. 2019) and on the hydroid *Hydractinia symbiolongicarpus* (Quiroga-Artigas et al. 2020). This technology would mark a huge advance if it could be developed for broadcast spawning corals such as *Acropora*, as it would allow processing of hundreds of embryos, and testing of multiple genes, in the short annual time window that eggs are available.

Another recently reported innovation, which may prove important for future studies, is gel immobilization (Randall et al. 2019), in which developmental stages of corals are embedded in low-melting-point agarose. The authors used this on developmental stages of five species of corals, including *A. millepora*, and obtained good survival in all species when embedding was done after larvae had become ciliated. This technique could prove particularly valuable for experimental studies since it allows larvae to be individually tracked, manipulated and photographed.

Living *Acropora muricata* colonies were recently imaged in unprecedented detail using light sheet illumination (Laissue et al. 2020). This technique allows the study of any processes in the living coral that would be interfered with by bright light. Unfortunately, it requires a rather specialized optical setup, so it probably will not be widely available, but it may enable certain observations that would not otherwise be possible.

10.8 CHALLENGING QUESTIONS

10.8.1 How Can We Deal with Hybridization and the Species Problem?

The taxonomic problems outlined in Section 10.1 may cause issues with reproducibility and will have to be taken into consideration as possible causes of differing experimental

results. For this reason, careful documentation of specimens is of the utmost importance.

10.8.2 What Is the Genomic Basis of the Differing Morphologies of Different Species of *Acropora* and Other Corals?

Presumably the answer to this question lies in gene regulation, as there are few genes involved in skeletogenesis that are species specific, especially if we limit consideration to the genus *Acropora*. So, this will be an interesting, but probably difficult-to-resolve, question.

10.8.3 What Determines the Time and Place at Which Coral Larvae Settle and Undergo Metamorphosis?

Settlement and metamorphosis in *Acropora* are obviously critical for completion of the life cycle and survival of the species but are surprisingly poorly understood. A first important question is what triggers the process of searching and settlement. Some of the temporal variability has a genetic basis, with 47% of variation due to parental effects (Kenkel et al. 2011), but what is it that sends some larvae into searching behavior (a dramatic behavioral change in which larvae go from horizontal swimming to corkscrew swimming into the bottom, apparently testing for chemical cues) in a few days, while others take weeks?

In an early effort to identify the inducer, Morse et al. (1996) surveyed the responses of ten species of Indo-Pacific *Acropora* and found that for all of them, an unidentified sulfated glycosaminoglycan emanating from crustose coralline algae (CCA) was the settlement inducer. While this compound may be the most effective settlement cue, it appears from several lines of evidence that there may be more than one cue that induces settlement and that there is a hierarchy of such cues in relation to their effectiveness in inducing the normally combined processes of settlement and metamorphosis. For instance, Negri et al. (2001) reported that it was actually inducers from the bacterium *Pseudoalteromonas* growing on the CCA that were responsible for settlement. Tebben et al. (2011) took this analysis further, establishing that it was tetrabromopyrrole (TBP) produced by the *Pseudoalteromonas* that was the critical compound for successful metamorphosis of *A. millepora*. However, 90% of the larvae induced to metamorphose by application of TBP did so in the water column and did not successfully attach to the substratum. Successful completion of the entire sequence of settlement, metamorphosis and attachment was only observed in the presence of two species of CCA, and it was determined in a later paper (Tebben et al. 2015) that in order to produce the complete normal sequence of going to the bottom, metamorphosing and attaching, the presence of CCA cell-wall–associated glycoglycerolipids and polysaccharides was required.

10.8.4 What Are the Receptor Molecules Driving Metamorphosis and How Is the Signal Transduced?

There are further related questions about how the larva receives and processes the information relating to settlement and metamorphosis. First, what is the receptor (or receptors) for the CCA compounds that stimulate settlement and metamorphosis? Second, what is the chain of transduction between this receptor and the effector molecules that produce the morphological changes of metamorphosis? There are some clues relating to the answer to the second question in that Iwao et al. (2002) tested the effect of several GLWamide peptides on larvae of *Acropora* and found that the *Hydra* peptide Hym-248 (EPLPIGLWa) induced metamorphosis in all of them but not in the other corals tested, while Erwin & Szmant (2010) found that the same peptide induced metamorphosis in *A. palmata* but not in *Orbicella (Montastrea) faveolata*. The cell bodies of cells expressing the *A. millepora* LWamide gene lie on the mesoglea but project to the surface of the planula larva (Attenborough et al. 2019), but whether these cells also contain the unknown metamorphosis receptors is unknown. A final puzzle is how the signal to metamorphose is distributed to the cells that must respond in larvae that lack a circulatory system.

10.8.5 There Are Many Questions Relating to the Symbiosis between Corals and Their Photosynthetic Dinoflagellate Endosymbionts Belonging to the Family Symbiodinaceae

The ecological success of reef-building corals in nutrient-poor tropical waters is due to their symbiosis with photosynthetic dinoflagellates belonging to the family Symbiodinaceae. These dinoflagellates are remarkable in that many or all occur in both a free-living, flagellated form and a coccoid symbiotic form, with individuals capable of switching between these forms depending on their environment. The relationship with the coral has been assumed to be a classical symbiosis (i.e. a mutualism) from which both partners benefit, with the coral receiving the energy for growth from the dinoflagellate's photosynthate, while the latter utilizes the nitrogenous and phosphate-containing waste produced by the coral, as well as obtaining what is normally a secure place to live. However, the assumption of mutualism as a general property of Symbiodiniaceae is currently being revisited (LaJeunesse et al. 2018; Liu et al. 2018; Mohamed et al. 2020b).

Understanding of the relationship between corals and their symbionts has grown explosively in the last few years, driven by the worldwide breakdown in this symbiosis reflected in widespread coral bleaching, which occurs when the symbionts leave or are expelled by the coral. Bleaching is most commonly caused by thermal stress, as most corals live very near their upper thermal limit and will die if the heating is prolonged.

Progress and problems in studying the symbiosis between cnidarians and their photosynthetic endosymbionts were summarized in a comprehensive review by Davy et al. (2012), and while considerable progress has been made in the intervening years, most of the questions raised in that review are still under investigation using newly developed molecular techniques which have opened the way to a much greater understanding of the symbiotic relationship and its complexity. So, just in the last 20 years, the field has gone from lumping all of the endosymbionts into a common basket, to recognizing a steadily increasing number of clades, to realizing that members of these clades differed in their physiology, to most recently classifying these clades into different genera (LaJeunesse et al. 2018). In the space available, it is only possible to outline some of the most active areas of research and some key literature references. These involve all aspects of the relationship between host and symbiont, including establishment, maintenance and breakdown. Unfortunately, the literature is full of apparently contradictory results which are difficult to interpret because of differing combinations of corals and their potential symbionts and differing experimental techniques. Some of the areas under most active investigation are the following. When and how is symbiosis established in *Acropora*? What is the mechanism of symbiont uptake and retention or rejection? What do the host and symbiont contribute to each other? What happens when corals bleach—does the coral evict its symbionts, or do they flee? Recent summaries of research in these areas include Morrow et al. (2018) and van Oppen and Medina (2020).

10.8.6 How Does the Coral Interact with Its Non-Dinoflagellate Endosymbionts and They with Each Other?

The coral is a metaorganism, playing host to many microorganisms in addition to the members of the Symbiodinaceae on which it is reliant for much of its energy. These include bacteria, viruses and other microbes such as apicomplexans. Recently, many techniques, including genomics and metabolomics, have been developed that facilitate study of these interactions. Deep sequencing enabled Robbins et al. (2019) to assemble "complete" metagenomes for 52 bacterial and archaeal taxa associated with in the coral *Porites lutea*, and analyses of these reveal numerous ways in which they could be contributing to the success of the metaorganism. Now it is a matter of establishing actual, as opposed to theoretical, contributions. Similarly, certain micro-organisms seem to be associated with coral diseases, but is the relationship causal, or is it just a reflection of stress? A few of the many recent reviews of this area include O'Brien et al. (2019), Matthews et al. (2020) and McIllroy et al. (2020).

10.8.7 Can Coral Reefs Be Restored, and What Is the Best Way to Accomplish This?

Due to their morphology, corals belonging to the genus *Acropora* are among the most sensitive to bleaching and death induced by global warming and, as pointed out in earlier sections, they are among the most important structural constituents of many reef systems. As a result of this, a great deal of effort is going into reef restoration, with much of it centered on *Acropora*. Three approaches which we will discuss here are assisted settlement, planting of nubbins and assisted evolution. A comprehensive summary and evaluation of reef restoration techniques is given by Boström-Einarsson et al. (2018) and Zoccola et al. (2020). In the following, we have discussed examples particularly involving *Acropora*.

10.8.7.1 Assisted Settlement

Optimal laboratory conditions have been determined for culture of larvae, induction of settlement and infection with symbiont (Pollock et al. 2017). In field applications of this technique, eggs and sperm are trapped in large floating traps, moved to enclosed rearing pens and then moved on to the desired site of settlement. This technique was pioneered in the Philippines (dela Cruz & Harrison 2017) and on the southern Great Barrier Reef by Peter Harrison and his colleagues and has now moved to a larger scale project near Cairns (https://citizensgbr.org/p/larval-restoration-project). The greatest effectiveness of this technique will almost certainly be in restoration of relatively small areas of high tourist value or for seeding source reefs for recolonization, for example, following a cyclone.

10.8.7.2 Planting of Nubbins

This technique has been attempted in several parts of the world, most notably in the Caribbean and in the waters surrounding Okinawa. There is no doubt that, although it is expensive, it can be successful, at least in limited areas, especially where reefs have suffered physical damage due to hurricanes or cyclones. However, it is difficult to judge success objectively since successes are considered newsworthy, while failures are generally ignored. Efforts over many years in the Caribbean are summarized by Calle-Triviño et al. (2020), and there are certainly examples of success. However, in Okinawa, restoration efforts seem to have been much less successful. For example, 89.2 % of the 79,487 corals transplanted in the Onna village area of Okinawa died within the first five years due to typhoons, bleaching and for

"unknown reasons" (Nature Conservation Division D.o.E.A. 2017).

10.8.7.3 Assisted Evolution

These approaches, which have been championed by Madeleine van Oppen and colleagues (van Oppen et al. 2015), were nicely summarized by Zoccola et al (2020) as follows:

> The authors propose to promote resilience/resistance of coral colonies by (1) inducing laboratory stress and selecting the colonies that survive, (2) actively modifying the coral-associated microbiota, (3) applying environmental stress hardening to generate more resistant phenotypes, and (4) genetically enhancing coral host-associated microalgae by means of mutation and selection using artificial evolution. Subsequently, methods for active modification of the coral genome through approaches such as CRISPR and synthetic biology were suggested.

While these methods may have some success, they may be outrun by climate change, and selection in the lab may not be relevant to survival in the field due to fitness tradeoffs.

10.8.7.4 Conclusions

While the previous measures may have some success, economics limits their application to relatively small scales. Experiments conducted under the umbrella of "assisted evolution" will be useful in delivering basic science outcomes, but their real-world relevance has yet to be demonstrated. Technical solutions would be much closer if coral holobionts comprised "plug-and-play" components, but this is clearly not the case (see, for example, Herrera et al. 2020). Moreover, there is a real danger that by focusing attention on reef restoration efforts, perspective on the big picture is lost—ultimately, there is only one solution to the problem of coral bleaching and death, and that means dealing with the anthropogenic impacts of pollution, coastal runoff and climate change. In the meantime, conservation of genetic resources is of critical importance in ensuring the long-term survival of coral reefs in anything like their current state.

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