Pure & Appl. Chem., Vol.53, pp.819-835.
Pergamon Press Ltd. 1981. Printed in Great Britain.

0033-4545/81/040819-17$02.00/0

CELLULAR AND MOLECULAR INTERACTIONS IN SYMBIOSES BETWEEN DINOFLAGELLATES AND MARINE INVERTEBRATES

Robert K. Trench

Department of Biological Sciences and the Marine Science Institute, University of California at Santa Barbara, Santa Barbara, California 93106

Abstract - Evidence on the possible role of cellular and molecular interactions during the establishment and perpetuation of symbioses involving dinoflagellates and marine invertebrates is presented. The symbioses are shown to demonstrate a high degree of specificity which may be expressed as selectivity during intercellular contact or physiological adjustments following the establishment of a potential association.

INTRODUCTION

The coexistence of organisms of distinct genetic and evolutionary histories is the fundamental phenomenon in symbiosis. Whether an initial association results in a mutually beneficial interaction or some level of integration less than mutualism, is the end result of a series of adjustments on the part of both partners in the association (1,2). The processes whereby two originally independent organisms initiate and perpetuate a symbiosis are very complex, and the study of such processes transcends classical disciplines. Hence, an investigator must be able to place each separate organism in its evolutionary and ecological contexts as well as consider the cell biology, physiology and ecology of the established consortium. Within aquatic environments, examples of symbioses between unicellular algae and invertebrates are numerous, but the level of integration between the partners in the associations often lacks in depth experimental analysis.

In the initiation of symbioses, particularly intracellular symbioses, the "infecting" organism must gain access to the cells of the host organism. Since all organisms possess the capability to distinguish between "self" and "non self", such an invasion might predictably result in the destruction of the invader. Obviously, since many well integrated symbioses do exist, some organisms are able to circumvent defence mechanisms of their hosts. Very little is known about the time and mechanism of initiation of most symbiotic associations (1,2). After an association between two organisms is established, it is possible to analyse functional parameters of the interaction in order to ascertain the relative degree to which the organisms have become mutually dependent, and this in turn can be viewed as an indication of the level of integration achieved between the two components.

In this paper, I shall restrict myself to a discussion of plant-animal symbioses involving 'zooxanthellae' and marine invertebrates. An interested reader may refer to a series of other articles which deal with a wider range of plant-animal symbioses (1-6).

DISTRIBUTION OF PLANT-ANIMAL SYMBIOSES

The distribution of plant-animal symbioses involving "zooxanthellae" within the marine environment can be discussed from both environmental and phyletic viewpoints. Examples of symbioses can be found in all the world oceans, in benthic and pelagic environments, and involve unicellular algae ranging from diatoms and red algae to dinoflagellates and invertebrate hosts ranging from the Protozoa to the Mollusca.

Temperate oceans provide fewer examples of plant-animal symbioses. On the European coast *Anemonia sulcata* and *Convoluta convoluta* are the two best known examples. On the N.E. coast of the U.S., some populations of *Astrangia danae* possess symbiotic dinoflagellates, while on the West coast, *Anthopleura elegantissima, A. xanthogramica* and *Clytia bakeri* are the only coelenterates with harbor "zooxanthellae". The pelagic coelenterate *Velella velella* also harbors "zooxanthellae" and can sometimes be found in temperate waters.

The most impressive array of associations between algae and marine invertebrates is found in warm, shallow water benthic communities comprising coral reef ecosystems. Within such environments, examples of pelagic invertebrates harboring "zooxanthellae" may also be found. Among coral reef biologists and invertebrate paleontologists, there is a concensus that the dramatic rise in importance of reef building Scleractinia as major contributors to carbonate

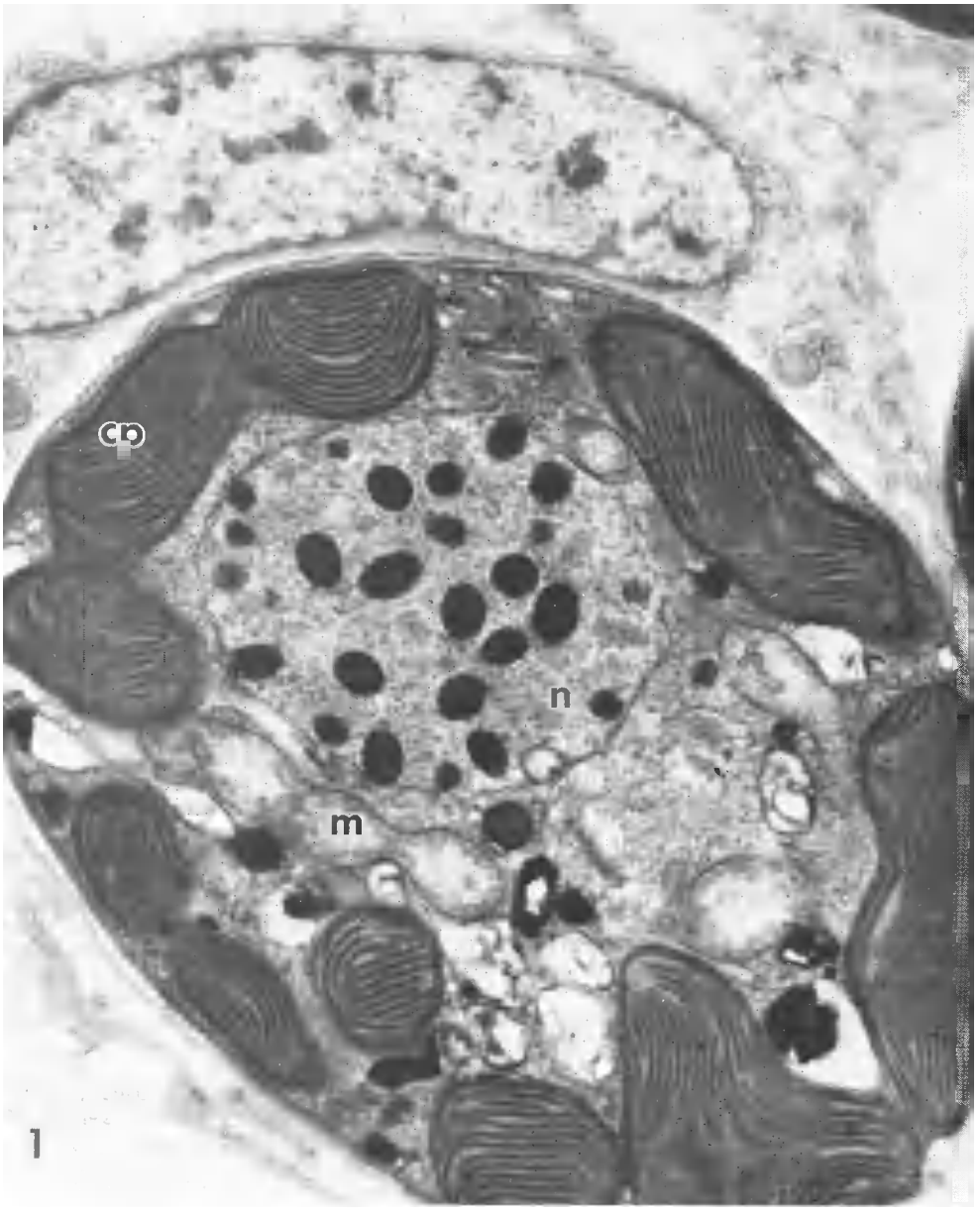

Fig. 1. Transmission electron micrograph of *S. microadriaticum* in the hypertrophied siphonal tissue of the clam *Tridacna maxima*. n, nucleus; m, mitrochondrion; cp, chloroplast. Magnification approximately 14,000 x.

accretions that was initiated in the Triassic was in all probability a result of the establishment of symbioses with "zooxanthellae" (7, 8). Similarly, the large size attained by some tridacnid bivalves has been attributed to their association with "zooxanthellae" (9). In light of current concepts on the role of "zooxanthellae" in the nutrition of the animals harboring them (1, 3, 4, 5, 6, 10), and in ameliorating the rate at which corals and forams deposit limestone skeletons (11, 12, 13) the selective advantage of symbiosis between "zooxanthellae" and reef-dwelling invertebrates becomes apparent.

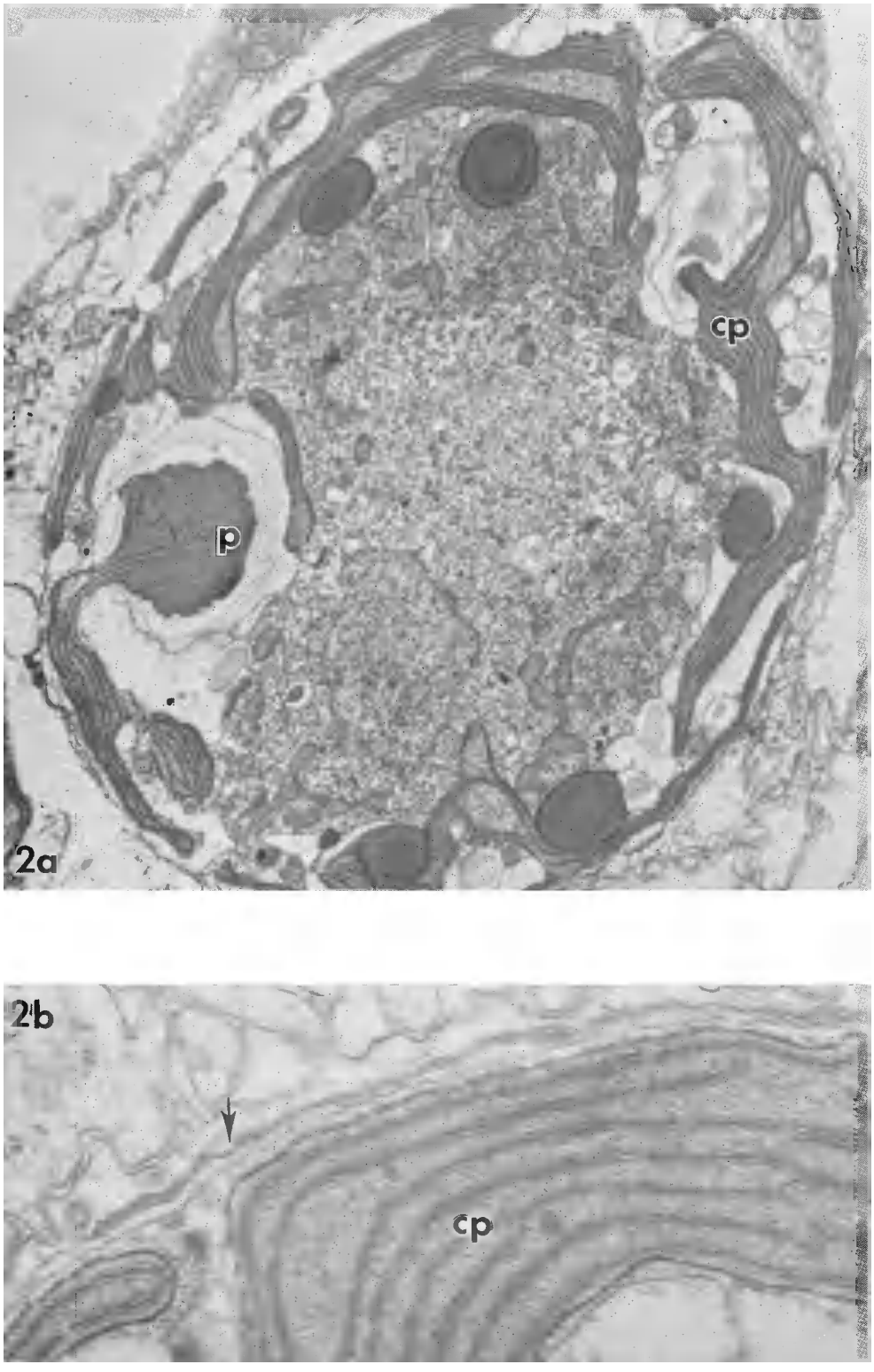

Fig. 2. a. Transmission electron micrograph of the algal symbiont, *Endodinium (=Amphidinium) chattonii* in the chondrophore *Velella velella*, cp. chloroplast, p, pyrenoid. Magnification approximately 10,000 x.
b. Transmission electron micrograph of the chloroplast and the alga-host cell interface (arrow) in *V. velella*. Magnification approximately 52,000 x.

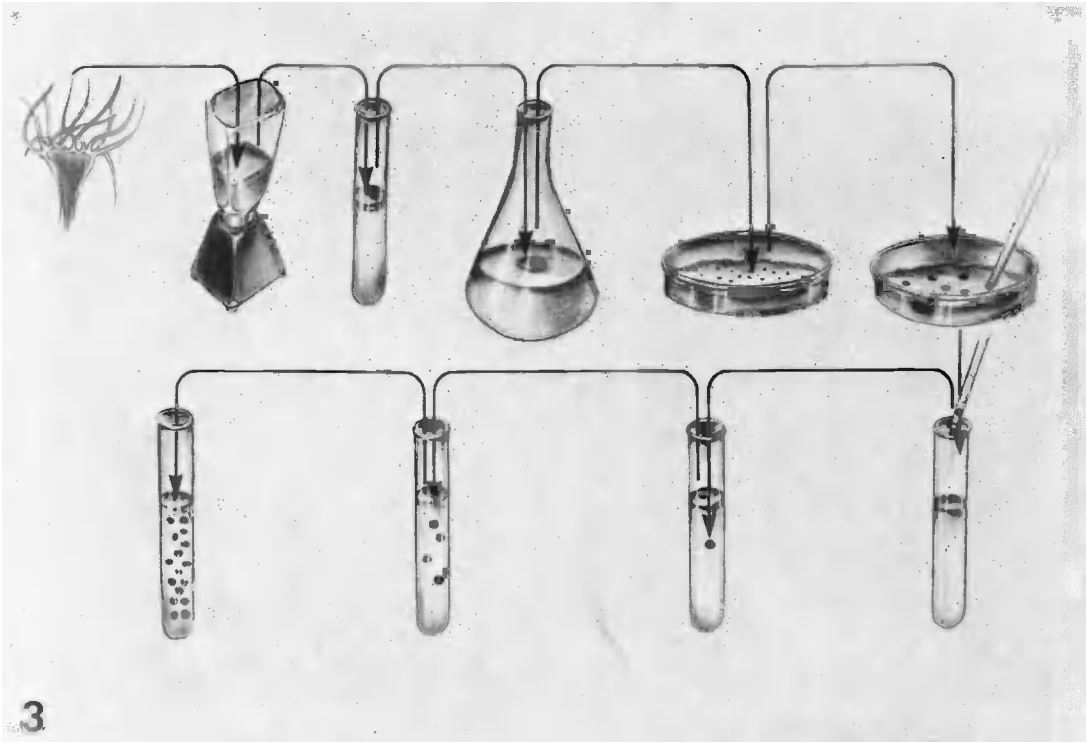

Fig. 3. Schematic representation of the process of isolation and cloning of *S. microadriaticum*. The algae are isolated by blending the host animal and cells are inoculated into growth media. After growth, the cells are dispersed again and individuals are transferred to a second agar plate. Colonies produced from single cells are then transferred to liquid media.

From a phyletic viewpoint, the distribution of symbioses involving "zooxanthellae" is quite random. Lists of invertebrates harboring "zooxanthellae" may be found in Droop (14), McLaughlin and Zahl (15), Taylor (16) and Trench (1). "Zooxanthellae" are known to occur as symbionts in invertebrate phyla including the Protozoa, Porifera, Cnidaria, Platyhelminthes and Mollusca. It should be emphasized however, that the term "zooxanthellae" as used until recently, refers to any brown alga in symbiosis. Hence, the diatom *Licmophora* found in the flatworm *Convoluta convoluta* (17) is often referred to as "zooxanthellae" as are the unicellular red algae or diatoms found in some foraminifera (18, 19, 20). In light of such obvious confusion, I shall attempt to discontinue the use of the term "zooxanthellae" in the remainder of this paper, and restrict the discussion to the amphidinioid and gymnodinioid dinoflagellates found in association with marine invertebrates.

Among the Protozoa, foraminiferans (e.g. *Sorites marginalis)* harbor a gymnodinioid dinoflagellate resembling *Symbiodinium microadriaticum* (21), while the radiolarian *Collosphaera* sp. harbors a dinoflagellate of uncertain taxonomic status called *Endodinium nutricola* (22).

Coelenterates may harbor either gymnodinioid or amphidimioid dinoflagellates, and there is no direct correlation between algal taxa and host taxa. The best known associations involve the gymnodinioid dinoflagellate *S. microadriaticum*, until recently believed to be a single genetic unit (see Fig. 1), but the chondrophore *Velella velella* harbors a dinoflagellate believed to be amphidinioid, called *Endodinium (=Amphidinium) chattonii* (23) (Fig. 2). Some sponges appear to harbor *S. microadriaticum* while flatworms (e.g. *Amphiscolops langerhansi)* harbor *Amphidinium klebsii* (24). Among the molluscs, *S. microadriaticum* may be found in some nuchibranch gastropods and in the bivalves *Tridacna, Hippopus* and *Corculum* (25, 26).

A point that is often overlooked in face of the plethora of symbioses, is that there is very little relationship between host taxa and the existence of symbioses with algae. For example, within the scleractinian Family Caryophyllidae, five of the six subfamiles are represented

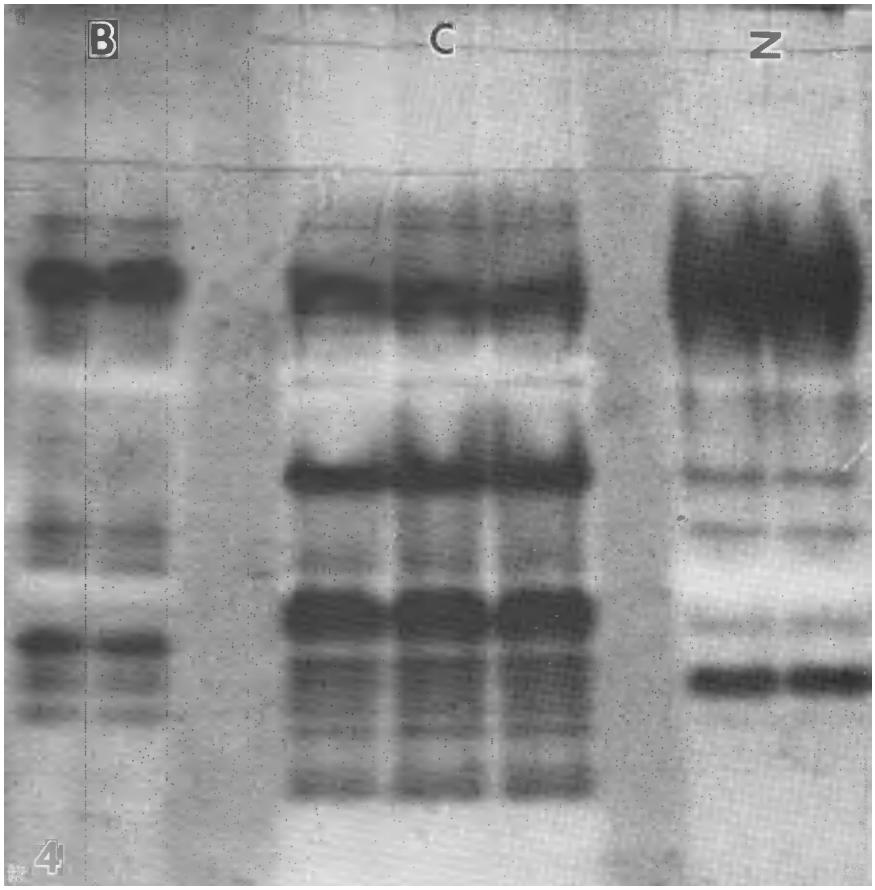

Fig. 4. An electrophoretogram of isoenzymes separated from three strains of *S. microadriaticum*. B, strain B algae from *Bartholomea annulata;* C strain C algae from *Cassiopeia xamachana*; Z, strain Z algae from *Zoanthus sociatus*. The darkly stained bands indicate isoenzymes of malate dehydrogenase while the white bands indicate isoenzymes of superoxide dismutase. The enzyme proteins were separated on 7% polyacrylamide gels.

by corals that are not symbiotic. Only corals in the Subfamily Eusmiliinae harbor symbiotic algae. Similarly, most of the dendnophyllid corals are non-symbiotic with the exception of *Turbinaria* (7). Again, among the large numbers of bivalve molluscs, only the tridacnids and *C. cardissa* in the Superfamily Cardiacea are symbiotic with dinoflagellates.

TAXONOMY OF ALGAL SYMBIONTS

In most of the well investigated marine algal-invertebrate symbioses, the algal partner has been shown to be a dinoflagellate. However, in many instances, the specific identity of the algae remains a source of confusion. However, at this point it is clear that there are at least two groups of dinoflagellates involved in symbioses with marine invertebrates, amphidinioid and gymnodinioid.

Taylor (24) unambiguously demonstrated that the dinoflagellate symbiont of the flatworm *Amphiscolops langerhansi* is the amphidinioid dinoflagellate *Ampidinium klebsii*. Taylor (23) concluded that the symbiont of *V. velella* is also amphidinioid, and identified the alga as *A. chatonii*. The symbiont isolated from the radiolarian *Colozoum interme* was also identified as *Amphidinium* sp. (16). However, in the case of the symbionts from *V. velella* and *C. interme*, Holland and Carré (22) disagree that either of these symbionts are amphidinioid. The specific identity of these algal symbionts should probably be given further attention.

Until very recently, the gymnodinioid dinoflagellate *S. microadriaticum*, the most frequently encountered symbiont of marine coelenterates and bivalve molluscs was believed to represent a single species population. This belief was based on the uniformity of structure (16, 27, 29). However, Schoenberg and Trench (30, 31, 32) have produced evidence which suggest that there is much variation in populations of *S. microadriaticum* and that such variation is very

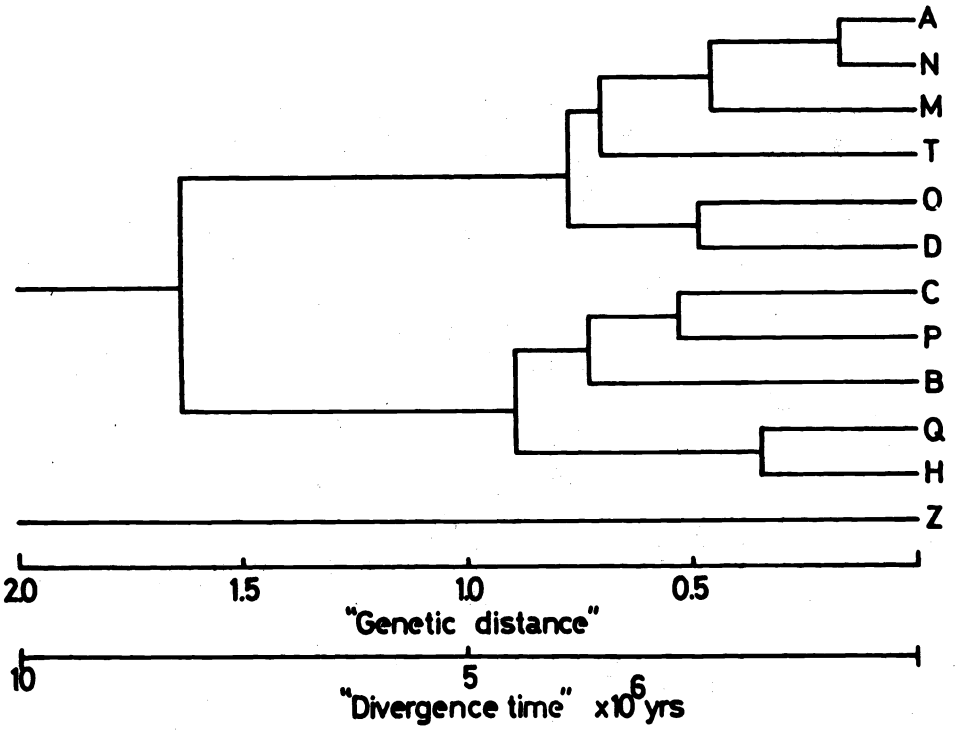

Fig. 5. A dendrogram illustrating the relatedness between different strains of *S. microadriaticum*, based on the similarities and differences in their isoenzyme patterns. For details see Schoenberg and Trench (30).

likely based on genetic differences. I shall summarize the evidence showing genetic differences in different populations of *S. microadriaticum*.

The approach taken was to isolate *S. microadriaticum* from a variety of marine invertebrate hosts and bring them into axenic culture in the same artificial medium, ASP-8A. From crude isolates, cloned populations of algae were produced (Fig. 3). All the algal cultures were maintained under identical conditions of illumination, temperature, and photoperiod. Uniform culture conditions circumvent the possibility that any observed variation could be the result of different environmental conditions.

Analyses of the algae were based on the electrophoretic separation of isoenzymes and on morphology. In the electrophoretic analyses, enzyme proteins were separated on undenatured gels, where the proteins migrate through the gel as a function of their molecular mass and net electrical charge (Fig. 4). Differences in mobility patterns of specific proteins then reflect possible differences in the amino acid composition of those proteins which may be related to possible differences in the genetic code directing their synthesis. From such data, it is possible to calculate the similarities and differences among the different strains of *S. microadriaticum* (Fig. 5). The evidence from biochemical analyses, were corroborated by examination of the morphology of the different strains (31).

More recently, S. Chang (unpublished) exploited the comparative biochemistry of the light harvesting complex, peridinin-chlorophyll a-protein (PCP), characteristically found in dinoflagellates (33), as a potential genetic marker, and finds (Fig. 6) that different strains of *S. microadriaticum* possess characteristic conformers of this pigment-protein complex after separation by isoelectric focusing. It should be emphasized that the algal strains retain the characteristic biochemical attributes after extended maintenance in

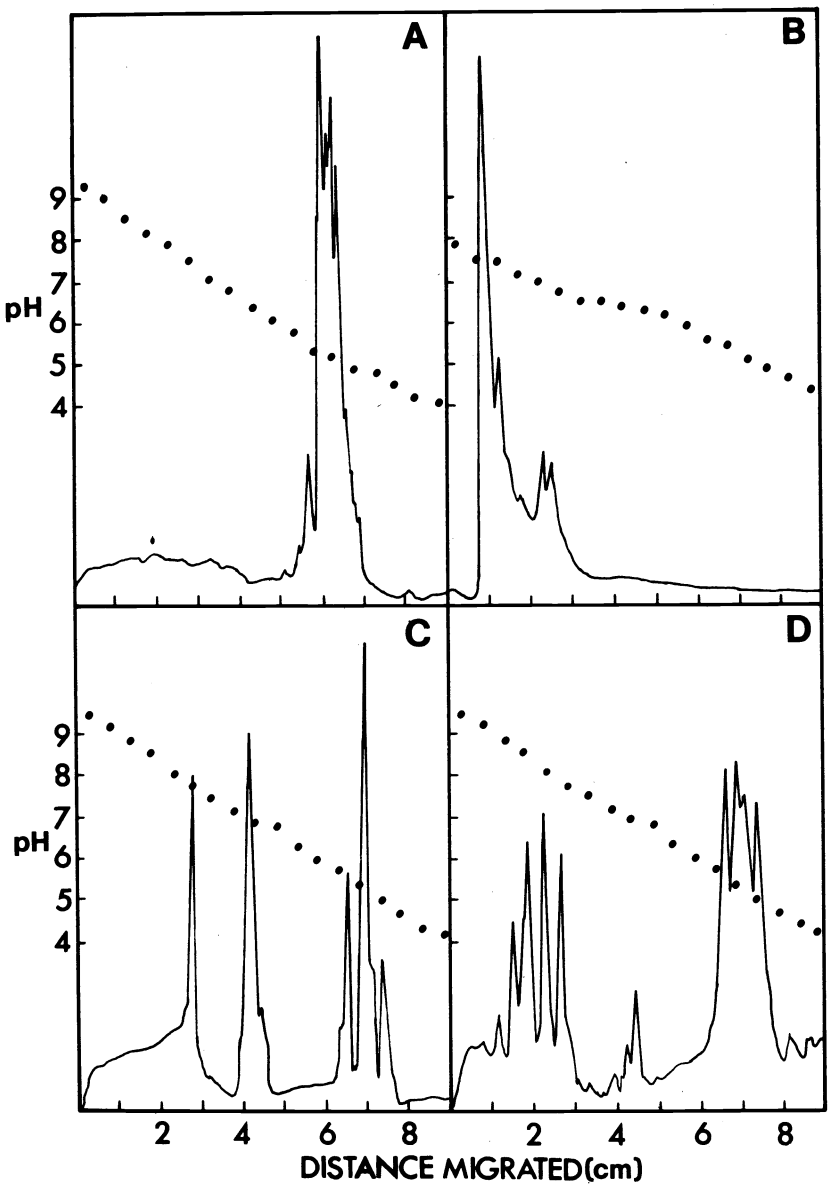

Fig. 6. The separation on conformers of PCP (peridinin-Chl. a-protein) from different strains of *S. microadriaticum*. a, algae from the coral *Montastrea annularis;* b, algae from the anemone *Anthopleura elegantissima;* c, algae from *Condylactis gigantea* and d, algae from the clam *Tridacna maxima*. Data obtained by S. Chang.

culture and after cloning, indicating that the criteria used in the assays are stable. In addition, different strains of *S. microadriaticum* demonstrate intrinsic differences in motility (Fig. 7) under the same constant conditions of culture (34).

All of the evidence cited above, taken together, support the concept that *S. microadriaticum* does not represent a single, genetically homogeneous population. However, it is not known whether each identified strain is equivalent to a distinct species or not, since our knowledge of possible gene flow between these algae is non-existent.

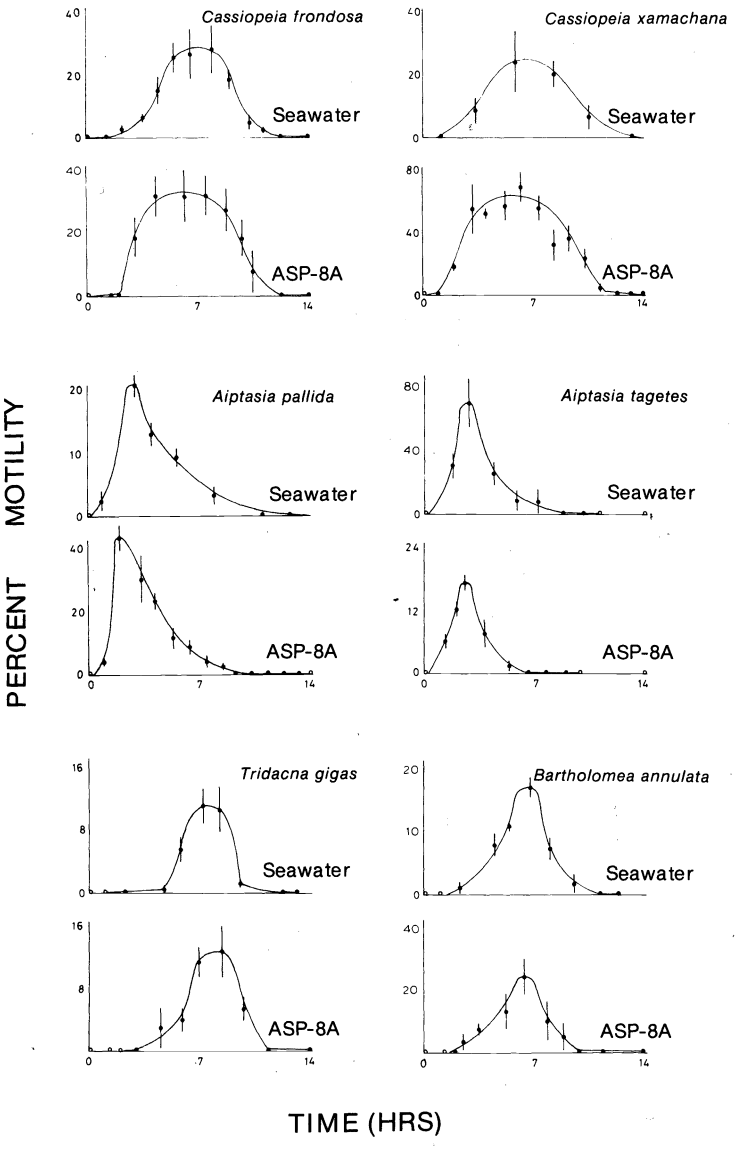

Fig. 7. Motility patterns for six isolates of *S. microadriaticum* tested in culture medium ASP-8A and filtered sea water. Time "zero" represents the start of the 14:10 h photoperiod. The dark cycle, during which the cells were non-motile is not included (for details see 34).

Recently, Loeblich and Sherley (35) conducted ultrastructural studies on *S. microadriaticum* isolated from *C. xamachana* and were able to demonstrate the existence of thecal plates associated with the amphiesma. Dinoflagellates in the Genus *Gymnodinium* do not possess thecal plates, so a strong argument was made to remove these symbiotic algae from the Genus *Gymnodinium* as proposed by Taylor (23). However, Loeblich and Sherley proposed reversion to the Genus *Zooxanthella* as originally proposed by Brandt (36). This change creates new problems, as the organism described as *Zooxanthella nutricola* by Brandt was from the radiolarian *Collozoum*, and Hollande and Carré (22) described the ultrastructure of this symbiont, referring to it as *Endodinium nutricola*. Its ultrastructure is quite distinct from that of

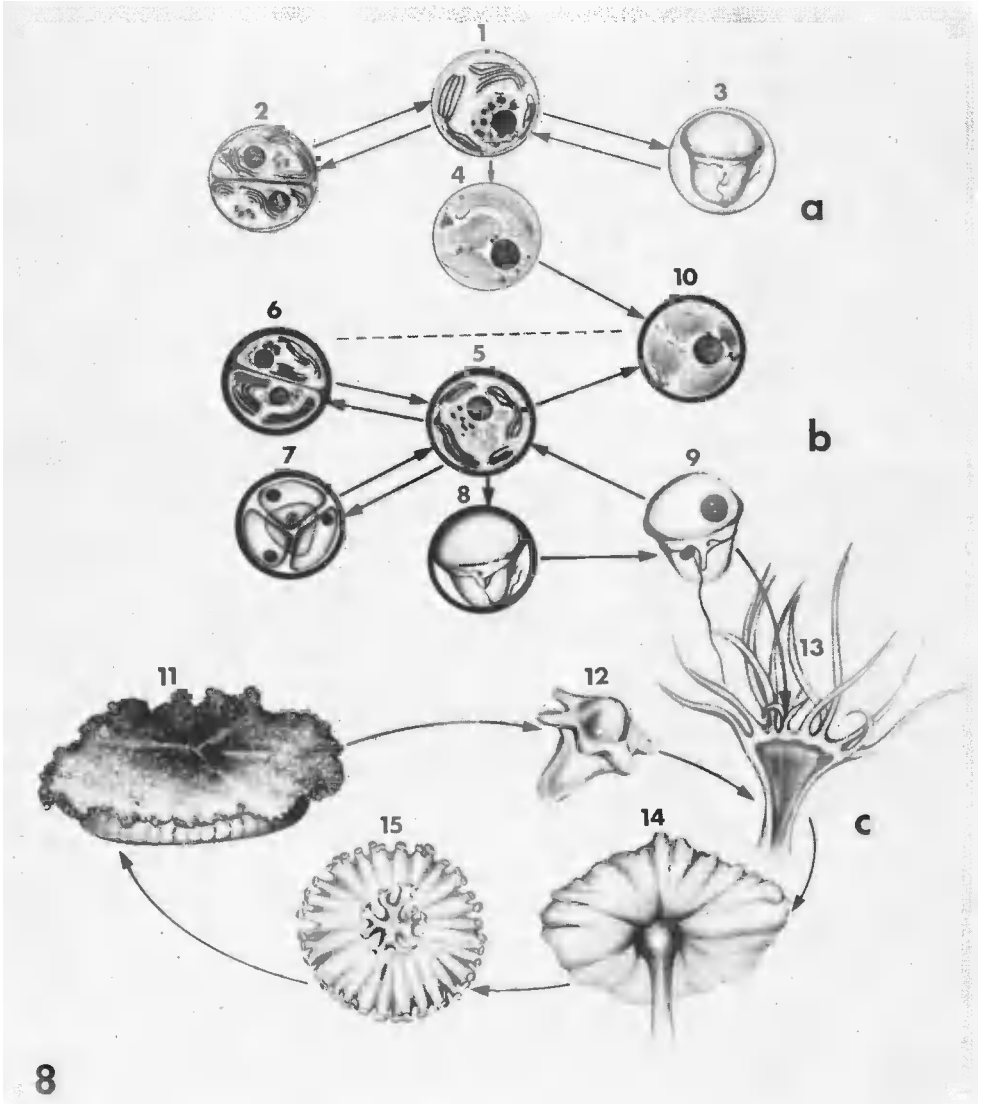

Fig. 8. Diagramatic representation of observed stages in the life cycle of *S. microadriaticum* (a) *in situ*, (b) in culture, and (c) of the jellyfish *Cassiopeia xamachana*. (1) the coccoid alga in a host cell, (2) binary fission by the alga in a host cell, (3) a motile cell produced in a host cell, (4) a senescent alga in a host cell; (5) an "encysted" alga in culture, (6) binary fission; (7) tetraspore formation; (8) production of motile cell; (9) the released gymnodimioid swarmer; (10) a senescent alga in culture; (11) a sexually mature *C. xamachana* produces an aposymbiotic planula which settles (12) and grows to the polypoid state (13); when infected with algae, strobilation occurs (14); and the ephyrae (15); harboring algal symbionts are released.

S. microadriaticum. In fact, Taylor (16) reported isolating an *Ampidinium* sp. from *Collozoum*. It is therefore abundantly clear that the systematics of symbiotic dinoflagellates is in a rather chaotic state, and in light of this, it is probably best to continue to refer to the gymnodinioid dinoflagellate symbionts as *S. microadriaticum*, bearing in mind that there *may* be different species within this group.

LIFE CYCLES OF SYMBIONTS AND HOSTS

Since the propensity to form symbiotic associations is an inheritable trait, the adjustment of life cycles by the separate partners to enhance the perpetuation of the symbiosis through generations is very important. There is no published information on the life cycle of symbiotic amphidinioid dinoflagellates, but some of these algae, e.g. *A. klebsii*, appear to retain their "freeliving" morphology when in symbiosis. Gymnodinioid dinoflagellates by contrast, are coccoid when in their animal hosts, and alternate between coccoid and motile gymnodinioid states in culture (34).

The life cycle of *S. microadriaticum* has been described by Freudenthal (29) and by Taylor (3). Unfortunately, there is disagreement on some of the finer details. For example, both authors illustrate the presumed gamete, but the illustrations are different. Again, assuming that the vegetative stage was diploid, Taylor (3) suggested that meiosis occurred prior to gametogenesis. However, although the exact ploidy of the vegetative stages of *S. microadriaticum* is unknown, several studies (37-40) have suggested that the vegetative stages of several dinoflagellates are haploid. If this is also true for *S. microadriaticum*, then meiosis would have to occur after gametic fusion (31). Very few investigators have reported actually seeing the fusion of gametes of *S. microadriaticum*.

Schoenberg and Trench (32) illustrated the various stages in the life cycle of *S. microadriaticum* that they observed *in situ* and in culture (see Fig. 8). *In situ*, the algae are usually coccoid vegetative cells with highly reduced or non existent "cell walls". The algae may undergo binary fission within the hosts, whether they are intercellular or intracellular (Fig. 9), and may also produce the microtubular apparatus associated with the flagella.

In culture, the coccoid cells are enclosed in a thick "cell wall", and they alternate between the coccoid non motile and the motile gymnodinioid states (34). Tetraspore production has only been observed in culture.

Many of the invertebrates harboring symbiotic algae reproduce sexually and asexually. Algae may be transmitted from parent to offspring directly during budding. However, in sexual reproduction, mechanisms have to develop whereby the progeny may acquire the algal symbionts. The life cycle of some coelenterates alternates between sexually reproducing and asexually reproducing forms. During sexual reproduction, there are two different ways in which the progeny may become infected with algae. In the first instance, referred to as the "closed system" (31) inheritance is maternal, and the algae are transmitted directly from the parent to the developing egg (Fig. 10). Examples of this method of inheritance can be found in some hydroids, corals and zoanthids. In these cases, the offspring inherit the same population of algae harbored by the parent.

In the second instance, referred to as the "open system", the offspring are released from the parents devoid of algae, and they subsequently become infected by algae from the ambient environment. Examples of this may be found in the jellyfish *Cassiopeia xamachana* (Fig. 8), several corals and in tridacnid bivalves (41, 42).

ACQUISITION OF SYMBIONTS BY HOSTS

In cases where the algae are maternally inherited, the animal hosts do not need to become "infected" by algae unless some perturbation in the environment causes loss of the algae, as occurred in some coral species following a lowering of salinity associated with heavy rainfall (43). Whether such animals become repopulated by algae from the surrounding environment or by proliferation of the algae remaining in the tissues, is unknown.

In "open systems" the algae must be acquired from the ambient environment. The mechanism through which infection is achieved in Nature is unknown, but there are three possibilities. First, motile gymnodinioid "swarmers" may infect the juvenile hosts. Second, faecal pellets containing algae (44) may be released by some hosts and incorporated by the juveniles. Third, the algae may be preyed upon by some herbivorous zooplankton which cannot digest them. When that zooplankton itself falls prey to a coelenterate, the final host may acquire the algae from the "intermediate host" after digestion (3, 5).

All the above three mechanisms imply that symbiotic dinoflagellates from any source could infect any potential host. This view is not supported by the observations that coelenterates such as *V. velella* have never been reported to have *S. microadriaticum* as symbiont, and corals and giant clams have never been reported to have amphidinioid symbionts. Clearly, there is selective discrimination in the establishment of symbioses. The details of the cellular and molecular mechanisms which modulate selectivity are at present not well understood (45).

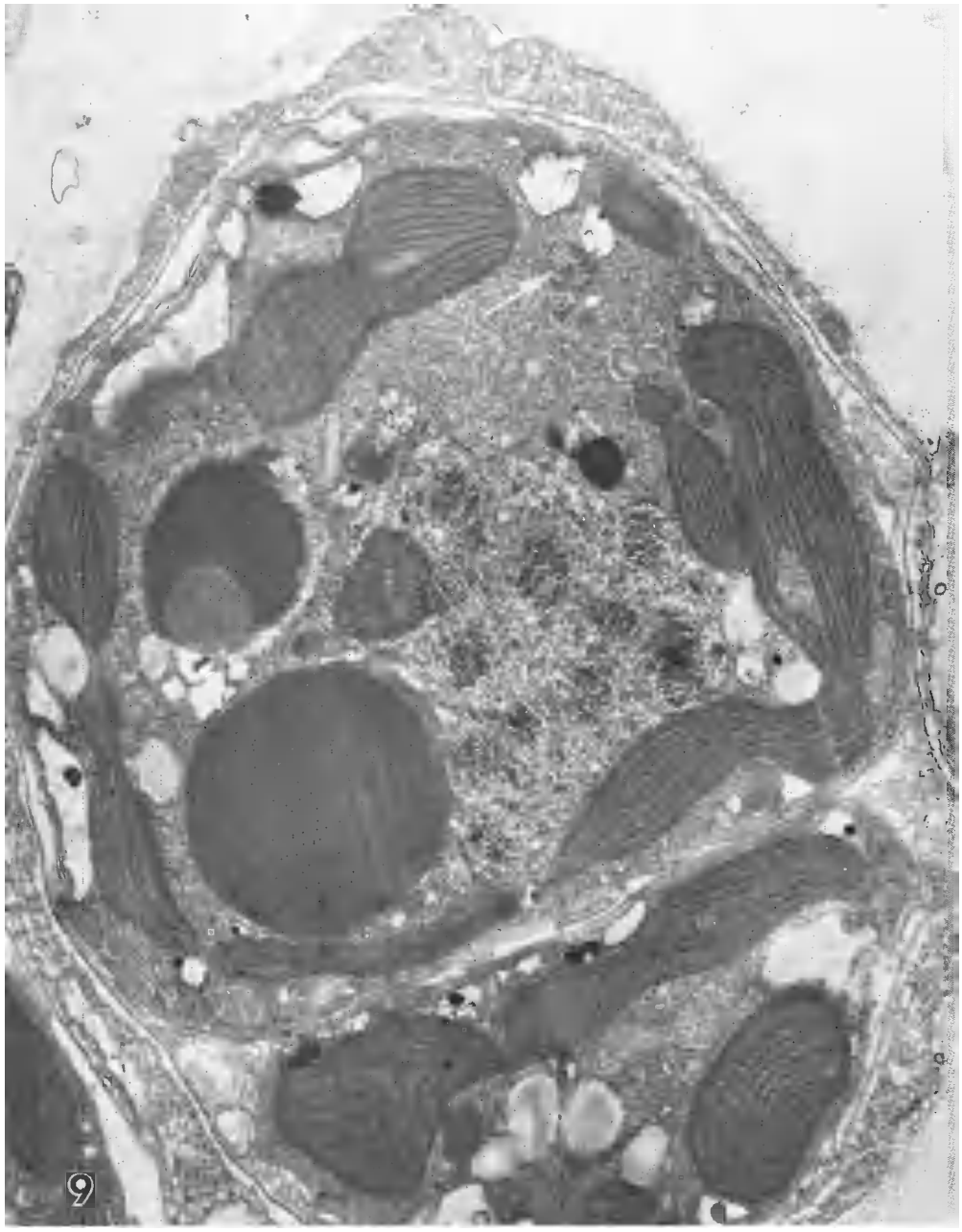

Fig. 9. Transmission electron micrograph of *S. microadriaticum* in *Xenia*, demonstrating binary fission and the synthesis of the flagellar microtubular apparatus (arrow). Magnification approximately 14,400 x.

The jellyfish *Cassiopeia* provides an excellent example of an "open system" as the palnulae are devoid of algae and develop into the polypoid scyphistomae which are also free of algae. Several observations point to selectivity in the process of infection in this and other invertebrates with "open systems" and I shall briefly relate these.

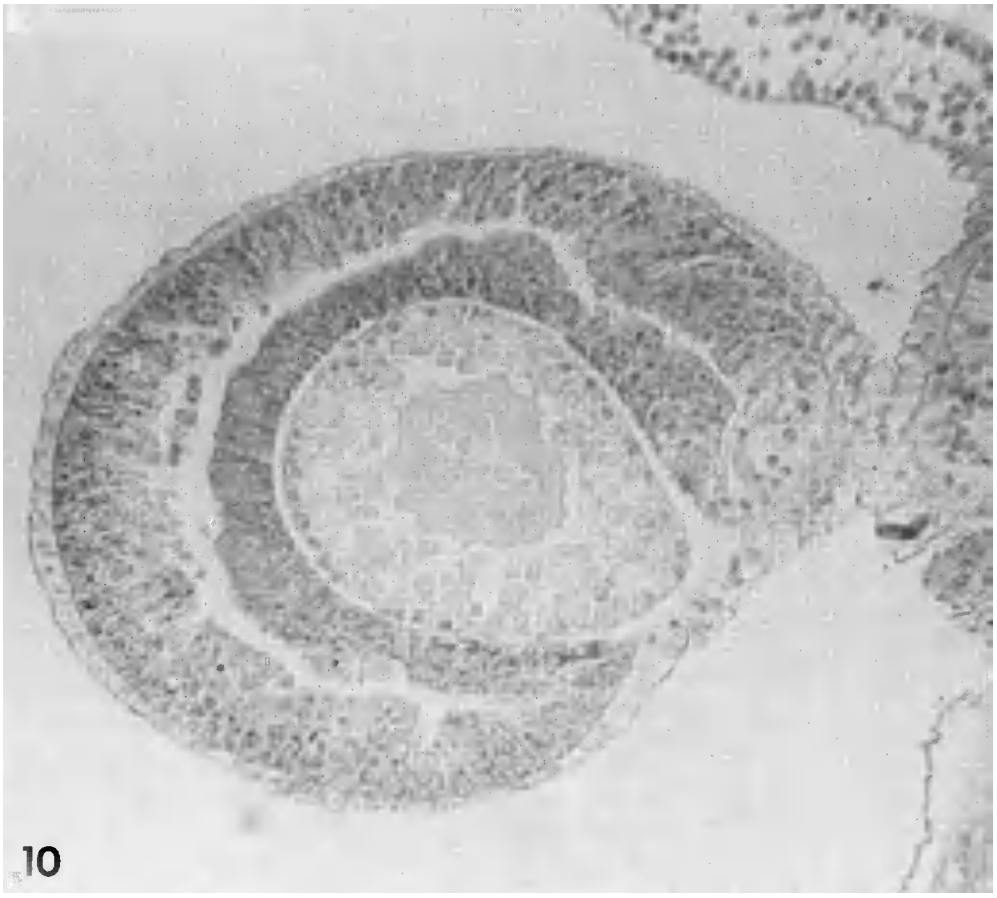

Fig. 10. Light micrograph of a developing egg on the hydroid *Myrionemia amboinense* showing the infection of the egg by algae derived from the parent hydroid. Magnification approximately 2,500 x.

First, several free living dinoflagellates can be found in the same environments where juvenile *Cassiopeia* settle, yet only one dinoflagellate, *S. microadriaticum* has ever been reported as establishing symbioses with this organism.

Second, Schoenberg and Trench (30) reported that they detected a single strain of *S. microadriaticum* in *A. tagetes* collected 1600 Km apart. Similarly, in a study conducted in Hawaii, Trench (unpublished) found that the algae isolated from *C. mertensii*, collected from different locations, demonstrated identical isoenzyme patterns for five enzyme systems, suggesting a uniformity of algal populations within the hosts. Considering that different invertebrate hosts from the same locations harbored algal populations that were distinct from those found in *C. mertensii*, one is forced to conclude that the scyphistomae were highly selective with respect to the strain of *S. microadriaticum* that they acquired.

In the laboratory, it is possible to experimentally infect the scyphistomae of *C. xamachana* or polyps of *A. tagetes* by injection of supernumerary algae into the coelenteric cavity. Such algae are endocytosed by the endodermal cells (e.g. Fig. 11). It is also possible to infect aposymbiotic hosts with motile *S. microadriaticum*.

Laboratory experiments on the resynthesis of symbioses between aposymbiotic clones of *A. tagetes* and strains of *S. microadriaticum* have been reported (32) The algae isolated from *A. tagetes* showed the highest potential to reassociate with *A. tagetes*. Although some other strains of *S. microadriaticum* also "infected" *A. tagetes*, the rate at which the heterologous algae grew in the "unnatural" host was lower than the rate of proliferation of the homologous algae. Some other strains were completely rejected.

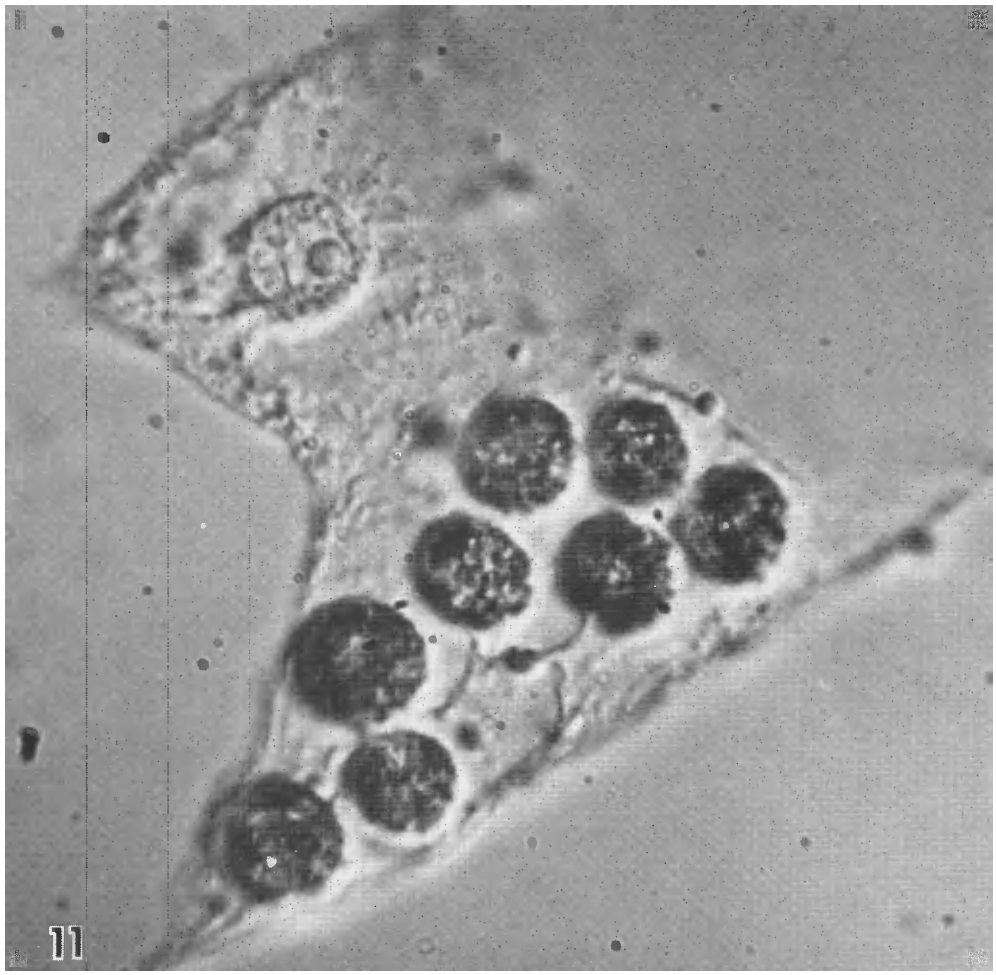

Fig. 11. Light micrograph taken with Nomarski interference optics showing the symbiotic algae within an endodermal cell isolated from *M. amboinense*. Magnification approximately 5,700 x.

The results of some recent experiments involving clones of the scyphistomae of *C. xamachana* and strains of *S. microadriaticum* demonstrate a similar phenomenon. In these studies, the assay used was the rate of uptake of algae by the endoderm cells after the injection of supernumerery algae into the coelenteric cavity of the host. Table 1 shows that freshly isolated homologous algae are taken up at a higher rate than cultured or dead algae. In fact, when isolated algae are maintained in culture for as brief a period as 24 h and then injected into the scyphistomae, their rate of uptake was significantly reduced. Electron microscope examination of the algae after 24 h in culture showed that by comparison with freshly isolated algae, aspects of the surface of the cultured algae were altered following cytokinesis, binary fission and the sheding of the mother cell wall (45).

When the rates of uptake of freshly isolated or cultured homologous algae were compared with the rate of uptake of heterologous algae (Table 2), even freshly isolated heterologous algae were taken up at higher rates than cultured algae. It should be emphasized that cultured strain Z algae, identified as the most distinct strain of *S. microadriaticum* (see Fig. 5) were never endocytosed, and although freshly isolated algae from *Z. pacificus* and *A. elegantissima* were endocytosed, neither of these algae persisted and formed a stable association, but were expelled within 24 to 72 h.

Laboratory experiments on infection using different strains of *S. microadriaticum* demonstrates some degree of specificity. Infection in Nature is not likely to occur by the uptake of supernumerary algae. In fact it is possible that only a single event occurs, and that the final population of algae harbored by an adult host results from the proliferation of that initial alga . This would be consistent with the observation that several of the hosts

Table 1

Influence of algal history on uptake by endoderm cells of the scyphistomae of *C. xamachana.**

Algal source	Strain	History	No. of host cells with algae†	Distribution of algae/ host endoderm cell (percent) 1	2	3	4(+)	N
C. xamachana	C	Freshly isolated	309 ± 129	51	29	13	7	5
C. xamachana	C	Freshly isolated, heat killed	129 ± 116	59	28	3	0	3
C. xamachana	C	Freshly isolated, heat killed	159 ± 122	68	24	7	0	5
C. xamachana	C	Cultured	10 ± 12	86	10	4	0	5
C. xamachana	C	Cultured algae + host homogenate	8 ± 10	97	3	0	0	5

*Scyphistomae of 0.3mm oral disk diameter only were used in this series of experiments. Cultured algae were maintained in culture medium ASP-8A. Animals were macerated 2 h after injection with the algae.
†Values represent mean ± 1 standard deviation.
Date obtained by N.J. Colley.

tested by Schoenberg and Trench (30) appeared to harbor clonal populations of *S. microadriaticum*.

Although laboratory tests show that a given host may acquire more than one strain of *S. microadriaticum*, usually only a few of these strains persit (1, 2) in a symbiosis. These observations imply that selectivity is a spectrum of processes beginning with discrimination at the level of cell-cell contact followed by subsequent adjustments between the two components (46). The final result is that the algal strain most compatible with a particular host persists and proliferates (32, 45, 47). Similar observations have been reported on experiments involving *Convoluta roscoffensis* (48) and *Amphiscolops langerhansi* (24).

Table 2

The rates of uptake of homologous and heterologous strains of *S. microadriaticum* by the endoderm cells of the scyphistomae of *C. xamachana.**

Algal source	Strain	History	No. of host cells with algae†	Distribution of algae/ host endoderm cell (percent) 1	2	3	4(+)	N
Cassiopeia xamachana	C	Freshly isolated	309 ± 129	51	29	13	7	5
C. xamachana	C	Cultured	10 ± 12	86	10	4	0	5
Aiptasia tagetes	A	Freshly isolated	68 ± 27	86	13	1	0	9
A. tagetes	A	Cultured	8 ± 7	88	12	0	0	5
Zoanthus pacificus	U	Freshly isolated	23 ± 13	93	6	1	0	5
Z. sociatus	Z	Cultured	0	0	0	0	0	5
Anthopleura elegantissima	U	Freshly isolated	44 ± 34	91	9	0	0	14

* The scyphistomae used in these experiments were 0.3 mm oral disk diameter. All other conditions were as previously described.

† Strain C algae, derived from *C. xamachana* are homologous; all other strains are heterologous. "U" denotes incompletely characterized strains.

Data obtained by N.J. Colley

METABOLIC INTERACTIONS

(i) Primary metabolites

Photosynthesis in endosymbiotic dinoflagellates has been studied in some depth, and an interested reader is referred to papers by Trench (1) and Muscatine (10). Two approaches have been taken in analyzing the fixation products of symbiotic dinoflagellates; (i) the analysis of photosynthetic products after *in vitro* fixation of $^{14}CO_2$ and (ii) analysis of ^{14}C-labelled compounds after fixation by the algae in the hosts' tissues. It should be recognized that *in situ* studies have to take into account material released by the algae and subsequently modified by the animal.

The studies reported by Muscatine (49) and Muscatine et al. (50, 51) as well as those of Trench (52-55) demonstrate that symbiotic gymnodinioid dinoflagellates incorporate photosynthetically fixed $^{14}CO_2$ into a wide range of compounds. Glucose was identified as a major intracellular product of photosynthesis. The major product released by the algae was, in most instances, glycerol, often accompanied by varying quantities of alamine, glucose and some organic acids. There are no published reports of the photosynthetic products of symbiotic amphidinioid dinoflagellates but a recent study in Palau on the amphidinioid dinoflagellate found in an unidentified pelagic flatworm demonstrated that glycerol was the major photosynthetic product released (56) by the algae *in vitro*.

Using the intact coral *Acropora scandens*, Schmitz and Kremer (57) confirmed many of the previous reports on carbon fixation products, and in addition demonstrated the incorporation of fixed ^{14}C into mannose, which was probably the result of host modification of substances translocated from the algae.

(ii) Secondary metabolites

It is quite clear that metabolites may move from symbiotic algae to their hosts, and that these substances may be utilized by the host in a variety of ways. The reverse pathway has not been investigated in as much detail (12) but some recent studies have thrown some new light on this subject. Patton et al. (58) and Blanquet et al. (59) have described experiments involving coelenterate hosts with gymnodinioid endosymbionts wherein it appears that acetate moves from the animals to the algae via a light-enhanced process, and that the acetate is incorporated into saturated fatty acids which are then transferred to the animals for use in the synthesis of wax esters and triglycerides.

There is growing interest in the role of symbiotic dinoflagellates in the biosynthesis of sterols in marine invertebrates (60) but this is a topic under review elsewhere (61-63).

From the data available, it would appear that secondary metabolites such as sterols are produced by symbiotic associations as a result of the metabolic cooperation of both organisms in the association. The algae apparently synthesize and release an intermediate which is further modified by the host. The final product is only expressed by the intact association. However, direct evidence of the release of sterols or sterol intermediates by symbiotic algae is still lacking.

CONCLUSIONS

From an examination of the many symbioses involving dinoflagellates and marine invertebrates, it is clear that these associations are not the result of random nonselective processes. Some animals form associations with gymnodinioid dinoflagellates while others establish symbioses with amphidinioid dinoflagellates. In Nature, this distinction appears to be exclusive.

The mechanisms through which speicficity is established are at present not completely understood, but the evidence available suggests that a spectrum of processes are involved. These include (a) possible ecological, behavioral, and physiological factors that influence the distribution of the algae and their potential hosts, (b) cellular and molecular events occuring on intercellular contact, (c) physiological processes modulating the integration of the consortium which is under the influence of "natural selection" (32) and (d) possible competitive exclusion between different algae in the same host. Any combination of these factors could unpredictably lead to an integrative or disintegrative association (2), or the expression of specificity.

Some progress is now being made on aspects of the cellular and molecular mechanisms which may determine specificity (45) and on possible forces of selection that may act on an established association (2, 24, 32, 45, 47) enhancing the perpetuation of the most "efficient" consortium. However, much of the studies of symbiosis is hampered, in the final analysis, by inadequate taxonomy. Repeated examples can be found in the literature where genetically distinct organisms are referred to by the same name, often resulting in confusion and conflicting

reports on their physiology and biochemistry. Unfortunately, in the current climate, support for systematic studies is not readily forthcoming.

ACKNOWLEDGEMENTS

I would like to thank my graduate students, S. S. Chang, N. J. Colley and W. K. Fitt for providing some of the experimental data presented in this paper. Studies in our laboratory are supported by NSF grant PCM78-15209.

REFERENCES

1. R. K. Trench, *Ann. Rev. Plant Physiol. 30*, 485-531 (1979).

2. R. K. Trench, in: *Cellular Interactions in Symbiosis and Parasitism* (C. B. Cook, P. W. Pappas and E. D. Rudolph, eds.), Ohio State University Press, Columbus (1980).

3. D. L. Taylor, *Adv. Mar. Biol. 11*, 1-56 (1973).

4. D. L. Taylor, *Ann. Rev. Microbiol. 27*, 171-187 (1973).

5. L. Muscatine, in: *Biology and Geology of Coral Reefs*, Vol. II, Biology I. (O. A. Jones and R. Endean, eds.), Academic Press, New York, London (1973).

6. L. Muscatine, in: *Coelenterate Biology*, (L. Muscatine and H. M. Lenhoff, eds.), Academic Press, New York, San Francisco and London (1974).

7. J. W. Wells, in: *Treatise on Invertebrate Paleontology*, (R. C. Moore, ed.), University of Kansas Press, Lawrence (1956).

8. P. H. Heckel, in: *Reefs in Time and Space*. Spec. Publs. Soc. Econ. Paleout. Miner. Vol. 18 (1974).

9. C. M. Yonge, *Proc. Zool. Soc. Lond. 123*, 551-561 (1953).

10. L. Muscatine, in: *Brookhaven Symp. in Biology, 31*, (in press).

11. T. F. Goreau, *Ann. New York Acad. Sci. 209*,127-167 (1963).

12. J. H. Vandermeulen and L. Muscatine, in: *Symbiosis in the Sea*, (V. B. Bernberg, ed.), University of South Carolina Press, Columbia (1974).

13. L. E. Dugay and D. L. Taylor, *J. Protozool. 25*, 356-361 (1978).

14. M. R. Droop, *Symp. Soc. Gen. Microbiol. 13*, 1717-199 (1963).

15. J. J. A. McLaughlin and P. A. Zahl, in: *Symbiosis*, (S. M. Henry, ed.), Vol. I. Academic Press, New York. (1966).

16. D. L. Taylor, in: *Symbiosis in the Sea*, (V. B. Vernberg, ed.), University of South Carolina Press, Columbia (1974).

17. G. Apelt, *Mar. Biol. 3*, 105-187 (1969).

18. S. Leutenegger, *Cahiers de Micropaleontologie. 3*, 1-55 (1977).

19. R. Röttger, *Naturwiss. Rundsch. 32*, 12 (1979).

20. J. J. Lee, C. W. Reimer and M. E. McEnery, *Botanica Marina 23*, 41-48 (1980).

21. E. Muller - Menz and J. J. Lee, *J. Protozool. 23*, 390-396 (1976).

22. A. Hollande and D. Carré, *Protistologica. 10*, 573-601 (1974).

23. D. L. Taylor, *J. mar. biol. Ass. U. K. 51*, 227-234 (1971).

24. D. L. Taylor, *J. mar. biol. Ass. U. K. 51*, 301-313 (1971).

25. C. M. Yonge, *Sci. Rep. Great Barrier Reef Exped. 1*, 283-321 (1936).

26. S. Kawaguti, *Biol. J. Okayama University. 14*, 1-12 (1968).

27. D. L. Taylor, *J. Phycol. 5,* 336-340 (1969).

28. M. Kevin, W. T. Hall, J. J. A. McLaughlin and P. A. Zahl. *J. Phycol. 5,* 341-350 (1969).

29. H. D. Freudenthal, *J. Protozool. 9,* 45-52 (1962).

30. D. A. Schoenberg and R. K. Trench, *Proc. R. Soc. Lond. B. 207,* 405-427 (1980).

31. D. A. Schoenberg and R. K. Trench, *Proc. R. Soc. Lond. B. 207,* 429-444 (1980).

32. D. A. Schoenberg and R. K. Trench, *Proc. R. Soc. Lond. B. 207,* 445-460 (1980).

33. H. W. Siegelman, J. H. Kycia and F. T. Haxo. *Brookhaven Symp. in Biol. 28,* 162-169 (1977).

34. W. K. Fitt, S. S. Chang, and R. K. Trench, *Bull. Mar. Sci.* (in press).

35. A. R. Loeblich and J. L. Sherley, *J. mar. biol. Ass. U. K. 59,* 195-205 (1979).

36. K. Brandt, *Arch. Anat. Physiol.* 570-574 (1881).

37. R. C. Tuttle and A. R. Loeblich, *J. Protozool. 24,* 313-316 (1977).

38. R. C. Tuttle and A. R. Loeblich, *Science 185,* 1061-1062 (1974).

39. L. A. Pfiester, *J. Phycol. 11,* 259-265 (1975).

40. L. A. Pfiester, *J. Phycol. 12,* 234-238 (1976).

41. M. LaBarbara, *Malacologia 15,* 69-79 (1975).

42. S. C. Jameson, *Pacific Science 30,* 219-233 (1976).

43. T. F. Goreau, *Science 145,* 383-386 (1964).

44. R. D. Steele, *Biol. Bull. 149,* 590-600 (1975).

45. R. K. Trench, N. J. Colley, and W. K. Fitt, *Symp. Acad. Wiss. Gottingen.* (in press).

46. R. Dubos and A. Kessler, *Symp. Soc. Gen. Microbiol. 13,* 1-11 (1963).

47. R. A. Kinzie, III and G. S. Chee, *Biol. Bull. 256,* 315 327 (1979).

48. L. Provasoli, T. Yamasu, and I. Manton, *J. mar. biol. Ass. U. K. 48,* 465-479 (1968).

49. L. Muscatine, *Science 156,* 516-519 (1967).

50. L. Muscatine and E. Cernichiari, *Biol. Bull. 137,* 506-523 (1969).

51. L. Muscatine, R. R. Pool and E. Cernichiari, *Mar. Biol. 13,* 298-308 (1972).

52. R. K. Trench, *Proc. R. Soc. Lond. B. 177,* 225-235 (1971).

53. R. K. Trench, *Proc. R. Soc. Lond. B. 177,* 237-250 (1971).

54. R. K. Trench, *Proc. R. Soc. Lond. B. 177,* 251-264 (1971).

55. R. K. Trench, *Helgolander, wiss. Meeresunters. 26,* 174-216 (1974).

56. R. K. Trench, Unpublished observations.

57. K. Schmitz and B. P. Kremer, *Mar. Biol. 42,* 305-313 (1977).

58. J. S. Patton, S. Abrahm, and A. A. Benson, *Mar. Biol. 44,* 235-247 (1977).

59. R. S. Blanquet, J. C. Nevenzel and A. A. Benson, *Mar. Biol. 54,* 185-194 (1979).

60. W. C. M. C. Kokke, N. W. Withers, I. J. Massey, W. Fenical and C. Djerassi. *Tetrahedron Lett.,* 3601-3604 (1979).

61. C. Djerassi, *3rd Int. Symp. on Marine Natural Products,* (this volume).

62. L. J. Goad, *3rd Int. Symp. on Marine Natural Products,* (this volume).

63. N. M. Withers, *Comp. Biochem. Physiol.* (In prep.)