CHAPTER 14
DINOFLAGELLATE REPRODUCTION

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1 INTRODUCTION

Early descriptions of both marine and freshwater dinoflagellates were based on living or preserved field samples. Only a small percentage of these organisms are even now available in culture. Thus, the older descriptions of their life cycles are only complete to the degree that the author was able to sample frequently and/or be fortuitous enough to have collected a population in all stages of its life history. Thus, researchers who are able to culture dinoflagellates and follow their life history in the laboratory are finding previously unknown vegetative and sexual stages not contained in original descriptions. In some instances these stages have been described as separate taxa.

2 VEGETATIVE REPRODUCTION

Bold & Wynne (1978) distinguish between vegetative or asexual cell division in which the products of cell division are either naked or surrounded completely by new cell walls that are not intimately related with the parental cell walls (*eleutheroschisis*) and that in which the development of the cell walls of the newly divided protoplast is initiated adjacent to, and continuous with, the

parental cell wall; each of the young protoplasts forms a wall over its entire surface; the walls of the young protoplasts remain closely contiguous with the parental cell wall, for at least a short period; and immediate rupture or hydration of the parental cell wall does not occur liberating the contained division products (*desmoschisis*). Since there is confusion in the literature concerning the term 'vegetative cell division' (Smith 1950 and Fritsch 1935) the authors have used the terms desmoschisis and eleutheroschisis to distinguish between methods of non-sexual reproduction.

Dinoflagellates either have a cell covering consisting only of membranes (unarmoured forms) or have structural cellulose or other polysaccharides in vesicles (armoured forms) which form plates (see Chapters 2 and 3). In 1970, Loeblich reintroduced Schutt's term 'amphiesma' for the dinoflagellate peripheral complex to replace the term 'theca', which is still used by some authors to designate dinoflagellate cell coverings ranging from those with no plates to those with many (Dodge & Crawford 1970; Steidinger & Cox 1980).

Armoured taxa which shed both epitheca and hypotheca at cell division often have a pellicular layer internal to the plates and external to the plasma membrane called a pellicle.

2.1 Desmoschisis

Three types of desmoschitic reproduction are known to occur in dinoflagellates. The most common is a simple pinching and splitting of one cell into two (binary fission) while continuously synthesizing the cell covering in the course of daughter cell separation (Fig. 14.1). This occurs in unarmoured or so called 'naked forms' such as *Gymnodinium* (Ehrenberg 1838) where it is oblique. Oblique division always occurs from the upper right cell quadrant to the lower left, even in dinophysoids (Taylor 1973). There is evidence that in some species of *Gymnodinium* the nuclear division which occurs at right angles to the plane of mitosis is unequal, resulting in daughter cells having different numbers of chromosomes. There is also evidence of nuclear fragmentation or amitosis occurring within a cell (Shyam & Sarma 1978).

In *Ceratium* the division of the cell wall and protoplast is oblique. Each daughter cell was thought to reproduce the other half (Wetherbee 1975a,b). Plate separation occurs along predetermined sutures thus separating adjacent thecal plates (Fig. 14.2). Dürr & Netzel (1974) have shown that in *Ceratium* the cell eventually sheds the half of the parental theca it inherited; this produces an entire new wall. Nuclear division in *C. tripos* usually begins during the last 2 hours of the light period. The cytoplasm splits by binary fission, with development of the missing half of the two new daughter cells beginning immediately. Non-disjunction may occur following division. This results in chains of two or more cells which remain attached during and after maturity (Wetherbee 1975b and Chapter 2). *Prorocentrum* and *Dinophysis* divide

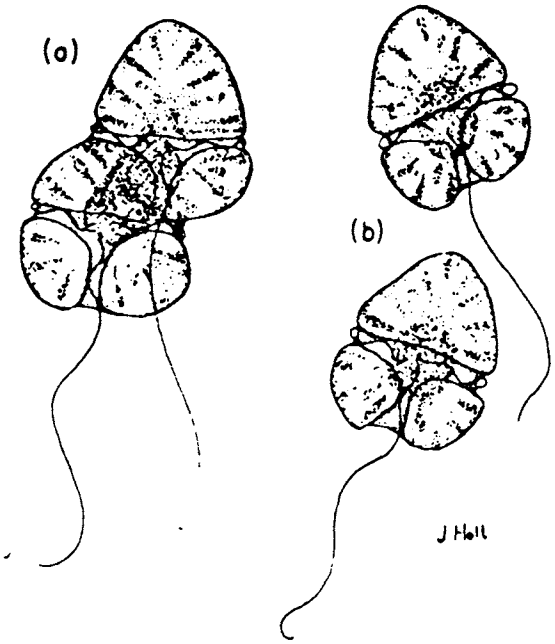

Fig. 14.1. Cell division in *Gymnodinium* sp. (a) Mitosis in oblique plane of motile parent cell (b) Daughter cells.

longitudinally, separating the two large valves of the parent cell such that each daughter cell retains one valve. The new wall, however, is almost fully formed before the separation of the parent wall along the 'megacytic zone' as Taylor (1973) showed in *Ornithocerus*. Whether a dinoflagellate divides by longitudinal or oblique division, the plane of division passes through the region of the cell from which the flagella emerge.

Dinoflagellate cells increase in volume during the course of the division cycle. Armoured species then must have some mechanism for expansion. In the Dinophysiales this is accomplished by growth of the cell wall along the margins of the sagittal fission line that divides the cell wall into two portions. Cells exhibiting this secondary growth are called megacytic. Some secondary growth does occur in young cells (Taylor 1973) (Fig. 14.3). Secondary growth is said to dissolve during cytokinesis or just following cytokinesis (Pavillard 1915; Tai & Skogsberg 1934; Taylor 1973).

Dinothrix is one of the few dinoflagellate genera that has a filamentous organization. In cell division the protoplast contracts slightly and divides obliquely into two (Fig. 14.4). The parent cell wall remains but the new daughter cells become enveloped in their own cell wall. Filaments thus formed are immobile and may branch sparingly.

2.2 Eleutheroschisis

There are two basic forms of asexual reproduction that occur in the dinoflagellates. The theca may be shed prior to division (ecdysis) as in

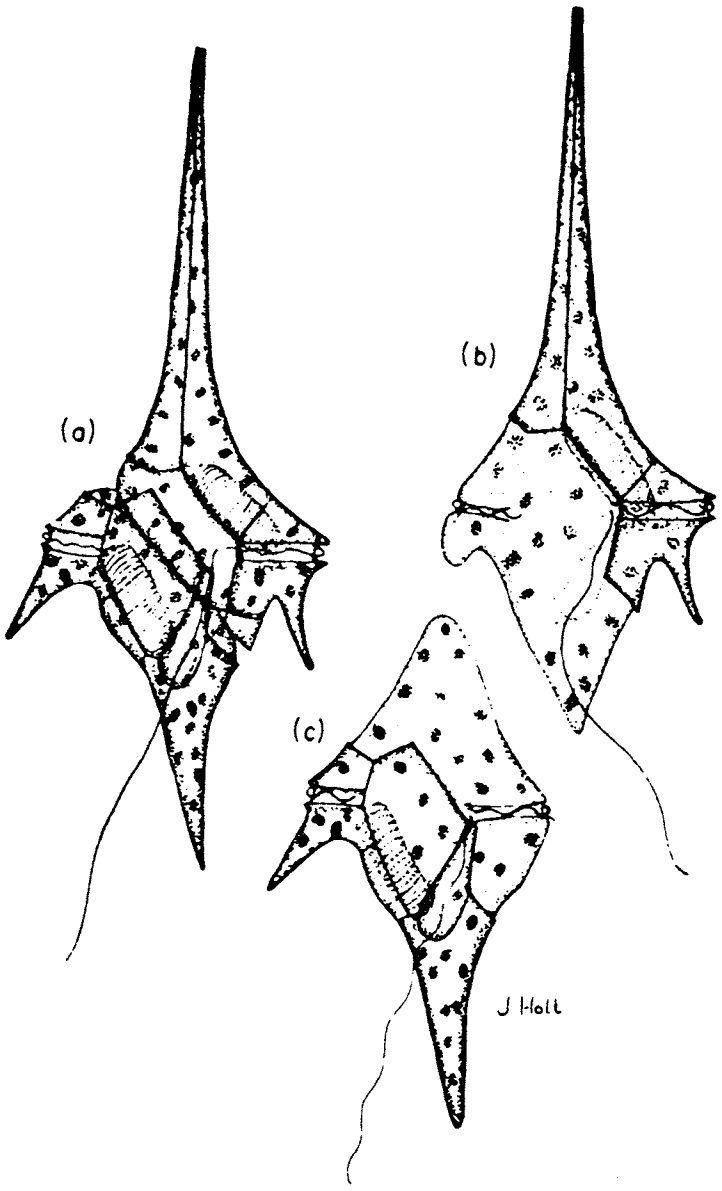

Fig. 14.2. Cell division in *Ceratium* (a) Mitosis in oblique plane of motile parent cell. (b) Daughter cell with parent epitheca (c) Daughter cell with parent hypotheca.

Symbiodinium and *Peridinium sanguineum* (Carter 1858) (Fig. 14.5) or it may undergo cytokinesis within the old wall which is shed by the daughter cells; these then form completely new walls (Fig. 14.6). Loeblich (1969) has provided tables (Tables 14.1, 14.2) listing the dinoflagellates that undergo each of the above-mentioned types of reproduction.

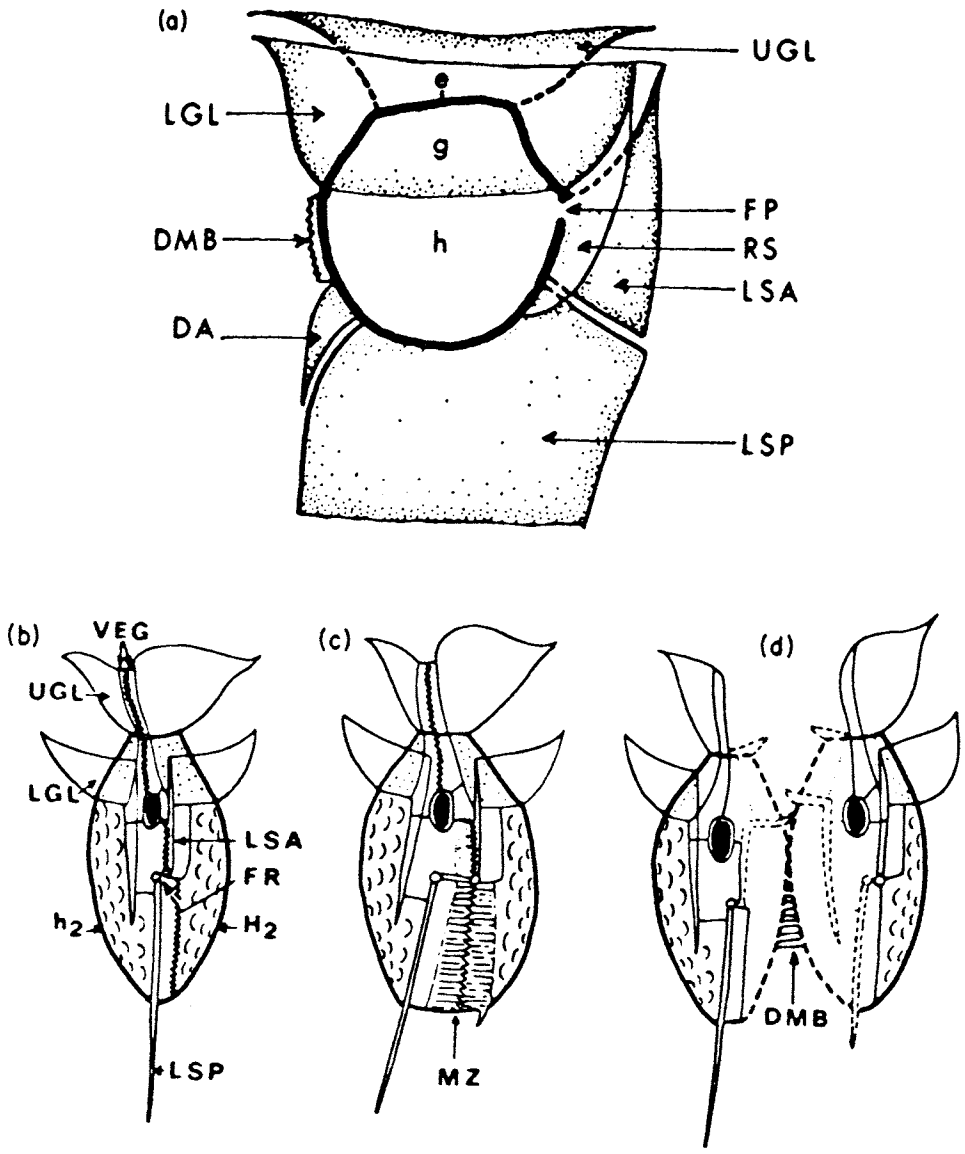

Fig. 14.3. (a) Diagrammatic illustration of the principal thecal components of *Ornithocerus*, seen from the right side. The cell body is shown in heavy outline; the lists are indicated: e, epitheca; g, girdle; h, hypotheca; DA, dorsal accessory list; DMB, dorsal megacytic bridge; FP, flagellar pore; LGL, lower girdle list; LSA, left sulcal list, anterior moiety; LSP, left sulcal list, posterior moiety; UGL, upper girdle list. (b–d) A diagrammatic representation of stages in division of a member of *Ornithocerus* as seen from the ventral (flagellar pore) side: (c) would be termed a megacytic cell. The incomplete lines in (d) represent newly formed material. The dotted part of the central region in (c) indicates a region of lateral growth hypothesized but not observed as yet. New list material forms between the two parts of the fission rib (FR) as they separate. DMB, dorsal megacytic bridge; LGL, lower girdle list; LSA, left sulcal list, anterior moiety; LSP, left sulcal list, posterior moiety; MZ, megacytic zone. (From F. J. R. Taylor (1973) Topography of cell division in the structurally complex genus *Ornithocerus*. *J. Phycol.* **9**, 1–10.)

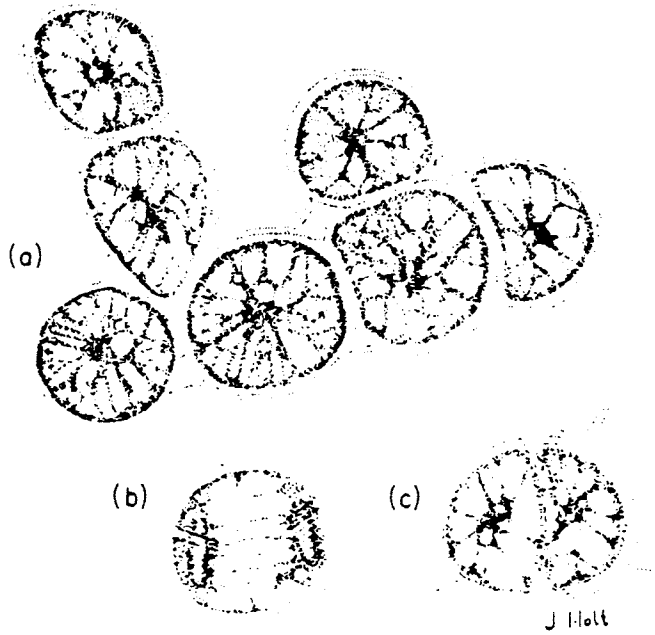

Fig. 14.4. Cell division in *Dinothrix*. (a) Filamentous growth habit (b) Mitosis within parent cell wall. (c) Daughter cell formation and enlargement within old parent cell wall.

In the Peridiniales, the cell wall accommodates increased cell volume during the mitotic cycle by thecal growth in the sutural area between plates, producing intercalary bands between adjacent plates. Pfiester & Skvarla (1979, 1980) have shown that such growth is slight in vegetative thecae but great in the thecae of plano- and hypnozygotes.

Yet another type of asexual reproduction occurs in those dinoflagellates known to have amoeboid forms. For a detailed description of these forms see

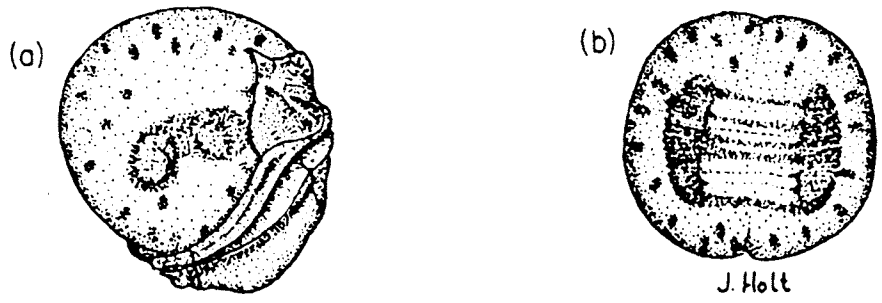

Fig. 14.5. Cell division in *Peridinium sanguineum*. (a) Ecdysis of parent theca. (b) Mitosis of protoplast.

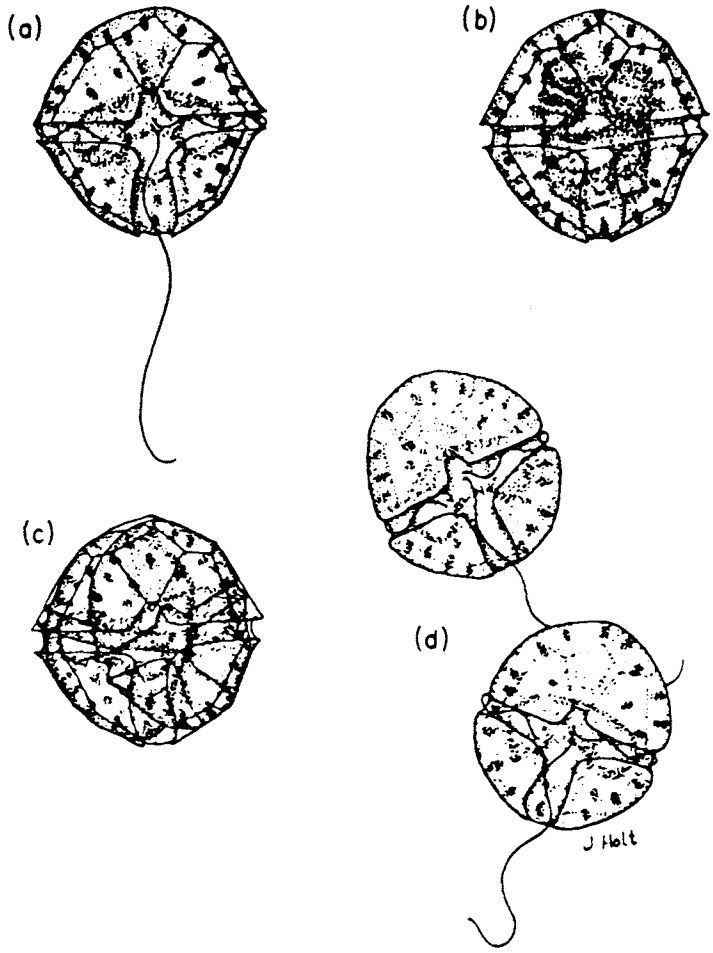

Fig. 14.6. Cell division in *Peridinium tolzii*. (a) Vegetative cell. (b) Mitosis. (c) Daughter cells within parent theca. (d) Daughter cells forming armoured theca.

the chapter on parasitic dinoflagellates by Cachon & Cachon (Chapter 13). *Stylodinium* and *Cystodinedria* both have non-motile, walled stages that are epiphytic on freshwater filamentous algae. The protoplast of this non-motile stage at times divides into two to four amoebae which are released, phagocytize intact filamentous algal cells and eventually develop back into their non-motile form. At some stage in development the protoplast of the non-motile stage divides producing gymnodinoid-like cells which are probably gametes (Pfiester & Popovský 1979). Fusion of these cells has been observed in *Cystodinium* (Pfiester & Lynch 1980), a genus similar to the non-motile stage of *Dinamoebidium* (Pascher 1930), another amoeboid-producing dinoflagellate (Fig. 14.7).

Table 14.1. Dinoflagellate species retaining one-half of the parent wall and regenerating the missing half (type 2)*

Species	Reference
Order Prorocentrales	
Exuviaella ssp.	Lebour 1925
Prorocentrum spp.	Lebour 1925
Order Dinophysiales	
Amphisolenia spp.	Kofoid and Skogsberg 1928
Dinophysis spp.	Schütt 1895
Ornithocercus spp.	Kofoid and Skogsberg 1928
Oxphysis oxytoxoides Kofoid, 1926	Kofoid 1926
Order Peridiniales	
Cachonina niei Loeblich, 1968	This paper
Ceratium spp.	Stein 1883; Lauterborn 1895
Ceratocorys spp.	Kofoid & Rigden 1912
Gonyaulax catenata (Levander) Kofoid, 1911	Kofoid & Rigden 1912
G. fragilis (Schütt) Kofoid, 1911	Kofoid & Rigden 1912
G. polygramma Stein, 1883	Kofoid 1911
G. triacantha Jörgensen, 1899	Kofoid 1906
Heteraulacus polyedricus (Pouchet) Drugg and Loeblich, Jr, 1967	Schütt 1895; Nie & Wang 1942
Protoceratium reticulatum (Claparède and Lachmann) Butschli, 1885	Braarud 1945
Pyrodinium bahamense Plate, 1906	
Spiraulaxina kofoidii (Graham) comb. nov.	Kofoif 1911

*From Loeblich (1969).

2.3 Cellular processes involved in vegetative reproduction

Vegetative reproduction involves cytokinesis and karyokinesis. Nuclear organization in the free-living dinoflagellates is unusual in that the chromosomes lack regular histones (Chapter 4), maintain the same appearance throughout the cell cycle and are visible as rod-shaped bodies within the interphase nucleus (Kubai & Ris 1969; Ris & Kubai 1974). Chromosome numbers of only 77 of the more than 2000 dinoflagellate taxa have been reported (Holt & Pfiester 1982). Dinoflagellate chromosome numbers range from 5 for *Syndinium turbo* (Chatton 1920) to approximately 274 for *Ceratium hirundinella* (Entz 1921). Recent studies have shown that dinoflagellates undergo polyploidy and/or aneuploidy in culture. Loper *et al.* (1980) demonstrated polyploidy in the unarmoured dinoflagellate, *Ptychodiscus brevis* in culture. Shyam & Sarma (1978) have suggested a possible polyploid or aneuploid series within the genus *Peridinium* and Holt & Pfiester (1982) have shown a relationship between chromosome numbers and years in culture in six freshwater dinoflagellates.

Table 14.2. Dinoflagellate species forming an entire new wall after cytokinesis (type 3)*

Species	Reference
Order Peridiniales	
Crypthecodinium cohnii (Seligo) Chatton in Grasse, 1952	Kubai & Ris 1969
Dissodium assymetricum (Mangin) comb. nov.	Lebour 1925
D. lenticulum (Bergh) comb. nov.	Lebour 1925
Gonyaulax polyedra Stein, 1883	This paper
G. series Kofoid and Rigden, 1912	Kofoid & Rigden 1912
Helgolandinium subglobosum von Stosch, 1969	von Stosch 1969
Heteraulacus sp.	Sousa & Silva 1969
Peridinium bipes Stein, 1883	Lefèvre 1932
P. cinctum (Muller) Ehrenberg, 1830	Lefèvre 1932
P. foliaceum (Stein) Biecheler, 1952	Sousa & Silva 1962
P. nudum Meunier, 1919	Paredes 1962
P. raciborskii var. *palustre* (Lindemann) Lindemann, 1928	Lindemann 1929
'P sanguineum' Carter, 1858	Carter 1858
'P. tabulatum' Ehrenberg, 1832	Schilling 1913
P. trochoideum (Stein) Lemmermann, 1910	Braarud 1957
Protoperidinium minutum (Kofoid) comb. nov. (cited as *Peridinium monospinum* Paulsen, 1907)	Lebour 1925
P. ovatum Pouchet, 1883	Schütt 1883
Pyrophacus horologium Stein, 1883	Stein 1883
Woloszynskia coronatum (Woloszynska) Thompson, 1950	Woloszynska 1917

*From Loeblich (1969).

In dinoflagellates the onset of cell division is marked by the duplication of the two flagellar bases from which the new flagella emerge. At this same time the nucleus enlarges and some chromosomes are found with Y-shapes and some with Vs. Nuclear division is unusual in that the nuclear envelope to which the chromosomes are attached remains intact, but a few to many cytoplasmic

Fig. 14.7. *Cystodinium* vegetative cell.

channels containing microtubules run completely through the nucleus (Leadbeater & Dodge 1967). V-shaped chromosomes make contact at their apices with the nuclear membrane where it surrounds cytoplasmic channels. Thus, the nuclear membrane is probably involved in some way in daughter chromosome separation (Kubai & Ris 1969). Division of the chromosome into two chromatids starts at one end of the chromosome and works toward the other. Once the chromosomes are separate the two longitudinal flagella move slightly apart and the nucleus becomes laterally invaginated by cytoplasmic streams from both sides. These invaginations become continuous through the nucleus and contain many microtubules (Fig. 14.8). Free-living and parasitic dinoflagellates have been found to have kinetochores though they are not all structurally identical (Oakley & Dodge 1974). In *Amphidinium* the nuclear envelope remains intact (Oakley & Dodge 1974), while in *Syndinium* the kinetochores are found in perforations or pores in the envelope (Ris & Kubai 1974; Fig. 13.18). In *Amphidinium* the kinetochore is completely outside the nuclear membrane. Thus, dinoflagellates do have a type of spindle but it is topologically outside the nucleus. As the chromatids separate, the nucleus becomes dumbell shaped and eventually separates in the middle forming two daughter nuclei. The cytoplasmic invaginations eventually disappear in the daughter nuclei. The nucleolus persists throughout mitosis (Leadbeater & Dodge 1967). The cytoplasm cleaves, separating the two nuclei and thus forming two separate protoplasts. As described above, thecal division occurs at this time in some dinoflagellates and not in others.

2.4 Cysts

As previously mentioned the term 'cysts' has different meanings for the palynologists and the neontologists. Palynologists use the term dinoflagellate cyst to refer to the fossilized forms of dinoflagellates (Chapter 15) while

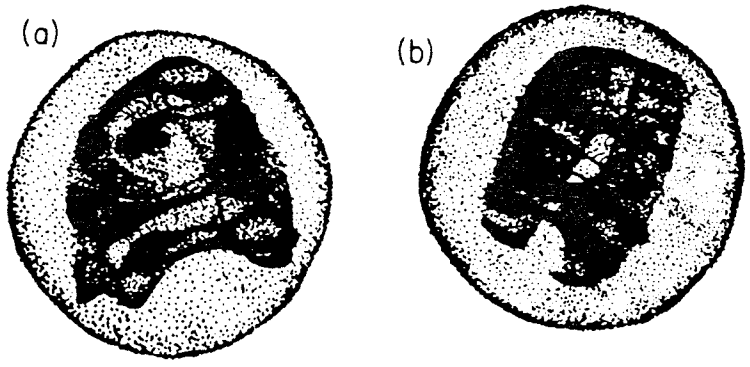

Fig. 14.8. Nuclear division by cytoplasmic invagination. (a) Cytoplasmic invagination. (b) Daughter nuclei separating.

neontologists have used it to refer to (i) a temporary resting state (temporary or digestive cysts), (ii) what is now known to be a dormant zygote (resting cyst), or (iii) a coccoid condition in which the cells are still photosynthetically active, e.g. *Pyrocystis*. Since we now know that some (probably many) of the fossilized cysts studied by palynologists are hypnozygotes their meaning of the term cysts will be discussed later under dinoflagellate sexual reproduction.

The occurrence of temporary cysts or spores has long been known and documented in numerous dinoflagellate studies. Fritsch (1935) referred to them as 'thin-walled' cysts. They are formed in *Peridinium cinctum* when cessation of movement is followed by a marked contraction of the protoplast. The contracted protoplast acquires a new wall before its release from the old theca, and the thin-walled spherical cysts thus formed may rest for a varying length of time. When placed in fresh media at 20°C the cyst will divide and germinate within 24 hours. It remains viable, however, for at least 5 months at 4°C in the dark. Thus, freshwater dinoflagellates may overwinter as thin-walled temporary cysts (Pfiester 1975). However, Anderson & Wall (1978) found that temporary cysts of *Gonyaulax tamarensis* which occur by ecdysis (Chapter 2) could not survive beyond a month at low temperatures. This may represent a basic difference between marine and freshwater dinoflagellate taxa. Evitt (pers. comm.) has found that the 'thin-walled' cyst of *P. limbatum* is fossilizable.

3 SEXUAL REPRODUCTION

Dinoflagellate sexual reproduction has long been disputed in the literature (Grell 1973) but has now been well documented and established by Von Stosch (1965, 1969, 1972, 1973), Cao Vien (1967a,b, 1968), Zingmark (1970), Tuttle & Loeblich (1975), Beam & Himes (1974, 1980), Pfiester (1975, 1976, 1977), Pfiester & Skvarla (1979, 1980), Pfiester & Lynch (1980), Walker & Steidinger (1979), Spero (1980), Anderson (1980), Turpin *et al.* (1978), and Yoshimatsui (1981). While a general pattern of sexual reproduction appears to be emerging it is important to remember that the sexual life histories of only twenty-two of the approximately 2000 extant species of dinoflagellates have been observed and described in the literature. Researchers are now only in the beginning stages of studying dinoflagellate sexual life histories. Developments leading to the culture of sensitive dinoflagellates have been of major importance in the discovery of and the ability to induce their sexual reproduction. Sexual reproduction appears to be of common occurrence in cultures but has not been recognized because (i) gametes usually look similar to regular cells, (ii) fusion has been confused with division, and (iii) 'warty' zygotes have been interpreted as aberrant cells. Given the previous studies on sexuality and further efforts to refine culture techniques, we should expect an increase in the number of sexual life histories known. Mechanisms of sexual reproduction in the algae are varied. Some of the life cycle types present in algae are: (i) haplontic (vegetative cells

are haploid with the zygote being the only diploid cell in the life cycle), (ii) diplontic (all cells are diploid except the gametes), and (iii) diplohaplontic (an alternation of haploid and diploid generations). All dinoflagellates studied thus far exhibit the haplontic type of life cycle with the exception of *Noctiluca*. For that reason Zingmark (1970) maintains that it is not a true dinoflagellate.

Haplontic dinoflagellates differ in the types of gametes produced. That is, in many species the gametes are *hologamous*, (Beam & Himes 1974; Cao Vien 1967a,b), i.e. gametes do not differ morphologically from vegetative cells. Some species are *isogamous*. That is, while the two gametes which fuse may differ morphologically from vegetative cells by size and/or amount of pigmentation and presence of theca they are morphologically identical to each other (von Stosch 1964, 1972, 1973; Pfiester 1975, 1976, 1977; Pfiester & Skvarla 1980; Anderson 1980; Spero & Moree 1981; Walker & Steidinger 1979). When fusing gametes differ from each other morphologically a species is said to be *anisogamous*. *Ceratium cornutum*, *C. horridum*, *Helgolandinium subglobosum* (Stosch 1972), *Protogonyaulax* (=*Gonyaulax*) *tamarensis* (Turpin *et al.* 1978), and *Coccidinium mesnili* (Chatton & Biecheler 1936) produce anisogamous gametes. Algal species that produce isogamous or anisogamous gametes are considered to be monoecious if sexual reproduction may occur within a clone such as *Peridinium cinctum* (Pfiester 1975) and dioecious if two different mating strains (usually designated plus and minus) must be combined in order for sexual reproduction to occur. Most dinoflagellates studied are monoecious. Dioecism has been reported for *Glenodinium lubiniensiforme* (Diwald 1937), *Woloszynskia apiculata* (Stosch 1973), *C. cornutum* (Stosch 1972), *Peridinium volzii* (Pfiester & Skvarla 1979), *Protogonyaulax catenella* (Yoshimatsu 1981) and one population of *Noctiluca scintillans* (=*miliaris*) (Hoefker 1930).

3.1 Nuclear phenomena associated with the sexual process

As discussed elsewhere in this chapter and book the dinoflagellate nucleus is unique in that it has features in common with both prokaryotes and eukaryotes. With the exception of *Noctiluca* the vegetative cells of dinoflagellates studied thus far are haploid, although the chromosome numbers may be high. Nuclear fusion has been observed to occur during plasmogamy but prior to its completion in fusing gametes of most species studied (Spector *et al.* 1981; Pfiester 1976, 1977; Pfiester & Skvarla 1979). The resulting nucleus is large and occupies a considerable part of the cell volume. In a growing number of described dinophycean zygotes the nucleus has been seen to enlarge further and to rotate rapidly within the cell. This phenomenon termed 'cyclosis', was first described by G. Pouchet (1883). Later Biecheler (1952) described the same process in six species of *Peridinium*. She showed that some time after the nucleus had come to rest cell division followed. Biecheler postulated that the phenomenon might be associated with meiosis. Von Stosch (1972, 1973) has clearly shown this to be

the case. The exact phase of the life cycle at which this occurs differs slightly for the organisms in which it has been noted. In *Ceratium horridum* nuclear cyclosis occurs during the planozygotic (motile zygote) stage. Von Stosch was able to show the double nature of the chromosomes during the late stages of cyclosis. Thus, nuclear cyclosis corresponds to the late zygotene or postzygotene of meiosis. In *C. cornutum* however, nuclear cyclosis occurred in the cell which emerged from the hypnozygote (non-motile zygote). In *Wolosynskia apiculata* it occurs for 9 hours before the onset of meiosis (von Stosch 1973). On excystment one cell or two may emerge.

Dinoflagellates have many characteristics in common with the ciliates (Phylum Protozoa). For this reason some (Taylor 1980) believe that they may be more closely related than previously supposed. While the ciliate macronucleus does not undergo a true cyclosis during division it enlarges greatly, nuclear reorganization occurs forming two zones with each zone moving toward the centre (Kudo 1966). Cyclosis does occur in ciliates' endoplasm. In *Paramecium* the endoplasm moves along the aboral side to the anterior region and down the other side, with a short cyclosis in the posterior half of the body (Kudo 1966).

Beam & Himes (Himes & Beam 1975; Beam & Himes 1980) have proposed an unusual one-division meiosis in dinoflagellates resulting from their work on the sexual life history of *Crypthecodinium cohnii* (Beam *et al.* 1977). Working with motility mutants that show complementation shortly after zygote formation they showed that segregations were always 1:1, i.e. in all tetrads showing recombination, only the two reciprocal recombinant genotypes were found; there were no tetratypes. They postulated that this could result from (i) centromere linkage, (ii) the absence of crossing over in an otherwise conventional meiosis, or (iii) an unusual one-division 'meiosis'. They concluded that reduction in *C. cohnii* does not employ a second meiotic division on the basis of two-celled zygotic cysts, many of which showed recombination, and the presence of eight-celled cysts. Von Stosch, Theil and Happach-Kasan (pers. comm.) recently found evidence for a two-step meiosis in *Ceratium cornutum*, a heterothallic freshwater dinoflagellate that reproduces anisogamously. Its planozygote increases in size for several weeks, eventually developing into a dormant hypnozygote. Under laboratory conditions one uninucleate swarmer escapes. Its size, shape and flagellation differs from vegetative cells. The two subsequent nuclear divisions of this cell are thought to be meiotic. Aberrant thecal halves of the meiocyte are transmitted to the offspring. These aberrant halves were used as markers. Von Stosch *et al.* were able to isolate ordered tetrads and to raise meiospore clones from them. Their sexual determination was then tested by subjecting them to conditions favourable to sexual reproduction in clonal cultures or in combination with each of the two standard female and male clones and determining the number of hypnozygotes formed. Of the 125 complete tetrads analysed, 69 had the sex factors segregated in the first meiotic division, 59 in the second meiotic division, while 7 tetrads were either 1–3

segregants or non-classifiable. Von Stosch *et al.* concluded from these data that meiosis is a two-step process in *C. cornutum* as opposed to the one-step division of Beam & Himes. Von Stosch *et al.* did observe parthenogenesis in male haploid and diploid clones. This phenomenon has also been observed in *Peridinium volzii* (L. A. Pfiester, pers. obs.) and in *Protogonyaulax tamarensis* (D. M. Anderson, pers. obs.).

3.2 Examples of sexual reproduction

NON-CYST FORMERS

There are dinoflagellates studied thus far that do not produce resting zygotes (cysts) in their sexual cycle: *Noctiluca scintillans* (Zingmark 1970), *Ceratium tripos* (von Stosch 1969), and *Peridinium gatunense* (Pfiester 1977).

Noctiluca (Fig. 14.9 and Chapter 2) is a marine dinoflagellate that may range up to 2 mm in size. It is a naked, spherical, non-photosynthetic colourless cell that is usually capable of luminescence. *Noctiluca* has a conspicuous nuclear mass near the oral groove, a cytostome and extended tentacle. Food vacuoles extend into the large cell vacuole but are separated from it.

Noctiluca's morphology changes during gametogenesis. Its tentacle disappears and the cell becomes more spherical. At this stage the nuclear mass is located at the periphery of the cell and food vacuoles have disappeared. The nucleus divides within a few hours, only to divide again in about 45 min. The four nuclei thus formed are arranged in a tetrad. This appears to signal the end

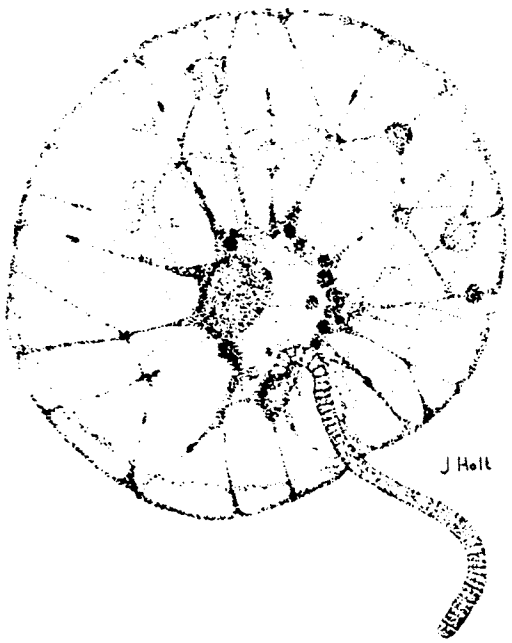

Fig. 14.9. *Noctiluca scintillans* vegetative cell.

of meiosis. The tetrad continues to divide every hour until it reaches the 256–1024 stage, depending on the original gametocyte size. Uniflagellated gametes are formed and released by a budding process. These gametes have a prominent constriction on one side of the flattened surface which has been interpreted as showing morphological affinity with dinoflagellates. Gametes fuse with the flattened side of one individual touching the narrow edge side of another. As fusion continues the gametes become non-motile as their flagella appear shortened. The nuclei have fused at this stage. Zygotes measure about 25μm in diameter when first formed. All zygotes formed during Zingmark's (1970) research died shortly after formation except for one, which increased in size, became vacuolated and developed a tentacle and nuclear mass. It too died, after reaching about 200μm in size.

Vegetative cells of *Noctiluca* are thus diploid, with meiosis occurring during gamete formation. Gametes are isogamous, Zingmark's strain was monoecious. *Noctiluca* thus has a diplontic life cycle (Fig. 14.10). The vegetative nucleus is the eukaryotic type (the chromosomes dispersing at interphase) and thus not typical of dinoflagellates, but the nucleus of the gametes is typically mesokaryotic. Thus, it appears that in the case of *Noctiluca* the two types of nuclei found in the dinoflagellates occur together in the same life history. Zingmark (1970) who named this type of nuclear condition 'noctikaryotic' did not believe that *Noctiluca* was a true dinoflagellate because: (i) while the gamete's body is partially constricted in the middle resembling a *Gymnodinium* cell, closer examination reveals that this is not a transverse girdle and a transverse flagellum is lacking; (ii) the vegetative nucleus is eukaryotic rather than mesokaryotic as in the dinoflagellates. However, as illustrated in Chapter 2, a ribbon-like flagellum is present in the feeding stage ('trophont').

CYST-FORMERS: HYPNOZYGOTES

Increasing numbers of dinoflagellate life cycles are being reported in the literature in which the zygote undergoes a long resting stage before germination similar to other algal groups such as the Volvocalan green algae. The life histories described to date have been cited elsewhere in this chapter, so that the serious student of dinoflagellate sexuality should be able to pursue this phenomenon in detail should he/she wish. Here we select two 'typical' taxa for which we describe the phenomenon in detail. The first is *Peridinium cinctum*.

Peridinium cinctum *life history*

The sexual life history of *P. cinctum* has been reported in detail at the light (Pfiester 1974, 1975), scanning (Pfiester & Skvarla 1980), and transmission electron microscopical levels (Spector *et al.* 1981). It is isogamous and monoecious (see Chapter 11B for its autecology).

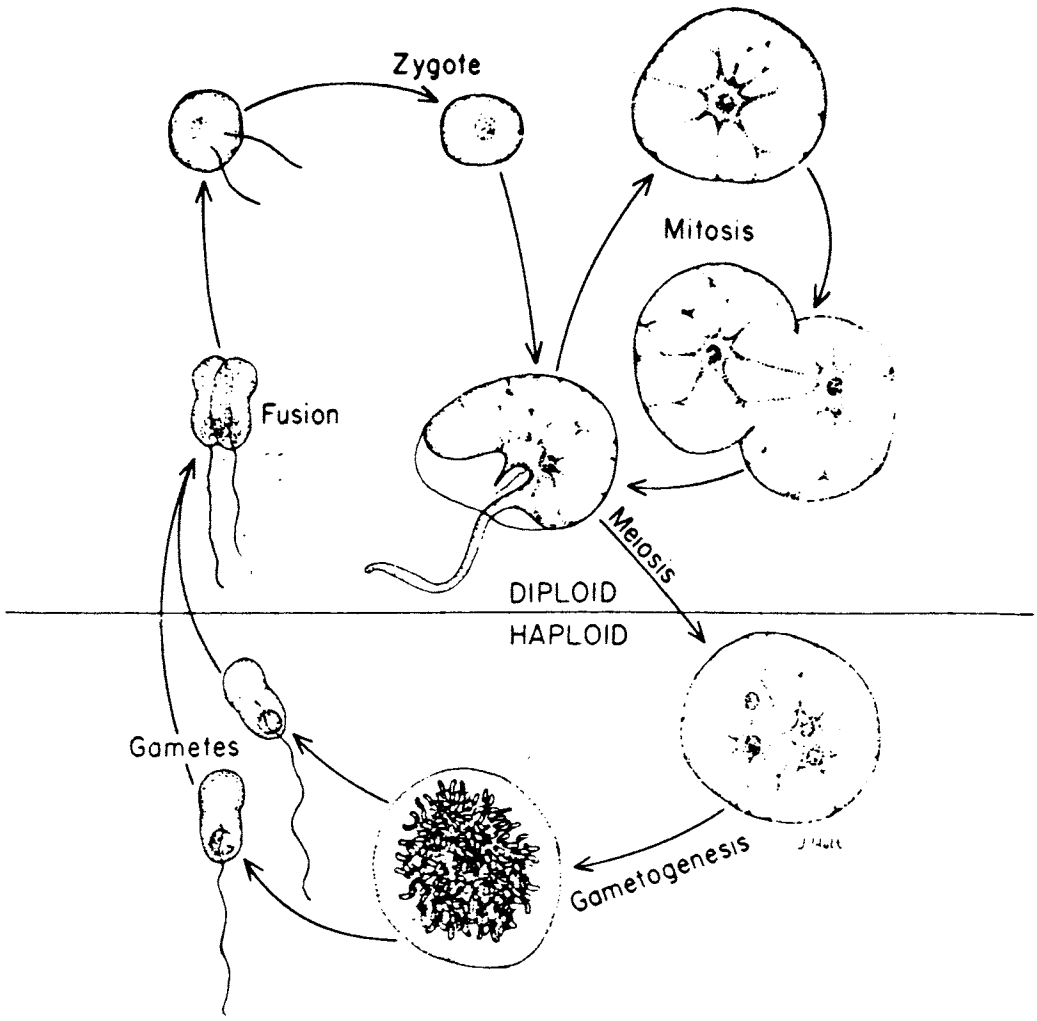

Fig. 14.10. Life cycle of *N. scintillans*

Peridinium is a thecate genus, i.e. its cell wall has a number of plates joined by what have been called intercalary bands (Chapter 2). The girdle (cingulum) divides the cell into two halves, the epi- and hypo-theca. The number and arrangements of the plates in these two halves has thus far been the major taxonomic character used in classifying these organisms and is referred to as a tabulation or formula. The plate pattern formula for *Peridinium* is 4′, 3a, 5c, ?s, 7″, 5‴, 2⁗ (Bourrelly 1968). Members of the genus tend to be flattened dorsoventrally. Vegetative cells are approximately 35–38 μm in length and have discoid, parietal chloroplasts. Cells contain large amounts of a brownish pigment, peridinin, the name derived from the generic name, giving a light to dark brown appearance.

Gamete formation, structure and development. When a vegetative population is placed in a nitrogen deficient medium, gametes are produced by the longitudinal mitotic division of small vegetative cells, not involving the parent theca (Pfiester 1975). Gametes so formed turn before being released so that the plane of cell division appears to be horizontal. Maximum numbers of gametes are observed 5–7 days following inoculation into the N-deficient medium. Transmission electron microscopy shows that the parent protoplast is surrounded by three membranes internal to the thecal plates (Spector & Treimer 1979) and that mucilage is deposited between the outer two membranes during gamete formation (Spector *et al.* 1981). Gametes, approximately 25 μm in diameter, are released by the breakdown of the parent theca. Initially gametes are naked, i.e. they lack a theca. However, a theca begins forming shortly after release. Gametes may fuse immediately upon release from the parent cell or remain motile for 24–36 hours, during which time they develop a cell wall. The final thickness of this wall depends upon the length of time the gametes remain motile before fusing (L. A. Pfiester, pers. obs.). Fusing gametes with thecal plates measuring 20–200 nm in thickness have been observed with the TEM (Spector *et al.* 1981) (Fig. 14.11). Fusing thecate gametes have also been

Fig. 14.11. Section through the fertilization tube (FT) of *Peridinium cinctum* connecting two gametes. Thecal plates (TP) are continuous between the gamete and appear to have fused. One nucleus (N), containing fibrous chromosomes, is present in the fertilization tube while the other nucleus has not yet migrated into the fertilization tube (FT) (× 3200). From Spector *et al.* (1981) *Amer. J. Bot.* **68**, 34–43.

observed with the light microscope (Pfiester 1976). However, at this observational level thecate gametes were never seen to complete fusion. Gametes have fewer chloroplasts than vegetative cells, many membrane-bound storage bodies and starch grains. The nucleus is large in proportion to the cell size and contains many slightly unwound chromosomes with 'arms' (loops) extending from them. As the gametes fuse laterally, a fertilization tube is formed which widens along the sulcus as fusion continues. At the level of the basal bodies the sulcus is devoid of thecal plates (in thecate gametes) and covered by a membrane. Initially the fertilization tube is located beneath the basal bodies. At that stage a fibrous chromosome is usually present in the tube. At later stages the two nuclei fuse in the fertilization tube. Fusion between naked gametes occurs as described for thecate gametes except that the fusing pair are surrounded by three membranes. Fusion takes approximately 45 minutes.

Planozygote formation, structure and development. The zygote is at first spherical but within 45 minutes resembles a *P. cinctum* vegetative cell 38 × 35 μm. A theca develops within 24 hours. Some time later a wall is laid down internal to the thecal plates. At this stage the planozygote, which has two trailing flagella, measures approximately 40 μm. It remains motile for approximately 2 weeks, enlarging during this time to a maximum size of 70 × 75 μm. The planozygote appears 'warty' as it enlarges, partly because of the protrusions present on the thecal plates (Spector *et al.* 1981) and partly because of an unequal widening of suture bands between plates (Pfiester & Skvarla 1980). At this stage the chromosomes are similar to those of vegetative cells. Once the maximum size is reached the planozygote loses motility, its protoplast contracts, one or more large red bodies develop and a third wall develops.

Hypnozygote structure, germination and meiosis. The non-motile zygote is referred to as a hypnozygote. It has been reported to contain chitin in its outer wall, which is extremely thick and is called the exospore (Pfiester 1975). The thin middle wall is referred to as the mesospore and the inner wall the endospore. The endospore has been reported to contain sporopollenin (Spector *et al.* 1981). The red body resists acetolysis and is fossilizable. Thus, it too may contain sporopollenin (W. Evitt, pers. comm.).

Meiosis has not been observed in *P. cinctum*. Nuclear stains will not penetrate the hypnozygote until just prior to germination. At that stage the cell has been shown to contain four nuclei (Pfiester 1975). On that basis meiosis is thought to have been completed before germination of the hypnozygote. It is highly possible that the binucleated planozygote observed by Pfiester (1975) was the result of the first meiotic division and that the second division was delayed until just prior to germination from the hypnozygotic resting stage. Thus, vegetative cells and gametes are said to be haploid. This is supported by the report (Pfiester 1975) of isolated gametes placed in N-enriched medium giving rise to vegetative populations which can later be induced sexually.

Ceratium cornutum *sexual life history*

Von Stosch (1964, 1965), in his work on the genus *Ceratium*, was the first to recognize that the 'Knauelstadium' (knotty stage) described by Borgert (1910) represented the postzygotene stage of meiosis in the *C. cornutum* zygote. Von Stosch, Theil and Happach-Kasan (pers. comm.) have recently completed further work on the sexual life history of *C. cornutum* wherein they have described meiosis in detail. *C. cornutum* is dioecious and anisogamous. Male gametes are considerably smaller than female gametes. They are engulfed by the female gamete through the sulcal area. Plates in this region appear 'hinged' and open for the male gamete and close again when syngamy is complete. The planozygote grows in size for several weeks until it develops into a dormant hypnozygote. If maintained at 4°C for at least 3 months the hypnozygotes will then germinate, producing a uninucleate swarmer. This meiocyte differs in size, shape and flagellation from vegetative cells. Von Stosch *et al.* (pers. comm.) have shown that its subsequent two nuclear divisions are meiotic. The thecal halves of the meiocyte are aberrant and are transmitted to the offspring of both steps of meiotic cell divisions. Thus, ordered tetrads are produced. From a detailed study of these tetrads von Stosch *et al.* have shown that meiosis is a two-division process in *C. cornutum* as opposed to the one-division process proposed for *Crypthecodinium*. They have shown further that some male clones may undergo haploid and diploid parthenogenesis.

A generalized dinoflagellate life cycle is shown in Fig. 14.12. Such a diagram may change in time as more dinoflagellates are cultured and their vegetative and sexual life histories elucidated.

4 SIGNIFICANCE OF SEXUAL REPRODUCTION TO DINOFLAGELLATE SYSTEMATICS

The earliest documented report of sexual reproduction in the Dinophyceae was Joseph's (1879) description of pairing and fusion of swimming cells of *Peridinium stygium*. The development of culture methods and techniques has enabled researchers to study and document sexual life histories in detail. While these studies are in themselves valuable and interesting, their effects on dinoflagellate systematics make them even more so. From these studies we now know that many (perhaps nearly all) of the fossil forms are remains of hypnozygotes rather than vegetative cells as previously thought. Their excellent preservation in the fossil record may indeed be due to the presence of sporopollenin in the cell wall as Spector *et al.* (1981) and earlier authors have postulated. Further, in the laboratory, researchers have been able to trigger the sexual process by drastically lowering the phosphorus and/or nitrogen concentrations in the culture media (see next section). While this may not be the only mechanism that induces sexuality in nature, it is not unreasonable to speculate that at least some of the

fossil hypnozygotes were initially formed under conditions of nutrient limitation. Thus, the knowledge of the sexual process in dinoflagellates may lead to a further understanding of the environment where fossil or extant hypnozygotes are found.

A number of descriptions and figures of taxa at various levels discuss and show extremely widened intercalary bands. We now know that in the armoured dinoflagellates the planozygote theca accommodates protoplast enlargement by increased width of intercalary bands (Pfiester & Skvarla 1979, 1980) (Fig. 14.12). Thus, these descriptions are not of normal vegetative cells as the authors

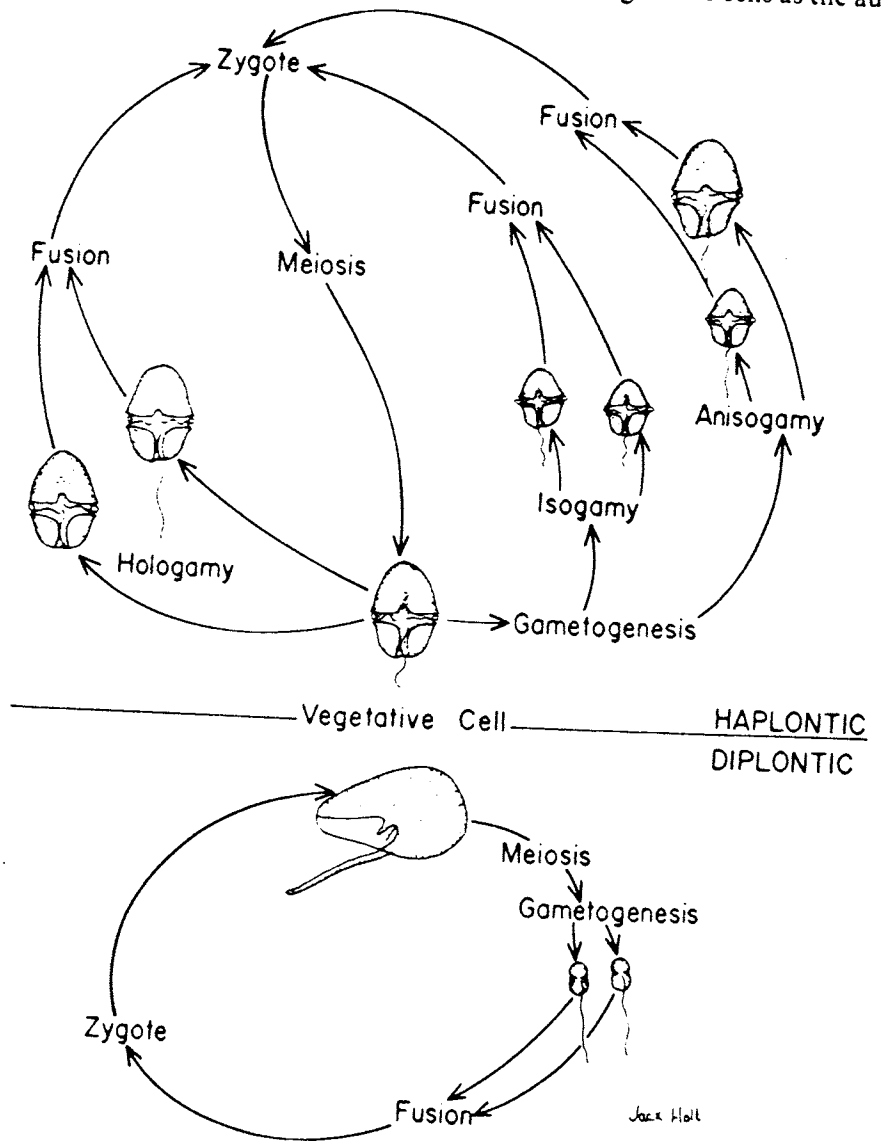

Fig. 14.12. Generalized dinoflagellate life cycle.

thought but are merely stages in a long-lived sexual cycle. Many described taxa may thus be invalid and simply represent a stage in the sexual life history of an already validly described taxon. Such is the case with *Pyrocystis margalefii* Leger and its connection with *Dissodinium pseudolunula* (Drebes 1981). Drebes (1981), in describing resting spores of *D. pseudolunula*, has presented strong evidence to show that *P. margalefii* (described in 1973) is but a description of the resting spores of *D. pseudolunula* and thus an invalid taxon. He further presents information that suggests that *Palaeocystodinium gozlowense* Alberti, might indeed be a fossil form of *D. pseudolunula*. To show further the effects of culturing and life-history studies on dinoflagellate systematics, photographs of *D. pseudolunula*, a marine species, appear similar to stages in the life history of *Cystodinium bataviense* (L. A. Pfiester, unpub. obs.).

Studies of life histories have shown that a number of dinoflagellates, e.g. *Symbiodinium microadriaticum* (Freudenthal 1962), have gymnodinoid stages in their life cycles. However, the motile stage appears to be transitory in the life history of *S. microadriaticum* while it is the dominant phase in the life history of *Gymnodinium* species, and division occurs in the cyst stage in the former and the motile phase of the latter. Thus, on the basis of life-history studies Freudenthal (1962) placed *Symbiodinium* in the family order Dinococcales, family Blastodiniaceae, recognizing the transient nature of the motile stages. D. Taylor (1971a,b) however, has renamed it *Gymnodinium microadriaticum* on the basis of the morphology of the motile phase. Thus, once again systematists must in some way weigh characters in classifying living organisms (see also the taxonomic appendix here).

The more characters a systematist can analyse in classifying an organism the more valid that classification should be. Thus, the knowledge of sexual life histories gives the systematist a vast array of new morphological information to help probe more accurately into phylogenetic relationships. There is no reason why the whole life cycle should not be taken into consideration for taxonomic purposes (F. Taylor 1979).

5 ENVIRONMENTAL CONTROL

Dinoflagellate life cycles are affected by a variety of environmental factors, with the major effects being changes in asexual growth rates and control of specific stages in sexual reproduction. Since the former topic is covered in another chapter (11), this section will focus primarily on environmental control of sexual reproduction.

5.1 Induction of sexuality

Environmental factors such as day length, temperature, nutrient depletion, light intensity, and dissolved gases have been suggested as possible causes of sexuality

in dinoflagellates (von Stosch 1967). However, many studies conducted to date were designed to demonstrate sexuality for a particular species, with little or no emphasis on underlying mechanisms.

The most common culture manipulation that induces sexuality in autotrophic species is nutrient starvation, and more specifically, nitrogen depletion (Tables 14.3, 14.4). Typically, exponentially growing cells are removed from their growth medium and resuspended in similar medium with no added nitrogen (e.g. Pfiester 1975; Turpin *et al.* 1978). Deletion of this single nutrient has induced sexuality in seven of the fourteen species on which culture manipulations have been attempted. Two studies used simultaneous reductions in both N and P. Sexuality and cyst formation have also been observed in ageing cultures with no manipulations, presumably due to the depletion of one nutrient (Cao Vien 1967a,b; Von Stosch 1973; Pfiester 1976).

Table 14.3. Carbon and nitrogen pools during sexual induction of *G. tamarensis**

	Days after inoculation (batch culture)					
	0	6	7	8	9	12
Inoculum: from f/2 into f/2						
cell conc. (ml^{-1})	85	936		2840		4000 (no zygotes)
external N (μM)	883	686		592		
N cell (pg/cell)	371	653		512		515
C cell (pg/cell)	1505	3284		2510		1920
C:N (by weight)	4·1	5·1		4·9		3·7
Inoculum: from f/2 into f/2 with f/20 N						
cell conc. (ml^{-1})	85		1341		5010	4500 (zygotes present)
external N (μM)	70		15		—	0·2
N cell (pg/cell)	371		519		189	188
C/cell (pg/cell)	1505		2692		1469	2584
C:N (by weight)	4·1		5·2		7·8	13·8

*Data from D. M. Anderson (unpubl.).

The time to gamete formation varies, but induction periods as short as 30 min or as long as 5–7 days have been reported, following the change in external nutrients (Pfiester 1975; Walker & Steidinger 1979). Once gametogenesis has occurred, addition of nutrients may have no effect on sexuality (Zingmark 1970; Von Stosch 1973; Pfiester 1976; Pfiester & Skvarla 1979), or it may cause the gametes to revert to vegetative cells (Von Stosch 1973; Pfiester 1975).

Since the objective of many studies was to document sexuality for a particular species, success with nitrogen starvation often precluded the testing of other nutrients. It would thus be incorrect to conclude from Table 14.4 that nitrogen is the most important nutrient in sexuality. For three species, (*Peridinium willei*, *Scrippsiella trochoidea*, and *Gonyaulax/Protogonyaulax tamarensis*), separate

manipulations of nitrogen and phosphorus induced sexuality (Pfiester 1976; Watanabe 1981; Anderson *et al.* 1984; Anderson & Linquist 1985).

In most of the studies listed in Table 14.4, details of the nutrient depletion are lacking. External nutrient concentrations were not measured, nor were the internal storage pools of the inoculum cultures or the sexually active cells. Significant changes in cellular metabolism are associated with variations in nutrient supply—changes mediated in part by internal storage pools that enable cells to divide for several generations after external nutrients disappear (Fogg 1959). An example of the importance of stored reserves is seen in Table 14.3 for *G. tamarensis*. In cultures grown in nutrient rich f/2 medium (Guillard & Ryther 1962), sexuality does not occur, whereas it is readily induced in the same medium with 90% less nitrogen. The major changes in metabolism are: (i) a rapid increase in N/cell following inoculation (luxury uptake), with only the nitrogen-depleted culture showing a significant decrease thereafter; and (ii) a relatively constant C/N ratio in the presence of excess nutrients, but a sharp increase as external nitrogen disappears.

Similar changes in internal nutrient pools are possible with phosphorus as the limiting nutrient (Anderson *et al.* 1984; Anderson & Lindquist 1985), and also with changes in physical conditions such as temperature or light (Goldman 1977, 1979). In this context, it is noteworthy that Von Stosch (1964) induced sexuality in *Ceratium cornutum* without altering external nutrient levels but instead by decreasing temperature, day length and light intensity. Since three factors were varied concurrently, individual effects remain obscure. In a recent attempt to isolate the effects of low light levels on sexuality, Anderson *et al.* (1984) found that cyst formation of *G. tamarensis* was negligible in nutrient-replete medium, even with a 50% reduction in growth rate due to non-optimum lights. Recent field data suggest that day length is also not a factor in sexuality for this species since sexual stages have been observed during blooms in two estuaries 40 km apart but at times that differed by over 1 month (Anderson *et al.* 1983).

Temperature alone has never been shown to be a factor that induces sexuality directly, but it can alter the process once initiated by nutrient depletion. For example, Anderson *et al.* (1984) showed that encystment of *G. tamarensis* was more sensitive to temperature than was growth rate, with optimal cyst production occurring over a relatively narrow temperature range and no encystment at some temperatures that permitted growth. Apparently some metabolic process unique to gamete formation, fusion, or encystment requires higher temperatures than those that support division of this species.

Another factor recently linked to sexuality is dissolved CO_2. When *S. trochoidea* was stressed with separate N and P depletion, sexuality and cyst production were observed, but the addition of less than 1 μM bicarbonate enhanced the phenomenon significantly (Watanabe *et al.* 1982). Perhaps the change in total carbonate is important because this marine species forms calcitic

Table 14.4. Induction of sexuality

Organism	Method	Comments	References
Amphidinium carterae (marine)	Ageing cultures		Cao Vien 1967
Ceratium cornutum (freshwater)	21–12°C LD 14/10–10/14 High light to low light	More recent data suggests reduced nutrients (N + P together) successful; also that reduced temperature and shorter day each work independently	von Stosch 1965; von Stosch, pers. comm.
Ceratium horridum (freshwater)	Ageing cultures	Produces gametes, but copulations relatively scarce	von Stosch 1972
Crypthecodinium cohnii (marine)	(a) None	(a) Heterotrophic nutrition; spontaneous induction of sexuality	Beam & Himes 1974, 1977
	(b) P + N depletion	(b) Nutrient depletion implicated	Tuttle & Loeblich 1974
Glenodinium lubiniensiforme (freshwater)	Nutrient depletion; ageing cultures	Best results from older, high nutrient cultures at high light intensities	Diwald 1937
Gonyaulax monilata (marine)	N-depletion	P-depletion not tested	Walker & Steidinger 1979
Gonyaulax tamarensis (marine)	(a) N-depletion	(a) Zygotes formed were irregular and warty; unsuccessful transition to resting cyst	Turpin *et al.* 1978
	(b) N-depletion; P-depletion	(b) Viable resting cysts formed	Anderson *et al.*, in press
Gymnodinium fungiforme (marine)	Decrease in concentration of algal food source	Phagotrophic nutrition	Spero & Moree 1981
Gymnodinium pseudopalustre (freshwater)	21–15°C LD 14/10–10/14	More recent data suggest N and P depletion successful; some sexuality in old cultures	von Stosch 1973; von Stosch, pers. comm.
Helgolandinium subglobosum (marine)	Ageing cultures		von Stosch 1972; von Stosch, pers. comm.
Noctiluca scintillans (marine)	None	Phagotrophic nutrition; spontaneous induction of sexuality	Zingmark 1970
Oxyrrhis marina (marine)	Change in food source from *Pyraminonas* to *Dunaliella*	Phagotrophic nutrition	von Stosch 1972; von Stosch, pers. comm.
Peridinium cinctum (freshwater)	N-depletion; P-depletion	Light necessary; other nutrients tested, but N depletion gave optimal response	Pfiester 1975
Peridinium gatunense (freshwater)	N-depletion	Other nutrients tested, but N depletion gave optimal response	Pfiester 1977
Peridinium volzii (freshwater)	N-depletion	Other nutrients tested, but N depletion gave optimal response	Pfiester & Skvarla 1979
Peridinium willei (freshwater)	N-depletion	Other nutrients tested, but N depletion gave optimal response	Pfiester 1976
Polykrikos kofoidi (marine)	None	Phagotrophic nutrition; spontaneous induction of sexuality	Morey-Gaines & Ruse 1980
Scrippsiella trochoidea (= *Peridinium trochoidium*) (marine)	(a) N-depletion; P-depletion; Vitamin and $NaHCO_2$ additions	(a) Encystment induced by either N or P starvation; addition of $NaHCO_3$ beneficial; vitamin additions inhibitory. Light and temperature optima were same for encystment as for maximum vegetative growth.	Watanabe *et al.* 1982
	(b) None	(b) Spontaneous induction of sexuality	Wall *et al.* 1970
Woloszynskia apiculata (freshwater)	Lower light (14 000–2000 lux) Reduced N + P		von Stosch 1973

cysts (Wall *et al.* 1970), but there are other possible effects, including those associated with changes in alkalinity that must be considered and evaluated for other organisms.

For some species, sexuality does not require direct environmental changes, although mostly heterotrophic or phagotrophic species are included in this category thus far. Zingmark (1970) observed gamete fusion and zygote formation in *Noctiluca scintillans* cultures fed on *Dunaliella*. Morey-Gaines & Ruse (1980) reported spontaneous cyst formation in phagotrophic *Polykrikos* cultures, while Beam & Himes (1974, 1980) describe sexuality in heterotrophic *Crypthecodinium cohnii* cultures without manipulation (Tuttle & Loeblich (1974) used N and P depletion to induce sexuality in the same species). Sexuality in a third phagotrophic species, *Gymnodinium fungiforme*, was recently observed following depletion of the algal food source (Spero & Moree 1981).

Experiments by Wall *et al.* (1970) demonstrated that the photosynthetic dinoflagellate *Peridinium trochoideum* (= *Scrippsiella trochoidea*) formed cysts in culture without any apparent stress. Although details of the induction experiments were not given, the authors suggested that for this species, encystment does not occur in response to an adverse environment but is a naturally occurring stage in the life history, favoured rather than inhibited by optimal conditions for vegetative growth. Watanabe *et al.* (1982) also demonstrated encystment in control (nutrient-rich) cultures of this same species, but increased the magnitude of the encysting fraction of the population by using nutrient limitation. Thus, an apparent spontaneous encystment process could be enhanced by nutrient depletion.

Field observations linking sexual stages to environmental parameters are limited, but there is evidence for a succession in encystment of different species throughout the year, especially in temperate regions. West (1909) found a strong correlation between temperature and encystment for four freshwater dinoflagellates, concluding that two species were 'winter forms' that encysted in response to the vernal rise of temperature while the other two were dominant during the summer and encysted during the autumnal temperature decrease. There may also be a difference between tropical and temperate species in this regard (Chapter 11B). Wall & Dale (1968) observed this successional phenomenon for marine species. They reported cysts in the water column during the declining stages of three dinoflagellate blooms, but did not attempt to explain the reasons for the declines. Anderson & Morel (1979), reported cyst formation for natural populations of *G. tamarensis* in the presence of relatively high levels of nitrate ($>5\,\mu M$) and with phosphate consistency above $0{\cdot}1\,\mu M$. It is not known whether such levels are limiting to this organism.

Despite the emphasis on nutrient depletion in experimental studies to date, it seems that a variety of factors could be important in the induction of sexuality in dinoflagellates. In a search for common mechanisms, it is difficult to reconcile reports of sexuality in some species under apparently optimal growth conditions

with the need for nutrient stress in other species. In this respect it is noteworthy that Watanabe *et al.* (1982) observed cyst formation in nutrient-rich control cultures of *Scrippsiella trochoidea* but enhanced the process through nutrient limitation. It seems prudent to emphasize that: (i) all studies to date have been conducted in batch cultures where growth conditions may not be as 'optimal' as they appear; (ii) that no measurements of the physiological status of the cells has been provided; and (iii) that within any culture there may well be some cells growing slower and responding differently to their environment than the bulk of the population. Until these issues are addressed, spontaneous sexuality without an applied stress must remain an unproven hypothesis.

The bulk of the reports of sexuality in dinoflagellates involve changes in growth conditions. One possibility is that gametogenesis occurs when cellular nitrogen or phosphorus drops to a critical or threshold level, either through a change in uptake rate due to low ambient concentrations, or to a change in the relative rates of uptake, storage, and metabolism under environmental stress. In *G. tamarensis* there are indications that when levels fall below the subsistence cell quota of 27 pg cell^{-1} phosphorus, gametes may be induced (Anderson & Lindquist 1985). Since several species can be forced into sexuality by at least two different stresses (N and P starvation) (Pfiester 1976; Watanabe *et al.* 1982; Anderson *et al.* 1984) such a direct link between sexuality and nutrient limitation requires that the pathways for uptake and utilization of nitrogen or phosphorus each include a mechanism for the shift to sexual metabolism.

Alternatively, it is tempting to hypothesize that general conditions resulting in slower growth induce sexuality, without specific emphasis on nutrients. Thus, the control of sexuality would lie at a stage more closely associated with cellular division. Although this has considerable appeal from an ecological standpoint, it is already evident from Table 14.3 that populations in nutrient-rich batch cultures have reduced growth rates as they reach maximum cell concentrations, but are not induced to sexuality. In this case, at least some of the non-nutritional factors that limit growth in the presence of excess nutrients (e.g. pH, self-shading, or excreted metabolites) do not trigger a sexual response.

One hypothesis advanced to explain the induction of gametogenesis in a green alga is consistent with the range of observations described above, but has yet to be tested for dinoflagellates. Cain & Trainor (1976) argued that sexual induction in *Scenedesmus obliquus* was inhibited by nitrogen concentrations above a specific threshold, but readily occurred at lower levels that were apparently not limiting. In other words, no gametes were formed at high nutrient concentrations, but they were plentiful as nutrients dropped below a critical level that seemed sufficient for continued vegetative division.

The key concept here is the 'limiting' concentration of nutrients. The uptake characteristics of cyst-forming dinoflagellates are not yet known, but it is probable that at the optimal temperatures typically used for batch culture

studies, growth rate can exceed the rate of nutrient uptake at seemingly adequate extra-cellular concentrations (Anderson & Lindquist 1985). The cells could thus be dividing at the expense of internal nutrient pools until they reach a threshold cell quota that triggers sexuality. What at first glance appears to be sexuality without stress might in actuality be a response to nutrient limitation caused by the dinoflagellate's relatively inefficient nutrient uptake capability.

It is evident from the foregoing discussion that the links between nutrient uptake, internal storage pools and sexuality are important and complex. Despite the difficulties in growing dinoflagellates in continuous cultures, a detailed understanding of these processes awaits such studies in which the cells can be maintained under physiological and environmental steady state conditions.

In overview, the environmental control of sexuality in many dinoflagellate species is both intuitively appealing and consistent with most laboratory results. Studies on field populations are too few, however, and the laboratory experiments too limited in scope to define the detailed mechanisms. Since spontaneous encystment has been reported (i.e. without any apparent stimulus), internal control such as that from a metabolic clock may be important for some species. However, it is still likely that such a process would be mediated in part by light, temperature or other environmental factors. Resolution of the complex interactions between external nutrients, internal storage pools, and the mediating effects of physical variables such as temperature, salinity or light require more comprehensive studies than have been attempted in the past.

5.2 Encystment and dormancy

Once sexuality is induced and gametes have fused, the product is generally a swimming zygotic cell (planozygote). Cao Vien (1967a, 1968) reported a non-motile cell immediately after fusion in *Amphidinium carteriæ*. In most cases, dinoflagellate planozygotes are large and deeply pigmented, but little is known of their physiology or the factors affecting longevity. In most cases, this cell swims for a variable length of time before becoming a non-motile resting cyst. The swimming stage can be as short as 3–5 days when sexuality leads directly to either division and haploid vegetative cells (e.g. *C. horridum*; von Stosch 1972) or to a very short dormancy in hypnozygote form (*P. gatunense*; Pfiester 1977). When a long-lived resting cyst or hypnozygote is the result, the planozygote swims from 1 to 3 weeks (Von Stosch 1973; Pfiester 1975, 1976, 1977; Walker & Steidinger 1979; Anderson *et al.* 1984). The reasons for this prolonged swimming phase without division are unknown but presumably relate to the metabolic changes associated with transition from active photosynthesis and nutrient uptake to prolonged dormancy. No experiments have examined the effects of environmental variations on this life cycle stage.

It should be emphasized that not every resting cyst need be sexual. H. A. Von Stosch (pers. comm.) reports male haploid 'parthenozygotes' in *Ceratium*

cornutum cultures, and Happach-Kasan (1980) describes vegetative cysts in *Ceratium hirundinella*.

It is important to define the terminology of dormancy carefully, recognizing that much of the dinoflagellate cyst literature is less rigorous. Borrowing from the terminology used for seeds of higher plants (Jann & Amen 1977), 'dormancy' is considered the suspension of growth by active endogenous inhibition, and 'quiescence' as the suspension of growth by unfavourable environmental conditions. Thus, it is not possible to germinate a dormant cyst until a mandatory resting period is completed. Similarly, a quiescent cyst cannot germinate until an applied external constraint (such as cold temperature) is removed.

Once a resting cyst is formed, the length of dormancy is highly variable (Table 14.5). In *P. gatunense*, the hypnozygote can excyst within 12 hours of formation (Pfiester 1977). In most other species, the mandatory dormancy period lasts from several weeks to 6 months, during which germination is either not possible under optimal conditions or occurs at a very low rate (Wall & Dale 1968; Dale *et al.* 1978; Anderson 1980). The effect of temperature on this process can be significant. For *G. tamarensis*, the first germination of new cysts was possible after 1·5 months of storage at 22°C or after 6 months at 5°C (Anderson 1980). In contrast, maturation of *S. trochoidea* cysts is equally long (3 weeks) at high and low temperatures (B. J. Binder, pers. comm.).

The factors that initiate excystment are obscure since previous studies have emphasized the process of sexuality rather than the underlying mechanisms. When cysts of several freshwater dinoflagellate species were stored continuously under normal culture conditions, spontaneous germination occurred after 2–4 months (Von Stosch 1973; Pfiester 1975, 1976, 1977; Pfiester & Skvarla 1979). This type of storage has not been tested for other species, so the generalization that excystment simply occurs without stimulus if suitable conditions are continuously maintained is not yet justified.

Several species have been stored at cold (4–5°C) or high (22°C) temperatures and tested for germination by raising or lowering the temperature thereafter (*P. cinctum* and *C. hirundinella* (Huber & Nipkow 1922, 1923); *C. cornutum* (Von Stosch 1965); *G. pseudopalustre* and *W. apiculata* (Von Stosch 1973); *G. tamarensis* (Anderson & Wall 1978; Anderson 1980). One generalization holds for all but one of the species tested: storage of cysts at low temperatures maintains quiescence until the temperature is increased. One exception is *C. cornutum* which, after a mandatory resting period, excysted spontaneously at 4°C in a laboratory refrigerator (H. A. von Stosch, pers comm.).

Long-term survival is possible at low temperatures. Huber & Nipkow (1922, 1923) found that constant exposure to cold temperatures in deeper sections of Lake Zurich suppressed cyst germination of *P. cinctum* for as long as 16·5 years. The only species tested at high temperatures (22°C) was *G. tamarensis*, which remained quiescent for 1 year as storage conditions were held constant (Anderson & Morell 1979; Anderson 1980).

Table 14.5. Dormancy and excystment

Organism	Method	Comments	References
Ceratium cornutum (freshwater)	Cold storage (0–4°C)	Spontaneous excystment; even in cold; laboratory cultures	von Stosch 1965; von Stosch, pers. comm.
Ceratium hirundinella (freshwater)	Temperature change (0 to 4–7°C successful, but 0 to 20°C optimal)	Excystment of planktonic cysts retarded for 1½ months, optimal after 7·5 months; excystment rate of cysts from sediments accelerated by high temperature; older cysts took longer to excyst; no effect from water type, potassium, salinity or light; excystment retarded by 5% glucose or various qualities of monochromatic light; cysts killed by freezing or drying.	Huber & Nipkow 1922, 1923; Morey-Gaines & Ruse 1980
Gonyaulax tamarensis (marine)	Temperature increase (4–15°C) Temperature decrease	Cysts from sediments were sonicated prior to testing; no effect from nutrients, or light; constant low or high temperature maintains dormancy; intermediate temperature not tested; mandatory dormancy 1–6 months, depending on storage temperature.	Anderson 1980; Anderson & Wall 1978; Anderson & Morell 1979
Gymnodinium pseudopalustre (freshwater)	No change from normal culture conditions; or storage at 3°C followed by higher light, temperature	Some spontaneous excystment after several weeks; more complete and better synchronized encystment if cold conditioned; laboratory cultures.	von Stosch 1973
Noctiluca scintillans (marine)	Normal culture conditions	Zygotes remain non-motile for several days without visual development, then enlarge; diplontic life history.	Zingmark 1970
Peridinium cinctum (freshwater)	No change from normal culture conditions; also tested storage at 4°C then increase to 20°C	Spontaneous excystment after 7–8 weeks with constant 20°C; no germination at 4°C for 5 months; laboratory cultures.	Pfiester 1975
Peridinium gatunense (freshwater)	No change from normal culture conditions	Spontaneous excystment after 12 h; laboratory cultures.	Pfiester 1977
Peridinium volzii (freshwater)	No change from normal culture conditions	Spontaneous excystment after 4 months; laboratory cultures.	Pfiester & Skvarla 1979
Peridinium willei (freshwater)	No change from normal culture conditions	Spontaneous excystment after 7–8 weeks; laboratory cultures.	Pfiester 1976
Woloszynskia apiculata (freshwater)	No change from normal culture conditions; also tested cold storage at 6°C	Some spontaneous excystment, but more complete and better synchronized with cold storage	von Stosch 1973

As demonstrated by Von Stosch (1973), cysts of some species are capable of limiting germination several weeks after formation, without an external stimulus but storage in the cold results in more complete and better synchronized excystment when warmer temperatures are restored. This emphasizes another common characteristic: increasing temperatures above cold storage levels breaks quiescence. In a similar manner, *G. tamarensis* cysts stored at high temperatures excyst if the temperature is lowered into an optimum range (Anderson & Morell, 1979, Anderson 1980). This is consistent with the existence of a temperature 'window' for excystment. High or low temperatures maintain quiescence, but once the temperature drops or rises into the permissive range, germination occurs.

Temperature is thus very important throughout dormancy. It can maintain quiescence for extended periods, determine the duration of dormancy after cyst formation, synchronize or entrain cyst populations for more uniform germination, and initiate the excystment process.

The effects of other environmental factors on dormancy and excystment are poorly understood. The first and most comprehensive study was that of Huber & Nipkow (1922, 1923) on *C. hirundinella* cysts from lake sediments. They found that manipulation of growth medium (well water, distilled water, filtered lake water, dilutions of KNO_3, Knop's and Kleb's artificial media, weakly saline medium) and incubation in the dark had little effect on the germination of cysts. A 5% glucose solution (as a non-electrolyte) and various qualities of monochromatic light (green, blue and red) retarded germination. Freezing and drying killed the cysts. These researchers also noted the adverse effect of non-optimum temperatures on the viability and shape of the germinated cells.

Another study examined the effects of light, nutrients, and trace metals on germination of *G. tamarensis* cysts (Anderson & Wall 1978). Unlike Huber & Nipkow (1922, 1923) who placed small quantities of sediment directly into culture medium, experiments with *G. tamarensis* used cysts isolated by micropipette from sediment samples after sonication and size fractionation (Wall & Dale 1968). These methods may have introduced artefacts associated with sonication, bacterial regeneration of nutrients, and light exposure during isolation. Laboratory incubation at 16°C after storage for 6 months at 5°C initiated excystment with no appreciable effect from light regime, nutrient concentration, or even toxic metal concentrations. Motility and viability of the excysted germlings, however, required highly chelated medium and light. In general, once initiated by the temperature increase, excystment proceeded through emergence regardless of the suitability of the ambient environment.

In comparing these various laboratory studies with the few field observations that have been made on cyst populations, one discrepancy is noteworthy. For certain species, between 80 and 100% of the cysts isolated in the laboratory will germinate during incubation at higher temperature (Von Stosch 1973, Anderson & Wall 1978, Anderson & Morell 1979). Examination of sediments during

blooms when temperatures have risen significantly reveals an abundance of viable cysts which will excyst rapidly when brought to the laboratory (Anderson & Morell 1979). It seems clear that there are factors that can override the temperature stimulus after overwintering and prevent excystment (e.g. anoxia), and/or that laboratory incubations are not adequate simulations of natural conditions. Here again the resolution of this important issue awaits further study.

There are also environmental factors that influence sexuality in dinoflagellates by operating indirectly on the behaviour or physical location of the organisms. One example is light, which induces either positive or negative photoactic movement (see Chapter 10) and thus acts to concentrate a population in dense accumulations at specific depths. On theoretical grounds, this could facilitate gamete pairing and may even contribute to the rapid decrease of external nutrients. Gametes of *W. apiculata* are negatively photoactic (Von Stosch 1973), and thus would accumulate away from a light source, while gametes of the other species that were examined exhibit the same positive photoaxis as vegetative cells and accumulate near the highest intensity (H. A. Von Stosch, pers. comm.).

A variety of hydrographic or wind-related features also accumulate dinoflagellates in surface or subsurface patches. An example of the importance of these mechanisms on a cyst-forming dinoflagellate is seen for *Gyrodinium uncatenum* in the Potomac River. In this system, a persistent estuarine front serves to concentrate and recirculate the motile population in downstream-flowing surface waters and upstream-flowing bottom waters (Tyler *et al.* 1982). Sexual stages also accumulate within this 'conveyor belt', and the resulting cysts accumulate in highest numbers along the subsurface transport pathway. Clearly these frontal regions and convergence zones are areas of increased sexual activity.

If we examine the accumulated information on environmental control of sexuality in dinoflagellates, it is clear that generalizations are not readily apparent. This is due in part to the paucity of specific experimental data and partly to the difference between species. Sexuality is not a simple, automatic response to adverse environmental conditions. It involves complex interaction between physical or chemical parameters and the metabolism of the organisms. Conceivably there are a variety of combinations of these factors that produce the physiological state that dictates the shift into sexual metabolism. Thus, low temperatures with high nutrient concentrations may have the same effect as the combination of high temperatures and low nutrients. Variations in these interactions at the species level can further explain the temporal distribution and succession of co-occurring organisms.

The environmental control of dormancy and excystment is equally complex. Temperature seems to be the most obvious controlling parameter, but it is clear that other factors contribute to the timing and magnitude of germination as

well. The existence of living dinoflagellate species whose cysts can be found in early Tertiary sediments (Wall & Dale 1970) or earlier, is strong evidence of the effectiveness of sexuality and cyst formation in responding to short- and long-term environmental fluctuations.

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