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## The roles of resting spores and akinetes in chlorophyte survival

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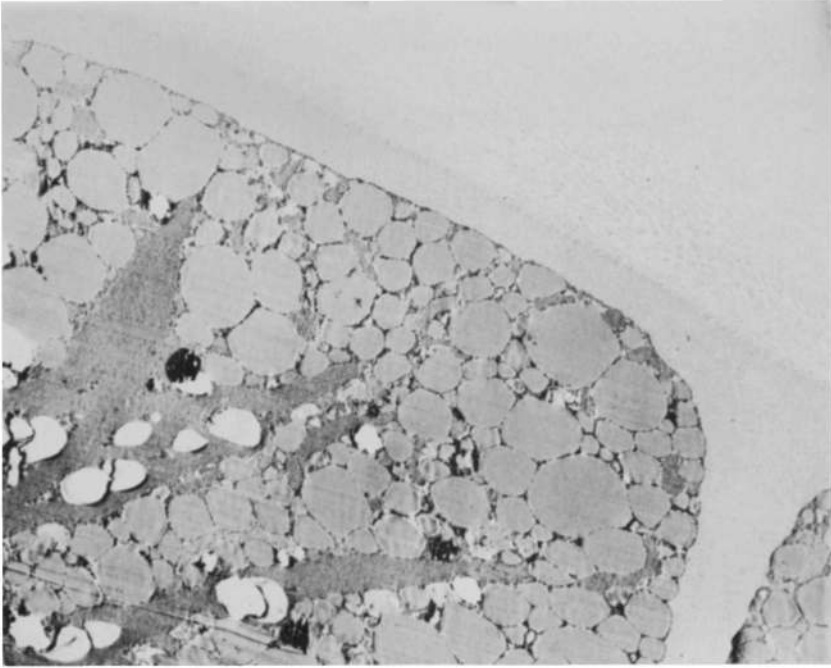
The majority of the green algae are freshwater or terrestrial organisms. They are subject to the same environmental stresses as their marine cousins: competition for nutrients, escape from poisons and predators, and fluctuations in light and temperature. The overriding problem in their survival and dissemination, however, is water loss. It is not surprising, then, that specialized thick-walled cells of one sort or another occur, not just in the majority, but in the preponderance of the freshwater genera. Among marine forms such cells are almost unknown. In fact, among the predominantly marine orders of Chlorophytes, *Dichotomosi-phon* is the only genus known to form akinetes, and it is an inhabitant of freshwater. This chapter concentrates on the thick-walled cell, its nature, and its roles in both short-term and long-term survival of freshwater Chlorophyta.

### 1.1 Thick-walled cell = resting cell = resistant cell

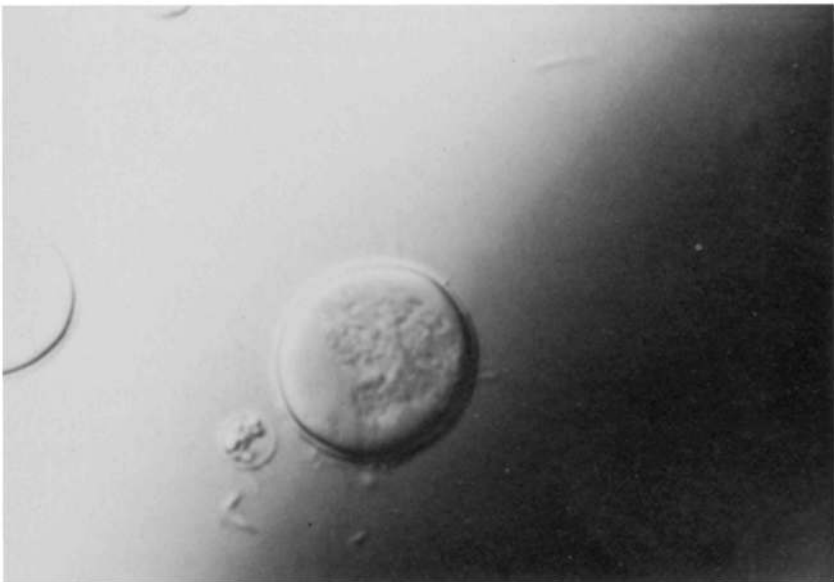
In classical terminology there are two major types of specialized thick-walled cells among the algae. One, the *akinetete*, is a modified vegetative cell in which the wall continues to thicken far more than in metabolically active cells and may also incorporate additional kinds of wall materials. The point of emphasis is that the original cell wall is incorporated into and forms the basis of the final thick wall (Fritsch, 1945). Classic examples are the akinetes of *Pithophora*, *Zygnema* (Fig. 1), *Tetraspora*, *Pedias-trum*, and *Spongiochloris*.

The second kind of thick-walled cell is represented by the *hypnospor*e and *hypnozygote*, both being protoplasts that have separated from their parental wall and participated in some further activity. This may only be formation of a sedentary zoospore or it may involve gametogenesis and

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**Fig. 1.** Electron micrograph of a portion of an akinete of *Zygnema* showing thickened wall and lipid accumulation in the cell interior. Plastid lamellae are no longer distinct. From McLean and Pessoney (1971).



**Fig. 2.** Living zygospore of *Pandorina* as seen in optical section with Nomarski optics. Large homogeneous-appearing region is lipoidal material.



Fig. 3. Light micrograph of a living *Oedogonium* zygote. From Hoffman (1965).

fusion of gametes; these cells then secrete a new, very heavy and usually specially decorated wall. Examples of this behavior include the hypno-spores of *Chlorococcum hypnosporum* Starr and *Ulothrix fimbriata* Bold, and the thick-walled zygotes of the Volvocales (Fig. 2), Oedogoniales (Fig. 3), Zygnematales (Figs. 4, 5), and Chlorococcales.

More difficult to define and identify are those usually small, relatively thick-walled vegetative cells imbedded in a heavy gelatinous matrix that must contribute heavily to the survival of their species. In many cases neither specialized akinetes nor hypno-spores are known for the species, and yet viable cells can be isolated from air currents or from dried material. Mattox (1971) describes relatively unspecialized cells in *Klebsormidium flaccidum* (Kützing) Silva, Mattox, et Blackwell that resist temperatures up to 100°C, at least briefly, and remain viable after air drying for more than 2 years. Proctor (1966) found that vegetative cells of some desmids could survive passage through the alimentary canal of waterfowl. These are exceptional cases, however, for the vast majority of vegetative cells are far more susceptible to environmental stress than are the specialized thick-walled cells in their life cycles.

The records of akinete and hypnozygote survival are very impressive, but limited so far, primarily by lack of older material to examine. Hoshaw's collection of Zygnematacean zygotes is now more than 20 years old and still viable, as are Hoffman's stocks of *Oedogonium foveolatum* Wittrock (personal communication). Coleman's *Pandorina* zygote collection germinates abundantly after 24 years. Other examples of long-lived zygospores and akinetes stored in the laboratory and remaining viable for periods of years are cited in Coleman (1975). Clear-cut records for field survival are much more difficult to obtain, but Pessoney (1968) suggests that akinetes are adequate for survival capacity in *Zygnema*, whereas zygospores, rather than akinetes, have greater survival capacity

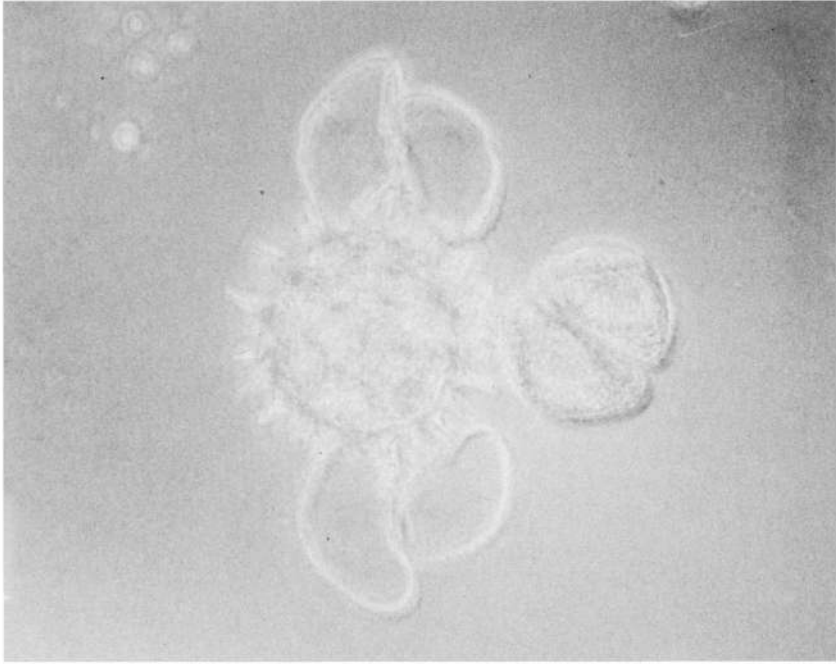


Fig. 4. Zygote, empty vegetative cell walls, and one vegetative cell of *Cosmarium*.

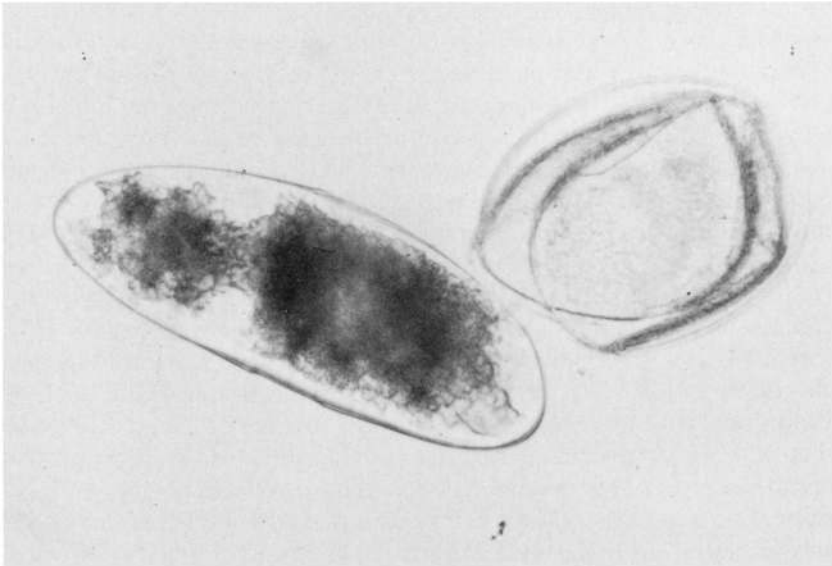


Fig. 5. Germling cell with empty zygospore wall of *Sirogonium*, illustrating the two wall layers left behind at germination. From Hoshaw (1968).

in *Spirogyra*. In a direct study, Lembi et al. (1980) germinated *Pithophora* akinetes cored from a depth of 15 cm below an Indiana lake, a depth that suggests a deposition time of 2–3 years. These reports help to confirm the commonly held presumptions concerning the efficacy of thick-walled spores but also indicate the paucity of direct experimental work in the field. Coring soil samples from recently created lakes, burying identifiable spores or vegetative cells in containers open to the subterranean environment, and even inoculation of single clones or compatible pairs of clones identifiable by mating type into experimental ponds for later recovery (Coleman, 1973) are all possible experimental approaches that would aid our understanding of the importance of thick-walled cells to population survival.

### 1.2 Dispersal of algae

The groups in which “vegetative cells” contribute heavily to survival under extreme conditions may be more obvious from an examination of the sources of colonizing algae. Over the short term of just a season or a few years, survival is a question of invading new habitats and establishing and maintaining the population from year to year. Geological and climatological events, not to speak of human activities, constantly alter the surface of the earth, destroying old niches as well as creating new ones. No open body of freshwater remains devoid of algae for much longer than the first gust of wind. Algae lurking in the soil, brought in by flood waters or conveyed by animals, including birds, insects, and even raccoons (Bassett, 1963), rapidly contribute to a diverse population.

Although no one has succeeded yet in identifying the precise sources of inocula for a body of water in nature, there has been extensive sampling of viable algae found floating in the atmosphere, on the presumption that atmospheric disturbances, particularly catastrophic ones, could provide adequate inocula of either soil or planktonic forms. Results of three of these studies (Brown et al., 1964; Schlichting, 1964; Brown, 1971) are tabulated in columns A, B, and C of Table 1, along with two results of soil samplings (Holm-Hansen, 1964; MacEntee et al., 1972) in columns E and G, a list of the phytoplankton genera present in more than 10% of water samples taken in the eastern United States (Taylor et al., 1979) in column F, and in column D, a composite listing of those Chlorophyta most tolerant of polluted waters, genera gleaned from more than 100 reports (Palmer, 1969). The genera are listed by orders according to the taxonomic treatment of Bourrelly (1966). Handling such lists by generic name alone begs many questions concerning species diversity and variation, but there is no alternative at present. Similarly, one must recognize that each author who cultivated samples used but a single medium and set of culture conditions, which are predominantly biased

Table 1. *Aerial, terrestrial, and aquatic sampling of chlorophytes*

	A	B	C	D	E	F	G
Chaetophorales							
				<i>Stigeoclonium</i>	+		
Cladophorales							
				<i>Cladophora</i>	+		
Chlorococcales							
				<i>Actinastrum</i>	+	+	
			+	<i>Ankistrodesmus</i>	+	+	
		+	+	<i>Bracteacoccus</i> -like			+
	+	+	+	<i>Characium</i>			+
	+	+	+	<i>Chlorella</i>	+	+	+
	+	+	+	<i>Chlorococcum</i>	+	+	+
	+			<i>Coelastrum</i>	+	+	
	+			<i>Crucigenia</i>	+	+	
	+			<i>Dictyochloris</i>			
				<i>Dictyococcus</i>			+
				<i>Dictyosphaerium</i>	+	+	
				<i>Golenkinia</i>	+	+	
				<i>Kentrosphaera</i>		+	
				<i>Kirchneriella</i>		+	
				<i>Lagerheimia</i>		+	
				<i>Micractinium</i>	+		
	+	+	+	<i>Neochloris</i> -like			+
				<i>Neosporangiococcum</i>			+
	+			<i>Oocystis</i>	+	+	+
	+			<i>Palmellococcus</i>			
				<i>Pediastrum</i>	+	+	
	+			<i>Planktosphaeria</i>			+
	+		+	<i>Protosiphon</i>			+
	+			<i>Radiococcus</i>			+
	+			<i>Radiosphaera</i>			+
	+		+	<i>Scenedesmus</i>	+	+	
				<i>Schroederia</i>		+	
				<i>Selenastrum</i>	+		
	+		+	<i>Spongiocloris</i> -like			+
	+			<i>Spongiococcum</i>			
			+	<i>Tetraedron</i>	+	+	+
	+			<i>Trebouxia</i>			+
			+	<i>Treubaria</i> -like		+	
	+			<i>Westella</i>			
Chlorosarcinales							
	+			<i>Borodinella</i> and <i>Borodinellopsis</i>			+
	+			<i>Chlorosarcina</i>		+	
	+			<i>Chlorosarcinopsis</i>			+
	+			<i>Chlorosphaeropsis</i>			
				<i>Coccomyxa</i>		+	
	+			<i>Friedmannia</i>			
	+		+	<i>Nannochloris</i>			
	+			<i>Ourococcus</i>			

	A	B	C		D	E	F	G
				<i>Tetracystis–</i> <i>Pseudotetracystis</i>				+
Oedognoiales	+	+						
			+	<i>Oedogonium</i>				
Siphonocladales								
			+	<i>Rhizoclonium</i>				
Tetrasporales								
			+	<i>Asterococcus</i> -like				
				<i>Gloeococcus</i>				+
			+	<i>Gloeocystis</i>				+
	+			<i>Hormotilopsis</i>				
	+		+	<i>Palmella</i> -like				+
				<i>Sphaerello cystis</i>				+
			+	<i>Sphaerocystis</i>				
Ultrichales	+			<i>Tetraspora</i>				
				<i>Binuclearia</i>		+		
	+		+	<i>Klebsormidium</i>				+
			+	<i>Microspora</i>				
	+			<i>Pleurastrum</i>				
	+		+	<i>Pleurococcus–</i> <i>Protococcus</i>				+
				<i>Pseudulvella</i> -like				
				<i>Raphidonema</i>		+		
	+			<i>Stichococcus</i>		+		+
Ulvaes	+		+	<i>Ulothrix</i>	+		+	
			+	<i>Prasiola</i> -like				
Volvocales								
	+	+	+	<i>Chlamydomonas</i>	+	+	+	+
				<i>Chlorogonium</i>	+		+	
				<i>Eudorina</i>	+			
				<i>Gonium</i>	+			+
				<i>Pandorina</i>	+		+	
			+	<i>Pleodorina</i>				
				<i>Pyrobotrys</i>	+			
Zygnematales				<i>Spondylomorom</i>	+			
				<i>Closterium</i>	+		+	
	+			<i>Cosmarium</i>	+		+	
	+			<i>Cylindrocystis</i>				
				<i>Euastrum</i>			+	
				<i>Penium</i>				+
	+			<i>Roya</i>				
				<i>Spirogyra</i>	+			
				<i>Staurastrum</i>			+	

Note: Air samples: A, B, and C. Water samples: D and F. Soil samples: E and G.  
 Sources: A, Brown et al. (1964); B, Brown (1971); C, Schlichting (1964); D,  
 Palmer (1969); E, Holm-Hansen (1964); F, Taylor et al. (1979); G, MacEntee et  
 al. (1972). Asterisks mark genera present in more than five samples.

toward the cultivation of common green algae. Nevertheless, the table offers some interesting comparisons.

The five genera of airborne algae in column B are all also represented in column A and, with one exception, in column C, suggesting that air sampling is remarkably repeatable, and that Michigan, Texas, and Hawaii samples are not particularly different. Half the genera in column C are also listed in column A. Even more striking is the fact that only 8 of the 28 algae found in dry forest soils (column G) are not listed among the air samplings. This suggests that viable soil algal cells are continuously introduced into the air. By contrast, 13 of the 20 most common components of the phytoplankton are not mentioned among either the airborne or the dry soil algae; for most of these, one might merely assume that their numbers were not sufficient to be detected, that is, they exhibit no unique characteristics.

Among the seven most common phytoplankton genera also trapped in air samples, four are listed only among the airborne algae, and the other three, *Oocystis*, *Tetraedron*, and *Chlamydomonas*, are common to air, soil, and water samples. In view of the number of species in the last-named genus, and their frequent production of heavy-walled resting cells, it comes as no surprise to find *Chlamydomonas* among the most commonly sampled algae. However, the tolerance of *Oocystis* and *Tetraedron* to desiccation during air transport must result, at least in part, from their heavy gelatinous sheaths.

Finally, all but six of the common phytoplankton genera (column F) are also listed among the 27 genera tolerant of polluted waters (column D). Perhaps the simplest interpretation would be that the physiological variation within genera, if not species, may be so great as to make pointless the search for genera that can serve as an index to pollution.

More to the point of our inquiry, only 2 of the 10 most common atmospheric algae are known to have heavy-walled cells in their life cycles, and only 6 out of the 14 most common soil forms. One must conclude that, at least for such small-celled, matrix-imbedded genera, the classic hypnospores and akinetes are not required for short-term survival in stressful situations. In this context, such a conclusion seems valid even if thick-walled cell stages exist of which we are not yet aware (Trainor & Burg, 1965), for they must be relatively rare. Thus, the most frequently transported organisms are not those with unique thick-walled akinetes or spores; they are merely the most abundant algae in soil and water. For these small-celled, gelatinous populations, abundance in numbers may play the same role in dissemination and maintenance of the species that akinetes and hypnospores play for the less-gelatinous forms, or for those algae with larger cells, particularly those with central vacuoles. Without exception, the genera listed in Table 1 that have vegetative cells over 15  $\mu\text{m}$  in any dimension are also known to be able to form specialized thick-walled cells.



### 1.3 Stable populations of algae

It might be assumed, from the many genera in Table 1, that the algal population of a continent is in a continual whirl, constantly being depleted at a site and being replenished by new immigrants. For several groups, however, there is evidence to suggest that this is not so. Many investigators have their favorite *Spirogyra* pond or some constant yearly source of a particular algal form. That these may represent the same genetic population is clear from the report by Proctor (1975) of recollecting the same unusual *Chara* from an isolated pond in South America that had been described decades earlier, from Biebel's (1973) report on the extremely rare species *Mesotaenium dodekahedron* Geitler, and from the recapture of identical mating types of *Pandorina morum* in the same ponds 18 and 19 years after the original study had been made (Coleman, 1977). This latter study and the one by Stein and McCauley (1976) show that even rather small ponds may have more than one population of a single species, a fact that would not be recognized on morphological grounds alone. Additional identifying characters are necessary to distinguish genetically isolated populations of the same species. Thus, we find that opportunities for immigration are frequent but also that unique and identifiable populations can inhabit the same pond for decades. There seems no basis on which to decide whether the most common algae are good migrators or good adaptors.

Among the orders represented in Table 1, only the Volvocales are outstandingly common in polluted environments, although relatively poorly represented in aerial samples, dry land samples, or even the plankton in general. They also are the only nonfilamentous order in which the majority of genera are known to have hypnospores, hypnozygotes, or akinetes. Particularly here, the thick-walled cell may play a major role in the spread and maintenance of populations. In fact, there are examples of successful laboratory intercrosses between organisms from geographically isolated sites for six species of this order (cited in Coleman, 1977). Although we know now that many morphological species are not cosmopolitan in their hybridization potential, some genetically compatible subpopulations of species may approach being worldwide in distribution.

### 1.4 Thick-walled cell formation as a developmental pathway

It is well to emphasize that the developmental pathway leading to the production of thick-walled cells in Chlorophytes is not primarily reproductive, in the sense of producing many new organisms. The thick-walled cells, akinetes, hypnospores, or hypnozygotes, are all formed in response to conditions of adequate photosynthesis but limiting nitrogen. Experimental manipulation of sporulation has been documented strik-

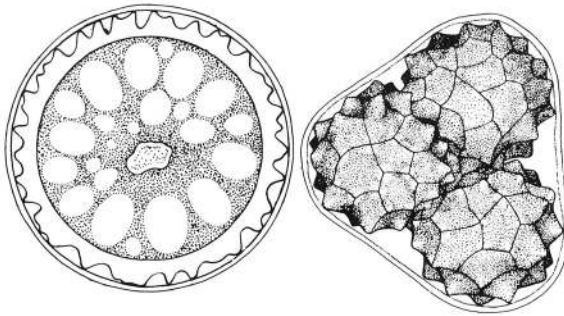


Fig. 6. Group of hypnospores of *Chlorococcum hypnosporum* and median optical section of such a hypnosporangium. From Starr (1955a).

ingly for akinetes of *Spongiocloris* by McLean (1968), for akinetes and hypnozygotes of Zygnematales by Hoshaw (1968), and for the hypnozygotes of *Chlamydomonas* by Sager and Granick (1954). Similarly, drying of algal mats promotes akinete formation in *Pithophora* (Lembi et al., 1980). Reviews and further citations of methods of inducing production and germination of resistant cells can be found in Coleman (1962), Erben (1962), and Dring (1974). Sager and Granick (1954) pointed out that the same stimulus is recognized by the spore-forming bacteria and that in both cases, resistant spores form just as population numbers peak. Unfortunately, the more specific biochemical nature of the trigger mechanism eludes us, so that we continue to describe the physiological manipulations in terms similar to those used by Klebs (1896) in his classic description of research on algal reproduction.

A factor that has lent confusion to investigations of the physiology of resting cell formation may be the concentration of research on organisms like *Chlamydomonas* that combine sexual reproduction with resting cell formation, the result being the hypnozygote. This type of life cycle, which is the most common among the freshwater Chlorophyta, can be contrasted with that of *Cladophora* or *Ulothrix*: These also make thick-walled cells (akinetes) as the environment becomes less hospitable for growth, but their reproductive activity occurs at a physiologically quite different portion of the life cycle, at the time when signals of a fresh growth period appear in their environment or when light, but not nitrogen, may be limiting.

The notion that resting cell formation represents a unique and separable developmental pathway can be inferred not only from the variations in life cycles of different genera but also experimentally. In *Chlamydomonas* (Ebersold, 1967), diploid cells can be obtained by nutritional selection methods. The cells are the product of normal gamete pairing and fusion but then omit the resting spore stage and its usual outcome, meiosis at zygote germination. Instead, such cells remain vegetative and