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Extraordinary Dinoflagellates: Past and Present

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ABSTRACT

This paper highlights the astonishing diversity of both form and function in a group of protists that were once obscure, but which now attract considerable attention from cell biologists, evolutionists, ecologists, aquaculturists and even public health authorities. They surprise and confuse all those who learn about them. For example, having only 50% of the species photosynthetic, they have been claimed to be plants (algae) by botanists and animals (protozoa) by zoologists and were separately classified as such, but both were wrong: they are flagellated protists belonging to the Alveolates and evolutionarily diverged in the Neoproterozoic, long before true animals and plants. However, they left little trace until the Mesozoic when, according to the record left by their highly resistant resting cysts, they radiated rapidly. The most likely explanation for this is the development of shallow continental shelves. Unlike many other groups they show little signs of extinction at the Cretaceous/Tertiary boundary, most major dinoflagellate lineages having persisted through more than 100 million years of Earth's history. As zooxanthellae they have contributed significantly to the success of reef-building corals.

Present-day dinoflagellates are unusual in many ways. Their chromosomes are not constructed like all other eukaryotes, lacking histones. The nuclear envelope does not break down during mitosis and the spindle is extranuclear. They have multiple chloroplast types. The genome of the commonest type has fragmented into minicircles and only two genes have been identified in the mitochondrial genome. Their morphology is astonishingly diverse, ranging from unicellular to multicellular and even coenocytic. The heterotrophic forms have various, complex feeding mechanisms and even the photosynthetic ones may engulf prey (mixotrophy). They show remarkable examples of form mimicking multicellular organisms, such as resemblances to tapeworms, jellyfish, nematocysts and even eyes. They can be beneficial symbionts or deadly parasites. More than 70 species can produce deadly toxins which can kill marine life or, if concentrated by shellfish or fish, can kill humans.

Keywords: Dinoflagellate, Protist, Alveolate, Evolution, Diversity, Fossil, Cysts

INTRODUCTION

Dinoflagellates are flagellated protists belonging

to the Alveolata, a well defined group characterized by the presence of a single layer of vesicles, termed amphiesmal vesicles in the case of dinoflagellates,

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or cortical alveoli, lying just beneath the cell membrane [1]. Structurally their outer layers most resemble ciliates, with numerous alveoli and rod-like trichocysts [2]. Molecular phylogenetics [3, 4] indicates a closer relationship to the sporozoans (Apicomplexa). They are the only group of the alveolates to have photosynthetic members, although this applies to only half of them, causing much confusion in the early days when all protists were assigned to either animals or plants. With the breakdown of this artificiality [5] their relationships have become much more clearly resolved. The discovery of vestigial plastids in their sporozoan sister-group [6] raised the possibility that the two may have had a common photosynthetic ancestry [7], but to this author the general organisation of dinoflagellates seem to be primordially heterotrophic [8]. Like euglenoids, those with plastids often retain a phagotrophic capability. Also, those genera that are basal to the dinoflagellates *sensu stricto* (*Oxyrrhis*, the perkinsids), dubbed the "predinoflagellates" by us, are all non-photosynthetic [9]. The "Chromalveolate hypothesis" of Cavalier-Smith [10] places a photosynthetic ancestry even earlier, at the branch between the Chromists and the Alveolates as a single secondary symbiotic event. This would require losses of plastids not only in the predinoflagellates but also in the deeper branching ciliates (which so far have no trace of plastids), a highly unparsimonious proposal [8]. There is good evidence for a common origin of the "red type" plastids of chromists and dinoflagellates [11], with xanthophyll differences arising later, but the question of single or multiple symbiotic events is open [12] and multiple symbiotic events are evident even within one group: the dinoflagellates (see below).

More than 2000 living, and 2500 fossil species, have been described. The ratio of photosynthetic to non-photosynthetic living species is almost 1:1. They are found in both marine and fresh waters, and can be benthic and planktonic. The great majority are marine planktonic species. The biology of the group has been reviewed by Spector [13] and Taylor [14], and a unified classification of fossil and recent dinoflagellates was first published by Fensome et al. [15].

THE PRESENT: MORPHOLOGICAL DIVERSITY

The typical dinoflagellate is a biflagellated, eukaryotic unicell. Fensome et al. [15] used the distinctive, ribbon-like transverse flagellum [16] as the

defining character of the Dinoflagellata *sensu stricto*, this being more inclusive than the dinokaryon. The general morphology of dinoflagellates has been reviewed by Taylor [17]. Surface grooves are associated with both flagella but the cingulum has been lost in the podolampids and both are absent in procoenocysts. In the more elaborate dinophysoids such as *Ornithocercus*, the edges of these grooves are extended greatly as elaborate "lists" and symbiotic, coccoid cyanobacteria live within the list space and even enclosed in a special chamber formed by the cingular lists e.g. *Histioneis*. The gradients of form within this complex group are almost continuous, suggesting a rapid evolution in which the ancestral forms have survived into the present [18]. A resistant-walled cyst is present in the life-cycle of 10–15% of extant species [19] and it is this stage which fossilizes well. Wall structures are present in the motile cells of many dinoflagellates: usually this consists of multiple cellulose plates constituting the theca which disintegrates on death. However some, such as *Balechina*, have a flexible, continuous wall layer, the pellicle, and this seems to have fossilized in some cases, e.g. *Dinogymnium*. Some dinoflagellates, such as *Pyrocystis*, have adopted the coccoid state as their primary life form, remaining photosynthetically active in this "vegetative cyst" [17]. Another is *Thoracosphaera*, which has added an outer calcite crystal layer greatly resembling a coccolithophorid (and mistaken for one for many years).

Chain formation occurs in a few species and there is a curious link with toxicity in several of these, such as *Alexandrium catenella*, *A. monilatum*, *Pyrodinium bahamense* var. *compressum* and *Gymnodinium catenatum*. Horns and spines are also common in thecate species [17]. Multicellularity is found in some genera, even with differentiation of specialised cells, e.g. the parasite *Haplozoon*, in which the anterior cell used for attachment to the prey has a spike, the stylet. A more well known coenocyte is *Polykrikos*, which resembles a fused chain of individuals, having multiple external units, such as girdles, sulci and pairs of flagella, but only a single internal compartment and half the number of nuclei as external units. It also contains multiple nematocysts that greatly resemble those of cnidarians, including the coiled up tube.

The warnowiaceans possess an extraordinary "eye", the ocellus or ocelliod, complete with a hyaline lens that focuses a beam of light onto a retina-like array of precisely arranged membranes backed by a dark red pigment layer [20]. The ocellus shows amazing structural features that argue for an ability

to focus an image on the surface of the retinoid. This raises the obvious and baffling question as to how subcellular components could “interpret” such an image.

The heterotrophic dinoflagellates have a variety of feeding mechanisms that include phagotrophic engulfment, a feeding tube (the peduncle) and the feeding veil or pallium (found in protoperidinioids and podolampids), which can surround multiple diatom cells in a chain and digest them in situ. There are many parasitic dinoflagellates, the majority belonging to the basal groups: the syndinians and blastodineans [21].

The “zooxanthellae” in all hermatypic corals are dinoflagellates, once thought to all be one species: *Symbiodinium microadriaticum*. Now a diversity of multiple genera and species have been identified in hosts ranging from protists (foraminiferans, polycystine radiolarians and acantharians) to medusae such as *Mastigias* and *Cassiopeia*, to molluscs such as the giant clam *Tridacna*.

UNUSUAL CELL BIOLOGICAL AND BIOCHEMICAL FEATURES

It was the discovery of the unique state of the chromosomes that sparked much interest in the group by cell biologists in the 1970s. The chromosomes of dinoflagellates are permanently condensed, lacking nucleosomes (balls of histones around which the DNA is coiled). Under the electron microscope each chromosome has a dense, fibrillar appearance, the DNA being arranged in banded whorls that are thought to be part of a single circular loop. This organization is sufficiently distinct to be referred to as a dinokaryon. Often very large amounts of DNA are present, between 10 and 100 times more than in other protists (excluding specialized states such as the macronuclei of ciliates) and human cells. Despite this, genetically, the swimming cells (mastigotes, not zoospores as sometimes stated) are haploid. It is commonly agreed that the absence of histones is due to loss at an early but unknown time in the evolution of the group, but histone-like proteins are present in some basal branches, such as the syndinians. Another oddity is the relatively high amount of modified bases, with the unique presence of 5-hydroxymethyluracil [22]. The mechanism of mitosis is also bizarre, being closed with an external spindle, similar to that found in the unrelated hypermastigote flagellates. The mitochondrial cristae are tubular, a feature shared by many other protists, including other alveolates and the chromists. On the

other hand, only two genes, *cob* and *cox1*, have been identified from the mitochondrial DNA so far [23], the rest presumably residing in the nucleus. If these are the only genes in the mitochondrion this would make dinoflagellates the group with the smallest mitochondrial genome (not counting those that have lost mitochondria completely). Also, there is very unusual, extensive substitutional editing of the mitochondrial mRNAs [24]. Plastid DNA is also unusual, in that it occurs in multiple minicircles [25], with only a single gene in each, another unique feature.

The predominant plastid type contains chlorophylls a and c₂ plus the unique accessory pigment peridinin, but the group is notorious for the variety of its plastid types, no other group having had the ability to acquire so many other group plastids secondarily or even tertiarily [12, 26]. In some of these cases the peridinin type seems to have been replaced by a foreign type [26].

Luminescence is relatively common in both photosynthetic and non-photosynthetic taxa and dinoflagellates are the most common sources of coastal luminescence. The former include the genera *Alexandrium*, *Pyrodinium*, *Gonyaulax*, *Lingulodinium*, *Ceratium* and *Pyrocystis* (all gonyaulacoids); the latter are primarily species of *Proto-peridinium* and the famous *Noctiluca*. Interestingly, many strains of the latter in the North Pacific are not luminescent. The light is a blue-white flash, associated with subcellular structures termed scintillons. The luciferin is unique in being an open tetrapyrrole.

The group is notorious for involvement in various types of “harmful algal blooms” (HABs) in both marine and freshwaters. More than 70 species produce toxins. These are primarily small molecular weight guanidium-containing neurotoxins and polyethers of various types. The toxins in the cells can be concentrated by filter-feeding shellfish, producing various types of shellfish poisoning [27], of which paralytic shellfish poisoning (PSP), produced primarily by species of *Alexandrium* and *Pyrodinium*, and diarrhetic shellfish poisoning (DSP) from *Dinophysis* species, are the most common. A tropical fish poison known as ciguatera is produced by *Gambierdiscus toxicus*, a dinoflagellate with a strong affinity for seaweeds, upon which it attaches. Other tropical benthic dinoflagellate genera may possibly also be involved in producing ciguatera poisoning. Others release toxins into the surrounding water, killing marine life, including fish, shellfish, seabirds and marine mammals. Some fish killers, such as *Karenia brevis* or *Pfiesteria piscicida*, can also affect hu-

mans through their toxins in aerosols (some of the effects of the latter species and its very complex putative life-cycle, are controversial).

THE PAST: EARLIEST ORIGINS

Alveolates are believed to have diverged from their large sister group, the chromists, in the Neoproterozoic, as part of the eukaryotic "Crown" radiation [28]. This is supported by calculations of molecular divergence which, if the assumed substitution rate in SSU rDNA over a long time is correct, places the divergence of ciliates at 1317 Ma and the sporozoon/dinoflagellate divergence at 900 Ma [29]. Also, the presence in Late Proterozoic and Paleozoic, as well as in more recent sediments, of a biomarker, dinosterane [30], derived from dinosterol which is unique to dinoflagellates, supports this conclusion. Either of these alone might not be convincing but together their coherence makes a good case. So too, might the putative dinoflagellate fossils of similar age, reported by Butterfield & Rainbird [31] but their group identity is equivocal. In fact, this is true for all putative dinoflagellate fossils throughout the Paleozoic.

There are numerous spiny acritarchs (organic-walled structures of unknown affinity) that *might* be dinoflagellates, but they lack the features used to recognize dinoflagellate cysts when they lack clear paratabulation, e.g. an angular archeopyle (exit opening) whose shape corresponds to thecal plates or process patterns that correspond to tabulations (see [32] for extensive explanation). Some modern dinoflagellate cysts are like this, such as *Alexandrium* cysts. The few Paleozoic fossils identified as dinoflagellates are now not accepted as such [33]. In the future it may be possible to identify fossil dinoflagellates by a signature in the wall composition, i.e. be able to recognize dinosporin, and attempts are presently under way to do this. Of course we have no knowledge of what these first members of the lineage were like and it is also possible that they did not produce dinosporin-walled cysts. Only 10–15% of modern dinoflagellates produce such structures [34]. They could have resembled present-day "predinoflagellates" such as *Oxyrrhis* [9], which also lacks a resistant cyst stage.

THE PAST: THE MESOZOIC EXPLOSION, THE K/T BOUNDARY AND BEYOND

There is a rich fossil record of undoubted dinoflagellates from the early Mesozoic onwards. Nearly

all are remnants of the benthic, resting cyst stage, which has a tough, chemically-resistant wall that preserves after the dinoflagellate has emerged (excysted) to rise to the plankton above. It is believed that this dormant benthic stage can only colonise the water above if the sediments are not too deep, i.e. not deeper than the continental shelf. Ancient relatives of those dinoflagellates which do not produce a benthic resting cyst would not have left a fossil record.

Given the "silence" of the Paleozoic record and the fact that more than 80% of modern dinoflagellates do not produce fossilizable structures, it seems reasonable to argue, as Evitt [35] has done, that it is fruitless to try to interpret too closely the patterns of evolution from their fossil record. However, Fensome et al. [36], in reviewing the observed patterns, noted that the overall percentage of cyst formation is not typical of the groups well represented in the Mesozoic record (gonyaulacoids and peridinioids) and so the relatively clear patterns observable in the record, with notable exceptions, are probably real. MacRae et al. [19] described the overall species abundance of the existing fossil record with relation to a variety of factors, such as sea level (which would influence the amount of shallow coastal shelf), and even possible influence of discovery (number of publications per period).

The first unquestioned dinoflagellate fossils occur in the Triassic and by the Late Jurassic there had been a massive radiation, particularly of gonyaulacoids, with peridinioids reaching their maximum in the Cretaceous. After the Maastrichtian there was a decline in the total number of taxa, which continued through the Tertiary [19]. Interestingly, this is the opposite of diatoms, which took over dominance of the oceans in the Tertiary [37]. In terms of the major families of dinoflagellates, all were established by the Jurassic, and most persisted through to the present day. There are decreases just after the Cretaceous in the spindle plots of the families shown by Fensome et al. [36], but two studies that explicitly focused on the K/T bolide impact extinction event [38, 39] could see little or no evidence of major impact on the dinoflagellates. The records of two small families of fossils, the Pareodiniaceae and Ceratiaceae, stop at the K/T boundary [36] but modern ceratiaceans are abundant and so their loss of record was apparently due to a cessation of resistant cyst formation (many are oceanic today). Certainly the K/T impact on dinoflagellates seems to have been less than on other protist groups such as foraminiferans and coccolithophorids [37].

MOLECULAR PHYLOGENETIC STUDIES: THE NEW SYNTHESIS

The techniques of molecular phylogenetics, starting at first with amino acid sequencing and now using gene sequence comparisons, are powerful tools for resolving relationships among dinoflagellates and determining the polarity of lineages proposed on the basis of morphoclines. However, interpreting the gene trees generated by the various methods and different genes (ribosomal DNAs, actins, tubulins etc.) has proved far from simple. We have recently reviewed the results and their congruence (or lack of it) with morphologically-based phylogenies [8, 21] and so only some primary features will be noted here. The dinoflagellate ribosomal DNA trees are characterised by very poor resolution of a large section of the main radiation: the so-called "GPP complex" [40]. Adding many more species has not helped resolve the branching sequence greatly [21]. Early branching taxa, the "predinoflagellates", are quite reasonably resolvable [9] and the gonyaulacoid lineage is clearly and coherently resolved. In a novel, progressive exercise John et al. [41] have used a combination of molecular and fossil record data to calibrate a molecular clock, using it, together with plate tectonics and paleo-oceanography, to construct the probable paleodistribution of a species complex within the toxic dinoflagellate genus *Alexandrium*.

ENVOI

It is the author's hope that this brief summary of some of the features of this extraordinary group of protists explains why dinoflagellates have held his otherwise easily distractible attention for more than 40 years. Much remains to be learned about them and further study will surely be as rewarding as in the past.

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