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Part I

Chlorophylls and carotenoids

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Microalgal classes and their signature pigments

S. W. JEFFREY[1], SIMON W. WRIGHT AND MANUEL ZAPATA

1.1 Introduction

The microalgae that make up the extensive phytoplankton pastures of the world's oceans originated in ancient evolutionary times. They obtained their primitive 'plastids' from an unknown ancestral cyanobacterium with photosynthetic oxygen-evolving capabilities (Bhattacharya, 1997; Delwiche, 1999; McFadden, 2001; Palmer, 2003; Keeling, 2004a, b). Serial symbioses within heterotrophic hosts gave rise to the present wide diversity of photosynthetic microalgae, which evolved a range of photosynthetic pigments capable of collectively harvesting most of the wavelengths of light available to them in underwater marine habitats (Jeffrey and Wright, 2006).

At the present time, the marine phytoplankton contribute at least a quarter of the biomass of the world's vegetation, and constitute the base of the food web that supports either directly or indirectly all the animal populations of the open sea. Some microalgae also contribute significantly to climatic processes, providing nuclei for atmospheric water condensation (Aiken *et al.*, 1992). All microalgae, by their photosynthetic activities, contribute to atmospheric carbon dioxide 'draw-down' (Jeffrey and Mantoura, 1997), thus helping to ameliorate green-house gases, by removing nearly a third of the anthropogenic carbon released to the atmosphere (Sabine and Feely, 2007).

Because of the important global role of phytoplankton, monitoring their biomass by measuring ocean colour from space (Sathyendranath, 1986; Sathyendranath *et al.*, 2004; Nair *et al.*, 2008) and increasing the accuracy of *in situ* pigment measurements to determine algal types in the water column (Jeffrey *et al.*, 1997b), have become high priority areas for oceanographic research. Indeed, UNESCO and the Scientific Committee on Oceanic Research (SCOR) were pivotal in funding our first (1997) volume on this topic: *Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods*.

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Since 1997 a number of new algal classes have been erected (reviewed by Huisman and Saunders, 2007), and the availability of new algal cultures and improved HPLC techniques have allowed identification of many new pigments (e.g. Britton *et al.*, 2004; Garrido and Zapata, 2006). In the following sections, we indicate the range of these pigments across the known microalgal classes, and address their value in the developing science of phytoplankton pigment oceanography.

1.2 Algal classification

1.2.1 The 'protistan perspective'

An excellent guide to the classification of the tens of thousands of present-day and fossil phytoplankton species is given by Huisman and Saunders (2007) and Garcia and Playford (2007). These authors summarize a brief history of algal classification since the times of Kützing (1843), when the first microscopes revealed the astonishing world of minute plants and animals. The Kingdom Protista was subsequently proposed by Haeckel (1866) to accommodate all microscopic organisms (both pro- and eukaryotes), placing the protozoa, eukaryotic algae, slime moulds and some lower fungi in a coherent scheme. Acceptance of protistan concepts have fluctuated over time, but recently received additional support from evidence that eukaryotic cells gained mitochondria and plastids by endosymbiosis (Bhattacharya, 1997). A few 'lower' animals, such as protozoa 'enslaved' organelles such as plastids from other taxa and so became 'plants', but earlier classification schemes, which were devised in ignorance of this process, resulted in phylogenetically incoherent groups. The resurgence of 'protistology' (Corliss, 1986; 1994) as a field of study has provided a new means of putting these microscopic organisms and their inter-relationships into a phylogenetic perspective.

Adl *et al.* (2005) have published a new scheme of restructuring a higher-level classification of eukaryotes from a protistan perspective. They define 'protist' as a eukaryote with a unicellular level of organization, but accept that forms of vegetative cell differentiation may exist. We include here a short discussion of this topic since the new protistan classification schemes and terminology are starting to pervade the oceanographic literature (e.g. Gast *et al.*, 2006), and may cause confusion in relation to traditional pigment classification schemes. The 28 authors responsible for Adl *et al.* (2005) (phycologists, mycologists, parasitologists and protistologists) have adopted a different hierarchical system. Traditional 'Kingdoms', such as Metazoa, Fungi and Plantae are recognized as deriving from monophyletic protist lineages. The authors grouped molecular protistan phylogenies into six clusters (as shown below), which included both photosynthetic and non-photosynthetic (heterotrophic) organisms:

(1) ***Opisthokonta:*** animals, fungi, choanoflagellates and Mesomycetozoa;
(2) ***Amoebozoa:*** traditional amoebae, slime moulds etc.;
(3) ***Rhizaria:*** foraminifera, radiolaria, heterotrophic flagellates etc.;
(4) ***Archaeplastida:*** red algae, green algae, Glaucophyta and Plantae;

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Table 1.1. *The protist perspective: highest ranks of photosynthetic eukaryotes are included, excluding all heterotrophic taxa. Adapted from the 'protist perspective' classification scheme of Adl* et al. *(2005).*

Super groups	First rank	Second rank (examples of photosynthetic eukaryotes)
Rhizaria	Cercozoa	Chlorarachniophyta, *Paulinella*
Archaeplastida	Glaucophyta	Glaucophyceae
	Rhodophyceae	Subdivisions uncertain according to Adl *et al*. (2005)
	Chloroplastida	Charophyta*, Chlorophyta, *Mesostigma*, Prasinophyta
Chromalveolata	Cryptophyceae	Cryptomonadales
	Haptophyta	Pavlovophyceae, Prymnesiophyceae
	Stramenopiles	Bacillariophyta, *Bolidomonas*, Chrysophyceae, Dictyochophyceae, Eustigmatales, Pelagophyceae, Phaeophyceae*, Phaeothamniophyceae, Pinguiochrysidales, Raphidophyceae, Synurales, Xanthophyceae
	Alveolata	Apicomplexa, Dinozoa
Excavata	Euglenozoa	Euglenida

* Clades with multicellular groups.

(5) ***Chromalveolata:*** alveolata, apicomplexa, stramenopiles, Cryptophyta, Haptophyta;
(6) ***Excavata:*** includes Euglenozoa.

For the purposes of pigment oceanography, we have modified this scheme of Adl *et al*. (2005) (see Table 1.1), to include only those photosynthetic microalgal groups relevant to the pigment oceanographer. This scheme necessarily omits the non-photosynthetic (heterotrophic) eukaryotes.

1.2.2 The classical 'pigment perspective'

Early studies using thin-layer chromatography (TLC) clearly showed pigments associated with several microalgal classes in phytoplankton field populations (e.g. Jeffrey, 1974), building on the previous recognition of the importance of pigments in algal taxonomy. Macro-chromatographic methods (large columns) had been used to separate pigments from seaweed extracts (Strain *et al*., 1944; Smith and Benitez, 1955; Strain, 1958) which showed clearly defined pigment suites across the red, green and brown algae (see Jeffrey, 1997). A number of algal divisions/classes were erected on the basis of macrophyte pigment phylogeny. The colloquial term 'chromophyte' (Chadefaud, 1950; Bourrelly, 1957) at first referred to those algae that were 'coloured differently from green' (!) but was subsequently limited to those golden-brown algae containing chlorophylls *a* and *c* (Christensen, 1989). Patterson (1989) and others thought that 'the concept of chromophytes as pigment-based was restrictive and phylogenetically unsatisfactory'; he recommended that 'the concept of Chromophyta be relinquished in favour of an extended protistan assemblage'. The presence of tripartite flagellar hairs was chosen instead of pigments, as the defining characteristic of these groups (see the glossary at the end of this chapter).

Because of growing confusion about the classification of chromophyte algae, Dr J. C. Green of the Plymouth Marine Laboratory (UK) hosted a conference on Chromophyte Algae in 1987 (Green *et al.*, 1989). Key issues discussed included: an historical perspective (Christensen, 1989); carotenoids (Bjørnland and Liaaen-Jensen, 1989); chlorophyll *c* pigments (Jeffrey, 1989); the Kingdom Chromista (Cavalier-Smith, 1989); the protistan perspective (Patterson, 1989); flagellar-based ultrastructure (Preisig, 1989); flagellar hairs (Leadbeater, 1989) and many other topics. Patterson urged the adoption of the term 'stramenopile' based on flagellar structure (*stramen* (Latin) = straw; *pilus* = hair) for those golden-brown 'chromophyte' algae previously characterized by the plastid-based pigments, chlorophylls *a* and *c*. However, the character of tripartite flagellar hairs is not shared by all stramenopiles (e.g. bipartite hairs, *Pelagomonas*; absence of flagellar hairs, some Pinguiophyceae; Dr R. A. Andersen, pers. comm.). Before long, another conference followed, this time on the Haptophyte algae (Green and Leadbeater, 1994), which added to the resolution of this difficult polyphyletic group.

In recent years, the use of pigment data to map microalgal populations in the water column has become an established and convenient way of studying field phytoplankton populations (Jeffrey and Wright, 2006). The increasing success of microalgal culture has also allowed many more species to be isolated and studied, and the range of pigments available for characterizing algal groups, even down to genera (i.e. the Haptophyta) has escalated dramatically (Zapata *et al.*, 2004). The increased precision of current HPLC pigment methods (Garrido and Zapata, 2006 and Chapter 4, this volume) has also allowed many new members of the chlorophyll *c* and fucoxanthin families to be identified (Zapata *et al.*, 2006; Airs and Llewellyn, 2006).

Table 1.2 identifies 27 pigmented photosynthetic microalgal classes from 11 divisions, listed independently from their heterotrophic protistan counterparts. This scheme is based on that of Huisman and Saunders (2007). Several macrophyte classes could have been included, whose pigmented zoospores released seasonally into the water column include a brief spell in the planktonic mode. The green algal class Ulvophyceae is one such example (Van den Hoek *et al.*, 1995a). Several freshwater lineages are also included for completeness, thus recognizing the application of pigment techniques to freshwater and estuarine environments.

The above represents the authors' understanding of the rapidly changing field of algal taxonomy, in which further advances, largely driven by algal genomics, are already occurring (Brodie and Lewis, 2007).

Table 1.2. *Classification of photosynthetic microalgal classes found in marine phytoplankton. Size classes are indicated. + indicates presence and – indicates absence from the various size classes. (Adapted from Huisman and Saunders, 2007.)*

Algal division	Class	Common name	Microplankton >20 µm	Nanoplankton 2–20 µm	Picoplankton 0.2–2 µm
PROKARYOTES					
Division: Cyanophyta					
Class:	Cyanophyceae, includes species previously known as Prochlorophyceae	Cyanophyte, cyanobacteria, blue-green algae, prochlorophytes	+	+	+
EUKARYOTES					
Division: Glaucocystophyta					
Class:	Glaucocystophyceae	Glaucocystophyte	+	+	–
Division: Rhodophyta					
Class:	Rhodophyceae*	Red algae	Macrophyte	+	–
Division: Heterokontophyta (≡ **Chromista, Chromophyta, Stramenopiles)**					
Class:	Bacillariophyceae	Diatom	+	+	–
Class:	Bolidophyceae	Bolidophyte	–	–	+
Class:	Chrysophyceae	Golden-brown algae	+	+	+
Class:	Dictyochophyceae	Silicoflagellate	+	+	+**
Class:	Eustigmatophyceae	Eustigmatophyte	–	+	–
Class:	Pelagophyceae	Pelagophyte	–	+	+
Class:	Phaeothamniophyceae	Phaeothamniophyte	–	+	–
Class:	Pinguiophyceae	Pinguiophyte	+	+	–
Class:	Raphidophyceae	Raphidophyte	+	+	–
Class:	Synurophyceae	Synurophyte	+	–	–
Class:	Xanthophyceae	Xanthophyte	+	+	–
Division: Haptophyta					
	Pavlovophyceae	Golden-brown algae	–	+	+
Class:	Prymnesiophyceae (≡ Coccolithophyceae[1])				

Table 1.2. (*cont.*)

Algal division		Class	Common name	Microplankton >20 µm	Nanoplankton 2–20 µm	Picoplankton 0.2–2 µm
			Golden-brown algae	–	+	+
			(coccolithophorid)	+	+	–
Division:	**Cryptophyta**					
Class:		Cryptophyceae	Cryptomonad	–	+	–
Division:	**Dinophyta**					
Class:		Dinophyceae	Dinoflagellate	+	+	–
Division:	**Euglenophyta**					
Class:		Euglenophyceae	Euglenophyte	+	+	–
Division:	**Chlorarachniophyta**					
Class:		Chlorarachniophyceae	Chlorarachniophyte	+	–	–
Division:	**Chlorophyta**					
Class:		Chlorophyceae	Chlorophyte/green algae	–	+	–
Class:		Prasinophyceae	Prasinophyte	–	+	+
Class:		Trebouxiophyceae	Green algae/lichen algae	+	+	–
Division:	**Streptophyta**					
Class:		Mesostigmatophyceae	Mesostigmatophyte	–	+	–
Division:	**Unknown**					
Class:		Unknown (1)	'Picobiliphytes'[2]	–	+	+
		Unknown (2)	Chlorophyll *d*-containing[3]	Unknown taxa		

* Includes macrophytes

** Includes one picoplanktonic species (Eikrem *et al*., 2004)

[1] Silva *et al*. (2007)

[2] Not yet formally described (Not *et al*., 2007);

[3] Kashiyama *et al*. (2008)

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1.3 Origins of microalgal plastids

It is now generally accepted that plastids (chloroplasts) of eukaryotic algae are endosymbiotic organelles, originally derived from a previously free-living ancestral cyanobacterium that developed the capacity for oxygenic photosynthesis (Bhattacharya, 1997; Delwiche, 1999; McFadden, 2001; Palmer, 2003). The host cell was a non-photosynthetic (heterotrophic) protist of unknown origin. Development of this ancient symbiosis over eons of time eventually resulted in reduction of the size of the plastid genome, by gene transfer, loss and substitution, until the majority of the plastid proteins were encoded in the nuclear genome of the host. Further evolutionary development of this early endosymbiosis resulted in three major primary lineages, each clearly monophyletic: the glaucocystophytes, and the green and red radiations (Moreira *et al.*, 2000). The modern cyanobacterial radiation was derived directly from the ancestral cyanobacterium and its relatives without undergoing any further symbioses (Figure 1.1).

Many other photosynthetic algae arose from secondary (or even tertiary) endosymbioses of cells from these lineages (Delwiche and Palmer, 1997). In these cases an alga already equipped with a primary (or secondary) plastid was engulfed by a non-photosynthetic host cell, entering into a permanent or semi-permanent association with it. The history of these events can be seen in present day microalgae by the presence of vestigial nuclei (e.g. the nucleomorph), loss of cell compartments and organelles, variations in the number of residual membranes surrounding the plastid (two, three or four – Figure 1.1), and analysis of nuclear and plastid genomes. Such attributes supported the hypothesis (Gibbs, 1981; 1993) that plastids of heterokonts (diatoms, brown algae, chrysophytes etc.) and haptophytes, cryptophytes and dinoflagellates all arose from ancestral red algae by various secondary and tertiary endosymbioses (Figure 1.1). By a similar process euglenophytes, chlorarachniophytes and green dinoflagellates acquired their plastids from ancestral prasinophytes/chlorophytes. New evidence is also suggesting that in some groups (e.g. dinoflagellates), plastid losses and replacements have occurred during algal evolution (Saldarriaga *et al.*, 2001). Living dinoflagellates in modern oceans may be found with either no pigments (heterotrophs), their own unique plastid (containing the dinoflagellate carotenoid, peridinin), or with plastids derived from cryptomonads, pennate or centric diatoms, prymnesiophytes or prasinophytes/chlorophytes. Some dinoflagellates may even harbour intact symbiotic cells (e.g. cyanobacteria) rather than enslaved plastids (Hallegraeff and Jeffrey, 1984).

Within present-day phytoplankton populations, members of most microalgal lineages and their taxonomic branches may be found. In the ancient oceans, the fossil record clearly shows the initial dominance of the green algal superfamily, but in later evolutionary times, the balance has switched to ecological dominance of chromophyte algae from the red algal radiation. Establishing which forces have promoted these changes in the modern oceans is an active area of current research (Quigg *et al.*, 2003, Grzebyk *et al.*, 2003; Falkowski *et al.*, 2004a, b). Grzebyk *et al.* (2003) suggest that ‘whereas all algal plastids possess a core set of genes, red plastids

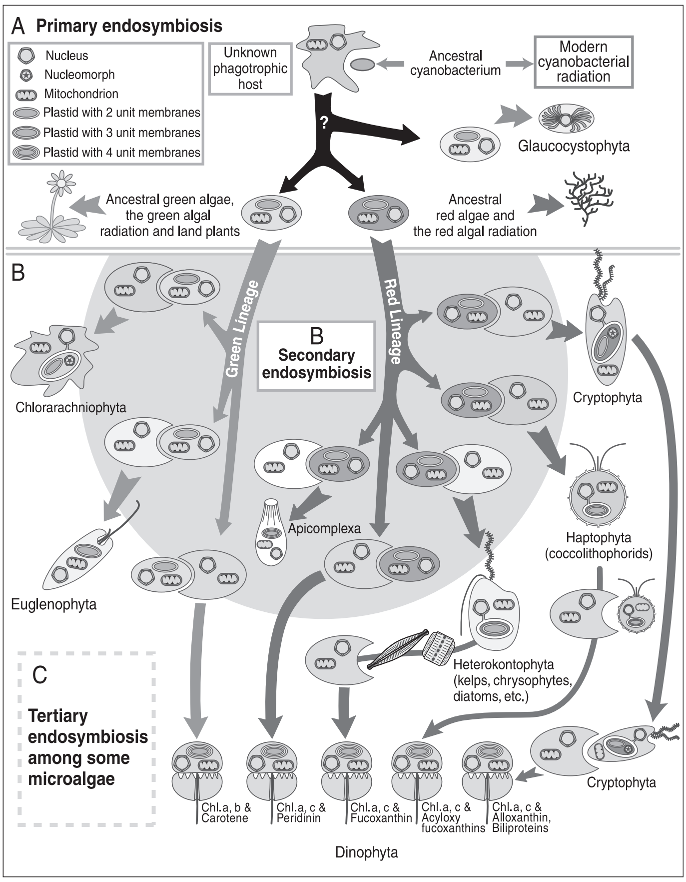

Figure 1.1. Diagram showing the hypothetical evolution of algal plastid diversity via serial endosymbiosis, based on Delwiche (1999), modified to show a common red algal origin of the plastids of apicomplexans, peridinin – containing dinoflagellates and heterokonts (Sanchez-Puerta and Delwiche, 2008; Janouškovec *et al.*, 2010). The evolutionary relationships among cryptophytes, haptophytes, heterokonts and alveolates are still controversial (Sanchez-Puerta and Delwiche, 2008), as are the number of tertiary endosymbioses (Bodył and Moszcyńksi, 2006). See colour plate section.

retain a complementary set of genes that potentially confer more capacity to express proteins regulating oxygenic photosynthetic and energy transduction pathways'.

1.4 Biological characteristics of currently recognized photosynthetic microalgal classes

Since publication of the SCOR-UNESCO volume: *Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods* (Jeffrey *et al.*, 1997b), additional