CHAPTER NINE

Genomics of Algal Host–Virus Interactions

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Abstract

Viruses in Earth's aquatic environment outnumber all other forms of life and carry a vast reservoir of genetic information. A large proportion of the characterized viruses infecting eukaryotic algae are large double-stranded DNA viruses, each of their genomes carrying more than a hundred genes, but only a minority of their genes resemble genes with known biological functionalities. Unusual forms of single-stranded DNA and single- and double-stranded RNA viral genomes have been characterized

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over the last 10 years, and the number of novel taxa of viruses being discovered continues to increase. Although viral infections are usually specific to certain host strains in a species, lytic viral infections nevertheless affect a large proportion of algae and have a global impact, for example in the termination of blooms. Resistance to viruses is thus subject to strong selection, but little is known about its mechanism. Lateral gene transfer between host and virus has been shown by comparisons between their complete genomes and must play an important role in coevolution in the microbial world. Recent advances in bioinformatics and the possibility of amplifying complete genomes from single cells promise to revolutionize analyses of viral genomes from environmental samples.

1. INTRODUCTION

When life was born in the oceans, so were viruses. All known life forms are infected, either chronically or lytically, at certain or all stages of their lifetimes, by their specific viruses. When terrestrial life forms evolved, viruses became hitchhikers that were forced to adapt to a drastically different environment. They could no longer diffuse or be carried to another host cell by diffusion or turbulence, so new means for transmission were required. Additionally, terrestrial plant cells have developed a rigid cell wall to resist the reduced osmotic pressure of a freshwater environment, a formidable barrier to viral ingress. Arguably, one of the selective pressures acting in adaptation of marine life to terrestrial conditions may have been to escape viral attack in an environment teeming with viruses that outnumber host populations by an order of magnitude. Nowadays, vegetal viruses are usually carried from plant to plant by sucking or biting insects or less frequently by the mechanical contacts with animals harvesting or moving through vegetation.

In this review, we will turn our attention to viruses of photosynthetic eukaryotes in the euphotic zone of aquatic environments, namely that depth of water that receives enough light for photosynthesis, on average down to about 200 m below the surface in the open sea, and at very variable depths in coastal or freshwater lakes and rivers, because of variable levels of turbidity. The term 'algae' will be used to regroup these organisms, although it has no phylogenetic significance, spanning at least four kingdoms in the tree of life (see Not *et al.* (2012), De Clerck, Bogaret, & Leliaert (2012) and Archibald (2012) in this volume for a review on the diversity of algae). In the context of this volume, we will furthermore consider only viruses whose complete genomic sequences have been analysed, giving clues about their biological functionalities, and apologize for not including important data about partial sequences, individual genes or the growing number of genomes being

assembled from metagenomic data (whose host species are not usually known). We will not include much detail about individual viruses but rather refer the reader to more detailed reviews and original research articles. Chloroviruses and other large viruses of protists have been reviewed extensively (Van Etten, 2003; Van Etten and Dunigan, 2011; Yamada, Onimatsu, & Van Etten, 2006), and a comprehensive review of dinoflagellate and diatom viruses is also available (Nagasaki, 2008), though this does not include the most recently discovered viruses. The largest viruses known, such as mimivirus (microbe-mimicking virus), infect non-photosynthetic protists, so we considered them outside of the scope of this botanical journal, although we note that they are in the very diverse family of double-stranded DNA (dsDNA) viruses that includes 'phycodnaviruses'. These giant viruses (giruses) are the subjects of several other reviews (Claverie *et al.*, 2006; Forterre, 2010; Van Etten, 2011; Van Etten *et al.*, 2010).

1.1. What Are Viruses?

Viruses consist of a nucleic acid sequence enclosed within a protein and/or lipid envelope. The simplest viral genomes thus may encode only two biological functionalities – a polymerase to ensure replication of their nucleic acid sequence and a capsid protein (CP) that is produced abundantly and coats the nucleic acid to provide protection in the period when a virus is not within its natural host. The nucleic acid component can be RNA or DNA, single or double stranded, and is a characteristic of the type of virus. Such simple viruses are completely dependent on host cell functionalities (such as protein synthesis), but algal viral genomes can also be very large, encoding hundreds of functionalities (Table 9.1). Terrestrial eukaryotes are dominated largely by only two kingdoms of organisms, animals and plants, but all of five kingdoms among the currently recognized eukaryotic divisions of life (Fig. 9.1) are well represented in aquatic environments. Whereas all the *Plantae* possess plastids, many lineages within the other four kingdoms can harbour photosynthetic plastids. The extent of such endosymbioses varies between kingdoms, most chromalveolates being photosynthetic, and symbiotic associations in the other kingdoms are more or less common depending on the lineage (reviewed in Johnson (2011) and Archibald (2012) in this volume).

1.2. Why Are Algal Viruses Important?

Phytoplankton is responsible for about half of the photosynthetic activity of the planet (Field, Behrenfeld, Randerson, & Falkowski, 1998), the second

Table 9.1 Algal Viruses Whose Complete Genomic DNA or RNA Sequences Are Known

Full name	GenBank Accession	Abbreviation	Source Information	Genome Size, Nucleotides
Acanthamoeba polyphaga mimivirus	NC_014649	APMV	Raoult *et al.* (2004)	1,181,549
Acanthocystis turfacea chlorella virus 1	NC_008724	ATCV-1	Fitzgerald *et al.* (2007)	288,047
Bathycoccus sp. RCC1105 virus BpV1	NC_014765	BpV1	Moreau *et al.* (2010)	198,519
Bathycoccus sp. RCC1105 virus BpV2	HM004430	BpV2	Moreau *et al.* (2010)	187,069
Cafeteria roenbergensis	GU244497	CroV	Fischer *et al.* (2010)	617,453
Chaetoceros lorenzianus DNA virus	NC_015211	ClorDNAV01	Tomaru *et al.* (2011)	5813
Chaetoceros salsugineum DNA virus	NC_007193	CsalDNAV	Nagasaki *et al.* (2005b)	6000
Chaetoceros tenuissimus DNA virus	NC_014748	CtenDNAV06	Shirai *et al.* (2007)	5639
Chaetoceros tenuissimus RNA virus	AB375474	CtenRNAV01	Shirai *et al.* (2008)	9431
Chara australis virus	JF824737	CAV	Gibbs *et al.* (2011)	9065[a]
Ectocarpus siliculosus virus 1	NC_002687	EsV-1	Delaroque *et al.* (2001)	335,593
Emiliania huxleyi virus 84	JF974290	EhV-84	Nissimov *et al.* (2011a)	395,820[a]
E. huxleyi virus 86	NC_007346	EhV-86	Wilson *et al.* (2005)	407,339
E. huxleyi virus 88	JF974310.1	EhV-88	Nissimov *et al.* (2012)	397,298
E. huxleyi virus 163	DQ127552–127818	EhV-163	Allen *et al.* (2006)	400,000[b]
E. huxleyi virus 201	JF974311.1	EhV-201	Nissimov *et al.* (2012)	407,301
E. huxleyi virus 203	JF974291	EhV-203	Nissimov *et al.* (2011b)	400,520[a]
E. huxleyi virus 207	JF974317.1	EhV-207	Nissimov *et al.* (2012)	421,891
E. huxleyi virus 208	JF974318.1	EhV-208	Nissimov *et al.* (2012)	411,003
Feldmannia species virus	NC_011183	FsV-158	Schroeder *et al.* (2009)	154,641
Heterocapsa circularisquama RNA virus	NC_007518	HcRNAV34	Nagasaki *et al.* (2005)	4375
H. circularisquama RNA virus	AB218609	HcRNAV109	Nagasaki *et al.* (2005)	4391

Heterosigma akashiwo RNA virus SOG263	NC_005281	HaRNAV-SOG263	Lang *et al.* (2004)	8587
Megavirus chiliensis	NC_016072	MGVC	Arslan *et al.* (2011)	1,259,197
Micromonas pusilla reovirus	NC_008171–8181	MpRV	Attoui *et al.* (2006)	25,563
Micromonas sp. RCC1109 virus MpV1	NC_014767	MpV1	Moreau *et al.* (2010)	184,095
Ostreococcus lucimarinus virus OlV1	NC_014766	OlV1	Moreau *et al.* (2010)	194,022
Ostreococcus tauri virus 1	NC_013288	OtV1	Weynberg *et al.* (2009)	191,761
O. tauri virus 2	NC_014789	OtV2	Weynberg *et al.* (2011)	184,409
Ostreococcus virus OsV5	NC_010191	OtV5	Derelle *et al.* (2008)	185,373
Paramecium bursaria chlorella virus 1[c]	NC_000852	PBCV-1	Yanai-Balser *et al.* (2010)	330,611
P. bursaria chlorella virus AR158	NC_009899	PBCV-AR158	Fitzgerald, Graves, Li, Feldblyum, *et al.* (2007)	344,691
P. bursaria chlorella virus NY2A	NC_009898	PBCV-NY2A	Fitzgerald, Graves, Li, Feldblyum, *et al.* (2007)	368,683
P. bursaria chlorella virus FR483	NC_008603	PBCV-FR483	Fitzgerald, Graves, Li, Hartigan, *et al.* (2007)	321,240
P. bursaria chlorella virus MT325	DQ491001	PBCV-MT325	Fitzgerald, Graves, Li, Hartigan, *et al.* (2007)	314,335
Rhizosolenia setigera RNA virus	AB243297	RsRNAV	Nagasaki *et al.* (2004)	11,200[b]
Schizochytrium sp. single-stranded RNA virus	NC_007522	SsSRVAV	Takao *et al.* (2006)	9035

Rows on a grey background are examples of viruses from non-photosynthetic protists.
[a]Almost complete.
[b]About 80% of this length complete.
[c]The most recent version of the genome, but this sequence was published in several parts previously.

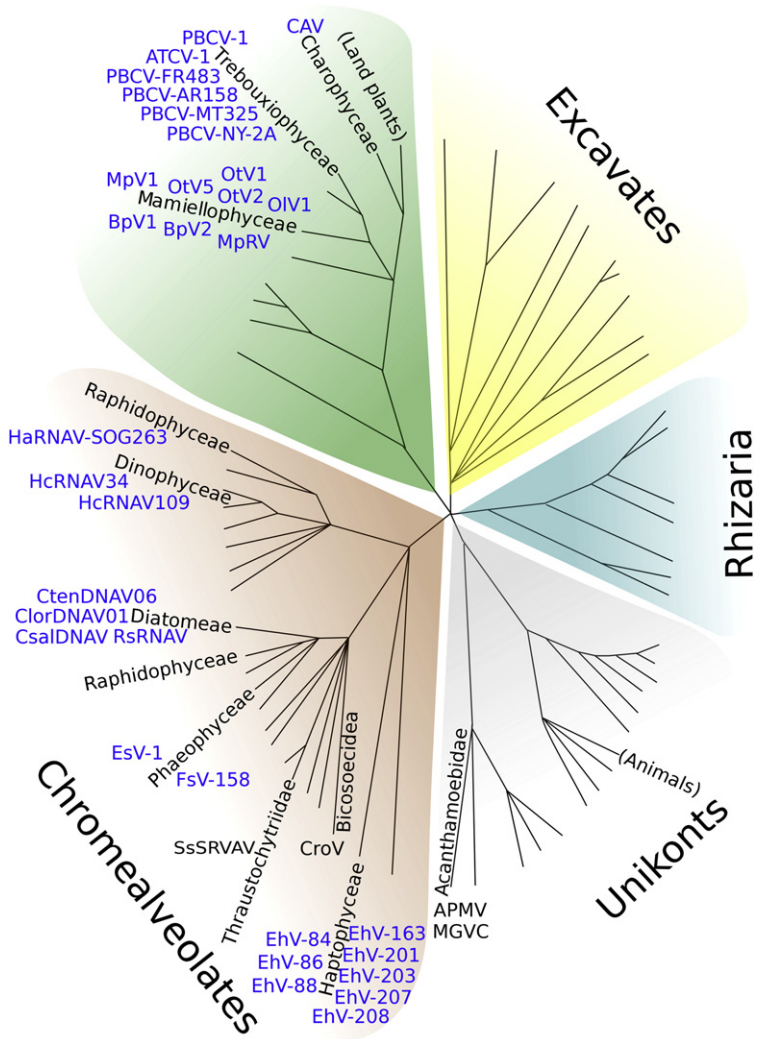

Figure 9.1 ***Algal viruses whose genomes have been sequenced.*** Known genomes of viruses infecting photosynthetic algae (text in blue, please refer to Table 9.1 for the names of hosts and viruses) lie mainly in two of the five eukaryotic kingdoms (coloured backgrounds) of life shown (most Unikonts, grey background, do not carry plastids, so their viruses are not included in this review). Only taxa with viruses mentioned in this review are labelled. Many lineages in Rhizaria, Metazoans (Unikonts) and Excavates can form symbioses with photosynthetic organisms (Johnson *et al.*, 2011). Algae of the Trebouxiophyceae infected by PBCV are usually symbionts of *Paramecium bursaria* (Alveolata) in nature. The positions of land plants and animals are shown for reference (tree simplified from Keeling *et al.*, 2005). See the colour plate.

half being ensured by terrestrial plants. Algae are at the base of the global food web, nourishing all aquatic life. In aquatic environments, while bacteria represent the largest biomass of organisms present, viruses outnumber them by 10 to 1. Algal viruses control blooms and shape the evolution of biodiversity in phytoplankton, yet little is known about their biological functions. In the oceans, viruses are thus the most abundant and diverse biological entities (Fuhrman, 1999; Wommack & Colwell, 2000) and infect all organisms from bacteria to whales (Suttle, 2005). The marine environment contains an estimated 10^{30} virus-like particles (Suttle, 2007). Most of the viruses described to date are species specific, infecting a single host species and sometimes even a single strain within a species. Due to their immobility, viruses depend on passive movement to contact a suitable host (Brussaard, 2004; Weinbauer, 2004). Consequently, the encounter rate between a virus and a host is directly affected by their relative abundances.

Several studies have shown the infection of a wide range of aquatic algae (Van Etten, Lane, & Meints, 1991; Van Etten & Meints, 2003) including bloom-forming marine phytoplankton (Suttle & Chan, 1995, Jacobsen, Bratbak, & Heldal, 1996; Sandaa, Heldal, Castberg, Thyrhaug, & Bratbak, 2001) like *Phaeocystis globosa* (Brussaard *et al.*, 2005), *Heterosigma akashiwo* (Nagasaki *et al.*, 1994a, 1994b; Nagasaki & Yamaguchi, 1997), *Aureococcus anophagefferens* (Gobler *et al.*, 1997, 2004, 2007), *Emiliania huxleyi* (Bratbak *et al.*, 1993) and *Ostreococcus* sp. (Countway & Caron, 2006).

When host organisms are lysed, nutrients are released into the surrounding environment and thus influence biogeochemical and ecological processes (Fuhrman, 1999; Gobler *et al.*, 1997; Sandaa, 2008; Wilhelm & Suttle, 1999). Viral lysis affects the efficiency of the biological pump by increasing or decreasing the relative amount of carbon in exported production (Suttle, 2007). This so-called 'viral shunt' moves material from heterotrophic and phototrophic microorganisms into particulate organic matter and dissolved organic matter (Gobler *et al.*, 1997; Middelboe *et al.*, 1996), which is mostly converted to CO_2 by respiration and photodegradation (Fuhrman, 1999; Suttle, 2005; Weinbauer, 2004; Wilhelm, 1999). Furthermore, in the sea the accelerated sinking rates of virus-infected cells increase the transport of organic molecules from the photic zone to the deep ocean (Lawrence & Suttle, 2004; Lawrence *et al.*, 2002).

Marine microbial virology has mainly concentrated on the infection of marine bacteria regarding abundance, genetic diversity, host specificity and genomics (Sullivan *et al.*, 2006). In comparison to prokaryotic viruses, less is known about viruses that infect marine eukaryotic phytoplankton, although

viral proliferation can trim populations or terminate phytoplankton blooms (Wommack & Colwell, 2000; Gobler *et al.*, 2004) and shuttle genetic material (Brown *et al.*, 2007; Rohwer & Thurber, 2009).

Most of the identified eukaryotic phytoplankton viruses are members of the family Phycodnaviridae, a diverse group of large icosahedral viruses with dsDNA genomes ranging from 160 to 560 kb with 100- to 220-nm-sized capsids (Van Etten *et al.*, 2002). Their importance in aquatic environments become clear in several independent studies (Monier, Claverie, & Ogata, 2008; Monier, Larsen, *et al.*, 2008; Short & Short 2008; Short & Suttle, 2002; Fischer, Allen, Wilson, & Suttle, 2010). Members of the Phycodnaviridae are currently grouped into six genera (named after the hosts they infect): *Chlorovirus, Coccolithovirus*, *Prasinovirus*, *Prymnesiovirus*, *Phaeovirus* and *Raphidovirus* (Wilson *et al.*, 2009). Complete genomes have been sequenced from representatives of the *Chlorovirus, Coccolithovirus, Phaeovirus* and *Prasinovirus* genera (Dunigan *et al.*, 2006).

Viruses affect host population dynamics and nutrient flow in aquatic food webs. However, only a small portion of marine viruses has been isolated and described so far, revealing that marine virology is still in its infancy. Each infection has the potential to introduce new genetic information in an organism or progeny virus, thereby driving the evolution of both host and viral assemblages (Suttle, 2007).

Marine viruses have been mainly studied for socio-economic reasons linked to massive and sudden death of microalgae or metazoan host organisms in natural marine environments or in aquacultures (Brussaard, 2004; Nagasaki, 2008). Microalgal bloom is a phenomenon characterized by a rapid increase in population of microscopic unicellular algae. When their pigments discolour the water, blooms are called 'red tide', 'brown tide' or 'green tide' depending on the colour of water. Harmful algal blooms cause large economic damage in fishery, aquaculture, leisure industries and other socio-economic activities in coastal areas. For instance, red tides of raphidophytes and dinoflagellates lead to recurrent serious mass mortality of cultured fishes and bivalves in Japan, Canada, New Zealand and Chile (K. Nagasaki, personal communication). Algal blooms often experience a sudden disintegration and disappearance, and in many cases, giant viruses are the agent infecting and killing bloom-forming algae. Viruses are thus one of the main regulators of the seasonal occurrence and termination of algal blooms. These viruses represent potential anti-algae agents (akin to 'phage therapy') in aquacultures. Algal blooms and associated viruses have been also implicated in other ecological/climatic processes. The cosmopolitan

coccolithophore *E. huxleyi* is known for its white blooms covering huge oceanic surfaces (Zondervan, 2007). This photosynthetic microalga contributes to the production of atmospheric dimethyl sulphide, which in turn leads to cloud formation. Coccolithophores drive massive sinking of calcium carbonate into deep oceanic lithosphere, thus contributing also to global carbon fluxes; for instance, Dover's chalk cliffs are largely (80%) made up of beautiful calcium carbonate scales of ancient coccolithophores (ca. 100 million years old) and illustrate the significant geochemical role of coccolithophores. The huge *E. huxleyi* blooms suddenly terminate due to the infection of marine viruses called EhVs (*E. huxleyi* viruses) with a large ~400-kb genome (Bratbak *et al.*, 1993; Wilson *et al.*, 2005b).

Global warming has now become unequivocal, after multiple and serious scientific surveys by international and intergovernmental organizations during the last two decades. The United Nation's Intergovernmental Panel on Climate Change reported significant increases in global average air and ocean temperatures, widespread melting of snow and ice and rising average sea level (the Fourth Assessment Report: http://www.ipcc.ch/pdf/assessment-report/ar4/syr/ar4_syr.pdf). The recent Tara-Arctic expedition (2007–2008) also revealed serious melting of the ice in the most northerly latitudes. Recent studies show that marine viral abundance increases with temperature (see Danovaro *et al.* [2011] for a review), but many other aspects of this complex ecosystem are also affected.

2. WHAT IS KNOWN ABOUT AQUATIC ALGAL VIRUS GENOMICS?

Although interest in aquatic algal viruses is growing (Fig. 9.2), for reasons mentioned above, relatively few algal viruses have been characterized at the level of their genomes. There are several reasons for this paucity of knowledge. The majority of algae have little direct economic importance, and unlike land plants, the large majority of algae are not easily observable, most species being microscopic and distributed around remote regions of the planet. In addition, most algal species are not easily cultured in the laboratory, usually a necessary condition to perform the molecular biological steps required for sequencing the genome. However, given the increased interest in aquatic life arising because of global climate change and the reduction in cost of next-generation sequencing, numerous new algal and viral genomes are now being sequenced.

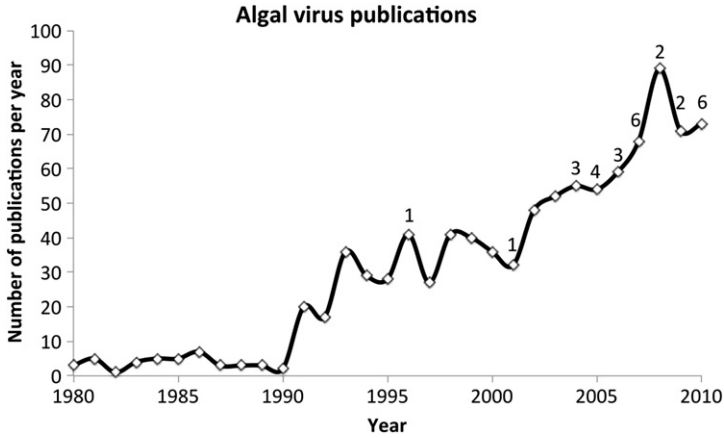

Figure 9.2 The growing interest in algal viruses is witnessed by an increasing number of publications. The curve shows new journal articles appearing each year as seen by an internet keyword search (Web of Knowledge) by combining the key words 'alga' and 'virus' over the 20-year period 1980–2010. Figures above the curve indicate the number of new complete genomes reported for the corresponding year (listed in Table 9.1). In 2011 (data not shown), 99 new articles and 6 complete genomes were published. Four more genomes have been described in the first 3 months of 2012.

2.1. Genomics

A new era is dawning for research on viral genomes with the advent of 'Next Generation Sequencing' (NGS) technologies (Gilbert & Dupont, 2011; Nowrousian *et al.*, 2010; Rodriguez-Brito *et al.*, 2010). Whole genomes of very large viruses can be sequenced more easily at a lower overall cost, and sequence analyses are facilitated by a growing number of bioinformatics programs for assembling and annotating data, permitting prediction of some of the encoded biological functionalities. In addition, the size ranges of viruses in general falls into those classes of organisms that can be collected from diverse environments by filtration (Lauro *et al.*, 2011) or flocculation (John *et al.*, 2011) and the relatively small size of their genomes, much less complex than those of eukaryotes, facilitates interpretation of sequences from metagenomic data or deep sequencing of given polymerase chain reaction-amplified marker genes. NGS technology thus allows the exploration of viral diversity in a range of environments since complete genome data from laboratory-cultured strains can be used to probe and assess the distribution of a species between these environments. The exponentially increasing amount of metagenomic data from diverse environments can thus be exploited. This kind of approach first enabled the distributions of

prokaryotic viruses (bacteriophages) to be analysed, revealing an unprecedented abundance and diversity, including astronomic numbers of unknown biological functionalities in these 'viromes' (Bench *et al.*, 2007), but more recently, attention is turning to eukaryotic viruses (e.g. Kristensen, Mushegian, Dolja, & Koonin, 2010; Monier Claverie, J.-M. & Ogata, H. 2008).

In the following sections, we will firstly summarize very briefly some of the history of aquatic algal virus genomes before discussing the evolution of host–virus interactions, finishing with some perspectives about how the field is developing.

2.1.1. dsDNA Viruses (Giruses) Abound in the Aquatic World

The first reports of viruses or viral-like particles in green and brown algae date back as far back as 1958, but further confirmations about their nature, with large particle sizes (100–200 nm) came in the 1970s (reviewed in Brown, 1972; Van Etten *et al.*, 1991). To date, the majority (about two thirds, 23/33 listed in Table 9.1) of algal viruses whose genomes are characterized are phycodnaviruses.

2.1.1.1. Chlorella Viruses

While most species of the unicellular green alga chlorella are free living, certain of them can form symbioses. The freshwater unicellular protozoan *Paramecium bursaria*, or the metazoan *Hydra viridis*, for example, can harbour symbiotic chlorella-like 'zoochlorellae'. In paramecium, each algal cell is enclosed in a perialgal vacuole, and all chlorellae in the host cell are inherited to the progeny, undergoing coordinated division with the host cells, giving a constant population density of several hundred per cell. When such hosts are cultured for some time under suitable conditions without light, the zoochlorellae are released and can be cultured independently on liquid or solidified media. Zoochlorellae in culture are susceptible to lytic attack from phycodnaviruses.

In native freshwater, the titre of PBCV-1 (*P. bursaria* chlorella virus) particles may attain 100,000 plaque-forming units (PFUs) per millilitre but more typically are found to be around 1–100 PFU/mL (Van Etten *et al.*, 1985).

Over the last 30 years, research on PBCV-1 has revealed some fascinating features about the structure and biological functionalities encoded by such large viruses (several reviews are available, Yamada *et al.*, 2006; Van Etten *et al.,* 2010; Van Etten and Dunigan, 2012). Analyses of chlorella virus genomes were pioneered by the assiduous work of J. Van Etten's laboratory,

who after beginning work on PBCV-1 in the 1980s sequenced several large regions of the PBCV-1 genome in the 1990s, before publishing an updated corrected version of the complete genome (Kutish *et al.*, 1996; Li *et al.*, 1995, 1997; Lu *et al.*, 1995, 1996; Yanai-Balser *et al.*, 2010). PBCV-1 is a member of the supergroup of viruses known as 'nuclear–cytoplasmic large DNA viruses' (NCLDV; Iyer, Aravind, & Koonin, 2001; Iyer, Balaji, Koonin, & Aravind, 2006) that includes viruses infecting metazoans (such as poxviruses) and viruses infecting algae (phycodnaviruses, see Table 9.1). In contrast to viruses of land plants, phycodnaviruses are really huge. PBCV-1, for example, encodes 365 predicted proteins and 11 transfer RNAs (tRNAs; Yanai-Balser *et al.*, 2011). The molecular structure of PBCV-1 has been examined in detail (Kuznetsov, Gurnon, Van Etten, & McPherson, 2005; Zhang *et al.*, 2011); the virion consists of an icosahedral particle made of glycoproteins containing a membrane-bounded dsDNA genome. After attachment to the wall of its specific host algal cell, the host cell wall is digested and the virion DNA is injected before a lytic infection cycle starts, the infection process thus resembling those of bacteriophages. Several complete genomes of chlorella viruses have now been sequenced and described (Fitzgerald *et al.*, 2010a, 2010b). Biological functionalities encoded by its 330-kb-long genome to govern the host cell during its lytic life cycle include (i) methylation of host histones, (ii) a restriction enzyme/DNA methylation system, (iii) sugar metabolizing enzymes, (iv) channel/transporter proteins, (v) DNA replication enzymes and (vi) polyamine metabolism enzymes, to mention but a few. Several other chlorella virus genomes have now been analysed (ATCV-1, AR158, NY2A, FR483, MT325, see Table 9.1), revealing new gene functionalities and a high genetic diversity within this group.

2.1.1.2. Viruses of Heterotrophic Protists

We mention these giruses here because of their exceptional sizes, remarkable panoplies of biological functionalities and phylogenetic relationship to viruses of algae (Monier, Claverie, & Ogata, 2008; Monier, Larsen, *et al.*, 2008, and see below), but they infect non-photosynthetic unicellular eukaryotes, and we will not review them here. The NCLDV group also includes largest known viruses, whose genome size exceeds those of the smallest bacteria. The first of these, mimivirus, that infects the freshwater amoeba *Acathamoeba polyphaga*, encodes 1018 predicted proteins (Raoult *et al.*, 2004; Renesto *et al.*, 2006; Legendre, Santini, Rico, Abergel, & Claverie, 2011), but an even larger virus of this kind has recently been

reported (Arslan, Legendre, Seltzer, Abergel, & Claverie, 2011). In the sea, *Cafeteria roenbergensis* is a common bactivorous flagellate (Fig. 9.1) and is also infected by a girus (Fischer *et al.*, 2010). Infections of both CroV and mimivirus are sometimes accompanied by virophages that depend on girus for growth in the host. Like satellite viruses of high plants, these virophages affect the severity of the girus infection in a host cell.

2.1.1.3. Phaeoviruses

Viruses infecting multicellular brown algae in the order Ectocarpales were recognized over 30 years ago (reviewed in Brown, 1972; Oliveira & Bisalputra, 1978), and the first genome of a virus in this group, EsV-1 (*Ectocarpus siliculosus* virus 1), was analysed in 2001 (Delaroque *et al.*, 2001) and a second complete genome for this group, FsV-158 (*Feldmannia* species virus 158), being published more recently (Schroeder *et al.*, 2009). The life cycles of phaeoviruses are particularly well adapted to those of the brown algae in this group, which undergo an alternation of generations (see Chapter 5, The Ectocarpus Genome Consortium, 2012, and Peters *et al.*, 2008 for further details of the life cycle). Whereas these algae spend most of their lifetimes as sessile filamentous forms, their cells being protected by a cellulose/alginate cell wall, their motile zoospores and gametes are naked cells that can be infected by specific phaeoviruses (Müller, 1991a). Once infected, the virus can be integrated into the host genome and is subsequently inherited in a Mendelian manner (Müller, 1991b). The filamentous plant then developing from the zooid or gamete shows no symptoms until it produces sporangia (fruiting bodies), in which infectious viral particles are produced, these being released under in certain environmental conditions (Müller, 1991a). Filamentous sporophyte plants carrying an integrated phaeovirus thus have reduced fertility and transmit viruses to other plants at propitious times during gamete release. The EsV-1 and FsV-158 genomes are strikingly different in size (336 and 155 kb, see Table 9.1), probably reflecting ancient evolutionary paths since their last common ancestors, providing interesting models for host–virus evolution. This genetic system resembles those of human herpesviruses in some ways. Herpesviruses are likewise large dsDNA viruses that are transmitted vertically to offspring and are integrated in the host genome, remaining latent until their outbreak in certain diseases (e.g. 'Chicken Pox' may reappear as 'Shingles' in later life), but good animal models for studying this disease are lacking (Kennedy, 2002).

2.1.1.4. Coccolithoviruses

Haptophyte algae (see Fig. 9.1) are common and abundant worldwide, some of them forming blooms which may be terminated by viral infections (see above).

In contrast to chloroviruses, phaeoviruses and prasinoviruses (see below), the large *E. huxleyi virus 86* genome (Wilson *et al.*, 2005) carries an RNA polymerase gene, suggesting that at least the virus more directly controls some of its own gene expression. Several other features also set this virus apart from the other phycodnaviruses. *Emiliania huxleyi* viruses are surrounded by a lipid membrane rather than a rigid capsid and enter their host cells via endocytosis (Mackinder *et al.*, 2009). After about 4.5 h, new virions are released by budding from the host cell. In all these characteristics, coccolithoviruses and other NCLDV of non-photosynthetic protists (shaded lines in Table 9.1) more closely resemble animal viruses, such as poxviruses. Remarkably, coccolithoviruses have acquired numerous genes, most likely from their host, that encode the synthesis of complex sphingolipids (Monier *et al.*, 2009). Recent work suggests that syringolipid signalling might play a role in controlling host cell death during infection (Han *et al.*, 2006; Monier *et al.*, 2009; Pagarete, Allen, Wilson, Kimmance, & de Vargas, 2009; reviewed in Michaelson, 2010; Bidle & Vardi, 2011). The life cycle of *E. huxleyi* is known (Laguna, Romo, Read, & Wahlund, 2001), and in nature, the diploid form carrying many coccoliths is far more abundant than its haploid (gametic) form, but both haploid and diploid forms can be grown in culture. Frada, Probert, Allen, Wilson, & de Vargas (2008) showed that only diploid cells were susceptible to viral attack and that this species might escape viral infection by meiosis, producing resistant gametic cells. Eight complete (or nearly complete) genomes are now available for viruses infecting *E. huxleyi* (Allen, Schroeder, Donkin, Crawfurd, & Wilson, 2006; Nissimov *et al.*, 2011a, 2011b, 2012) and a complete host genome is also available for *E. huxleyi* (see the website of the Joint Genome Institute [JGI]: http://www.jgi.doe.gov/).

2.1.1.5. Prasinoviruses

Green algae in the class Mamiellophyceae (formerly Prasinophyceae, see Marin and Melkonian, 2010) are globally distributed in aquatic environments. In coastal regions and marine lagoons, picoplanktonic algae of the order Mamiellales are often dominant, common genera including the genera *Micromonas*, *Ostreococcus* and *Bathycoccus*, the composition of their diversity

depending on the environment. In high latitudes, *Micromonas* often prevails (Lovejoy, 2007; Not *et al.*, 2004), whereas *Ostreococcus* is more prevalent in temperate latitudes (Zhu, Massana, Not, Marie, & Vaulot, 2005; Viprey, Guillou, Ferréol, & Vaulot, 2008; Demir-Hilton *et al.*, 2011). These species are among the smallest unicellular organisms known, and complete algal host genomes are available for six species, three *Ostreococcus* (Derelle *et al.*, 2006; Palenik *et al.*, 2007; Grigoriev *et al.*, 2012, see http://genome.jgi-psf.org/OstRCC809_2/OstRCC809_2.home.html), two *Micromonas* (Worden *et al.*, 2009) and one *Bathycoccus* (Moreau *et al.* 2012). Viruses of *Micromonas pusilla* were among the first to be observed in the Phycodnaviridae (Mayer & Taylor, 1979), but the first genome of a *Micromonas* sp. virus became available only recently (Moreau *et al.*, 2010). However, the first sequenced viral genome available in this group was that of OtV5, a virus infecting *Ostreococcus tauri* (Derelle *et al.*, 2008). This virus was chosen first because *O. tauri* is the species of the Mamiellales for which the most physiological data currently exist, including a completely sequenced genome. Eight complete genomes of prasinoviruses are currently available (Fig. 9.1; Derelle *et al.* 2008; Moreau *et al.*, 2010; Weynberg, Allen, Ashelford, Scanlan, & Wilson, 2009; Weynberg, Allen, Gilg, Scanlan, & Wilson, 2011). Perhaps the most surprising finding from comparative genomics within the prasinoviruses is that their genomes show less divergence than those of their host genomes (Moreau *et al.*, 2010, and see below), despite them being mainly species specific (Clerissi *et al.*, 2012), in contrast to classical dogma about the fast evolution of viral genomes. Prasinoviruses were also found to have several genes encoding enzymes for amino acid synthesis not found in other viruses, but it is not clear why these particular pathways have been recruited into the viral genome. Complete genomes are currently available for seven *Prasinovirus* strains (BpV1, BpV2, MpV1, OlV1, OtV1, OtV2 and OtV5, Table 9.1), but this figure will probably double within the next 2 years.

2.1.1.6. Unassigned DNA Viruses – *Chaetoceros salsugineum* Nuclear Inclusion Virus

Chaetoceros is one of the most abundant and widespread genera of diatoms known, with approximately 400 species described (Rines & Theriot, 2003). Temperature, climate, salinity, nutrients and predators are regarded as important factors controlling its abundance and population dynamics. *Chaetoceros salsugineum* nuclear inclusion virus (CsNIV) is a 38-nm icosahedral virus that replicates within the nucleus of *C. salsugineum*. CsNIV has a novel partially dsDNA genome, being a single molecule of covalently

closed circular single-stranded DNA (ssDNA; 6005 nucleotides), together with a piece of linear ssDNA (997 nucleotides) that is complementary to a portion of the closed circle (Nagasaki *et al.*, 2005). The putative polymerase shows low but significant similarity that of circoviruses (e.g. beak and feather virus disease of birds). Two other viruses of this kind have now been reported (Table 9.1, CtenDNAV06 and ClorDNAV01).

2.1.2. RNA Viruses

Several kinds of RNA viruses have been found to infect algae. While some of these have been loosely regrouped with previously classified viruses, others have not yet been classified or represent new groups of viruses.

2.1.2.1. Picorna-Like Viruses

Picornaviruses are small positive-strand RNA viruses with icosahedral particles (about 30 nm diameter). In mammals, specific picornaviruses cause diseases such as polio, common colds and foot-and-mouth disease. Their genomes are about 7- to 11-kb long with a long 5' untranslated leader sequence, and they are translated to produce a polyprotein that is proteolytically processed to produce CPs and a replicase (RNA-dependent RNA polymerase or RdRp). Picorna-like viruses are abundant in aquatic environments (Culley *et al.*, 2003; Culley, Lang, & Suttle, 2006; Koonin, Wolf, Nagasaki, & Dolja, 2008). Several species of dinoflagellates and raphidophytes are toxic bloom-forming algae that are ecologically and economically important because they can cause major fish kills. Tai *et al.* (2003) first visualized HaRNAV (*H. akashiwo* RNA virus) as 25-nm diameter particles that can form crystalline lattices in the cytoplasm of its Raphidophyte host *H. akashiwo* during infection, before host cells are lysed. Its positive-strand RNA genome (Lang, Culley, & Suttle, 2004) resembles tomato ringspot virus and certain insect viruses but its overall identity to these is <30% at the amino acid level. Translation probably produces a single polyprotein that is processed to yield three CPs and a replicase. Picorna-like viruses such as RsRNAV (*Rhizosolenia setigera* RNA virus), a 11.2-kb-long single-stranded RNA virus (Nagasaki, K., Tomaru, Y., Katanozaka, N., Shirai, Y., Nishida, K., Itakura, S., *et al.*, 2004), infects *R. setigera,* a diatom. Diatoms (Bacillariophyceae) are among the most widespread organisms on the Earth (Not *et al.* (2012) and Mock and Medlin (2012) in this volume), so it is not surprising that viruses of this kind are found to be abundant in metagenomic data (Culley *et al.*, 2006). Shirai *et al.* (2008) characterized another picorna-like virus, CtenRNAV01 (*Chaetoceros tenuissimus* RNA virus), monophyletic with RsRNAV.

2.1.2.2. dsRNA Viruses

The first double-stranded RNA virus to be sequenced (Attoui, 2006) has a multipartite genome of 11 segments ranging from 0.8 to 5.8 kb, similar to the family Reoviridae and infects the unicellular green alga *M. pusilla* (Brussaard *et al.*, 2004). It is the founder member of the genus *Mimoreovirus*. Phylogenetic analysis using RdRp, the longest predicted protein, revealed only distant relationships to other Reoviruses such as fijivirus (infecting graminaceous plants) and bluetongue virus (infecting cattle).

2.1.2.3. Unassigned RNA Viruses

Two other kinds of single-stranded RNA viruses that have not yet been classified have been discovered. *Heterocapsa circularisquama RNA virus* is a ~4.4-kb virus infecting the dinoflagellate *H. circularisquama*. The virus contains just two open-reading frames (ORFs), one encoding a polyprotein (~3 kb, with two predicted proteins, a protease and an RdRp) and the other encoding a CP (~1 kb). Its closest relatives are higher plant viruses in the Tetravirideae and Luteovirideae (such as beet chlorosis virus and rice yellow mottle virus).

The recently described genome of *Chara australis* virus, whose host is within the Phylum Streptophyta, much more closely related to land plants, has recently become available (Gibbs *et al.*, 2011). Predicted biological functionalites of proteins encoded by 4 of the 12 ORFs identified include RdRp, movement protein (MP), coat/CP and helicase. The closest similarities of the individual proteins were to very divergent groups of viruses (RdRp similarities to benyviruses such as beet necrotic yellow vein virus and helicase similar to pestiviruses such as bovine viral diarrhoea virus) suggesting ancient divergence from known groups. However, the coat protein showed some homology to tobamoviruses (such as tobacco mosaic virus [TMV]), highlighting the relationship of its host with land plants. Indeed, the MP gene also shows homology to the TMV MP group, perhaps not surprising because, as the authors point out, charophyte algae have plasmodesmata-like intercellular connections, and these connections may be used by the virus for systemic spread in the host (Gibbs *et al.*, 2011).

2.2. Transcriptomics

Only a few genomes of algal viruses have been available until recently, so it is not surprising that this field of work is in its infancy. Analyses of the expressed genes of a complete virus permit the activity of the viral coding

sequences to be verified *in vivo* in culture. It can further be developed to examine the temporal dynamics of gene expression in a viral life cycle or to examine gene expression in water samples collected from the environment and becomes an even more powerful tool when complete host genomes are also available since the changes in host gene expression in response to viral attacks can also be investigated.

2.2.1. Laboratory-Grown Cultures

Initial analyses of viral expression have usually involved the synthesis of a custom-made set of oligonucleotides representing all the genes of the virus spotted in a microarray on a solid support. RNA is then extracted from infected host cells and is used to make a fluorescently marked complementary DNA library that can be hybridized to the microarray and scanned to assess the level of expression of individual genes by their intensities of fluorescence. Using this technique, expression of 65% of EhV-86 viral genes could be detected in *E. huxleyi* (Wilson *et al.*, 2005), although the biological function of the large majority of the gene products remains unknown.

More recently, using a similar technique, studies aimed at describing the temporal sequence of viral gene expression have been undertaken. Yanai-Balser *et al.* (2010) performed a detailed analysis of infection of PBCV-1 transcription using RNA extracted from seven time points during infection, permitting them to show that 99% of the 365 predicted genes and 11 tRNAs are expressed at some time point during the life cycle, being classed as early (66% of genes before DNA replication) and late (36% after DNA synthesis begins). Many early expressed genes were involved in DNA replication and metabolism, while some of the late expressed genes included proteins known to be virion structural genes (CPs) and others packaged on the virion such as enzymes degrading polysaccharides, necessary for the virus to penetrate a new host cell and enzymes required for injection of its DNA into a new host cell.

A time course of expression of EhV-86 was examined at three time points but this time using RNA expressed sequence tags (ESTs) cloned by production from both virus and hosts. The ESTs were then sequenced and compared to the viral sequence (Wilson *et al.*, 2005; the draft host sequence is available at the JGI website: http://www.jgi.doe.gov/), and grouped in functional categories. In a second set of experiments, oligonucleotide arrays were used to confirm the expression of genes identified by EST analyses from both host and viral genomes and showed that 12% of the assembled ESTs (223 clusters of clones) were similar to the viral genome (Kegel *et al.*, 2007, 2010).

A preliminary transcriptomic analysis of host and viral genes expressed after infection of the unicellular green alga *O. tauri,* comparing healthy and OtV5-infected cells, has been done and used to establish the codon usage preferences in host and virus-infected cells (Michely, S., Toulza, E., Subirana, L., John, U., Cognat, V., Maréchal-Drouard, L., Grimsley, N., Moreau, H., & Piganeau, G., unpublished). Codon usage bias increases with overall gene expression levels, and the tRNAs carried by the virus may help to optimize viral protein translation given the available host tRNAs.

Several questions, such as response to abiotic stress (Dittami *et al.*, 2009) and developmental changes (Peters *et al.*, 2008), are being addressed by transcriptomic analyses of the brown alga *Ectocarpus siliculosus* (The Ectocarpus Genome Consortium, 2012). Since this genome contains an integrated copy of the virus EsV-1, transcriptional analyses also include those viral genes expressed from the latent viral genome. Detailed studies aimed at studying the virus, which would require the study of viral proliferation in the gametophytic stage of the host, have however not yet been attempted.

2.2.2. Environmental Samples

Using a mesocosm (an enclosure of seawater under natural conditions), Pagarete *et al.* (2011) examined the propagation of *E. huxleyi* viruses in a bloom produced after addition of phosphate to enrich natural seawater. The bloom of *E. huxleyi* that resulted, and its subsequent demise by viral lysis, thus occurred with the mixture of strains found at the start of the experiment with the natural populations present in the sampled water (six mesocosms of 11 m^3). Although the transcriptomic probes used to monitor this phenomenon were necessarily limited to those with available ESTs, and the infection could not be synchronous, 'early' and 'late' patterns of gene expression could nevertheless be distinguished during the massive viral lysis observed.

3. HOW DO ALGAE SURVIVE IN THE PRESENCE OF VIRUSES?

While much is known about resistance to viruses in higher plants, different evolutionary paths have been taken in the recruitment of resistance genes and different resistance mechanisms are found (Chapman and Carrington, 2007). We will not review this subject area here but take a more limnological

approach to the problem, for which a broader review can be found elsewhere (Thomas, Jacquet, Grimsley, & Moreau, 2012).

Coexistence of phytoplankton hosts and their viruses is a major question to understand how the equilibrium between host and viral populations can be maintained. There are several mechanisms by which a host can avoid interacting with its viruses, including passive or active mechanisms. Among the more passive mechanisms used by the hosts, the 'escape' strategies are well documented in several studies. For example, virus-infected *H. akashiwo* cells sink more rapidly than healthy cells, then moving out of the euphotic zone because of their higher density (Lawrence & Suttle, 2004; Lawrence, Chan, & Suttle, 2001). This may prevent viral infection of conspecifics. This 'altruist' strategy can be explained in terms of evolution for clonal conspecifics. Another example of escape is the narrow host specificity associated with a high host diversity. In this case, the large dilution of infectable hosts reduces their accessibility by viruses (Suttle and Chan, 1994; Suttle, 2005). A third example is the 'Cheshire Cat' strategy (Frada *et al.*, 2008). Here, the haploid phase of the haptophyte *E. huxleyi* does not calcify (i.e. it no longer has hard calcium carbonate scales, but instead has organic surface scales), in contrast to the diploid stage. Frada *et al.* (2008) showed that such 'naked' forms were indeed resistant to EhVs and that exposure of diploid cells to viruses promoted transition to the haploid stage 6 days after infection. These authors suggested that the host's molecules recognized by EhV capsids may be modified or absent in haploid cells, allowing them to become 'invisible' to viruses. Zoochlorellae (such as *P. bursaria* chlorella) are protected from viral attacks (e.g. by PBCV-1) when they are taken up into their host symbiont cells (see above).

More active mechanisms have also been described which can affect each step in the viral cycle, including interaction with the host cell surface, viral DNA entry, viral replication or viral release, as any of these steps might form a barrier to viral propagation.

Adsorption is mediated by receptors used by viruses and which belong to widely different families of proteins, carbohydrates or lipids, often in complex cell surface matrix structures (Baranowski, 2001). Viral receptors are naturally occurring cellular molecules that serve physiological functions for the cell, which have been hijacked by the virus for adsorbing to the cell. Resistance to viral adsorption conferred by host cell mutations could include (i) receptor structure modification, (ii) alteration of receptor accessibility, (iii) decrease in the number of receptors on the cell surface and/or (iv) loss of receptor sites. However, cellular or molecular descriptions of these four processes are rather scarce (Labrie *et al.*, 2010). For eukaryotic

phytoplankton, Tarutani, Nagasaki, and Yamaguchi (2006) demonstrated the importance of adsorption in determining viral susceptibility and specificity since viruses of the red tide-forming Raphidophyceae *H. akashiwo* could not adsorb to resistant cells. In another example, Waters and Chan (1982), using a high multiplicity of infection, found that only 50% of the green microalga *M. pusilla* cells lysed after 9 h and a high proportion of viruses did not adsorb while all susceptible cells should have been infected during the first hour. They cloned virus-resistant cells and showed that a mutant virus was able to infect these resistant cells. They suggested that hosts may change composition or conformation of their surface molecules to resist viral pressure, through spontaneous mutation. Another mechanism could be the secretion of extracellular viral inhibitors such as the cell wall sulphated polysaccharide of *Porphyridium* sp., which prevents viral access to cell receptors (Huheihel *et al.*, 2002). Brussaard *et al.* (2007) showed that colonial forms of *Phaeocystis pouchetii* are resistant to viral infection, in contrast to individual cells. Thus, colonial forms, which are surrounded by an 'outer skin', were protected from viral adsorption. In *O. tauri*, two types of resistance were described but in both cases viruses could absorb to the host membrane. However, the mechanism of this immunity to infection remains unknown (Thomas *et al.*, 2011). Overall, the resistance mechanisms of blocking viral entry remain unknown in marine organisms.

Little is known about resistance mechanisms operating at the level of the next step of infection, i.e. the viral replication inside the host cells. The only example described so far for marine phytoplankton is for the dinoflagellate *H. circularisquama* (Tomaru *et al.,* 2009). They transfected the viral RNA genome into resistant cells to see whether this host was permissive or not to viral replication and found that intracellular viral RNA replication was interrupted in virus-resistant cells, but the molecular mechanism responsible is unknown.

The last step of the lytic cycle is the release of viral particles, which in known protist–virus interactions most frequently occurs as a 'burst' from the host cell, at a point when the cell is full of new virus particles. Viral dissemination is thus usually rapid and intense. However, infected host cells can survive and replicate due to lysogeny or chronic infection. Although lysogeny frequently occurs in prokaryotes, a chronic type of infection has only recently come to light in eukaryotic phytoplankton (Thomas *et al.*, 2011). This could be due to an experimental bias since most of the approaches being based on observation of lysis to track viruses. Chronic infections, where viruses adopt a regulated replication within their host and

where their expulsion operates by simple diffusion through the cell wall or by budding has been reported in the green picoeukaryote *O. tauri*. In this species, a virus-resistant cell type, named 'resistant producer (R^P)', was able to produce one to three viruses per cell and per day without lysing. Viruses were released through vesicles formed at host membrane (Thomas *et al.*, 2011). R^P cells were so named, rather than chronically infected because they appeared to grow at a similar rate to wild-type cells in parallel cultures. However, competition experiments, mixing R^P and resistant non-producing (immune) cells, did reveal them to have a small reduction in fitness (Thomas *et al.*, 2011). In the *E. huxleyi*–EhV-86 interaction, Pagarete *et al.* (2009) suggested that sphingolipids promote the formation of lipid rafts in the membrane of the alga *E. huxleyi,* which can then become focal points on the membrane for viral budding and release. As the infection progresses, there is a massive increase in sphingolipid requirement and the accumulation of sphingolipids within infected cells triggers virion release through host programmed cell death (PCD). The expression pattern of the sphingolipids pathway in coccolithophorids suggests that sphingolipid biosynthesis driven by the virus is a crucial factor for successful dissemination of viruses. Pagarete *et al.* (2009) speculated that a host-specific sphingolipid could be involved as a bioactive lipid-signalling molecule among host cells, able to trigger meiosis in a fraction of the cell population and allowing them to escape viral infection (Frada *et al.*, 2008).

How can viruses persist in the environment if spontaneous resistance to them occurs frequently? Theory suggests that the coexistence of susceptible and resistant cells may be due to a trade-off between competitive ability and reduced mortality (Lenski, 1988) and that development of viral resistance has physiological costs related to a decrease in fitness of other functionalities (Weinbauer, 2004). Bohannan and Lenski (2000) confirmed this fitness cost associated with the evolution of resistance to viruses. The cost of resistance is often observed to be reduced growth rate. As an example, virus-resistant *P. pouchetii* cells grow 50% more slowly than control cells (Haaber & Middelboe, 2009). Resource uptake might also be reduced by mutations affecting the host cell surface, lowering competitiveness (Bohannan, Travisano, & Lenski, *et al.*, 1999). Susceptible cells allow the virus to persist and the virus in turn prevents the susceptible cell population from competitively excluding the resistant cells, establishing a dynamical equilibrium between hosts and viruses in the environment. The fitness cost of resistance can be eliminated through the reversion of resistance into a susceptible phenotype. When the virus is absent, the resistant organisms are less fit than their

susceptible counterparts, increasing the probability of extinction for resistant cells. Variations of fitness associated with virus resistance could then structure microbial communities in aquatic ecosystems.

An adaptation in a virus lineage may change the selection pressure on the host lineage, giving rise to a counter-adaptation. If this occurs reciprocally, an unstable runaway escalation or 'arms race' may result (Comeau & Krisch, 2005; Dawkins & Krebs, 1979) producing strong influences on the dynamics of viruses and their hosts in natural systems (Middelboe *et al.*, 2001). Adaptative responses include viral change to recognize new host receptors, production of proteins by the host that mask the phage receptor and production of extracellular matrices as physical barrier between phages and their receptors (Labrie *et al.*, 2010). Coexistence of hosts and viruses may thus lead to clonal successions of specific host–virus systems over time. In a study of *H. akashiwo,* Tarutani, Nagasaki, and Yamaguchi (2000) observed three periods in which hosts and viruses differed. At first, susceptible cells were dominant and decreased with time due to the viral pressure, then virus-resistant cells dominated, consequently reducing the viral population, and finally, susceptible host cells dominated presumably because a decrease in the abundance of the lytic viruses might allow the growth of susceptible cells.

4. DO VIRUSES AND HOSTS SHARE THEIR GENETIC INFORMATION BY LATERAL GENE TRANSFER?

Viruses are frequently seen as 'gene robbers' (Moreira & López-García, 2009), containing a more or less important fraction of genes acquired from an external source, eukaryotic, prokaryotic or viral (see Gogarten & Gogarten, 2009). This might perhaps explain the huge variation in genome sizes of viruses (Monier *et al.*, 2007). However, the amount of transferred genetic material in viral genomes is still subject to intense debate (Monier *et al.*, 2007; Moreira & Brochier-Armanet, 2008), owing to the very high number of genes with no known homologues (ORFans) in viruses. Another explanation of these discrepancies is linked to the different methods used to uncover lateral (or horizontal) gene transfer (LGT): compositional methods can lead to an overestimation of the number of transferred genes (Monier *et al.*, 2007), while phylogenetic approaches may underestimate it (Fischer *et al.,* 2010). In all cases, LGT is acknowledged to be of primary importance in marine viruses, including NCLDV (Iyer *et al.*, 2006, Filée and Chandler, 2010) within which the Phycodnaviridae, algal dsDNA viruses, form a very

diverse group (Bidle & Vardi, 2011). Among the organisms that might donate genes to viruses, their hosts are prime candidates because of the intimacy of this relationship and the evolutionary pressure that each partner exerts on the other. Here, we would like to underline a fundamental difference between prokaryotic and eukaryotic host–virus systems. In prokaryotes, viruses (bacteriophages), genetic transduction and the widespread high frequency of homologous recombination facilitates gene flow (see Abedon (2009) for a review). In eukaryotic systems, the genetic material is 'compartmentalized' and species' barriers are usually strict, limiting interspecies genetic flux. Host cells are larger and the virus life cycle usually occurs in specific compartments (nucleus, organelles or cytoplasmic regions), depending on the host–virus interaction in question and on the stage of the viral infection. Only in the last few years, as complete genomes for some eukaryotic hosts and their viruses have become available (Filée, Pouget, & Chandler, 2008), has it been possible to question the presence and amount of LGT between eukaryotic hosts and their viruses. We will focus here on this particular category of LGT, involving algal viruses and their hosts. The amount of gene transferred to viruses from their hosts appears to be low (Monier *et al.,* 2007), whether the number of total LGT is considered to be important or not (most of the 'foreign' genes likely to have been transferred to virus genomes are from bacterial origin). Several studies have suggested the occurrence of gene transfers between phages and their cyanobacterial hosts (see Lindell *et al.*, (2004)), but such transfers between viruses and eukaryotic phototrophic hosts take place too and have only recently started to be documented. Certainly, the most spectacular example suggested for such transfers involves an entire metabolic pathway for sphingolipid biosynthesis in the coccolithophore *E. huxleyi* and its virus EhV (Monier *et al.*, 2009; Pagarete *et al.*, 2009). This pathway seems to be functional in the virus and its host, though its exact role in the infection is still not clear but may involve PCD process. Because of its extent (seven genes are involved) and its functional activity in the virus and its host, this LGT likely reflects the evolutionary arms race within this association (Bidle & Vardi, 2011). Viral sphingolipids are suspected to be involved in virion release (Pagarete *et al.*, 2009), while PCD induced by host sphingolipids could limit the spread of viral infection (Vardi *et al.*, 2009). In *P. bursaria* and its viruses, host—virus LGT are also suspected (Filée *et al.*, 2008). In the Mamiellales–prasinovirus system (Derelle *et al.,* 2008; Moreau *et al.*, 2010), Weynberg *et al.* (2011) have also shown good support for host–virus LGTs, such as the transfer of a heat-shock protein between *Bathycoccus prasinos* and its virus BpV (Moreau

et al., 2010) and a gene encoding a phosphate transporter in OtV–2 from its *Ostreococcus* host (Weynberg *et al.*, 2011). However, it is generally difficult to infer whether transfers happened from virus to host or from host to virus. It seems likely that both processes can take place (Monier *et al.*, 2009), making viruses potential shuttles to convey genes from a host to another. This could have some important evolutionary consequences, especially if one viral strain can infect several host species.

5. ARE VIRUSES SPECIFIC TO ONE OR MORE HOST SPECIES, AND HOW ARE THESE PARTNERS EVOLVING TOGETHER?

Most viruses appear to be fairly specific for their host species, though they might infect several host strains within the same species (Clerissi *et al.,* 2012; Nagasaki & Yamaguchi, 1998; Nagasaki *et al.*, 2003; Derelle *et al.,* 2008; Sahlsten, 1998; Sandaa, 2008; see Wommack & Colwell (2000), for a review). However, a problem here is to define the species status of hosts, which in the case of unicellular clonal populations is not usually straightforward because there are few algae for which good genetic data are available. In some cases, algal viruses infect hosts from different geographic regions or from more different phylogenetic clades (e.g. Bellec, Grimsley, & Desdevises, 2010; Zingone *et al.,* 2006). This ability for the same viral clone to infect different host strains can have important consequences in planktonic ecosystems, in their role in the maintenance of genetic diversity in hosts and regarding the possibility to transfer genes between related hosts. The mechanisms for host specificity are still unknown but this subject is being investigated at a molecular level (Tomaru, Shirai, & Nagasaki, 2008), in particular in relation to surface proteins (Nagasaki *et al.*, 2005). This generally high host specificity (at least at the level of a species) suggests the existence of cospeciation patterns between algal viruses and their hosts, but this has never been properly investigated. Cospeciation between viruses and their hosts is often assumed, and information about host divergence is used to infer speciation events in viruses and to estimate their evolutionary rates (Firth *et al.*, 2010). However, this should first be assessed, and previous studies on host–virus evolutionary interactions (Gottschling *et al.,* 2011; Jackson & Charleston, 2004) suggest that a significant cospeciation signal is generally present in the coevolutionary history of viruses and their hosts (but not always, see Nemirov, Leirs, Lundkvist, & Olsson, 2010). However, strict

cospeciation is not the rule (e.g. Ramsden, Holmes, & Charleston, 2009) even in the terrestrial ecosystems investigated, where hosts can be separated by physical barriers limiting or precluding contact between host species and then host-switching events (such as in the papillomavirus–host association [Rector *et al.*, 2007]). In the aquatic, and particularly marine, environment, such barriers are more diffuse or absent, and the occurrence of a significant cophylogenetic signal would reflect the close adaptation of viruses to their hosts more than the absence of opportunity to host switch. In the Mamiellales–prasinovirus system, currently available data suggest that cospeciation is not very strict (see Moreau *et al.*, 2010) but that it is probably significant (Bellec, Grimsley, Moreau, & Desdevises, 2009). Further work is needed to clarify this point.

6. RED QUEENS AND WHITE PAWNS – WHICH PARTNER IS EVOLVING THE FASTEST?

Moreau *et al.* (2010) showed from genomic data that the evolutionary divergence between prasinoviruses and their hosts is clearly higher than that between their respective viruses. If significant cospeciation is indeed occurring in this association, this would support the 'White Pawn' hypothesis (see below) that Mamiellales evolve faster than their viruses, a very surprising situation considering current knowledge on virus evolutionary rates (see Duffy & Holmes, 2008). Moreau *et al.* (2010) also hypothesized that one reason for the lower evolutionary divergence of the viruses might be that since viruses must remain dormant (e.g. in the sediment) until they find a suitable host cell, and since host cells must divide frequently to remain viable, then the host genomes would be replicated less frequently than the viral genomes – the so-called 'White Pawn' hypothesis. Providing that coevolution of hosts and viruses has occurred, a topsy–turvy situation could thus arise; in contrast to viruses attacking relatively long-lived animal or multicellular plant hosts, protist viruses are much more likely to attack a host genome that has already undergone numerous replications since its ancestor was infected by the same virus. While there is no concrete evidence that this may be the case, it is known that viral lysates remain infective after many years in the refrigerator and that viruses of toxic dinoflagellates return to infect seasonal algal blooms (Nagasaki *et al.*, 2004). It is difficult to predict what the possible effect of the White Pawn hypothesis might be, especially given the lack of knowledge about effective

viral population sizes and mutation rates. However, Ogata and Claverie (2007) found that in contrast to bacterial genomes where ORFs with no predicted biological functionalities (called ORFans) evolve faster than non-ORFans, in large dsDNA viruses, ORFans appear to evolve in a similar way to non-ORFans. This observation thus supports the notion of ancient viral genes but could also be a possible effect of White Pawn evolution.

The high number of ORFans in virus genomes, mentioned above, can be explained in at least two different ways: (1) it reflects the great ancestry of viruses that could have appeared before, and could be at the origin of, the cellular world (Forterre, 2006), and cellular life forms have gained from viruses only a subset of these diverse genes. This would explain why so many genes in viruses have no cellular homologues; and (2) viruses evolve very fast in comparison to their hosts, so that any gene gained from a host quickly accumulates so many substitutions and changes that any homology with the original cellular gene is more or less quickly lost. This hypothesis is compatible with the view that viruses are by-product of cells, mobile genetic elements (Moreira & López-García, 2009). In order to assess the contributions of these two alternatives, that are not necessarily mutually exclusive to the process of evolution, it thus becomes crucial to assess the relative rates of evolution in viruses and their hosts. Ideally, this requires these rates to be independently estimated, for example using fossil or geological calibrations or time-structured data from samples obtained at different dates (Firth *et al.*, 2010). In the absence of such data and as an approximate solution, if a very clear cospeciation pattern exists between viruses and their hosts, temporal information about hosts (which is generally more readily available than for their viruses) can be transposed to corresponding viruses, and relative rates of homologous genes can be compared. To our knowledge, none of these data exist yet for algal viruses, but large dsDNA viruses are frequently assumed to evolve slowly than other kinds of viruses (e.g. Drake, 1991; Gago *et al.*, 2009; Rector *et al.*, 2007). From time-structured data, Firth *et al.* (2010) have suggested that dsDNA viruses may evolve much faster than previously thought, at rates comparable to those of RNA viruses.

7. WHAT IS NEXT?

Historically, the ease of growth of the host organism under laboratory conditions has always played a determinant role for the discovery of new viruses. This was true for bacteriophages and has been important for algal

viruses. *Chlorella* and *Emiliana*, for example, are easily grown in liquid media and can be plated out in soft agar. However, culture techniques for the great majority of microbial species are not yet developed (Amann *et al.*, 1990; Cuvelier *et al.*, 2010), and this remains an important hurdle for the analysis of viruses. Until now, it has not usually been possible to produce a sufficient quantity of pure clonal virus particles for sequencing from microbial eukaryotes, except for when the host cells can be cultured in the laboratory.

7.1. Single-Cell Genomics

The technology for sequencing the DNA of individual cells by multiple displacement amplification (MDA; Blanco, Bernad, Esteban, & Salas, 1992, Hellani et al., 2004) is progressing, and this technique promises to yield much information about uncultured and unculturable microbial species. Under carefully controlled laboratory conditions, it is possible to isolate individual microbial cells by flow cytometry and serial dilutions, extract their DNAs and randomly amplify their DNAs. Single bacterial cells were first used to demonstrate the feasibility of this procedure (Martinez-Garcia *et al.*, 2012; Raghunathan *et al.*, 2005; Stepanauskas & Sieracki, 2007). In a similar way, for flow-sorted eukaryotic phytoplankton, MDA has recently permitted whole-genome amplification from a group of cells (Lepere *et al.*, 2011) and genome amplification from single cells (Heywood, Sieracki, Bellows, Poulton, & Stepanauskas, 2011). However, for technical reasons, only partial genomes of the eukaryotic host cells can be amplified at present (M. Sieracki, personal communication). Yoon *et al.* (2011) amplified genomes from single picobiliphyte cells and found that these cells were infected with either large dsDNA viruses or ssDNA viruses. The assembly of new viral genomes from single uncultivated cells thus holds much promise for the future since an appreciable proportion of host cells may be present in a population, and many copies of complete viral are captured within the cell, providing a natural over-representation of these sequences relative to those of the host. In addition, even if the host species is uncultivable, its sequence data should permit its phylogenetic classification to be determined, a cherry on the cake.

7.2. Metagenomics

Using the mimivirus genome sequence as a probe to find sequences in public databases with the BLAST software tool revealed a surprising number of matches, hinting that NCLDV may be common in seawater (Monier, Claverie, & Ogata, 2008; Monier, Larsen, *et al.*, 2008), an observation supported by

the recent single-cell genomics analyses (see above). Assembly of complete algal viral genomes from metagenomic data is now a realistic approach to find new viral genomes since new bioinformatics methodologies for assembling complete genomes from paired-end reads promise to revolutionize metagenomics (Iverson *et al.*, 2012, and following chapter on Environmental Genomics, Toulza, Blanc-Mathieu, Gourbiere, & Piganeau, 2012). Given the further development of NGS techniques, sufficient computing capacities, and adequate software programs, the description of the genomes of algae and their viruses in aquatic environments is set to make a quantum leap, but will experimental biology be able to reveal some of the unknown biological functionalities encoded in this bottom line of life on Earth?

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