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Anne-Claire Baudoux, H Lebredonchel, H Dehmer, M Latimier, R Edern, Fabienne Rigaut-Jalabert, P Ge, Laure Guillou, Elodie Foulon, Yves Bozec, et al.

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A-C. Baudoux[1,2*], H. Lebredonchel[3,4], H. Dehmer[1,2], M. Latimier[1,2], R. Edern[1,2], F. Rigaut-Jalabert[1,2], P. Ge[1,2], L. Guillou[1,2], E. Foulon[1,2], Y. Bozec[1,2], T. Cariou[1,2], Y. Desdevises[3,4], E. Derelle[3,4], N. Grimsley[3,4], H. Moreau[3,4], and N. Simon[1,2]

[1] Sorbonne University, UPMC Paris 06, Station Biologique de Roscoff, UMR7144, Roscoff, France

[2] CNRS, Station Biologique de Roscoff, UMR7144 and FR2424, Roscoff, France

[3] Sorbonne University, UPMC Paris 06, Observatoire Océanologique de Banyuls-sur-mer, UMR7232, Banyuls-sur-mer, France

[4] CNRS, Observatoire Océanologique de Banyuls-sur-mer, UMR7232, Banyuls-sur-mer, France

Running title: *Micromonas* – virus interactions

For correspondence. Email acbaudoux@sb-roscoff.fr; Tel (+33)2 98 29 25 37; Fax (+33)2 98 29 23 24

Abstract

The genus *Micromonas* comprises distinct genetic clades that commonly dominate eukaryotic phytoplankton community from polar to tropical waters. This phytoplankter is also recurrently infected by abundant and genetically diverse prasinoviruses. Here we report on the interplay between prasinoviruses and *Micromonas* with regards to the genetic diversity of this host. During one year, we monitored the abundance of 3 clades of *Micromonas* and their viruses in the Western English Channel both in the environment, using clade-specific probes and flow cytometry, and in the laboratory, using clonal isolates of *Micromonas* clades to assay for their viruses by plaque-forming units. We showed that the seasonal fluctuations of *Micromona*s clades were closely mirrored by the abundances of their corresponding viruses, indicating that the members of *Micromonas* genus are susceptible to viral infection, regardless of their genetic affiliation. The characterization of 45 viral strains revealed that *Micromonas* clades are attacked by specific virus populations , which exhibit distinctive host clade specificity, life history traits, and genetic diversity. However, some viruses can also cross-infect different host clades suggesting a mechanism of horizontal gene transfer within the genus *Micromonas* . This study provides novel insights into the impact of viral infection for the ecology and evolution of the prominent phytoplankter *Micromonas*.

Key words: Mamiellophyceae, Phycodnaviridae, Prasinovirus, host specificities, life history traits, DNA polymerase, English Channel

Introduction

Viruses are undoubtedly the most abundant biological entities in the ocean and they intimately interact with every facet of the marine biosphere. Through these interactions, viruses profoundly influence the global biogeochemical cycles by altering the structure and the function of marine communities and by contributing to the cycling of major elements (for review see Suttle, 2005, 2007; Sime-Ngando, 2014). In spite of their global-scale implications, the nature and the dynamics of virus-host interactions in marine environments are far from understood and many basic, yet fundamental, questions remain unsolved: Who are the hosts infected by marine viruses? What are the infection strategies evolved by viruses? How do virus-host interactions vary in time and space? In this respect, characterizing the interplay of ecologically relevant virus-host model systems is a prerequisite for advancing our understanding of virus impacts in nature.

Viruses that infect the cosmopolitan green picoalga *Micromonas* were first reported by (Pienaar, 1976) and they were found to co-occur with their hosts in a wide range of marine environments, from temperate to cold waters (e.g., Cottrell and Suttle, 1991, 1995a; Sahlsten, 1998; Zingone *et al.*, 1999). The majority of known *Micromonas* viruses belong to the genus *Prasinovirus* in the Phycodnaviridae family. Virions are tailless, they exhibit icosahedral capsids of 100 -125 nm in diameter and dsDNA genome of approx. 200 kb (Mayer and Taylor 1979; Cottrell and Suttle 1991; Zingone *et al.*, 1999). Yet, these prasinoviruses have variable tolerance to chloroform (Martinez et al. 2015) suggesting ultrastructural divergence and they exhibit significant level of genetic variation (Cottrell and Suttle, 1995b). These viruses display a marked seasonal dynamics (Sahlsten, 1998; Zingone *et al.*, 1999) and they were shown to cause considerable amount of mortality in their host population (Cottrell and Suttle, 1995a; Evans *et al.*, 2003). *Micromonas* strains show a great variability with respect to their susceptibility to infection, indicating that viruses do not only quantitatively but also

qualitatively regulate their host populations (Sahlsten, 1998; Zingone *et al.*, 1999, 2006). So far, the infection patterns and processes underlying these complex interactions are poorly understood. Of particular interest, the relation between the susceptibility to infection and the genetic diversity of *Micromonas* has seldom been investigated (Zingone *et al.*, 2006).

The ubiquitous genus *Micromonas* is genetically diverse and comprises an assemblage of 3 (Guillou *et al.*, 2004) to 5 (Šlapeta *et al.*, 2006; Worden, 2006) discrete phylogenetic clades (or lineages) of flagellated cells that correspond, at least, to 3 distinct species (Simon, unpublished). These clades are often sympatric in marine ecosystems (Foulon *et al.*, 2008; Šlapeta *et al.*, 2006). Yet, their relative contribution to total *Micromonas* abundance varies in time and space, suggesting that they occupy specific niches (Foulon *et al.*, 2008). The factors that regulate this clade dynamics are not clearly understood. In all likelihood, *Micromonas* clades exhibit differential responses to abiotic factors but they might also respond differently to predation risks, including those imposed by viruses.

To test whether *Micromonas* clades display differential sucspetibility to virus infection, we combined field and laboratory experiments on 45 novel viral isolates in order to characterize the interactions between the genetic clades of *Micromonas* and their viruses in the Western English Channel (WEC) throughout the year 2009. The WEC constitutes an ideal study site where *Micromonas* is known to dominate the picophytoplankton community (Not *et al.*, 2004). The three main *Micromonas* genetic types, designated as clades [A.ABC.12], [B.E.3], and [C.D.5] (Worden, 2006) and here referred to as clades A, B, and C, respectively, are recorded year round in this ecosystem (Foulon *et al.*, 2008). Our study revealed that *Micromonas* clades interact with specific viral populations that display distinctive dynamics and life history traits.

Results and Discussion

Physico-chemistry at the sampling station

Strong tidal mixing produces a permanently mixed water column at the long term monitoring coastal station SOMLIT-Astan (48°46′N, 3°58′W, Marrec *et al.*, 2013). During the sampling period (February to December 2009), the water temperature progressively increased from 8.8°C in February to a maximum of 16.9°C in September while salinity varied between 34.8 (March) and 35.3 (September). Nutrient dynamics varied according to the classical pattern observed in this area (Not *et al.*, 2004). Phosphate and nitrate concentrations showed comparable dynamics with minima recorded in summer (0.16 and 0.7 µM, respectively) and maxima during the winter period (0.53 and 11 µM, respectively). Detailed dynamics of biogeochemical variables at SOMLIT-Astan are publicly available on the observatory website (http://somlit-db.epoc.u-bordeaux1.fr/bdd.php).

Dynamics of *Micromonas* clades in the Western English Channel

The phytoplankton community at SOMLIT-Astan during this period was numerically dominated by picophytoplanktonic cells, which comprised, on average, 90% of the total community in 2009 (data not shown). Among these, the abundance of the picoeukaryotes varied between 2.1 and 16.5 × 10^3 cells mL^{-1} with *Micromonas* spp. accounting for 20% to 80% of the total counts as shown by a combination of flow cytometry (FCM) and TSA-FISH analyses (Fig. 1A). As reported previously, this prominent genus displayed marked seasonal dynamics with major peaks of abundance recorded in late spring (6.6 × 10^3 cells mL^{-1}), early summer (6.3 × 10^3 cells mL^{-1}), and mid-autumn (4.4 × 10^3 cells mL^{-1}) (Foulon *et al.*, 2008; Not *et al*., 2004).

The 3 main *Micromonas* genetic clades (hereafter referred to as clades A, B, and C) exhibit recurring seasonal dynamics in the WEC according to previous time series (Foulon *et*

al., 2008). We confirmed this pattern of succession in 2009 (Fig. 1B). *Micromonas* clade A numerically dominated the bloom from February to mid-May comprising 63 to 77% of the total counts. From June to December, both genetic types A and B equally contributed to *Micromonas* counts, although clade B cells showed a sudden, yet unexplained, drop in abundance on June 16. Cells that belonged to clade C were the least abundant accounting, on average, for 7 ± 6% of the total counts with somewhat higher contribution during the winter period (14%) than the remainder of the year (5%).

The ecological processes that influence the shifts in the relative abundance of *Micromonas* clades are unclear. It is traditionally believed that global-scale distribution and diversity of species are mostly driven by ability to withstand abiotic controls (Wiens et al. 2011). Previous study on the global distribution of *Micromonas* clades suggested that clade B tend to thrive in warmer coastal waters whereas clade C mostly occur during low-light conditions (Foulon *et al.*, 2008). At local spatial extents, it is however well accepted that species distribution is also influenced by biotic controls (Wiens et al. 2011). It is thus likely that besides eco-physiological adaptations, biotic interactions also regulate the observed *Micromonas* clade dynamics in the Western English Channel.

Dynamics of *Micromonas* viruses in the Western English Channel

Viruses that infect *Micromonas* (hereafter referred to as MicV), mainly prasinoviruses, have been observed and isolated from different oceanic regions (Cottrell and Suttle, 1991, 1995a; Sahlsten, 1998; Zingone *et al.*, 1999; Short and Short, 2008). FCM analyses of cultured isolates showed that these prasinoviruses consistently cluster within a well-defined population based on their nucleic acid fluorescence (upon Sybr green I staining) and side scatter properties (Fig. S1). In the WEC, this specific population accounted for a small but significant proportion (on average 10%) of the total virus counts and displayed a marked seasonal dynamics with concentration ranging from 3.4×10^5 mL^{-1} in February to 8.8×10^5

143 mL^{-1} in July 2009 (Fig. 2A). This FCM population probably does not exclusively comprise
144 *Micromonas* viruses, but counts of this viral population covary strikingly well with
145 *Micromonas* abundances ($r=0.675$, $n=20$, $p<0.005$), suggesting that viruses might perhaps
146 control abundance of this genus during the sampling period.

147 To further investigate this hypothesis, we quantified infectious MicV in the WEC by
148 plaque assay using cultures of each 3 *Micromonas* clades. Significant differences in the
149 number of PFU were detected depending on the phylotype of the host culture (Fig. 2B). As
150 also recorded for their hosts, PFU obtained on *Micromonas* clade A and B (hereafter, PFU-A
151 and PFU-B, respectively) were substantially more abundant than PFU formed on clade C-
152 hosts (hereafter PFU-C). During the sampling period, PFU-A abundance increased
153 concomitantly with the development of *Micromonas* clade A. Their maximum abundance (8
154 $\times 10^2$ virus mL^{-1}) was observed 2 weeks after the peak of host abundance (Figs. 1B and 2B).
155 The monitoring of PFU-B showed low concentration during the winter-spring period but their
156 dynamics was tightly coupled to their host abundance from the summer period until the end of
157 the year, reaching a peak of 11×10^2 virus mL^{-1} in autumn. Regarding PFU-C, infectious
158 particles were detected year round but their abundance was 10 to 100-fold lower than PFU-A
159 and -B with no clear relation to their specific host dynamics. Because of variable strain
160 susceptibilities to viral infection, it is difficult to compare our results to former PFU
161 monitoring on *Micromonas* host cell clades although maximal abundance were within the
162 same range ($1.0 - 4.6 \times 10^3$ PFU mL^{-1}, Cottrell and Suttle, 1991; Sahlsten, 1998; Zingone *et*
163 *al.*, 1999). For the same reason, this assay most likely underestimates the global abundance of
164 viruses that infect each *Micromonas* phylotype. Nonetheless, it provides unequivocal
165 evidence that *Micromonas*, regardless of their genetic clade, were subject to viral attack year
166 round in the WEC.
167

Micromonas virus - host interactions

The above results led us to question whether specific virus populations are associated to *Micromonas* clades and, if so, how these virus populations interact with their hosts. To tentatively address this question, 45 viruses (14-16 per host clade) were isolated throughout the sampling period and they were characterized. These viruses were selected randomly from a collection of 176 viral isolates obtained from plaques on *Micromonas* clade A, B, and C along the year 2009. Regardless of the host on which they were isolated, MicVs exhibited sizeable icosahedral capsids (110 to 130 nm in diameter) as determined by negative staining using transmission electron microscopy, and large dsDNA genome, as suggested by their flow cytometry signature upon Sybr green I staining (Fig. S1). We therefore assigned our isolates to the virus family Phycodnaviridae and the genus *Prasinovirus*.

Host specificities - The host range of these 45 prasinoviruses was then determined by pair-wise infection using 14 strains belonging to the 3 *Micromonas* clades (Table 1, Table S1). The large majority of our isolates (35 MicVs) were strictly clade-specific. They infected host strains that belonged only to the clade of their initial host. The remaining 10 viruses could propagate at comparable yield on hosts belonging to clades A and B. Six viruses (out of 15) isolated on a clade A strain could also infect clade B strains, and 4 viruses (out of 14) isolated on a clade B strain could also infect clade A strains. Within each main pattern of specificity (A, B, AB, and C), a remarkable clonal diversity was observed with 26 unique specificity profiles that included variable virus infection types ranging from generalist to specialist.

In order to test the structure of this infection network, we applied a network-based analysis according to Flores *et al.* (2011) and Weitz *et al.* (2013). As observed previously for phage-bacteria infection networks (Flores *et al.*, 2011), the structure of *Micromonas* - virus interactions was statistically modular. This statistical analysis applied at the whole matrix

scale and intra-module scale discriminated 3 modules, which comprised the viruses isolated on hosts from clade A, clade B, and clade C, respectively (Fig. S2). Altogether, these results suggest that *Micromonas* clade A, B, and C interact with distinct, yet diversified, viral populations. The observed variability in strain-specificity indicates that viruses influence the intraspecific diversity within each *Micromonas* clades. We also showed that host switches can occur between the more closely related host clades (those that belong to clades A and B), suggesting that viruses could also promote *Micromonas* diversity through horizontal gene transfers within this genus.

Virus infection strategies - One-step growth experiments conducted on 4 to 5 representative viruses per host clade indicated a similar clade-specific grouping of our isolates. This assay provided estimates of the virus burst size and latent period, which can be related to the virus life strategy. By analogy to the theory of the r- and K-selection (MacArthur, 1967), opportunistic (the most r-selected) viruses are those that exhibit short generation times and high burst sizes whereas less virulent (the most K-selected) viruses are those that induce low mortality in their host population (Suttle, 2007) and they propagate through latent or chronic infection as observed for specific *Ostreococcus* viruses (Thomas *et al.*, 2011). Based on these parameters, we detected divergent life strategies in *Micromonas* viruses depending on the host clades that they attack (Fig. 3). The viruses isolated on clade B hosts tended to be the most virulent with short latent period and relatively high progeny production, regardless of their infection range types (i.e. specialist or generalist) whereas the viruses isolated on clade C strains tended to be the least virulent with generally extended latent period (up to 30 h) and moderate to high burst size (Fig. 3). Viruses isolated on clade A hosts exhibited an intermediate phenotype with moderate latent period and low burst sizes.

In contrast to past theories (trade-offs hypothesis, (Poulin, 1998)), generalism (i.e., viruses that propagate on several host clades) was not associated with any compensatory effect on the burst size or on the latent period. The infection kinetics of the selected viral isolates does not correlate to the growth rates of their host (Fig. S3). , The observed differences in infection strategies could be explained by the dynamics of their respective host clade in the studied area. For example, *Micromonas* clade C is present year round at low abundance. It is thus tempting to speculate that viruses specific to this host clade had to evolve long latent periods to be maintained within the host population and fairly high progeny production to increase the probability of encounter with their host (Abedon *et al.*, 2001). By contrast, *Micromonas* clade B displays a marked seasonal dynamics in the WEC with high abundance recorded between mid-June and end-September. We could thus expect that corresponding viruses had to evolve an opportunist lifestyle to efficiently exploit their transient resources.

Phylogenetic relationships between *Micromonas* viruses

To investigate the phylogenetic relationships between these viral isolates, we amplified a fragment of the gene encoding their DNA polymerase (*polB*, Chen and Suttle, 1996, Table S2). This sequence is well conserved among Phycodnaviridae, yet there is sufficient sequence variability to build phylogenetic relationships between viruses from the same genus (Bellec *et al.*, 2009). In spite of their affiliation to the Phycodnaviridae family, the amplification of the *polB* gene using the AVS1/AVS2 primers failed for 8 of the 45 selected isolates including B-, C- and AB-specific viruses. The reason for this failure is unclear. We cannot exclude that the sequence targeted by the degenerated primers might be too divergent. It is also possible that these polymerases carry an intein that makes the PCR product too long to amplify easily under the tested conditions (Clerissi *et al.*, 2013).

The partial *polB* sequences of the remaining 37 isolates fell into 29 haplotypes (Fig. 4). The large majority of these haplotypes (24 out of 29) were new, i.e. different from *polB* sequences in public databases (BLAST analyses, http://www.ncbi.nlm.nih.gov/). In most cases, viral isolates that belonged to the same haplotype exhibited different specificity patterns, suggesting that *polB* sequencing underestimates the functional diversity of phycodnaviruses as reported previously (Baudoux and Brussaard, 2005; Bellec *et al.*, 2009; Clerissi *et al.*, 2012). In the phylogenetic trees, *polB* sequences of viruses infecting clade C hosts formed a well-supported cluster while viruses that infect clade A and clade B hosts distributed into multiple branches suggesting a higher level of genetic diversity. Interestingly, regardless of the host strain they infect, viruses that were highly specific (that infect one single strain among all strains tested) fell apart from other viruses (i.e. RCC4232 and RCC4240, 4242, 4243, Fig. 4 and Table 1). The prasinoviruses that infect *Ostreococcus tauri* showed a similar phylogenetic pattern and tended to cluster with counterparts that exhibit similar infection range types (Clerissi *et al.*, 2012).

This recurrent correlation between *polB* phylogeny and the number of hosts infected by a given prasinovirus is somewhat puzzling. The *polB* gene is indeed used as a neutral marker of evolution in Phycodnaviridae (Chen and Suttle, 1996; Dunigan *et al.*, 2006), that is, this gene is well-conserved among this virus family whereas specificity markers are expected to be more variable genes (Clerissi *et al.*, 2012). Furthermore, virus-host specificity is typically determined during events upstream the virus DNA replication. Several studies reported that the virus attachment on host cell surface determines the susceptibility of marine plankton to infection (e.g., Tarutani *et al.*, 2006; Mizumoto *et al.*, 2007; Stoddard *et al.*, 2007). In other words, the evolution of resistance to viral infection is mostly due to changes in host surface properties that limit viral attachment. Recently, Thomas *et al.* (2011) evidenced an intriguing mode of resistance in the prasinophyte *Ostreococcus tauri.* This study

demonstrated that prasinoviruses could still attach on resistant strains but they did not produce viral progeny. It is thus likely that the resistance mechanism was not due to a change in the host receptor but rather occurred at the DNA entry or DNA replication stage, in which the *polB* gene product would be involved. Alternatively, viral attachment and internalisation might be a two-stage process as in reoviruses (Reiter *et al.*, 2011). The notion that DNA replication machinery might be involved in host specificity is of course highly speculative but the repeatedly observed link between *polB* clustering and prasinovirus infectivity range certainly deserves appropriate investigation.

Concluding remarks

In the Western English Channel, a complex assemblage of viruses is associated to the dominating picophytoplankton species *Micromonas spp*. Our study revealed, for the first time, that the main *Micromonas* genetic clades (clade A, clade B, and clade C) are attacked by specific virus populations which displayed distinctive dynamics and life history traits. The apparently high variability in *Micromonas* virus specificities suggests that viruses maintain a high genetic diversity within each of these clades. However, host switching can occur (particularly for hosts that belong to the clade A and B) suggesting that virus could also influence *Micromonas* diversity through horizontal gene transfers. The complete genome sequencing of representative viruses and hosts should provide insightful information on the extent of these putative genetic exchanges. It is of course critical to keep in mind that our model-based approach only reflects a partial picture of the actual diversity of virus-host interactions. Still, these approaches provide invaluable information to improve our understanding of the mechanisms of virus-host interactions as well as the evolution and outcomes of these interactions in natural environments.

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