

# Micromonas versus virus: New experimental insights challenge viral impact

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## Summary

Viruses have recurrently been hypothesized as instrumental in driving microbial population diversity. Nonetheless, viral mediated co-existence of r/k-strategists, predicted in the Killing-the-Winner (KtW) hypothesis, remains controversial and demands empirical evidence. Therefore, we measured the life strategy parameters that characterize the relevant system *Micromonas*-*Micromonas* Virus (MicV). A large number of host and viral strains (37 and 17, respectively) were used in a total of 629 cross-infectivity tests. Algal and viral abundances were monitored by flow cytometry and used to calculate values of growth rate, resistance capacity, and viral production. Two main assumptions of the KtW model, namely (1) a resistance-associated cost on growth and (2) a negative correlation between resistance and viral production capacity, were mildly observed and lacked statistical significance. *Micromonas* strains infected by more MicV strains presented higher lysis and viral production rates as the number of infectious virus strains increased, suggesting a ‘one-gate’ regulation of infection in this system. MicV strains demonstrated a vast range of virion production capacity, which unexpectedly grew with increasing host-range. Overall, the significant trends observed in here demonstrate strong co-interactions at different levels between *Micromonas* and MicV populations, however, the role of viruses

as major driving force in phytoplankton fitness wasn't explicitly observed.

## Introduction

It is now well acknowledged that marine viruses interact with their cellular counterparts in the microbial kingdom on an unforeseen scale (Fuhrman, 1999; Brussaard, 2004; Suttle, 2007; Breitbart, 2012). An estimated  $10^{23}$  new infections occur every second in the ocean, and induce the mortality through cell lysis of an important fraction of marine microbes, which include bacteria, phytoplankton and zooplankton (Suttle and Chan, 1994; Suttle, 2005; Baudoux and Brussaard, 2008; Mojica *et al.*, 2015). The extent of viral mediated mortality can be remarkably high during phytoplankton blooms where it can reach near 100% cell mortality (Bratbak *et al.*, 1993; Brussaard *et al.*, 1996a,b; Baudoux *et al.*, 2006). Viral lysis is of fundamental importance to global biogeochemical cycling and ecosystem function (Fuhrman, 1999). Understanding viral impact on marine microbes is hence an indispensable part in our efforts to model marine ecosystem functioning. Although it has become evident that viruses can contribute to the structure and diversity of microbial communities (Waterbury and Valois, 1993; Suttle, 1994; Brussaard, 2004), the extent to which they impact their eukaryotic hosts remains unknown. These relentless viral interactions with their cellular hosts represent, at least theoretically, an important factor regulating phytoplankton fitness (Thingstad and Lignell, 1997).

In the pelagic realm, where microorganisms have a life expectancy of hours to days before they are either consumed by a predator or lysed by a virus (Våge *et al.*, 2013), selection pressure for efficient life strategies including competition for nutrients and defence against predation or parasitism is likely to be high. In theory at least, if viruses are major drivers of phytoplankton fitness, then the expectation would be to observe the existence of r and k-selected cells, demonstrating a clear trade-off between their capacity to grow or to fight viral infection (resistance) (Thingstad, 2000; Suttle, 2007). Several studies suggested that viral infection induces morphological and metabolic changes in host population and, in most cases, the development of resistance is associated with a fitness penalty

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for the resistant microbes (Brockhurst *et al.*, 2004; Brockhurst *et al.*, 2005; Benmayor *et al.*, 2008; Riemann and Grossart, 2008; Middelboe *et al.*, 2009). For example, in the marine cyanobacteria *Synechococcus*, such cost of resistance (COR) was manifested by a 20% reduction in fitness, in terms of growth rate and competitive capacity, compared to ancestral susceptible strains (Lennon *et al.*, 2007). In the case of *Prochlorococcus*, another major cyanobacterial group, fitness costs have been associated with resistance-rendering mutations in genomic island regions (Avrani *et al.*, 2011). If the existence of a COR seems to be well acknowledged for some bacterial strains, the question remains open regarding eukaryotic phytoplankton.

Dynamic models of viral-host interactions in marine environments have, at their core, the same set of assumptions as were made for dynamics of *Escherichia coli* and associated phage in chemostats (Levin *et al.*, 1977). These 'box' models consider how abundances of hosts, viruses and resources change with time due to the effects of resource uptake, cell division, viral-induced mortality of hosts, and viral reproduction via lysis. Extensions of these models to the ocean have taken into account a greater complexity of factors, including the possible covariation of bacterial and viral life history traits (Middelboe, 2000; Middelboe *et al.*, 2001; Weitz, 2008). The most prominent one is the Killing the Winner model (KtW), which predicts that viruses represent a balancing factor that allows the co-existence of host species with different growth rates (Thingstad and Lignell, 1997). Viruses that specifically kill (or control) fast-growing host cells provide niches for the development of slower-growing cells. This would be the *sine qua non* condition that allows the maintenance of highly dynamic and diverse microbial communities (Thingstad, 2000).

In this study, we present the first extensive estimation of viral resistance-associated fitness costs, in terms of growth rate, in the relevant marine phytoplankton *Micromonas*. This prominent phytoplankton recurrently dominates coastal picoeukaryotic communities in a wide range of marine environments (Knightjones and Walne, 1951; Thomsen and Buck, 1998a,b) and represents an important contributor to global primary production (Marañón *et al.*, 2001; Worden *et al.*, 2004). Recent phylogenetic studies have demonstrated the existence of at least three major *Micromonas* clades (van Baren *et al.*, 2016). *Micromonas* cells are recurrently infected by viruses in contrasted marine ecosystems. The vast majority of *Micromonas* viruses (hereafter referred to as MicV) belong to the genus *Prasinovirus*, within the *Phycodnaviridae* family (double stranded DNA viruses that infect marine or freshwater eukaryotic algae) (Dunigan *et al.*, 2006; Bellec *et al.*, 2009). These lytic viruses are wide-spread (Cottrell and Suttle, 1991), abundant, genetically diverse (Cottrell and Suttle, 1995), and exhibit variable host specificity and life strategies (Baudoux and Brussaard, 2005). Taking

advantage of the large number of different but closely related host and virus strains for this host-virus system, an extensive array of cross-infectivity experiments was conducted in order to investigate the existence of a COR and the applicability of the KtW model in this eukaryotic phytoplankton-virus system.

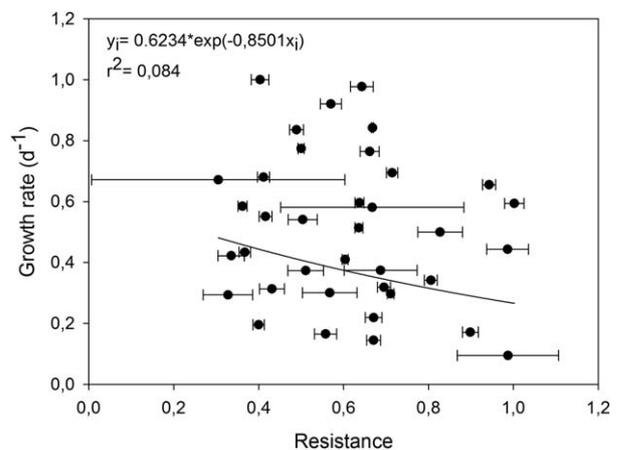
## Results

### Host-based parameters

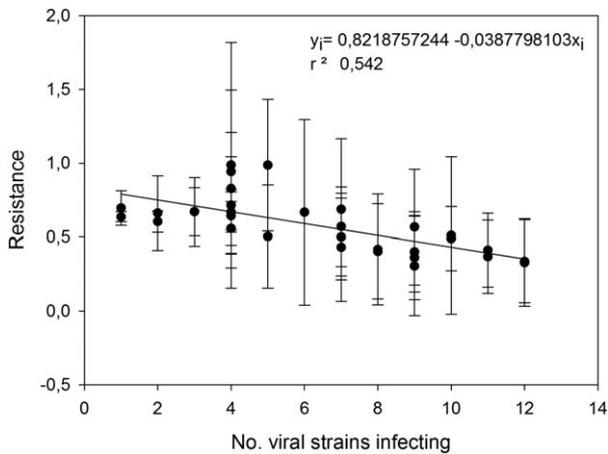
*Micromonas* average growth rate ( $\mu$ ) varied among strains from 0.09 (SD  $\pm$  0.11) to 0.98 (SD  $\pm$  0.02)  $\text{d}^{-1}$ . Variability in growth rate was not correlated to phylogenetic clade affiliation ( $F(2, 34) = 0.095$ ,  $p = 0.909$ ) (Supporting Information Fig. S1).

In order to investigate the existence of a trade-off between growth and resistance capacities, we measured the level of resistance of each *Micromonas* strain to viral infection in two manners: (1) percentage of cells that were not lysed after incubation with viruses (Fig. 1) and (2) the number of MicV strains that successfully produced progeny on that host (Supporting Information Fig. S2). Using either type of resistance measurement we did not identify a significant correlation between growth rate and resistance (Pearson's  $r = -0.174$ ,  $p = 0.302$ , and Pearson's  $r = 0.143$ ,  $p = 0.397$ , respectively). Yet, a tendency for resistance to decrease as growth rate increased was observed, but the level of variation was high and the trend not statistically significant (Fig. 1). Curiously, in three cases out of 201 successful infections the algae grew faster with viral inoculum than in the control.

*Micromonas* strains with lower levels of viral-provoked cell lysis were infected by a significantly lower number of viral strains (Pearson's  $r = -0.737$ ,  $p < 0.01$ ) (Fig. 2). Also



**Fig. 1.** Growth rates of different *Micromonas* strains as a function of resistance to viral infection (as % of surviving cells). Resistance tended to increase as growth rate ( $\mu$ ) decreased but the correlation was not statistically significant.

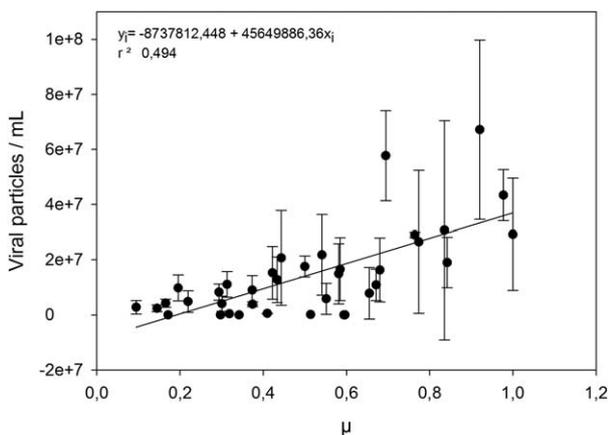


**Fig. 2.** Resistance (as % of surviving cells) of different *Micromonas* strains as a function of the number of viral strains that induced cell lysis. The number of viruses infecting each *Micromonas* strain clearly decreased as their capacity to survive infection increased.

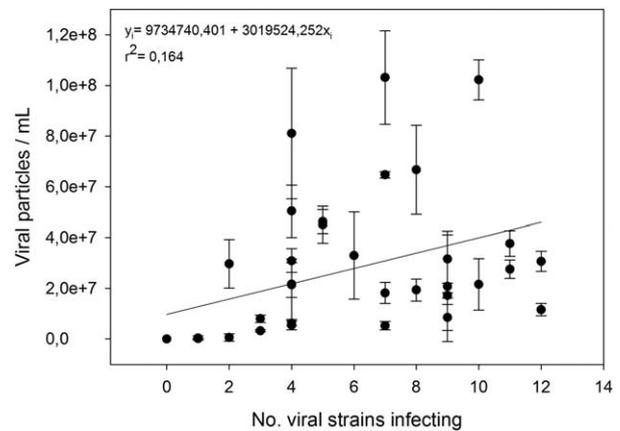
significant (Pearson's  $r = 0.697$ ,  $p < 0.01$ ) was the correlation between growth rate and viral production, with the fastest growing cells being capable of producing more virions (Fig. 3). Maximum viral production was positively correlated with the number of viral strains infecting each algal strain (Pearson's  $r = 0.406$ ,  $p = 0.0128$ ) (Fig. 4).

#### Virus-based parameters

We observed an important variation in virion production capacity among the different MicV strains, which was clearly reflected in the 'Maximal Viral Production' (Supporting Information Fig. S3b). On the other hand, 'Average Viral Production' (Supporting Information Fig. S3a) indicated that while some viral strains tend to be consistent regarding the amount of progeny they produce on each



**Fig. 3.** A positive correlation was observed between growth rate ( $\mu$ ) and viral production capacity among the *Micromonas* strains under study.



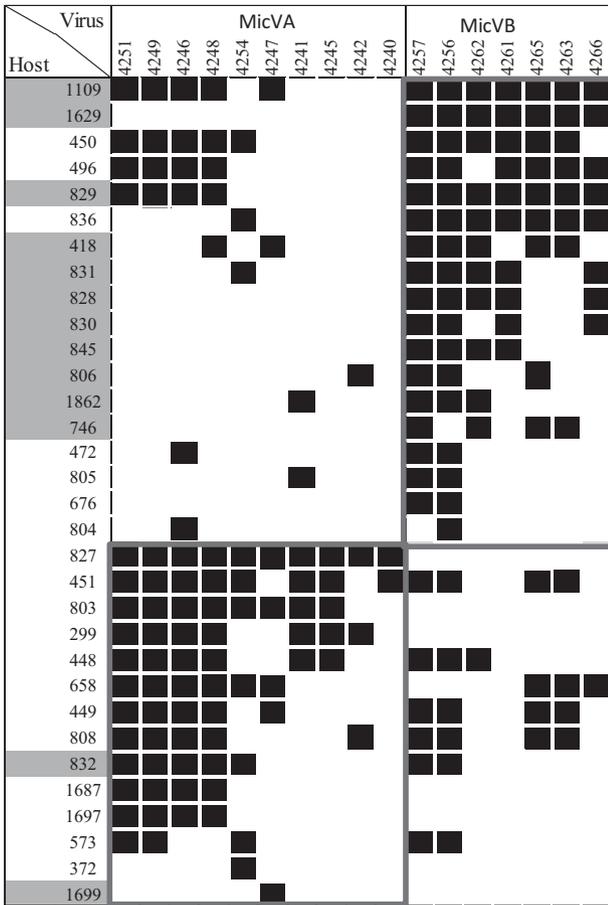
**Fig. 4.** Correlation between number of viral strains infecting each *Micromonas* strain and maximum viral production. Note that when an algal strain is infected by more viruses, the maximum viral production is also higher.

different host (small error bars), other MicV strains displayed variable virion production depending on the host (large error bars). There were no significant relationships in average and maximal viral production associated with clade affiliation of host strains as determined by one-way ANOVA ( $F(1, 15) = 0.042$ ,  $p = 0.839$ ;  $F(1, 15) = 0.253$ ,  $p = 0.621$ , respectively). Among the 17 MicV strains used in this study only 2, RCC4240 and RCC4245 were strictly clade-specific (both isolated in *Micromonas* clade A) (Guilou *et al.*, 2004; Baudoux *et al.*, 2015). The remaining MicV isolates could infect hosts from clades A and B (Fig. 5). Host range among MicV strains was very variable, from generalists who could infect up to 23 host strains (e.g., RCC4256 and RCC4247) to specialists that could infect only two strains (e.g. RCC4240). Viruses isolated from *Micromonas* clade A strains tended to be more clade-specific than those isolated from clade B (Fig. 5) ( $F(1, 15) = 12.21$ ,  $p = 0.0033$ ).

We observed an unexpected tendency for more generalist viruses to display higher viral production of new progeny than viruses with narrow host ranges (Fig. 6). This was supported by significant positive correlations observed between average/maximum viral production and the number of different algal strains that a virus can infect (Pearson's  $r = 0.680$ ,  $p = 0.00268$  and Pearson's  $r = 0.842$ ,  $p < 0.01$ , respectively).

#### Modularity and nestedness analysis

The bipartite network analysis applied to the whole host-range matrix displayed a combination of nestedness and modularity levels. Two modules were clearly discriminated, and comprised viruses isolated from hosts that belong to clades A and B, respectively (Fig. 5). At the same time, each module showed an intra-modular nested structure. In

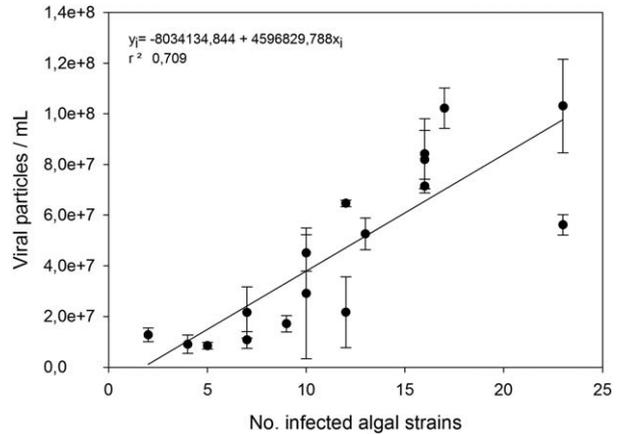


**Fig. 5.** Host range of the 17 MicV strains. Both, viral and algal strains, are organized according to the modular structure derived from the modularity analysis (see Methods for details). Black squares indicate successful infections. White and grey colours in the host column correspond to the phylogenetic clades A and B, respectively. The only *Micromonas* strain from clade C that was used was not infected by any of the viruses and hence is not represented.

that nested pattern specialist viruses tended to infect the most susceptible hosts, while the viruses with broader host-range infected hosts with higher resistance (Fig. 5).

**Discussion**

In this study, we performed an extensive array of cross-infectivity experiments between *Micromonas* and MicV to investigate viral impact on phytoplankton fitness and the applicability of the KtW model to this eukaryotic phytoplankton-virus system. To our knowledge, this is the first study where KtW has been empirically tested using a large collection of eukaryotic phytoplankton strains and viruses. One of the most consensual hypotheses about viral impact on aquatic microbial organisms (including phytoplankton) is the co-existence of fast-growers along with others that specialized in resisting viral infection with incurring costs on their growth capacity. This trade-off, often



**Fig. 6.** Maximal viral production increased significantly with expanding host-range. We can appreciate the increasing of the viral production as the virus increases its host range. The same results were obtained with average viral production (Fig. S4).

called Cost of Resistance, is also one of the pillars of the KtW model (Thingstad, 2000; Short, 2012; Våge *et al.*, 2013). The existence/magnitude of this trade-off has not yet been demonstrated in eukaryotic phytoplankton (Heath and Collins, 2016). It was reasonable to expect that a trend should emerge when analysing resistance capacity among a large number of hosts with variable growth rates as attempted here. In our study we observed a tendency for growth rate to decrease with increasing resistance (Fig. 1), which would point towards the existence of a trade-off between these two parameters; as the KtW model predicts. However, and most noticeable, that tendency was not significant. Hence, our observations contribute with scepticism on the prevalence of COR in this phytoplankton-virus system. Nonetheless, and despite the lack of statistical significance, the mild decreasing slope observed between growth and resistance capacities would agree rather with a lower than a high COR value (Våge *et al.*, 2013). The identification of a COR in phytoplankton-virus systems is a demanding challenge. Phytoplankton cell fitness results from different selective pressures, such as: nutrient harvesting capacity, resistance to grazing, or tolerance to abiotic factors (Lythgoe and Chao, 2003; Brockhurst *et al.*, 2004; Meaden *et al.*, 2015). Viruses should hence be only another selective force amongst a pool of vectors that are not all pulling cell fitness in the same direction. This can, and most probably will, cloud the trace of COR in any phytoplankton-virus system. The impact of other selective forces on *Micromonas* could explain the pronounced levels of variation measured, and why the correlation between growth rate and resistance observed in this study was not significant. In this eukaryotic phytoplankton system, viruses do appear to have a measurable impact on host fitness, but they are probably not the only or main source of selection. If they were, that

'growth rate versus resistance' decreasing trend would have been significant.

In rare occasions (3 out of 201) we observed increased algal growth when in the presence of viruses. This interesting phenomenon has also been previously reported in the related phytoplankton *Ostreococcus tauri* (Thomas *et al.*, 2011). We can speculate that this could be linked to a group response to viral presence, where infected cells could use cell signalling warning mechanisms that would speed up cell division in other cells. However, to these days we do not have an answer to this question.

In the *Micromonas* - MicV system the capacity of a cell to produce virions was strongly linked to its growth rate ( $\mu$ ) (Fig. 3). This was theoretically expected and reported previously for other host/virus systems (Moebus, 1996a,b; Middelboe, 2000; Parada *et al.*, 2006; Motegi and Nagata, 2007). Cell growth rate is generally related to the number of ribosomes, which can vary significantly between rapid and slow-growing cells (Knoll *et al.*, 1999). The number of ribosomes is responsible for the protein synthesizing capacity at the time of infection, which can then condition the rate of virus production (Hadas *et al.*, 1997). Less intuitive is the KtW prediction that resistance should be correlated with viral production. Namely, that the higher the resistance the smaller the number of virions produced (Våge *et al.*, 2013). We did not observe (with statistical significance) such correlation in our data.

Resistance (percentage of surviving cells) did significantly decrease with the number of viral strains capable of infecting the host (Fig. 2). This means that, in strains that are susceptible to fewer viruses, the few viruses that infect them do not provoke severe cell lysis. On the other extreme, we have strains that not only are incapable of keeping viruses outside their gates, but also provide a viral-friendly intracellular environment that will lead to significantly increased cell lysis. In some co-evolution experiments with marine bacteria, it has been proven that the gain of resistance to a virus after infection leads to a broader resistance to many other viruses (Middelboe *et al.*, 2009; Avrani *et al.*, 2011; Flores *et al.*, 2011; Marston *et al.*, 2012). In those cases, immunity seems to be primarily regulated in one (or few) main gate(s). If one viral strain is capable of breaching that gate, then the probably that another viral strain does too increases. This is what we observed in *Micromonas* too. One should expect, if viruses were the main selective force driving *Micromonas* fitness, that those extremely immune-diminished strains would compensate with significantly higher growth rates. Our results show that that is not always the case, suggesting that other factors are probably as important trimming the adaptive space of this phytoplankton species.

From the virus perspective, our results show that there are hosts that offer better conditions for viral replication, allowing a broader range of viral strains to maximize their

viral production. We also observed increased viral production with increasing number of possible hosts. This is curious for it means that generalist viruses produce more progeny than specialist viruses. This apparently is a paradox. How can then specialist viruses compete with viruses that have a bigger host pool and can produce more virions? And if being a generalist virus is such an advantage, why did we register the existence of so many specialized MicV strains? In several cases a generalist strategy has been reported to have inherent disadvantages, especially due to antagonistic pleiotropy (Duffy *et al.*, 2006; Elena *et al.*, 2009; Nikolin *et al.*, 2012; Keen, 2014), accumulation of neutral mutations that are deleterious in alternative niches (Kawecki, 1994), and slower evolutionary rates compared with specialists (Whitlock, 1996). In the case of *Micromonas*, a recent study indicates that the thermal stability of a generalist virus (RCC4265) was significantly lower when compared to the one displayed by a specialist virus (RCC4229) (Demory *et al.*, 2017), suggesting structural differences. These type of trade-offs would explain why a great variation in host-ranges is observed in nature, instead of a predominance of generalist viruses. It should also be mentioned that in recent studies with *Tobacco etch potyvirus* (TEV), Bedhomme *et al.* (2012) questioned this classical theoretical need for a trade-off. They have done this by presenting 'un-costly' strong adaptive potential to new hosts.

The MicV strains demonstrated capacity to infect hosts isolated from contrasted marine ecosystems and different oceans. This reveals the maturity of this co-evolutionary interaction (Thingstad *et al.*, 2014) and possibly implies strong stabilizing selection of host defence mechanisms and/or the loss of resistance mechanisms (Waterbury and Valois, 1993). In the virus-host interaction network (VHIN) obtained in our study (Fig. 5), there was a significant modularity match between viral strains and the host clade they were isolated from. This modularity is mostly likely related to the phylogenetic distances between hosts (Baudoux *et al.*, 2015; Weitz, 2016). A high degree of intra-modular nestedness was also revealed in the *Micromonas* - MicV system. Such nested structure is characteristic of VHINs that form ordered subsets of each other (Flores *et al.*, 2016). Two popular models attempt to explain the process that would lead to such co-evolutionary pattern: sequential gene-for-gene adaptations (GFG) or the appearance of viral alleles that facilitate infection against specific host defensive alleles (Matching Allele, MA) (Flor, 1955; Agrawal and Lively, 2002). These two models lead to different outcomes. The MA model implies that COR is similar among all alleles, predicting local adaptation and specialization, and resulting in a Red Queen scenario where frequency-dependent selections favour rare genotypes. The GFG model would result in an Arms Race dynamics where one genotype replaces another leading to continual

improvements in both populations. Weitz *et al.* (2013) debate why this last scenario is more suitable to explain nestedness in planktonic VHINs. The intra-modular nestedness observed is hence consistent with the KtW hypothesis where co-infection is taken into consideration, leading to nested interaction matrices and viruses with broader host ranges (Thingstad *et al.*, 2014). Finally, it should be noted that the VHIN analysis presented here would potentially benefit from the use of quantitative data, instead of its binary form that not only loses information as it introduces bias that will accentuate some features and mask others (Beckett and Williams, 2013).

## Conclusions

Overall, the major findings in this study are in agreement with the trends expected theoretically, notably those supporting the KtW model. However, the incapacity to unequivocally demonstrate the existence of a strong resistance-associated trade-off or shows that there is large space for improvement not only of this model, but also of our theories on phytoplankton-virus interactions in the oceans. The unexpected higher viral production registered for generalist viruses also adds in that need to better understand the parameters that regulate phytoplankton-virus relationships. Those efforts should also consider the incorporation of increased complexity and relevant ecological context, namely in situations where cells have to compete for limited resources.

## Materials and methods

### *Micromonas* and *MicV* strains

Algal and viral strains were obtained from the Roscoff Culture Collection, France. A total of 37 *Micromonas* strains were maintained in 30 mL crystal flasks with IMR  $\frac{1}{2}$  medium (Klochova *et al.*, 2006) at 16°C and a 14:10 h light:dark illumination cycle at 155  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  irradiance. The strains belonged to the three major phylogenetic clades previously identified for this species (22 from clade A, 14 from clade B and 1 from clade C) (Guillou *et al.*, 2004) and have been isolated from a wide geographic range (Supporting Information Table S1). *MicV* viral strains have been previously assigned to two main groups, *MicV*-A and *MicV*-B, according to the clade affiliation of the host from which they were isolated (Baudoux *et al.*, 2015). We selected 17 *MicV* strains, with 10 and 7 belonging to groups A and B, respectively (Supporting Information Table S2). For all viral isolates, viral stocks were produced by infection of exponentially growing *Micromonas* RCC827, RCC451 and RCC829 strains (Supporting Information Table S2). Viral lysates were centrifuged at 12 000  $\times g$  for 20 min and the supernatant was filtered through a 0.45  $\mu\text{m}$  syringe filter (Whatman plc, GE Healthcare Life Sciences, Kent, England) to remove cellular debris. Stocks were kept at 4°C in the dark and were renewed so often as to never be more than 2 weeks old before inoculation.

### Cross-infectivity experiments

Cross-infectivity experiments were performed between all the *Micromonas* and *MicV* strains and represented a total of 629 crossings. Prior to the experiment, *Micromonas* cultures were maintained in exponential growth phase with cell concentrations ranging from  $10^5$  to  $10^6$  cells  $\text{mL}^{-1}$ . The experiments were performed in 24 well culture plates under the same temperature and light conditions mentioned above. Two mL of each algal culture at  $1 \times 10^5$  cells  $\text{mL}^{-1}$ , were inoculated in triplicate with each of the 17 viral strains at a concentration of  $1 \times 10^6$  viral particles  $\text{mL}^{-1}$ , resulting in multiplicity of infection (MOI) of 10. Three replicates of uninfected culture were also used as a control for each *Micromonas* strain. Cultures were incubated for 72h.

### Enumeration of algae and viruses

At times 0 h and 72 h, samples (500  $\mu\text{l}$ ) were taken for algal and viral counting using a FACSCalibur BC flow cytometer (Becton–Dickinson, Biosciences, NJ, USA) provided with an air-cooled laser procuring an excitation beam of 15 mW at 488 nm. Viral samples were fixed with 20  $\mu\text{l}$  of glutaraldehyde (final concentration 1%) for 30 min at 4°C, and frozen at  $-80^\circ\text{C}$  until further use. For flow cytometry analysis, samples were thawed, diluted 500-fold in TE buffer (10:1 mM Tris:EDTA, pH 8, filtered through 0,2 $\mu\text{m}$ ), and stained with SYBR Green I 10000x diluted (Invitrogen, 1600 Faraday Avenue, PO Box 6482, Carlsbad CA, 92008 United States) for 10 min at 80°C before analysis. Algal enumeration was conducted on fresh samples and cell population were discriminated using chlorophyll auto-fluorescence (670 LP) and SSC signals. Viruses were discriminated using the green fluorescence (530/30) and SSC signals.

### Growth rate, resistance, viral production

Growth rates ( $\mu$ ) were calculated for each *Micromonas* strain using the control non-inoculated incubations according to the following formula (Levasseur *et al.*, 1993):

$$\mu = \text{Ln} (N_2/N_1)/t$$

Where  $N_1$  and  $N_2$  were the cell concentrations at the beginning and end of the experiment, respectively, and  $t$  was the incubation time, in days. Growth rate values were normalized to values between 0 and 1.

The level of resistance of each *Micromonas* strain to viral infection was measured in two manners. First manner was the percentage of cells that were not lysed after incubation with viruses, by comparison with the non-inoculated controls. For each *Micromonas* strain a resistance value was hence calculated against each of the 17 *MicV* strains. Those 17 resistance values were then averaged to obtain an overall resistance capacity for each alga strain. Resistance was also estimated as the number of *MicV* strains that successfully produced progeny on that host.

Viral production was estimated as the capacity of each viral strain to produce new progeny in a specific host. This was calculated as the difference between final and initial viral concentrations. These values were averaged to obtain an

average viral production capacity for each viral strain. The maximum amount of viruses that each MicV strain could produce was also registered as 'Maximum viral production.'

Potential correlations between the different parameters (growth rate, resistance, and viral production) were investigated with regression slopes and statistical probability analyses, using either Anova (*F*) or Pearson analysis.

#### Host-virus network analysis

In order to test the structure of the infection network, we used the BiMat package for Matlab (Flores *et al.*, 2016). This network-based analysis was applied on a binary matrix where 0 referred to no lysis and 1 to lysis. The unique *Micromonas* strain from clade c was not included in this analysis. The NODF algorithm was used to measure nestedness and is based on overlap and decreasing fill (Almeida-Neto *et al.*, 2008). It returns a score between 0 and 1, where 1 corresponds to a perfectly nested structure. Modularity (Q<sub>b</sub>) was calculated using the Leading-Eigenvector algorithm (Newman, 2006). The value Q<sub>b</sub>, introduced by Barber (2007), is calculated using the standard bipartite modularity function. To quantify the statistical significance of the nestedness (NODF) and modularity (Q<sub>b</sub>), 100 null random matrices (for each) were created with the null model Equiprobable (a random matrix in which all the interactions are uniformly permuted).

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### Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's website.

**Table S1.** *Micromonas* strain information, including geographical origin and taxonomical clade based on previously determined 18S rRNA sequence data (Guillou *et al.*, 2004; Slapeta *et al.*, 2006; Lovejoy *et al.*, 2007). NI = No information.

**Table S2.** MicV strains used in this study, their isolation information, along with the respective *Micromonas* strain in which each viral stock is prepared.

**Fig. S1.** Growth rate of the *Micromonas* strains used in the infection experiment, calculated according to Levasseur *et al.* (1993). Values correspond to the control samples and *Micromonas* clades names A, B and C correspond to those of Guillou *et al.* (2004).

**Fig. S2.** Growth rate ( $\mu$ ) and number of viral strains infecting each algal strain correlation. Growth rate values were normalized between 0 and 1. We can observe that a higher number of viruses infect the algae as the growth rate of these increases.

**Fig. S3.** Viral production (A) and maximum viral production per MicV strain (B).

**Fig. S4.** As with maximal viral production, average viral production also increased significantly with expanding host-range.