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REVIEW AND SYNTHESIS

Making sense of virus size and the tradeoffs shaping viral fitness

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Abstract

Viruses span an impressive size range, with genome length varying a thousandfold and virion volume nearly a millionfold. For cellular organisms the scaling of traits with size is a pervasive influence on ecological processes, but whether size plays a central role in viral ecology is unknown. Here, we focus on viruses of aquatic unicellular organisms, which exhibit the greatest known range of virus size. We outline hypotheses within a quantitative framework, and analyse data where available, to consider how size affects the primary components of viral fitness. We argue that larger viruses have fewer offspring per infection and slower contact rates with host cells, but a larger genome tends to increase infection efficiency, broaden host range, and potentially increase attachment success and decrease decay rate. These countervailing selective pressures may explain why a breadth of sizes exist and even coexist when infecting the same host populations. Oligotrophic ecosystems may be enriched in "giant" viruses, because environments with resource-limited phagotrophs at low concentrations may select for broader host range, better control of host metabolism, lower decay rate and a physical size that mimics bacterial prey. Finally, we describe where further research is needed to understand the ecology and evolution of viral size diversity.

Keywords

Allometric scaling, bacteria, burst size, competition, decay rate, diffusivity, giant virus, host range, phage, phytoplankton.

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INTRODUCTION

Viruses are ubiquitous and abundant molecular symbionts that influence individual health, population and community dynamics, evolution, and biogeochemistry, across the tree of life. By nature, viruses are smaller than the cells they infect, but the range of virus sizes is nonetheless substantial, with lengths of viral particles (virions) varying from 17 nm to *c.* 1.5 µm, and genome size varying from *c.* 1 kb to 2.5 Mb (Campillo-Balderas *et al.*, 2015). The largest 'giant' viruses have primarily been isolated from unicellular protists (Campillo-Balderas *et al.*, 2015; Wilhelm *et al.*, 2017), although there is metagenomic evidence for 'megaphages' of prokaryotes with genomes up to 716 kb (Devoto *et al.*, 2019; Al-Shayeb *et al.*, 2020), and a chaetognath appears to be infected by viruses 1.25 µm in length (Shinn and Bullard, 2018). In contrast, the known viruses of plants and fungi have genomes < 30 kb (Campillo-Balderas *et al.*, 2015). At a finer phylogenetic scale, particular species or strains of prokaryotes and eukaryotes can be infected by viruses of very different size. For example, the marine bacterium *Cellulophaga baltica* is infected by phages ranging from 6.5 to 242 kb (Holmfeldt *et al.*, 2007) and the marine dinoflagellate *Heterocapsa circularisquama* is infected by a 4.4 kb ssRNA virus and a 365 kb dsDNA virus (Tomaru *et al.*, 2009).

For cellular life, body size is a 'master trait' that influences numerous organismal properties, such as metabolic rate, nutrient uptake affinity, predator-prey linkages, and population growth rate (Finkel 2001, Brown *et al.*, 2004; Fuchs and Franks, 2010; Edwards *et al.*, 2012). The substantial variation in virus size raises the question of whether size plays a similar central role in virus ecology and evolution (Record *et al.*, 2016). For example, are there general relationships between virus size and key viral traits? Do size-related tradeoffs lead to selection for different sizes of viruses infecting different kinds of hosts, or under different environmental conditions? How do viruses of different size coexist when infecting the same host population? There are straightforward physical reasons why being smaller should be advantageous for a virus: smaller particles should encounter hosts faster due to greater diffusivity, at least in aquatic systems or aqueous microenvironments, and limited host resources during infection can be partitioned among a greater number of 'offspring'. The existence of a spectrum of virus sizes implies that the costs of increased size can be offset by countervailing benefits. Virion size and genome size are tightly correlated (Cui *et al.*, 2014), and benefits of increased size likely derive from the functions encoded by viral genes, including better control of attachment to the host, replication, transcription, translation and host metabolism; strategies countering host antiviral defenses; and repair of damaged viral nucleic acids (Sharon *et al.*, 2011; Samson *et al.*, 2013; Fischer *et al.*, 2014; Koonin and Yutin, 2019, Mendoza *et al.*, 2020). These functions could increase the probability of successful infection, the number of virions produced per infection, the range of hosts than can be successfully infected, and/or the persistence of virions in the extracellular environment.

In this study, we focus on the question of how virus size affects key viral traits, and how these traits affect viral fitness. We utilize models of viral population biology and transport, synthesize and analyse available data, and outline major knowledge gaps and future research directions. We focus primarily on viruses that infect aquatic microbes (unicellular prokaryotes and eukaryotes), as these viruses are known to vary greatly in size and have been relatively well-studied in culture. However, most of the concepts we develop should be useful for understanding viruses in general. The remainder of the paper is structured as follows: first we introduce a quantitative framework for relating viral traits to fitness; then we focus on individual traits, reviewing the literature to outline hypotheses for how each trait may vary with virus size, and presenting analyses of existing data to test the hypotheses when possible; finally we summarize our findings and suggest the necessary next steps for advancing our understanding of virus size diversity. Details of data compilation and statistical methods are presented in Methods S1 and Table S1.

THEORY FOR HOW VIRAL FITNESS IS DETERMINED BY KEY VIRAL TRAITS

A variety of theoretical work has considered how viral traits may evolve under realistic constraints, such as the optimal latent period (Wang *et al.*, 1996; Bull, 2006; Edwards and Steward, 2018) or lytic vs. temperate life history strategies (Stewart and Levin, 1984, Maslov and Sneppen, 2015; Weitz *et al.*, 2019). Here, we review how readily measurable viral traits affect fitness, to provide a quantitative framework for how size may influence fitness via these traits. Future work could analyse how virus size is predicted to evolve, depending on specific environmental conditions and trait-size relationships.

In order to connect a metric of fitness to virus traits (burst size, latent period, contact rate, decay rate, etc.) we imagine a lytic virus population that may compete for hosts with one or more additional virus populations. Using a simple model of virus-microbe population dynamics, at steady state the density of the limiting resource, uninfected cells, is $S^* = \frac{d}{k(be^{-mL}-1)}$ (Eqn.1; Eqn. S4 in Appendix S1; Levin *et al.*, 1977). Here, S is the density of uninfected cells, m is the host mortality rate from causes other than viral infection, b is the burst size (new virions produced per infection), L is the latent period, d is the viral decay rate and k is the effective adsorption rate—the rate at which successful new infections are formed. The parameter k can be decomposed into subprocesses, and here we define $k = caw$, where c is the contact rate at which host and virus encounter each other, a is the attachment efficiency (probability that encounter leads to successful attachment) and w is the probability that attachment leads to a successful infection (eventual lysis of the host, releasing new virions). The distinction between contact rate and attachment efficiency will be important when considering the role of virus size.

The quantity S^* is the uninfected host density at which growth of the viral population balances the decay rate. Therefore, this is also the host density threshold required for persistence of a viral population, and if the host density is initially greater it will be cropped down to this level at steady state. S^* can be used as a measure of viral fitness because if there are two viral strains, and $S_1^* < S_2^*$, then strain 1 will drive the host to a lower density and competitively exclude the other strain at equilibrium (Tilman, 1982). Bonachela and Levin (2014) used a very similar model and proved that the strategy that minimizes S* is the evolutionarily stable strategy (ESS), meaning it cannot be invaded by any alternative strategy. It is more intuitive to consider the inverse of S^* as a metric of fitness, because a smaller S* equates to greater competitive ability; the inverse of S^* is $\frac{k\left(be^{-mL}-1\right)}{d}$. This quantity is the average net number of new virions produced per infection $(be^{-mL}-1)$, scaled by the rate of successfully encountering a new host (k) relative to the rate of 'dying' while waiting to encounter a new host (d). This analysis assumes that host-virus dynamics reach a steady-state attractor, which may not be true, but the S^* quantity is still a useful index of competitive ability. Furthermore, in a simpler model with no latent period it can be shown that S^* predicts the winner in competition even if populations fluctuate (Appendix S2). This analysis relates traits to fitness for lytic viruses, which we focus on in this study due to a greater accumulation of relevant trait data, but potential effects of size on viruses with temperate strategies will be discussed as well.

In Appendix S3 we extend this analysis to ask under what conditions a broader host range is selected for. The main result is that fitness (measured in terms of competitive outcomes) is proportional to host range (measured as the number of host strains that can be infected). This means that a generalist virus and a specialist virus will have similar fitness when the cost of generalism is directly proportional to host range breadth. For example, if a generalist virus can infect twice as many strains as specialist viruses, but has an adsorption rate that is 50% lower on each strain, it will be competitively equivalent to the specialists. If the cost of generalism is lower then generalism will be favoured, and vice versa. These results are derived from a simple model but they allow us to quantify, as a first approximation, how tradeoffs involving host range and other viral traits may affect selection on virus size.

HOW DOES VIRUS SIZE AFFECT CONTACT RATE AND ATTACHMENT TO HOST CELLS?

We now consider key viral traits individually, to outline expectations for how virus size may affect each trait and analyse relevant data where it is available. Adsorption rate is important for viral fitness (eqn. 1) because it determines the rate at which new infections can be established, as well as the time a viral particle spends in the extracellular environment where it may be exposed to UV radiation, adsorption to non-host material, ingestion, etc. (Suttle and Chen, 1992; Noble and Fuhrman, 1997). As described above, it is useful to separate the adsorption rate into the contact rate c (per capita rate at which hosts and viruses encounter each other) and the attachment efficiency a (probability of successful attachment to the host). In theory the contact rate will depend on physical processes of Brownian motion, advection and turbulence, while attachment efficiency will be a function of host receptor availability, affinity of the receptor for the virus, and mechanisms such as reversible binding by viral fibres that keep the virus from diffusing away from the host (Schwartz, 1976; Wickham *et al*., 1990; Storms and Sauvageau, 2015).

Physical theory for contact rate typically starts by asking what the rate would be if viruses relied solely on molecular diffusion to encounter their hosts, and if all viruses that contact the host are adsorbed. Under pure diffusion the contact rate is predicted to be:

$$c = 4\pi r_H D_V \tag{2}$$

where r_H is host radius and D_V is the diffusivity of the virus (Murray and Jackson, 1992). The diffusivity of a spherical virus is predicted to be:

$$D_V = \frac{k_B T}{6\pi\eta r_V} \tag{3}$$

where k_B is the Boltzmann constant, T is temperature, η is dynamic viscosity of water and r_V is the virion radius. Therefore, the diffusion-limited contact rate is predicted to be inversely proportional to virus diameter, which is a substantial fitness cost of increasing size.

The diffusivity of viruses due to Brownian motion is low enough that contact rates could be increased considerably by processes that create fluid motion relative to the host cell. Rates of diffusion can be enhanced by advective flow arising from host motility, feeding currents or host sinking, and diffusion can also be enhanced by turbulence, which causes shear around the host cell (Murray and Jackson, 1992). Host motility, feeding currents, sinking and turbulence can also lead to the host cell encountering the virus by direct interception (i.e., without the aid of Brownian motion), which is the mechanism by which small flagellates are thought to encounter immotile prey (Shimeta, 1993; Kiørboe, 2008).

The results in Appendix S4 show that diffusion enhanced by advection is the primary mechanism that could significantly increase virus contact rates beyond the 'pure diffusion' scenario (eqn. 2), and so we consider here how that mechanism depends on virus size. An approximate formula for contact rate when advection enhances diffusion is:

$$c = 4\pi r_H D_V 0.5\left[1 + \left(1 + 2\frac{u_H r_H}{D_V}\right)^{1/3}\right] \tag{4}$$

where u_H is the velocity of the host relative to the surrounding water (Murray and Jackson, 1992). Because the formula includes the term $\left(1 + 2\frac{u_H r_H}{D_V}\right)^{1/3}$, the enhancement of contact rates due to advection is greater as u_H and r_H increase, and also as r_V increases (because D_V is in the denominator, and $D_\mathrm{V} \sim 1/r_\mathrm{V}$). In other words, host motility matters more for bigger hosts, for hosts that swim faster, and for bigger, less diffusive viruses. These effects are visualized in Fig. 1a, which shows that contact rate always declines with virus size, but the penalty for large size is slightly less when hosts are motile. The effect of host motility on contact rates ranges from modest (~2-fold increase for a 20 nm virus infecting a 1 μm host swimming at 30 μm s^{-1}) to large (>10-fold increase for a 300 nm virus infecting a 20 μm host swimming at 250 μm s^{-1}). Langlois *et al.* (2009) use numerical simulation to show that the effect of swimming on diffusion may be underestimated by the formulas used here, but the effect is only *c*. 2-fold for the relevant particle sizes and swimming speeds.

Although Brownian motion, potentially enhanced by advection, is expected to drive contact rates, it is possible that direct interception is important for particularly large viruses encountering hosts that generate a strong current (Figs S1 and S2). The simple model of interception based on Stokes flow may underestimate particle contact rates (Langlois *et al*., 2009), and if interception is great enough then 'giant' viruses could have greater contact rates than slightly smaller viruses (Figs S1 and 2). Therefore, a better understanding of the physics of particle encounter will be important for understanding virus ecology and size evolution, in addition to understanding predator-prey dynamics among microbes.

We have compiled published data on the adsorption rates of viruses of aquatic microbes (cyanobacteria, heterotrophic bacteria, eukaryotic phytoplankton, heterotrophic protists; Table S2; Methods S1) to ask whether virus size has any relation to contact rate or attachment efficiency. There is a tendency for adsorption rate to be greater for larger viruses (Fig. 1b), but this may be due to larger viruses having hosts that are larger, more motile, or both. We therefore used eqns 2–4 to ask how observed adsorption rates compare to the theoretical maximum (Fig. 1c). Predictions and observations are positively correlated ($r = 0.68$), but many of the observations are 10-100x lower than predicted. This is consistent with a previous analysis of adsorption rates of phages (Talmy *et al*., 2019) and could be due to sparse host receptors, a low binding affinity of the virus ligand to the host receptor or a lack of mechanisms for keeping the virus from diffusing away from the host before irreversible binding to the receptor occurs (Storms and Sauvageau, 2015). Several of the large eukaryotic viruses have adsorption rates that are higher than the prediction based on Brownian motion alone (eqn. 2), but accounting for host swimming (eqn. 4) brings them closer to the 1:1 line (Fig. S3). However, a number of the phages also have motile hosts, and including reasonable numbers for host swimming speed moves them further below the 1:1 line (Fig. S3).

Fig. 1D shows the proximity of adsorption rate to the theoretical maximum as a function of virus size. Although the sample size is limited, it is noteworthy that the five largest viruses are all fairly close to the theoretical maximum. It is possible that some of the functions encoded in larger genomes increase attachment efficiency, such as the synthesis of proteins that aid attachment to host glycans (Rodrigues *et al*., 2015), or a greater diversity of proteins for binding host receptors (Schwarzer *et al*., 2012). If attachment efficiency is promoted strongly by certain genes, this could outweigh the reduction in diffusivity associated with larger size, increasing the actual adsorption rate. If a virus is large enough to induce phagocytosis this could also potentially increase encounter efficiency relative to other mechanisms of entry, and some of the largest viruses have been shown to enter their amoeba hosts via phagocytosis (Rodrigues *et al*., 2016). If phagocytosis is in fact a more efficient entry mechanism then large size

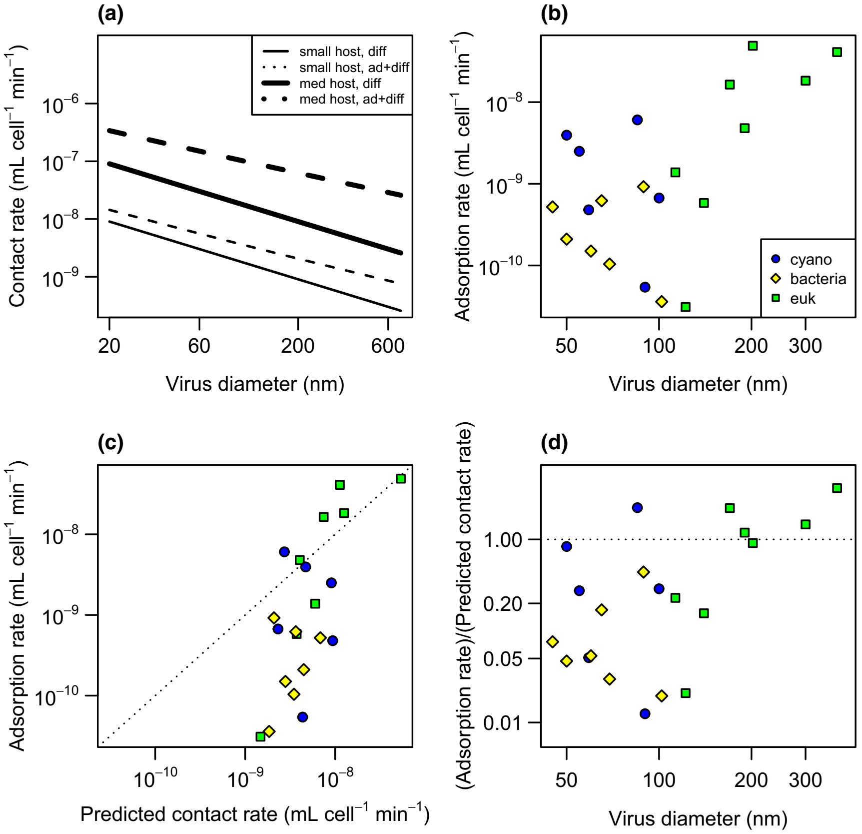

Figure 1 Model predictions and observations of contact rate and adsorption rate. (a) Predicted contact rate as a function of virus size. The 'small host' lines correspond to a host with diameter 1 μm and swimming speed 10 μm s^{-1}, and the 'medium host' lines correspond to a host with diameter 10 μm and swimming speed 100 μm s^{-1}. The solid lines are the pure diffusion prediction (no advection), and the dashed lines are for swimming hosts (advection + diffusion). (b) Observed adsorption rates for viruses of aquatic bacteria, phytoplankton, and the protist *Cafeteria roenbergensis* (Table S2; Methods S1). (c) Observed rates vs. theoretical predictions described in Methods S1. (d) Observed adsorption rate, relative to the theoretical maximum contact rate, as a function of virus size. 'cyano' = virus infecting cyanobacterium, 'bacteria' = virus infecting heterotrophic bacterium, 'euk' = virus infecting unicellular eukaryote.

could be selected for, in order to induce phagocytosis (Rodrigues *et al.*, 2016). Protists that eat bacteria-sized prey are known to ingest larger prey at higher rates, which could be due to differences in contact rates or size preferences during ingestion (Chrzanowski and Šimek, 1990; Holen and Boraas, 1991; Šimek and Chrzanowski, 1992; Epstein and Shiaris, 1992). Additional measurements of adsorption rates for viruses across the full size spectrum will be needed to test whether larger size is on average an advantage or disadvantage, and whether rates of successful encounter and infection differ for viruses that enter by phagocytosis.

HOW DOES VIRUS SIZE AFFECT VIRAL PRODUCTION DURING INFECTION?

Burst size and latent period of a lytic virus are determined by the rate at which new virions are created during an infection and the timing of cell lysis (You *et al.*, 2002). The production of virions may decline as host resources are depleted, and the timing of cell lysis may evolve in response to intracellular conditions, host density and other factors (Wang *et al.*, 1996; Abedon *et al.*, 2003). A previous analysis of phytoplankton viruses showed that burst size of dsDNA viruses may be limited by the host resources used in virus genome replication, with lysis occurring once those resources are exhausted (Edwards and Steward, 2018). By contrast, small ssDNA and ssRNA viruses that infect large hosts may maximize fitness by lysing the host before those resources are exhausted (Edwards and Steward, 2018).

In light of these results, we focus here on the role of viral genome size in constraining burst size and the rate of viral replication. eqn 1 shows that burst size and latent period are expected to play a large role in virus fitness, and therefore viral size evolution may be driven in part by its effects on these life history parameters. We previously showed that burst size is correlated with the host:virus genome size ratio (Edwards and Steward, 2018), and this relationship can be decomposed into the effects of host genome size and virus genome size (Methods S1; Table S3). Of the total variation in burst size across phytoplankton viruses, 48% is explained by host and virus genome sizes in combination, with a partial R^2 of 30% for host genome size and a partial R^2 of 14% for virus genome size (4% of variation cannot be uniquely attributed to either predictor because host and virus genome sizes are partially correlated). Although burst size tends to decline for larger viruses, the effect of virus genome size is less than proportional, i.e., a tenfold increase in genome size leads to a less than tenfold decrease in burst size (Fig. 2a). The estimated slope for virus genome size is −0.52 (95% CI = [−0.225, −0.911]) when host and virus taxonomy are included as random effects, or a slope of −0.3 (95% CI = [−0.11, −0.55]) when host and virus taxonomy are not included. This means that a tenfold increase in virus genome size would be expected to reduce burst size by a factor of $1/10^{-0.52} = 3.3$ or $1/10^{-0.3} = 2.0$.

A less-than-proportional relationship between virus genome size and burst size suggests that larger viruses are producing more total viral material per infection, which could happen if viruses with larger genomes are better at extracting resources from their hosts, better at maintaining metabolic processes that fuel replication, more efficient at transcription or translation, etc. For another perspective on the same processes we can consider how quickly new virions are produced during an infection. All else equal, we would expect that larger virions take longer to construct, due to rate limitation by protein elongation, supply of amino acids or dNTPs, or other processes (You *et al.*, 2002; Birch *et al.*, 2012). The data for phytoplankton viruses exhibit a weak trend of virion production rate declining with virus size ($F_{1,36} = 2.3$, $P = 0.14$; Fig. 2b), which is consistent with a penalty for larger size that is not directly proportional to size. Finally, rather than looking at production rate on a per virion basis we can consider production rate on a per nucleotide basis, to quantify the total rate at which viral nucleotides are produced during an infection. Nucleotide production rate increases strongly with viral size, with the largest viruses on average producing viral nucleotides c. 100x faster than the smallest viruses (Fig. 2c; $F_{1,11} = 6.5$, $P = 0.027$). In combination with Fig. 2a and b, this argues that larger size does incur a cost of producing fewer offspring per infection, but that the cost is partially mitigated by a more effective infection process facilitated by the functions encoded in larger genomes. Repeating these analyses using virion volume instead of genome size produces similar results, but the slope of burst size vs. virus genome size is shallower (a 10-fold increase in virion volume leads to a 1.5-fold decrease in burst size; results not shown), and the increase in production rate with virion volume is steeper (the largest viruses create virion volume *c.* 700x faster than the smallest viruses; results not shown). These difference in scaling when using virus genome size vs. virion volume as the predictor are expected, because the genome is a smaller proportion of total virion volume for larger viruses (discussed further in the section *Are larger viruses more persistent in the environment?*). An important question for future research is how resource limitation affects these relationships. Nutrient or light limitation have been shown to reduce burst size and lengthen latent period (Wilson *et al.*, 1996, Maat and Brussard 2016, Thamatrakoln *et al.*, 2019), but the data analysed here are from experiments under resource-replete conditions. It is possible that the advantage of large genome size is greater under resource limitation, due to a greater control of rate-limiting metabolic reactions.

DO LARGER VIRUSES HAVE BROADER HOST RANGES?

Relatively large viruses could be favoured in competition with smaller viruses if a larger genome size is associated with a broader host range (Appendix S3; Chow and Suttle, 2015). There is substantial evidence that the ability to attach to host cells plays a major role in defining viral host range (Tétart *et al.*, 1996; Tarutani *et al.*, 2006; Stoddard *et al.*, 2007; Lin *et al.*, 2012; Le *et al.*, 2013), and laboratory experiments often find that hosts evolve resistance by limiting attachment (Lenski, 1988; Stoddard *et al.*, 2007). Therefore, virus size may be correlated with host range if having more genes facilitates attachment to a broader range of receptors. For example, the large myovirus phi92 possesses a 'Swiss army knife' of multiple tail fibres and/or spikes that appears to facilitate a relatively broad host range encompassing diverse *Escherichia coli* and *Salmonella* strains (Schwarzer *et al.*, 2012). In addition, if the largest viruses are typically ingested by their hosts then

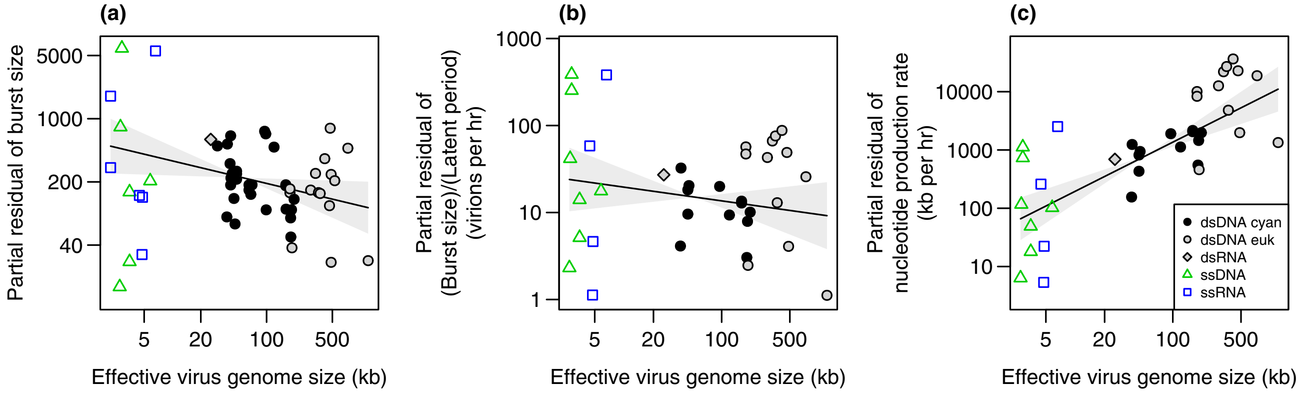

Figure 2 Burst size and production rates as a function of virus genome size for viruses of phytoplankton and the protist *Cafeteria roenbergensis*. (a) Burst size vs. virus genome size. Partial residual burst size is plotted, which removes variation in burst size explained by host genome size. Effective virus genome size is plotted, which divides genome size by 2 for single-stranded viruses. (b) Virion production rate, (burst size)/(latent period), vs. virus genome size. Partial residual production rate is plotted, which removes variation explained by host growth rate. (c) Nucleotide production rate (kb per hr) vs. virus genome size. Partial residual production rate is plotted, which removes variation explained by host growth rate. Plotted lines are fitted smoothers ± 95% CI from generalized additive mixed models. 'dsDNA cyan' = dsDNA viruses infecting cyanobacteria; 'dsDNA euk' = dsDNA viruses infecting unicellular eukaryotes. dsRNA, ssDNA, and ssRNA viruses all infect eukaryotes in this dataset. Data and data sources are presented in Table S3 and described in Methods S1.

they may have a broader host range than smaller non-ingested viruses, due to the fact that ingestion of prey tends to be a less specific interaction than ligand-receptor binding.

Although attachment is known to be important in defining host range, in some cases viruses can attach with equal or lesser efficiency to related strains or taxa that do not yield productive infections (Samimi and Drews, 1978; Thomas *et al.*, 2011; Yau *et al.*, 2018). Presumably the infections were not productive in these cases because of many processes downstream of attachment that can limit infection success, such as successful entry into the host, intracellular host defense mechanisms, and effective meshing with host replication and translation machinery (Samson *et al.*, 2013). Furthermore, attaching to a relatively narrow range of cell types in the environment can be adaptive if broader attachment results in a loss of virions to hosts that cannot be infected as productively (Heineman *et al.*, 2008). Therefore, the ability to create productive infections once attached may play a large role in defining host range, and viruses with more genes that provide greater autonomy during infection, via control of replication, transcription, translation or metabolism, or by evading host defenses, may achieve productive infections in a broader range of hosts.

To assess whether there is an association between genome size and host range we re-analysed data from two studies of marine phages. Holmfeldt and colleagues characterized a taxonomically diverse collection of 40 phages isolated on 21 strains of the marine bacterium *Cellulophaga baltica* (Holmfeldt *et al.*, 2007; Holmfeldt *et al.*, 2016, Šulčius and Holmfeldt, 2016). Across these phages there is an overall positive correlation between host range and genome size, if host range is quantified as the proportion of 21 *Cellulophaga* strains infected (Fig. 3a; $r = 0.49$, $P = 0.001$). We also quantified host range in a way that incorporates phylogenetic relatedness of the host strains, using the summed branch lengths of the infected strains, calculated from a phylogeny estimated from ribosomal internal transcribed spacer (ITS) sequences; this yielded similar results (results not shown). A positive relationship also appears to occur within phage families, with larger isolates of myovirus, siphovirus, and podovirus having a greater host range than smaller isolates within the same groups (Fig. 3a). However, when phage family and phage genus (as classified by Holmfeldt et al. [2016]) are both included as random effects in a mixed model, in order to account for phylogenetic non-independence in host range (Felsenstein, 1985), the effect of host genome size is less clear ($\chi_1 = 2.45$, $P = 0.12$). This suggests that an even greater phylogenetic diversity of viruses may be needed to robustly test such relationships using a comparative approach. Wichels *et al.* (1998) characterized 22 phages from the North Sea that infect the bacteria *Pseudoalteromonas*. Across the phages in this study there is also a positive correlation between genome size and host range ($r = 0.79$, $P < 0.001$), and evidence for such a relationship remains after taxonomic random effects are included in a generalized additive mixed model ($\chi_{1.2} = 7.2$, $P = 0.007$; taxonomic terms include family, morphotype and species as defined by the authors).

In sum, it may be the case that viruses with larger genomes tend to infect a broader range of hosts, and future analyses from diverse host-virus systems would help test the generality of this pattern. At the same time, it is noteworthy that among *Cellulophaga* phages the smallest phage family, the Microviridae, exhibit relatively broad host range on average (Fig. 3a). A large study of *Vibrio* phages also found that small phages, which the authors classified as Autolykiviridae, had broader host ranges than larger Caudovirales (Kauffman *et al.*, 2018). Future work incorporating a quantitative metric of viral fitness on each host strain would help test whether small, broad-range viruses suffer a cost of lower fitness on each individual host strain (Jover *et al.*, 2013, Record *et al.*, 2016). Tradeoffs affecting viral traits, including those related to size and those orthogonal to size, are likely multidimensional (Goldhill and Turner, 2014), and therefore it will be important to measure multiple traits on diverse viruses to better understand the constraints on viral evolution.

ARE LARGER VIRUSES MORE PERSISTENT IN THE ENVIRONMENT?

The rate at which free virions are lost from a viral population (whether by inactivation or disintegration) is as important for fitness as adsorption rate, burst size or host range (eqn S4 in Appendix S1). However, the effects of virus size on loss rates are poorly known. Decay of phage infectivity in marine systems has been shown to be influenced by sunlight, adsorption to particles, high molecular weight dissolved material such as enzymes and ingestion by protists (Suttle and Chen, 1992, Noble and Furhman 1997). Although a number of studies have estimated decay rates and how they vary across environmental gradients, we are not aware of studies that look at whether these rates vary systematically with size. Heldal and Bratbak (1991) noted that viruses >60 nm disappeared more slowly when viral production was halted with cyanide, but they presented no quantitative data.

The physical forces that affect virion stability likely vary with size. For viruses with larger double-stranded genomes the capsid can be highly pressurized due to the dense packaging of negatively charged, dehydrated, curved nucleic acids (Purohit *et al.*, 2003; Li *et al.*, 2008; Molineux and Panja, 2013). In a comparative study of coliphages, De Paepe and Taddei (2006) found that phages with a faster multiplication rate in culture had a faster decay rate as well. Faster decay was also associated with a higher nucleotide packaging density and a lower surfacic mass (capsid molecular weight per capsid surface area), suggesting that greater pressure makes capsids less stable, and this can be partially mitigated by increased capsid thickness. A re-analysis of their data show that virus diameter is also negatively correlated with decay rate ($r = -0.64$; mixed model with virus family random effect – $F_{1,13} = 9.1$, $P = 0.01$; Fig. 4a), which could be due to larger phages having lower packaging density, higher surfacic mass or other causes.

We have compiled measurements of genome length and virion dimensions for 193 viruses (Table S4; Methods S1). Among dsDNA viruses infecting unicellular organisms, the fraction of the virion volume occupied by the viral genome declines systematically with increasing virion size (Fig. 4b), although there are notable differences among virus types. The tailless viruses infecting prokaryotes tend to have a lower fractional volume than other dsDNA viruses of the same size, and genome fractional volume declines steeply with increasing

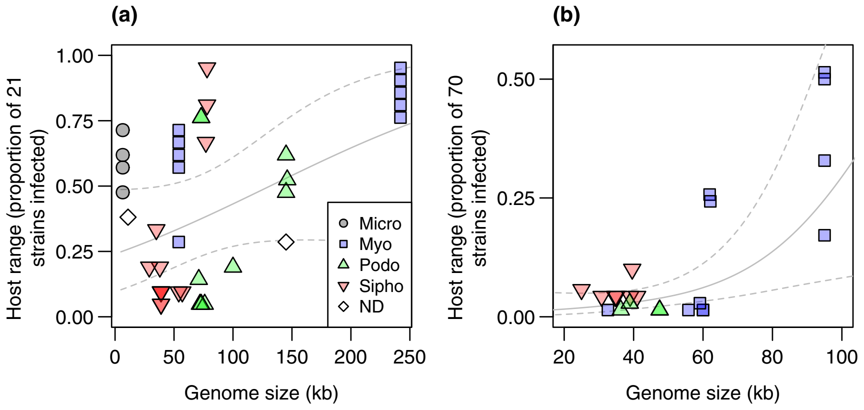

Figure 3 Host range vs. genome size in marine bacteriophages. (a) Host range (proportion of 21 strains infected) vs. genome size for *Cellulophaga baltica* phages (Šulčius and Holmfeldt, 2016). (b) Host range (proportion of 70 strains infected) vs. genome size for *Pseudoalteromonas* phages (Wichels *et al.*, 1998). Lines depict fitted smoothers and 95% CI from generalized additive mixed models that include taxonomic random effects to account for related viruses having similar host ranges. 'Micro' = Microviridae, 'Myo' = Myoviridae, 'Podo', = Podoviridae, 'Sipho' = Siphoviridae, 'ND' = taxonomy not determined. Statistical methods are described further in Methods S1.

virion size for this group and for the eukaryote-infecting dsDNA viruses, which vary more than tenfold in diameter. In contrast, genome fractional volume of the tailed viruses infecting prokaryotes (members of the family *Caudovirales*) is uniformly high and weakly correlated with virion size. Regression analyses indicate that the slope of log(genome fractional volume) vs. log(equivalent spherical diameter) is *c.* −1.7 for dsDNA eukaryote viruses and tailless dsDNA viruses infecting prokaryotes, while the slope for tailed phages is −0.24 (Fig. S4a). It is also noteworthy that the largest viruses overlap with small prokaryote and eukaryote cells, both in diameter and genome fractional volume, and that the largest viruses tend to infect phagotrophic eukaryotes.

A decline in genome fractional volume with virion size could be driven by selection for virion stability, because the pressure at which a capsid bursts is expected to be inversely proportional to capsid radius (Aznar *et al.*, 2012). If dsDNA viruses generally evolve to have an internal pressure near the burst limit, then the packaging density of the genome would have to decline such that internal pressure is inversely proportional to capsid radius. However, without direct measurements it is unclear whether the observed decline in density is sufficient to equalize the stability of larger and smaller viruses, or whether larger viruses tend to be more or less stable on average. In addition, for tailed dsDNA bacteriophages the injection of the viral genome into the host cell may be driven by high genome packaging density, likely due to hydrodynamic effects of the osmotic imbalance with the host cytoplasm (Molineux and Panja, 2013). In contrast, eukaryote-infecting viruses and tailless prokaryote viruses often use membrane fusion, endocytosis or phagocytosis as an entry mechanism, although some have a more phage-like strategy (Nurmemmedov *et al.*, 2007; Wulfmeyer *et al.*, 2012; Mäntynen

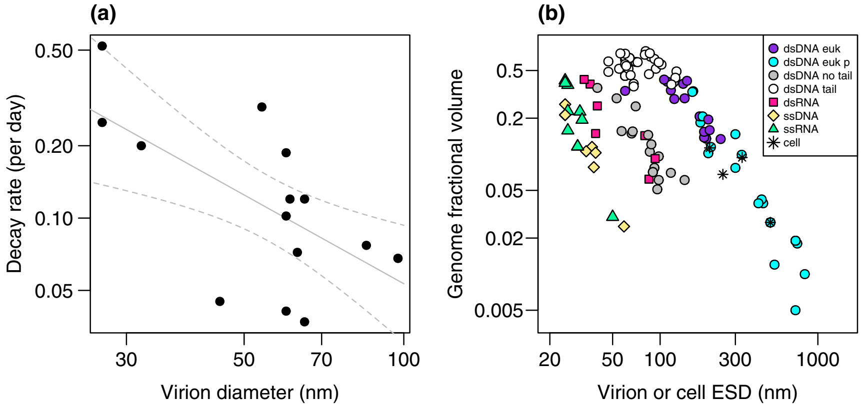

Figure 4 (a) Decay rate vs. virion diameter, for phages that infect *E. coli* (data from Table 1 in De Paepe and Taddei, 2006). Lines are linear regression fit ± 95% CI. (b) Genome fractional volume vs. equivalent spherical diameter, for viruses that infect unicellular organisms, and for four representative cellular organisms. Genome fractional volume is the estimated nucleic acid volume divided by total virion volume. Cell or virion volumes were estimated from reported outer dimensions (including outer envelope, if present, but excluding tails or fibrils) using volume formulae for simplified approximate shapes as indicated in Table S4. Nucleic acid volume is estimated as volume of a cylinder with diameter being 2.37 nm for double-stranded nucleic acid and 1.19 nm for single-stranded and 0.34 nm per nt or bp. 'dsDNA euk' = dsDNA viruses infecting non-phagotrophic eukaryotes, 'dsDNA euk p' = dsDNA viruses infecting phagotrophic eukaryotes, 'dsDNA no tail' = tailless dsDNA viruses infecting prokaryotes, 'dsDNA tail' = tailed dsDNA viruses infecting prokaryotes, 'dsRNA' = dsRNA viruses, 'ssDNA' = ssDNA viruses, 'ssRNA' = ssRNA viruses, 'cell' = cellular organisms (one archaeon, two bacteria, one eukaryote). Data and data sources are presented in Table S4 and described in Methods S1.

et al., 2019). Therefore, while tailed phages may require dense packaging of nucleic acids, many of the largest eukaryote-infecting viruses may reap little benefit from a pressurized capsid. The presence of a lipid envelope around the capsid may contribute to a lower genome fractional volume for many of the tailless dsDNA prokaryote viruses, but the large difference in fractional volume between these viruses and the tailed phages indicates that the envelope itself is likely not the sole explanation (Fig. S4b). The dsRNA viruses may follow a scaling relationship similar to the tailless dsDNA phages, but the number of representatives in the dataset is relatively small (Fig. 4b).

Fractional genome volume also declines with virion size for ssRNA and ssDNA viruses, and these two kinds of viruses appear to follow a similar scaling relationship (Fig. 4b). This similarity is also apparent when viruses infecting multicellular organisms are included in the comparison (Fig. S4c). The slope of the relationship is *c.* −2.8 for the single-stranded viruses infecting unicells (Fig. S4a). This systematic size scaling may also be attributable to selection equalizing the stability of larger and smaller viruses, although the scaling relationship and mean fractional volume likely differ between single-stranded viruses and double-stranded viruses a result of different physical processes underlying virion assembly and stability (Šiber *et al.*, 2012).

Finally, physical instability may not be the primary cause of losses of infectious virions, at least in environments where solar radiation and/or non-specific adsorption are high (Suttle and Chen, 1992, Noble and Furhman 1997). Eukaryotic viruses with larger genomes have the capacity to code for and package protective enzymes such as photolyase (Fischer *et al.*, 2014), which could result in larger viruses losing infectivity at slower rates. This may be of less consequence in bacteriophages, which were observed to exploit the host DNA repair mechanisms (Weinbauer *et al.*, 1997). To understand the consequences of these patterns for viral fitness, and to test whether rates of inactivation and decay generally change with virus size, future work should investigate decay rates in the laboratory and in natural systems for a broad size range of viruses.

SYNTHESIS AND OUTLOOK

Here, we summarize our findings on the relationships between virus size and important virus traits, and we discuss implications and future research directions.

The physics of Brownian motion predicts that smaller viruses should encounter their hosts at a faster rate (Fig. 1a), but observed adsorption rates are often much lower than theoretical contact rates (Fig. 1c), and it is possible that larger viruses have a greater attachment efficiency (Fig. 1d). New measurements of adsorption rates of large viruses are needed to test this possibility. Furthermore, entering the host cell via phagocytosis may be a particularly good strategy for ensuring that encounter leads to infection, but demonstrating this quantitatively will require studies on the mechanism of entry, and efficiency of attachment and entry, for diverse viruses.

Burst size is lower for larger viruses (Fig. 2a), which is expected if host materials and energy limit viral production, but the cost is less than would be expected if viral production was inversely proportional to genome size. This discrepancy is likely due to greater control of viral replication and host physiology by viruses with larger genomes, as evidenced by their greater nucleotide production rate (Fig. 2c). Host range may generally increase with virus size, as observed for two diverse groups of phages infecting aquatic bacteria (Fig. 3), and this may be a result of greater autonomy during replication, a greater range of counter-defenses, or ability to attach to a greater diversity of receptors. Finally, decay rate has not been widely studied for viruses with unicellular hosts, but a comparison of coliphages suggests that larger viruses could have a lower decay rate (Fig. 4 a). In sum these observations suggest that larger viruses experience reductions in burst size, a modest or negligible reduction in adsorption rate, an increase in host range, and potentially a decline in decay rate. These countervailing selection pressures may explain how viruses of very different size have evolved and can persist when infecting the same host population.

Are there particular host traits or environmental conditions that could select for larger or smaller viruses? The relative fitness of different virus sizes could shift between contexts if the relationship between size and virus traits shifts between contexts, or if there is a shift in the relative importance of different size-related traits for fitness. For example, are there conditions under which the reduction in burst size with increased virus size is lessened? The data synthesized in Figure 2 come from experiments with resource-replete host cultures, and it is possible that under host resource limitation a greater virus size is more costly (due to less energy and materials available for replication) or less costly (due to greater control of host metabolism). Testing this possibility will require measurements of the infection cycle of diverse viruses under different resource conditions. Although contact rates are predicted to decline for larger viruses because of reduced diffusivity, the magnitude of the decline is somewhat less when hosts are motile or generate feeding currents (Fig. 1a). Therefore, larger viruses may be more common among hosts that are highly motile. Likewise, if phagotrophy is an effective means of entering host cells, for viruses large enough to induce phagotrophy, then the largest viruses may be particularly prevalent among phagotrophic hosts (Fig. 4b).

If larger viruses tend to have a broader host range, the benefits of broad host range may be greatest when hosts are at low abundance. In our model of host range evolution, a virus will be able to persist if the sum of its host populations, in the absence of viral mortality, exceeds the minimum persistence threshold S^* (Appendix S3). Therefore, oligotrophic environments may be enriched in larger viruses if smaller viruses with narrower host ranges cannot persist. Low host density is also expected to select for lysogeny (Stewart and Levin, 1984, Weitz *et al.*, 2019), and so environments with a greater proportion of temperate viruses may also tend to have larger viruses in the lytic fraction. Finally, if larger size is associated with reduced inactivation and decay rates, then larger viruses may be favoured under conditions that promote rapid decay, such as exposure to high insolation.

If we combine several of the conditions that could favour large viruses, it may be that motile, phagotrophic protists with low population densities are particularly likely to host giant viruses. Low population densities are characteristic of the oligotrophic open ocean and other environments with low nutrient or energy supply. These environments are also relatively enriched in motile, phagotrophic eukaryotes, including mixotrophic phytoplankton and heterotrophic protists (Edwards, 2019), compared to more productive environments where immotile, non-phagotrophic diatoms often dominate microbial biomass. Therefore, the effects of low resource supply on both population densities and community structure may cause larger viruses to be favoured in oligotrophic environments. If larger eukaryotic viruses are also better able to repair DNA damage, this could augment selection for larger viruses in near-surface waters with higher UV iradiance.

Testing for patterns in virus size distributions across environmental conditions or host types will require a suite of methods, including substantial new isolation efforts, as well as metagenomic protocols that can capture the full size spectrum of viruses in the environment while quantitatively comparing viruses with RNA, ssDNA and dsDNA genomes. For example, two studies compared RNA and DNA viral metagenomes in coastal ocean environments in Hawai'i (Steward *et al.*, 2013) and Antarctica (Miranda *et al.*, 2016) and found that the abundance of RNA viruses rivals that of DNA viruses. The RNA viruses identified were exclusively eukaryote-infecting, while most of the DNA viruses were likely phages. This implies that RNA viruses, which tend to be smaller, are more prevalent than larger DNA viruses among eukaryotic viruses in coastal environments. No comparable studies have been performed in the open ocean, but we predict that in the pool of eukaryote-infecting viruses, larger DNA viruses, and 'giant' *Mimiviridae* in particular, are more prevalent than small ssDNA or ssRNA viruses in open ocean environments that tend to be more oligotrophic. It is less clear whether one should expect the size structure of prokaryote-infecting viruses to vary as much across environmental gradients in the ocean, because the abundance of prokaryotes varies much less than the abundance and biomass of unicellular eukaryotes (Li *et al.*, 2004). A study of virus morphology across ocean regions found little variation in the structure of the bulk viral community, which is thought to be numerically dominated by bacteriophages (Brum *et al.*, 2013). However, the locations compared in this study were open ocean environments that varied little in chlorophyll-a, a proxy for community biomass; comparing these environments to productive coastal locations may show that smaller phages become more prevalent in coastal systems.

Compilations of virus isolates across the tree of life show that bacteria and archaea are mainly infected by dsDNA viruses with a range of sizes, while eukaryote viruses primarily have RNA genomes that tend to be small, although there is a substantial minority of eukaryote-infecting DNA viruses that tend to be larger (Koonin *et al.*, 2015, Campillo-Balderas *et al.*, 2015). The genome composition and size distribution of eukaryote viruses may reflect alternative strategies responding to the barrier of the eukaryote nucleus, which restricts access to the host's DNA replication and transcription machinery. Larger DNA viruses typically replicate partially or entirely in the cytoplasm, producing their own replication 'factories', while positive-sense single-stranded RNA viral genomes can immediately act as a template for translation (Schmid *et al.*, 2014, Koonin *et al.*, 2015). In our analyses of burst size and decay rate we have treated viruses with different genome types as comparable data points along the virus size spectrum (Figs 2 and4), but future work may consider whether there are important trait or niche differences between these groups that are not explained by size alone. It could also be the case that viruses of different size or different genome type differ in evolutionary rates of 'speciation' or extinction, for example if size or genome type affect the probability of host range shifts, or success in coevolutionary arms races with hosts (Lenski and Levin, 1985). Effects of size or genome type on diversification rates could vary with host type or environmental conditions, leading to patterns of virus diversity that vary with host type or environment. Future work may address these possibilities by applying macroevolutionary phylogenetic models to diverse viral clades (e.g., Caetano *et al.*, 2018).

Finally, we have focused here on lytic viruses, because a substantial number of lytic viruses infecting unicellular aquatic organisms have been isolated and characterized. Selection for a temperate strategy of integrating into the host genome may also lead to important constraints on virus size evolution. The temperate strategy is a form of vertical symbiont transmission and is expected to be selected for when host densities are low (making horizontal transmission less likely) and when the virus presents a low fitness cost or even a benefit (Weitz *et al.*, 2019). Considering the potential costs and benefits of prophage or provirus in the host genome, a larger virus genome will incur greater material and energy costs but will also be able to encode more functions that could benefit the host. Future work may consider how the size distribution of temperate viruses compares to lytic viruses, across environmental gradients or different host characteristics.

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AUTHORSHIP

All authors contributed to the conception and outline of the work. K.F.E wrote the initial manuscript, analysed mathematical models, and contributed to data compilation and analysis. G.F.S and C.R.S. both contributed to manuscript writing and to data compilation and analysis.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Should the manuscript be accepted, the data supporting the results will be archived in an appropriate public repository and the data DOI will be included at the end of the article (https://doi.org/10.5061/dryad.0gb5mkkzw).

REFERENCES

Abedon, S.T., Hyman, P. & Thomas, C. (2003). Experimental examination of bacteriophage latent-period evolution as a response to bacterial availability. *Appl. Environ. Microbiol.*, 69(12), 7499–7506.

Al-Shayeb, B., Sachdeva, R., Chen, L.X., Ward, F., Munk, P., Devoto, A. *et al.* (2020). Clades of huge phages from across Earth's ecosystems. *Nature*, 578(7795), 1–7.

Aznar, M., Luque, A. & Reguera, D. (2012). Relevance of capsid structure in the buckling and maturation of spherical viruses. *Phys. Biol.*, 9(3), 036003.

Birch, E.W., Ruggero, N.A. & Covert, M.W. (2012). Determining host metabolic limitations on viral replication via integrated modeling and experimental perturbation. *PLoS Comput. Biol.*, 8(10), e1002746.

Bonachela, J.A. & Levin, S.A. (2014). Evolutionary comparison between viral lysis rate and latent period. *J. Theor. Biol.*, 345, 32–42.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G.B. (2004). Toward a metabolic theory of ecology. *Ecology*, 85(7), 1771–1789.

Brum, J.R., Schenck, R.O. & Sullivan, M.B. (2013). Global morphological analysis of marine viruses shows minimal regional variation and dominance of non-tailed viruses. *ISME J.*, 7(9), 1738–1751.

Bull, J.J. (2006). Optimality models of phage life history and parallels in disease evolution. *J. Theor. Biol.*, 241(4), 928–938.

Caetano, D.S., O'Meara, B.C. & Beaulieu, J.M. (2018). Hidden state models improve state-dependent diversification approaches, including biogeographical models. *Evolution*, 72(11), 2308–2324.

Campillo-Balderas, J.A., Lazcano, A. & Becerra, A. (2015). Viral genome size distribution does not correlate with the antiquity of the host lineages. *Frontiers in Ecology and Evolution*, 3, 143.

Chow, C.E.T. & Suttle, C.A. (2015). Biogeography of viruses in the sea. *Annual review of virology*, 2, 41–66.

Chrzanowski, T.H. & Šimek, K. (1990). Prey-size selection by freshwater flagellated protozoa. *Limnol. Oceanogr.*, 35(7), 1429–1436.

Cui, J., Schlub, T.E. & Holmes, E.C. (2014). An allometric relationship between the genome length and virion volume of viruses. *J. Virol.*, 88 (11), 6403–6410.

De Paepe, M. & Taddei, F. (2006). Viruses' life history: towards a mechanistic basis of a trade-off between survival and reproduction among phages. *PLoS Biol.*, 4(7), e193.

Devoto, A.E., Santini, J.M., Olm, M.R., Anantharaman, K., Munk, P., Tung, J. *et al.* (2019). Megaphages infect Prevotella and variants are widespread in gut microbiomes. *Nature Microbiology*, 4(4), 693.

Edwards, K.F. (2019). Mixotrophy in nanoflagellates across environmental gradients in the ocean. *Proc. Natl. Acad. Sci. USA*, 116 (13), 6211–6220.

Edwards, K.F. & Steward, G.F. (2018). Host traits drive viral life histories across phytoplankton viruses. *Am. Nat.*, 191(5), 566–581.

Edwards, K.F., Thomas, M.K., Klausmeier, C.A. & Litchman, E. (2012). Allometric scaling and taxonomic variation in nutrient utilization traits and maximum growth rate of phytoplankton. *Limnol. Oceanogr.*, 57(2), 554–566.

Epstein, S.S. & Shiaris, M.P. (1992). Size-selective grazing of coastal bacterioplankton by natural assemblages of pigmented flagellates, colorless flagellates, and ciliates. *Microb. Ecol.*, 23(3), 211–225.

Felsenstein, J. (1985). Phylogenies and the comparative method. *Am. Nat.*, 125(1), 1–15.

Finkel, Z.V. & Irwin, A.J. (2001). Light absorption by phytoplankton and the filter amplification correction: cell size and species effects. *J. Exp. Mar. Biol. Ecol.*, 259(1), 51–61.

Fischer, M.G., Kelly, I., Foster, L.J. & Suttle, C.A. (2014). The virion of Cafeteria roenbergensis virus (CroV) contains a complex suite of proteins for transcription and DNA repair. *Virology*, 466, 82–94.

Fuchs, H.L. & Franks, P.J.S. (2010). Plankton community properties determined by nutrients and size-selective feeding. *Mar. Ecol. Prog. Ser.*, 413, 1–15.

Goldhill, D.H. & Turner, P.E. (2014). The evolution of life history trade-offs in viruses. *Curr. Opin. Virol.*, 8, 79–84.

Heineman, R.H., Springman, R. & Bull, J.J. (2008). Optimal foraging by bacteriophages through host avoidance. *Am. Nat.*, 171(4), E149–E157.

Heldal, M. & Bratbak, G. (1991). Production and decay of viruses in aquatic environments. *Mar. Ecol. Prog. Ser*, 72(3), 205–212.

Holen, D.A. & Boraas, M.E. (1991). The feeding behavior of *Spumella* sp. as a function of particle size: Implications for bacterial size in pelagic systems. *Hydrobiologia*, 220(1), 73–88.

Holmfeldt, K., Middelboe, M., Nybroe, O. & Riemann, L. (2007). Large variabilities in host strain susceptibility and phage host range govern interactions between lytic marine phages and their *Flavobacterium* hosts. *Appl. Environ. Microbiol.*, 73(21), 6730–6739.

Holmfeldt, K., Solonenko, N., Howard-Varona, C., Moreno, M., Malmstrom, R.R., Blow, M.J. & *et al.* (2016). Large-scale maps of variable infection efficiencies in aquatic Bacteroidetes phage-host model systems. *Environ. Microbiol.*, 18(11), 3949–3961.

Jover, L.F., Cortez, M.H. & Weitz, J.S. (2013). Mechanisms of multi-strain coexistence in host–phage systems with nested infection networks. *J. Theor. Biol.*, 332, 65–77.

Kauffman, K.M., Hussain, F.A., Yang, J., Arevalo, P., Brown, J.M., Chang, W.K. *et al.* (2018). A major lineage of non-tailed dsDNA viruses as unrecognized killers of marine bacteria. *Nature*, 554(7690), 118.

Kiørboe, T. (2008). *A Mechanistic Approach to Plankton Ecology*. Princeton: Princeton University Press.

Koonin, E.V., Dolja, V.V. & Krupovic, M. (2015). Origins and evolution of viruses of eukaryotes: the ultimate modularity. *Virology*, 479, 2–5.

Koonin, E.V. & Yutin, N. (2019). Evolution of the large nucleocytoplasmic DNA viruses of eukaryotes and convergent origins of viral gigantism. *Adv. Virus Res*, 103, 167–202.

Langlois, V.J., Andersen, A., Bohr, T., Visser, A.W. & Kiørboe, T. (2009). Significance of swimming and feeding currents for nutrient uptake in osmotrophic and interception-feeding flagellates. *Aquat. Microb. Ecol.*, 54(1), 35–44.

Le, S., He, X., Tan, Y., Huang, G., Zhang, L., Lux, R. *et al.* (2013). Mapping the tail fiber as the receptor binding protein responsible for differential host specificity of *Pseudomonas aeruginosa* bacteriophages PaP1 and JG004. *PLoS One*, 8(7), e68562.

Lenski, R.E. & Levin, B.R. (1985). Constraints on the coevolution of bacteria and virulent phage: a model, some experiments, and predictions for natural communities. *Am. Nat.*, 125(4), 585–602.

Lenski, R.E. (1988). Experimental studies of pleiotropy and epistasis in *Escherichia coli*. I. Variation in competitive fitness among mutants resistant to virus T4. *Evolution*, 42(3), 425–432.

Levin, B.R., Stewart, F.M. & Chao, L. (1977). Resource-limited growth, competition, and predation: a model and experimental studies with bacteria and bacteriophage. *Am. Nat.*, 111(977), 3–24.

Li, W.K., Head, E.J. & Harrison, W.G. (2004). Macroecological limits of heterotrophic bacterial abundance in the ocean. *Deep Sea Res. Part I*, 51(11), 1529–1540.

Li, Z., Wu, J. & Wang, Z.G. (2008). Osmotic pressure and packaging structure of caged DNA. *Biophys. J.*, 94(3), 737–746.

Lin, T.-Y., Lo, Y.-H., Tseng, P.-W., Chang, S.-F., Lin, Y.-T. & Chen, T.-S. (2012). A T3 and T7 recombinant phage acquires efficient adsorption and a broader host range. *PLoS One*, 7(2), e30954.

Maat, D.S. & Brussaard, C.P.D. (2016). Both phosphorus-and nitrogen limitation constrain viral proliferation in marine phytoplankton. *Aquat. Microb. Ecol.*, 77(2), 87–97.

Mäntynen, S., Sundberg, L.R., Oksanen, H.M. & Poranen, M.M. (2019). Half a century of research on Membrane-Containing bacteriophages: bringing new concepts to modern virology. *Viruses*, 11(1), 76.

Maslov, S. & Sneppen, K. (2015). Well-temperate phage: optimal bet-hedging against local environmental collapses. *Sci. Rep.*, 5, 10523.

Mendoza, S.D., Nieweglowska, E.S., Govindarajan, S., Leon, L.M., Berry, J.D., Tiwari, A. *et al.* (2020). A bacteriophage nucleus-like compartment shields DNA from CRISPR nucleases. *Nature*, 577(7789), 244–248.

Miranda, J.A., Culley, A.I., Schvarcz, C.R. & Steward, G.F. (2016). RNA viruses as major contributors to Antarctic virioplankton. *Environ. Microbiol.*, 18(11), 3714–3727.

Molineux, I.J. & Panja, D. (2013). Popping the cork: mechanisms of phage genome ejection. *Nat. Rev. Microbiol.*, 11(3), 194–204.

Murray, A.G. & Jackson, G.A. (1992). Viral dynamics: a model of the effects of size, shape, motion and abundance of single-celled planktonic organisms and other particles. *Mar. Ecol. Prog. Ser.*, 103–116.

Noble, R.T. & Fuhrman, J.A. (1997). Virus decay and its causes in coastal waters. *Appl. Environ. Microbiol.*, 63(1), 77–83.

Nurmemmedov, E., Castelnovo, M., Catalano, C.E. & Evilevitch, A. (2007). Biophysics of viral infectivity: matching genome length with capsid size. *Q Rev Biophys*, 40, 327–356.

Purohit, P.K., Kondev, J. & Phillips, R. (2003). Mechanics of DNA packaging in viruses. *Proc. Natl Acad. Sci.*, 100(6), 3173–3178.

Record, N.R., Talmy, D. & Våge, S. (2016). Quantifying tradeoffs for marine viruses. *Front. Mar. Sci.*, 3, 251.

Rodrigues, R.A.L., Abrahão, J.S., Drumond, B.P. & Kroon, E.G. (2016). Giants among larges: how gigantism impacts giant virus entry into amoebae. *Curr. Opin. Microbiol.*, 31, 88–93.

Rodrigues, R.A.L., dos Santos Silva, L.K., Dornas, F.P., de Oliveira, D.B., Magalhães, T.F.F., Santos, D.A. *et al.* (2015). Mimivirus fibrils are important for viral attachment to the microbial world by a diverse glycoside interaction repertoire. *J. Virol.*, 89(23), 11812–11819.

Samimi, B. & Drews, G. (1978). Adsorption of cyanophage AS-1 to unicellular cyanobacteria and isolation of receptor material from *Anacystis nidulans*. *J. Virol.*, 25(1), 164–174.

Samson, J.E., Magadán, A.H., Sabri, M. & Moineau, S. (2013). Revenge of the phages: defeating bacterial defences. *Nat. Rev. Microbiol.*, 11(10), 675.

Schmid, M., Speiseder, T., Dobner, T. & Gonzalez, R.A. (2014). DNA virus replication compartments. *J. Virol.*, 88(3), 1404–1420.

Schwartz, M. (1976). The adsorption of coliphage lambda to its host: effect of variations in the surface density of receptor and in phage-receptor affinity. *J. Mol. Biol.*, 103(3), 521–536.

Schwarzer, D., Buettner, F.F., Browning, C., Nazarov, S., Rabsch, W., Bethe, A. *et al.* (2012). A multivalent adsorption apparatus explains the broad host range of phage phi92: a comprehensive genomic and structural analysis. *J. Virol.*, 86(19), 10384–10398.

Sharon, I., Battchikova, N., Aro, E.M., Giglione, C., Meinnel, T., Glaser, F. *et al.* (2011). Comparative metagenomics of microbial traits within oceanic viral communities. *ISME J*, 5(7), 1178.

Shimeta, J. (1993). Diffusional encounter of submicrometer particles and small cells by suspension feeders. *Limnol. Oceanogr.*, 38, 456–465.

Shinn, G.L. & Bullard, B.L. (2018). Ultrastructure of Meelsvirus: A nuclear virus of arrow worms (phylum Chaetognatha) producing giant "tailed" virions. *PLoS One*, 13(9), e0203282.

Šiber, A., Božič, A.L. & Podgornik, R. (2012). Energies and pressures in viruses: contribution of nonspecific electrostatic interactions. *Physical Chemistry Chemical Physics*, 14(11), 3746–3765.

Šimek, K. & Chrzanowski, T.H. (1992). Direct and indirect evidence of size-selective grazing on pelagic bacteria by freshwater nanoflagellates. *Appl. Environ. Microbiol.*, 58(11), 3715–3720.

Steward, G.F., Culley, A.I., Mueller, J.A., Wood-Charlson, E.M., Belcaid, M. & Poisson, G. (2013). Are we missing half of the viruses in the ocean? *ISME J*, 7(3), 672–679.

Stewart, F.M. & Levin, B.R. (1984). The population biology of bacterial viruses: why be temperate. *Theor. Popul. Biol.*, 26(1), 93–117.

Stoddard, L.I., Martiny, J.B.H. & Marston, M.F. (2007). Selection and characterization of cyanophage resistance in marine Synechococcus strains. *Appl. Environ. Microbiol.*, 73(17), 5516–5522.

Storms, Z.J. & Sauvageau, D. (2015). Modeling tailed bacteriophage adsorption: Insight into mechanisms. *Virology*, 485, 355–362.

Šulčius, S. & Holmfeldt, K. (2016). Viruses of microorganisms in the Baltic Sea: current state of research and perspectives. *Mar. Biol. Res.*, 12(2), 115–124.

Suttle, C.A. & Chen, F. (1992). Mechanisms and rates of decay of marine viruses in seawater. *Appl. Environ. Microbiol.*, 58(11), 3721–3729.

Talmy, D., Beckett, S.J., Zhang, A.B., Taniguchi, D.A.A., Weitz, J.S. & Follows, M.J. (2019). Contrasting controls on microzooplankton grazing and viral infection of microbial prey. *Front. Mar. Sci*, 6, 182.

Tarutani, K., Nagasaki, K. & Yamaguchi, M. (2006). Virus adsorption process determines virus susceptibility in *Heterosigma akashiwo* (Raphidophyceae). *Aquat. Microb. Ecol.*, 42(3), 209–213.

Tétart, F., Repoila, F., Monod, C. & Krisch, H.M. (1996). Bacteriophage T4 host range is expanded by duplications of a small domain of the tail fiber adhesin. *Journal of Molecular Biology*, 258(5), 726–731.

Thamatrakoln, K., Talmy, D., Haramaty, L., Maniscalco, C., Latham, J.R., Knowles, B. *et al.* (2019). Light regulation of coccolithophore host–virus interactions. *New Phytol.*, 221(3), 1289–1302.

Thomas, R., Grimsley, N., Escande, M., Subirana, L., Derelle, E. & Moreau, H. (2011). Acquisition and maintenance of resistance to viruses in eukaryotic phytoplankton populations. *Environ. Microbiol.*, 13(6), 1412–1420.

Tilman, D. (1982). *Resource Competition and Community Structure*. Princeton: Princeton University Press.

Tomaru, Y., Mizumoto, H., Takao, Y. & Nagasaki, K. (2009). Co-occurrence of DNA-and RNA-viruses infecting the bloom-forming dinoflagellate, *Heterocapsa circularisquama*, on the Japan coast. *Plankton and Benthos Research*, 4(4), 129–134.

Wang, N., Dykhuizen, D.E. & Slobodkin, L.B. (1996). The evolution of phage lysis timing. *Evol. Ecol.*, 10(5), 545–558.

Weinbauer, M.G., Wilhelm, S.W., Suttle, C.A. & Garza, D.R. (1997). Photoreactivation compensates for UV damage and restores infectivity to natural marine virus communities. *Appl. Environ. Microbiol.*, 63, 2200–2205. https://doi.org/10.1128/AEM.63.6.2200-2205.1997.

Weitz, J.S., Li, G., Gulbudak, H., Cortez, M.H. & Whitaker, R.J. (2019). Viral invasion fitness across a continuum from lysis to latency. *Virus evolution*, 5(1), vez006.

Wichels, A., Biel, S.S., Gelderblom, H.R., Brinkhoff, T., Muyzer, G. & Schütt, C. (1998). Bacteriophage diversity in the North Sea. *Appl. Environ. Microbiol.*, 64(11), 4128–4133.

Wickham, T.J., Granados, R.R., Wood, H.A., Hammer, D.A. & Shuler, M.L. (1990). General analysis of receptor-mediated viral attachment to cell surfaces. *Biophys. J.*, 58(6), 1501–1516.

Wilhelm, S.W., Bird, J.T., Bonifer, K.S., Calfee, B.C., Chen, T., Coy, S.R. *et al.* (2017). A student's guide to giant viruses infecting small eukaryotes: from Acanthamoeba to Zooxanthellae. *Viruses*, 9(3), 46.

Wilson, W.H., Carr, N.G. & Mann, N.H. (1996). The effect of phosphate status on the kinetics of cyanophage infection in the oceanic cyanobacterium *Synechococcus* sp. WH7803. *J. Phycol.*, 32(4), 506–516.

Wulfmeyer, T., Polzer, C., Hiepler, G., Hamacher, K., Shoeman, R., Dunigan, D.D. *et al.* (2012). Structural organization of DNA in chlorella viruses. *PLoS One*, 7(2), e30133.

Yau, S., Caravello, G., Fonvieille, N., Desgranges, É., Moreau, H. & Grimsley, N. (2018). Rapidity of genomic adaptations to prasinovirus infection in a marine microalga. *Viruses*, 10(8), 441.

You, L., Suthers, P.F. & Yin, J. (2002). Effects of *Escherichia coli* physiology on growth of phage T7 in vivo and in silico. *J. Bacteriol.*, 184(7), 1888–1894.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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