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The viriosphere, diversity, and genetic exchange within phage communities

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Natural phage communities are reservoirs of the greatest uncharacterized genetic diversity on Earth. Yet, identical phage sequences can be found in extremely different environments, which implies that there is wide circulation of viral genes among distantly related host populations. Further evidence of genetic exchange among phage and host communities is the presence in phage of genes coding for proteins that are essential for photosynthesis. These observations support the idea that a primary role of host populations in phage ecology and evolution is to serve as vectors for genetic exchange.

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Introduction

The viriosphere, the portion of the Earth where viruses interact with their hosts, probably spans all environments in which life occurs. Our understanding of the viriosphere has evolved greatly over the past 15 years. Initial observations that viruses are extremely abundant and infectious in seawater [1–3] resulted in many studies that have led to the paradigm that viruses are major players in many processes ranging from microbial mortality to global geochemical cycles. Environmental viruses are undoubtedly the largest reservoir of genetic diversity on the planet; they structure microbial communities, cause the lysis of a large portion of the ocean biomass on a daily basis, transfer genetic material among host organisms, and shunt nutrients between particulate and dissolved phases. Much (but not all) of this understanding has been derived from work carried out on marine ecosystems.

Viruses are ubiquitous, abundant and temporally dynamic members of aquatic communities. In marine and fresh waters there are, on average, about ten million viruses per mL, and as viruses depend upon their hosts for replication, the relative abundance of specific virus types roughly parallels that of the organisms they infect. Consequently, phages are by far the most abundant viruses in the ocean: there are $\sim 10^{27}$ phage particles in the ocean and they comprise ~270 Mt of C [4]. Although it is clear that viruses can be significant agents of microbial mortality, demonstration of their direct role in nutrient and energy cycles, in the control of community composition and in lateral gene transfer has been more difficult to measure directly. Nonetheless, many studies support the view that marine viruses are major players in the marine milieu. These studies and the resulting observations are summarized in numerous excellent reviews [4–12].

Phages share a long evolutionary history with their hosts. Given that the same three families of double-stranded DNA phages (*Podoviridae*, *Siphoviridae* and *Myoviridae*) infect cyanobacteria and other Gram-negative bacteria, but as far as we know do not infect Archaea, one can speculate that the existence of phage predated the split of the cyanobacteria from the rest of the Gram-negative bacteria 3–3.5 billion years ago. This also suggests that their emergence was subsequent to the divergence of Archaea from Bacteria. As the emergence of prokaryotes probably predates that of the first eukaryotes by about a billion years [13], phage–host interactions were the dominant biological interactions during early life on Earth. Phages were probably the primary agents of mortality, and were the vectors for gene transfer and the drivers of nutrient and energy cycling. These same phage-mediated processes still characterize the principle environmental interactions of phages with their hosts. In this review we focus on two closely linked areas that are being actively explored — diversity and lateral gene transfer in environmental phage communities.

Temporal and spatial variation in the diversity of environmental phages

Even before the emergence of interest in the high abundance of viruses in the sea, electron microscopy revealed that marine virus communities are morphologically diverse [14]. Most of the putative phages resemble small podoviruses, although myoviruses are most commonly isolated (reviewed in [8,15]). Given the large contribution of unicellular cyanobacteria to photosynthesis in the sea, it is not surprising that cyanophages that infect *Synechococcus* were among the first viruses isolated that infect marine primary producers [3,16,17]. Similar to isolates that infect marine heterotrophic bacteria, myoviruses were most commonly isolated, although podoviruses

and siphoviruses also occurred. However, given the vast diversity of phages in seawater, and that only a tiny fraction of potential hosts have been cultured, culture-independent methods have been used to estimate the genetic variation in natural virus communities. In particular, advances in techniques and the identification of suitable targets have propelled us into an era in which genomic approaches for interrogating viral communities are becoming routine [18].

Pulse field gel electrophoresis is a culture-independent approach that has been used to examine how diverse the genome sizes of the dominant members of virioplankton communities are. The studies have shown substantial temporal and spatial changes in genome sizes [19–22], which emphasizes that these highly dynamic communities are tightly linked to host populations. Pulse-field gel electrophoresis has the advantage of not targeting specific subsets of phage communities but it has limited sensitivity, which has led to the generation of other DNA-based approaches.

Unfortunately, there is no universally present gene that can be used to make inferences regarding diversity in natural phage communities. However, several genes can be targeted that are associated with specific subsets of phage communities. The genetic diversity of these genes can be examined by denaturing gradient gel electrophoresis and subsequent sequence analysis [23]. The first attempts of this method in phage communities targeted a fragment of the structural gene (*g20*) in T4-like cyanophage that infect marine cyanobacteria of the genus *Synechococcus* [24]. The studies examined variations in genetic composition that occur with depth along a transect bisecting the North and South Atlantic [25] and in fjords in British Columbia [26•]. The results from these studies revealed pronounced genetic differences in the genotypic composition of viral communities that were separated by even a few meters. Clearly, there is tremendous spatial heterogeneity in marine phage communities.

Subsequent work targeted a much longer fragment of *g20* to obtain data that was more conducive to phylogenetic analysis. These studies showed that there is much greater variation in *g20* sequences from marine [27••,28] and freshwater [29] viral communities than those that have been recovered from phages in culture [30]. Moreover, all of the sequences from cultured cyanophages fell within a few well-defined clusters [28,30], and all of these clusters were within a well-supported monophyletic group (cultured *Synechococcus* phage; CSP) [27••]. Some environmental sequences also fell within CSP, which suggests that these sequences originated from cyanophages. However, most sequences fell outside of CSP, and as CSP contained no cultured representatives there is little evidence to support the fact that these sequences were derived from cyanophages. One surprising result from these analyses was that indistinguishable *g20* sequences were recovered from samples collected in the Southern Ocean, the Gulf of Mexico, Lake Constance in Germany, and a melt-water pond on an ice shelf in the Canadian Arctic [27••]. Similar results have been reported for DNA polymerase sequences from putative viruses that infect eukaryotic algae [31], and also for podoviruses, where in one case indistinguishable podovirus-like sequences occurred in samples collected from freshwater as well as from marine sediment and water samples [32••]. Such results are significant because they imply that phage genes are moving among viruses from widely different environments in which closely related hosts are unlikely to occur. This means either that phages that have broad host ranges are able to infect bacteria in very different environments or that phage genes are circulating quite quickly through many host intermediates. The latter explanation seems most plausible, given the relatively narrow host range of most phages.

Cyanophage-encoded photosynthesis genes

Perhaps the most interesting example of lateral gene transfer among phages and their hosts to date is the discovery of cyanophages that contain homologs to genes that encode key components of the photosynthetic machinery found in cyanobacteria [33••]. These proteins, D1 and D2, which are encoded by *psbA* and *psbD*, respectively, form a hetero-dimer that is involved in charge separation in photosystem II. D1 is common to all oxygenic phototrophs and has a high turnover rate as a result of photodamage. Photodamage to D1 is inexorable, regardless of prevailing light conditions or acclimation of the cell (see [34•] for a review of this system in *Synechococcus*).

At least one of these genes, as well as a varying number of other related photosynthesis genes, have been reported in cyanophages that infect *Synechococcus* [35••] and/or *Prochlorococcus* [36••]. They have also been identified in BAC (bacterial artificial chromosome) clones and amplicons from environmental samples [37•]. The presence of these genes in cyanophages appears to be widespread, with 37 of 68 cyanomyoviruses isolated from the Red Sea carrying *psbA* [35••].

Phylogenetic reconstructions based on the inferred amino-acid sequences of PsbA and PsbD show that the virus and host sequences are clustered together for both marine *Synechococcus* [35••,38•] and *Prochlorococcus* [36••] relative to freshwater cyanobacteria and various eukaryotic sequences. This is interpreted as being evidence for horizontal acquisition from their hosts. Differences in the genetic organization of the photosynthesis genes between *Synechococcus* and phages have been interpreted as implying that multiple transfer events occur between the two groups in a potentially ongoing process [35••]. However, within the *Synechococcus* amino-acid based analyses, cya-

nophage PsbA and PsbD sequences were observed to form distinct subclades [35••,36••], which suggests that this acquisition was not recent. PsbA sequences from *Prochlorococcus* and their infecting cyanophages do not segregate in this way [36••]. A more recent phylogenetic reconstruction of *psbA* DNA sequences from *Synechococcus* and infecting phages shows strong support that the phages form a separate clade to the host sequences [39••]. However when *Prochlorococcus* and their phages are included in an analogous analysis (Figure 1), they are not distinguishable in the same manner. This pattern is also observed for *psbD* sequences (data not shown). It is interesting to note that the freshwater cyanobacterial sequences cluster together in both the *psbA* and *psbD* analyses (Figure 1 and Hambly and Suttle, unpublished).

The separation of host and phage *psbA* sequences is reflected by the GC content of the gene. Zeidner *et al.* [39••] postulate that many cyanophage sequences were constructed from fragments of both *Prochlorococcus* and *Synechococcus psbA* sequences as a result of horizontal gene transfer. They observed that *Prochlorococcus* phage sequences are not as patchy as those of *Synechococcus* phages, and conclude that this represents a less promiscuous history of recombination.

Interpretation of these results is complicated because some cyanophages that contain homologs of *psbA* and/or *psbD* (e.g. S-WHM1) infect strains of both *Synechococcus* and *Prochlorococcus* [40]. Interestingly, cyanophages that infect *Synechococcus* have broader host ranges than those that infect *Prochlorococcus*. It is not clear why phages that infect *Prochlorococcus* should be less promiscuous than those that infect *Synechococcus*. It could be an artifact of the isolation process, or there could be intrinsic differences between these phages that are reflected in the isolations to date. It seems possible that this apparent promiscuity and the associated GC content variation might be related to the isolation of the host and phage strains tested. For instance, some *Prochlorococcus* strains and their phages were isolated in close temporal and spatial proximity, whereas other virus–host combinations were not. Clearly, there is a greater chance of genetic exchange between co-occurring hosts and phages than between temporally and spatially distant communities. Ultimately, more sampling is required to determine whether there are intrinsic differences in the host range of cyanophages that infect strains of *Synechococcus* and *Prochlorococcus*.

Lindell *et al.* [36••] extensively studied the rates of fixation of synonymous and non-synonymous mutations of seven photosynthesis-related genes, including *psbA*, from several *Prochlorococcus* strains and their phages. They observed that the cyanophage sequences have diverged further than those of their hosts (the rate of substitutions of the cyanophage *psbA* sequences being up to about three times that of their hosts), although most of the nucleotide substitutions did not cause changes in the amino-acid sequence. This conservation of amino-acid sequence implies maintenance of protein functionality [36••], potentially as a means to prevent photoinhibition during infection [36••]. More recently, Zeidner *et al.* [39••] carried out comparable analyses for the *psbA* sequences in *Synechococcus* and their phages, and observed similar divergence in the phage sequences compared to those of the hosts. This has led to the conclusion that strong purifying selection was occurring in both. A further analysis of the *psbA* sequences tested for rates of recombination based upon analysis of the position of the third codon [39••]. The results of these tests implied that there is recombination between *Synechococcus* and phages that infect it, but not between *Prochlorococcus* and *Synechococcus*.

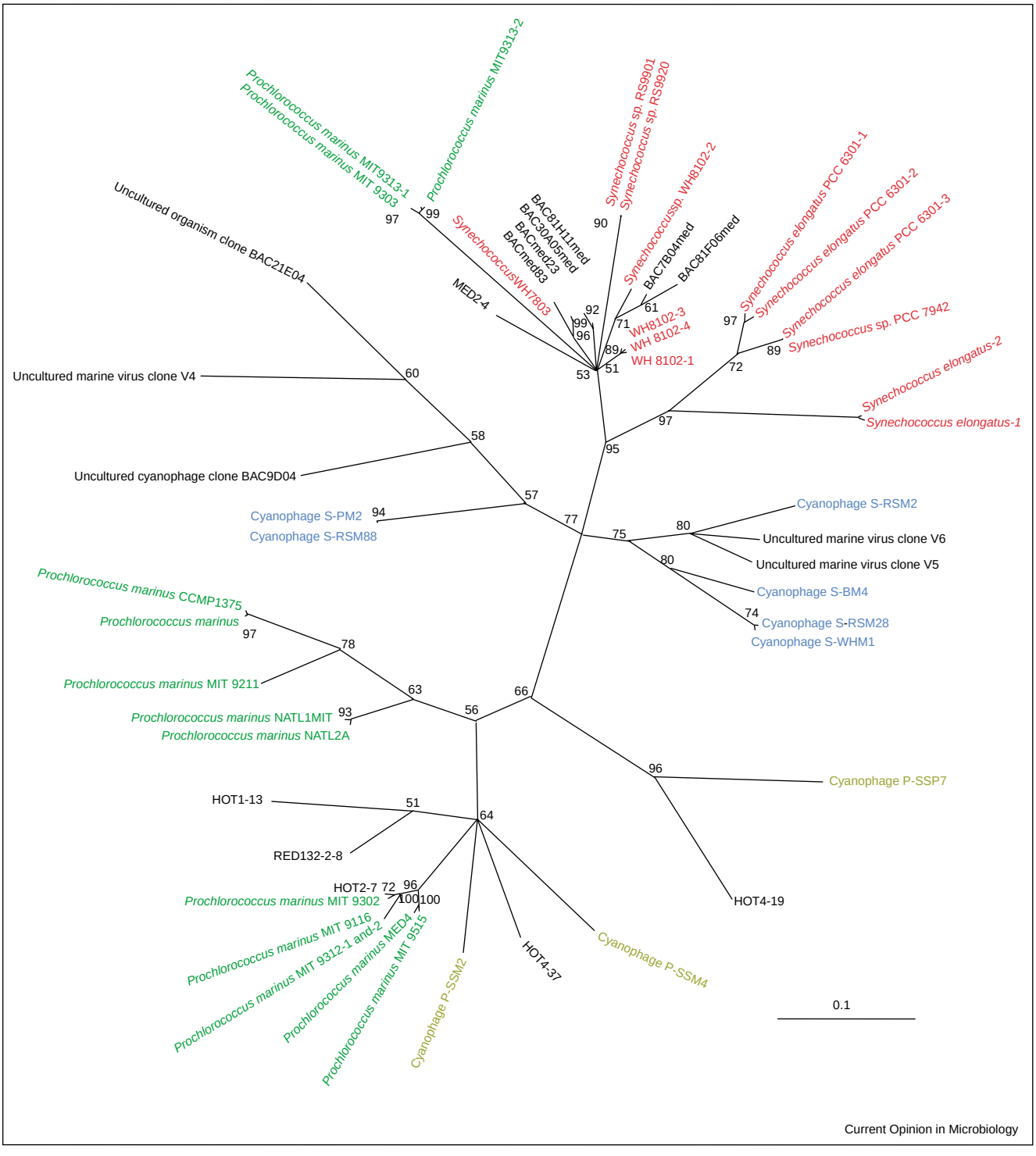
Horizontal gene transfer undoubtedly occurs in natural microbial communities; however, the scale of the process, the benefit to hosts and viruses, and the implications for the evolution of the organisms involved, are poorly comprehended. The transfer of biochemically important genes from host to virus and back through this process has undoubtedly shaped the microbial biosphere as we know it today. The maintenance of functionality of such genes in this process results in the potential for acquisition of immediately useful genetic material by the recipient. The transfer of, for example, photosynthesis genes in this way will continue to act as a force on evolution in the natural environment, potentially promoting the capabilities of the organisms involved, and will act to shape the biosphere of the future.

The metagenomic approach to phage diversity

The above studies emphasize the enormous genetic diversity of phages, even within highly conserved genes that represent a small subset of marine viruses. However, because these studies target genes that are known to be present in cultured phages, they cannot address the question of the overall diversity of phage communities. Quantification of the genetic diversity of the entire viral community can be approached by sequence analysis of shotgun libraries of the total viral DNA. This metagenomic approach has provided the first insight into the extent of the genetic diversity in natural viral communities.

In a study [41] that examined two uncultured coastal viral communities from the water column, the majority (>65%) of 1061 sequences were not significantly similar to any other sequences deposited in databases and only about a third of the sequences with recognizable similarity had their closest match to phage sequences. A subsequent study that examined a coastal sediment sample revealed even greater uncharacterized diversity [42•],

Figure 1



An unrooted phenogram derived by maximum likelihood using TREEPUZZLE 5.2 with default settings from an alignment of 53 *psbA* sequences (732 nucleotides) from a range of cyanobacteria, cyanophages and environmental samples. All sequences were obtained from GenBank. The scale bar represents 0.1 substitutions per site, and quartet puzzling values are shown (all are >50). Quartet puzzling values provide an estimate of support of a given branch, and can be interpreted in much the same way as bootstrap values. Values >90 indicate very strongly supported branches. Cyanobacterial sequences are labeled by name and strain designation followed by an arbitrary copy number where appropriate. Cyanobacterial sequences amplified from the environment are labeled with their clone designations as provided in the GenBank database (BAC, MED, RED and HOT). Cyanophage names are prefixed depending on the host used for original isolation (P, *Prochlorococcus*; S, *Synechococcus*). The colours are as follows: black, environmental sequence from a clone library; green, *Prochlorococcus* isolate; red, *Synechococcus* isolate; blue, cyanophage isolated using *Synechococcus* as a host; taupe, cyanophage isolated using *Prochlorococcus* as a host.

with 75% of the sequences showing no significant similarity to other deposited sequences and yielding an estimate of 10 000 different viral genotypes in a kg of sediment.

One of the most striking differences between the metagenomes from the sediment and the water-column samples was the relatively greater representation in the sediment sample of sequences that had higher similarity to siphoviruses. Culture work has shown that a higher proportion of siphoviruses are temperate relative to other phage, which raises the possibility that lysogeny and the potential for genetic exchange among phage populations and between phage and prokaryotes might be more common in sediments. Although metagenomics has the potential to reveal much about the genetic richness of marine viral communities, the large diversity of these communities means that one would have to sample these communities in considerable depth to begin to assemble complete genomes of uncultured phage. This appears to be especially true in the sediment viral communities, in which the most abundant phage were estimated to make up only 0.01–0.1% of the total viral community [42•]. By contrast, the water-column communities were estimated to be considerably less even, with the dominant members estimated to constitute 2–3% of the total community [41]. Nonetheless, even with relatively shallow sampling we can begin to appreciate the composition and complexity of natural phage communities. As metagenomic data accumulate, more sophisticated tools are evolving. Already, innovative approaches have been developed that allow inferences to be made regarding the structure and diversity of viral communities [43], as well as of networks of biochemical pathways [44]. Ultimately, it is bioinformatics tools such as these that will allow us to build the bridges between the composition of viral communities and their interaction with the prokaryotic communities.

Conclusions

Recent observations provide clear evidence of the enormous genetic diversity within phage communities, and also demonstrate that genes that are indistinguishable at the sequence level are present in phage populations from very different environments. These data imply that, at least for some phage populations, the viriosphere is a single community through which phage genes circulate relatively freely. This suggests that a primary function of the host community in phage ecology and evolution is to serve as a vehicle for genetic exchange among phage populations.

Update

As discussed above, diversity in phage communities occurs over a wide range of spatial and temporal scales. One would also expect these changes to be associated with changes in the host communities. Evidence of this can be seen in data that show seasonal changes in co-occurring communities of marine cyanophages and *Synechococcus* [45]. In this study, it was found that both the abundance and the diversity of cyanophages and *Synechococcus* co-varied. However, the story is made more complicated by observations that some phages have broad host ranges and are able to infect other species or genera. This is true not only of cyanophages [40] but also of vibriophages. Vibriophages that were isolated on *Vibrio parahaemolyticus* were found to infect more than one species of *Vibrio*, including *V. alginolyticus*, *V. natriegens* and *V. vulnificus* [46]. High titers of phages that infect *V. parahaemolyticus* occur in oysters all year round, even when the host is undetectable. These changes in host abundance were accompanied by changes in the host range of the viral communities, which suggests that infection of other species is sustaining the viral community [46].

The story of cyanophage diversity and genes for photosynthetic proteins continues to unfold with the sequencing of three more cyanophage genomes [47]. These phage also contain psbA homologues (Figure 1), as well as numerous other genes associated with photosynthesis (e.g. petE, petF and psbD). In addition, homologues of genes associated with phosphate stress and LPS biosynthesis were present. These data reinforce ideas of the strong co-evolutionary relationship between phages and the hosts they infect. Over evolutionary time, ancestors of these phages have clearly picked up functional metabolic genes from their hosts. Yet, as we see for *psbA*, these genes have their own evolutionary history within phage populations. They should not be thought of as host genes, but as phage genes that were co-opted over evolutionary time to maximize their own fitness.

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