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ORIGINAL PAPER

High diversity and potential origins of T4-type bacteriophages on the surface of Arctic glaciers

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Abstract Tailed bacteriophages are the most abundant viruses in the biosphere. Here we examined the T4-type bacteriophage community inhabiting the surface of two glaciers in Svalbard. We used a molecular approach to target *g23*, the major capsid protein gene, to demonstrate that in the extreme cryoconite hole habitats the T4-type phages are surprisingly diverse. Phylogenetic analysis revealed that cryoconite hole sediments harbour a mixed phage community spanning multiple T4-type phage subgroups. The majority (71 %) of phage sequences clustered into three novel phylogenetically distinct groups, whilst the remainder clustered with known marine and soil derived phage sequences. The meltwater in cryoconite holes also contained a further distinct phage community which was related to previously detected marine phage variants. The ability of phages to move between marine and glacial habitats was tested in a transplantation experiment. Phages from the nearby marine fjord were found to be capable of initiating infection of supraglacial bacteria, suggesting suitable hosts could be found by non-native phages. Together this evidence suggests that the surface of glaciers contain both novel and cosmopolitan phages, some of which may have arrived in the cryosphere from other biomes.

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Introduction

Environmental virus diversity has been estimated to be remarkably high. Metagenomic investigations have estimated that there are several thousand viral genotypes per litre of marine water (Breitbart et al. 2002) and possibly up to a million genotypes in a kilogram of marine sediment (Breitbart et al. 2004a). Despite this enormous proposed phage diversity, studies targeting conserved phage genes have revealed that a proportion of phages are globally distributed. Identical or almost identical phage genes are detected across multiple biomes and large geographical distances (Breitbart et al. 2004b; Short and Suttle 2005). This has led to speculation that certain phages, or their genes, are constantly moving around the biosphere. Hence, whilst phage diversity may be extremely high on local scales, it may be globally more limited (Breitbart and Rohwer 2005).

The tailed bacteriophages are amongst the most abundant form of virus in aquatic ecosystems (Wommack and Colwell 2000). Amongst the three tailed phage families, the Myovirus major capsid protein gene has been found to be the most abundant protein in the Global Ocean Survey metagenome (Yooseph et al. 2007). Myoviruses are currently divided up into eight genera (King et al. 2011), with the enterobacteriophage T4 being the archetype of the Myovirus family. PCR-based investigations targeting the conserved *g23* region, coding for the major capsid protein, have found T4-type viruses to be ubiquitous in the biosphere. This suggests that their host range is not as narrow or geographically limited as previously thought (Filée et al. 2005).

T4-type phages are now considered a superfamily of viruses which to date has at least 18 proposed subgroups (Filée et al. 2005; Jia et al. 2007; Wang et al. 2009a, b, 2011; Butina et al. 2010). However, only five of these groups contain known cultured representatives. Because of the abundance of tailed phages in the environment, and the large datasets of T4-type phage sequences available from multiple environments across the globe, the T4-type phages were targeted in this study for the first investigation of viral diversity in glacial ecosystems.

Glaciers and ice sheets cover 10 % of the Earth's surface during interglacial periods (Benn and Evans 1997), and covered up to 30 % during the Last Glacial Maximum (Paterson 1994). Once thought devoid of life, the surfaces of glaciers are now known to host active microbial communities (Hodson et al. 2008) that are important for the development of glacial forefields (Stres et al. 2010) (the region between the current glacial edge and the moraines from the last glacial maximum). Supraglacial ecosystems include a wide variety of habitats such as wet surface snow, streams, meltwater lakes, pools and cryoconite holes. Cryoconite holes on the surface of glaciers and ice sheets (Fig. 1) are particularly interesting because they are considered hot spots of microbial activity in the cryosphere (Hodson et al. 2008). They are small water filled depressions, typically tens of centimetres wide and deep with a thin layer of sediment at the bottom. They may cover 1–10 % of the ablation zone of glaciers (Hodson et al. 2007). Cryoconite ecosystems are an extreme environment for life to exist; they are oligotrophic environments (Stibal et al. 2006; Säwström et al. 2007a), subjected to persistent low temperatures of ~0.1 °C (Hodson et al. 2008) and annual freeze–thaw cycles. Despite this, they harbour abundant microbial communities dominated by bacteria, including filamentous cyanobacteria (Stibal et al. 2006), fungi, algae, ciliates, rotifers and tardigrades (as reviewed in Hodson et al. 2008). Carbon and nitrogen are actively fixed by the cyanobacterial community in cryoconite holes (Telling et al. 2011), which also may contain a reservoir of carbon from allochthonous habitats (Stibal et al. 2008). These carbon sources are important to adjacent habitats (Hodson et al. 2008) and other hydrologically connected ecosystems (Hood et al. 2009). Virus-like particles (VLP) have also been found to be abundant in cryoconite holes in recent years, with 10^6–10^7 VLP ml^{-1} reported in the waters and sediments of cryoconite holes, respectively (Anesio et al. 2007). Viruses are implicated in significant bacterial mortality in supraglacial ecosystems, and as much as 20 % of the heterotrophic bacterial community has been reported to contain mature phage particles at any one time (Säwström et al. 2007b). The highly truncated nature of cryoconite ecosystems and the high rates of phage infection have led to speculation that the role phages play in the recycling of carbon and nutrients may be greater than in other ecosystems (Anesio et al. 2007). The extreme nature of cryoconite ecosystems means they are ideal places to probe for novel phage sequences, which may be unique to such habitats or part of a subset of total global ubiquitous phage diversity.

Fig. 1 Cryoconite holes on the surface of a glacier. A group of cryoconite holes are shown where the dark material has melted into the surface of the ice owing to its lower albedo. This melting forms a pool of water above the sediment

Methods

Study area and sample collection

This study was centred on two valley glaciers near Ny-Ålesund, in the Northwest of the Svalbard archipelago (78.55°N 11.55°E): Austre Brøggerbreen (AB) and Västre Brøggerbreen (VB) (Fig. 2). Both glaciers terminate within 3 km of Kongsfjorden and are ca. 50 m above sea level to ~600 m elevation at the headwall. They are undergoing significant retreat and thinning, with much of the ice below the equilibrium line where snow input is equal to melting through ablation (Hagen et al. 2003). This leads to large ablation areas in the summer months which provide liquid water that flows through cryoconite holes and the surface layer of ice in an interconnected drainage system. Samples were collected from supraglacial ecosystems for molecular analysis between 25th July and 4th August 2009 from point AB and VB (Fig. 2). Water samples were also collected from a small proglacial lake (PRO) and Kongsfjorden (KF1) to assess whether there is overlap between supraglacial, proglacial and marine communities. Kongsfjorden is a marine fjord surrounded by glaciers and is subjected to large ice inputs from several calving glaciers fronts; hence, it represented the end point of the glacial drainage system. Samples were collected from the surface marine waters approximately 2 km offshore. At points AB and VB,

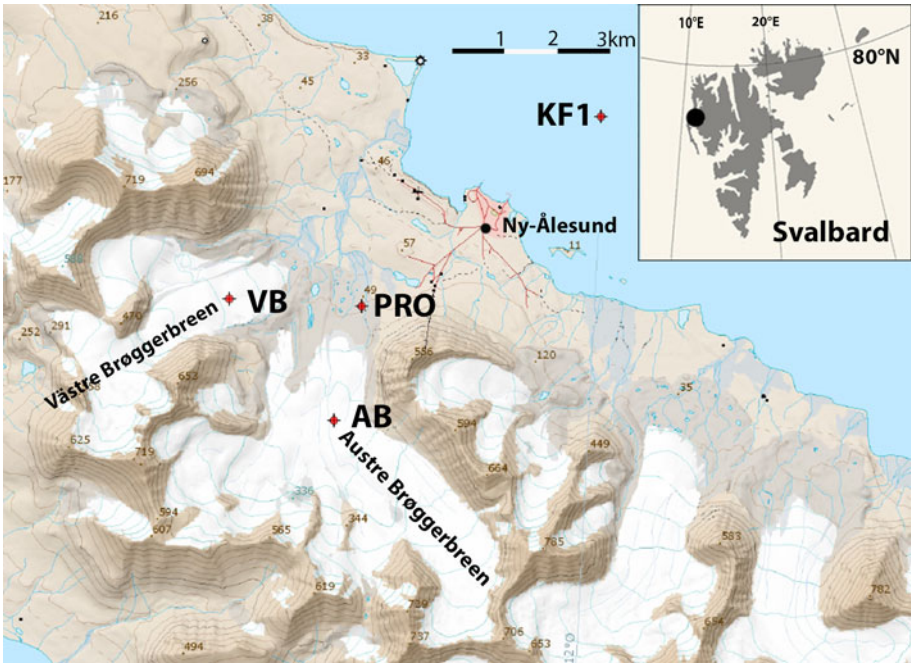


Fig. 2 Map of sampling area and the glaciers Austre Brøggerbreen and Västre Brøggerbreen around Ny-Ålesund (78.53°N 12.04°E), in the Northwest of the Svalbard archipelago. The marked sites indicate the location of sampling. Map DataCopyright© Norwegian Polar Institute

cryoconite sediment was sampled and pooled from several cryoconites within a 10 m radius of the sampling point with a sterile 50 ml syringe and transferred to sterile 50 ml tubes. This sampling strategy was chosen as a previous study on bacterial 16S rRNA diversity has found a low degree of spatial scaling on the same individual Svalbard glaciers, most likely owing to meltwater redistribution and continuous mixing of the cryoconite material on the glacier surface (Edwards et al. 2011). Samples were frozen within 2 h of collection at −20 °C and stored for up to 9 months before molecular analysis. A cryoconite water sample was collected in 2 × 10 litre acid and Milli-Q washed polycarbonate bottles from the cryoconite holes from point AB (designated CRY). Water samples from the proglacial lake (PRO) and Kongsfjorden (KF1) were also collected in 10 l containers as before. Viruses were concentrated from water samples before extraction of DNA. Samples were pre-filtered through Stericap PLUS 0.22 µm PES filters (Millipore) before being concentrated to ∼200 ml via tangential flow filtration (TFF) using a 30 kDa cut-off filter. Viral concentrates were further concentrated to 100 µl using 20 ml Vivaspin centrifugal concentrators with a 30 kDa cut-off (Sartorius-Stedim Biotech). Viral concentrates were stored in the dark at 4 °C before further analysis.

DNA extraction and PCR

Total DNA from 250 mg of pooled cryoconite sediment was extracted using a MoBio Powersoil DNA Isolation Kit, according to manufacturer's instructions. DNA was extracted from the aquatic viral concentrates by performing 2× hot–cold extraction cycles (Chen et al. 1996) and using this concentrate directly in PCR reactions. Polymerase chain reaction (PCR) was performed using primer MZIA1bis and MZIA6 (Filée et al. 2005) to target *g23* which encodes the major capsid protein gene of the T4-type bacteriophages. Using the reaction conditions previously described (Filée et al. 2005), 2 µl of cryoconite DNA extract from the sediment or 10 µl of viral concentrate from the water samples was used as PCR template. DNA bands of between 200 and 600 bp were excised under UV light and purified using a Wizard SV Gel and PCR Clean-Up kit (Promega) according to manufacturer's instructions.

Cloning and sequencing

Excised DNA was cloned into the pGEM-T Easy Vector (Promega) and transformed into JM109 competent cells (Promega). For each sample 90–180 white colonies were picked and subjected to further colony PCR with a reduction to 25 cycles to confirm the correct insert. All correctly sized colony PCR products were digested with restriction enzyme *Rsa*1 (Promega) for 4 h at 37 °C before being run on a 2 % agarose gel. Restriction mapping of *Rsa*1 digests allowed differentiation of several of the known marine virus groups (Filée et al. 2005) by gel electrophoresis (data not shown). Up to 50 colonies per sample were selected for sequencing which were to include representatives from all unique banding patterns present on the gel. This allowed a much greater diversity of clones to be sequenced in each environment but precluded the interpretation of diversity coverage as repeated sequencing of the same clones was

deliberately reduced. Plasmid DNA was sequenced by Geneservice (http://www.lifesciences.sourcebioscience.com).

Phylogenetic analysis

Nucleotide sequences were manually edited and trimmed before being translated to amino acids and subjected to a BLASTp search. Similar sequences were downloaded from the NCBI database and a multiple sequence alignment was performed using MUSCLE (http://www.ebi.ac.uk/Tools/msa/muscle/) on all sequences. The *g23* gene fragment amplified by these primers is known to contain a hypervariable loop (Filée et al. 2005), thus regions of low conservation (amino acid residues 16–52 and 72–174) were removed using Jalview (Waterhouse et al. 2009) (online resource 1) before phylogenetic analysis to maintain consistency with all other *g23* phylogenetic studies. A Neighbour-Joining analysis was performed on the alignment using MEGA version 4 (Tamura et al. 2007). All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons. Bootstrap proportions were calculated by analysis of 2,000 bootstrap replicates.

Transplantation of the virus community experiment

To determine whether phages from cryoconite holes are capable of initiating infection in bacteria from the marine environment, a transplantation experiment was performed where a concentrated virus community from cryoconites was used to infect bacteria from the marine fjord in Svalbard (Treatment $V_{AB}P_{KF1}$; Table 2), and vice versa (Treatment $V_{KF1}P_{AB}$; Table 2). The experimental design ensured that the addition of a viral concentrate from the alien habitat enhanced the natural levels of viruses by approximately one order of magnitude. A cryoconite viral concentrate was created by vigorously mixing 500 g of pooled cryoconite sediment AB with 5 l of virus-free water (30 kDa ultrafiltrate from glacial meltwater). This cryoconite slurry was filtered successively through 47 mm GF/F filters (Whatman) and 0.22 µm Stericap^{TM} PLUS (Millipore) filters. The filtrate was then concentrated to approximately 200 ml via tangential flow filtration (TFF) using a 30 kDa cut-off filter. This concentrate was re-filtered through a 0.2 µm Isopore filter (Millipore) before successive 10 ml aliquots were loaded onto a Vivaspin^{TM} 30 kDa centrifugal concentrator (Sartorius Stedim) and concentrated further to $\sim$1 ml. To begin the transplantation treatments, 166 µl of cryoconite viral concentrate was added to 6 replicates of 1.5 ml unfiltered seawater from Kongsfjorden. Control samples consisted of 6 seawater replicates with 166 µl virus-free cryoconite water added only (30 kDa ultrafiltrate). One of the control replicates was killed with the addition of 100 µl Glutaraldehyde. Treatments were incubated at 4 °C for 12 h before bacterial carbon production (BCP) was measured over a further 3 h according to the methods of Smith and Azam (1992).

The reverse experiment was performed by filtering and concentrating 20 l of seawater from the surface waters of Kongsfjorden (KF1) via TFF as before to create 1 ml of marine viral concentrate. To prepare bacterial hosts from cryoconite sediment, 1 ml cryoconite sediment from AB was diluted with 9 ml virus-free glacial water and vortexed. 1.5 ml aliquots of this sediment slurry were removed and added to each of 12 microcentrifuge tubes. These were divided into 6 controls which were supplemented with 166 µl virus-free seawater (30 kDa ultrafiltrate), and 6 virally enriched samples where 166 µl of marine viral concentrate was then added to the cryoconite communities. Samples were incubated at <1 °C for 12 h before BCP was then measured as before. Treatments were compared against control incubations with a *t* test in SPSS.

Results

Sequencing and alignment

A total of 43 unique *g23* clones from cryoconites were sequenced, these comprised of 31 clones from cryoconite sediments and 12 clones from cryoconite water. Another 20 unique clones from the proglacial lake and 15 from the marine site in Kongsfjorden were also sequenced to assess the potential for phage communities to overlap between supraglacial and other nearby environments. A number of PCR products from cryoconite sediments were not viral, these were easily identified and removed by the notable absence of highly conserved regions in the first 14 and last 47 amino acid residues (online resource 1). All *g23* sequences were deposited in GenBank under accession numbers JQ996716–JQ996802. T4-type *g23* sequences from all sample points appeared diverse upon initial inspection, with amino acid lengths between 121 and 180 residues. Cryoconite sediment and water *g23* sequences had amino acid lengths of 121–159 residues and shared identities of between 60 and 99 % with previously detected environmental phages (Table 1; online resource 2). Sequence similarity between clones from this study showed surprising diversity, with some clones from cryoconites sharing as little as 25 % amino acid identity (clones CRY2 and VB19).

Phylogenetic analysis

Phylogenetic analysis (Fig. 3) revealed there were no sequences from any sample locations that grouped with

Table 1 Summary of *g23* sequence fragment similarity to GenBank clones

Location	# Clones sequenced	# Unique clones	# Unique clones with similarity to GenBank (% identity)			# Clones in novel phylogenetic groups
			<70 %	70–90 %	>90 %	
Cryoconite sediment	99	31	7	21	3	30
Cryoconite melt	25	12	2	9	1	4
Proglacial	26	20	6	8	6	19
Marine	25	15	6	4	5	5

previously cultured or detected phages in the T-evens, pseudo T-evens, schizo T-evens and thermo T-evens. Only one marine clone (KF36) grouped with representatives from the cyanophage group the exoT-evens. Cryoconite sediment and meltwater clones as a whole fell into 10 groups, 2 of which were the previously known Marine Groups II and IV (Filée et al. 2005). There were different groupings of sequences between cryoconite meltwaters (CRY labelled clones in Fig. 3) and cryoconite sediment (AB and VB labelled clones). The majority of cryoconite meltwater clones (8 out of 12) clustered into a subgroup of the previously defined Marine Group II. Here, clone CRY5 also shared up to 99 % identity with marine clone KF12 detected from Kongsfjorden in this study. The majority of cryoconite sediment clones (22 out of 31) formed three new monophyletic subgroups upon phylogenetic analysis, here labelled Polar I, Polar IIa and Env Ia. Polar group I formed with 100 % bootstrap support from 15 unique cryoconite sediment clones, whilst it shared up to 75 % amino acid identity with borehole water and paddy soil clones; bootstrap support for its placement near Paddy group IX was low. Polar group IIa contained three clones isolated from cryoconites which showed only ~60 % identity to environmental sequences detected in borehole waters (online resource 2). Env Ia is a subgroup that contained four phylogenetically distinct sediment clones from the two glaciers that were most closely grouped to black soil sequences, sharing 70 % amino acid identity. One sediment clone (VB1) grouped with marine sequences into Marine group IV, with 95 % bootstrap support. This clone was also identical to Kongsfjorden clone KF86. The rest of the cryoconite sediment clones grouped with previously ungrouped clones from mainly soil environments. The four sediment clones in A, B and C (Fig. 3) clustered with sequences detected from Chinese mollisols and Chinese and Japanese paddy field soils. These clones grouped with 88–99 % bootstrap support and shared up to 92 % identity with these previously detected soil sequences. Two sediment clones (AB13 and VB3) clustered in the proposed group Env II with black soil sequences, sharing over 90 % amino acid identity. A further two clones (VB22 and VB26) clustered in the most unique proposed group, Env III. Env III is a deep branching group also containing two cryoconite meltwater clones (CRY2 and CRY4) which group with a wide range of previously ungrouped representatives from soils, temperate and Antarctic lakes and marine waters. All members of this group only showed approximately 40 % amino acid identity to other phage variants in the GenBank database.

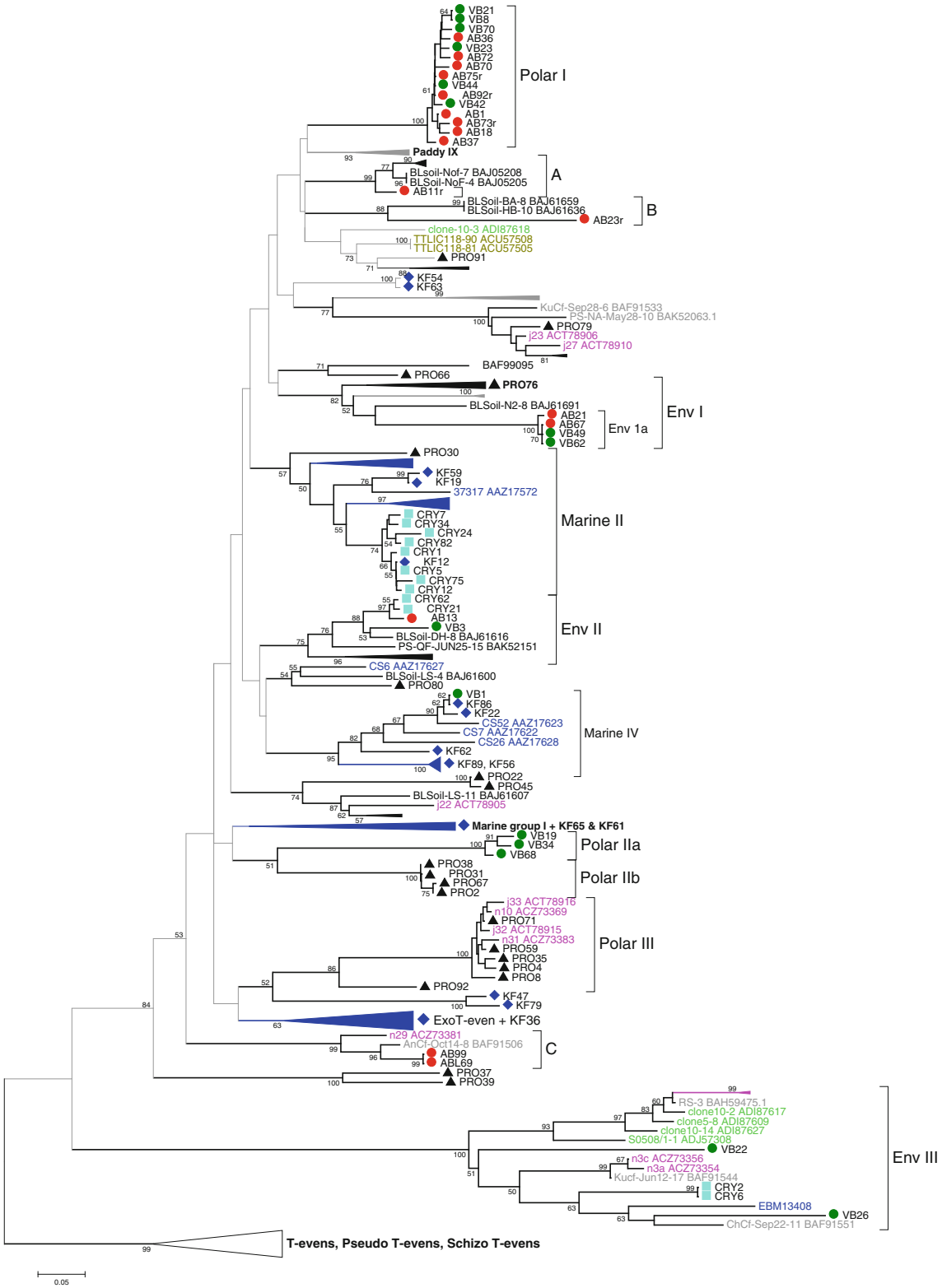
Proglacial lake *g23* clones fell into ten groups. In the Polar III group a cluster of six proglacial lake clones shared amino acid identities of 97–99 % with clones isolated from an Antarctic lake (López-Bueno et al. 2009). Four proglacial lake clones also formed the subgroup Polar IIb. The majority of marine clones (11 out of 15) sequenced grouped into previously proposed Marine groups I, II and IV.

Transplantation of the viral community

Bacterial carbon production (BCP) values in treatment $V_{KF1}P_{AB}$ (Table 2) were 42 % of control (*t* test $P < 0.05$) when a viral concentrate was added, suggesting that viruses from the fjord could infect bacteria from cryoconites. On the other hand, BCP was not significantly different when a cryoconite viral concentrate was added to the marine bacterial community sampled from KF1 ($V_{AB}P_{KF1}$), (*t* test $P > 0.05$). The viral concentrates contained only concentrated viruses and dissolved organic matter. Any dissolved organic matter additions would be expected to increase bacterial production (Judd et al. 2006).

Discussion

This study examined the bacteriophage communities in cryoconite holes on the surface of glaciers in Svalbard. Using a set of PCR primers to target the *g23* region, coding for the major capsid protein of the T4-type bacteriophages, it has been demonstrated that the T4-type phages are present and potentially diverse in these ecosystems. The phylogenetic analysis presented here suggests that the community has mixed origins from a variety of environments, alongside a number of novel phages.



Novel T4-type sequences

Many clones from cryoconites grouped into 3 novel phage subgroups, tentatively labelled Polar group I, Polar group IIa and Env 1a which contain clones hitherto unique to Arctic environments (Fig. 3). Several studies using *g23* sequence fragments have demonstrated that uncultured T4-type phages from marine, freshwater and terrestrial environments generally form unique clusters specific to their environment when phylogenetic analysis is performed.

◀ **Fig. 3** Neighbour-Joining tree of T4-type viruses inferred using the amino acid sequences of *g23* gene fragments. Bootstrap proportions >50 % are denoted and represented by a *bold line*. *Red* and *green circles* represent clones isolated from Svalbard glaciers: Austre Brøggerbreen (AB) and Västre Brøggerbreen (VB) respectively. *Light blue squares* cryoconite meltwater. *Dark blue diamonds* represent Kongsfjorden (KF) and *black triangles* a proglacial lake (PRO). Polar groups I–III refer to new groups composed from only g23 sequences found in the Arctic or Antarctic. Environmental groups (Env groups) I–III are groups proposed which contain sequences from a variety of environments. Marine I, II, IV, Exo-T and T-evens groups are described previously (Filée et al. 2005), as is Paddy group IX (Wang et al. 2009a). Colour coding of the text and collapsed groups denotes GenBank sequence origins: *Blue* represents marine clones, *grey* represents paddy field clones, *black* represents soil (or a mixture of paddy and soil) clones, *pink* represents Antarctic clones, *green* represents freshwater clones

For example, whilst five unique marine phage groups have been proposed (Filée et al. 2005), several studies on Japanese paddy fields proposed a further nine groups of soil phages (Jia et al. 2007; Wang et al. 2009a, b). A recent study on Lake Baikal in East Siberia showed that freshwater phages from the lake also formed several unique and distinct clusters (Butina et al. 2010) as did a study on Chinese mollisols (Wang et al. 2011). Further analysis on all available T4-type *g23* sequences from varied environments has shown that T4-type phage communities as a whole are distinctly different between habitats (Liu et al. 2011). The polar groups proposed here contain unique sequences and appear to follow this trend of novel phage groups in different environments, presumably associated with the available host population found in cryoconites.

In a recent study of cryoconite bacterial 16S rRNA diversity, several bacterial strains were identified to be closely related to Proteobacteria, Actinobacteria and Cyanobacterial strains isolated only from other cold freshwater environments (Edwards et al. 2011). This suggests novel cold adapted phage-host communities could be present, however, this has yet to be determined as no phage-host systems have as yet been cultured and studied from glacial ecosystems. The relationship between phages and their environment is not straightforward in cryoconite ecosystems, as when the whole phage community is considered there are many clones that also group and overlap with those from other environments.

Known viral groups

None of the *g23* sequence fragments detected in this study fell into the T-evens or pseudo T-evens subgroups. Although members of these groups are frequently detected in environmental samples from marine and soil environments, e.g. (Filée et al. 2005; Wang et al. 2009b), the only known hosts for these groups are enterobacteria in mammalian guts. Apart from marine clone KF36, there was also a lack of *g23* sequences that clustered with the exo T-evens phage group; among the known hosts for these phages are marine and freshwater cyanobacteria. As cyanobacteria are dominant primary producers in cryoconite ecosystems (Stibal et al. 2006), it was expected that a proportion of the virus community in cryoconites would be cyanophages. The PCR primers used in this study have primarily detected cyanophages infecting unicellular cyanobacteria, particularly marine strains (Filée et al. 2005; Deng and Hayes 2008). The cyanobacterial community in cryoconites is overwhelmingly filamentous, making up 90–99 % (mean 95.7 %) of cyanobacteria by abundance in Svalbard cryoconites (Kastovská et al. 2005). In previous studies, another important capsid protein assembly gene used in phage fingerprinting (*g20*) could not be identified in freshwater phages infecting filamentous cyanobacteria (Baker et al. 2006). Concurrently, a study on UK freshwater lakes found that most cyanophages isolated from the water column were unable to be amplified by targeting *g20* or *g23* genes (Deng and Hayes 2008). It is probable therefore that cyanophages infecting filamentous freshwater species have highly novel capsid assembly protein genes, thus the PCR primers selected in this study were unable to anneal to cyanophages DNA from cryoconites.

Table 2 Transplantation of viruses between habitats experimental setup

Treatment		Prokaryote source		Viral concentrate			VLP Enrichment		Incubation	BCP**
Code	Type			Source		VLP* added (10^7 VLP)	VLP in prokaryote source (10^7 ml^{-1})	VLP Enrichment factor (in 1.66 ml)	Temp °C	% of control
V_0P_{KF1}	Control	Marine	KF1	–	–	–	4.9	–	4	
$V_{AB}P_{KF1}$		Marine	KF1	Cryo.	AB	62	4.9	8.5	4	107 % (NS)
V_0P_{AB}	Control	Cryo.	AB	–	–	–	5.1	–	<1	
$V_{KF1}P_{AB}$		Cryo.	AB	Marine	KF1	3221	5.1	380	<1	42 %

NS not significant (*t* test $P > 0.05$)

* VLP virus-like particles

** BCP bacterial carbon production

Diverse origins and movement between biomes

The finding of a cryoconite hole sediment clone (VB1) that grouped strongly with Marine group IV phages, and was identical to marine clone KF86 in this study, suggests an overlap and movement of some of the viral community between marine and glacial habitats. The detection of highly similar virus sequences across biomes is not uncommon in PCR-based studies on viral diversity and has led to speculation that some viruses are able to move around the biosphere, either infecting similar hosts or being more opportunistic in nature and having a broad host range (Breitbart et al. 2004b; Short and Suttle 2005). The mixing of phage communities between glacial and marine habitats would not be unexpected given their close proximity (Fig. 2) and a likely virus transport pathway through sea spray or precipitation. Obligate marine bacterial strains have been reported in snowfall onto glaciers in the high Canadian Arctic (Jungblut et al. 2010) supporting the existence of such a pathway. The greatest number of clones related to marine phage sequences in this study were found in cryoconite hole meltwaters (CRY labelled clones in Fig. 3). Cryoconite hole meltwater is derived from the melting snowpack and ice itself. Phages present here may reflect those present in precipitation onto the glacier which may explain their grouping into Marine group II. The potential mixing of phages raises the possibility that phages from marine environments could successfully colonise glacial ecosystems. Indeed this is further reinforced with data from the virus transplantation experiment (Table 2) which demonstrated that addition of a concentrate of marine viruses to cryoconite hole bacteria caused a substantial decrease in bacterial carbon production up to 42 % of control. The addition of a concentrate of viruses was designed to show if infection was possible, but not to simulate the true rate of virus addition to the habitat. However, from this result it is probable that some marine phages are not simply mixed and utilised as an inactive carbon source in cryoconites but are actively infecting supraglacial bacteria. The ability of phages to infect hosts from different biomes has been previously reported experimentally (Sano et al. 2004; Bonilla-Findji et al. 2009). Thus, the viruses from marine waters entering glacial ecosystems could bring novel virally encoded genes to supraglacial ecosystems.

There is also the possibility that cryoconite virus communities can be washed off glaciers and enter hydrologically connected ecosystems, initiating infection in downstream hosts. However, no reduction in bacterial carbon production was observed when a concentrate of cryoconite viruses was added to marine bacteria in this study. The viral concentrate made from cryoconites was only an eightfold enrichment of the marine natural virus levels (compared to 380-fold enrichment for the reverse experiment) which could explain the lack of response by marine bacteria. However, the lack of response may also reflect a more specialised phage community inhabiting cryoconites being unable to infect diverse marine hosts.

The grouping of some cryoconite sediment clones with those sequenced from non-marine habitats suggests further diverse origins for the phage component from these ecosystems. Six cryoconite sediment clones grouped with those from terrestrial ecosystems. The 4 cryoconite clones in clusters A, B and C (Fig. 3) plus the 2 clones in Env II grouped with soil-phage sequences, suggesting shared ancestry for some cryoconite and soil-based virus sequences. Similarly, the four clones in Env Ia, although phylogenetically distinct, also displayed a shared ancestry with soil-phage sequences by forming a subgroup in the Env I cluster. Further to this, Env III contained the most unique phages in this study which grouped with several outliers from multiple environments. Env III members were characterised by a lack of conservation in one of the most conserved regions in the *g23* sequence, the last 47 amino acid residues. Representatives from cryoconites clustered here alongside representatives from Siberian, Chinese and Antarctic freshwater lakes, Japanese paddy field soils and the marine environment; implying a common ancestor for all its members which originate from across the globe and span three biomes.

Opportunities for genetic exchange

The relatedness of certain cryoconite hole derived sequences to those that are globally distributed and from distant biomes suggests there may be opportunities for phages from diverse origins to colonise glacial ecosystems. This could be either through individual phages infecting a diverse range of hosts, or phages finding similar hosts between these ecosystems. There are several lines of evidence for the latter option. The similarities between some cryoconite hole and soil-phage sequences may reflect the nature of the inoculating cryoconite material itself, much of which may be derived from the surrounding moraines and tundra (Stibal et al. 2008). Indeed, several species of cyanobacteria found previously in Svalbard cryoconites correspond to those found in nearby soils and proglacial streams and represent opportunistic cosmopolitan taxa (Stibal et al. 2006). Similarly, the heterotrophic bacterial diversity on Austre Brøggerbreen and Västre Brøggerbreen contains 16S rRNA sequences that closely match those from other cold polar freshwater habitats, alongside a large proportion of sequences that show close identity (>97 %) with those commonly found in temperate soils and humic lakes (Edwards et al. 2011). This suggests that components of the heterotrophic prokaryotic community on glaciers are

cosmopolitan in their distribution. Such an occurrence of widely distributed hosts may account for presence of phages similar to those from other distant habitats. Such cosmopolitan phage groups in the same habitat may provide opportunity for genetic exchange of phages from distinct types of biomes (e.g., terrestrial and marine) in cryoconite ecosystems.

Bipolar lake communities

Most proglacial lake *g23* sequences fell into different subgroups relative to those sequenced from cryoconites. A surprising find was a cluster of Arctic proglacial lake phages in Polar group III that shared up to 99 % amino acid identity to those found in an Antarctic lake on Livingston Island (López-Bueno et al. 2009). Both lakes are oligotrophic environments, frozen for long periods of the year and are characterised by microbially dominated ecosystems. The near identical nature of these T4-type pages between the poles could indicate movement and exchange of virus genes between the Arctic and Antarctic on recent evolutionary timescales. Such similar strains in widely separated but analogous habitats fit the longstanding conjecture that "everything is everywhere, but the environment selects" (Baas-Becking 1934). The cold biosphere is an ideal place to test such a conjecture as parallel environments are separated by large distances, with equatorial regions serving as potential barriers to dispersal of cold adapted organisms (Staley and Gosink 1999). The bipolar distribution of near identical phages could also suggest that they infect similar hosts found at both poles, hence analogous habitats and host availability could exert a strong selection pressure on the virus capsid. Indeed identical bacterial strains have previously been found between high Arctic and Antarctic lakes (Pearce et al. 2007). It follows that if potential cold adapted hosts are selected by the environment then their associated phages may follow the same distribution.

Conclusions

The molecular evidence in this study suggests that there is a diverse bacteriophage community present in supraglacial environments which span several T4-type virus subgroups. Three novel clusters of *g23* sequence fragments were formed from cryoconite sediment clones which represent novel phage strains so far unique to the polar environments. These may be phages infecting unique hosts of polar regions or part of a larger but under-sampled global phage diversity. Many sequences also grouped with representatives from distant environments, suggesting a cosmopolitan component of phages are also present. This mixed community appears to agree with a recent study on bacterial diversity which also demonstrated the presence of a number of cold adapted taxa alongside a large component of cosmopolitan species previously found in temperate regions (Edwards et al. 2011). Complementing this detection of cosmopolitan phages in cryoconite holes, marine phages were found to be capable of initiating infection of cryoconite hole bacteria in this study, hence the presence of marine and other potentially globally distributed phages may provide opportunities for recombination amongst diverse phages in cryoconite ecosystems. Further to this it may enhance opportunities for transduction between bacteria from a broad range of environments and psychrophilic hosts in supraglacial ecosystems.

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