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Temporal Dynamics and Decay of Putatively Allochthonous and Autochthonous Viral Genotypes in Contrasting Freshwater Lakes

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Aquatic viruses play important roles in the biogeochemistry and ecology of lacustrine ecosystems; however, their composition, dynamics, and interactions with viruses of terrestrial origin are less extensively studied. We used a viral shotgun metagenomic approach to elucidate candidate autochthonous (i.e., produced within the lake) and allochthonous (i.e., washed in from other habitats) viral genotypes for a comparative study of their dynamics in lake waters. Based on shotgun metagenomes prepared from catchment soil and freshwater samples from two contrasting lakes (Cayuga Lake and Fayetteville Green Lake), we selected two putatively autochthonous viral genotypes (phycodnaviruses likely infecting algae and cyanomyoviruses likely infecting picocyanobacteria) and two putatively allochthonous viral genotypes (geminiviruses likely infecting terrestrial plants and circoviruses infecting unknown hosts but common in soil libraries) for analysis by genotype-specific quantitative PCR (TaqMan) applied to DNAs from viruses in the viral size fraction of lake plankton, i.e., 0.2 μm > virus > 0.02 μm. The abundance of autochthonous genotypes largely reflected expected host abundance, while the abundance of allochthonous genotypes corresponded with rainfall and storm events in the respective catchments, suggesting that viruses with these genotypes may have been transported to the lake in runoff. The decay rates of allochthonous and autochthonous genotypes, assessed in incubations where all potential hosts were killed, were generally lower (0.13 to 1.50% h^{-1}) than those reported for marine virioplankton but similar to those for freshwater virioplankton. Both allochthonous and autochthonous viral genotypes were detected at higher concentrations in subsurface sediments than at the water-sediment interface. Our data indicate that putatively allochthonous viruses are present in lake plankton and sediments, where their temporal dynamics reflect active transport to the lake during hydrological events and then decay once there.

Viruses play crucial roles in the ecology and biogeochemistry of aquatic ecosystems by causing mortality of bacteria, archaea, and eukaryotes (23, 64). Viruses maintain large abundances in both freshwater and salt water (4, 46), typically exceeding bacterial abundances by 10- to 100-fold. Viral lysis accounts for a significant percentage of bacterial biomass daily in marine and freshwater ecosystems (57, 64, 66), releasing particulate organic matter within bacterioplankton into the dissolved organic matter pool. Since bacteria are the most abundant hosts, most virioplankton are believed to infect cooccurring bacterioplankton (45). However, in coastal waters and particular freshwater habitats, viruses may also be produced by allochthonous (terrestrial) hosts and may be transported to aquatic habitats through rainfall events and runoff (38), groundwater discharge (22), sewage outfall (32, 33), or aerosols (1). Because the abundance of viruses in plankton does not typically vary strongly over time (24, 43), the production and decay of viruses are balanced in coastal ocean (28) and lake (59) habitats, but they may be unbalanced in eutrophic marine waters (8). While there have been several studies examining the fate and persistence of human-pathogenic viruses in aquatic habitats (7, 52), few have addressed viruses of other terrestrial hosts.

In contrast to studies of aquatic ecosystems, there have been few studies of the diversity and distribution of viruses in soils. Williamson et al. (67) optimized extraction protocols for viruses across several soil types and found that viral abundances ranged from 10^8 to 10^9 virus-like particles (VLPs) g^{-1} dry weight, with the highest abundances in forest soils and lower abundances in agricultural soils. Interestingly, viral abundance was correlated with soil moisture and land use type but was not related to soil texture (68, 69). Relatively few studies have investigated the diversity of viruses in soils by using metagenomic approaches, and only one of these (21) targeted viruses during sequencing. A comparison of rainforest, desert, and prairie soils demonstrated that viral assemblages are unique, with the highest level of diversity occurring in rainforest soils (~10^6 viral genotypes) (21). Viral assemblages in Delaware and Wisconsin soils were compared to aquatic viruses and reflected the host composition (*Actinobacteria* in soils and cyanobacteria in aquatic habitats), with a lack of homology to known viruses or sequences in environmental sequence databases (53). Similarly, analyses of soil and aquatic viral communities demonstrated that these communities harbor mostly genetically distinct assemblages (53).

Viral decay in aquatic ecosystems is the result of UV light exposure (58), attachment to particles and sinking (29), heat-labile organic matter (notably nucleases) (42), and consumption by heterotrophic nanoflagellates (27). The magnitudes of these factors in aquatic habitats have been subject to significant study in the past 2 decades, with most studies reporting decay rates of 2 to 4% of viruses h^{-1} (64). VLPs in sediment and soil habitats may have lower decay rates due to chelating properties of organic matter that inhibit extracellular nucleases (35). For example, viable cya-

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nophages have been recovered from deep sediments in the Saanich Inlet (56). The decay rates of viruses of eukaryotic hosts have not been studied extensively in natural aquatic settings, with the exception of pathogenic human viruses in groundwater (6) and nucleopolyhedroviruses of insects (31).

The aim of this study was to examine the dynamics of allochthonous and autochthonous viruses in two contrasting freshwater habitats, Cayuga Lake (CL) and Fayetteville Green Lake (FGL), and their respective catchments. First, we elucidated putatively autochthonous and allochthonous viral genotypes in the epilimnia of these lakes by shotgun metagenomic sequencing, which provided targets for quantitative approaches to study the dynamics of specific viral genotypes. Second, we analyzed the abundances in surface waters of several viral genotypes presumably infecting allochthonous and autochthonous hosts over a 6-week period in summer to study the dynamics during rainfall events. We also examined the presence of viral genotypes within sediments as a possible environmental reservoir. Finally, we estimated decay rates of viral genotypes in incubations to compare the persistence of allochthonous and autochthonous genotypes within the lake habitat.

MATERIALS AND METHODS

Lake settings and sampling locations. Water column and soil samples were collected from two locations—FGL and CL (Fig. 1). FGL is a small (0.258 km^2), deep (mean depth, 52 m), meromictic (permanently stratified) marl lake in Onondaga County, NY. The epilimnion (surface water) of FGL is unusually oligotrophic due to permanent chemical stratification and a small watershed area (4.33 km^2) which is mainly state parkland (15). The lake has been the subject of several paleolimnological studies in the past 5 decades because the monimolimnion (subthermocline water) of the lake is believed to represent an early earth analogue (72). The lake also contains littoral laminated $CaCO_3$ structures (bioherms) (14), which are believed to be caused by precipitation from photosynthesizing benthic microalgae. Dense blooms of coccoid cyanobacteria also cause $CaCO_3$ precipitation during whiting events (60). One hundred liters of epilimnion water was collected from a concrete pier located on the northeast arm of FGL on 1 October 2010. Water column samples were collected using a sample-rinsed plastic bucket and transferred to 5 20-liter HDPE Cubitainers, which were placed into a cooler for transport to Cornell University (Ithaca, NY). Soil samples were collected on the same date from agricultural cropland in the northeastern area of the catchment (Fig. 1B). Five soil cores (3 cm^3) were collected using syringe corers from surface soil at randomly selected locations within a 10-m radius. Care was taken to avoid live vegetation (roots and leaves) within the cores, although soil cores contained leaf litter and humus from plants. Vegetation was primarily grasses (*Poaceae*). The soil cores were immediately placed into sterile 15-ml centrifuge tubes, which were subsequently frozen in liquid N_2 before transport to the laboratory.

CL is the largest of the New York Finger Lakes, at 172 km^2, and the second deepest, at 133 m (mean depth, 55 m), and is a glacially formed warm polymictic (mixing multiple times per year) lake (Fig. 1C). The lake experiences moderate productivity from the fall through spring, during ice-free months (42a). The lake receives substantial inorganic nutrient inputs from the surrounding catchment (~1,150 km^2), including point source inputs from tertiary treated sewage from the city of Ithaca in the southern basin and non-point-source inputs from several small towns that line its shores (25). Water quality has, however, improved over the past 2 decades as a consequence of upgraded sewage treatment plants and invasion by zebra mussels (36). The lake is generally P limited (25). Epilimnion water column samples (40 liters) were collected from a pier in Stewart Park, Ithaca, NY (approximately 3 m from shore), on 10 October 2010, using a sample-rinsed bucket (Fig. 1C). Five soil cores were collected from pasture and croplands of the Estuarine Research Park, which is within the catchment of Cascadilla Creek, on the same date. The locations of soil core collection were chosen to reflect agricultural land use (*Sorghum* cropland and soils beneath a livestock pen). Soil cores were collected by syringe corers, transferred to sterile 15-ml tubes, and frozen at −80°C in the laboratory.

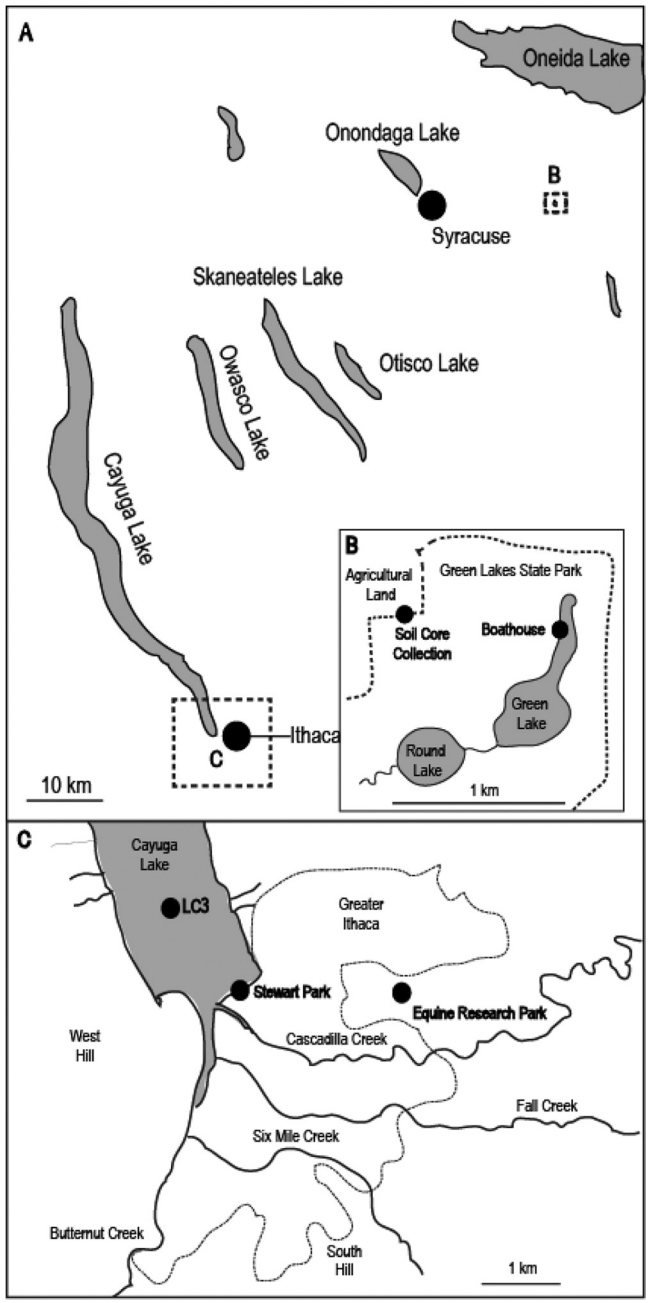

FIG 1 (A) Map of sampling locations in the Finger Lakes region of upstate New York. Samples were collected from two contrasting lakes: Fayetteville Green Lake, within the Green Lakes State Park, at the boathouse for plankton and sediments and on agricultural land nearby for soil (B); and Cayuga Lake, in Stewart Park and at site LC3 for plankton and at the equine research park for soil (C).

Water column virus preparation. Water samples from both FGL and CL were sequentially filtered through 142-mm-diameter 10-μm-pore-size Nuclepore and 0.2-μm-pore-size Durapore filters, using positive-air-

TABLE 1 Sequences for quantitative PCR primers and probes used in this study[a]

Genotype	Primer or probe sequence (5′–3′)		
	Forward primer	Reverse primer	Probe
FGL phycodnavirus	TTTCATTTTTGCCGATGGAT	TTGTGCGTACAATTCGTCGT	TCGCCAAGCCCCATATCAGGA
FGL cyanomyovirus	GCACAGATCAGCACCAGTGT	GGATTAGCAGGCAGACGAAG	CACTGGCGCTACATCTGGATCGA
FGL circovirus	CCATCCCACCATTTATTTGC	GGGTCCATCTGGAACTGGTA	GGCATTGGGAAAAAGCTCTCTTGC
FGL geminivirus	TCCGAGGAGCAGAGTATCGT	ATGCTAATATCGGGCGAGTG	TTCACCGTCCTTGCGGGCAT
CL phycodnavirus	GCAGGCCGAACAGAAGATAC	AAGGCACTGCGACAGGTTAT	GGCGCTTCTCCAGCATACAGCA
CL cyanomyovirus	ACGGTATCAAGGCCAATGAG	CGACCACCGAAGTAGAAGGA	TGTCCAACTGTTAGGTCAGTGGGGT
CL circovirus	GGAAGTCAAGGGTTCGTCAA	TACCATTCTCGGGGATCAAG	GCCGAGGTTATCTGGATCACCAGC
CL geminivirus	GGAATGCACCTCCGATAAGA	AATGTCGTACCGTTGGAAGC	GCCTGTGTCTTCGTACGTAAGCTTCC

[a] The sequences for oligonucleotide standards are presented in Table S1 in the supplemental material. Primers were designed using Primer3, based on assembled contiguous sequences from the water columns and soils of Fayetteville Green Lake (FGL) and Cayuga Lake (CL).

pressure filtration. The filtrate was subjected to tangential flow ultrafiltration using a recirculating Prep/Scale tangential flow ultrafilter (Millipore) with a 30-kDa molecular mass cutoff. The concentrate volume was 175 ml for both lakes. The presence of virus particles (and absence of bacterial cells) was confirmed by SYBR green I staining and epifluorescence microscopy (41, 44).

Soil virus preparation. Samples (1 cm^3) from each of 5 soil cores were pooled for each metavirome, representing 2.0 g and 4.9 g dry weight of soil for CL and FGL, respectively. Soil was homogenized in 35 ml 0.02-μm-filtered phosphate-buffered saline (PBS) by use of a sterilized mortar and pestle for 2 min, following the protocols of Thurber et al. (63), which were based on a previous work (70). After homogenization, the sample was briefly centrifuged at 3,000 × *g* for 5 min, after which the supernatant was syringe filtered through 0.2-μm polyethylsulfone (PES) filters (VWR) to remove larger particles. The soil extracts were checked for the presence of viruses by SYBR green I epifluorescence microscopy.

Metavirome preparation. Samples were prepared for sequencing following the protocols of Thurber et al. (63). Water column concentrates were amended with 1 M NaCl before further processing. Both soil and water column concentrates were precipitated by the addition of 10% polyethylene glycol 8000 (PEG 8000) at 4°C overnight in sterilized round-bottom tubes (Oak Ridge). After precipitation, samples were centrifuged at 13,000 × *g* for 30 min in a fixed-angle rotor (Sorvall). The supernatant was decanted, and viral pellets were resuspended in 3 ml 0.02-μm-filtered PBS. Resuspended viruses were then filtered through 0.2-μm PES syringe filters (Acrodisc). The filtrate was subjected to density gradient ultracentrifugation according to the protocols of Thurber et al. (63), using density steps of 1.3, 1.5, and 1.7 g ml^{-1} and a swinging-bucket rotor at 60,000 × *g* for 2 h. Viruses in the 1.5- to 1.7-g ml^{-1} fraction (~1 ml) were removed using a sterile syringe and refiltered through a 0.2-μm PES filter. The concentrate was treated with nucleases (2.5 U of DNase and 0.25 U of RNase) for 2 h at 37°C to eliminate nucleic acids not incorporated into viral capsids (40). DNA was extracted from 500 μl of each sample by use of the cetyltrimethylammonium bromide (CTAB)-EDTA extraction procedure as previously described (63). Extracted DNA was tested for the presence of cellular DNA by amplification of both 16S rRNA and 18S rRNA genes following previously described protocols (63). For all samples, there were no amplicons generated after 30 cycles of PCR, indicating that cell lysates were clear of contaminating cellular DNA.

DNA metaviromes were prepared for each sample by use of a Genomiphi kit, with 1 μl of purified viral DNA used as the template. Five Genomiphi reaction mixtures were pooled and purified using a DNeasy tissue kit (Qiagen). Cleaned Genomiphi reaction mixtures were quantified by PicoGreen fluorescence and submitted for sequencing at EnGenCore (University of South Carolina), where each sample was run on 1/8 of a picotiter plate using Titanium pyrosequencing chemistry.

Bioinformatic analyses. Reads were initially trimmed to remove low-quality sequence reads and sequencing and amplification adapters (~2% of sequence reads in each library), using CLC Genomics Workbench 4.0. In addition, reads of <100 bp were discarded. False duplicate reads (26), which represented only 0.2 to 1.0% of viral reads, were not removed from our data set. Analysis of cleaned-up sequence libraries followed two approaches. First, sequence reads were analyzed using the VIROME pipeline (http://virome.diagcomputing.org/), which compares open reading frames (ORFs) by BLASTp searches ($E < 0.001$) against several databases of reference peptides (Uniprot, SEED, ACLAME, COG, GO, and KEGG databases). Second, libraries were assembled into contiguous sequences (contigs) by use of CLC Genomics Workbench 4.0 to increase confidence in the annotation of short reads. Reads were assembled using the following stringent assembly parameters: minimum overlap of 0.2, minimum similarity of 0.95, penalty for mismatch of 2, penalty for insertion of 3, and penalty for deletion of 3. We used an approach similar to that of the VIROME pipeline to assign contig phylogeny. ORFs were extracted from assembled contigs by use of the GetORF algorithm (http://emboss.sourceforge.net/apps/cvs/emboss/apps/getorf.html) and were subsequently compared by BLASTx searches against the nonredundant (nr) protein database at NCBI, using an E value cutoff of 10^{-3}. Contigs containing at least 1 viral gene ORF (referred to as "viral contigs" throughout this study) were subjected to further tBLASTx comparison against viral genomes at the CAMERA website. Phylogeny in all cases was taken as the BLASTx or tBLASTx hit (with the lowest E value) in the respective protein or genome database.

Selection of targets for qPCR and primer design. Candidate allochthonous viral genotypes were identified in soil metaviromes, and candidate autochthonous viral genotypes were identified within water column metaviromes. Candidate contigs were identified based on observations of sequence read phylogeny variation between soil and water column metaviromes, confidence of contig annotation (i.e., lowest E value), putative host organism, and contig sequence coverage. Selected candidate allochthonous and autochthonous contigs were used to design quantitative PCR (qPCR) primers and TaqMan probes, using the Primer3 program (Table 1) (50). Oligonucleotide standards were used for generation of a qPCR standard curve for quantification of abundance (see Table S1 in the supplemental material). The linearity of standards was established for each primer-probe pair prior to downstream analyses of absolute viral abundance.

qPCR. qPCRs were carried out in duplicate, with duplicate standards and at least 2 negative controls per run, in an ABI 7300 real-time PCR machine. qPCR mixtures (25 μl) comprised 1× TaqMan master mix (Applied Biosystems International), 10 pmol each of forward and reverse primers and probes (Table 1), and 2 μl template DNA. qPCR mixtures were subjected to an initial heating step at 50°C for 10 min, followed by a hot start at 95°C for 5 min. The reaction mixtures were then thermally cycled at 95°C for 30 s followed by 1 min at 60°C for a total of 60 cycles. The cycle threshold for calculation of gene abundance was calculated automatically by the ABI 7300 software. The R^2 values of standards were >0.97. The number of genome copies per reaction mix was determined by comparison of cycle threshold crossing based on 8 standards, from 10^8 to

TABLE 2 Viral metagenomic characteristics of CL and FGL soil and freshwater libraries

Location	Habitat	No. of raw reads	No. of reads after trimming	Avg read length (bp)	Avg G+C%
CL	Soil	1,714	1,673	320	48
	Water	11,901	11,671	349	44
FGL	Soil	6,406	6,288	321	48
	Water	16,648	16,365	317	43

10^1 copies reaction mix^{-1}. Data on the number of genotype copies per milliliter were converted to absolute abundances per ml lake water by dividing the number of genome copies per reaction mix by 2 and then multiplying the result by the total DNA extract volume and dividing that number by the volume in ml. qPCR results were recorded only if duplicate reaction mixtures both demonstrated amplification.

Survey of viral genotype abundance. Samples for isolation of viral nucleic acids were collected every 7 days for a total of 35 days, at the same location as that used for metavirome preparation for FGL and from LC3, a site offshore from the metavirome collection site, for CL. Samples were collected from surface waters by use of a sample-rinsed bucket. Sixty milliliters of surface water was then syringe filtered through 0.2-μm PES and 0.02-μm Anotop filters, and the samples were frozen in Whirlpak bags before transport to Cornell University. Corresponding meteorological data for FGL (Fayetteville, NY) and CL (Ithaca, NY) were obtained from the National Climatic Data Service (http://www.ncdc.noaa.gov/).

Viral decay experiments. To examine the ambient decay rates of viral genotypes within each lake environment, lake surface water was collected using a sample-rinsed bucket and dispensed into two acid-washed and sample-rinsed polycarbonate bottles (2 liters). Bottles were kept cool for transport to Cornell University, where they were immediately treated with 2% NaN_3 to inhibit aerobic respiration (29). The bottles were incubated at ~25% attenuated surface irradiance on an array within Beebe Lake (an impoundment of Cascadilla Creek on the Cornell University campus), which has light and temperature conditions similar to those of the two lakes from which water was collected. Samples (50 ml) were collected daily for the determination of viral genotype abundance, following the approach used in the survey of viral genotype abundance. The decay rates of selected viruses were determined by first calculating the proportion of viruses relative to initial abundance at each time point and then conducting linear regression of proportions against time by use of the XLStat plug-in (Addinsoft SARL) in Microsoft Excel.

Survey of environmental reservoirs. We examined the abundance of viruses in benthos, which may serve as a reservoir of settled viruses. Five sediment cores were collected from waters adjacent to the sampling location in FGL. Syringe corers were used to collect sediments from surface (0 to ~2 cm) and deep (2 to ~5 cm) sediments, and these samples were immediately placed into sterile 15-ml centrifuge tubes and frozen in liquid nitrogen. DNAs from the sediments were extracted using a Zymo soil DNA kit (Zymo Research) applied to 200 mg of starting material.

Nucleotide sequence accession number. Sequences of metagenomic libraries and contigs have been deposited in the CAMERA database under accession number CAM_P_0000919.

RESULTS

Soil and plankton metaviromic libraries. A total of 35,997 sequence reads across all 4 libraries were obtained, representing 11.8 Mbp of genomic information (Table 2). Between 35 and 67% of reads assembled into contiguous sequences, resulting in 174 to 2,103 contigs per library, with a mean contig length of 400 to 518 bp, representing 0.07 to 1.08 MBp per library (see Table S2 in the supplemental material).

The majority of viral reads in DNA libraries did not match genomes or proteins of any organisms (Fig. 2A). Among protein hits, those for viruses comprised 21 to 29% of total annotations, which is less than the amount for bacteria but more than that for eukaryotes. Viral contigs (i.e., those harboring at least 1 ORF which matched viral proteins with an E value of <0.001) represented 2 to 10% of total contigs. The lower percentage of viral contigs than viral reads suggests that there was greater coverage of viral contigs than of contigs for organisms in other domains. The phylogenetic affiliations of viral contigs and sequence reads were similar and were dominated by bacteriophages (73 to 84% of viral reads and 50 to 89% of viral contigs). Among viral annotations, siphoviruses comprised the greatest proportion of hits (33.7% ± 6.1% [mean ± standard error {SE}] of viral reads), followed by myoviruses (in the water column, these were mostly cyanomyoviruses; 28.2% ± 5.4% of viral reads), podoviruses (9.3% ± 2.3% of viral reads), and microviruses (7.0% ± 5.6% of viral reads). Eukaryotic viruses were also detected and included phycodnaviruses (1.3% ± 0.2% of viral reads) and circoviruses (0.1% ± 0.1% of viral reads) (Fig. 2B). Sequence reads most closely matching geminiviruses were weakly detected only in the CL soil library (5.3% of

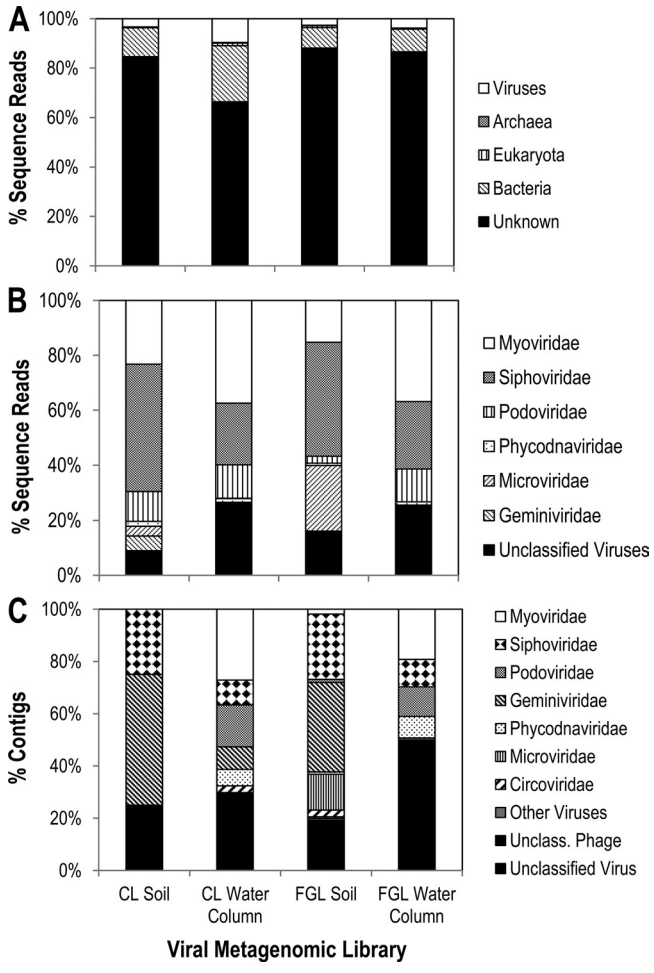

FIG 2 Phylogenetic annotation of metaviromes of sequence reads (A and B) and contiguous sequences (C). The read annotations were performed using the VIROME pipeline (http://virome.diagcomputing.org/), while the contig annotation was performed based on BLASTx searches of contig ORFs against viral, bacterial, archaeal, and eukaryotic databases at CAMERA (http://camera.calit2.net), using an E value cutoff of 10^{-3}. (A) Affiliation of reads by kingdom. (B) Viral read annotation by family. (C) Viral contig affiliation by family.

TABLE 3 Predicted diversity and richness of metaviromic libraries[a]

Lake	Habitat	Richness	Evenness	Prevalence of most abundant genotype (%)	Shannon-Wiener index
FGL	Water	10,000	0.8786	10.8	8.0922
	Soil	20,000	0.2692	44.2	2.6660
CL	Water	13	0.9759	15.5	2.5032
	Soil	1,452	0.2844	67.1	2.0707

[a] Assessed using the phage communities from contig spectra (PHACCS) algorithm (2). The analysis was based on contigs assembled from sequence reads by CLC Genomics Workbench 4.0, using a minimum overlap of 0.8 and a similarity of 95%.

viral reads) but comprised a larger proportion of contigs (34 to 50% of viral contigs). Most geminiviruses are very similar by nucleotide identity (49), while most geminivirus annotated reads in this study shared 26 to 49% (mean ± SE, 38% ± 1%) amino acid identity to the closest geminivirus genome. Hence, the geminiviruses observed in this study may represent distant relatives of known geminiviruses or representatives of closely related families (i.e., cycloviruses or circoviruses) (48). An estimate of viral diversity in each habitat was obtained by PHACCS (2). Generally, soil communities were more even than those in the water column, and the predicted richness of communities was lower for CL soil and water column libraries than for the FGL libraries (Table 3).

Quantitative PCR primer design. Based on our analysis of metaviromic read and contig libraries, we selected candidate viral genotypes that were more strongly represented in terrestrial libraries than in water column libraries (i.e., allochthonous viruses) and vice versa (autochthonous viruses) (see Fig. S1 in the supplemental material). In both lakes, geminivirus and circovirus contigs were targeted as allochthonous viral genotypes, and phycodnavirus and cyanomyovirus contigs were targeted as autochthonous viral genotypes. The geminivirus contigs selected most closely matched bean yellow dwarf virus and tomato mottle virus for FGL and CL, respectively, while the circovirus contigs most closely resembled two uncultivated circovirus genomes retrieved from the Chesapeake Bay (CB-A) and reclaimed water (RW-E) (Table 4). The cyanomyovirus contigs targeted were most similar to sequences of *Prochlorococcus* phage P-SSM4, and those of phycodnaviruses were most similar to sequences of *Acanthocystis turfacea* chlorella virus 1 (Table 4). The genes on contigs targeted by quantitative PCR encoded primarily replication-associated proteins, but a predicted protein gene (gp7 gene) was targeted for the FGL cyanomyovirus genotype, a cyanophage-encoded phosphorus uptake gene (*phoH*) was targeted for the CL cyanomyovirus genotype, and a hypothetical protein gene was used for both FGL and CL phycodnavirus genotypes.

Time series analysis of viral genotype abundance. All 4 viral genotypes from FGL and 3 viral genotypes from CL were detected in extracted virus-sized DNA; however, the geminivirus genotype was not detected in CL (Fig. 3). The greatest genotype abundance was obtained for cyanomyoviruses ($4.1 \times 10^5 \pm 0.9 \times 10^5$ copies ml^{-1} in FGL and $1.5 \times 10^3 \pm 0.9 \times 10^3$ copies ml^{-1} in CL, representing ~6% and 0.001% of total virioplankton in the lakes, based on SYBR green I counts in 2010 from a nearby location), and the lowest genotype abundance was obtained for the phycodnavirus genotype in FGL ($4.0 \times 10^1 \pm 0.9 \times 10^1$ copies ml^{-1}, or 0.00006% of total virioplankton abundance) and the circovirus genotype in CL (6.2 ± 3.4 copies ml^{-1}, or 0.0000001% of virioplankton abundance).

The abundance of viral genotypes in the lakes changed over time (Fig. 3B and C). In FGL, the two allochthonous genotypes were below detection thresholds on the first sampling date, and after day 7, they gradually decreased over the remainder of the sampling period. The cyanomyovirus genotype maintained its abundance until mid-August, when the abundance fell precipitously. Phycodnaviruses declined throughout the sampling period. In CL, the circovirus genotype was detected only over the first 3 sampling dates, at very low abundances, and then fell below the detection threshold of 1 copy ml^{-1}. The geminivirus genotype was never detected. The cyanomyovirus genotype decreased in abundance over time, while the phycodnavirus genotype increased throughout the sampling period. The abundance of all viral genotypes in CL was much lower than that in FGL.

Total precipitation in upstate New York (http://www.ncdc.noaa.gov/) was episodic during the sampling period and was preceded by a dry period in both catchments (Fig. 3A). Rainfall events were more frequent toward the end of the survey period. Significant rainfall events occurred after the first week's sampling, when >8 cm of rain fell in the FGL catchment (but was not measured in the CL catchment). Less-than-trace amounts (i.e., <0.25 cm in Fig. 3A) of precipitation were recorded at the Ithaca weather station over the course of the sampling period, though the region experienced frequent storm events.

Environmental reservoirs. All four viral genotypes probed in FGL were detected in sediment samples (Fig. 4); however, their distribution in the sediment column varied with depth. The abundances of all four viral genotypes were greater in deeper sediments (2 to 5 cm) than in surface sediments (0 to 2 cm). The phycodnavirus genotype was the only genotype that was highly abundant in

TABLE 4 Closest matches in the nr database at GenBank for putatively autochthonous and allochthonous viral contigs by BLASTx searches using an expect score cutoff of 10^{-3}

Genotype	Contig length (bp)	Avg coverage	Closest match	Protein	GenBank accession no.	% amino acid identity
FGL phycodnavirus	1,558	9.4	*Acanthocystis turfacea* chlorella virus 1	Hypothetical protein ATCV1_Z838L	ABT16972	54
FGL cyanomyovirus	1,825	7.0	*Prochlorococcus* phage P-SSM4	gp7	AAX46881	32
FGL circovirus	364	4.0	Circovirus-like genome CB-A	Replicase	ACQ78166	47
FGL geminivirus	2,180	26.4	Bean yellow dwarf virus	Replication-associated protein	CAA71908	28
CL phycodnavirus	471	9.6	*Acanthocystis turfacea* chlorella virus 1	Hypothetical protein ATCV1_Z838L	ABT16972	72
CL cyanomyovirus	3,269	8.0	*Prochlorococcus* phage P-SSM4	PhoH	AAX46998	44
CL circovirus	1,302	2.7	Circovirus-like genome RW-E	Replicase	ACQ78164	59
CL geminivirus	1,194	3.5	Tomato mottle virus	Replicative protein	AAC32414	33

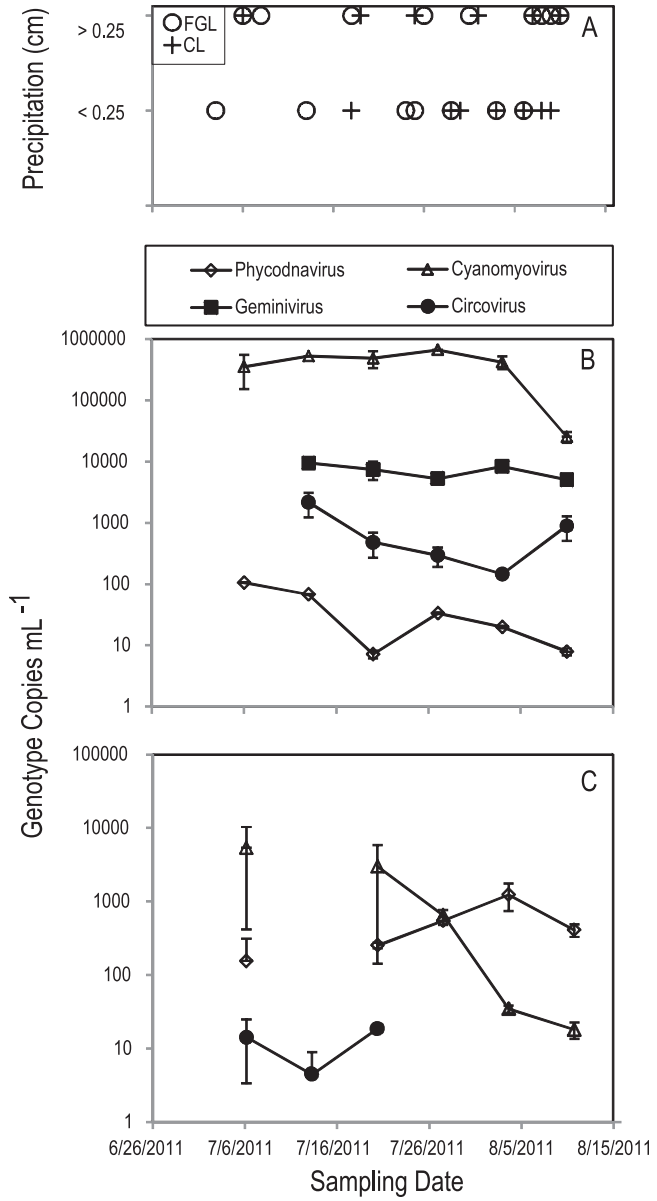

FIG 3 Catchment precipitation (A) and viral genotype abundances in Fayetteville Green Lake (FGL) (B) and Cayuga Lake (CL) (C) during summer 2011. Error bars show SE for duplicate samples. Autochthonous viral genotypes are indicated by open symbols, while allochthonous viral genotypes are indicated by closed symbols. Precipitation data were obtained for Ithaca and Syracuse weather stations from the National Climatic Data Service. Missing data at any time point indicate that abundances were below the detection threshold.

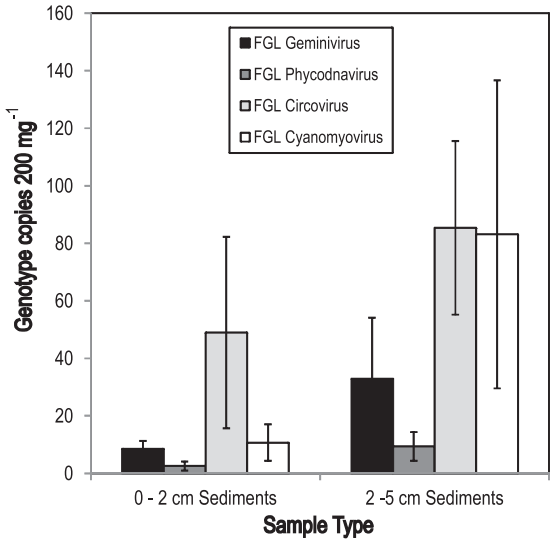

FIG 4 Abundance of viral genotypes in surface and deep sediments and within net plankton (>64 μm) in Fayetteville Green Lake. Error bars show SE for duplicate samples.

the sediments (200 mg sediment ≈ 0.5 ml lake water) compared to lake water.

Decay experiment. Phycodnavirus (autochthonous) and geminivirus (allochthonous) genotypes were detected in decay experiments using FGL water, while all four genotypes were detected in the CL decay incubations (see Fig. S2 in the supplemental material). Phycodnavirus, cyanomyovirus, and circovirus genotypes had large increases in abundance at the beginning or middle of the experiment. The CL cyanomyovirus genotype demonstrated a very rapid increase in viral abundance within the first 5 days of incubation, followed by a decline over the remainder of the experiment (see Fig. S2). Because this likely represented release of progeny virus from dead or dying cells over the initial part of the experiment, we calculated viral genotype decay rates for these genotypes from days 5 to 20. There was significant linear regression ($P < 0.05$) for only 2 of the 6 detected genotypes (Table 5). The highest decay rate was for the CL circovirus genotype (5.87% h^{-1}); however, the regression was not significant. The CL cyanomyovirus genotype had the second highest decay rate (1.50% h^{-1}), while the FGL phycodnavirus genotype and both the CL and FGL geminivirus genotypes had similar decay rates (0.13 to 0.17% h^{-1}).

DISCUSSION

Viruses play crucial roles in the ecology and biogeochemistry of aquatic ecosystems, hence the need for understanding their diversity, origins, and fates. Our data illustrate that virioplankton in freshwater ecosystems may represent a convergence of aquatic and terrestrial production, and their dynamics represent a confluence of terrestrial, limnological, and meteorological phenomena.

Diversity of lake viruses and comparison to soil viral communities. Among reads and contigs matching viral genomes, those that were well represented across all libraries were consistent with previous studies of phage diversity in seawater, sediments, and elsewhere (3, 10, 12, 13, 18, 21, 37). The large proportion of

TABLE 5 Regression statistics for CL and FGL viral genotypes in decay incubations

Lake	Genotype	Slope	Intercept	r^a	Decay rate (% h^{-1})	Significance
FGL	Geminivirus	−0.04	0.84	0.60	0.17	*
	Phycodnavirus	−0.03	0.43	0.40	0.13	NS
CL	Circovirus	−1.41	24.44	0.54	5.87	NS
	Geminivirus	−0.03	1.02	0.22	0.13	NS
	Cyanomyovirus	−0.36	6.63	0.59	1.50	*

[a] r = regression coefficient; *, $P < 0.05$; NS, not significant.

reads matching cyanomyoviruses for the FGL and CL water columns may reflect strong homology among cyanophages which infect picocyanobacteria (54, 55) or a poor representation of myovirus genomes from other hosts in the databases used for comparison. The Φ29 amplification used in this and previous studies strongly selects for single-stranded circular genomes (48). This bias may have caused our observation of circoviruses, microviruses, and geminiviruses in metaviromic libraries. The large proportion of unidentifiable reads based on alignment to genomes of sequenced viruses, bacteria, or eukaryotes agrees with the results of previous viral metagenomic studies (3, 10–12, 20, 37, 62). The unannotated sequence space may represent novel diversity, which in turn reflects the lack of representative genomes of viruses from lake and soil habitats (19, 53).

Geminiviruses are single-stranded circular viruses that infect a wide range of plants, including grasses (*Poaceae*) and nightshades (*Solanaceae*). They are transmitted primarily by insect vectors, including whiteflies and leafhoppers. A recent survey of whitefly-associated viruses revealed substantial diversity in geminiviruses across several sampling locations (39). Their presence in soil may reflect accumulation of infected plant material in humus or insect-derived matter in litter. Soils may form a reservoir of geminiviruses, which may be transported to plants by vectors or other means. Geminiviruses of plants are typically highly conserved in nucleotide sequences (39). These viral genotypes may represent viruses infecting other terrestrial hosts, since our annotation was based on translated nucleotide-protein comparisons (i.e., BLASTx) in which average homology to geminiviruses was low. Phycodnaviruses are large double-stranded DNA (dsDNA) viruses that typically infect eukaryotic algae (17, 51). Circoviruses, which are circular single-stranded DNA (ssDNA) viruses (48), were large constituents of soil libraries and were less common in freshwater libraries. Most commonly known to infect vertebrate hosts, they have also recently been observed in plankton of the Chesapeake Bay, near Bermuda, and in reclaimed wastewater (48).

Based on metaviromic analyses, we identified groups of viruses that were of putatively allochthonous (geminivirus and circovirus) and autochthonous (phycodnavirus and cyanomyovirus) origins (Table 4; see Fig. S1 in the supplemental material). We chose contiguous sequences of these viral types that were detected in soil libraries and freshwater libraries. However, there were representatives of each of these groups in contig libraries from both habitat types. The allochthonous and autochthonous viruses differed in nucleic acid structure (both allochthonous viruses are ssDNA viruses, while both autochthonous viruses are dsDNA viruses) and targeted different host kingdoms (both allochthonous viruses infect eukaryotic organisms, while the autochthonous cyanomyovirus infects cyanobacteria). Therefore, our choice of viruses represents a range of infection dynamics and physical characteristics.

Temporal dynamics of viral genotypes. The abundance of viral genotypes reflected the abundance of putative hosts, with the FGL and CL cyanomyovirus genotypes having the greatest overall abundance (~5 to 10% of virioplankton abundance by SYBR green microscopy, which was 6.3×10^6 VLPs ml^{-1} in August 2009 [our unpublished data]). This observation agrees with PHACCS analysis (Table 3), which found that the dominant viral genotype within FGL and CL plankton represented 10 to 15% of total viral abundance. FGL is characterized by very large populations of pelagic cyanobacteria, which cause whiting events in spring and fall due to photosynthesis-induced pH changes and their subsequent impacts on carbonate chemistry (60). Picocyanobacteria comprise ~1% of total bacterioplankton in FGL (1.7×10^7 cells ml^{-1} in August 2009 [our unpublished data]). The abundance of the cyanomyovirus genotype in FGL was higher than that in CL. The low abundance of the phycodnavirus genotype in FGL relative to those of the other genotypes, including putative allochthonous viruses, was expected. Phytoplankton communities in the lake are dominated by cyanobacteria, while eukaryotic algae such as *Chlamydomonas* and small pennate diatoms typically comprise a much smaller proportion of lake microbial flora (61).

The temporal dynamics of putatively allochthonous and autochthonous viral genotypes may reflect both rainfall events and the dynamics of phytoplankton over the sampling period. The circovirus genotypes were observed only after the first week of sampling, when a large rainfall event occurred in the FGL catchment (delivering ~8 cm of rain in 24 h after a dry period of several weeks), and were detected in CL only at the start of the sampling period. The FGL geminivirus genotype followed a similar trend to that of the FGL circovirus genotype, but the CL geminivirus genotype was not detected. The absence of CL geminiviruses may be related to differences in autochthonous viral composition between 2010, when metagenomes were prepared, and 2011, when the sampling was conducted. In contrast, the cyanomyovirus and phycodnavirus genotypes were detected on most sampling dates in both lakes. Our consistent autochthonous viral detection but ephemeral allochthonous viral detection suggests that the presence of the latter may reflect a balance between the arrival of viruses to the catchment (possibly via rainfall) and their decay or sedimentation within the lake environment.

Viral presence in environmental reservoirs. Viral genotypes of both allochthonous and autochthonous origins were detected within sediments and, interestingly, were more abundant (per unit weight of sediment) in deeper sediment horizons. These results suggest that sediments may be a sink for both putatively allochthonous and autochthonous virus particles within lake habitats, as has been observed in coastal marine ecosystems. For example, an investigation of cyanophages within sediments off the Saanich Peninsula, which is considered a high-deposition area, revealed the presence of infective cyanophages 20 m beneath the sediment surface and estimated to be several hundred years old (56). The greater abundance of viral genotypes in deeper sediments than in shallow ones suggests that they may be entrained and concentrated there, possibly on particle surfaces or colloids. Hence, sediments may form environmental reservoirs of allochthonous and autochthonous viral genotypes. We speculate that these may become resuspended when sediments are disturbed, in a manner previously observed for human viruses (9). These results raise interesting questions about the physical and infectivity decay of viruses in sediment habitats.

Decay of viral genotypes. Viral decay in aquatic ecosystems may occur via several mechanisms (42). By stopping virus production and the production of new ectoenzymes by heterotrophic bacteria and then monitoring the decrease in viral abundance, it is possible to estimate viral decay rates (28). We used a similar approach to estimate decay of allochthonous and autochthonous viral genotypes in incubations treated with NaN_3 (c.f. KCN, used in previous studies but not used in our study due to environmental concern). To the best of our knowledge, this investigation represents the first genotype-specific investigation of decay by use of

this approach. Previous studies have observed the abundance of decay-resistant polyhedra of nucleopolyhedroviruses in water troughs over time and detected their presence for several weeks (31). However, investigations of native phages and viruses or ssDNA viruses via our approach have not been conducted previously.

The large increase in cyanophage, algal virus, and circovirus genotypes over the first 5 days of sampling suggests that they may have been released by decaying organisms within the incubations. To account for this, decay rates were calculated for the latter part of the experiments by use of a previously established approach (30). The rates of virus production across 5 genotypes detected over the course of the experiment were generally lower than those reported for entire virioplankton communities in most marine waters (5, 28, 42) and were in line with decay rates estimated for Lake Bourget (59). The CL circovirus genotype decayed more rapidly than marine and lake virioplankton; however, our estimates are in line with a previous study of cyanophage decay in freshwater lakes (34). Previous studies of individual viruses have reported a range of decay rates, depending on the physicochemical conditions of the waters that the phages inhabit (16, 65). The regression used to calculate the decay rate was not significant for the CL circovirus, FGL phycodnavirus, or CL geminivirus genotype. For the two genotypes with significant regressions, the decay rate of the cyanophage genotype was almost an order of magnitude higher than that for the FGL geminivirus genotype. The high degree of variability in genotype decay rates suggests that there is no difference between the decay rates of ssDNA and dsDNA eukaryotic virus genotypes.

The stability and persistence of viruses of allochthonous origin have implications for lake water use, as potentially pathogenic viruses of agriculturally important species may be present in lake virioplankton for several days after introduction. For example, the use of lake water containing viruses of agriculturally important crops for irrigation may give rise to new terrestrial infections (73). It is important, however, that our data apply to the detection of viral nucleic acids only. Infectivity of virus particles may be lost well before physical loss of the viruses (42, 58, 71). The extent to which the reemergence of allochthonous aquatic viruses in terrestrial habitats will occur remains unknown.

Conclusions. To the best of our knowledge, this study is the first to synchronously investigate the dynamics of allochthonous and autochthonous viruses in aquatic habitats through a combined shotgun metagenomic and quantitative PCR approach. Our data suggest that putatively allochthonous viruses comprise a component of virioplankton in lakes and that their abundance may be linked to local factors influencing transport from the catchment. The decay rates of the allochthonous viral genotypes examined in this study were the same as those for the autochthonous genotypes, which were generally lower than those reported for seawater but similar to those reported for lakes. The detection of genotypes within sediments of the lake suggests that this compartment may be a reservoir of allochthonous and autochthonous viruses. This study raises interesting questions about the possibility of transport of allochthonous viruses back into the terrestrial environment from aquatic habitats, perhaps by anthropogenic processes such as irrigation or by vector transport.

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