Bacteriophage Ecology Group (BEG) News

Dedicated to the ecology and evolutionary biology of the parasites of unicellular organisms (UOPs)

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July 1, 2002 issue (volume 13)

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Editorial

Editorials should be written on subjects relevant to The Bacteriophage Ecology Group as an organization, to *BEG News* (either the concept or a given issue of *BEG News*), or the science of Bacteriophage Ecology. While my assumption is that I will be writing the bulk of these editorials, I wish to encourage as many people as possible to seek to relieve me of this duty, as often as possible. Additionally, I welcome suggestions of topics that may be addressed. Please address all correspondences to abedon.1@osu.edu or to "Editorials," *Bacteriophage Ecology Group News*, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. Please send all submissions as Microsoft Word documents, if possible (I'll let you know if I have trouble converting other document formats), and in English.

Calling a Phage a "Phage"

by Stephen T. Abedon

Assembling the Bacteriophage Ecology Group Bibliography can be challenging, particularly since not all phage-ecology references are obviously phage-ecology references. Generally my strategy has been to do "phage" searches on the various online databases. For example, with Medline I use this search:

$phage$ not $phageal not macrophage$

which assures that I catch references by all those individuals who insist on referring to "bacterial viruses" as "phages" or "bacteriophage" or "actinophages," etc., rather than simply as "phage." Typically I customize the output of my search results so that 400 references are displayed per page. Still, even though I often don't need to go more than 1,000 references into these lists before I start seeing references I caught during the last quarter's search, that's a lot of references to consider. Thus, to save time, I've attempted to eliminate a few very common terms that contain "phage", such as "macrophage," but which often have nothing to do with bacterial viruses.

Consistently, what I don't do are searches for the terms "virus" or "viral" since the number of phage papers I would find that I

wouldn't find using only a "phage" search would be small. Still, it bothers me that clearly I must be missing at least some phage-ecology papers because, as I've found, sometimes authors neglect to call a phage a phage. The purpose of this editorial, therefore, is to suggest that it would be helpful if papers that considered phages actually had the term "phage," or a derivative (e.g., phages or bacteriophage or, indeed, all three), somewhere in their title, or, at the very least, in their abstract. Not only would this be helpful to *me*, but consider everyone else who might need to wade through endless "virus" searches to find the few papers that refer to "the viruses of bacteria" but not to "phage."

Is this really a problem? To attempt to address this question I have employed my handy-dandy BEG bibliography to do a "virus" or "viral" but not "phage" or "bacteriophage" search. Considering only the more modern references (i.e., 1998 through 2001; see below), there are over 40 seemingly phage-ecology (or evolution) references that do not use the word "phage" in their title nor, if I had it to search, in their abstract as well. That's an average, of course, of over 10 "phage"-less phage-ecology papers per year. I observe that *avoidance* of "phage" is particularly common among ecosystem ecologists. I note that if I am having trouble finding (or noticing) these or, particularly, *other* "phage"-less references, then clearly at least some of our more "phage"-minded colleagues might as well. What have we missed?

Special thanks to Steven McQuinn for the wonderful "phage-virus" gif found at the top of this editorial.

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New BEG Members

The BEG members page can be found at www.phage.org/beg_members.htm. There are two ways of "joining" BEG. One, the "traditional" way, is to have your name listed on the web page and on the list server. The second, the "non-traditional" way, is to have your name only listed on the list server. The latter I refer to as "non-members" on that list. Members, e.g., individuals listed on the BEG members list page, should be limited to individuals who are actively involved in science (research, instruction, outreach, industry) and who can serve as a phage ecology resource to interested individuals. If you have an interest in phage ecology but no real expertise in the area, then you should join as a non-member. To join as a member, please contact BEG using the following link: abedon.1@osu.edu. Include:

- your name
- your e-mail address
- your snail-mail address
- the URL of your home page (if you have one)
- a statement of whether or not you are the principal investigator
- a statement of your research interests (or phage ecology interests)
- a list of your phage ecology references, if any

Note that it is preferable that you include the full reference, including the abstract, if the reference is not already present in the BEG bibliography. Responsibility of members includes keeping the information listed on the BEG members page up to date including supplying on a reasonably timely basis the full references of your new phage ecology publications. Reprints can also be sent to *The Bacteriophage Ecology Group*, care of Stephen Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. To join BEG as a non-member, please contact BEG using the following link: abedon.1@osu.edu and minimally include your name and e-mail address.

Please welcome our newest members

name (home page links)	status	e-mail	address
Tom Chen	---	r90241213 @ms90.ntu.edu.tw or bacteriophage605 @yahoo.com.tw	Institute of Oceanography, National Taiwan university, PO BOX 23-13, Taipei, Taiwan
	interests:	(contents | BEG members | top of page)	
Pierre Rossi	PI	pierre.rossi @unine.ch	University of Neuchâtel, Microbiology Laboratory, CP2, 2007 Neuchâtel, Switzerland
	interests:	Use of phages as water tracers: ground water (porous and fractured media) as well as rivers and lakes. Use of phages as tools for biological control of bacterial diseases. (contents | BEG members | top of page)	
Michael H. Walter	PI	michael.walter @uni.edu	Dept. of Biology, MSH 2438, UNI, Cedar Falls, IA 50614-0421
	interests:	Diversity and taxonomy of phages of the *Bacillus cereus* group and phages of plant pathogenic bacteria. What non-DNA based tools can be used to distinguish these phages from one another? We're also investigating possibilities for phage control of bacteria and the saccharide binding specificity of phages. (contents | BEG members | top of page)	

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Meetings

The BEG Meetings link will continue. Reminders of upcoming meetings will be placed in this section of *BEG News*. If you know of any meetings that might be of interest to BEG members, or would like to recap a meeting that you've attended, then please send this information for posting to abedon.1@osu.edu or to "BEG Meetings," *Bacteriophage Ecology Group News*, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906.

Please send photos, etc. from meetings for inclusion in this section.

Evergreen International Phage Meeting

Next Summer's phage meeting has been scheduled for July 23-27, 2003. Information pertaining to the meeting may be found at http://www.evergreen.edu/phage/. This meeting will bring together phage people with the widest possible array of interests - from the ecological to the molecular - in a setting of rain forest spender. Click here for a tour of The Evergreen State College.

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Jobs

Looking for job? Looking to fill a position? Please send advertisement and information to abedon.1@osu.edu or to "Jobs", Bacteriophage Ecology Group News, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. Please send all information as text (e.g., as an e-mail) or as Microsoft Word documents, if possible (I'll let you know if I have trouble converting any other document formats), and in English. I will update this section as I receive material, regardless of what date this issue of BEG News goes live.

Click here for International Society for Microbial Ecology Employment Listings.

Click here for American Association for the Advancement of Science Employment Listings.

Click here for AAAS "Microbial Ecology" Search.

Click here for AAAS "Ecology and Microbiology" Search.

Position Announcement for anticipatory staffing:

Research Assistant/Post-doc-Microbiology

(Research Assistant 2-B/H)

This anticipated position provides laboratory and field support to research the microbiology and epidemiology of foodborne pathogens (such as *E. coli* O157, *Salmonella*, *Campylobacter* and antibiotic resistant bacteria) in animals and the environment. Primary responsibilities for this position include the isolation, culture and molecular characterization of temperate bacteriophages. Other duties include collection of field samples; processing and analyzing of samples for microorganisms using traditional and molecular techniques; communicating with livestock producers and other scientists; summarizing experimental data for analysis.

Bachelor's degree (or equivalent combination of education and experience) in a field of the Biological Sciences required, MS preferred. Preference will also be given to those candidates that have experience in the isolation and culture of bacteriophages, molecular biology techniques, computer skills, and handling livestock.

This position is located at the Food Animal Health Research Program, Ohio Agriculture Research and Development Center, The Ohio State University, located in Wooster Ohio. Salary: $22,000-31,000. Interested individuals should forward a CV and names of three references the address below. E-mail-based communication is encouraged. This is a temporary appointment for approximately 1 year, with possible extension. This position may also be filled as a post-doctoral or visiting scientist with some modification to required duties. This position is open until a suitable candidate is identified. The Ohio State University is an equal-opportunity employer. Women and minorities are encouraged to apply.

Dr. Jeffrey LeJeune
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1680 Madison Ave.
Wooster, OH 44691
(330)-263-3739
lejeune.3@osu.edu

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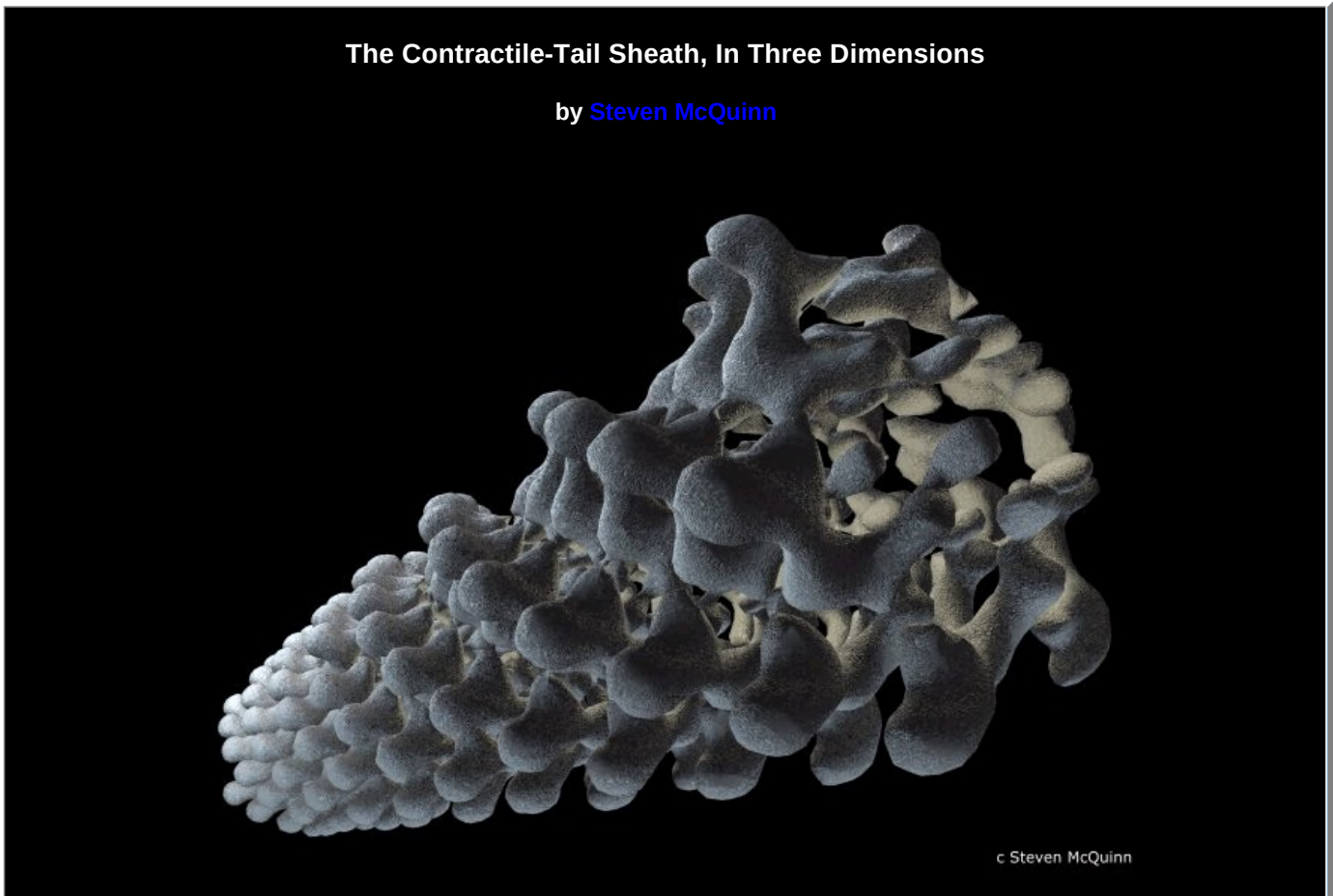
Submissions

Submissions are non-editorial items describing or highlighting some aspect of bacteriophage ecology including news pieces, historical pieces, reviews, and write-ups of research. Peer review of submissions is possible and a desire for peer review should be indicated. Send all submissions to abedon.1@osu.edu or to "Submissions", Bacteriophage Ecology Group News, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. Please send all submissions as Microsoft Word documents, if possible (I'll let you know if I have trouble converting any other document formats), and in English.

The Contractile-Tail Sheath, In Three Dimensions

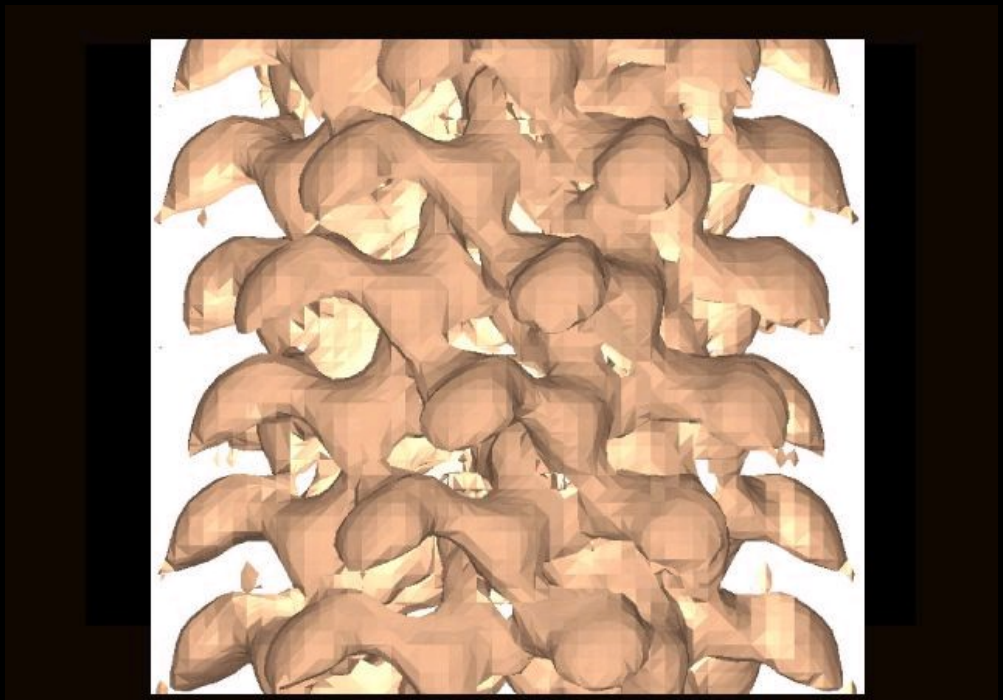
by Steven McQuinn



Phage T4 contractile-tail sheath (with surface texture and fancy lighting).

What You Are Seeing...

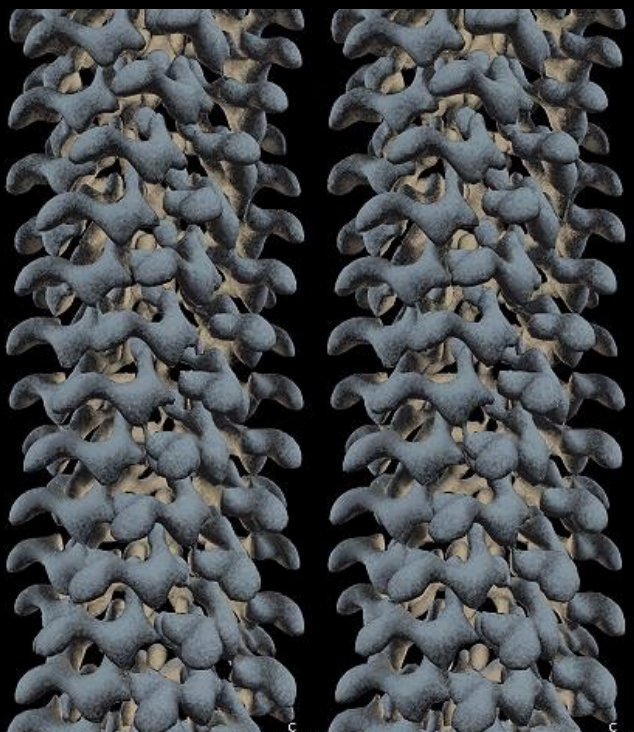
These are synthetic photographs of data. While it is tempting to say, "this is what the extended tail sheath of bacteriophage T4 really looks like," such a statement makes no sense in the nanoscale microcosm where visible light washes over phage the way ocean swells move through plankton. Rather, phage must be probed using the severely short end of the electromagnetic spectrum. Electron microscopists and x-ray crystallographers examining phage details compile data sets of infinitesimal measurements and clever mathematical calculations. Such data sets can be visualized in various ways to illustrate protein morphology, even protein molecular structure. When data is furnished thus to the eye it becomes comprehensible in the most fundamental way, though the caveats of method should never be slighted. We are not looking at a thing, we are looking at the result of a process.

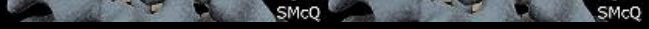


Straight-forward, unadorned image of one density surface data set for the T4 tail sheath, extended configuration. Note the fusion between gp18 proteins.

Where It Came From...

Anyone in middle age can appreciate how reassuring it is to see 20-year-old data looking splendid when dressed up in modern fashion. The images here were created from cryo-electron microscopy density surfaces calculated for a paper published in 1985 in the Journal of Molecular Biology. Kevin Leonard kindly dug up the old mag tapes, converted the files for use with contemporary visualization software (AVS), exported them as VRML files, compressed and sent them to me via email and ftp. All this over his weekend and during his busy workweek.

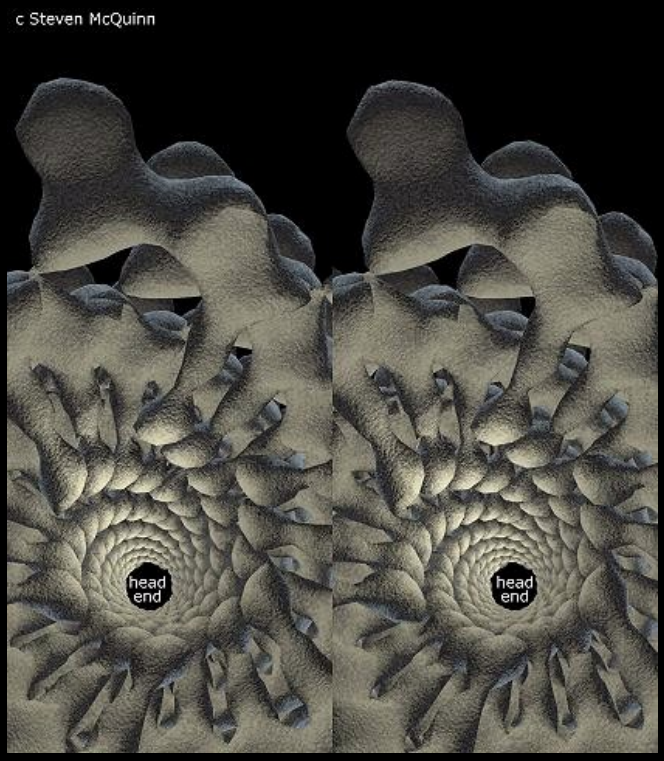




A side view of the sheath, in stereo, with exaggerated depth. See below for how to visualize stereo pairs.

How Much Artistic License...

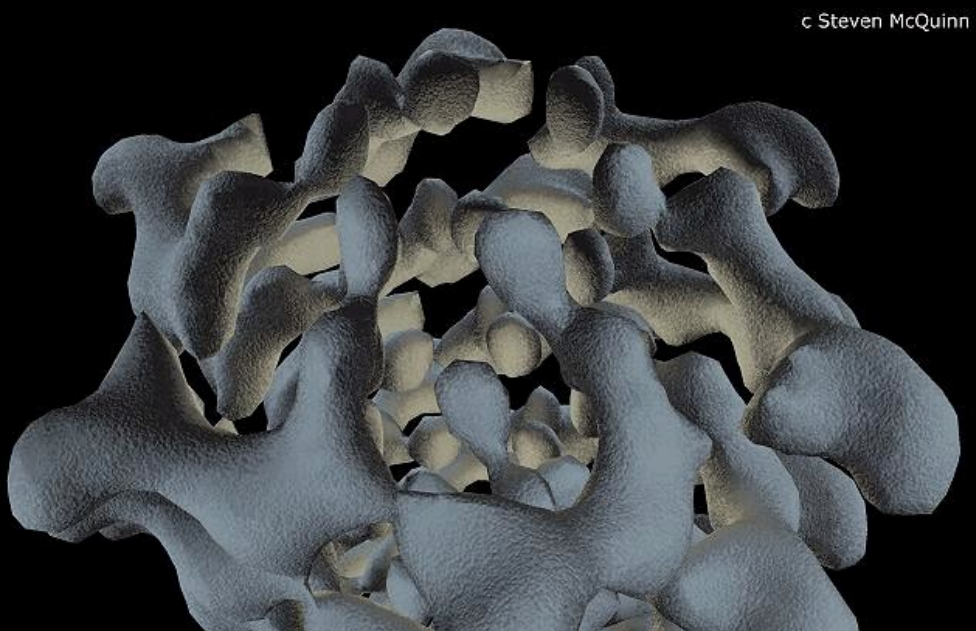
The VRML files, imported into my 3D graphics software as a polygon mesh, defined 4 annular rings in a stack, the top and bottom rings clipped somewhat. I trimmed the geometry down to the fully intact middle two rings, assembling duplicates to make a full helical stack 24 rings high. The bump map supplying texture to the gp18 proteins serves purely for displaying the surface curvature and has no structural significance. Ideally, the surface of the protein would show the lumpiness of constituent atoms with van der Waal radii and be colored to indicate surface charge, but I cannot find any such data; apparently the molecular structure of gp18 has not yet been worked out. The 3D synthetic lighting sources consist of a warm-colored tube light extending up the middle of the tail sheath and a cool-colored ring light encircling the sheath.



Looking up through the sheath from the baseplate position toward the head. The tail tube fits inside.

How To View The Stereo Pairs...

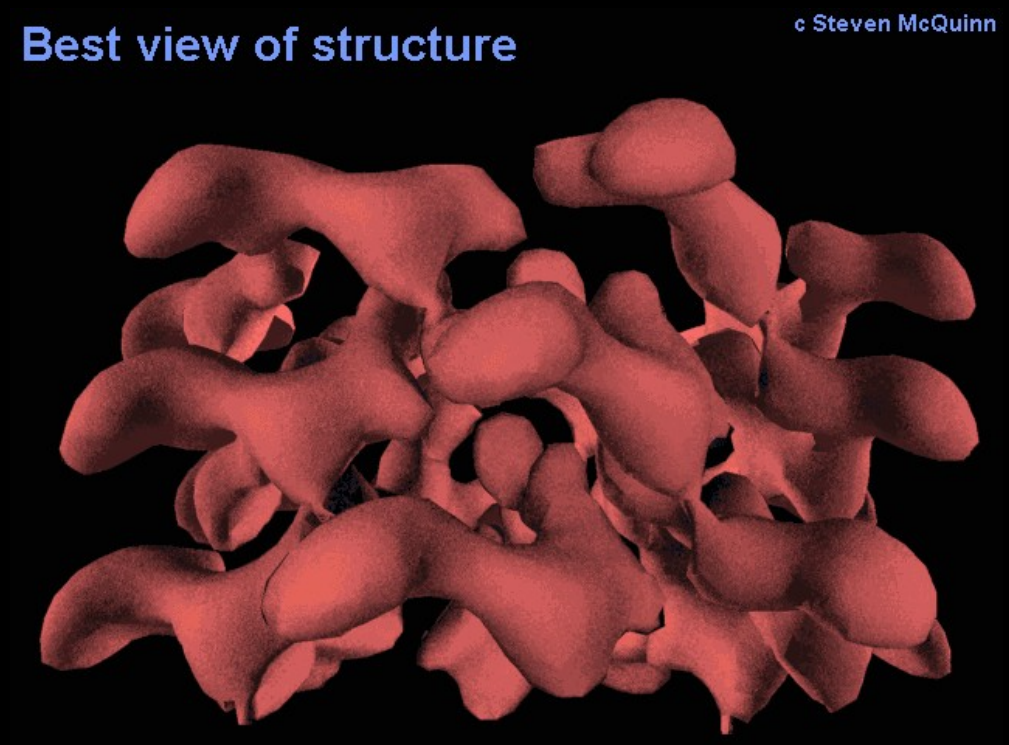
Stare through the stereo pairs as if your thoughts were lost in a distant daydream, gazing off into space; suddenly the right/left images will fuse into one. Stereo fusion requires the eyes to drift apart, exactly the opposite of looking cross-eyed. To help you achieve this fusion, the paired images here are set apart the same distance as the separation of your two eyes--if you view the images on my high resolution monitor. However, it may well be that your monitor displays lower resolution than mine, making the paired images more widely separated and thus harder to fuse. In this case, open the link to the PDF version and use the percentage controls in Acrobat Reader to resize the images for best effect. (It is possible to look distantly while focusing closely if you wear strong reading glasses, which you can borrow from someone nearby who is older than 50.)



The gp18 proteins in a ring kick up their legs like synchronized swimmers. The "feet" (upper, middle of image) connect with the gp19 proteins of the tail tube inside the sheath (gp19 is not shown).

What It All Means...

You can see how the gp18 proteins arranged in a helical stack seem to link bulge-to-bulge with their immediate neighbors. Depending how the data is visualized, these bulges can appear as fusions, and likely represent the bonds between the proteins that hold the extended sheath together. However, the extended sheath would not be stable were it not for the tail tube which extends down through the sheath. The tail tube is not included in these visualizations but you can infer it by the arrangement of the inner ends of gp18 positioned like legs with knees and toes pointed upward. Each end "foot" of gp18 is matched by a corresponding gp19 protein in the tail tube. It is thought that the attraction between the tail tube and the inner structure of the tail sheath holds the sheath in extended position. When bacteriophage T4 infects its *E. coli* host, the baseplate at the bottom of the sheath (not illustrated) springs open, initiating an upward cascade of broken gp18/gp19 connections, allowing the sheath to contract into a different helical arrangement with shorter length and greater radius. It is thought that sheath contraction physically drives the tail tube tip into the host cell wall.



Animated comparison of two data sets from Lepault and Leonard. The one without correction (red) shows a slim version of gp18, allowing a view of how distinct units fit together. The version with phase-contrast correction (blue) is thought to be the best representation of shape and size, but the units are hard to distinguish. Shown are three annular rings, essentially, of 6 gp18 subunits each. The red one is a little clipped on top, the blue a little extended on the bottom.

Remember Those Caveats...

If only I could simply leap to the conclusion that these crisp, clear images of structure represent Truth, my task in animating tail sheath contraction would be a bit easier. The particular data set I used for the above views shows a slim, distinct gp18; however, the data are uncorrected. Lepault and Leonard determined that a different data set, corrected for the phase-contrast transfer function, represents the best display of gp18 size and shape, though the unit proteins in that view are too fused to be distinct. Superposition of the two data sets shows that they are very similar in general structure, with ambiguity about how the gp18 bond together. My challenge as a 3D animator will be to separate artifact from architecture, basing my interpretations on a synthesis of both data sets. It seems that even with data-derived imagery there is no escaping the need for creativity.

Credits

Lepault J, Leonard K. Three-dimensional structure of unstained, frozen-hydrated extended tails of bacteriophage T4. J Mol Biol 1985 Apr 5;182(3):431-41 [PRESS FOR ABSTRACT]

Kevin Leonard (leonard@embl-heidelberg.de), Group Leader (http://mac-leonard4.embl-heidelberg.de/index.html)with the European Molecular Biology Laboratory (http://www.embl-heidelberg.de/)

Jean Lepault (LEPAULT@frcgm51.bitnet), Group Leader in the Methods and Electron Microscopy division of Le Laboratoire de Virologie Moleculaire & Structurale (formerly Le Laboratoire de Genetique Des Virus,

http://www.gv.cnrs-gif.fr/) of CNRS (http://www.cnrs.fr/index.html)

"Phage"-less References (1998-2001)

1. Blum, H., W. Zillig, S. Mallock, H. Domdey, and D. Prangishvili. 2001. The genome of the archael virus SIRV1 has features in common with genomes of eukaryal viruses. Virology 281:6-9. abstract: The virus SIRV1 of the extremely thermophilic archaeon *Sulfolobus* has a double-stranded DNA genome similar in architecture to the genomes of eukaryal viruses of the families Poxviridae, Pycodnaviridae, and Asfarviridae: the two strands of the 32,301 bp long linear genome are covalently connected forming a continuous polynucleotide chain and 2029 kb long inverted repeats are present at the termini. Very likely it also shares with these viruses mechanisms of initiation of replication and resolution of replicative intermediates.

2. Hewson, I., J. M. O'Neil, C. A. Heil, G. Bratbak, and W. C. Dennison. 2001. Effects of concentrated viral communities on photosynthesis and community composition of co-occurring benthic microalgae and phytoplankton. Aquat.Microb.Ecol. 25:1-10. abstract: Marine viruses have been shown to affect phytoplankton productivity; however, there are no reports on the effect of viruses on benthic microalgae (microphytobenthos). Hence, this study investigated the effects of elevated concentrations of virus-like particles on the photosynthetic physiology and community composition of benthic microalgae and phytoplankton. Virus populations were collected near the sediment surface and concentrated by tangential flow ultrafiltration, and the concentrate was added to benthic and water column samples that were obtained along a eutrophication gradient in the Brisbane River/Moreton Bay estuary, Australia. Photosynthetic and community responses of benthic microalgae, phytoplankton and bacteria were monitored over 7 d in aquaria and in situ. Benthic microalgal communities responded to viral enrichment in both eutrophic and oligotrophic sediments. In eutrophic sediments, Euglenophytes (Euglena sp.) and bacteria decreased in abundance by 20 to 60 and 26 to 66%, respectively, from seawater controls. In oligotrophic sediments, bacteria decreased in abundance by 30 to 42% from seawater controls but the dinoflagellate Gymnodinium sp. increased in abundance by 270 to 3600% from seawater controls, The increased abundance of Gymnodinium sp. may be related to increased availability of dissolved organic matter released from lysed bacteria. Increased (140 to 190% from seawater controls) initial chlorophyll a fluorescence measured with a pulse-amplitude modulated fluorometer was observed in eutrophic benthic microalgal incubations following virus enrichment, consistent with photosystem II damage. Virus enrichment in oligotrophic water significantly stimulated carbon fixation rates, perhaps due to increased nutrient availability by bacterial lysis. The interpretation of data from virus amendment experiments is difficult due to potential interaction with unidentified bioactive compounds within seawater concentrates. However, these results show that viruses are capable of influencing microbial dynamics in sediments.

3. Hofer, J. S. and R. Sommaruga. 2001. Seasonal dynamics of viruses in an alpine lake: Importance of filamentous forms. Aquat.Microb.Ecol. 26:1-11. abstract: Viruses are an important component of the planktonic food web in freshwater and marine systems, but most studies have been done in the ocean and in lowland lakes. In this work, the seasonal dynamics and structure of the virioplankton as well as their impact on bacteria during a day/night cycle were studied in an alpine lake located 2417 m above sea level. The abundance of virus-like particles (VLP) was determined at 5 discrete depths (0.5 to 8 m) by direct counts with a TEM in samples collected from May to November 1998 at weekly to bi-weekly intervals. Viruses reached the highest abundances under ice (4.6 X 10^6 VLP ml-1) with a second maximum in autumn. After ice-break, the VLP abundance decreased to undetectable values (<2 X 10^4 VLP ml-1) probably because of the negative effect of solar radiation that was negatively correlated with the viral abundance in the upper 2 m of the water column (Spearman rank correlation, rs = -0.773, $p < 0.01$). The virioplankton was morphologically diverse, consisting of forms commonly found in other aquatic systems, but unlike other studies, we found filamentous VLP (FVLP) 450 to 730 nm long that attained abundances of up to 1.3 X 10^6 ml-1 and accounted for 7 to 100% of the total viral abundance. These FVLP were found occasionally inside filamentous heterotrophic bacteria (> 10 mum) and their respective abundances were positively correlated (rs = 0.728, $p < 0.01$). The absence of these conspicuous forms in other aquatic ecosystems suggests that FVLP are well adapted to the harsh environmental conditions or are specific to bacterial hosts found in alpine lakes. Finally, between 5 and 28% of the newly produced bacteria were killed by non-filamentous viruses, which therefore are a modest cause of bacterial mortality in this lake.

4. Larsen, A., T. Castberg, R. A. Sandaa, C. P. D. Brussaard, J. K. Egge, M. Heldal, A. Paulino, R. Thyrhaug, E. J. van Hannen, and G. Bratbak. 2001. Population dynamics and diversity of phytoplankton, bacteria and viruses in a seawater enclosure. Mar.Ecol.Prog.Ser. 221:47-57. abstract: We now know that the abundance of free viruses in most marine environments is high. There is still, however, a lack of understanding of their occurrence and distribution and of in situ relationships between viral and host communities in natural environments. This may be partly due to methodological limitations. Our main aim was therefore to perform a case study in which a variety of methods were applied in order to give an improved, high-resolution description of the microbial communities in a natural environment, In order to do this we combined light microscopy (LM), transmission electron microscopy (TEM), flow cytometry (FCM), PCR denaturing gradient gel electrophoresis (PCR-DGGE) and pulsed-field gel electrophoresis (PFGE) and studied the diversity and succession of algae, bacteria and viruses in a nutrient enriched seawater enclosure. In the enclosure we experienced a situation where the development of the dominating algal population, which consisted of several flagellate species, was followed by proliferation of several different size-classes of viruses. The total bacterial number decreased markedly during the flagellate bloom but the community composition was maintained and the diversity remained high. Our results indicate a close linkage between various algal, bacterial and viral populations and show that virioplankton do not necessarily terminate algal and bacterial blooms but that they keep the host populations at non-blooming levels.

5. Prangishvili, D., K. Stedman, and W. Zillig. 2001. Viruses of the extremely therophilic archaeon □*Sulfolobus*. Trends Microbiol. 9:39-42. abstract: Viruses of *Sulfolobus*□ are highly unusual in their morphology, and genome structure and sequence. Certain characteristics of the replication strategies of these viruses and the virus-host interactions

suggest relationships with eukaryal and bacterial viruses. Moreover, studying these viruses led to the discovery of archaeal promoters and has provided tools for the development of the molecular genetics of these organisms. The *Sulfolobus* viruses contain unique regulatory features and structures that undoubtedly hold surprises for researchers in the future.

6. Simek, K., M. G. Weinbauer, K. Hornak, J. R. Dolan, J. Nedoma, M. Masin, and R. Amann. 2001. Changes in bacterial community composition and dynamics and viral mortality rates associated with enhanced flagellate grazing in a mesoeutrophic reservoir. Appl.Environ.Microbiol. 67:2723-2733. abstract: Bacterioplankton from a meso-eutrophic dam reservoir was size fractionated to reduce (<0.8-mum treatment) or enhance (<5-mum treatment) protistan grazing and then incubated in situ for 96 h in dialysis bags. Time course samples were taken from the bags and the reservoir to estimate bacterial abundance, mean cell volume, production, protistan grazing, viral abundance, and frequency of visibly infected cells. Shifts in bacterial community composition (BCC) were examined by denaturing gradient gel electrophoresis (DGGE), cloning and sequencing of 16S rDNA genes from the different treatments, and fluorescence in situ hybridization (PISH) with previously employed and newly designed oligonucleotide probes, Changes in bacterioplankton characteristics were clearly linked to changes in mortality rates. In the reservoir, where bacterial production about equaled protist grazing and viral mortality, community characteristics were nearly invariant, In the "grazer-free" (0.8-mum-filtered) treatment, subject only to a relatively low mortality rate (similar to 17% day(-1)) from viral lysis, bacteria increased markedly in concentration. While the mean bacterial cell volume was invariant, DGGE indicated a shift in BCC and FISH revealed an increase in the proportion of one lineage within the beta proteobacteria, In the grazing-enhanced treatment (5-mum filtrate), grazing mortality was similar to 200% and viral lysis resulted in mortality of 30% of daily production. Cell concentrations declined, and grazing-resistant flocs and filaments eventually dominated the biomass, together accounting for > 80% of the total bacteria by the end of the experiment. Once again, BCC changed strongly and a significant fraction of the large filaments was detected using a FISH probe targeted to members of the Flectobacillus lineage, Shifts of BCC were also reflected in DGGE patterns and in the increases in the relative importance of both beta proteobacteria and members of the Cytophaga-Flavobacterium cluster, which consistently formed different parts of the bacterial flocs. Viral concentrations and frequencies of infected cells were highly significantly correlated with grazing rates, suggesting that protistan grazing may stimulate viral activity.

7. Brussaard, C. P. D., D. Marie, and G. Bratbak. 2000. Flow cytometric detection of viruses. Journal of Virological Methods 85:175-182. abstract: Representatives from several different virus families (Baculoviridae, Herpesviridae, Myoviridae, Phycodnaviridae, Picornaviridae, Podoviridae, Retroviridae, and Siphoviridae) were stained using a variety of highly fluorescent nucleic acid specific dyes (SYBR Green I, SYBR Green II, OliGreen, PicoGreen) and examined using a standard flow cytometer equipped with a standard 15 mW argon-ion laser. The highest green fluorescence intensities were obtained using SYBR Green I. DNA viruses with genome sizes between 48.5 and 300 kb could easily be detected. The fluorescence signals of the small genome-sized RNA viruses (7.4□14.5 kb) were found at the limit of detection. No significant linear relationship could be found between genome size and the green fluorescence intensity of the SYBR Green I stained virus preparations. To our knowledge, this is the first report of detecting and discriminating between a wide range of different viruses directly using flow cytometry. This rapid and precise assay represents a new and promising tool in the field of virology.

8. Diez, B., J. Anton, N. Guixa-Boixereu, C. Pedros-Alio, and F. Rodriguez-Valera. 2000. Pulsed-field gel electrophoresis analysis of virus assemblages present in a hypersaline environment. International Microbiology 3:159-164. abstract: A method for analyzing virus assemblages in aquatic environments was developed and used for studying the highest-salinity ponds (from 13.4 to 35% salinity) from a multi-pond solar saltern in Alicante, Spain. The protocol consisted of a series of concentration and purification steps including tangential flow filtration and ultracentrifugation, followed by the preparation of total viral nucleic acids that were subsequently separated by pulsed-field gel electrophoresis. For every sample analyzed, a characteristic DNA pattern was obtained, whose complexity was related to viral diversity. The comparison of our results with a similar analysis carried out with marine virus assemblages shows that, as expected, the viral diversity corresponding to the analyzed hypersaline environment is considerably lower than that of a marine environment

9. Jeffrey, W. H., J. P. Kase, and S. W. Wilhelm. 2000. Ultraviolet radiation effects on bacterioplankton and viruses in marine ecosystems, p. 206-236. *In* S. J. De Mora and et al. (eds.), Effects Of UV Radiation On Marine Ecosystems. Cambridge University Press, Cambridge.

10. Lukasik, J., T. M. Scott, D. Andryshak, and S. R. Farrah. 2000. Influence of salts on virus adsorption to microporous filters. Appl.Environ.Microbiol. 66:2914-2920. abstract: We investigated the direct and indirect effects of mono-, di-, and trivalent salts (NaCl, MgCl(2), and AlCl(3)) on the adsorption of several viruses (MS2, PRD-1, phiX174, and poliovirus 1) to microporous filters at different pH values. The filters studied included Millipore HA (nitrocellulose), Filterite (fiberglass), Whatman (cellulose), and 1MDS (charged-modified fiber) filters. Each of these filters except the Whatman cellulose filters has been used in virus removal and recovery procedures. The direct effects of added salts were considered to be the effects associated with the presence of the soluble salts. The indirect effects of the added salts were considered to be (i) changes in the pH values of solutions and (ii) the formation of insoluble precipitates that could adsorb viruses and be removed by filtration. When direct effects alone were considered, the salts used in this study promoted virus adsorption, interfered with virus adsorption, or had little or no effect on virus adsorption, depending on the filter, the virus, and the salt. Although we were able to confirm previous reports that the addition of aluminum chloride to water enhances virus adsorption to microporous filters, we found that the enhanced adsorption was associated with indirect effects rather than direct effects. The increase in viral adsorption observed when aluminum chloride was added to water was related to the decrease in the pH of the water. Similar results could be obtained by adding HCl. The increased adsorption of viruses in water at pH 7 following addition of aluminum chloride was probably due to flocculation of aluminum, since removal of flocs by filtration greatly reduced the enhancement observed. The only direct effect of aluminum chloride on virus adsorption that we observed was interference with adsorption to microporous filters. Under conditions under which hydrophobic interactions were minimal, aluminum chloride interfered with virus adsorption to Millipore, Filterite, and 1MDS filters. In most cases, less than 10% of the viruses adsorbed to filters in the presence of a multivalent salt and a compound that interfered with hydrophobic interactions (0.1% Tween 80 or 4 M urea).

11. Middelboe, M. 2000. Bacterial growth rate and marine virus□host dynamics. Microb.Ecol. 40:114-124. abstract: The dynamics of a marine virus□host system were investigated at different steady state growth rates in chemostat

cultures and the data were analyzed using a simple model. The virus□host interactions showed strong dependence on host cell growth rate. The duration of the infection cycle and the virus burst size were found to depend on bacterial growth rate, and the rate of cell lysis and virus production were positively correlated with steady state growth rate in the cultures ($r^2 > 0.96$, $p < 0.05$). At bacterial growth rates of 0.02 to 0.10 h^{-1} in the chemostats the virus burst size increased from 12 □ 4 to 56 □ 4, and the latent period decreased from 2.0 to 1.7 h. Resistant clones of the host strain were present in the cultures from the beginning of the experiment and replaced the sensitive host cells following viral lysis in the cultures. Regrowth of resistant cells correlated significantly ($r^2 = 1.000$, $p < 0.02$) with the lysis rate of sensitive cells, indicating that release of viral lysates stimulated growth of the non-infected, resistant cells. The constructed model was suitable for simulating the observed dynamics of the sensitive host cells, viruses and resistant clones in the cultures. The model was therefore used in an attempt to predict the dynamics of this virus□host interaction in a natural marine environment during a certain set of growth conditions. The simulation indicated that a steady state relationship between the specific viruses and sensitive and resistant bacterial clones may occur at densities that are reasonable to assume for natural environments. The study demonstrates that basic characterization and modeling of specific virus□host interactions may improve our understanding of the behavior of bacteria and viruses in natural systems.

12. Riemann, L., G. F. Steward, and F. Azam. 2000. Dynamics of bacterial community composition and activity during mesocosm diatom blooms. Appl.Environ.Microbiol. 66:578-587. abstract: Bacterial community composition, enzymatic activities, and carbon dynamics were examined during diatom blooms in four, 200 liter laboratory seawater mesocosms.□ The objective was to determine whether the dramatic shifts in growth rates and ectoenzyme activities, which are commonly observed during the course of phytoplankton blooms and their subsequent demise, could result from shifts in bacterial community composition.□ Nutrient enrichment of metazoan-free seawater resulted in diatom blooms dominated by *Thalassiosira sp*. which peaked nine days after enrichment (24 g chl *a* l^{-1}). At this time bacterial abundance abruptly decreased from 2.8 to 0.75 x 10^6 ml^{-1} and analysis of bacterial community composition, by denaturing gradient gel electrophoresis (DGGE) of PCR-amplified, 16S rRNA gene fragments, revealed a disappearance of three dominant phylotypes.□ Increased viral and flagellate abundance suggested that both lysis and grazing could have played a role in the observed phylotype-specific mortality.□ Subsequently, new phylotypes appeared and bacterial production, abundance and enzyme activities shifted from being predominantly associated with the <1.0 m size-fraction towards the >1.0 m size-fraction indicating a pronounced microbial colonization of particles.□ Sequencing of DGGE bands suggested that the observed rapid and extensive colonization of particulate matter was mainly by specialized ??Proteobacteria and Cytophagales-related phylotypes.□ These particle-associated bacteria had high growth rates as well as high cell specific aminopeptidase, ??glucosidase and lipase activities.□ Rate measurements as well as bacterial population dynamics were almost identical among the mesocosms indicating that the observed bacterial community dynamics were systematic and repeatable responses to the manipulated conditions.

13. Rodriguez, F., E. Frenandez, R. N. Head, D. S. Harbour, G. Bratbak, M. Heldal, and R. P. Harris. 2000. Temporal variability of viruses, bacteria, phytoplankton and zooplankton in the western English Channel off Plymouth. Journal of the Marine Biological Association of the United Kingdom 80:575-586. abstract: The temporal distribution of autotrophic and heterotrophic components of the planktonic community was studied from samples collected weekly at station L4, located to the south of Plymouth, UK, from October 1992 to January 1994. Phytoplankton succession followed the typical pattern of temperate waters. the development of a summer *Gyrodinium aureolum* bloom being the most prominent feature. Bacterial numbers were significantly correlated with temperature during autumn and winter, whereas resource availability and predation, including viruses, appear to be the most important controlling factors in spring and summer. High mesozooplankton densities, mainly copepods, were observed throughout most of the study associated with a series of diatom blooms, and also during autumn when low phytoplankton biomass was measured. This data set was analysed in order to build up conceptual trophodynamic models whereby the role of biological communities on the cycling of organic matter could be inferred. The results obtained in this study provide empirical evidence supporting the existence of a succession of trophic organization patterns in a coastal temperate environment. Classical models (herbivorous or microbial webs) appeared episodically whereas transition models (multivorous web) dominated throughout most of the seasonal cycle.

14. Steward, G. F. and F. Azam. 2000. Analysis of marine viral assemblages, p. 159-165. *In* C. R. Bell, M. Brylinski, and P. Johnson-Green (eds.), Microbial Biosystems: New Frontiers. Atlantic Canada Society for Microbial Ecology. abstract: Viruses are the numerically dominant microbes in every oceanic environment from the surface into the sediments. A liter of surface seawater from a typical mesotrophic area contains 10^{10} of them, about ten times more than bacteria. While total counts of viruses are becoming easier to make, we still know very little about the viruses that comprise a given assemblage.□ Infectivity assays are extremely useful and still the best way to assay for infectious viruses for any particular host.□ However, this approach requires that each potential host organism be cultured, making it impractical if not impossible to completely characterize natural assemblages.□ Morphological studies have been enlightening, but are time consuming and difficult to do quantitatively.□ Here we report a fingerprinting approach to characterize natural viral assemblages.□ In this approach, viruses are concentrated and intact viral genomes are separated based on their size via pulsed-field gel electrophoresis.□ The number of distinguishable bands provides a minimum estimate of the number of different viruses, while band position and staining intensity reveal the genome size distribution within the assemblage.□ With this technique we have detected spatial and temporal differences, as well as many similarities, in viral assemblages among a variety of marine habitats. Current efforts are directed toward combining this technique with other methods of fractionation and sequence analysis to allow both morphological and genetic description of uncultivated marine viruses.□ Direct investigation of dominant or particularly widespread viruses may ultimately provide clues as to which marine organisms contribute most to the viral pool, and which organisms are likely to be significantly influenced by viral mortality.

15. Suttle, C. A. 2000. The ecology, evolutionary and geochemical consequences of viral infection of cyanobacteria and eukaryotic algae, p. 248-286. *In* C. J. Hurst (ed.), Viral Ecology. Academic Press, New York.

16. Thingstad, T. F. 2000. Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. Limnol.Oceanogr. 45:1320-1328. abstract: Mechanisms controlling virus abundance and partitioning of loss of bacterial production between viral lysis and

protozoan predation are discussed within the framework of an idealized Lotka-Volterra-type model. This combines nonselective protozoan predation with host-selective viral lysis of bacteria. The analysis leads to a reciprocal relationship between bacterial diversity and viruses, in which coexistence of competing bacterial species is ensured by the presence of viruses that "kill the winner," whereas the differences in substrate affinity between the coexisting bacterial species determine viral abundance. The ability of the model to reproduce published observations, such as an approximate 1:10 ratio between bacterial and viral abundance, and the ability of viral lysis to account for 10-50% of bacterial loss are discussed.

17. Wilhelm, S. W. and R. E. H. Smith. 2000. Bacterial carbon production in Lake Erie is influenced by viruses and solar radiation. Canadian Journal of Fisheries and Aquatic Sciences 57:317-326. abstract: Bacterial production is an integral recycling mechanism that facilitates carbon flow through aquatic food webs. Factors influencing bacterial activity therefore impact carbon flow. Although ecologists consider grazing and dissolved organic carbon flux to be the major regulators of bacterial activity, we explored two other important pressures. Virus-like particle abundance ranged from 3.7 to 37.9 x 10^{10} L-1 in samples collected during August 1997 and July 1998. Bacterial abundance during these periods ranged from 1.8 to 4.6 x 10^{9} L-1. Based on electron microscopic analysis, viruses in Lake Erie would have been responsible for 12.1 to 23.4 % of bacterial mortality and, in quasi-steady state conditions, a comparable loss of bacterial productivity. In the central basin, solar radiation was also demonstrated to regulate bacterial productivity. Ultraviolet radiation (UVR, 295-400 nm) was shown to inhibit bacterial productivity according to a cumulative exposure kinetic model, and biological weighting functions were derived to enable calculation of time- and depth-integrated photoinhibition. The daytime photoinhibitory loss of bacterial carbon production was estimated to be 14 to 30% over the upper 5 m, primarily due to UVR > 320 nm. Viruses and sunlight are therefore of comparable importance as regulators of bacterial activity in this system.

18. Wommack, K. E. and R. R. Colwell. 2000. Virioplankton: viruses in aquatic ecosystems. Microbiol.Mol.Biol.Rev. 64:69-114.

19. Binder, B. 1999. Reconsidering the relationship between virally induced bacterial mortality and frequency of infected cells. Aquat.Microb.Ecol. 18:207-215. abstract: The relative contribution of viral lysis to overall mortality in aquatic bacterial populations is often estimated as twice the frequency of infected cells (FIC). The `factor-of-two rule' upon which this estimate is based assumes (1) steady-state conditions, (2) that latent period is equivalent to generation time, and (3) that infected cells are not grazed. FIC values for this calculation are themselves derived from measurements of the frequency of visibly infected cells (FVIC) by the use of a simple conversion factor. A steady-state model was developed to more rigorously define the relationships between FIC, FVIC, and the fraction of mortality from viral lysis (FMVL). This model shows that even under the restrictive assumptions listed above, the factor-of-two rule systematically overestimates FMVL for typically reported values of FVIC. The model also shows that although grazing on infected cells further reduces FMVL for a given estimate of FIC, at the same time such grazing increases FIC for a given measurement of FVIC. In combination, these 2 effects minimize the influence of grazing on the calculation of FMVL from FVIC. Overall, the relationship between FMVL and FVIC is well approximated as follows: FMVL epsilon FVIC/[gamma ln(2) (1 - epsilon - FVIC)], where gamma = the ratio between the latent period and generation time, and \g?\ = the fraction of the latent period during which viral particles are not yet visible. Using typically observed values of FVIC, and assuming that gamma = 1 (per assumption 2, above) and \g?\ = 0.186 (per literature estimates), the model suggests that, on average, viral lysis accounts for approximately 22% (range: 4.5 to 45%) of total bacterial mortality in a range of aquatic environments, corresponding to a mean overestimate of 24% (range: 4 to 44%) by the factor-of-two rule. Perhaps most importantly, the model shows that calculations of FMVL from FIC or FVIC are very sensitive to changes in the relative length of the latent period (gamma) and in the assumed proportion of the latent period during which viral particles are not recognizable (epsilon). Constraining these 2 factors would greatly improve the reliability of FMVL calculations.

20. Fuhrman, J. A. 1999. Marine viruses and their biogeochemical and ecological effects. Nature 399:541-548. abstract: Viruses are the most common biological agents in the sea, typically numbering ten billion per litre. They probably infect all organisms, can undergo rapid decay and replenishment, and influence many biogeochemical and ecological processes, including nutrient cycling, system respiration, particle size-distributions and sinking rates, bacterial and algal biodiversity and species distributions, algal bloom control, dimethyl sulphide formation and genetic transfer. Newly developed fluorescence and molecular techniques leave the field poised to make significant advances towards evaluating and quantifying such efforts.

21. Guixa-Boixareu, N., K. Lysnes, and C. Pedros-Alio. 1999. Viral lysis and bacterivory during a phytoplankton bloom in a coastal water microcosm. Appl.Environ.Microbiol. 65:1949-1958. abstract: The relative importance of viral lysis and bacterivory as causes of bacterial mortality were estimated. A laboratory experiment was carried out to check the kind of control that viruses could exert over the bacterial assemblage in a non-steady-state situation. Virus-like particles (VLP) were determined by using three methods of counting (DAPI [4',6-diamidino-2-phenylindole] staining, YOPRO staining, and transmission electron microscopy). Virus counts increased from the beginning until the end of the experiment. However, different methods produced significantly different results. DAPI-stained VLP yielded the lowest numbers, while YOPRO-stained VLP yielded the highest numbers. Bacteria reached the maximal abundance at 122 h (3 x 10 super(7) bacteria ml super(-1)), after the peak of chlorophyll a (80 mu g liter super(-1)). Phototrophic nanoflagellates followed the same pattern as for chlorophyll a. Heterotrophic nanoflagellates showed oscillations in abundance throughout the experiment. The specific bacterial growth rate increased until 168 h (2.6 day super(-1)). The bacterivory rate reached the maximal value at 96 hours (0.9 day super(-1)). Bacterial mortality due to viral infection was measured by using two approaches: measuring the percentage of visibly infected bacteria (%VIB) and measuring the viral decay rates (VDR), which were estimated with cyanide. The %VIB was always lower than 1% during the experiment. VDR were used to estimate viral production. Viral production increased 1 order of magnitude during the experiment (from 10 super(6) to 10 super(7) VLP ml super(-1) h super(-1)). The percentage of heterotrophic bacterial production consumed by bacterivores was higher than 60% during the first 4 days of the experiment; afterwards, this percentage was lower than 10%. The percentage of heterotrophic bacterial production lysed by viruses as assessed by the VDR reached the highest values at the beginning (100%) and at the end (50%) of the experiment. Comparing both sources of mortality at each stage of the bloom, bacterivory was found to be higher than viral lysis at days 2 and 4, and viral lysis was higher than bacterivory at days 7 and 9. A balance between bacterial losses and bacterial production was calculated for each sampling interval. At intervals of 0 to 2 and 2 to 4 days, viral lysis and bacterivory accounted for all the bacterial losses. At intervals of 4 to 7 and 7 to 9 days, bacterial losses were not balanced by the sources of mortality measured. At these time points, bacterial abundance was about

20 times higher than the expected value if viral lysis and bacterivory had been the only factors causing bacterial mortality. In conclusion, mortality caused by viruses can be more important than bacterivory under non-steady-state conditions.

22. Marie, D., C. P. D. Brussaard, G. Thyrhaug, G. Bratbak, and D. Vaulot. 1999. Enumeration of marine viruses in culture and natural samples by flow cytometry. Appl.Environ.Microbiol. 65:45-52. abstract: Flow cytometry (FCM) was successfully used to enumerate viruses in seawater after staining with the nucleic acid-specific dye SYBR Green-I. The technique was first optimized by using the Phaeocystis lytic virus PpV-01. Then it was used to analyze natural samples from different oceanic locations. Virus samples were fixed with 0.5% glutaraldehyde and deep frozen for delayed analysis. The samples were then diluted in Tris-EDTA buffer and analyzed in the presence of SYBR Green-I. A duplicate sample was heated at 80 degree C in the presence of detergent before analysis. Virus counts obtained by FCM were highly correlated to, although slightly higher than, those obtained by epifluorescence microscopy or by transmission electron microscopy (r = 0.937, n = 14, and r = 0.96, n = 8, respectively). Analysis of a depth profile from the Mediterranean Sea revealed that the abundance of viruses displayed the same vertical trend as that of planktonic cells. FCM permits us to distinguish between at least two and sometimes three virus populations in natural samples. Because of its speed and accuracy, FCM should prove very useful for studies of virus infection in cultures and should allow us to better understand the structure and dynamics of virus populations in natural waters.

23. Noble, R. T., M. Middelboe, and J. A. Fuhrman. 1999. The effects of viral enrichment on the mortality and growth of heterotrophic bacterioplankton. Aquat.Microb.Ecol. 18:1-13. abstract: The direct effects of viral enrichments upon natural populations of marine viruses and bacteria were studied in seawater from Santa Monica Bay, CA, USA. Active virus concentrates, or control additions (ultrafiltered seawater or autoclaved virus concentrate) were added to 2 l incubations of protist-free seawater, and the effects were monitored for about 3 d. At the beginning of the experiments, the virus numbers reflected the expected addition of intact virus particles as determined by transmission electron microscopy (TEM). Subsequently, the mean frequency of visibly infected bacteria (FVIB; % bacteria which were visibly infected with 5 or more virus-like particles) was greater in the enriched incubations than in the controls. In controls, the estimated percent of bacteria that were infected remained constant at about 5 to 10% of the total bacterial population, but with active enrichment, 10 to 35% of the total bacterial population was infected at a given time. Therefore, by increasing the concentration of active viruses in seawater incubations we were able to increase the amount of bacterial mortality attributed to virus infection. Even with the presumed increase in bacterial mortality, the net increases in bacterial abundance in the samples that were enriched with active virus concentrate were higher than those seen in the controls. The viral abundance in bottles that were enriched with the active virus concentrate was significantly higher than that in the controls in Expts 2 and 3 ($p < 0.05$), but by the end of the experiments, viral abundances in the enriched incubations approached control levels. In Expts 1 and 2, rates of DOP hydrolysis were higher in the samples enriched with the active virus concentrate, and may have been due to an increase in the incidence of viral lysis. However, overall analysis of DCAA, DFAA, and DOP hydrolysis were quite variable and difficult to interpret. Results indicate that viral enrichment increased the incidence of bacterial infection and consequently stimulated the growth of subpopulations of non-infected heterotrophic bacterioplankton.

24. Paul, J. H. 1999. Microbial gene transfer: an ecological perspective. J.Mol.Microbiol.Biotechnol. 1:45-50. abstract: Microbial gene transfer or microbial sex is a means of exchanging loci amongst prokaryotes and certain eukaryotes. Historically viewed as a laboratory artifact, recent evidence from natural populations as well as genome research has indicated that this process may be a major driving force in microbial evolution. Studies with natural populations have taken two approaches-either adding a defined donor with a traceable gene to an indigenous community, and detecting the target gene in the indigenous bacteria, or by adding a model recipient to capture genes being transferred from the ambient microbial flora. However, both approaches usually require some cultivation of the recipient, which may result in a dramatic underestimation of the ambient transfer frequency. Novel methods are just evolving to study □*in situ* gene transfer processes, including the use of green fluorescent protein (GFP)-marked plasmids, which enable detection of transferrants by epifluorescence microscopy. A transduction-like mechanism of transfer from viral-like particles produced by marine bacteria and thermal spring bacteria to *Escherichia coli* has been documented recently, indicating that broad host range transduction may be occurring in aquatic environments. The sequencing of complete microbial genomes has shown that they are a mosaic of ancestral chromosomal genes interspersed with recently transferred operons that encode peripheral functions. Archaeal genomes indicate that the genes for replication, transcription, and translation are all eukaryotic in complexity, while the genes for intermediary metabolism are purely bacterial. And in eukaryotes, many ancestral eukaryotic genes have been replaced by bacterial genes believed derived from food sources. Collectively these results indicate that microbial sex can result in the dispersal of loci in contemporary microbial populations as well as having shaped the phylogenies of microbes from multiple, very early gene transfer events.

25. Prangishvili, D., H. P. Arnold, D. Gotz, U. Ziese, I. Holz, J. K. Kristjansson, and W. Zillig. 1999. A novel virus family, the Rudiviridae: Structure, virus-host interactions and genome variability of the sulfolobus viruses SIRV1 and SIRV2. Genetics 152:1387-1396. abstract: The unenveloped, stiff-rod-shaped, linear double-stranded DNA viruses SIRV1 and SIRV2 from Icelandic *Sulfolobus* isolates form a novel virus family, the Rudiviridae. The sizes of the genomes are 32. 3 kbp for SIRV1 and 35.8 kbp for SIRV2. The virions consist of a tube-like superhelix formed by the DNA and a single basic 15.8-kD DNA-binding protein. The tube carries a plug and three tail fibers at each end. One turn of the DNA-protein superhelix measures 4.3 nm and comprises 16.5 turns of B DNA. The linear DNA molecules appear to have covalently closed hairpin ends. The viruses are not lytic and are present in their original hosts in carrier states. Both viruses are quite stable in these carrier states. In several laboratory hosts SIRV2 was invariant, but SIRV1 formed many different variants that completely replaced the wild-type virus. Some of these variants were still variable, whereas others were stable. Up to 10% nucleotide substitution was found between corresponding genome fragments of three variants. Some variants showed deletions. Wild-type SIRV1, but not SIRV2, induces an SOS-like response in *Sulfolobus*. We propose that wild-type SIRV1 is unable to propagate in some hosts but surmounts this host range barrier by inducing a host response effecting extensive variation of the viral genome.

26. Short, S. M. and C. A. Suttle. 1999. Use of the polymerase chain reaction and denaturing gradient gel electrophoresis to study diversity in natural virus communities. Hydrobiologia 401:19-32. abstract: Viruses are abundant members of marine and freshwater microbial communities, and are important players in aquatic ecology and geochemical cycles. Recent methodological developments have allowed the use of the polymerase chain reaction (PCR) to examine the diversity of natural communities of viruses without the need for culture. DNA polymerase genes are highly conserved and are, therefore, suitable targets for PCR analysis of microbes that do not

encode rRNA. As natural virus communities are largely made up of dsDNA viruses, and as many dsDNA algal viruses encode their own DNA polymerase, PCR primers can be designed to amplify fragments of these genes. This approach has been used to examine the genetic diversity in natural communities of viruses that infect phytoplankton. Algal-virus-specific primers were used to amplify polymerase fragments from natural virus samples, demonstrating the presence of a diverse community of viruses closely related to those that are known to infect phytoplankton. We have modified this approach by using denaturing gradient gel electrophoresis (DGGE) to rapidly analyze PCR products. DGGE will permit rapid and efficient fingerprinting of natural marine viral communities, and allow spatial and temporal differences in viral community structure to be examined. This paper provides a brief overview of how PCR and DGGE can be used to examine diversity in natural viral communities drawing on viruses that infect phytoplankton as an example.

27. Sommaruga, R., B. Sattller, A. Oberleiter, A. Wille, S. Sommaruga-W□grath, R. Psenner, M. Felip, L. Camarero, S. Pina, R. GironTs, and J. Catal□n. 1999. An in situ enclosure experiment to test the solar UVB impact on plankton in a high altitude mountain lake: II) effects on the microbial food web. Journal of Plankton Research 21:859-879. abstract: We studied the impact of ambient levels of solar UVB radiation on the planktonic microbial food web (viruses, heterotrophic bacteria, heterotrophic flagellates and ciliates) of a high-mountain lake (2417 m above sea level) under in situ conditions for 16 days. Enclosures of 1 m3 receiving either the full sunlight spectrum or sunlight without UVB radiation were suspended at the lake surface.□ We found that the abundance of heterotrophic flagellates was always lower in the +UVB treatment than in the -UVB one. In addition, bacterial consumption, measured by the disappearance of fluorescently labelled bacteria, was significantly ($p<0.05$) reduced in the +UVB treatment. The abundance of non-filamentous bacteria (<10 □m long) was also lower in the +UVB treatment, suggesting a direct effect of UVB on their growth. This was supported by the significantly ($p<0.05$) lower cell-specific activity ([3H]-thymidine incorporation) found on the fifth day of the experiment. In contrast, UVB radiation had no effect on filamentous bacteria (>10 □m long) that represented only a small fraction of the total abundance (<4%) but up to ~70 % of the total bacterial biovolume. Ciliates, mainly Urotricha pelagica and U. furcata, were less impacted by UVB radiation although the net growth rate during the first week of the experiment was lower in the +UVB treatment than in the -UVB one (0.22 and 0.39 d-1, respectively). The abundance of virus-like particles during the first week of the experiment was higher in the -UVB treatment. After reaching the maximum value for the interaction viruses x bacteria, their number decreased dramatically (by ~85%) in both treatments with a decay rate of ~0.017 h-1. This study illustrates the complexity in assessing the impact of UVB radiation when more than one trophic level is considered and indicates the existence of different sensitivity to UVB radiation among components of the microbial food web.

28. van Hannen, E. J., G. Zwart, M. P. van Agterveld, H. J. Gons, J. Ebert, and H. J. Laanbroek. 1999. Changes in bacterial and eukaryotic community structure after mass lysis of filamentous cyanobacteria associated with viruses. Appl.Environ.Microbiol. 65:795-801. abstract: During an experiment in two laboratory-scale enclosures filled with lake water (130 liters each) we noticed the almost-complete lysis of the cyanobacterial population. Based on electron microscopic observations of viral particles inside cyanobacterial filaments and counts of virus-like particles, we concluded that a viral lysis of the filamentous cyanobacteria had taken place. Denaturing gradient gel electrophoresis (DGGE) of 16S ribosomal DNA fragments qualitatively monitored the removal of the cyanobacterial species from the community and the appearance of newly emerging bacterial species. The majority of these bacteria were related to the Cytophagales and actinomycetes, bacterial divisions known to contain species capable of degrading complex organic molecules. A few days after the cyanobacteria started to lyse, a rotifer species became dominant in the DGGE profile of the eukaryotic community. Since rotifers play an important role in the carbon transfer between the microbial loop and higher trophic levels, these observations confirm the role of viruses in channeling carbon through food webs. Multidimensional scaling analysis of the DGGE profiles showed large changes in the structures of both the bacterial and eukaryotic communities at the time of lysis. These changes were remarkably similar in the two enclosures, indicating that such community structure changes are not random but occur according to a fixed pattern. Our findings strongly support the idea that viruses can structure microbial communities.

29. Wilhelm, S. W. and C. A. Suttle. 1999. Viruses and nutrient cycles in the sea. BioScience 49:781-788. abstract: Viruses play critical roles in the structure and function of aquatic food webs.

30. Wommack, K. E., J. Ravel, R. T. Hill, and R. R. Colwell. 1999. Population dynamics of Chesapeake Bay virioplankton: total-community analysis by pulsed-field gel electrophoresis. Appl.Environ.Microbiol. 65:231-240. abstract: It has been hypothesized that, by specifically lysing numerically dominant host strains, the virioplankton may play a role in maintaining clonal diversity of heterotrophic bacteria and phytoplankton populations. If viruses selectively lyse only those host species that are numerically dominant, then the number of a specific virus within the virioplankton would be expected to change dramatically over time and space, in coordination with changes in abundance of the host. In this study, the abundances of specific viruses in Chesapeake Bay water samples were monitored, using nucleic acid probes and hybridization analysis. Total virioplankton in a water sample was separated by pulsed-field gel electrophoresis and hybridized with nucleic acid probes specific to either single viral strains or a group of viruses with similar genome sizes. The abundances of specific viruses were inferred from the intensity of the hybridization signal. By using this technique, a virus comprising 1/1,000 of the total virioplankton abundance (ca. 10 (4) PFU/ml) could be detected. Titers of either a single virus species or a group of viruses changed over time, increasing to peak abundance and then declining to low or undetectable levels, and were geographically localized in the bay. Peak signal intensities, i.e., peak abundances of virus strains, were 10-fold greater than the low background level. Furthermore, virus species were found to be restricted to a particular depth, since probes specific to viruses from bottom water did not hybridize with virus genomes from surface water at the same geographical location. Overall, changes in abundances of specific viruses within the virioplankton were episodic, supporting the hypothesis that viral infection influences, if not controls, clonal diversity within heterotrophic bacteria and phytoplankton communities.

31. Wommack, K. E., J. Ravel, R. T. Hill, and R. R. Colwell. 1999. Hybridization analysis of Chesapeake Bay Virioplankton. Appl.Environ.Microbiol. 65:241-250. abstract: It has been hypothesized that, by specifically lysing numerically dominant host strains, the virioplankton may play a role in maintaining clonal diversity of heterotrophic bacteria and phytoplankton populations. If viruses selectively lyse only those host species that are numerically dominant, then the number of a specific virus within the virioplankton would be expected to change dramatically over time and space, in coordination with changes in abundance of the host. In this study, the abundances of specific viruses in Chesapeake Bay water samples were monitored, using nucleic acid probes and hybridization analysis. Total virioplankton in a water sample was separated by pulsed-field gel electrophoresis and hybridized with nucleic

acid probes specific to either single viral strains or a group of viruses with similar genome sizes. The abundances of specific viruses were inferred from the intensity of the hybridization signal. By using this technique, a virus comprising 1/1,000 of the total virioplankton abundance (ca. 10(4) PFU/ml) could be detected. Titers of either a single virus species or a group of viruses changed over time, increasing to peak abundance and then declining to low or undetectable levels, and were geographically localized in the bay. Peak signal intensities, i.e., peak abundances of virus strains, were 10-fold greater than the low background level. Furthermore, virus species were found to be restricted to a particular depth, since probes specific to viruses from bottom water did not hybridize with virus genomes from surface water at the same geographical location. Overall, changes in abundances of specific viruses within the virioplankton were episodic, supporting the hypothesis that viral infection influences, if not controls, clonal diversity within heterotrophic bacteria and phytoplankton communities.

32. Bath, C. and M. L. Dyall-Smith . 1998. His1, and archaeal virus of the *Fuselloviridae* family that infects *Haloarcula hispanica*. J.Virol. 72:9392-9395. abstract: A novel archaeal virus, His1, was isolated from hypersaline waters in south-eastern Australia. It was lytic, grew only on *Ha. hispanica* (up to titres of 1011p.f.u./ml), and displayed a "lemon-shaped" morphology (74nm x 44nm) previously reported only for a virus of the extreme thermophiles (SSV1). The density of His1 was approximately 1.28g/ml - similar to that of SSV1 (1.24g/ml). Purified particles were resistant to low salt. The genome was linear, dsDNA and 14.9kb in size, which was similar in size to the genome of the SSV1 (ie. 15.5kb). Morphologically, this isolate clearly belongs to the recently proposed *Fuselloviridae* family of archaeal viruses. It represents the first member from the extremely halophilic archaea, and its host, *Ha. hispanica*, is one that can be readily manipulated genetically.

33. Clarke, K. J. 1998. Virus particle production in lysogenic bacteria exposed to protozoan grazing. FEMS Microbiol.Let. 166:177-180. abstract: Electron microscopy was used to investigate the apparent induction of virus particle production in bacteria undergoing digestion by ciliates. Results showed that numbers of bacteria containing virus particles increased by a factor of 25 when enclosed within ciliate food vacuoles. It was also found that 10% of these particles survived the digestion process to be released back into the aquatic habitat within faecal pellets. The possibility of virus gene transfer occurring between lysogenically infected bacteria that survive the ciliate digestive processes, is also considered.

34. Juniper, S. K., D. F. Bird, M. Summit, M. Pong Vong, and E. T. Baker. 1998. Bacterial and viral abundances in hydrothermal event plumes over northern Gorda Ridge. Deep-Sea Research 45:2739-2749. abstract: This study presents first-time observations of bacterial and viral abundances in hydrothermal event plumes. Two water-column event plumes were formed in conjunction with seismic events and seafloor volcanic eruptions on the northern Gorda Ridge in February--March 1996. Epifluorescence counts of bacteria and viruses were performed on water samples from 3 successive cruises staged in the 10--90 days that followed the onset of seismicity. Relative to background seawater at these 1800--3200 m depths, bacterial abundance was enhanced by 2-3 fold within both event plumes. In contrast, viral numbers were below background seawater values in the younger and more intense of the two event plumes (EP96A), and enhanced in the other (EP96B). Changes in viral abundance may be a secondary response to that of plume bacteria as well as being influenced by particle formation and precipitation within the plumes. Lower bacteria/heat, virus/heat and virus/bacteria ratios in EP96A versus EP96B confirm distinct differences in the microbial response to event plume formation, possibly related to observed differences in plume chemistry.

35. Noble, R. T. and J. A. Fuhrman . 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat.Microb.Ecol. 14:113-118. abstract: A new nucleic acid stain, SYBR Green I, can be used for the rapid and accurate determination of viral and bacterial abundances in diverse marine samples. We tested this stain with formalin-preserved samples of coastal water and also from depth profiles (to 800 m) from sites 19 and 190 km offshore, by filtering a few ml onto 0.02 mu m pore-size filters and staining for 15 min. Comparison of bacterial counts to those made with acridine orange (AO) and virus counts with those made by transmission electron microscopy (TEM) showed very strong correlations. Bacterial counts with AO and SYBR Green I were indistinguishable and almost perfectly correlated (r super(2) = 0.99). Virus counts ranged widely, from 0.03 to 15 x 10 super(7) virus ml super(-1). Virus counts by SYBR Green I were on the average higher than those made by TEM, and a SYBR Green I versus TEM plot yielded a regression slope of 1.28. The correlation between the two was very high with an r super(2) value of 0.98. The precision of the SYBR Green I method was the same as that for TEM, with coefficients of variation of 2.9%. SYBR Green I stained viruses and bacteria are intensely stained and easy to distinguish from other particles with both older and newer generation epifluorescence microscopes. Detritus is generally not stained, unlike when the alternative dye YoPro I is used, so this approach may be suitable for sediments. SYBR Green I stained samples need no desalting or heating, can be fixed with formalin prior to filtration, the optimal staining time is 15 min (resulting in a total preparation time of less than 25 min), and counts can be easily performed at sea immediately after sampling. This method may facilitate incorporation of viral research into most aquatic microbiology laboratories.

36. Pina, S., A. Creus, N. Ganz lez, R. GironTs, M. Felip, and R. Sommaruga. 1998. Abundance, morphology and distribution of planktonic virus-like particles in two high-mountain lakes. Journal of Plankton Research 20:2413-2421. abstract: Direct counts of virus-like particles (VLP) by transmission electron microscopy revealed abundances of up to 3×10^7 ml^{-1} in the plankton of two remote high-mountain lakes in the Alps and in the Pyrenees. Most VLP were icosahedric without tail and with diameters between 40 and 90 nm, but also very large ones with diameter of up to 325 nm were observed. VLP outnumbered bacteria by a factor of 4.2 to 42.8 and bacterial cells were infected with large numbers (>50) of viral particles. This study constitutes the first report on aquatic viruses for alpine lakes and it suggests that they may be an important additional source of bacterial mortality in these systems.

37. Prangishvili, D., H. P. Klenk, G. Jakobs, A. Schmiechen, C. Hanselmann, I. Holz, and W. Zillig. 1998. Biochemical and phylogenetic characterization of the dUTPase from the archaeal virus SIRV. Journal of Biological Chemistry 273:6024-6029. abstract: The derived amino acid sequence from a 474-base pair open reading frame in the genome of the *Sulfolobus islandicus* rod-shaped virus SIRV shows striking similarity to bacterial dCTP deaminases and to dUTPases from eukaryotes, bacteria, Poxviridae, and Retroviridae. The putative gene was expressed in *Escherichia coli*, and dUTPase activity of the recombinant enzyme was demonstrated by hydrolysis of dUTP to dUMP. Deamination of dCTP by the enzyme was not detected. Phylogenetic analysis based on amino acid sequences of the characterized enzyme and its homologues showed that the dUTPase-encoding dut genes and the dCTP deaminase-encoding dcd genes constitute a paralogous gene family. This report is the first identification and functional characterization of an archaeal dUTPase and the first phylogeny derived for the dcd-dut gene family.

38. Thingstad, T. F. 1998. A theoretical approach to structuring mechanisms in the pelagic food web. Hydrobioligia 363:59-72. abstract: In the literature there is a commonly used idealized concept of the food web structure in the pelagic photic zone food web, based to a large extent on size dependent relationships. An outline is here given of how the elementary size-related physical laws of diffusion and sinking, combined with the assumption of predators being size selective in their choice of prey, give a theoretical foundation for this type of structure. It is shown how such a theoretical fundament makes it possible to relate a broad specter of phenomena within one generic and consistent framework. Phenomena such as Hutchinson's and Goldman's paradoxes, the influence of nutrients and water column stability on the balance between microbial and classical food webs, bacterial carbon consumption, new production and export of DOC and POC to the aphotic zone, eutrophication and diversity, can all be approached from this perspective. By including host-specific viruses, this approach gives a hierarchical structure to the control of diversity with nutrient content controlling the maximum size of the photic zone community, size selectivity of predators regulating how the nutrient is distributed between size-groups of osmotrophic and phagotrophic organisms, and viral host specificity regulating how the nutrients within a size group is distributed between host groups. I also briefly discuss how some biological strategies may be successful by not conforming to the normal rules of such a framework. Analyzing the behavior of these idealized systems is thus claimed to facilitate our understanding of the behavior of complex natural food webs.

39. Weinbauer, M. G. and M. G. Hoefle. 1998. Size-specific mortality of lake bacterioplankton by natural virus communities. Aquat.Microb.Ecol. 15:103-113. abstract: The potential effect that viral lysis has on the cell size distribution of bacterioplankton was investigated during late summer stratification in Lake Plusssee, Germany. Size-specific bacterial mortality due to viral lysis was estimated from in situ samples by a transmission electron microscopy based examination of visibly infected cells (VIC) and in an experiment with varying concentrations of the natural virus community. In all depth layers the highest percentage of cells was found in a cell length class that was smaller for the entire bacterial community (0.3-0.6 mu m) than for VIC (0.6-0.9 mu m). For cells <2.4 mu m the highest frequency of VIC (FVIC) was detected in the size classes 0.6-0.9 and 0.9-1.2 mu m, and the FVIC was high in the size classes 1.2-1.5 (all depth layers) and 1.5-1.8 mu m (meta- and hypolimnion). The estimated mortality due to viral lysis in these size classes was significant with maxima of 29 to 55% in the epilimnion, 30 to 59% in the metalimnion and 56 to 107% in the hypolimnion. In all depth layers the FVIC of bacteria <0.3 mu m in length was ca 30% of that averaged for the entire bacterial community, and in the experiment the percentage of cells <0.3 mu m was highest in enclosures with high viral activity.

40. Weinbauer, M. G. and M. G. H□fle. 1998. Cell size-specific lysis of lake bacterioplankton by natural virus communities. Aquat.Microb.Ecol. 156:103-113. abstract: The potential effect that viral lysis has on the cell size distribution of bacterioplankton was investigated during late summer stratification in Lake Plusssee, Germany. Size-specific bacterial mortality due to viral lysis was estimated from in situ samples by a transmission electron microscopy based examination of visibly infected cells (VIC) and in an experiment with varying concentrations of the natural virus community. In all depth layers the highest percentage of cells was found in a cell length class that was smaller for the entire bacterial community (0.3-0.6 mu m) than for VIC (0.6-0.9 mu m). For cells <2.4 mu m the highest frequency of VIC (FVIC) was detected in the size classes 0.6-0.9 and 0.9-1.2 mu m and the FVIC was high in the size classes 1.2-1.5 (all depth layers) and 1.5-1.8 mu m (meta- and hypolimnion). The estimated mortality due to viral lysis in these size classes was significant with maxima of 29 to 55% in the epilimnion, 30 to 59% in the metalimnion and 56 to 107% in the hypolimnion. In all depth layers the FVIC of bacteria <0.3 mu m in length was ca 30% of that averaged for the entire bacterial community, and in the experiment the percentage of cells <0.3 mu m was highest in enclosures with high viral activity. In the experiment the average cell size was smaller in enclosures with high than in that with low viral activity. The data demonstrate that being small could be a strategy of cells to reduce mortality due to viral lysis probably by reducing the contact rates with viruses. Thus, viral lysis could be one of the mechanisms keeping the cell size small in aquatic ecosystems. In oxic water cells in the largest size class (>2.4 mu m) were not infected with viruses, and in enclosures with epilimnetic lake water the percentage of cells >2.4 mu m was highest in enclosures with highest viral abundance, suggesting that resistance against infection favored large cells. However, in the meta- and hypolimnion the FVIC was high for cells >2.4 mu m and, since the burst size increased with bacterial cell size, lysis of large cells could contribute significantly to viral production. Also, a major portion of biomass was found in cells >2.4 mu m. The finding that viral lysis is size-specific and can affect the cell size distribution of bacteria in lake water has important implications for our understanding of the mechanisms which regulate bacterial production and nutrient cycling in pelagic environments.

41. Weinbauer, M. G. and M. G. Hofle. 1998. Significance of viral lysis and flagellate grazing as factors controlling bacterioplankton production in a eutrophic lake. Appl.Environ.Microbiol. 64:431-438. abstract: The effects of viral lysis and heterotrophic nanoflagellate (HNF) grazing on bacterial mortality were estimated in a eutrophic lake (Lake Plussee in northern Germany) which was separated by a steep temperature and oxygen gradient into a warm and oxic epilimnion and a cold and anoxic hypolimnion. Two transmission electron microscopy-based methods (whole-cell examination and thin sections) were used to determine the frequency of visibly infected cells, and a model was used to estimate bacterial mortality due to viral lysis. Examination of thin sections also showed that between 20.2 and 29.2% (average, 26.1%) of the bacterial cells were empty (ghosts) and thus could not contribute to viral production. The most important finding was that the mechanism for regulating bacterial production shifted with depth from grazing control in the epilimnion to control due to viral lysis in the hypolimnion. We estimated that in the epilimnion viral lysis accounted on average for 8.4 to 41.8% of the summed mortality (calculated by determining the sum of the mortalities due to lysis and grazing), compared to 51.3 to 91.0% of the summed mortality in the metalimninon and 88.5 to 94.2% of the summed mortality in the hypolimnion. Estimates of summed mortality values indicated that bacterial production was controlled completely or almost completely in the epilimnion (summed mortality, 66.6 to 128.5%) and the hypolimnion (summed mortality, 43.4 to 103.3%), whereas in the metalimnion viral lysis and HNF grazing were not sufficient to control bacterial production (summed mortality, 22.4 to 56.7%). The estimated contribution of organic matter released by viral lysis of cells into the pool of dissolved organic matter (DOM) was low; however, since cell lysis products are very likely labile compared to the bulk DOM, they might stimulate bacterial production. The high mortality of bacterioplankton due to viral lysis in anoxic water indicates that a significant portion of bacterial production in the metalimnion and hypolimnion is cycled in the bacterium-virus-DOM loop. This finding has major implications for the fate and cycling of organic nutrients in lakes.

42. Wilhelm, S. W., M. G. Weinbauer, C. A. Suttle, and W. H. Jeffrey. 1998. The role of sunlight in the removal and repair of viruses in the sea. Limnol.Oceanogr. 43:586-592. abstract: We investigated the in situ destruction rates of marine

viral particles as well as the decay rates of infectivity for viral isolates along an similar to 400-km transect from oligotrophic offshore waters to productive coastal waters in the Gulf of Mexico. Light-mediated decay rates of viral infectivity averaged over the solar day ranged from 0.7 to 0.85 h super(-1) in surface waters at all stations and decreased with depth in proportion to the attenuation of UVB (305 nm). The destruction rates of viral particles also decreased with depth, although the rates of particle destruction were only 22-61% of infectivity when integrated over the mixed layer. The rates of viral particle destruction indicated that at three of four stations 6-12% of the daily bacterial production would have to be lysed in order to maintain ambient viral concentrations. At the fourth station, where there was a dense bloom of *Synechococcus* spp. and the mixed layer was shallower, 34-52% of the daily bacterial production would have to be lysed. A comparison of the difference between destruction rates of viral particles and infectivity integrated over the depth of the mixed layer implies that host-mediated repair must have restored infectivity to 39-78% of the sunlight-damaged viruses daily. The calculated frequency of contacts between viral particles and bacterial cells that resulted in infection (contact success) ranged from similar to 18 to 34% in offshore waters, where the frequency of contacts between viruses and bacteria was much lower, to similar to 1.0% at the most inshore station, where contact rates are much higher. This suggests that in offshore waters bacterial communities are less diverse, and that there is less selection to be resistant to viral infection. This paper provides a framework for balancing viral production, destruction, and light-dependent repair in aquatic viral communities.

43. Wilhelm, S. W., M. G. Weinbauer, C. A. Suttle, R. J. Pledger, and D. L. Mitchell. 1998. Measurements of DNA damage and photoreactivation imply that most viruses in marine surface waters are inefective. Aquat.Microb.Ecol. 14:215-222. abstract: The proportion of viruses in natural marine communities that are potentially infectious was inferred from the relationship between DNA damage and the loss of infectivity in marine viral isolates and measurements of the DNA damage in natural viral communities. Several viral isolates which infect marine Vibrio spp. were exposed to UV-C radiation and the concentration of cyclobutane pyrimidine dimers in the viral DNA was measured with a highly sensitive radioimmunoassay. The loss of infectivity in the UV-exposed isolates was also determined under conditions which either activated or repressed the blue light dependent photolyase enzyme in host cells in order to examine the damage-dependent response of this bacterial repair system. In addition, the accumulation of DNA photodamage during the solar day was measured in DNA isolated from natural viral communities collected along a transect in the western Gulf of Mexico. Using the correlation between DNA damage and infectivity for one of the viral isolates, we estimated the proportion of the natural viral community which was infective. The results imply that, due to light-mediated repair of damaged viral DNA by host-cell mechanisms (photoreactivation), greater than 50% of the viruses in natural communities are infective despite high rates of DNA damage. Furthermore, the accumulation of cyclobutane pyrimidine dimers was highest at the station where the surface mixed layer was shallowest, emphasizing the importance of mixing depth in relation to the accumulation of DNA damage. These experiments demonstrate that physical parameters such as mixing depth are critically interwoven with light penetration in influencing the infectivity of marine viral communities.

44. Wilson, W. H., S. Turner, and N. H. Mann. 1998. Population dynamics of phytoplankton and viruses in a phosphate-limited mesocosm and their effect on DMSP and DMS production. Estuarine, Coastal and Shelf Science 46 (Supplement a):49-59. abstract: The effect of phosphate limitation on viral abundance, phytoplankton bloom dynamics and production of dimethylsulphoniopropionate (DMSP) and dimethyl sulphide (DMS) was investigated in seawater mesocosm enclosures, in a Norwegian fjord, during June 1995. Daily estimates of viral concentrations, based on transmission electron microscope (TEM) counts, varied on an apparently random basis in each of the enclosures. A large *Synechococcus* spp. bloom developed in an enclosure which was maintained at a high N:P ratio, simulating phosphate-deplete growth conditions. Following phosphate addition to this enclosure, there was a large increase in estimated virus numbers shortly before an apparent collapse of the *Synechococcus* bloom. It is tentatively suggested that lysogenic viruses were induced following phosphate addition to the phosphate-limited enclosures, and that these observations add to a growing body of evidence which supports the hypothesis that nutrient availability may be responsible for the switch between lysogeny and lytic production. High DMS concentrations and viral numbers were observed on the demise of the flagellate (predominantly Emiliania huxleyi) and diatom blooms, but overall there was no significant correlation. Highest concentrations of DMSP were associated with blooms of E. huxleyi, for which an intracellular concentration of 0.5 pg cell-1 (SD, 0.06) was calculated. Good correlation of DMSP with *Synechococcus* spp. cell numbers was observed, suggesting that these species of picoplankton may be significant producers of DMSP. No effects of phosphate limitation on DMS and/or DMSP production were evident from the data.

45. Wilhelm, S. W., M. G. Weinbauer, C. A. Suttle, R. J. Pledger, and D. L. Mitchell. 1998. Measurements of DNA damage and photoreactivation imply that most viruses in marine surface waters are inefective. Aquat.Microb.Ecol. 14:215-222. abstract: The proportion of viruses in natural marine communities that are potentially infectious was inferred from the relationship between DNA damage and the loss of infectivity in marine viral isolates and measurements of the DNA damage in natural viral communities. Several viral isolates which infect marine Vibrio spp. were exposed to UV-C radiation and the concentration of cyclobutane pyrimidine dimers in the viral DNA was measured with a highly sensitive radioimmunoassay. The loss of infectivity in the UV-exposed isolates was also determined under conditions which either activated or repressed the blue light dependent photolyase enzyme in host cells in order to examine the damage-dependent response of this bacterial repair system. In addition, the accumulation of DNA photodamage during the solar day was measured in DNA isolated from natural viral communities collected along a transect in the western Gulf of Mexico. Using the correlation between DNA damage and infectivity for one of the viral isolates, we estimated the proportion of the natural viral community which was infective. The results imply that, due to light-mediated repair of damaged viral DNA by host-cell mechanisms (photoreactivation), greater than 50% of the viruses in natural communities are infective despite high rates of DNA damage. Furthermore, the accumulation of cyclobutane pyrimidine dimers was highest at the station where the surface mixed layer was shallowest, emphasizing the importance of mixing depth in relation to the accumulation of DNA damage. These experiments demonstrate that physical parameters such as mixing depth are critically interwoven with light penetration in influencing the infectivity of marine viral communities.

Submissions Archive

- On an Invisible Microbe Antagonistic to the Dysentery Bacillus by Felix d'Herelle
- Obituary: Hansjürgen Raettig - Collector of Bacteriophage References (October 12, 1911 - December 1, 1997)

- Some Quotations
- Bacteriophages: A Model System for Human Viruses
- How Big is 10^{30}?
- Selling Phage Candy
- A List of Phage Names
- An Expanded Overview of Phage Ecology
- Rendering Phage Heads
- A Few Thoughts on Phage Therapy

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Phage Images

Please send any phage images that you would like to present in this section to "Phage Images," *The Bacteriophage Ecology Group*, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. Alternatively, you may scan the images yourself and send them as an attachment to abedon.1@osu.edu. Please save all scans in gif or jpg formats and preferably with an image size (in terms of width, height, and kbytes) that will readily fit on a standard web page. No copyrighted material without permission, please!

Phage Image Archive

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- The Face of the Phage
- Bacteriophage T2
- SSV1-Type Phage
- Saline Lake Bacteriophage
- Coliphage LG1
- Bacteriophage HK97
- Phage T4 (art)
- Phage T4 on the pedestal outside of Barker Hall at Berkeley
- Electron micrograph of phage P22
- Thin section of T4 phages hitting a microcolony of *E. coli* K-12
- T4 phage v1
- T4 Tail Model
- Gingerbread phage

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New Publications

New bacteriophage publications are listed below. Each quarter not-yet-listed publications from the previous two years will be presented along with their abstracts. The indicator "???" denotes, of course, that specific information is not yet in the BEG Bibliography. Please help in the compilation of the BEG Bibliography by supplying any updated information, correcting any mistakes, and, of course, sending the references to your bacteriophage ecology publications, as well as the references to any bacteriophage ecology publications that you know of but which are not yet in the bibliography (send to abedon.1@osu.edu or to "BEG Bibliography," *Bacteriophage Ecology Group News*, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906). Also, be sure to indicate any listed publications that you feel should not be presented in the BEG Bibliography. This list is also present with available abstracts at the end of *BEG News*.

1. Epigenetics as a first exit problem. Aurell, E., Sneppen, K. (2002). *Physical Review Letters* 88:048101. [PRESS FOR ABSTRACT]

2. Microviridae, a family divided: isolation, characterization, and genome sequence of phiMH2K, a bacteriophage of the obligate intracellular parasitic bacterium *Bdellovibrio bacteriovorus*. Brentlinger, K. L., Hafenstein, S., Novak, C. R., Fane, B. A., Borgon,

R., McKenna, R., Agbandje-McKenna, M. (2002). *Journal of Bacteriology* 184:1089-1094. [PRESS FOR ABSTRACT]

3. On the stability properties of a stochastic model for phage-bacteria interaction in open marine environment. Carletti, M. (2002). *Mathematical Biosciences* 175:117-131. [PRESS FOR ABSTRACT]

4. [The antiviral activity of chitosan (review)]. Chirkov, S. N. (2002). *Prikladnaia Biokhimiia I Mikrobiologiia* 38:5-13. [PRESS FOR ABSTRACT]

5. Filamentous phage active on the gram-positive bacterium *Propionibacterium freudenreichii*. Chopin, M. C., Rouault, A., Ehrlich, S. D., Gautier, M. (2002). *Journal of Bacteriology* 184:2030-2033. [PRESS FOR ABSTRACT]

6. Snapshot of the genome of the pseudo-T-even bacteriophage RB49. Desplats, C., Dez, C., Tetart, F., Eleaume, H., Krisch, H. M. (2002). *Journal of Bacteriology* 184:2789-2804. [PRESS FOR ABSTRACT]

7. Biological properties and cell tropism of Chp2, a bacteriophage of the obligate intracellular bacterium *Chlamydophila abortus*. Everson, J. S., Garner, S. A., Fane, B., Liu, B. L., Lambden, P. R., Clarke, I. N. (2002). *Journal of Bacteriology* 184:2748-2754. [PRESS FOR ABSTRACT]

8. RS1 element of *Vibrio cholerae* can propagate horizontally as a filamentous phage exploiting the morphogenesis genes of CTX-Phi. Faruque, S. M., Kamruzzaman, M., Nandi, R. K., Ghosh, A. N., Nair, G. B., Mekalanos, J. J., Sack, D. A. (2002). *Infection and Immunity* 70:163-170. [PRESS FOR ABSTRACT]

9. Microbiology. A tail of two specifi-cities. Hatfull, G. F. (2002). *Science* 295:2031-2032. [no abstract]

10. Engineering a reduced *Escherichia coli* genome. Kolisnychenko, V., Plunkett, G., Herring, C. D., Feher, T., Posfai, J., Blattner, F. R., Posfai, G. (2002). *Genome Research* 12:640-647. [PRESS FOR ABSTRACT]

11. The activity of chosen bacteriophages on *Yersinia enterocolitica* strains. Kot, B., Bukowski, K., Jakubczak, A., Kaczorek, I. (2002). *Polish Journal of Veterinary Science* 5:47-50. [PRESS FOR ABSTRACT]

12. Viruses causing lysis of the toxic bloom-forming alga, *Heterosigma akashiwo* (Raphidophyceae), are widespread in coastal sediments of British Columbia, Canada. Lawrence, J. E., Chan, A. M., Suttle, C. A. (2002). *Limnology and Oceanography* 47:545-550. [PRESS FOR ABSTRACT]

13. Efficacy of bacteriophage use in complex treatment of the patients with burn wounds. Lazareva, E. B., Smirnov, S. V., Khvatov, V. B., Spiridonova, T. G., Bitkova, E. E., Darbeeva, O. S., Mayskaya, L. M., Parphenyuk, R. L., Menshikov, D. D. (2002). *Antibiotiki i Khimioterapiya* 46:10-14. [PRESS FOR ABSTRACT]

14. Reverse transcriptase-mediated tropism switching in *Bordetella* bacteriophage. Liu, M., Deora, R., Doulatov, S. R., Gingery, M., Eiserling, F. A., Preston, A., Maskell, D. J., Simons, R. W., Cotter, P. A., Parkhill, J., Miller, J. F. (2002). *Science* 295:2091-2094. [PRESS FOR ABSTRACT]

15. Lysogeny in marine *Synechococcus*. McDaniel, L., Houchin, L. A., Williamson, S. J., Paul, J. H. (2002). *Nature (London)* 415:496. [PRESS FOR ABSTRACT]

16. The genome of bacteriophage phiKZ of *Pseudomonas aeruginosa*. Mesyanzhinov, V. V., Robben, J., Grymonprez, B., Kostyuchenko, V. A., Bourkaltseva, M. V., Sykilinda, N. N., Krylov, V. N., Volckaert, G. (2002). *Journal of Molecular Biology* 317:1-19. [PRESS FOR ABSTRACT]

17. Uptake and processing of modified bacteriophage M13 in mice: implications for phage display. Molenaar, T. J. M., Michon, I., de Haas, S. A. M., van Berkel, T. J. C., Kuiper, J., Biessen, E. A. L. (2002). *Virology* 293:182-191. [PRESS FOR ABSTRACT]

18. Bacteriophage Mu genome sequence: analysis and comparison with Mu-like prophages in *Haemophilus*, *Neisseria* and *Deinococcus*. Morgan, G. J., Hatfull, G. F., Casjens, S., Hendrix, R. W. (2002). *Journal of Molecular Biology* 317:337-359. [PRESS FOR ABSTRACT]

19. Lysogeny and lytic viral production during a bloom of the cyanobacterium *Synechococcus* spp. Ortmann, A. C., Lawrence, J. E., Suttle, C. A. (2002). *Microbial Ecology* 43:225-231. [PRESS FOR ABSTRACT]

20. Experimental genomic evolution: extensive compensation for loss of DNA ligase activity in a virus. Rokyta, D., Badgett, M. R., Molineux, I. J., Bull, J. J. (2002). *Molecular Biology and Evolution* 19:230-238. [PRESS FOR ABSTRACT]

21. Bacteriophage SP6 is closely related to phages K1-5, K5, and K1E but encodes a tail protein very similar to that of the distantly related P22. Scholl, D., Adhya, S., Merril, C. R. (2002). *Journal of Bacteriology* 184:2833-2836. [PRESS FOR ABSTRACT]

22. Use of bacteriophage Ba1 to identify properties associated with *Bordetella avium* virulence. Shelton, C. B., Temple, L. M., Orndorff, P. E. (2002). *Infection and Immunity* 70:1219-1224. [PRESS FOR ABSTRACT]

23. Sequence analysis of marine virus communities reveals groups of related algal viruses are widely distributed in nature. Short, S. M., Suttle, C. A. (2002). *Applied and Environmental Microbiology* 68:1290-1296. [PRESS FOR ABSTRACT]

24. Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline waters. Sinton, L. W., Hall, C. H., Lynch, P. A., Davies-Colley, R. J. (2002). *Applied and Environmental Microbiology* 68:1122-1131. [PRESS FOR ABSTRACT]

25. Mobile elements as a combination of functional modules. Toussaint, A., Merlin, C. (2002). *Plasmid* 47:26-35. [PRESS FOR ABSTRACT]

26. Reconsidering transmission electron microscopy based estimates of viral infection of bacterio-plankton using conversion factors derived from natural communities. Weinbauer, M. G., Winter, C., Hofle, M. G. (2002). *Aquatic Microbial Ecology* 27:103-110. [PRESS FOR ABSTRACT]

27. Direct measurements of viral production in stratified and tidally mixed waters in the Strait of Georgia. Wilhelm, S. W., Brigden, S. M., Suttle, C. A. (2002). *Microbial Ecology* 43:168-173. [PRESS FOR ABSTRACT]

28. Effects of *Escherichia coli* physiology on growth of phage T7 in vivo and in silico. You, L., Suthers, P. F., Yin, J. (2002). *Journal of Bacteriology* 184:1888-1894. [PRESS FOR ABSTRACT]

29. Selecting a sensitive bacteriophage assay for evaluation of a prototype water recycling system. Brion, G. M., Silverstein, J. (2001). *Life Support Biosph. Sci.* 8:9-14. [PRESS FOR ABSTRACT]

30. [Current clinical application of bacteriophages and perspectives for their genetic modifications]. Dabrowska, K., Bus, R., Mazur, A., Weber-Dabrowska, B., Mulczyk, M., Gorski, A. (2001). *Polskie Archiwum Medycyny Wewnetrznej* 105:85-90. [no abstract]

31. Comparative genomics reveals close genetic relationships between phages from dairy bacteria and pathogenic streptococci: evolutionary implications for prophage-host interactions. Desiere, F., McShan, W. M., van, Sinderen, Ferretti, J. J., Brussow, H. (2001). *Virology* 288:325-341. [PRESS FOR ABSTRACT]

32. Identification of a genetic determinant responsible for host specificity in *Streptococcus thermophilus* bacteriophages. Duplessis, M., Moineau, S. (2001). *Molecular Microbiology* 41:325-336. [PRESS FOR ABSTRACT]

33. Comparative study of nine *Lactobacillus fermentum* bacteriophages. Foschino, R., Picozzi, C., Galli, A. (2001). *Journal of Applied Microbiology* 91:394-403. [PRESS FOR ABSTRACT]

34. Isolation of a lysogenic bacteriophage carrying the stx(1(OX3)) gene, which is closely associated with Shiga toxin-producing *Escherichia coli* strains from sheep and humans. Koch, C., Hertwig, S., Lurz, R., Appel, B., Beutin, L. (2001). *Journal of Clinical Microbiology* 39:3992-3998. [PRESS FOR ABSTRACT]

35. [Phagotherapy in terms of bacteriophage genetics: hopes, perspectives, safety, limitations]. Krylov, V. N. (2001). *Genetika* 37:869-887. [PRESS FOR ABSTRACT]

36. Where are the pseudogenes in bacterial genomes? Lawrence, J. G., Hendrix, R. W., Casjens, S. (2001). *Trends in Microbiology* 9:535-540. [PRESS FOR ABSTRACT]

37. Presence of bacteriophages in animal feed as indicators of fecal contamination. Maciorowski, K. G., Pillai, S. D., Ricke, S. C. (2001). *Journal of Environmental Science and Health Part B Pesticides* 36:699-708. [PRESS FOR ABSTRACT]

38. Filamentous bacteriophage stability in non-aqueous media. Olofsson, L., Ankarloo, J., Andersson, P. O., Nicholls, I. A. (2001). *Chemistry and Biology* 8:661-671. [PRESS FOR ABSTRACT]

39. [Bacteria-killing viruses, Stalinists and "superbugs"]. Olsen, I., Handal, T., Lokken, P. (2001). *Tidsskrift for den Norske Laegeforening* 121:3197-3200. [PRESS FOR ABSTRACT]

40. Modeling virus inactivation on salad crops using microbial count data. Petterson, S. R., Teunis, P. F., Ashbolt, N. J. (2001). *Risk Analysis* 21:1097-1108. [PRESS FOR ABSTRACT]

41. [Autoplaque formation in a *Pseudomonas fluorescens* strain: phage-like particles and transactivation of the defective phage]. Shaburova, O. V., Kurochkina, L. P., Krylov, V. N. (2001). *Genetika* 37:893-899. [PRESS FOR ABSTRACT]

42. Designing better phages. Skiena, S. S. (2001). *Bioinformatics* 17 Suppl 1:S253-S261. [PRESS FOR ABSTRACT]

43. Inactivation of bacteriophages in water by means of non-ionizing (UV-253.7 nm) and ionizing (gamma) radiation: a comparative approach. Sommer, R., Pribil, W., Appelt, S., Gehringer, P., Eschweiler, H., Leth, H., Cabaj, A., Haider, T. (2001). *Water Research* 35:3109-3116. [PRESS FOR ABSTRACT]

44. Characterization of a Shiga toxin-encoding temperate bacteriophage of *Shigella sonnei*. Strauch, E., Lurz, R., Beutin, L. (2001). *Infection and Immunity* 69:7588-7595. [PRESS FOR ABSTRACT]

45. Community Structure: Viruses. Suttle, C. A. (2001). pp. 364-370 in Hurst, C. J., Knudson, G. R., McInerney, M. J., Stezenbach, L. D., Walter, M. V. (eds.) *Manual of Environmental Microbiology (2nd Edition)*. ASM Press, Washington, DC. [no abstract]

46. Zoonotic *Escherichia coli*. Wasteson, Y. (2001). *Acta Veterinaria Scandinavica Supplement* 95:79-84. [PRESS FOR ABSTRACT]

47. Filamentous phage biology. Occurrence of coliphages in fish and aquaculture farms. Webster, R., Barbas, C. F., III, Burton, D. R., Scott, J. K., Silverman, G. J., Rao, B. M., Surendran, P. K. (2001). *Phage display: A laboratory manual.* 37:146-149. [PRESS FOR ABSTRACT]

48. A fast method for assessing rapid inactivation and adsorption kinetics of bacteriophages using batch agitation experiments and colloidal clay particles. Rossi, P., Aragno, M. (1999). *Canadian Journal of Microbiology* 45:9-17. [no abstract]

49. Different trajectories of parallel evolution during viral adaptation. Wichman, H. A., Badgett, M. R., Scott, L. A., Boulianne, C. M., Bull, J. J. (1999). *Science* 285:422-424. [PRESS FOR ABSTRACT]

50. Bacteriophage tracing techniques. Rossi, P., Käss, W. (1998). pp. 244-270 in Matthes, Käss (eds.) *Tracing Techniques in Geohydrology*. Balkema, Rotterdam. [no abstract]

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New Publications with Abstracts

For your convenience, a list of new publications without associated abstracts (but with links to abstracts) is found above. The list presented below is identical to the above list except that abstracts are included.

1. **Epigenetics as a first exit problem. Aurell, E., Sneppen, K. (2002). *Physical Review Letters* 88:048101.** We develop a framework to discuss the stability of epigenetic states as first exit problems in dynamical systems with noise. We consider in particular the stability of the lysogenic state of the lambda prophage. The formalism defines a quantitative measure of robustness of inherited states

2. **Microviridae, a family divided: isolation, characterization, and genome sequence of phiMH2K, a bacteriophage of the obligate intracellular parasitic bacterium *Bdellovibrio bacteriovorus*. Brentlinger, K. L., Hafenstein, S., Novak, C. R., Fane, B. A., Borgon, R., McKenna, R., Agbandje-McKenna, M. (2002). *Journal of Bacteriology* 184:1089-1094.** A novel single-stranded DNA phage, phiMH2K, of *Bdellovibrio bacteriovorus* was isolated, characterized, and sequenced. This phage is a member of the Microviridae, a family typified by bacteriophage PhiX174. Although *B. bacteriovorus* and *Escherichia coli* are both classified as proteobacteria, phiMH2K is only distantly related to fX174. Instead, phiMH2K exhibits an extremely close relationship to the Microviridae of *Chlamydia* in both genome organization and encoded proteins. Unlike the double-stranded DNA bacteriophages, for which a wide spectrum of diversity has been observed, the single-stranded icosahedral bacteriophages appear to fall into two distinct subfamilies. These observations suggest that the mechanisms driving single-stranded DNA bacteriophage evolution are inherently different from those driving the evolution of the double-stranded bacteriophages

3. **On the stability properties of a stochastic model for phage-bacteria interaction in open marine environment. Carletti, M. (2002). *Mathematical Biosciences* 175:117-131.** In this paper we extend the deterministic model for the epidemics induced by virulent phages on bacteria in marine environment introduced by Beretta and Kuang [Math. Biosci. 149 (1998) 57], allowing random fluctuations around the positive equilibrium. The stochastic stability properties of the model are investigated both analytically and numerically suggesting that the deterministic model is robust with respect to stochastic perturbations

4. **[The antiviral activity of chitosan (review)]. Chirkov, S. N. (2002). *Prikladnaia Biokhimiia I Mikrobiologiia* 38:5-13.** Data on the inhibitory effect of chitosan on viral infections in animals, plants, and microorganisms are reviewed. The effects of the physicochemical parameters and structure of chitosan on its antiviral activity are analyzed. Possible mechanisms of the inhibitory effect of chitosan on viral infections are discussed

5. **Filamentous phage active on the gram-positive bacterium *Propionibacterium freudenreichii*. Chopin, M. C., Rouault, A., Ehrlich, S. D., Gautier, M. (2002). *Journal of Bacteriology* 184:2030-2033.** We present the first description of a single-stranded DNA filamentous phage able to replicate in a gram-positive bacterium. Phage B5 infects *Propionibacterium freudenreichii* and has a genome consisting of 5,806 bases coding for 10 putative open reading frames. The organization of the genome is very similar to the organization of the genomes of filamentous phages active on gram-negative bacteria. The putative coat protein exhibits homology with the coat proteins of phages PH75 and Pf3 active on *Thermus thermophilus* and *Pseudomonas aeruginosa*, respectively. B5 is, therefore, evolutionarily related to the filamentous phages active on gram-negative bacteria

6. **Snapshot of the genome of the pseudo-T-even bacteriophage RB49. Desplats, C., Dez, C., Tetart, F., Eleaume, H., Krisch, H. M. (2002). *Journal of Bacteriology* 184:2789-2804.** RB49 is a virulent bacteriophage that infects *Escherichia coli*. Its virion morphology is indistinguishable from the well-known T-even phage T4, but DNA hybridization indicated that it was phylogenetically distant from T4 and thus it was classified as a pseudo-T-even phage. To further characterize RB49, we randomly sequenced small fragments corresponding to about 20% of the approximately 170-kb genome. Most of these nucleotide sequences lacked sufficient homology to T4 to be detected in an NCBI BlastN analysis. However, when translated, about 70% of them encoded proteins with homology to T4 proteins. Among these sequences were the numerous components of the virion and the phage DNA replication apparatus. Mapping the RB49 genes revealed that many of them had the same relative order found in the T4 genome. The complete nucleotide sequence was determined for the two regions of RB49 genome that contain most of the genes involved in DNA replication. This sequencing revealed that RB49 has homologues of all the essential T4 replication genes, but, as expected, their sequences diverged considerably from their T4 homologues. Many of the nonessential T4 genes are absent from RB49 and have been replaced by unknown sequences. The intergenic sequences of RB49 are less conserved than the coding sequences, and in at least some cases, RB49 has evolved alternative regulatory strategies. For example, an analysis of transcription in RB49 revealed a simpler pattern of regulation than in T4, with only two, rather than three, classes of temporally controlled promoters. These results indicate that RB49 and T4 have diverged substantially from their last common ancestor. The different T4-type phages appear to contain a set of common genes that can be exploited differently, by means of plasticity in the regulatory sequences and the precise choice of a large group of facultative genes

7. **Biological properties and cell tropism of Chp2, a bacteriophage of the obligate intracellular bacterium *Chlamydophila abortus*. Everson, J. S., Garner, S. A., Fane, B., Liu, B. L., Lambden, P. R., Clarke, I. N. (2002). *Journal of Bacteriology* 184:2748-2754.** A number of bacteriophages belonging to the Microviridae have been described infecting chlamydiae. Phylogenetic studies divide the Chlamydiaceae into two distinct genera, *Chlamydia* and *Chlamydophila*, containing three and six different species, respectively. In this work we investigated the biological properties and host range of the recently described bacteriophage Chp2 that was originally discovered in *Chlamydophila abortus*. The obligate intracellular development cycle of chlamydiae has precluded the development of quantitative approaches to assay bacteriophage infectivity. Thus, we prepared hybridomas secreting monoclonal antibodies (monoclonal antibodies 40 and 55) that were specific for Chp2. We demonstrated that Chp2 binds both *C. abortus* elementary bodies and reticulate bodies in an enzyme-linked immunosorbent assay. Monoclonal antibodies 40 and 55 also detected bacteriophage Chp2 antigens in chlamydia-infected eukaryotic cells. We used these

monoclonal antibodies to monitor the ability of Chp2 to infect all nine species of chlamydiae. Chp2 does not infect members of the genus *Chlamydia* (*C. trachomatis*, *C. suis*, or *C. muridarum*). Chp2 can infect *C. abortus, C. felis*, and *C. pecorum* but is unable to infect other members of this genus, including *C. caviae* and *C. pneumoniae*, despite the fact that these chlamydial species support the replication of very closely related bacteriophages

8. **RS1 element of *Vibrio cholerae* can propagate horizontally as a filamentous phage exploiting the morphogenesis genes of CTX-Phi. Faruque, S. M., Kamruzzaman, M., Nandi, R. K., Ghosh, A. N., Nair, G. B., Mekalanos, J. J., Sack, D. A. (2002). *Infection and Immunity* 70:163-170.** In toxigenic *Vibrio cholerae*, cholera toxin is encoded by the CTX prophage, which consists of a core region carrying ctxAB genes and genes required for CTX-Phi morphogenesis, and an RS2 region encoding regulation, replication, and integration functions. Integrated CTX-Phi is often flanked by another genetic element known as RS1 which carries all open reading frames (ORFs) found in RS2 and an additional ORF designated rstC. We identified a single-stranded circularized form of the RS1 element, in addition to the CTX-Phi genome, in nucleic acids extracted from phage preparations of 32 out of 83 (38.5%) RS1-positive toxigenic *V. cholerae* strains analyzed. Subsequently, the corresponding double-stranded replicative form (RF) of the RS1 element was isolated from a representative strain and marked with a kanamycin resistance (Km(r)) marker in an intergenic site to construct pRS1-Km. Restriction and PCR analysis of pRS1-Km and sequencing of a 300-bp region confirmed that this RF DNA was the excised RS1 element which formed a novel junction between ig1 and rstC. Introduction of pRS1-Km into a V. cholerae O1 classical biotype strain, O395, led to the production of extracellular Km(r) transducing particles, which carried a single-stranded form of pRS1-Km, thus resembling the genome of a filamentous phage (RS1-KmF). Analysis of *V. cholerae* strains for susceptibility to RS1-KmF showed that classical biotype strains were more susceptible to the phage compared to El Tor and O139 strains. Nontoxigenic (CTX(-)) O1 and O139 strains which carried genes encoding the CTX-Phi receptor toxin-coregulated pilus (TCP) were also more susceptible (>1,000-fold) to the phage compared to toxigenic El Tor or O139 strains. Like CTX-Phi, the RS1F genome also integrated into the host chromosomes by using the attRS sequence. However, only transductants of RS1-KmF which also harbored the CTX-Phi genome produced a detectable level of extracellular RS1-KmF. This suggested that the core genes of CTX-Phi are also required for the morphogenesis of RS1F. The results of this study showed for the first time that RS1 element, which encodes a site-specific recombination system in V. cholerae, can propagate horizontally as a filamentous phage, exploiting the morphogenesis genes of CTX-Phi

9. **Microbiology. A tail of two specifi-cities. Hatfull, G. F. (2002). *Science* 295:2031-2032.**

10. **Engineering a reduced *Escherichia coli* genome. Kolisnychenko, V., Plunkett, G., Herring, C. D., Feher, T., Posfai, J., Blattner, F. R., Posfai, G. (2002). *Genome Research* 12:640-647.** Our goal is to construct an improved *Escherichia coli* to serve both as a better model organism and as a more useful technological tool for genome science. We developed techniques for precise genomic surgery and applied them to deleting the largest K-islands of *E. coli*, identified by comparative genomics as recent horizontal acquisitions to the genome. They are loaded with cryptic prophages, transposons, damaged genes, and genes of unknown function. Our method leaves no scars or markers behind and can be applied sequentially. Twelve K-islands were successfully deleted, resulting in an 8.1% reduced genome size, a 9.3% reduction of gene count, and elimination of 24 of the 44 transposable elements of *E. coli*. These are particularly detrimental because they can mutagenize the genome or transpose into clones being propagated for sequencing, as happened in 18 places of the draft human genome sequence. We found no change in the growth rate on minimal medium, confirming the nonessential nature of these islands. This demonstration of feasibility opens the way for constructing a maximally reduced strain, which will provide a clean background for functional genomics studies, a more efficient background for use in biotechnology applications, and a unique tool for studies of genome stability and evolution

11. **The activity of chosen bacteriophages on *Yersinia enterocolitica* strains. Kot, B., Bukowski, K., Jakubczak, A., Kaczorek, I. (2002). *Polish Journal of Veterinary Science* 5:47-50.** The aim of the present study was to evaluate the lytic activity of three bacteriophages on *Yersinia enterocolitica* strains isolated from humans and pigs. The *Y. enterocolitica* strains tested belonged to 0:3, 0:9 and 0:2 serogroups. The ZD5 phage was obtained from a water sample, but remaining phages were obtained from the lysogenic *Y. frederiksenii* 7291 and *Y. enterocolitica* 8684 strains. All the *Y. enterocolitica* strains tested which belonged to 0:9 serogroup did not show any susceptibility to the bacteriophages used. The bacteriophages tested showed different lytic activity on the *Y. enterocolitica* 0:3 strains investigated. The phage susceptibility of *Y. enterocolitica* 0:3 strains revealed 9 different phage patterns. ZD5 phage showed the highest lytic activity, because it produced confluent lysis of the most *Y. enterocolitica* 0:3 strains tested. The *Y. enterocolitica* 0:2 strains isolated from pigs showed the similar phage susceptibility. The *Y. kristensenii* and *Y. pseudotuberculosis* strains tested were not sensitive to the bacteriophages used

12. **Viruses causing lysis of the toxic bloom-forming alga, *Heterosigma akashiwo* (Raphidophyceae), are widespread in coastal sediments of British Columbia, Canada. Lawrence, J. E., Chan, A. M., Suttle, C. A. (2002). *Limnology and Oceanography* 47:545-550.** Viruses that infect and cause lysis of the toxic alga *Heterosigma akashiwo* are abundant and widespread in the Strait of Georgia, Canada, and adjacent inlets during the summer months when blooms of this alga occur. Because viruses are subjected to many mechanisms of removal and their host is intermittently dormant, the persistence of viruses may be dependent on environmental reservoirs. We extracted pore water from sediments collected in the Strait of Georgia and screened for the presence of infectious agents that cause lysis of *H. akashiwo*. Lytic agents were widespread throughout the study region, being detected in 17 of 20 sites surveyed. Lytic agents were present in sediments ranging from highly organic to clay-rich and were retrieved from cores taken at water depths of 25-285 m. The highest concentration of lytic agents was found at the sediment-water interface; however, lytic agents were found as deep as 40 cm below the sediment-water interface. Examination of agents isolated from various sites revealed virus-like particles similar to50 nm in diameter. These are similar to other virus-like particles that have been isolated that infect this alga. This suggests that the most abundant lytic agents in the sediments are viruses and that these viruses may be long-lived once buried in the sediments. The widespread presence of viral-size lytic agents that infect H. akashiwo is consistent with viral infection being a mortality agent of this alga in the overlying waters and suggests that they may play in important role in regulating their population dynamics.

13. **Efficacy of bacteriophage use in complex treatment of the patients with burn wounds. Lazareva, E. B., Smirnov, S. V., Khvatov, V. B., Spiridonova, T. G., Bitkova, E. E., Darbeeva, O. S., Mayskaya, L. M., Parphenyuk, R. L., Menshikov, D. D. (2002). *Antibiotiki i Khimioterapiya* 46:10-14.** Results of clinical and laboratory evaluation of the treatment with pyobacteriophage in tablets of the patients with burn wounds are presented. It was shown that phagotherapy provided more rapid cure of pyoseptic complications, temperature normalization, wounds purification and lower lethality Bacteriological analysis of wound secretions revealed that after the treatment staphylococci and streptococci were cultured 2 times rarely, *Proteus* spp. Were isolated 1.5 times rarely, *E.coli* was not isolated. The amount of positive haemocultures also diminished. Investigation of

immunologic status demonstrated statistically significant normalization of immunity on cell level. Phagocytosis level didn't change while in control group (without bacteriophage use) it became lower. Antibody level enhanced but less extensively than in control group. The results of trial demonstrates positive effect of phagotherapy use at the patients with burns

14. **Reverse transcriptase-mediated tropism switching in *Bordetella* bacteriophage. Liu, M., Deora, R., Doulatov, S. R., Gingery, M., Eiserling, F. A., Preston, A., Maskell, D. J., Simons, R. W., Cotter, P. A., Parkhill, J., Miller, J. F. (2002). *Science* 295:2091-2094.** Host-pathogen interactions are often driven by mechanisms that promote genetic variability. We have identified a group of temperate bacteriophages that generate diversity in a gene, designated mtd (major tropism determinant), which specifies tropism for receptor molecules on host *Bordetella* species. Tropism switching is the result of a template-dependent, reverse transcriptase-mediated process that introduces nucleotide substitutions at defined locations within mtd. This cassette-based mechanism is capable of providing a vast repertoire of potential ligand-receptor interactions

15. **Lysogeny in marine *Synechococcus*. McDaniel, L., Houchin, L. A., Williamson, S. J., Paul, J. H. (2002). *Nature (London)* 415:496.** Viral infection of bacteria can be lytic, causing destruction of the host cell, or lysogenic, in which the viral genome is instead stably maintained as a prophage within its host. Here we show that lysogeny occurs in natural populations of an autotrophic picoplankton (*Synechococcus*) and that there is a seasonal pattern to this interaction. Because lysogeny confers immunity to infection by related viruses, this process may account for the resistance to viral infection seen in common forms of autotrophic picoplankton

16. **The genome of bacteriophage phiKZ of *Pseudomonas aeruginosa*. Mesyanzhinov, V. V., Robben, J., Grymonprez, B., Kostyuchenko, V. A., Bourkaltseva, M. V., Sykilinda, N. N., Krylov, V. N., Volckaert, G. (2002). *Journal of Molecular Biology* 317:1-19.** Bacteriophage phiKZ is a giant virus that efficiently infects *Pseudomonas aeruginosa* strains pathogenic to human and, therefore, it is attractive for phage therapy. We present here the complete phiKZ genome sequence and a preliminary analysis of its genome structure. The 280,334 bp genome is a linear, circularly permutated and terminally redundant, A+T-rich double-stranded DNA molecule. The phiKZ DNA has no detectable sequence homology to other viruses and microorganisms, and it does not contain NotI, PstI, SacI, SmaI, XhoI, and XmaIII endonuclease restriction sites. The genome has 306 open reading frames (ORFs) varying in size from 50 to 2237 amino acid residues. According to the orientation of transcription, ORFs are apparently organized into clusters and most have a clockwise direction. The phiKZ genome also encodes six tRNAs specific for Met (AUG), Asn (AAC), Asp (GAC), Leu (TTA), Thr (ACA), and Pro (CCA). A putative promoter sequence containing a TATATTAC block was identified. Most potential stem-loop transcription terminators contain the tetranucleotide UUCG loops. Some genes may be assigned as phage-encoded RNA polymerase subunits. Only 59 phiKZ gene products exhibit similarity to proteins of known function from a diversity of organisms. Most of these conserved gene products, such as dihydrofolate reductase, ribonucleoside diphosphate reductase, thymidylate synthase, thymidylate kinase, and deoxycytidine triphosphate deaminase are involved in nucleotide metabolism. However, no virus-encoded DNA polymerase, DNA replication-associated proteins, or single-stranded DNA-binding protein were found based on amino acid homology, and they may therefore be strongly divergent from known homologous proteins. Fifteen phiKZ gene products show homology to proteins of pathogenic organisms, including *Mycobacterium tuberculosis*, *Haemophilus influenzae*, *Listeria* sp., *Rickettsia prowazakeri*, and *Vibrio cholerae* that must be considered before using this phage as a therapeutic agent. The phiKZ coat contains at least 40 polypeptides, and several proteins are cleaved during virus assembly in a way similar to phage T4. Eleven phiKZ-encoded polypeptides are related to proteins of other bacteriphages that infect a variety of hosts. Among these are four gene products that contain a putative intron-encoded endonuclease harboring the H-N-H motif common to many double-stranded DNA phages. These observations provide evidence that phages infecting diverse hosts have had access to a common genetic pool. However, limited homology on the DNA and protein levels indicates that bacteriophage phiKZ represents an evolutionary distinctive branch of the Myoviridae family

17. **Uptake and processing of modified bacteriophage M13 in mice: implications for phage display. Molenaar, T. J. M., Michon, I., de Haas, S. A. M., van Berkel, T. J. C., Kuiper, J., Biessen, E. A. L. (2002). *Virology* 293:182-191.** Internalization and degradation of filamentous bacteriophage M13 by a specific target cell may have major consequences for the recovery of phage in in vivo biopanning of phage libraries. Therefore, we investigated the pharmacokinetics and processing of native and receptor-targeted phage in mice. ^{35}S-radiolabeled M13 was chemically modified by conjugation of either galactose (lacM13) or succinic acid groups (sucM13) to the coat protein of the phage to stimulate uptake by galactose recognizing hepatic receptors and scavenger receptors, respectively. Receptor-mediated endocytosis of modified phage reduced the plasma half-life of native M13 (t(1/2) = 4.5 h) to 18 min for lactosylated and 1.5 min for succinylated bacterophage. Internalization of sucM13 was complete within 30 min after injection and resulted in up to 5000-fold reduction of bioactive phage within 90 min. In conclusion, these data provide information on the in vivo behavior of wild-type and receptor-targeted M13, which has important implications for future in vivo phage display experiments and for the potential use of M13 as a viral gene delivery vehicle

18. **Bacteriophage Mu genome sequence: analysis and comparison with Mu-like prophages in *Haemophilus*, *Neisseria* and *Deinococcus*. Morgan, G. J., Hatfull, G. F., Casjens, S., Hendrix, R. W. (2002). *Journal of Molecular Biology* 317:337-359.** We report the complete 36,717 bp genome sequence of bacteriophage Mu and provide an analysis of the sequence, both with regard to the new genes and other genetic features revealed by the sequence itself and by a comparison to eight complete or nearly complete Mu-like prophage genomes found in the genomes of a diverse group of bacteria. The comparative studies confirm that members of the Mu-related family of phage genomes are genetically mosaic with respect to each other, as seen in other groups of phages such as the phage lambda-related group of phages of enteric hosts and the phage L5-related group of mycobacteriophages. Mu also possesses segments of similarity, typically gene-sized, to genomes of otherwise non-Mu-like phages. The comparisons show that some well-known features of the Mu genome, including the invertible segment encoding tail fiber sequences, are not present in most members of the Mu genome sequence family examined here, suggesting that their presence may be relatively volatile over evolutionary time.The head and tail-encoding structural genes of Mu have only very weak similarity to the corresponding genes of other well-studied phage types. However, these weak similarities, and in some cases biochemical data, can be used to establish tentative functional assignments for 12 of the head and tail genes. These assignments are strongly supported by the fact that the order of gene functions assigned in this way conforms to the strongly conserved order of head and tail genes established in a wide variety of other phages. We show that the Mu head assembly scaffolding protein is encoded by a gene nested in-frame within the C-terminal half of another gene that encodes the putative head maturation protease. This is reminiscent of the arrangement established for phage lambda

19. **Lysogeny and lytic viral production during a bloom of the cyanobacterium *Synechococcus* spp. Ortmann, A. C., Lawrence, J. E., Suttle, C. A. (2002). *Microbial Ecology* 43:225-231.** Lytic viral production and lysogeny were investigated in cyanobacteria and heterotrophic bacteria during a bloom of *Synechococcus* spp. in a pristine fjord in British Columbia, Canada.

Triplicate seawater samples were incubated with and without mitomycin C and the abundances of heterotrophic bacteria, cyanobacteria, total viruses and infectious cyanophage were followed over 24 h. Addition of mitomycin C led to increases in total viral abundance as well as the abundance of cyanophages infecting *Synechococcus* strain DC2. Given typical estimates of burst size, these increases were consistent with 80% of the heterotrophic bacteria and 0.6% of *Synechococcus* cells being inducible by the addition of mitomycin C. This is the highest percentage of lysogens reported for a natural microbial community and demonstrates induction in a marine *Synechococcus* population. It is likely that the cyanophage production following the addition of mitomycin C was much higher than that titered against a single strain of *Synechococcus*; hence this estimate is a minimum. In untreated seawater samples, lytic viral production was estimated to remove ca. 27% of the gross heterotrophic bacterial production, and a minimum of 1.0% of the gross cyanobacterial production. Our results demonstrate very high levels of lysogeny in the heterotrophic bacterial community, outside of an oligotrophic environment, and the presence of inducible lysogens in *Synechococcus* spp. during a naturally occurring bloom. These data emphasize the need for further examination of the factors influencing lytic and lysogenic viral infection in natural microbial communities.

20. **Experimental genomic evolution: extensive compensation for loss of DNA ligase activity in a virus. Rokyta, D., Badgett, M. R., Molineux, I. J., Bull, J. J. (2002). *Molecular Biology and Evolution* 19:230-238.** Deletion of the viral ligase gene drastically reduced the fitness of bacteriophage T7 on a ligase-deficient host. Viral evolution recovered much of this fitness during long-term passage, but the final fitness remained below that of the intact virus. Compensatory changes occurred chiefly in genes involved in DNA metabolism: the viral endonuclease, helicase, and DNA polymerase. Two other compensatory changes of unknown function also occurred. Using a method to distinguish compensatory mutations from other beneficial mutations, five additional substitutions from the recovery were shown to enhance adaptation to culture conditions and were not compensatory for the deletion. In contrast to the few previous studies of viral recovery from deletions, the compensatory changes in T7 did not restore the deletion or duplicate major regions of the genome. The ability of this deleted genome to recover much of the lost fitness via mutations in its remaining genes reveals a considerable evolutionary potential to modify the interactions of its elements in maintaining an essential set of functions

21. **Bacteriophage SP6 is closely related to phages K1-5, K5, and K1E but encodes a tail protein very similar to that of the distantly related P22. Scholl, D., Adhya, S., Merril, C. R. (2002). *Journal of Bacteriology* 184:2833-2836.** The lytic salmonella phage SP6 encodes a tail protein with a high degree of sequence similarity to the tail protein of the biologically unrelated lysogenic salmonella phage P22. The SP6 tail gene is flanked by an upstream region that contains a promoter and a downstream region that contains a putative Rho-independent transcription terminator, giving it a cassette or modular structure almost identical to the structure of the tail genes of coliphages K1E, K5, and K1-5. It now appears that SP6, K1-5, K5, and K1E are very closely related but have different tail fiber proteins, giving them different host specificities

22. **Use of bacteriophage Ba1 to identify properties associated with *Bordetella avium* virulence. Shelton, C. B., Temple, L. M., Orndorff, P. E. (2002). *Infection and Immunity* 70:1219-1224.** *Bordetella avium* causes bordetellosis, an upper respiratory disease of birds. Commercially raised turkeys are particularly susceptible. We report here on the use of a recently described *B. avium* bacteriophage, Ba1, as a tool for investigating the effects of lysogeny and phage resistance on virulence. We found that lysogeny had no effect on any of the in vivo or in vitro measurements of virulence we employed. However, two-thirds (six of nine) spontaneous phage-resistant mutants of our virulent laboratory strain, 197N, were attenuated. Phage resistance was associated, in all cases, with an inability of the mutants to bind phage. Further tests of the mutants revealed that all had increased sensitivities to surfactants, and increased amounts of incomplete (O-antigen-deficient) lipopolysaccharide (LPS) compared to 197N. Hot phenol-water-extracted 197N LPS inactivated phage in a specific and dose-dependent manner. Acid hydrolysis and removal of lipid A had little effect upon the ability of isolated LPS to inactivate Ba1, suggesting that the core region and possibly the O antigen were required for phage binding. All of the mutants, with one exception, were significantly more sensitive to naive turkey serum and, without exception, significantly less able to bind to tracheal rings in vitro than 197N. Interestingly, the three phage-resistant mutants that remained virulent appeared to be O antigen deficient and were among the mutants that were the most serum sensitive and least able to bind turkey tracheal rings in vitro. This observation allowed us to conclude that even severe defects in tracheal ring binding and serum resistance manifested in vitro were not necessarily indicative of attenuation and that complete LPS may not be required for virulence

23. **Sequence analysis of marine virus communities reveals groups of related algal viruses are widely distributed in nature. Short, S. M., Suttle, C. A. (2002). *Applied and Environmental Microbiology* 68:1290-1296.** Algal-virus-specific PCR primers were used to amplify DNA polymerase (*pol*) gene fragments from geographically isolated natural virus communities. Natural algal virus communities were obtained from coastal sites in the Pacific Ocean in British Columbia, Canada, and the Southern Ocean near the Antarctic peninsula. Genetic fingerprints of algal virus communities were generated using denaturing gradient gel electrophoresis (DGGE). Sequencing efforts recovered 33 sequences from the gradient gel. Of the 33 sequences examined, 25 encoded a conserved amino acid motif indicating that the sequences were *pol* gene fragments. Furthermore, the 25 *pol* sequences were related to *pol* gene fragments from known algal viruses. In addition, similar virus sequences (>98% sequence identity) were recovered from British Columbia and Antarctica. Results from this study demonstrate that DGGE with degenerate primers can be used to qualitatively fingerprint and assess genetic diversity in specific subsets of natural virus communities and that closely related viruses occur in distant geographic locations. DGGE is a powerful tool for genetically fingerprinting natural virus communities and may be used to examine how specific components of virus communities respond to experimental manipulations.

24. **Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline waters. Sinton, L. W., Hall, C. H., Lynch, P. A., Davies-Colley, R. J. (2002). *Applied and Environmental Microbiology* 68:1122-1131.** Sunlight inactivation in fresh (river) water of fecal coliforms, enterococci, *Escherichia coli*, somatic coliphages, and F-RNA phages from waste stabilization pond (WSP) effluent was compared. Ten experiments were conducted outdoors in 300-liter chambers, held at 14C (mean river water temperature). Sunlight inactivation (k(S)) rates, as a function of cumulative global solar radiation (insolation), were all more than 10 times higher than the corresponding dark inactivation (k(D)) rates in enclosed (control) chambers. The overall k(S) ranking (from greatest to least inactivation) was as follows: enterococci > fecal coliforms greater-than-or-equal *E. coli* > somatic coliphages > F-RNA phages. In winter, fecal coliform and enterococci inactivation rates were similar but, in summer, enterococci were inactivated far more rapidly. In four experiments that included freshwater-raw sewage mixtures, enterococci survived longer than fecal coliforms (a pattern opposite to that observed with the WSP effluent), but there was little difference in phage inactivation between effluents. In two experiments which included simulated estuarine water and seawater, sunlight inactivation of all of the indicators increased with increasing salinity. Inactivation rates in

freshwater, as seen under different optical filters, decreased with the increase in the spectral cutoff (50% light transmission) wavelength. The enterococci and F-RNA phages were inactivated by a wide range of wavelengths, suggesting photooxidative damage. Inactivation of fecal coliforms and somatic coliphages was mainly by shorter (UV-B) wavelengths, a result consistent with photobiological damage. Fecal coliform repair mechanisms appear to be activated in WSPs, and the surviving cells exhibit greater sunlight resistance in natural waters than those from raw sewage. In contrast, enterococci appear to suffer photooxidative damage in WSPs, rendering them susceptible to further photooxidative damage after discharge. This suggests that they are unsuitable as indicators of WSP effluent discharges to natural waters. Although somatic coliphages are more sunlight resistant than the other indicators in seawater, F-RNA phages are the most resistant in freshwater, where they may thus better represent enteric virus survival

25. **Mobile elements as a combination of functional modules. Toussaint, A., Merlin, C. (2002). *Plasmid* 47:26-35.** Prokaryotic mobile elements have traditionally been classified as bacteriophages, plasmids, and transposons. We propose here a global classification of these and other bacterial and archaeal mobile elements based on their modular structure. This would allow for setting up interconnected databases where mobile elements could be stored as combinations of functional modules. Such a database would be very helpful. It would, for instance, allow for analyzing the phylogeny of individual blocks within an element, to understand how modules get associated and properly express the functions they carry in various bacterial hosts. Modules of practical importance, as for instance those that encode toxins or other virulence factors, could be identified and compared, and probes devised to test bacterial populations for the presence of such modules

26. **Reconsidering transmission electron microscopy based estimates of viral infection of bacterio-plankton using conversion factors derived from natural communities. Weinbauer, M. G., Winter, C., Hofle, M. G. (2002). *Aquatic Microbial Ecology* 27:103-110.** The frequency of virus infected bacterial cells (FIC) was estimated in surface waters of the Mediterranean Sea, the Baltic Sea and the North Sea using the frequency of visibly infected cells (FVIC) as determined by transmission electron microscopy (TEM) and published average conversion factors (average 5.42, range 3.7 to 7.14) to relate FVIC to FIC. A virus dilution approach was used to obtain an independent estimation of FIC in bacterioplankton, and we provide evidence for the reliability of this approach. Across all investigated environments, FIC ranged from 2.4 to 43.4 %. FIC data using both approaches were well correlated; however, the values were higher using the virus dilution approach, This indicates that the TEM approach has the potential to reveal spatiotemporal trends of viral infection; however, it may underestimate the significance of viral infection of bacteria when average conversion factors are used. Using data from the virus dilution approach and the TEM approach, we calculated new conversion factors for relating FVIC to FIC (average 7.11, range 4.34 to 10.78). Virally caused mortality of bacteria estimated from published FVIC data of marine and freshwater systems and using the new conversion factors ranged from not detectable to 129 %, thus confirming that viral infection is a significant and spatiotemporally variable cause of bacterial cell death.

27. **Direct measurements of viral production in stratified and tidally mixed waters in the Strait of Georgia. Wilhelm, S. W., Brigden, S. M., Suttle, C. A. (2002). *Microbial Ecology* 43:168-173.** The abundance of heterotrophic bacteria and viruses, as well as rates of viral production and virus-mediated mortality, were measured in Discovery Passage and the Strait of Georgia (British Columbia, Canada) along a gradient of tidal mixing ranging from well mixed to stratified. The abundances of bacteria and viruses were approximately 10^6 and 10^7 mL^{-1}, respectively, independent of mixing regime. Viral production estimates, monitored by a dilution technique, demonstrated that new viruses were produced at rates of 10^6 to 10^7 mL^{-1} h^{-1} across the different mixing regimes. Using an estimated burst size of 50 viruses per lytic event, ca. 19 to 27% of the standing stock of bacteria at the stratified stations and 46 to 137% at the deep-mixed stations were removed by viruses. The results suggest that mixing of stratified waters during tidal exchange enhances virus-mediated bacterial lysis. Consequently, viral lysis recycled a greater proportion of the organic carbon required for bacterial growth under non-steady-state compared to steady-state conditions.

28. **Effects of *Escherichia coli* physiology on growth of phage T7 in vivo and in silico. You, L., Suthers, P. F., Yin, J. (2002). *Journal of Bacteriology* 184:1888-1894.** Phage development depends not only upon phage functions but also on the physiological state of the host, characterized by levels and activities of host cellular functions. We established Escherichia coli at different physiological states by continuous culture under different dilution rates and then measured its production of phage T7 during a single cycle of infection. We found that the intracellular eclipse time decreased and the rise rate increased as the growth rate of the host increased. To develop mechanistic insight, we extended a computer simulation for the growth of phage T7 to account for the physiology of its host. Literature data were used to establish mathematical correlations between host resources and the host growth rate; host resources included the amount of genomic DNA, pool sizes and elongation rates of RNA polymerases and ribosomes, pool sizes of amino acids and nucleoside triphosphates, and the cell volume. The in silico (simulated) dependence of the phage intracellular rise rate on the host growth rate gave quantitatively good agreement with our in vivo results, increasing fivefold for a 2.4-fold increase in host doublings per hour, and the simulated dependence of eclipse time on growth rate agreed qualitatively, deviating by a fixed delay. When the simulation was used to numerically uncouple host resources from the host growth rate, phage growth was found to be most sensitive to the host translation machinery, specifically, the level and elongation rate of the ribosomes. Finally, the simulation was used to follow how bottlenecks to phage growth shift in response to variations in host or phage functions

29. **Selecting a sensitive bacteriophage assay for evaluation of a prototype water recycling system. Brion, G. M., Silverstein, J. (2001). *Life Support Biosph. Sci.* 8:9-14.** A rapid, simple, and direct (RSD) assay of eluate from filter concentration was developed to enumerate low numbers of MS2 bacteriophage, used as a surrogate for enteric viruses, from samples collected from a prototype-sized water recycling system. The RSD assay utilized a 50-ml eluate volume in a modified single-layer assay, neutralizing eluate pH by buffered, double-strength agar. The RSD assay developed was simpler and minimized sample-handling steps compared with another published method. The RSD assay method showed greater sensitivity than the other published method for recovering phage from filter eluate while avoiding pH shifts, which can inactivate phage. Grant numbers: NAGW 2356

30. **[Current clinical application of bacteriophages and perspectives for their genetic modifications]. Dabrowska, K., Bus, R., Mazur, A., Weber-Dabrowska, B., Mulczyk, M., Gorski, A. (2001). *Polskie Archiwum Medycyny Wewnetrznej* 105:85-90.**

31. **Comparative genomics reveals close genetic relationships between phages from dairy bacteria and pathogenic streptococci: evolutionary implications for prophage-host interactions. Desiere, F., McShan, W. M., van, Sinderen, Ferretti, J. J., Brussow, H. (2001). *Virology* 288:325-341.** The genome of the highly pathogenic M1 serotype *Streptococcus pyogenes* isolate SF370 contains eight prophage elements. Only prophage SF370.1 could be induced by mitomycin C

treatment. Prophage SF370.3 showed a 33.5-kb-long genome that closely resembled the genome organization of the cos-site temperate Siphovirus r1t infecting the dairy bacterium Lactococcus lactis. The two-phage genomes shared between 60 and 70% nucleotide sequence identity over the DNA packaging, head and tail genes. Analysis of the SF370.3 genome revealed mutations in the replisome organizer gene that may prevent the induction of the prophage. The mutated phage replication gene was closely related to a virulence marker identified in recently emerged M3 serotype *S. pyogenes* strains in Japan. This observation suggests that prophage genes confer selective advantage to the lysogenic host. SF370.3 encodes a hyaluronidase and a DNase that may facilitate the spreading of *S. pyogenes* through tissue planes of its human host. Prophage SF370.2 showed a 43-kb-long genome that closely resembled the genome organization of pac-site temperate Siphoviridae infecting the dairy bacteria *S. thermophilus* and *L. lactis*. Over part of the structural genes, the similarity between SF370.2 and *S. thermophilus* phage O1205 extended to the nucleotide sequence level. SF370.2 showed two probable inactivating mutations: one in the replisome organizer gene and another in the gene encoding the portal protein. Prophage SF370.2 also encodes a hyaluronidase and in addition two very likely virulence factors: prophage-encoded toxins acting as superantigens that may contribute to the immune deregulation observed during invasive streptococcal infections. The superantigens are encoded between the phage lysin and the right attachment site of the prophage genome. The genes were nearly sequence identical with a DNA segment in *S. equi*, suggesting horizontal gene transfer. The trend for prophage genome inactivation was even more evident for the remaining five prophage sequences that showed massive losses of prophage DNA. In these prophage remnants only 13-0.3 kb of putative prophage DNA was detected. We discuss the genomics data from S. pyogenes strain SF370 within the framework of Darwinian coevolution of prophages and lysogenic bacteria and suggest elements of genetic cooperation and elements of an arms race in this host-parasite relationship

32. **Identification of a genetic determinant responsible for host specificity in *Streptococcus thermophilus* bacteriophages. Duplessis, M., Moineau, S. (2001). *Molecular Microbiology* 41:325-336.** Phage-host interactions remain poorly understood in lactic acid bacteria and essentially in all Gram-positive bacteria. The aim of this study was to identify the phage genetic determinant (anti-receptor) involved in the recognition of *Streptococcus thermophilus* hosts. The complete genomic sequence of the lytic *S. thermophilus* phage DT1 was determined previously, and bioinformatic analysis indicated that orf18 might be the anti-receptor gene. The orf18 of six additional *S. thermophilus* phages was determined (DT2, DT4, MD1, MD2, MD4 and Q5) and compared with the orf18 of DT1. The deduced ORF18 was divided into three domains. The first domain, which contains the N-terminal part of the protein, was conserved in all seven phages. The second domain was detected in only two phages and flanked by a motif called collagen-like repeats. The second domain also contained a variable region (VR1). All seven phages had a third domain that consisted of the C-terminal section of the protein as well as another variable region (VR2). Chimeric DT1 phages were constructed by recombination; a portion of its orf18 was replaced by the corresponding section in orf18 of the phage MD4. All DT1 chimeric phages acquired the host range of phage MD4. Analysis of the orf18 in the chimeric phages revealed that host specificity in phages DT1 and MD4 resulted from VR2. This is the first report on the identification and characterization of a phage gene involved in the host recognition process of Gram-positive bacteria

33. **Comparative study of nine *Lactobacillus fermentum* bacteriophages. Foschino, R., Picozzi, C., Galli, A. (2001). *Journal of Applied Microbiology* 91:394-403.** AIMS: To investigate the basic properties of six temperate and three virulent phages, active on *Lactobacillus fermentum*, on the basis of morphology, host ranges, protein composition and genome characterization. METHODS AND RESULTS: All phages belonged to the Siphoviridae family; two of them showed prolate heads. The host ranges of seven phages contained a common group of strains. SDS-PAGE protein profiles, restriction analysis of DNA and Southern blot hybridization revealed a high degree of homology between four temperate phages; partial homologies were also detected among virulent and temperate phages. Clustering derived from host range analysis was not related to the results of the DNA hybridizations. CONCLUSION: The phages investigated have common characteristics with other known phages active on the genus Lactobacillus. Sensitivity to viral infection is apparently enhanced by the presence of a resident prophage. SIGNIFICANCE AND IMPACT OF THE STUDY: These relationships contribute to the explanation for the origin of phage infection in food processes where Lact. fermentum is involved, such as sourdough fermentation

34. **Isolation of a lysogenic bacteriophage carrying the stx(1(OX3)) gene, which is closely associated with Shiga toxin-producing *Escherichia coli* strains from sheep and humans. Koch, C., Hertwig, S., Lurz, R., Appel, B., Beutin, L. (2001). *Journal of Clinical Microbiology* 39:3992-3998.** A specific PCR for the detection of a variant of the gene encoding Shiga toxin 1 (stx(1)) called stx(1(OX3)) (GenBank accession no. Z36901) was developed. The PCR was used to investigate 148 Stx(1)-producing *Escherichia coli* strains from human patients (n = 72), cattle (n = 27), sheep (n = 48), and a goat (n = 1) for the presence of the stx(1(OX3)) gene. The stx(1(OX3)) gene was present in 38 Shiga toxin-producing *E. coli* (STEC) strains from sheep belonging to serogroups O5, O125, O128, O146, and OX3 but was absent from Stx(1)-positive ovine STEC O91 strains. The stx(1(OX3)) gene was also detected in 22 STEC strains from humans with nonbloody diarrhea and from asymptomatic excreters. Serotypes O146:H21 and O128:H2 were most frequently associated with stx(1(OX3))-carrying STEC from sheep and humans. In contrast, Stx(1)-producing STEC strains from cattle and goats and 50 STEC strains from humans were all negative for the stx(1(OX3)) gene. The stx(1(OX3))-negative strains belonged to 13 serotypes which were different from those of the stx(1(OX3))-positive STEC strains. Moreover, the stx(1(OX3)) gene was not associated with STEC belonging to enterohemorrhagic *E. coli* (EHEC) serogroups O26, O103, O111, O118, O145, and O157. A bacteriophage carrying the stx(1(OX3)) gene (phage 6220) was isolated from a human STEC O146:H21 strain. The phage was able to lysogenize laboratory *E. coli* K-12 strain C600. Phage 6220 shared a similar morphology and a high degree of DNA homology with Stx(2)-encoding phage 933W, which originates from EHEC O157. In contrast, few similarities were found between phage 6220 and Stx(1)-encoding bacteriophage H-19B from EHEC O26

35. **[Phagotherapy in terms of bacteriophage genetics: hopes, perspectives, safety, limitations]. Krylov, V. N. (2001). *Genetika* 37:869-887.** The appearance and spreading of multidrug-resistant bacterial pathogens is a consequence of the large-scale use of antibiotics in medicine. In view of this, claims for the phage therapy were renewed: in recent studies, the natural phages and their products neutralizing various proteins, as well as the bacterial products often controlled by defective prophages (bacteriocins) were applied for treatment of bacterial infections. Constructs obtained by gene engineering are increasingly used to change some bacteriophage: properties to expand the spectrum of their lytic activity and to eliminate therapeutic drawbacks of some natural phages. In this review, the problem of phage therapy is discussed in general with respect to bacteriophage properties, their genetics, structure, evolution, taking into account long-term experience of the author in the field of bacteriophage genetics. Note that the general concept of phage therapy should be developed to ensure long-term, efficient and harmless phage therapy

36. **Where are the pseudogenes in bacterial genomes? Lawrence, J. G., Hendrix, R. W., Casjens, S. (2001). *Trends in Microbiology* 9:535-540.** Most bacterial genomes have very few pseudogenes; notable exceptions include the genomes of the

intracellular parasites *Rickettsia prowazekii* and *Mycobacterium leprae*. As DNA can be introduced into microbial genomes in many ways, the compact nature of these genomes suggests that the rate of DNA influx is balanced by the rate of DNA deletion. We propose that the influx of dangerous genetic elements such as transposons and bacteriophages selects for the maintenance of relatively high deletion rates in most bacteria; the sheltered lifestyle of intracellular parasites removes this threat, leading to reduced deletion rates and larger pseudogene loads

37. **Presence of bacteriophages in animal feed as indicators of fecal contamination. Maciorowski, K. G., Pillai, S. D., Ricke, S. C. (2001). *Journal of Environmental Science and Health Part B Pesticides* 36:699-708.** The objectives of this study were to determine if indigenous male specific and somatic bacteriophages could be detected in animal feeds and if isolated phages contained RNA or DNA. Seven fresh feeds, 2 fresh feed ingredients, 7 stored feeds, 2 stored feed ingredients, and 8 samples of poultry diets suspected to contain Salmonella spp. were enriched and spot plated for indigenous phages using *Escherichia coli* Famp and CN-13 as hosts. Bacteriophage numbers were below detection without enrichment, but both male specific and somatic coliphages were detected in all animal feeds, feed ingredients, and poultry diets after 16 h of enrichment, even after the samples had been stored for 14 months of storage at -20 C. Five out of 9 fresh feeds and 7 out of 8 stored feeds contained RNA somatic phages

38. **Filamentous bacteriophage stability in non-aqueous media. Olofsson, L., Ankarloo, J., Andersson, P. O., Nicholls, I. A. (2001). *Chemistry and Biology* 8:661-671.** BACKGROUND: Filamentous bacteriophage are used as general cloning vectors as well as phage display vectors in order to study ligand-receptor interactions. Exposure to biphasic chloroform-water interface leads to specific contraction of phage, to non-infective I- or S-forms. RESULTS: Upon exposure, phage were inactivated (non-infective) at methanol, ethanol and 1-propanol concentrations inversely dependent upon alcohol hydrophobicity. Infectivity loss of phage at certain concentrations of 1-propanol or ethanol coincided with changes in the spectral properties of the f1 virion in ultraviolet fluorescence and circular dichroism studies. CONCLUSIONS: The alcohols inactivate filamentous phage by a general mechanism--solvation of coat protein--thereby disrupting the capsid in a manner quite different from the previously reported I- and S-forms. The infectivity retention of phagemid pG8H6 in 99% acetonitrile and the relatively high general solvent resistance of the phage strains studied here open up the possibility of employing phage display in non-aqueous media

39. **[Bacteria-killing viruses, Stalinists and "superbugs"]. Olsen, I., Handal, T., Lokken, P. (2001). *Tidsskrift for den Norske Laegeforening* 121:3197-3200.** In June 2000, the WHO warned that the level of resistance to drugs used to treat common infectious diseases is now reaching a crisis point. If world governments do not control infections better in order to slow down the development of drug resistance, entire populations could be wiped out by superbugs against which there is no efficient treatment. Development of resistance is due to both underuse and overuse of drugs, and strategies have been worked out, to slow down the development of resistance for instance by the Norwegian Ministry of Health and Social Affairs. The present article deals with an old principle, mainly developed behind the Iron Curtain, which is now attracting renewed attention in the west: the application of bacterial viruses (bacteriophages) in the fight against bacteria. According to clinical trials in Eastern Europe, mostly uncontrolled, phages have been used successfully in treatments against antibiotic-resistant bacteria, for instance in suppurative wound infections, gastroenteritis, sepsis, osteomyelitis and pneumonia. These encouraging data are supported by recent findings in well-controlled animal models demonstrating that phages can rescue animals from a variety of fatal infections. The present review discusses possible advantages and limitations of phage treatment in humans

40. **Modeling virus inactivation on salad crops using microbial count data. Petterson, S. R., Teunis, P. F., Ashbolt, N. J. (2001). *Risk Analysis* 21:1097-1108.** Microbial counts of the persistent *Bacteroides fragilis* bacteriophage B40-8 from a virus decay experiment conducted under glasshouse conditions were used to model the decay of viruses on wastewater-irrigated lettuce and carrot crops. The modeling approach applied gave specific consideration to the discrete nature of microbial count data. The experimental counts were best fit by a negative binomial distribution indicating highly dispersed distribution of viruses on lettuce and carrot crops following irrigation with wastewater. In addition, there was evidence for biphasic inactivation of viruses, signifying the presence of a persistent subpopulation of viruses that decayed slowly, resulting in virus accumulation on the crop surface over subsequent irrigations. Maximum likelihood estimates of initial and persistent subpopulation inactivation rates were 2.48 day(-1) and 0.51 day(-1) for lettuces and 0.84 day(-1) and 0.046 day(-1) for carrots. Maximum likelihood estimates of the persistent virus subpopulation size were 0.12% and 2% for lettuce and carrots, respectively

41. **[Autoplaque formation in a *Pseudomonas fluorescens* strain: phage-like particles and transactivation of the defective phage]. Shaburova, O. V., Kurochkina, L. P., Krylov, V. N. (2001). *Genetika* 37:893-899.** Natural bacteriophages of *Pseudomonas fluorescens* are rare and its temperate phages have not been described so far. In search for these phages, we have found that one of the *P. fluorescens* strains forms numerous small transparent autoplaques of different size and shape, which contained material reproducible on the same strains. When centrifuged in a cesium chloride gradient, this material yielded a band in the density zone of about 1.3 g/cm3, where protein components or bacteriophages with a relatively low content of nucleic acid are usually located. In the band material, electron microscopy revealed phagelike particles with empty and mostly undamaged heads and tails carrying in their distal region a formation resembling contracted sheath. DNA isolated from the preparation consisted of two components: a distinct 54-kb fragment, and a diffuse fragment ranging in size from 20 to 9.5 kb. Treatment of the large DNA fragment with various endonucleases yielded 42.2- and 29.5-kb fragments (on average for different endonucleases); whereas the same treatment of the diffuse fragment yielded two- to three distinct fragments with the overall molecular sizes of 8.9 and 6.2 kb (for different nucleases). We have suggested that cells harbor two different genetic elements whose interaction results in the autoplaque appearance and in the formation of negative colonies after infection with the autoplaque material. One of the two elements displays properties of a defective prophage with disturbed DNA synthesis and assembly, whereas the other exhibits the properties of a transposable phage. After complementation or some other interaction between these elements (transactivation, prophage induction caused by repressor inactivation), a bulk of defective phage particles devoid of DNA and a few DNA-containing particles were produced. It remains unclear whether both DNA types are contained in the same or different particles. The phage (or a system of elements) referred to as PT3 is noninducible. The phage mutants forming larger negative colonies (NCs) were also revealed. Some of bacterial mutants resistant to PT3 infection produce the mutant phage with small and turbid NCs. PT3 produces no NCs on the lawns of other strains of the same or other pseudomonade species. This is the first case of describing a natural temperate bacteriophage in *P. fluorescens*. The two different elements of this phage may represent the same genome of the defective prophage divided into two portions within a bacterial chromosome, each of which is capable of packaging into the phage head

42. **Designing better phages. Skiena, S. S. (2001). *Bioinformatics* 17 Suppl 1:S253-S261.** We propose a method to engineer the genome of bacteriophages to increase their effectiveness as antibacterial agents. Specifically, we exploit the redundancy of the triplet code to design genomes that avoid restriction sites while producing the same proteins as wild-type phages. We give an efficient algorithm to minimize the number of restriction sites against sets of cutter sequences, and demonstrate that that

phage genomes can be significantly protected against surprisingly large sets of enzymes with no loss of function. Finally, we develop a model to explain why evolution has failed to eliminate many possible restriction sites despite selective pressure, thus motivating the need for genome-level sequence engineering

43. **Inactivation of bacteriophages in water by means of non-ionizing (UV-253.7 nm) and ionizing (gamma) radiation: a comparative approach. Sommer, R., Pribil, W., Appelt, S., Gehringer, P., Eschweiler, H., Leth, H., Cabaj, A., Haider, T. (2001). *Water Research* 35:3109-3116.** Thc inactivation behaviour of the bacteriophages PHI X 174 (ssDNA virus). MS2 (ssRNA virus) and B40-8 (dsDNA) toward non-ionizing (UV-253.7 nm) as well as to ionizing radiation (gamma radiation) was studied in order to evaluate their potential as viral indicators for water disinfection by irradiation. Previous findings of the high UV-253.7 nm resistance of MS2 were confirmed whereas an unexpected high sensitivity to gamma radiation compared to the two other phages was found. On the other hand, PHI X 174 revealed an enhanced UV sensitivity but a high resistance to ionizing radiation. B40-8 had an intermediate position between the other two bacteriophages relative to both types of radiation. As expected, the data of *E. coli* reconfirmed the unreliability of fecal indicator bacteria for the purpose of predicting responses of viruses to water treatment. In UV disinfection the influence of water matrix may be adequately controlled by considering the UV (253.7 nm) absorption of the water whereas so far no such parameter has existed for the influence of the water quality on ionizing irradiation with respect to the scavenger concentration

44. **Characterization of a Shiga toxin-encoding temperate bacteriophage of *Shigella sonnei*. Strauch, E., Lurz, R., Beutin, L. (2001). *Infection and Immunity* 69:7588-7595.** A Shiga toxin (Stx)-encoding temperate bacteriophage of *Shigella sonnei* strain CB7888 was investigated for its morphology, DNA similarity, host range, and lysogenization in *Shigella* and *Escherichia coli* strains. Phage 7888 formed plaques on a broad spectrum of *Shigella* strains belonging to different species and serotypes, including Stx-producing *Shigella* dysenteriae type 1. With *E. coli*, only strains with rough lipopolysaccharide were sensitive to this phage. The phage integrated into the genome of nontoxigenic *S. sonnei* and laboratory *E. coli* K-12 strains, which became Stx positive upon lysogenization. Moreover, phage 7888 is capable of transducing chromosomal genes in E. coli K-12. The relationships of phage 7888 with the *E. coli* Stx1-producing phage H-19B and the *E. coli* Stx2-producing phage 933W were investigated by DNA cross-hybridization of phage genomes and by nucleotide sequencing of an 8,053-bp DNA region of the phage 7888 genome flanking the stx genes. By these methods, a high similarity was found between phages 7888 and 933W. Much less similarity was found between phages H-19B and 7888. As in the other Stx phages, a regulatory region involved in Q-dependent expression is found upstream of stxA and stxB (stx gene) in phage 7888. The morphology of phage 7888 was similar to that of phage 933W, which shows a hexagonal head and a short tail. Our findings demonstrate that stx genes are naturally transferable and are expressed in strains of S. sonnei, which points to the continuous evolution of human-pathogenic Shigella by horizontal gene transfer

45. **Community Structure: Viruses. Suttle, C. A. (2001). pp. 364-370 in Hurst, C. J., Knudson, G. R., McInerney, M. J., Stezenbach, L. D., Walter, M. V. (eds.) *Manual of Environmental Microbiology (2nd Edition)*. ASM Press, Washington, DC.**

46. **Zoonotic *Escherichia coli*. Wasteson, Y. (2001). *Acta Veterinaria Scandinavica Supplement* 95:79-84.** *Escherichia coli* is a normal inhabitant of the gastrointestinal tract of all warm-blooded animals, but variants of this species is also among the important etiological agents of enteritis and several extraintestinal diseases. The *E. coli* strains that cause diarrhoeal illness are categorised into pathogenicity groups based on virulence properties, mechanisms of pathogenicity, clinical symptoms and serology. The five main categories include enterotoxinogenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAggEC), enteroinvasive *E. coli* (EIEC) and Shiga (Vero) toxin-producing *E. coli* (STEC/VTEC). From a zoonotic point of view, STEC is the only *E. coli* pathogenicity group of major interest, as the shiga toxin-producing strains are able to cause severe disease in humans when being transmitted through the food chain from their animal reservoirs. The focus of this manuscript is therefore on STEC; pathogenicity factors, disease, the reservoirs and on-farm ecology, transmission into the food chain, growth and survival in food and in the environment, and the shiga toxin-encoding bacteriophages

47. **Filamentous phage biology. Occurrence of coliphages in fish and aquaculture farms. Webster, R., Barbas, C. F., III, Burton, D. R., Scott, J. K., Silverman, G. J., Rao, B. M., Surendran, P. K. (2001). *Phage display: A laboratory manual.* 37:146-149.** Coliphages were detected in water samples collected from brackish water and fresh water fish farms. Coliphages were also detected in the farmed fresh water fish, common carp and marine fish, oil sardine, from local market. Coliphage levels obtained were as follows:- water from brackish water fish farm 3 pfu.ml-1, water from fresh water fish farm 23 pfu.ml-1, fresh water fish 240 pfu.g-1 and marine fish 3500 pfu.g-1

48. **A fast method for assessing rapid inactivation and adsorption kinetics of bacteriophages using batch agitation experiments and colloidal clay particles. Rossi, P., Aragno, M. (1999). *Canadian Journal of Microbiology* 45:9-17.**

49. **Different trajectories of parallel evolution during viral adaptation. Wichman, H. A., Badgett, M. R., Scott, L. A., Boulianne, C. M., Bull, J. J. (1999). *Science* 285:422-424.** The molecular basis of adaptation is a major focus of evolutionary biology, yet the dynamic process of adaptation has been explored only piecemeal. Experimental evolution of two bacteriophage lines under strong selection led to over a dozen nucleotide changes genomewide in each replicate. At Least 96 percent of the amino acid substitutions appeared to be adaptive, and half the changes in one line also occurred in the other. However, the order of these changes differed between replicates, and parallel substitutions did not reflect the changes with the largest beneficial effects or indicate a common trajectory of adaptation.

50. **Bacteriophage tracing techniques. Rossi, P., Käss, W. (1998). pp. 244-270 in Matthes, Käss (eds.) *Tracing Techniques in Geohydrology*. Balkema, Rotterdam.**

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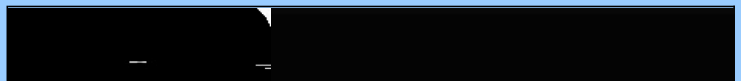
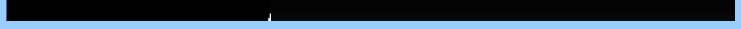
Acknowledgements





Thanks to Steve McQuinn!

Contact Steve Abedon (microdude+@osu.edu) with suggestions, criticisms, comments, or anything else that might help make this a better site.