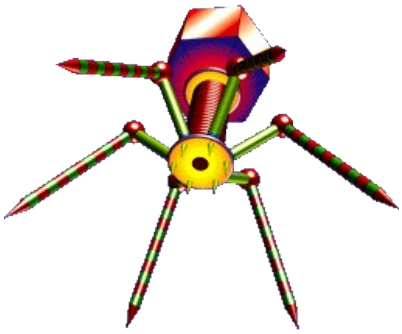

Bacteriophage Ecology Group (BEG) News

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

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October 1, 1999 issue (volume 2)

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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 correspondence 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 any other document formats), and in English.

When Grown *In Vitro*, do Parasites of Multicellular Organisms (MOPs) become Unicellular Organism Parasites (UOPs)?

The Bacteriophage Ecology Group (BEG) is concerned, of course, with the ecology of bacteriophages. When modeling bacteriophage growth, especially in liquid culture, the dynamics of host acquisition by bacteriophages should essentially resemble those of any organism that acquires its "prey" through random diffusion. Of organisms that obtain their resources directly from other living organisms, we may, of course, further subdivide into (i) those that acquire no more than one "prey" per lifetime (the parasites) and (ii) those that acquire more than one "prey" per lifetime (the predators). An individual bacteriophage clearly cannot acquire more than one "prey" (or host) in a lifetime so clearly, by these definitions, is more parasite-like than predator-like.

Among parasites we may further subdivide into (i) those parasites that infect multicellular organisms and (ii) those parasites that infect unicellular organisms. From the standpoint of a parasite, the former, but not the latter, is supplied with additional "prey" by the infected host. This is most easily visualized when considering obligately lytic intracellular parasites in which host cells represent discrete "prey." Thus, acquisition of a single host cell can lead to the generation of parasite progeny which can then go on to acquire new "prey," all without any parasite ever leaving the original host. This is not the case for the parasites of unicellular hosts (or unicellular organism parasites, i.e., UOPs). The progeny of UOPs must leave their host (e.g., an individual bacterium) to acquire new "prey." It has been my policy to define UOP rather broadly to include such parasites as *Bdellovibrio* as well as the viruses of yeasts and those of protozoa.

Nevertheless, ambiguity rears its ugly head in defining multicellularity. An important component of the first night of the 1999 Gordon Conference on

Microbial Population Biology (held July 18-23) was the argument that bacteria often exist effectively as multicellular organisms. That is, individual cells interact in ways such that the whole of a bacterial population is greater than (or, at least, different from) the sum of its parts. If bacteria can be multicellular, then are bacteriophages truly UOPs? Is anything?

I won't attempt to answer that question but instead will address the implications of bacterial multicellularity on bacteriophage replication, based on a contrast between the environments represented by multicellularity versus those represented by unicellularity. I will then question whether, from the standpoint of bacteriophage growth, eukaryotic cells growing in tissue culture are any more multicellular than, for example, bacteria growing in a biofilm or bacteria growing within an agar lawn or colony.

With regard to the population-wide growth of obligately intracellular parasites, two considerations may occur during the jump from unicellularity to multicellularity. First, the susceptibility of individual host cells to parasite infection my decline. Second, the dynamics of host-cell acquisition may change. Considering the former, we may envisage barriers to parasite diffusion, changes in host cell-surface markers, or even mechanisms of parasite inactivation both prior to and following host-cell infection (e.g., an immune system). Alternatively, with regard to the dynamics of host-cell acquisition, clearly with multicellularity the odds of finding two host cells that are adjacent is no longer a statistically independent product of global host-cell densities. Thus, within a multicellular system the acquisition of a single host cell increases the likelihood or rate with which progeny may find additional host cells. Clearly these two factors would result in (i) a decrease in host-cell susceptibility and (ii) an increase in host-cell clumping.

Together these factors affect parasite replication in opposite directions with the former decreasing and the latter increasing host susceptibility in terms of the spread of progeny parasites to adjacent cells. We may thus envision that if parasitism has significant negative impact, then the benefits of multicellularity to the host must either outweigh the costs of increased parasite susceptibility by multicellular organisms or that along with multicellularity comes at least an opportunity for increased defense against parasites. If the latter is the case then one might even go so far as to argue that multicellularity could have evolved in general as a mechanism of parasite (or predator) evasion. For example, the evolution of metazoa could have been motivated as a mechanism of protection against protozoa-mediated engulfment (i.e., big things are harder to engulf and multicellularity is one route toward bigness). Alternatively, as well as additionally, multicellularity may have evolved as a means toward more-effective predation of, for example, multicellular cyanobacterial mats.

Thus, a multicellular organism represents both a juicier, perhaps more obtainable target for parasitism, but simultaneously a less appetizing morsel to the extent that colonial living leads to mutual protection against parasitism. Since prokaryotes are probably as capable of at least some mutual protection as eukaryotes, then I suppose I must concede that bacteria, from the point of view of a bacteriophage, are not nearly as unicellular as I might otherwise like to think. How, then, might we classify eukaryotic cells living together in a tissue culture flask? Are such cells any less susceptible to a parasite than bacteria living in an agar lawn or within a biofilm? My intuition suggests no. I welcome (and would like to publish) any good arguments either for or against this assertion.

All of this pontification stems from a debate I've been having with myself over whether we should include an article-found in the September 10 issue of *Science* on vesicular stomatitis virus evolution-into the BEG bibliography: Rosario Miralles, Philip J. Gerrish, Andrés Moya, and Santiago F. Elena, Clonal Interference and the Evolution of RNA Viruses, *Science* 285(5434):1745-1747. Since the primary experimental technique used in this study involves little more than growing viruses on "baby hampster kidney cells... grown as monolayers under Dulbecco's modified Eagle's minimum essential medium," it strikes me that what we have here is an example of UOP-mediated experimental evolutionary biology. Therefore, along with UOP ecology and evolutionary biology in general, it is my tentative contention that Miralles *et al.* should be included in the BEG bibliography (along with, for example, papers on the viruses of protozoa). What do you think?

MicroDude, a.k.a., **Stephen T. Abedon**

Developer and Editor
The Bacteriophage Ecology Group
Dedicated to the ecology and evolutionary biology of the parasites of unicellular organisms (UOPs)

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New BEG Members

The BEG members list can be found on the BEG home page. As we add new members, these individuals will be introduced in this section. Note that, in fact, 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, i.e., individuals listed on the BEG home page, should be limited to individuals who are actively involved in science 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 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 list 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 T. 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.

name (home page links)	status	e-mail	address	other
Jeffrey L. Blanchard	---	jblancha @promega.com	Postdoctoral Fellow, Promega Corporation, 2800 Woods Hollow Rd., Madison, WI 53711-5399	interests
Jed Fuhrman	PI	fuhrman @usc.edu	Dept. of Biological Sciences, MC-0371, University of Southern California, Los Angeles, CA 90089-0371	interests, publications, home page
Paul E. Turner	---	paul.e.turner @uv.es	NSF-NATO Postdoctoral Fellow, Instituto Cavanilles de Biodiversidad y Biologfa Evolutiva, University of Valencia, P.O. Box 22085, 46071 Valencia, Spain	interests, publications
Holly A. Wichman	PI	hwichman @uidaho.edu	Dept. of Biological Sciences, University of Idaho, Moscow ID 83844-3051	interests, publications, home page
John Yin	PI	yin @mail.che.wisc.edu	Department of Chemical Engineering, University of Wisconsin-Madison, Madison, WI 53706-1691	interests, publications, home page

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New Links

Links relevant to The Bacteriophage Ecology Group fall into a number of categories (e.g., see Bacteriophage Ecology Links on The Bacteriophage Ecology Group home page). Listed below are those links that overtly deal with phage ecology issues. With each issue of *BEG News* this list will be included, *in toto*, but updated with new links and with no-longer-working links, both clearly indicated. If you know of a link that should be included on this page, or the whereabouts of a now-dead link, please let me know.

- Abundance and variety of bacteriophages [new]
- Assessment of MS2 Bacteriophage Adsorption to Koch Membrane
- Attachment and Replication of *Pseudomonas aeruginosa* Bacteriophages Under Conditions Simulating Aquatic Environments [new]
- Bacteriophage Ecology Bibliography
- Bacteriophages (an overview)
- BioVir Laboratories (an environmental testing laboratory)
- Characterization of Marine Viruses
- Determination of Optimal Conditions for Bacteriophage Lysis of *Janthinobacterium lividum* Broth Cultures
- The effect of phosphate status on virus populations during a mesocosm study
- G. Eliava Institute of Bacteriophage (Tbilisi)
- Host Interactions and Growth Strategy of Aquatic Bacteriophages
- How the cholera bacterium got its virulence
- Lactobacillus Bacteriophage, Lay Discussion
- *Lactococcus garvieae* Phage [new]
- The Microbe Zoo | Dirtland | House of Horrors [featuring the strangler fungus, *Vampirococcus* and *Bdellovibrio*]
- Molecular ecology and evolution of *Streptococcus thermophilus* bacteriophages in industrial milk fermentations [new]
- MS2 Inactivation by Chlorine during Microgravity
- Nature of Things - The Virus that Cures [10/29/98 CBC TV broadcast]
- Phage (an introduction to some basic phage biology) [new]
- PhageBiotics Foundation
- Phage Therapeutics, Inc.
- Phage Therapy
- Prevalence of Broad-Host-Range Lytic Bacteriophages of *Sphaerotilus natans*, *Escherichia coli*, and *Pseudomonas aeruginosa* [new]
- *Pseudomonas* Transduction in Wild (Fresh Water) [lost link?]
- Pseudolysogeny
- Return of a Killer [11/2/98 *U.S. News and World Report* article on phage therapy]
- Revenge of the Bug Zappers [new]
- A Review of the ASCRC Starter Strategy [new]
- Sunlight-induced DNA damage and in marine viral communities
- Tbilisi Institute for Bacteriophage
- Toward a Theory of Molecular Computing (includes Lambda-Phage Choice Between Lysis and Lysogeny Model) [new]
- The Curious Microbe: *Bdellovibrio*
- The Ecology of Computer Viruses
- The Isolation of T-Even Phages
- The Virus that Cures

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New Features

In this section I will highlight new or updated features of the BEG site. If you have any ideas of how either the BEG site or *BEG News* might be improved, please let me know.

phage-history links:

I've added a small section of phage-history links. This started out as part of my ongoing (with luck soon over) effort to figure out where coliphage T2 came from. If you know of any additional phage-history links, please let me know. Here are what I've found to date:

- *Arrowsmith* by Sinclair Lewis (the original phage-therapy novel)
- Fredrick John Babel (1911-1997)
- Jacques J. Bronfenbrenner (???-???)
- Cold Spring Harbor Laboratory
- Francis Harry Compton Crick (1916-present)
- Max Delbrück (1906-1981):
  - The Caltech Institute Archives
  - Cold Spring Harbor Laboratory
  - Nobel Prize
- Renato Dulbecco (1914-present)
- Emory L. Ellis (1906-present)
- *Felix D'Herelle and the Origins of Molecular Biology*
- Alfred Day Hershey (1908-1997)
  - Cold Spring Harbor Laboratory
  - Washington University
  - Nobel Prize
- Hershey-Chase Experiment
- History of Genetics Timeline
- History of Molecular Biology
- History of Bacteriophage Research
- The Isolation of T-Even Phages
- Frantois Jacob (1920-present)
- Salvador Luria (1912-1991)
  - Cold Spring Harbor Laboratory
  - Nobel Prize
  - A Slot Machine, a Broken Test Tube : An Autobiography
- André Michel Lwoff (1902-1994)
- Major Discoveries Made with Bacteriophages
- The Nobel Prize in Physiology or Medicine 1962
- The Nobel Prize in Physiology or Medicine 1965
- The Nobel Prize in Physiology or Medicine 1969
- On an Invisible Microbe Antagonistic to the Dysentery Bacillus. Felix d'Herelle
- *Phage and the Origins of Molecular Biology*, Expanded Edition
- Phage Course
- Phage Group
- Recipients of the Pasteur Award
- F. W. Twort (1877-1950)
- Urban Legend: Phage Isolated from Letter
- James D. Watson (1928-present)
  - Cold Spring Harbor Laboratory
  - Nobel Prize

meta names/tags:

Some time back I requested suggestions for meta names/tags to append to the BEG splash page. These are read by some search engines and thereby allow a broader indexing of a page than would occur if search engines considered only those words that were visible. Thanks to those of you who replied. If you know of any additional terms that you would like to add to this list, or have any suggestions, please let me know. Here is the current list:

1. acellular
2. actinophage
3. actinophages
4. bacteria sex
5. bacterial sex
6. bacteriophage
7. bacteriophage ecology
8. bacteriophage evolution
9. bacteriophage immunity
10. bacteriophage therapy
11. bacteriophage typing
12. bacteriophages
13. bacteriophagology
14. bdellovibrio
15. carrier state
16. conjugation
17. cryptic phage
18. cryptic prophage
19. cyanophage
20. cyanophages
21. ecology
22. environmental biology

23. environmental microbiology
24. evolution
25. evolutionary biology
26. fecal pollution
27. indicator
28. generalized transduction
29. generalized transducing phages
30. halophage
31. halovirus
32. heteroimmune phages
33. homoimmune phages
34. horizontal transfer
35. legionella
36. lysis
37. lysogen
38. lysogenic
39. lytic
40. marine viruses
41. microbial population biology
42. microbiology
43. mycovirus
44. mycoviruses
45. parasitism
46. phage
47. phage ecology
48. phage evolution
49. phage genetics
50. phage immunity
51. phage therapy
52. phage typing
53. phages
54. phagology
55. phycovirus
56. phycoviruses
57. population biology
58. predation
59. predator
60. prey
61. prophage
62. prophage derepression
63. prophage induction
64. phage as transposons recombination
65. specialized transducing phages
66. symbiosis
67. transduction
68. vampirococcus
69. virioplankton
70. viral dissinfection
71. virology
72. virus
73. virus ecology
74. virus evolution
75. viruses

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Upcoming Meetings

The BEG Meetings link will continue, but reminders about 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, 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.

Microbial Population Biology Gordon Research Conference:

The biannual Microbial Population Biology Gordon Research Conference was held July 18 through July 23, 1999, at Plymouth State College in Plymouth, New Hampshire. A list of sessions, speakers, and session chairs is available on the web. This year's meeting was lot's of fun, as usual. Important information relevant to the 2001 meeting includes: (i) yes, it looks very much as though there will be a meeting in two years, (ii) the new chair for the upcoming meeting will be Lin Chao (who a bacteriophage ecologist, though he denies this), and (iii) the location for the next meeting is uncertain. A majority voted to no longer hold the meeting in Plymouth, New Hampshire (which disappoints me since I really enjoy visiting New Hampshire every two years). Suggestions for new sites included: Rhode Island (too hot), California (which would be in the Winter rather than our traditional Summer), and Italy (which, I suppose, could very well be as much fun as New Hampshire). Below are shown a number of phage ecology/evolutionary biology types, past, present, and future(?) who were at the meeting including Greg Krukonis, Michael Feldgarden, Paul Turner, Christina Burch, Rich Lenski, Jim Bull, Fred Cohan, Brendan Bohannan,

Conrad Istock, Junetso Ito, Matt Brauer, Sharon Messenger, Holly Wichman, Steve Abedon, Camilla Ramg (with Jasper Chao), Bruce Levin, Allan Campbell, Julia Bell, Jeff Blanchard, Lin Chao, and Kathy Duncan.

Evergreen International Phage Meeting:

The above web link still refers to last year's (1998) meeting. Next year's meeting tentatively will be held August 3-8, 2000. The 1998 meeting had a strong bacteriophage ecology presence and with luck (i.e., with your participation!) we will do even better in 2000. A new, and rather interesting rumor, however, is that the meeting may not be held in Olympia, Washington, this coming year. With luck we'll know more come January 1.

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Jobs

The BEG Employment / Job Listings page will no longer be maintained. Instead, any job listings will be found in this section of *BEG News*. If you are looking to*fill* a bacteriophage-ecology related position or are in *search of* a bacteriophage-ecology related position, please feel free to advertise as such here (there will be no charge, of course). Legitimate information only, please, and *BEG News* cannot be held responsible for any incorrect information supplied by posters. Send any information for posting to abedon.1@osu.edu or to "BEG Jobs," *Bacteriophage Ecology Group News*, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906.

VIRUS-HOST INTERACTIONS AND ANTI-VIRAL STRATEGIES POST-DOC

As a team of chemical engineers, cell biologists, and molecular virologists, we are developing experimental and theoretical approaches to study the growth, adaptation, and inactivation of viruses (prokaryotic and eukaryotic). In the lab we are advancing methods to visualize and quantify the intracellular and population-level dynamics of virus-host interactions. At the same time, we are creating physical-chemical models and computer simulations to better understand how viral growth depends on the kinetics of its constituent processes. Our team of one post-doc and six graduate students pursues research in well-equipped facilities located in the Department of Chemical Engineering at University of Wisconsin-Madison. Our studies are enriched by collaborations with virologists, both on campus and at other institutions.

Madison is a safe, bicycle-friendly, affordable city surrounded by attractive lakes. It routinely ranks at or near the top of national surveys of 'best places to live,' and Madisonians thrive on its rich diversity of cultural and outdoors opportunities.

Experience in cell biology, molecular biology, or molecular virology is desirable. Skills in immuno-cytochemistry, in-situ hybridization, anti-sense methodologies, or digital imaging would be especially welcome. Interested candidates should forward their curriculum vitae and have three letters of reference sent to Dr. John Yin, either by e-mail (yin@engr.wisc.edu), fax (608 262-5434, attn: Dr. John Yin) or using the following address: Dept. of Chemical Engineering, University of Wisconsin, Madison, WI 53706-1691 USA.

References:

Spatially-resolved growth and evolution of viruses Yin, J. (1993) J. Bact. 175(5), 1272-1277; Lee, Y. and J. Yin (1996), Biotech. Bioeng., 52, 438-442; Lee, Y. and J. Yin (1996), Nature Biotech.14 : 491-493.

Viral Intracellular Kinetics Endy, D., D. Kong and J. Yin (1997), Biotech. Bioeng., 55(2), 375-389; Reddy, B. and J. Yin (1999), AIDS Research and Human Retroviruses, 15(3), 273-283.

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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 doc ument formats), and in English.

No entry.

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Letters

Letters should consist of comments, short statements, or personal editorials. Send all letters to abedon.1@osu.edu or to "Letters", Bacteriophage Ecology Group News, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansf ield, Ohio 44906. Please send all letters in English and all mailed or attached letters as Microsoft Word documents, if possible (I'll let you know if I have trouble converting any other document formats). In addition to standard letters, BEG receives questions on a regular basis that may be addressed by BEG members. These questions are listed below. Anybody interested in answering these questions through *BEG News*, e-mail me at the following address: abedon.1@osu.edu. Alternatively, answer through the prompt following each question. Please note that these questions have not been edited for grammar, spelling, or clarity.

No entry.

Questions

No entry.

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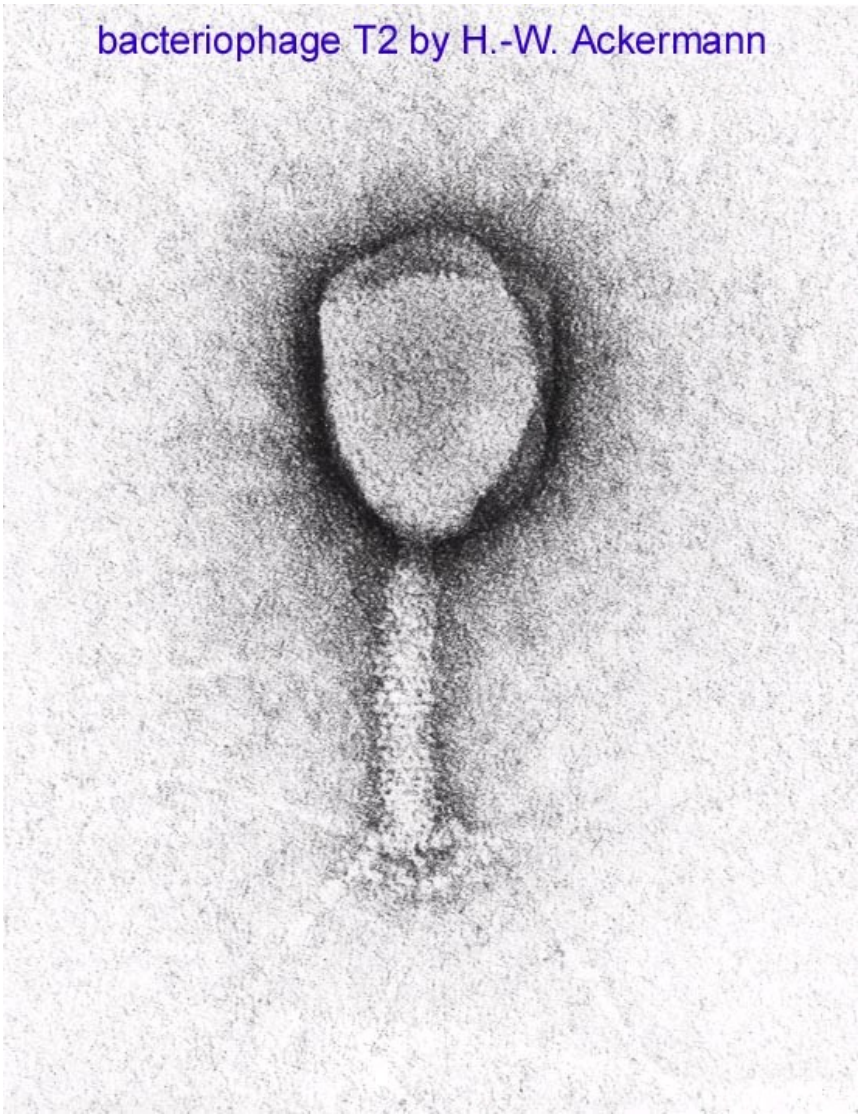
Phage Images

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.



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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. *Horizontal Gene Transfer*. Syvanen, M., Kado, C. I. (1998). Chapman & Hall, New York. [no abstract]

2. *Practical Phage Control*. ??? (1999). International Dairy Federation. (see: http://www.cheesereporter.com/micro_books.htm) [no abstract]

3. [Practical use of adapted Salmonella bacteriophage for the treatment and prevention of nosocomial infections]. Akimkin, V. G., Bondarenko, V. M., Voroshilova, N. N., Darbeeva, O. S., Baiguzina, F. A. (1998). *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii* ???:85-86. [no abstract]

4. Phage-encoded genes and Salmonella virulence [letter]. Ali, T. R., Pallen, M. J. (1998). *Molecular Microbiology* 28:1039-1041. [PRESS FOR ABSTRACT]

5. Stable expression of the Lactobacillus casei bacteriophage A2 repressor blocks phage propagation during milk fermentation. Alverez, M. A., Rodriguez, A., Suarez, J. E. (1999). *Journal of Applied Microbiology* 86:812-816. [PRESS FOR ABSTRACT]

6. Test tube evolution catches time in a bottle. Appenzeller, T. (1999). *Science* 284:2108-2110. [PRESS FOR ABSTRACT]

7. Evolution by small steps and rugged landscapes in the RNA virus phi6. Burch, C. L., Chao, L. (1999). *Genetics* 151:921-927. [PRESS FOR ABSTRACT]

8. Occurrence of male-specific bacteriophage in feral and domestic animal wastes, human feces, and human-associated wastewaters. Calci, K. R., Burkhardt, W. 3rd, Watkins, W. D., Rippey, S. R. (1998). *Applied and Environmental Microbiology* 64:5027-5029. [PRESS FOR ABSTRACT]

9. Fecal coliform-related bacterial and coliphage populations in five lakes of southeastern Spain. Calvo, C., Gomez, M. A., Gonzalez-Lopez, J. (1998).

Microbiological Research 153:283-288. [PRESS FOR ABSTRACT]

10. Prophage induction of indigenous marine lysogenic bacteria by environmental pollutants. Cochran, P. K., Kellogg, C. A., Paul, J. H. (1998). *Mar. Ecol. Prog. Ser.* 164:124-133. [PRESS FOR ABSTRACT]

11. Seasonal abundance of lysogenic bacteria in a subtropical estuary. Cochran, P. K., Paul, J. H. (1998). *Applied and Environmental Microbiology* 64:2308-2312. [PRESS FOR ABSTRACT]

12. An estimator of the mutant frequency in assays using transgenic animals. Delongchamp, R. R., Malling, H. V., Chen, J. B., Heflich, R. H. (1999). *Mutation Research* 440:101-108. [PRESS FOR ABSTRACT]

13. Causative agents of bacterial mortality and the consequences to marine food webs. Fuhrman, J. A.Noble, R. T. (1999). p. ???-??? in Bell, C. R., Brylinsky, M., Johnson-Green, P. (eds.) *Microbial Biosystems: New Frontiers. Proc 8th Int Symp Microb. Ecol.*. Atlantic Canada Societyfor Microbial Ecology, Halifax, Canada. [no abstract]

14. Impact of viruses on planktonic bacteria. Fuhrman, J. A. (1999). p. ???-??? in Kirchman, D. L. (ed.) *Microbial Ecology of the Oceans*. Wiley & Sons, ??? [no abstract]

15. Marine viruses and their biogeochemical and ecological effects. Fuhrman, J. A. (1999). *Nature (London)* 399:541-??? [PRESS FOR ABSTRACT]

16. [Comparative study of Morganella and Providencia bacteriophages]. Gabrilovich, I. M., Zarochentsev, M. V., Saimov, S. R. (1998). *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii* ???:20-22. [PRESS FOR ABSTRACT]

17. Detection of infectious enteroviruses, enterovirus genomes, somatic coliphages, and Bacteroides fragilis phages in treated wastewater. Gantzer, C., Maul, A., Audic, J. M., Schwartzbrod, L. (1998). *Applied and Environmental Microbiology* 64:4307-4312. [PRESS FOR ABSTRACT]

18. Targeted delivery of multivalent phage display vectors into mammalian cells. Ivanenkov, V. V., Felici, F., Menon, A. G. (1999). *Biochimica et Biophysica Acta* 1448:463-472. [PRESS FOR ABSTRACT]

19. Uptake and intracellular fate of phage display vectors in mammalian cells. Ivanenkov, V. V., Felici, F., Menon, A. G. (1999). *Biochimica et Biophysica Acta* 1448:450-462. [PRESS FOR ABSTRACT]

20. Combined use of bacteriophage typing and pulsed-field gel electrophoresis in the epidemiological analysis of Japanese isolates of enterohemorrhagic Escherichia coli O157:H7. Izumiya, A., Masuda, T., Ahmed, R., Khakhria, R., Wada, A., Terajima, A., Itoh, K., Johnson, W. M., Konuma, H., Shinagawa, K., Tamura, K., Watanabe, H. (1998). *Microbiology and Immunity* 42:515-519. [PRESS FOR ABSTRACT]

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23. Targeting bacteriophage to mammalian cell surface receptors for gene delivery [see comments]. Larocca, D., Witte, A., Johnson, W., Pierce, G. F., Baird, A. (1998). *Human Gene Therapy* 9:2393-2399. [PRESS FOR ABSTRACT]

24. Occurrence and levels of phages proposed as surrogate indicators of enteric viruses in different types of sludges. Lasobras, J., Dellunde, J., Jofre, J., Lucena, F (1999). *Journal of Applied Microbiology* 86:723-729. [PRESS FOR ABSTRACT]

25. [Reconstruction of possible paths of the origin and morphological evolution of bacteriophages]. Letarov, A. V. (1999). *Genetika* 34:1461-1469. [PRESS FOR ABSTRACT]

26. Bacteriophage and gene transfer. Lindqvist, B. H. (1998). *APMIS. SUPPLEMENTUM* 84:15-18. [no abstract]

27. Elevated production of dimethylsulfide resulting from viral infection of cultures of Phaeocystis pouchetii. Malin, G., Wilson, W. H., Bratbak, G., Liss, P. S., Mann, N. H. (1998). *Limnology and Oceanography* 43:1389-1393. [no abstract]

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29. Enumeration of marine viruses in culturea nd natural samples by flow cytometry. Marie, D., Brussaard, C. P. D., Thyrhaug, G., Bratback, G., Vaulot, D. (1999). *Applied and Environmental Microbiology* 65:45-52. [PRESS FOR ABSTRACT]

30. Cloning and sequencing of major capsid protein (mcp) gene of a vibriophage, KVP20, possibly related to T-even coliphages. Matsuzaki, S., Inoue, T., Kuroda, M., Kimura, S., Tanaka, S. (1998). *Gene* 222:25-30. [PRESS FOR ABSTRACT]

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32. Application and evaluation of male-specific bacteriophage as a process integrity or faecal contamination indicator in a pork slaughterhouse environment. Miller, A. J., Eblen, B. S., Oser, A., Burkhardt, W. 3rd (1998). *Journal of Applied Microbiology* 85:898-904. [PRESS FOR ABSTRACT]

33. The so-called chromosomal verotoxin genes are actually carried by defective prophages. Mizutani, S., Nakazono, N., Sugino, Y. (1999). *DNA Research* 6:141-143. [no abstract]

34. Intra-species host specificity of HaV (Heterosigma akashiwo virus) clones. Nagasaki, K., Yamaguchi, M. (1998). *Aquat. Microb. Ecol.* 14:109-112. [no abstract]

35. Filamentous phage fs1 of Vibrio cholerae O139. Nakasone, N., Honma, Y., Toma, C., Yamashiro, T., Iwanaga, M. (1998). *Microbiology and Immunity* 42:237-239. [PRESS FOR ABSTRACT]

36. The effects of viral enrichment on the mortality and growth of heterotrophic bacterioplankton. Noble, R. T., Middelboe, M., Fuhrman, J. A. (1999). *Aquat. Microb. Ecol.* ???:???-??? [no abstract]

37. The fates of viruses in the marine environment. Noble, R. T. (1999). Ph.D Dissertation, University of Southern California. [PRESS FOR ABSTRACT]

38. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Noble, R. T., Fuhrman, J. A. (1998). *Aquat. Microb. Ecol.* 14:113-118. [PRESS FOR ABSTRACT]

39. Phage-lift for game theory. Nowak, M. A., Sigmund, K. (1999). *Nature (London)* 398:367-368. [PRESS FOR ABSTRACT]

40. Phage viability in organic media: insights into phage stability. Olofsson, L., Ankarloo, J., Nicholls, I. A. (1998). *Journal of Molecular Recognition* 11:91-93. [PRESS FOR ABSTRACT]

41. Bacteriophage: tools toward a cell-targeted delivery [comment]. Paillard, F. (1999). *Human Gene Therapy* 9:2307-2308. [no abstract]

42. Targeted gene delivery to mammalian cells by filamentous bacteriophage. Poul, M. A., Marks, J. D. (1999). *Journal of Molecular Biology* 288:203-211. [PRESS FOR ABSTRACT]

43. A high incidence of prophage carriage among natural isolates of Streptococcus pneumoniae. Ramirez, M., Severina, E., Tomasz, A. (1999). *Journal of Bacteriology* 181:3618-3625. [PRESS FOR ABSTRACT]

44. Advances in the separation of bacteriophages and related particles. Serwer, P.,Griess, G. A. (1999). *Journal of Chromotography. B, Biomedical Sciences and Applications* 722:179-190. [PRESS FOR ABSTRACT]

45. [Vibrio cholerae temperate phage O139: characteristics and role in changing expression of chromosomal virulence genes]. Smirnova, N. I., Eroshenko, G. A., Shchelkanova, Elu., Livanova, L. F., Konnov, N. P. (1999). *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii* ???:3-9. [PRESS FOR ABSTRACT]

46. The complete genome sequence of the Streptomyces temperate phage straight phiC31: evolutionary relationships to other viruses. Smith, M. C., Burns, R. N., Wilson, S. E., Gregory, M. A. (1999). *Nucleic Acids Research* 27:2145-2155. [PRESS FOR ABSTRACT]

47. 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. Sommaruga, R., Sattller, B., Oberleiter, A., Wille, A., Sommaruga-W"grath, S., Psenner, R., Felip, M., Camarero, L., Pina, S., Giron□s, R., Catal n, J. (1999). *Journal of Plankton Research* 21:859-879. [PRESS FOR ABSTRACT]

48. ??? Steward, G. F., Azam, F. (1999). p. ???-??? in Bell, C. R., Brylinsky, M., Johnson-Green, P. (eds.) *Microbial Biosystems: New Frontiers. Proc. 8th Int. Symp. Microb. Ecol.*. Atlantic Canada Society for Microbial Ecology, Halifax, Canada. [no abstract]

49. Revenge of the bug zappers. Electrocuted flies spew viruses for feet - and into your food. Susman, E. (1999). *MSNBC Chicago* . [PRESS FOR ABSTRACT]

50. Bacteriophage inactivation at the air-water-solid interface in dynamic batch systems. Thompson, S. S., Yates, M. V. (1999). *Applied and Environmental Microbiology* 65:1186-1190. [PRESS FOR ABSTRACT]

51. Hybrid frequencies confirm limit to coinfection in the RNA bacteriophage phi-6. Turner, P. E., Burch, C., Hanley, K., Chao, L. (1999). *Journal of Virology* 73:2420-2424. [PRESS FOR ABSTRACT]

52. Prisoner's dilemma in an RNA virus. Turner, P. E., Chao, L. (1999). *Nature (London)* 398:441-443. [PRESS FOR ABSTRACT]

53. Sex and the evolution of intrahost competition in RNA virus ?6. Turner, P. E., Chao, L. (1998). *Genetics* 150:523-532. [PRESS FOR ABSTRACT]

54. Changes in bacterial and eukaryotic community structure after mass lysis of filamentous cyanobacteria associated with viruses. van Hannen, E. J., Zwart, G., van Agterveld, M. P., Gons, H. J., Ebert, J., Laanbroek, H. J. (1999). *Applied and Environmental Microbiology* 65:795-801. [PRESS FOR ABSTRACT]

55. Bacteriophage biology and bacterial virulence. Waldor, M. K. (1998). *Trends in Microbiology* 6:295-297. [no abstract]

56. 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. [no abstract]

57. Measurements of DNA damage and photoreactivation imply that most viruses in marine surface waters are inefective. Wilhelm, S. W., Weinbauer, M. G., Suttle, C. A., Pledger, R. J., Mitchell, D. L. (1998). *Aquat. Microb. Ecol.* 14:215-222. [PRESS FOR ABSTRACT]

58. The role of sunlight in the removal and repair of viruses in the sea. Wilhelm, S. W., Weinbauer, M. G., Suttle, C. A., Jeffrey, W. H. (1998). *Limnology and Oceanography* 43:586-592. [PRESS FOR ABSTRACT]

59. Hybridization analysis of Chesapeake Bay Virioplankton. Wommack, K. E., Ravel, J., Hill, R. T., Colwell, R. R. (1999). *Applied and Environmental Microbiology* 65:241-250. [PRESS FOR ABSTRACT]

60. Population dynamics of Chesapeake Bay virioplankton: total-community analysis by pulsed-field gel electrophoresis. Wommack, K. E., Ravel, J., Hill, R. T., Colwell, R. R. (1999). *Applied and Environmental Microbiology* 65:231-240. [PRESS FOR ABSTRACT]

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. ***Horizontal Gene Transfer*. Syvanen, M., Kado, C. I. (1998). Chapman & Hall, New York.**

2. ***Practical Phage Control*. ??? (1999). International Dairy Federation. (see: http://www.cheesereporter.com/micro_books.htm)**

3. **[Practical use of adapted Salmonella bacteriophage for the treatment and prevention of nosocomial infections]. Akimkin, V. G., Bondarenko, V. M., Voroshilova, N. N., Darbeeva, O. S., Baiguzina, F. A. (1998). *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii* ???:85-86.**

4. **Phage-encoded genes and Salmonella virulence [letter]. Ali, T. R., Pallen, M. J. (1998). *Molecular Microbiology* 28:1039-1041.**

5. **Stable expression of the Lactobacillus casei bacteriophage A2 repressor blocks phage propagation during milk fermentation. Alverez, M. A., Rodriguez, A., Suarez, J. E. (1999). *Journal of Applied Microbiology* 86:812-816.** A general strategy was applied to implement resistance against temperate bacteriophages that infect food fermentation starters through cloning and expression of the phage repressor. Lactobacillus casei ATCC 393 and phage A2 were used to demonstrate its feasibility as milk fermentation is drastically inhibited when the strain is infected by this phage. The engineered strain Lact. casei EM40::cI, which has the A2 repressor gene (cI) integrated into the genome, was completely resistant and able to ferment milk whether phage was present or not. In addition, viable phages were eliminated from the milk, probably through adsorption to the cell wall. Finally, the integration of cI in the genome resulted in a stable resistance phenotype, being unnecessary selective pressure during milk fermentation.

6. **Test tube evolution catches time in a bottle. Appenzeller, T. (1999). *Science* 284:2108-2110.** By running experiments on microbes for thousands of generations, researchers are exploring the roles of chance and history in evolution.

7. **Evolution by small steps and rugged landscapes in the RNA virus phi6. Burch, C. L., Chao, L. (1999). *Genetics* 151:921-927.** Fisher's geometric model of adaptive evolution argues that adaptive evolution should generally result from the substitution of many mutations of small effect because advantageous mutations of small effect should be more common than those of large effect. However, evidence for both evolution by small steps and for Fisher's model has been mixed. Here we report supporting results from a new experimental test of the model. We subjected the bacteriophage phi6 to intensified genetic drift in small populations and caused viral fitness to decline through the accumulation of a deleterious mutation. We then propagated the mutated virus at a range of larger population sizes and allowed fitness to recover by natural selection. Although fitness declined in one large step, it was usually recovered in smaller steps. More importantly, step size during recovery was smaller with decreasing size of the recovery population. These results confirm Fisher's main prediction that advantageous mutations of small effect should be more common. We also show that the advantageous mutations of small effect are compensatory mutations whose advantage is conditional (epistatic) on the presence of the deleterious mutation, in which case the adaptive landscape of phi6 is likely to be very rugged.

8. **Occurrence of male-specific bacteriophage in feral and domestic animal wastes, human feces, and human-associated wastewaters. Calci, K. R., Burkhardt, W. 3rd, Watkins, W. D., Rippey, S. R. (1998). *Applied and Environmental Microbiology* 64:5027-5029.** Male-specific bacteriophage (MSB) densities were determined in animal and human fecal wastes to assess their potential impact on aquatic environments. Fecal samples (1,031) from cattle, chickens, dairy cows, dogs, ducks, geese, goats, hogs, horses, seagulls, sheep, and humans as well as 64 sewerage samples were examined for MSB. All animal species were found to harbor MSB, although the great majority excreted these viruses at very low levels. The results from this study demonstrate that in areas affected by both human and animal wastes, wastewater treatment plants are the principal contributors of MSB to fresh, estuarine, and marine waters.

9. **Fecal coliform-related bacterial and coliphage populations in five lakes of southeastern Spain. Calvo, C., Gomez, M. A., Gonzalez-Lopez, J. (1998). *Microbiological Research* 153:283-288.** Aerobic heterotrophic bacteria, fecal and total coliforms, fecal streptococci and coliphages were isolated from five protected lakes in the Antequera area of Spain over the time from January to March (1994-96). The water samples contained large number of heterotrophic bacteria (mean counts 0.2 to 5.0 x 10(7) cfu per 100 ml). Most of the lakes contained fecal streptococci and a relationship between streptococci and salinity of the water samples was established. Coliphages were isolated from lakes containing fecal coliform and these bacteria were taxonomically identified as E. coli. Coliform bacilli do not seem to be an adequate indicator of fecal pollution for these ephemeral small lakes.

10. **Prophage induction of indigenous marine lysogenic bacteria by environmental pollutants. Cochran, P. K., Kellogg, C. A., Paul, J. H. (1998). *Mar. Ecol. Prog. Ser.* 164:124-133.** Lysogenic bacteria may be abundant components of bacterial assemblages in marine waters. The tremendous number of viruses found in estuarine and other eutrophic environments may be the result in part of induction of prophages. Mitomycin C is the inducing agent of choice for prophage induction; however this is not naturally found in the marine environment. We determined the capability of environmentally important pollutants to effect prophage induction in natural populations of marine bacteria. We investigated Aroclor 1248, a PCB mixture, bunker C fuel oil No. 6, and a pesticide mixture as inducing agents for natural bacterial communities from the Gulf of Mexico. Mitomycin C was also employed as a positive control for induction. Induction was determined as a significant increase in viral direct counts compared to control and ranged from 149 to 1336% of the controls. Two-thirds of the environments sampled showed prophage induction by one of the methods utilized, with the PCB mixture and Aroclor 1248 giving the highest percent efficiency (75%) of induction. This study shows that many environmentally important pollutants may be inducing agents for natural lysogenic viral production in the marine environment.

11. **Seasonal abundance of lysogenic bacteria in a subtropical estuary. Cochran, P. K., Paul, J. H. (1998). *Applied and Environmental Microbiology* 64:2308-2312.** Seasonal changes in the abundance of inducible lysogenic bacteria in a eutrophic estuarine environment were investigated over a 13-month period. Biweekly water samples were collected from Tampa Bay, Fla., and examined for prophage induction by mitomycin C treatment. At the conclusion of the study, we determined that 52.2% of the samples displayed prophage induction, as indicated by significant increases in viral direct counts compared with uninduced controls. Samples that displayed prophage induction occurred during the warmer months (February through October), when surface water temperatures were above 19 degree C, and no induction was observed in November, December, or January. This study presents clear evidence that there is seasonal variation in the number of inducible lysogenic bacteria in an estuarine environment.

12. **An estimator of the mutant frequency in assays using transgenic animals. Delongchamp, R. R., Malling, H. V., Chen, J. B., Heflich, R. H. (1999). *Mutation Research* 440:101-108.** The Poisson distribution is a fundamental probability model for count data, and is a natural model for the observed plaque counts in mutation assays using animals with lambda or PhiX174 transgenes. The Poisson likelihood for observed counts is a function of the mutant fraction, and it is straightforward to derive the associated maximum likelihood estimate of the mutant fraction and its variance. The estimate is easy to calculate, and if not the same, very similar to ad hoc estimates in current use. The model indicates the proper way to combine data from a number of plates, possibly prepared with different sample dilutions. The estimator of the mutant fraction is biased as a consequence of dividing by a random variable, the plaque count used to calculate the total recovered plaque-forming units. Fortunately, the bias becomes negligible as this count becomes large. On the other hand, increasing this count can

increase the variance by decreasing the amount of sample assayed for mutant phages. Concurrent heed to the bias and the variance provides some guidance as to the optimum allocation of a sample into portions assayed for mutant phages and total recovered phages. The distribution of the estimate of the mutant fraction is related to the binomial distribution. This relationship implies a binomial distribution for the mutant count conditional on an overall count (either the sum of mutant and counted total plaques or the sum of counted mutant and non-mutant plaques). A special but important case occurs when each plate can be evaluated for mutant plaques and non-mutant plaques. Then, the observed proportion of mutants estimates the mutant fraction. More generally, the relationship to a binomial distribution provides a procedure for calculating a confidence interval.

13. **Causative agents of bacterial mortality and the consequences to marine food webs. Fuhrman, J. A.Noble, R. T. (1999). p. ???-??? in Bell, C. R., Brylinsky, M., Johnson-Green, P. (eds.)** ***Microbial Biosystems: New Frontiers. Proc 8th Int Symp Microb. Ecol.*****. Atlantic Canada Societyfor Microbial Ecology, Halifax, Canada.**

14. **Impact of viruses on planktonic bacteria. Fuhrman, J. A. (1999). p. ???-??? in Kirchman, D. L. (ed.)** ***Microbial Ecology of the Oceans*****. Wiley & Sons, ???**

15. **Marine viruses and their biogeochemical and ecological effects. Fuhrman, J. A. (1999).** ***Nature (London)*** **399:541-???** 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.

16. **[Comparative study of Morganella and Providencia bacteriophages]. Gabrilovich, I. M., Zarochentsev, M. V., Saimov, S. R. (1998).** ***Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii*** **???:20-22.** 7 strains of M.morganii phages and 7 strains of P.rettgeri phages were isolated from lysogenic cultures and the environment. The main biological properties of these phages were studied. The phages under study formed independent taxonomic groups. These phages were found to be highly specific and capable of being used for the identification of bacteria.

17. **Detection of infectious enteroviruses, enterovirus genomes, somatic coliphages, and Bacteroides fragilis phages in treated wastewater. Gantzer, C., Maul, A., Audic, J. M., Schwartzbrod, L. (1998).** ***Applied and Environmental Microbiology*** **64:4307-4312.** In this study, three types of treated wastewater were tested for infectious enteroviruses, the enterovirus genome, somatic coliphages, and Bacteroides fragilis phages. The aim of this work was to determine whether the presence of the two types of bacteriophages or of the enterovirus genome was a good indicator of infectious enterovirus contamination. The enterovirus genome was detected by reverse transcription-polymerase chain reaction. Infectious enteroviruses were quantified by cell culturing (BGM cells), and the bacteriophages were quantified by plaque formation on the host bacterium (Escherichia coli or B. fragilis) in agar medium. Forty-eight samples of treated wastewater were analyzed. Sixteen samples had been subjected to a secondary treatment for 8 to 12 h (A), 16 had been subjected to a secondary treatment for 30 h (B1), and 16 had been subjected to both secondary and tertiary treatments (B2). The mean concentrations of somatic coliphages were 4.9 x 10(4) PFU . liter-1 for treatment line A, 9.8 x 10(3) PFU . liter-1 for B1, and 1.4 x 10(3) PFU . liter-1 for B2, with all the samples testing positive (100%). The mean concentrations of B. fragilis phages were 1.7 x 10(3) PFU . liter-1 for A (100% positive samples), 17 to 24 PFU . liter-1 for B1 (44% positive samples), and 0.8 to 13 PFU . liter-1 for B2 (6% positive samples). The mean concentrations of infectious enteroviruses were 4 most probable number of cytopathogenic units (MPNCU) . liter-1 for A (31% positive samples) and <1 MPNCU . liter-1 for B1 and B2 (0% positive samples). The percentages of samples testing positive for the enterovirus genome were 100% for A, 56% for B1, and 19% for B2. The percentages of samples testing positive for the enterovirus genome were significantly higher than those for infectious enteroviruses. This finding may have been due to the presence of noninfectious enteroviruses or to the presence of infectious enteroviruses that do not multiply in BGM cell cultures. However, under our experimental conditions, nondetection of the genome implies the absence of infectious viruses. There was a significant correlation between the concentration of somatic coliphages or B. fragilis phages and the presence of infectious enteroviruses or the presence of the enterovirus genome. However, the somatic coliphage concentration did not lead to fluctuations in the infectious enterovirus concentration, whereas the B. fragilis phage concentration did.

18. **Targeted delivery of multivalent phage display vectors into mammalian cells. Ivanenkov, V. V., Felici, F., Menon, A. G. (1999).** ***Biochimica et Biophysica Acta*** **1448:463-472.** Novel peptide motives targeting endocytosing receptors were isolated from phage display libraries of random peptides by recovering internalized phage from mammalian cells. The peptide-presenting phage selected by internalization in HEp-2 and ECV304 human cells were taken up 1000- to 100,000-fold more efficiently than their parent libraries, and from 10 to 100 times faster than phage particles displaying integrin-binding peptides. A high degree of selectivity of phage uptake was observed in these cells: phage selected in ECV304 cells were internalized approximately 100-fold more efficiently in ECV304 cells than in HEp-2 cells. Likewise, phage selected in HEp-2 cells were subsequently taken up approximately 40-fold more efficiently by HEp-2 cells than by ECV304 cells. In multiple independent trials using a cyclic peptide library, an identical peptide sequence displayed on phage was internalized by and recovered from ECV304 cells. These findings indicate that the internalization process is highly selective, and is capable of capturing a specific peptide from 2 x 10(7) peptide variants. Immunofluorescence microscopy showed juxtanuclear localization of internalized phage. These results demonstrate the feasibility of using multivalent phage-display libraries to identify new targeting ligands for the intracellular delivery of macromolecules.

19. **Uptake and intracellular fate of phage display vectors in mammalian cells. Ivanenkov, V. V., Felici, F., Menon, A. G. (1999).** ***Biochimica et Biophysica Acta*** **1448:450-462.** Receptor-mediated endocytosis is exploited in experimental systems for selective delivery of genes and drugs into specific cells. To improve targeting efficiency of delivery vectors, we have used phage display technology to isolate novel ligands for endocytosed receptors. We show here that phage vectors internalized by mammalian cells via integrin-mediated endocytosis can be rescued by cell lysis and quantitated by infection of bacteria. Immediately following uptake, phage enter an intracellular compartment where they remain intact, with phage titer unaffected by the addition of chloroquine. Phage are then translocated to a second intracellular compartment in which they are inactivated and their titer affected by chloroquine. Immunofluorescence microscopy showed an association of the second compartment with supranuclear organelles. The ability to recover internalized phage in an infectious form from two distinctive intracellular compartments provides a means to select novel ligands from phage libraries for targeted delivery of macromolecules into mammalian cells.

20. **Combined use of bacteriophage typing and pulsed-field gel electrophoresis in the epidemiological analysis of Japanese isolates of enterohemorrhagic Escherichia coli O157:H7. Izumiya, A., Masuda, T., Ahmed, R., Khakhria, R., Wada, A., Terajima, A., Itoh, K., Johnson, W. M., Konuma, H., Shinagawa, K., Tamura, K., Watanabe, H. (1998).** ***Microbiology and Immunity*** **42:515-519.** A total of 236 enterohemorrhagic Escherichia coli (EHEC) 0157:H7 isolates in Japan were investigated by bacteriophage typing, and the results were compared with those of pulsed-field gel electrophoresis (PFGE). Seven phage types (PTs) were observed in 71 isolates which were derived from 22 outbreaks. All of the isolates from ten outbreaks in the Kinki region (midwestern part of Japan) in July-August 1996 were grouped into the same PFGE type (IIa) and PT 32, while among total isolates, there were such varieties as PFGE type IIa containing five phage types and PT32 containing two PFGE types. These results suggest that the ten outbreaks should be considered to be a single outbreak, and show that the combined use of bacteriophage typing and PFGE enhances reliability in epidemiological surveys.

21. **Combined use of bacteriophage typing and pulsed-field gel electrophoresis in the epidemiological analysis of Japanese isolates of enterohemorrhagic Escherichia coli O157:H7. Izumiya, A., Masuda, T., Ahmed, R., Khakhria, R., Wada, A., Terajima, A., Itoh, K., Johnson, W.**

M., Konuma, H., Shinagawa, K., Tamura, K., Watanabe, H. (1998). ***Microbiology and Immunity*** **42:515-519.** A total of 236 enterohemorrhagic Escherichia coli (EHEC) 0157:H7 isolates in Japan were investigated by bacteriophage typing, and the results were compared with those of pulsed-field gel electrophoresis (PFGE). Seven phage types (PTs) were observed in 71 isolates which were derived from 22 outbreaks. All of the isolates from ten outbreaks in the Kinki region (midwestern part of Japan) in July-August 1996 were grouped into the same PFGE type (IIa) and PT 32, while among total isolates, there were such varieties as PFGE type IIa containing five phage types and PT32 containing two PFGE types. These results suggest that the ten outbreaks should be considered to be a single outbreak, and show that the combined use of bacteriophage typing and PFGE enhances reliability in epidemiological surveys.

22. **Anticodon nuclease: a bacterial RNA restriction enzyme. Kaufmann, G. (1999).** ***TIBS*** **???:???-???** The tRNALys-specific anticodon nuclease exists in an E. coli isolate in latent form, complexed with a DNA restriction enzyme. A phage T4-encoded alleviator of DNA restriction disrupts this masking interaction but other phage proteins repair the damaged tRNALys. Detection of a homologous system in Neisseria and a different anticodon nuclease in Colicin E5 suggest widespread occurrence of versatile tRNA restriction endonucleases. Studying them may provide new insights into the evolution of RNA recognition and cleavage mechanisms.

23. **Targeting bacteriophage to mammalian cell surface receptors for gene delivery [see comments]. Larocca, D., Witte, A., Johnson, W., Pierce, G. F., Baird, A. (1998).** ***Human Gene Therapy*** **9:2393-2399.** Filamentous bacteriophages represent one of nature's most elegant ways of packaging and delivering DNA. In an effort to develop novel methods for ligand discovery via phage gene delivery, we conferred mammalian cell tropism to filamentous bacteriophages by attaching basic fibroblast growth factor (FGF2), transferrin, or epidermal growth factor (EGF) to their coat proteins and measuring CMV promoter-driven reporter gene expression in target cells. In this system, FGF2 was a more effective targeting agent than transferrin or EGF. The detection of green fluorescent protein (GFP) or beta-galactosidase (beta-Gal) activity in cells required FGF2 targeting and was phage concentration dependent. Specificity of the targeting for high-affinity FGF receptors was demonstrated by competing the targeted phage with FGF2, by the failure of FGF2-targeted bacteriophage to transduce high-affinity FGF receptor-negative cells, and by their ability to transduce these same cells when stably transfected with FGFR1, a high-affinity FGF receptor. Long-term transgene expression was established by selecting colonies for G418 resistance, suggesting that with the appropriate targeted tropism, filamentous bacteriophage can serve as a vehicle for targeted gene delivery to mammalian cells.

24. **Occurrence and levels of phages proposed as surrogate indicators of enteric viruses in different types of sludges. Lasobras, J., Dellunde, J., Jofre, J., Lucena, F (1999).** ***Journal of Applied Microbiology*** **86:723-729.** A method based on the treatment of sludge with beef extract recovered, with similar efficiency, the three groups of bacteriophages studied from different kinds of sludges. The three groups of bacteriophages were found in high numbers in the different sludge types, the highest value being that of somatic coliphages in primary sludge of a biological treatment plant (1.1 x 10(5) pfu g-1) and the lowest being that of Bacteroides fragilis phages (110 pfu g-1) in de-watered, anaerobically, mesophilically-digested sludge. All phages studied accumulated in the sludges. In primary and activated sludges, all three types accumulated similarly but in lime-treated sludge and de-watered, anaerobically, mesophilically-digested sludge, the relative proportion of F-specific bacteriophages decreased significantly with respect to somatic coliphages and bacteriophages infecting B. fragilis. All phages survived successfully in stored sludge, depending on the temperature, and again, F-specific bacteriophages survived less successfully than the others.

25. **[Reconstruction of possible paths of the origin and morphological evolution of bacteriophages]. Letarov, A. V. (1999).** ***Genetika*** **34:1461-1469.** The problem of the origin and evolution of viruses and, in particular, the origin and evolution of bacteriophages is of considerable interest. However, so far, this problem has not been solved with quantitative methods of molecular systematics. In the present study, an attempt to reconstruct the possible paths of appearance and evolution of bacteriophages based on their structural features and morphogenesis, as well as general characteristics of their life cycles and genome organization, was carried out. A scheme describing phylogeny of the main bacteriophage groups and evolution of their life cycles is suggested. Existence of two independently evaluating types of morphogenesis ("budding outward" and "budding inward") is postulated.

26. **Bacteriophage and gene transfer. Lindqvist, B. H. (1998).** ***APMIS. SUPPLEMENTUM*** **84:15-18.**

27. **Elevated production of dimethylsulfide resulting from viral infection of cultures of Phaeocystis pouchetii. Malin, G., Wilson, W. H., Bratbak, G., Liss, P. S., Mann, N. H. (1998).** ***Limnology and Oceanography*** **43:1389-1393.**

28. **Taxonomy of bacterial viruses: establishment of tailed virus genera and the order Caudovirales [news]. Maniloff, J., Ackermann, H.-W. (1998).** ***Archives in Virology*** **143:2051-2063.**

29. **Enumeration of marine viruses in culturea nd natural samples by flow cytometry. Marie, D., Brussaard, C. P. D., Thyrhaug, G., Bratback, G., Vaulot, D. (1999).** ***Applied and Environmental Microbiology*** **65:45-52.** 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.

30. **Cloning and sequencing of major capsid protein (mcp) gene of a vibriophage, KVP20, possibly related to T-even coliphages. Matsuzaki, S., Inoue, T., Kuroda, M., Kimura, S., Tanaka, S. (1998).** ***Gene*** **222:25-30.** A large, tailed, prolate-headed vibriophage designated KVP20 was isolated from seawater. KVP20 was morphologically very similar to the previously described vibriophage, KVP40 (Matsuzaki, S., Inoue, T., Tanaka, S., 1998. Virology, 242, 314-318). However, they showed entirely different host specificities and could easily be differentiated from each other by their patterns of DNA restriction fragments. The major capsid protein (mcp) gene of KVP20 encoding the precursor of major capsid protein (pro-Mcp) was cloned and sequenced. The deduced amino-acid (aa) sequence of KVP20 pro-Mcp was compared with the reported aa sequences of KVP40 pro-Mcp, as well as of the equivalent proteins (gp23s) of coliphages T4 and RB49. There was 96.7, 57.5, and 55.2% homology to the corresponding proteins of KVP40, T4, and RB49, respectively. These data strongly suggest that the two vibriophages are closely related to each other and that they are both distantly, but definitely, related to coliphages T4 and RB49.

31. **Virulence evolution in a virus obeys a trade-off. Messenger, S. L., Molineux, I. J., Bull, J. J. (1999).** ***Proceedings of the Royal Society of London - Series B: Biological Sciences*** **266:297-404.** The evolution of virulence was studied in a virus subjected to alternating episodes of vertical and horizontal transmission. Bacteriophage f1 was used as the parasite because it establishes a debilitating but non-fatal infection that can be transmitted vertically (from a host to its progeny) as well as horizontally (infection of new hosts). Horizontal transmission was required of all phage at specific intervals, but was prevented otherwise. Each episode of horizontal transmission was followed by an interval of obligate vertical transmission, followed by an interval of obligate horizontal transmission etc. The duration of vertical transmission was eight times longer per episode in one treatment than in the other, thus varying the relative intensity of selection against virulence while maintaining selection for some level of virus production. Viral lines with the higher enforced rate of infectious transmission

evolved higher virulence and higher rates of virus production. These results support the trade-off model for the evolution of virulence.

32. **Application and evaluation of male-specific bacteriophage as a process integrity or faecal contamination indicator in a pork slaughterhouse environment. Miller, A. J., Eblen, B. S., Oser, A., Burkhardt, W. 3rd (1998).** ***Journal of Applied Microbiology*** **85:898-904.** A male-specific bacteriophage plaque assay was evaluated as a faecal contamination or process integrity indicator for aspects of the pork slaughter process. Over 400 samples were tested including: sponge swabs from animal hauling trailer floors and dressed carcass surfaces; faecal material; water from slaughter sites; and water from each stage of wastewater treatment. Bacteriophage were observed in wastewater, trailers, slaughter process water and swine faeces. No bacteriophage were observed on dressed carcasses. Numbers of phage plaque-forming units per gram or millilitre showed greater variation and were usually lower than standard indicators, including total coliform or Escherichia coli counts. Among the applications studied, male-specific bacteriophage appear to be best suited for process control verification for wastewater treatment.

33. **The so-called chromosomal verotoxin genes are actually carried by defective prophages. Mizutani, S., Nakazono, N., Sugino, Y. (1999).** ***DNA Research*** **6:141-143.**

34. **Intra-species host specificity of HaV (Heterosigma akashiwo virus) clones. Nagasaki, K., Yamaguchi, M. (1998).** ***Aquat. Microb. Ecol.*** **14:109-112.**

35. **Filamentous phage fs1 of Vibrio cholerae O139. Nakasone, N., Honma, Y., Toma, C., Yamashiro, T., Iwanaga, M. (1998).** ***Microbiology and Immunity*** **42:237-239.** Filamentous phage, fs1, was obtained from Vibrio cholerae O139. The lysogenized strains produced a large amount of fs1 phage in the culture supernatant. This phage was previously reported as novel fimbriae of that organism. The genome of the phage was a 6.5 kb single-stranded DNA. The capsid of fsl consists of a small molecule peptide (about 2.5 kDa).

36. **The effects of viral enrichment on the mortality and growth of heterotrophic bacterioplankton. Noble, R. T., Middelboe, M., Fuhrman, J. A. (1999).** ***Aquat. Microb. Ecol.*** **???:???-???**

37. **The fates of viruses in the marine environment. Noble, R. T. (1999). Ph.D Dissertation, University of Southern California.** Viruses are an important component of the marine microbial food web, as they are capable of contributing to a significant fraction of the mortality of heterotrophic bacterioplankton. To better understand the ecological roles of viruses in the ocean and their possible influences upon biogeochemical cycles, I studied the fates of viruses in relation to other components of the microbial food web. the fates of viruses were studied by examining loss of infectivity, biochemical degradation, the effects of viral enrichment on bacterial mortality, and virus production. Spatio-temporal analysis of surface seawater of Santa Monica Bay over five years demonstrated significantly higher viral and bacterial abundances during the rainy season, with nearly constant virus to bacterial ratios of about 10. Loss of infectivity was studied with the use of eight laboratory cultured host/virus systems. The decay of infectivity of these viruses was assessed in seawater, and was partitioned according to singular causative agents of decay, such as ultraviolet light, heat labile material such as extracellular enzymes, and/or particles for adsorption. Virus isolates native to Santa Monica Bay consistently degraded more slowly in full sunlight than bacteriophages isolated from the North Sea, and although sunlight was an important contributing factor to virus decay, decay due to particles and dissolved substances in seawater was also significant.

38. **Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Noble, R. T.Fuhrman, J. A. (1998).** ***Aquat. Microb. Ecol.*** **14:113-118.** 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.

39. **Phage-lift for game theory. Nowak, M. A., Sigmund, K. (1999).** ***Nature (London)*** **398:367-368.** The prisoner's dilemma is a classic of game theory in which acting for individual advantage is pitted against acting for collective benefit. An example has been identified among clones of a virus that infects bacteria.

40. **Phage viability in organic media: insights into phage stability. Olofsson, L., Ankarloo, J., Nicholls, I. A. (1998).** ***Journal of Molecular Recognition*** **11:91-93.** The stability of the filamentous phages derived from phagemid pG8H6 has been examined in a range of solvents and solvent mixtures. The results show an enhanced capacity to infect E. coli after exposure to various organic solvent-water mixtures. The dependence of stability upon solvent hydrophobicity was demonstrated. Furthermore, conditions have been identified which should allow the application of phage display libraries based upon pG8H6 in organic media.

41. **Bacteriophage: tools toward a cell-targeted delivery [comment]. Paillard, F. (1999).** ***Human Gene Therapy*** **9:2307-2308.**

42. **Targeted gene delivery to mammalian cells by filamentous bacteriophage. Poul, M. A., Marks, J. D. (1999).** ***Journal of Molecular Biology*** **288:203-211.** We report that prokaryotic viruses can be re-engineered to infect eukaryotic cells resulting in expression of a reporter gene inserted into the bacteriophage genome. Phage capable of binding mammalian cells expressing the growth factor receptor ErbB2 and undergoing receptor-mediated endocytosis were isolated by selection of a phage antibody library on breast tumor cells and recovery of infectious phage from within the cell. As determined by immunofluorescence, F5 phage were efficiently endocytosed into 100 % of ErbB2 expressing SKBR3 cells. To achieve reporter gene expression, F5 phage were engineered to package the green fluorescent protein (GFP) reporter gene driven by the CMV promoter. These phage when applied to cells underwent ErbB2-mediated endocytosis leading to GFP expression. GFP expression occurred only in cells overexpressing ErbB2, was dose-dependent reaching, 4 % of cells after 60 hours and was detected with phage titers as low as 2.0 x 10(7) cfu/ml (500 phage/cell). The results demonstrate that bacterial viruses displaying the appropriate antibody can bind to mammalian receptors and utilize the endocytic pathway to infect eukaryotic cells, resulting in expression of a reporter gene inserted into the viral genome. This represents a novel method to discover targeting molecules capable of delivering a gene intracellularly into the correct trafficking pathway for gene expression by directly screening phage antibodies. This should significantly facilitate the identification of appropriate targets and targeting molecules for gene therapy or other applications where delivery into the cytosol is required. This approach can be adapted to directly select, rather than screen, phage antibodies for targeted gene expression. The results also demonstrate the potential of phage antibodies as an in vitro or in vivo targeted gene delivery vehicle.

43. **A high incidence of prophage carriage among natural isolates of Streptococcus pneumoniae. Ramirez, M., Severina, E., Tomasz, A. (1999). *Journal of Bacteriology* 181:3618-3625.** The majority (591 of 791, or 76%) of Streptococcus pneumoniae clinical isolates examined showed the presence of two or more chromosomal SmaI fragments that hybridized with the lytA-specific DNA probe. Only one of these fragments, frequently having an approximate molecular size of 90 kb, was shown to carry the genetic determinant of the pneumococcal autolysin (N-acetylmuramic acid-L-alanine amidase). Strains carrying multiple copies of lytA homologues included both antibiotic-susceptible and -resistant isolates as well as a number of different serotypes and strains recovered from geographic sites on three continents. Mitomycin C treatment of strains carrying several lytA-hybridizing fragments caused the appearance of extrachromosomal DNA hybridizing to the lytA gene, followed by lysis of the bacteria. Such lysates contained phage particles detectable by electron microscopy. The findings suggest that the lytA-hybridizing fragments in excess of the host lytA represent components of pneumococcal bacteriophages. The high proportion of clinical isolates carrying multiple copies of lytA indicates the widespread occurrence of lysogeny, which may contribute to genetic variation in natural populations of pneumococci.

44. **Advances in the separation of bacteriophages and related particles. Serwer, P.,Griess, G. A. (1999). *Journal of Chromotography. B, Biomedical Sciences and Applications* 722:179-190.** Nondenaturing gel electrophoresis is used to both characterize multimolecular particles and determine the assembly pathways of these particles. Characterization of bacteriophage-related particles has yielded strategies for characterizing multimolecular particles in general. Previous studies have revealed means for using nondenaturing gel electrophoresis to determine both the effective radius and the average electrical surface charge density of any particle. The response of electrophoretic mobility to increasing the magnitude of the electrical field is used to detect rod-shaped particles. To increase the capacity of nondenaturing gel electrophoresis to characterize comparatively large particles, some current research is directed towards either determining the structure of gels used for electrophoresis or inducing steric trapping of particles in dead-end regions within the fibrous network that forms a gel. A trapping-dependent technique of pulsed-field gel electrophoresis is presented with which a DNA-protein complex can be made to electrophoretically migrate in a direction opposite to the direction of migration of protein-free DNA.

45. **[Vibrio cholerae temperate phage O139: characteristics and role in changing expression of chromosomal virulence genes]. Smirnova, N. I., Eroshenko, G. A., Shchelkanova, Elu., Livanova, L. F., Konnov, N. P. (1999). *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii* ???:3-9.** Restriction analysis of temperate cholera phage 139 isolated from Vibrio cholerae P16064, serogroup 0139, showed its DNA to be double-stranded linear with cohesive terminals. DNA-DNA hybridization on nylon membranes revealed that many V. cholerae strains of serogroup 0139 isolated in different regions contained a temperate cholera phage 139 in their genomes. Southern blot hybridization of chromosomal DNA PST-fragments with phage probe showed that the temperate phage 139 was identical to the temperate phage of serogroup II V. eltor. The phage integrated in the chromosome near genes encoding motility (mot) and production of the capsule (cap) and purine (pur). Phage genome is apparently responsible for instability of cap, pur, and mot genes whose products are important for the development of an infectious process in cholera.

46. **The complete genome sequence of the Streptomyces temperate phage straight phiC31: evolutionary relationships to other viruses. Smith, M. C., Burns, R. N., Wilson, S. E., Gregory, M. A. (1999). *Nucleic Acids Research* 27:2145-2155.** The completed genome sequence of the temperate Streptomyces phage straight phiC31 is reported. straight phiC31 contains genes that are related by sequence similarities to several other dsDNA phages infecting many diverse bacterial hosts, including Escherichia, Arthrobacter, Mycobacterium, Rhodobacter, Staphylococcus, Bacillus, Streptococcus, Lactobacillus and Lactococcus. These observations provide further evidence that dsDNA phages from diverse bacterial hosts are related and have had access to a common genetic pool. Analysis of the late genes was particularly informative. The sequences of the head assembly proteins (portal, head protease and major capsid) were conserved between straight phiC31, coliphage HK97, staphylococcal phage straight phiPVL, two Rhodobacter capsulatus prophages and two Mycobacterium tuberculosis prophages. These phages and prophages (where non-defective) from evolutionarily diverse hosts are, therefore, likely to share a common head assembly mechanism i.e. that of HK97. The organisation of the tail genes in straight phiC31 is highly reminiscent of tail regions from other phage genomes. The unusual organisation of the putative lysis genes in straight phiC31 is discussed, and speculations are made as to the roles of some inessential early gene products. Similarities between certain phage gene products and eukaryotic dsDNA virus proteins were noted, in particular, the primase/helicases and the terminases (large subunits). Furthermore, the complete sequence clarifies the overall transcription map of the phage during lytic growth and the positions of elements involved in the maintenance of lysogeny.

47. **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. Sommaruga, R., Sattler, B., Oberleiter, A., Wille, A., Sommaruga-W"grath, S., Psenner, R., Felip, M., Camarero, L., Pina, S., Giron□s, R., Catal n, J. (1999). *Journal of Plankton Research* 21:859-879.** 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.

48. **??? Steward, G. F.Azam, F. (1999). p. ???-??? in Bell, C. R., Brylinsky, M., Johnson-Green, P. (eds.) *Microbial Biosystems: New Frontiers. Proc. 8th Int. Symp. Microb. Ecol.*. Atlantic Canada Society for Microbial Ecology, Halifax, Canada.**

49. **Revenge of the bug zappers. Electrocuted flies spew viruses for feet - and into your food. Susman, E. (1999). *MSNBC Chicago*.** http://www.msnbc.com/news/276262.asp#BODY "Zzzzzap." Aah, the satisfying sound of a barbecue-crashing fly being electronically fried in a bug zapper. But, as it turns out, the insect may get revenge for its electrocution, spewing viruses and bacteria as far as 8 feet from the devices - and into your food. 'If you must use the units to control flies, at least use them far as possible from the food table inside the house, and away from the grill and condiment table outside.' - JOHN URBAN Kansas State University RESEARCHERS at Kansas State University in Manhattan said Wednesday that particles of electrocuted insects are spewed all over a room when the critters explode in a blaze of blue light. "Most people probably think that using electrocuting traps to control insects in one's backyard or around food-handling areas would improve sanitation, but the results of this study suggests their use actually spreads microorganisms," said John Urban, associate professor of microbiology. He spoke here at the general meeting of the American Society for Microbiology. But you don't need to abandon the devices altogether, he said. "If you must use the units to control flies, at least use them far as possible from the food table inside the house, and away from the grill and condiment table outside." Even hanging the bug zappers near a home pool would probably be safe, he said, because any viruses or bacteria blasted into the water would succumb to chlorine. His team had already shown that flies electrocuted in a common bug zapper shower bacteria for about 6 feet. The new study, he said, shows almost identical patterns of virus spread during electrocution. The zappers use light to attract pests to an electrified metal grid and then electrocute them - producing a sizzling snap, crackle and pop. For the experiments, they used a virus called FX174, which is similar in size and shape similar to the human

poliovirus but does not pose a risk to human health. The experiments were performed in an experimental chamber at the university. Team member Alberto Broce, a professor of entomology, chose houseflies as the insect model "because they are filth flies and are known to ingest microorganisms in the food they eat," Urban said. "Also, their surfaces are easily contaminated by the filth they walk upon." SURFACE MICROBES REAL DANGER When the houseflies hit the traps, their bodies literally exploded, Urban said. However, the electronic zap did not destroy all the viruses that had attached to the insect's body. As it turns out, microbes on the fly's surface were far more dangerous than any they had ingested. Overall, approximately one virus out of every 4,000 on a fly's surface was spread by electrocution, and virus was spread an average of 6 feet. Only about 1 in 1,000,000 of the viruses inside the fly were released upon electrocution, although they also spewed out as far as 6 feet, the study showed. "This is potentially significant since flies moving about on filth such as feces are most likely to become surface contaminated," he said. Feces can carry a vast number of organisms that are harmful to human health, causing disorders ranging from the severe diarrhea of roatvirus infection to the crippling cramps of shigella infection.

50. **Bacteriophage inactivation at the air-water-solid interface in dynamic batch systems. Thompson, S. S., Yates, M. V. (1999).** ***Applied and Environmental Microbiology*** **65:1186-1190.** Bacteriophages have been widely used as surrogates for human enteric viruses in many studies on virus transport and fate. In this investigation, the fates of three bacteriophages, MS2, R17, and phiX174, were studied in a series of dynamic batch experiments. Both MS2 and R17 readily underwent inactivation in batch experiments where solutions of each phage were percolated through tubes packed with varying ratios of glass and Teflon beads. MS2 and R17 inactivation was the result of exposure to destructive forces at the dynamic air-water-solid interface. phiX174, however, did not undergo inactivation in similar studies, suggesting that this phage does not accumulate at air-water interfaces or is not affected by interfacial forces in the same manner. Other batch experiments showed that MS2 and R17 were increasingly inactivated during mixing in polypropylene tubes as the ionic strength of the solution was raised (phiX174 was not affected). By the addition of Tween 80 to suspensions of MS2 and R17, phage inactivation was prevented. Our data suggest that viral inactivation in simple dynamic batch experiments is dependent upon (i) the presence of a dynamic air-water-solid interface (where the solid is a hydrophobic surface), (ii) the ionic strength of the solution, (iii) the concentration of surface active compounds in the solution, and (iv) the type of virus used.

51. **Hybrid frequencies confirm limit to coinfection in the RNA bacteriophage phi-6. Turner, P. E., Burch, C., Hanley, K., Chao, L. (1999).** ***Journal of Virology*** **73:2420-2424.** Coinfection of the same host cell by multiple viruses may lead to increased competition for limited cellular resources, thus reducing the fitness of an individual virus. Selection should favor viruses that can limit or prevent coinfection, and it is not surprising that many viruses have evolved mechanisms to do so. Here we explore whether coinfection is limited in the RNA bacteriophage phi6 that infects Pseudomonas phaseolicola. We estimated the limit to coinfection in phi6 by comparing the frequency of hybrids produced by two marked phage strains to that predicted by a mathematical model based on differing limits to coinfection. Our results provide an alternative method for estimating the limit to coinfection and confirm a previous estimate between two to three phages per host cell. In addition, our data reveal that the rate of coinfection at low phage densities may exceed that expected through random Poisson sampling. We discuss whether phage phi6 has evolved an optimal limit that balances the costly and beneficial fitness effects associated with multiple infections.

52. **Prisoner's dilemma in an RNA virus. Turner, P. E., Chao, L. (1999).** ***Nature (London)*** **398:441-443.** The evolution of competitive interactions among viruses was studied in the RNA phage phi6 at high and low multiplicities of infection (that is, at high and low ratios of infecting phage to host cells). At high multiplicities, many phage infect and reproduce in the same host cell, whereas at low multiplicities the viruses reproduce mainly as clones. An unexpected result of this study was that phage grown at high rates of co-infection increased in fitness initially, but then evolved lowered fitness. Here we show that the fitness of the high-multiplicity phage relative to their ancestors generates a pay-off matrix conforming to the prisoner's dilemma strategy of game theory. In this strategy, defection (selfishness) evolves, despite the greater fitness pay-off that would result if all players were to cooperate. Viral cooperation and defection can be defined as, respectably, the manufacturing and sequestering of diffusible (shared) intracellular products. Because the low-multiplicity phage did not evolve lowered fitness, we attribute the evolution of selfishness to the lack of clonal structure and the mixing of unrelated genotypes at high multiplicity.

53. **Sex and the evolution of intrahost competition in RNA virus ?6. Turner, P. E., Chao, L. (1998).** ***Genetics*** **150:523-532.** Sex allows beneficial mutations that occur in separate lineages to be fixed in the same genome. For this reason, the Fisher-Muller model predicts that adaptation to the environment is more rapid in a large sexual population than in an equally large asexual population. Sexual reproduction occurs in populations of the RNA virus phi6 when multiple bacteriophages coinfect the same host cell. Here, we tested the model's predictions by determining whether sex favors more rapid adaptation of phi6 to a bacterial host, Pseudomonas phaseolicola. Replicate populations of phi6 were allowed to evolve in either the presence or absence of sex for 250 generations. All experimental populations showed a significant increase in fitness relative to the ancestor, but sex did not increase the rate of adaptation. Rather, we found that the sexual and asexual treatments also differ because intense intrahost competition between viruses occurs during coinfection. Results showed that the derived sexual viruses were selectively favored only when coinfection is common, indicating that within-host competition detracts from the ability of viruses to exploit the host. Thus, sex was not advantageous because the cost created by intrahost competition was too strong. Our findings indicate that high levels of coinfection exceed an optimum where sex may be beneficial to populations of phi6, and suggest that genetic conflicts can evolve in RNA viruses.

54. **Changes in bacterial and eukaryotic community structure after mass lysis of filamentous cyanobacteria associated with viruses. van Hannen, E. J., Zwart, G., van Agterveld, M. P., Gons, H. J., Ebert, J., Laanbroek, H. J. (1999).** ***Applied and Environmental Microbiology*** **65:795-801.** 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.

55. **Bacteriophage biology and bacterial virulence. Waldor, M. K. (1998).** ***Trends in Microbiology*** **6:295-297.**

56. **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.**

57. **Measurements of DNA damage and photoreactivation imply that most viruses in marine surface waters are inefective. Wilhelm, S. W., Weinbauer, M. G., Suttle, C. A., Pledger, R. J., Mitchell, D. L. (1998).** ***Aquat. Microb. Ecol.*** **14:215-222.** 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.

58. **The role of sunlight in the removal and repair of viruses in the sea. Wilhelm, S. W., Weinbauer, M. G., Suttle, C. A., Jeffrey, W. H. (1998).** ***Limnology and Oceanography*** **43:586-592.** 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.

59. **Hybridization analysis of Chesapeake Bay Virioplankton. Wommack, K. E., Ravel, J., Hill, R. T., Colwell, R. R. (1999).** ***Applied and Environmental Microbiology*** **65:241-250.** 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.

60. **Population dynamics of Chesapeake Bay virioplankton: total-community analysis by pulsed-field gel electrophoresis. Wommack, K. E., Ravel, J., Hill, R. T., Colwell, R. R. (1999).** ***Applied and Environmental Microbiology*** **65:231-240.** 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.

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