

# Bacteriophage Ecology Group (BEG) News

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

© Stephen T. Abedon (editor)

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October 1, 2001 issue (volume 10)

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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, <u>I wish to encourage as many people as possible to seek to relieve me of this duty</u>, <u>as often as possible</u>. Additionally, I welcome suggestions of topics that may be addressed. Please address all correspondences to <u>abedon.1@osu.edu</u> or to "Editorials," *Bacteriophage Ecology Group News*, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. Please send all submissions as Microsoft Word documents, if possible (I'll let you know if I have trouble converting other document formats), and in English.

### **The Bacteriophage Rise**

At the level of individual infections our molecular understanding of phages integrates with our ecological understanding within a framework that I call phage organismal ecology (see Which Ecology are You?). The concerns of the phage organismal ecologist are those things that contribute to phage per-infection productivity. For example, the length of a phage's life cycle will be shorter (i) the more rapidly adsorption occurs, (ii) the shorter the eclipse period, (iii) the less time spent producing phages, and (iv) the more rapidly phage progeny are released once release (e.g., lysis) has been initiated.

The standard assays employed by phage organismal ecologists are single-step growth experiments and phageadsorption assays. Delbrück (1942) distinguished various aspects of single-step growth including "the *constant period*, the *rise period*, and the *burst size*." The rise period is "an indication of the latent periods of virus growth" and refers to the period beginning when populations of synchronously phage-infected bacteria begin lysing and which ends when these populations finish lysing. It is clear from Delbrück's Figure 1 that the rise period begins as the constant period (minimum latent period) ends.

© Phage et al.

Nearly a decade after Delbrück began, along with Ellis, his famous single-step growth experiments (1939), Doermann (1947; 1952; Doermann & Dissosway, 1948) figured out how to penetrate the mystery of phage intracellular development via artificial bacterial lysis. From these intracellular phage growth experiments Doermann was able to identify two additional aspects of phage single-step growth: (i) His famous eclipse period which is the time during which the artificial lysis of an infected bacterium does not release mature phage progeny and (ii) his less famous but no less important period of accumulation of phage progeny within not-yet-lysed phage-infected bacteria.

Unfortunately, unlike the eclipse, Doermann failed to supply us with a catchy moniker for the period during which the artificial lysis of an infected bacterium *does* result in the release of phage progeny, referring to this period instead only as "intracellular phage growth" (Doermann & Dissosway, 1948), "mature phage is found to accumulate" (Doermann, 1952), or "accumulation of infectious phage particles" (Doermann, 1967). Note that though Doermann and Dissosway (1948) at least once use the term "rise" ("This hypothesis would also predict that a curve describing intracellular phage growth in mass cultures should bend upward during the first portion of the rise..."), technically, this period is not equivalent to Delbrück's rise. Lately, though, I've noticed a tendency by a number of researchers to describe this period of intracellular phage growth as a rise.

The increase in phage density observed by Doermann (1947; 1952) during his intracellular growth experiments is an artifact of various methods of induced bacteria lysis, unlike the rise period of Delbrück (1942). During phage growth as it occurs under almost all other conditions there is no extracellular "rise" corresponding to this period of intracellular phage-progeny accumulation (or "multiplication proper" to use Ackermann & Dubow's, 1987, terminology). Consequently, I propose here that we avoid using one term to describe two distinct phage single-step events, particularly by not using the term *rise* to describe aspects of phage intracellular growth. Instead, I propose substituting a word or phrase that better captures the idea that this is a natural process that occurs within infected cells and that is highly relevant to phage per-infection productivity. Lately I have been using the phrase, "Period of (phage) progeny maturation" (Abedon *et al.*, 2001). What do you think?

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

is the Developer and Editor of The Bacteriophage Ecology Group web site which is dedicated to the ecology and evolutionary biology of the parasites of unicellular organisms (UOPs)

#### REFERENCES

Abedon, S.T., T.D. Herschler, and D. Stopar. 2001. Bacteriophage latent-period evolution as a response to resource availability. *Appl. Environ. Microbiol.* **67**:4233-4241.

Ackermann, H.-W. and M.S. DuBow . 1987. Viruses of Prokaryotes, Volume 1, General Properties of Bacteriophages. CRC Press, Boca Raton, Florida.

Delbrück, M. 1942. Bacterial viruses (bacteriophages). Adv. Enzymol. 2:1-32.

**Doermann, A.H.** 1947. Intracellular growth of bacteriophage. Year Book Carnegie Institute of Washington **47**:176-182.

**Doermann, A.H.** 1952. The intracellular growth of bacteriophages. I. Liberation of intracellular bacteriophage T4 by premature lysis with another phage or with cyanide. *J. Gen. Physiol.* **35**:645-656.

**Doermann, A.H.** 1967. The eclipse in the bacteriophage life cycle, p. 79-87. In J. Cairns, G.S. Stent, and J.D. Watson (eds.), *Phage and the Origins of Molecular Biology*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

**Doermann, A.H. and C. Dissosway**. 1948. Intracellular growth and genetics of bacteriophage. Year Book Carnegie Institute of Washington **48**:170-176.

Ellis, E.L. and M. Delbrück. 1939. The growth of bacteriophage. J. Gen. Physiol. 22:365-384.

### **Editorial Archive**

- BEG: What we are, Where we are, Where we're going by Stephen T. Abedon
- When Grown In Vitro, do Parasites of Multicellular Organisms (MOPs) become Unicellular Organism Parasites (UOPs)? by Stephen T. Abedon
- Bacteriophages as Model Systems by Stephen T. Abedon
- 2000 and Sun: A Phage Odyssey by Stephen T. Abedon
- Lytic, Lysogenic, Temperate, Chronic, Virulent, Quoi? by Stephen T. Abedon
- Which Ecology are You? by Stephen T. Abedon
- Science NetWatch October 13, 2000
- The Best of Times, the Worst of Times by Ry Young
- Naming Bacteriophages by Hans-Wolfgang Ackermann and Stephen T. Abedon
- The Bacteriophage Rise by Stephen T. Abedon

# **New BEG Members**

The BEG members list can be found at www.phage.org/beg\_members.htm as well as 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, e.g., 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 the full reference, including the abstract, if the reference is not already present in the **BEG bibliography**. Responsibility of members includes keeping the information listed on the **BEG members 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 Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. To join BEG as a non-member, please contact BEG using the following link: abedon.1@osu.edu and minimally include your name and e-mail address.

### Please welcome our newest members

name (home page links)	status	e-mail	address	
Tai-You Ha	PI	tyha77 @yahoo.com	Department of Microbiology and Immunology, Chonbuk National University Medical School, 2-20 Kumahm-dong sun, Duckjin-ku, Chonju, Chonbuk 561-182, South Korea	
	interests:	Vibrio vulnificus (a halophilic bacterium) phage. (contents   BEG members   top of page)		
	PI	ray.kepner @marist.edu	Biology Department, Marist College, 238 Donnelly Hall, 3399 North Road, Poughkeepsie, NY 12601	
Raymond L. Kepner, Jr.	interests:	Aquatic microbial ecology, freshwater cyanophage and bacteriophage, polar limnology, extremophiles. I am currently working on the isolation and characterization of UOPs from the Canadian High Arctic, Antarctica, and the Hudson River. (contents   BEG members   top of page)		
Cothoring Los Carrillo		sbxcml @nottingham.ac.uk	Division of Food Sciences, Sutton Bonington Campus, University of Nottingham. Loughborough. LE12 5RD United Kingdom	
Catherine Loc-Carrillo	interests:	Isolating and characterising Campylobacter bacteriophage from poultry. (contents   BEG members   top of page)		
Daniel Nelson	PI	nelsond @rockefeller.edu	The Rockefeller University, Laboratory of Bacterial Pathogenesis and Immunology, 1230 York Ave., Box 172, New York, NY 10021	
	interests:	Bacteriophage lytic enzymes and experimental bacteriophage therapies. (contents   BEG members   top of page)		
	PI	jpaul @seas.marine.usf.edu	University of South Florida, Department of Marine Sciences, 140 Seventh Avenue South, St. Petersburg, FL 33701	
John H. Paul	interests:	Viruses in the marine environment, focusing on lysogeny. We have determined a seasonality in lysogeny, with few inducible lysogens in winter, but a dramatic increase occurring in early spring (February in Florida) that continues throughout the summer. Another component of our research involves development of molecular methods to detect human pathogenic RNA viruses in coastal surface and ground waters. (contents   BEG members   top of 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 at www.phage.org/beg\_links.htm). Listed below are new links found on that page. 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.

# New Bacteriophage Ecology Links

- Bacteriophage multiplication in biofilm communities
- Combating Phage Rage
- Development and Application of Strategies to Generate Bacteriophage Resistant Strains for Use in Milk Fermentation Processes
- Effects of Polymer Soil Treatment on Subsurface Virus and Bacteria Transport
- The mode of action and risk assessment of bacteriophage associated with virulent strains of the marine pathogen, Vibrio harveyi
- Survival of the Fittest Bacteriophage

# **New Links to Phage-Ecology Abstracts**

- Application of *Streptococcus thermophilus* DPC1842 as an adjunct to counteract bacteriophage disruption in a predominantly lactococcal Cheddar cheese starter: use in bulk starter culture systems
- Effect of Lactococcus lactis ssp.lactis ml3 and c2 bacteriophage peptides and Lactobacillus plantarum yit0068 bacteriophage peptides on the growth of L.lactis ssp.lactis C2 and the inhibition of ml3 and c2 bacteriophage proliferation (PDF document)
- Inhibition of Lactobacillus plantarum yit0068 bacteriophage proliferation in L. plantarum host grown in medium containing Lactococcus lactis ssp. lactis c2 phage-peptide
- · Sanitation/Phage Control: The How, What, Why, Where, and When of Bacteriophage Control (PDF document)
  - Virus Attenuation at a Recharge Basin Receiving Reclaimed Water for Artificial Recharge

# **New Phage-Therapy Links**

- A few years ago there was a lot of publicity about phages as an alternative to antibiotics. With recent increases in antibiotic resistant strains what has happened to phages?
- An Alternative to Antibiotics
- Antibacterial therapy may only be a phage away
- Antibiotic resistance may see return to phage therapy
- Bacteriophage a new approach for combating nosocomial respiratory infections caused by *Pseudomonas* aeruginosa
- Bacteriophage (AgriPHAGE) Pesticide Petition Filing 4/00
- Bacteriophage Having Multiple Host Range
- Bacteriophage Therapy of Pseudomonas Burn Wound Sepsis
- Bacteriophages: An alternative to antibiotics?
- Biotech firm tries a novel tactic in fighting bacteria (Puget Sound Business Journal)
- Cocktail that cures
- Combating the antibiotic resistance crisis: Therapeutic use of Bacteriophages (Viruses) for Treating Acne, a
  Bacterial Disease
- Comparing bacteriophages to antibiotics
- Frequently Asked Questions
- Germ Warfare
- Georgian Republic Children's Hospital An antibiotic alternative from Russia (you will need to scroll down somewhat)
- History of the Bacteriophage and Phage Therapy
- The Hunt is On: For New Ways to Overcome Bacterial Resistance
- Microorganisms in foods and around them
- The Next Phage
- Novel Bacteriophage Therapies for Vibrio cholerae Infection
- An Old Fashion, Hi-Tech Answer to Antibiotic Resistance: Bacteriophage Therapies Come Roaring Back
- Phage Therapy
- Phage therapy--advantages over antibiotics?
- Phage Therapy: Merril & Scholl Interview's
- Phage Therapeutics lands \$5 million (Puget Sound Business Journal)
- Phages: Bacteria-Killing Viruses May Fight For Humankind Again
- Researchers Find Novel Way to Kill Streptococci Bacteria
- Southern Phage Group NZ (Phage Therapy Project)
- Study focuses on natural way to fight bacterial infection in cattle
- 'Superbug' victim saved by killer virus treatment
- Therapeutic Use of Bacteriophages in Bacterial Infections
- Therapy of infections in cancer patients with bacteriophages
- Turn of a Phrase Section: Phage Therapy (Wide World of Words)
- The use of a bacteriophage of *Vibrio vulnificus* for the potential treatment of bacterial infection (search for "antibacterials")
- The use of bacterial viruses in the treatment of *C. difficile* associated disease and *Psuedomonas aeruginosa* infections of burn wounds
- Viruses that attack bacteria may help meet the challenge of antibiotic resistance
- Viruses May Replace Some Antibiotics (will need to scroll down some)
- Where communism succeeded and capitalism failed
- Yahoo! Phage Therapy and Molecular Microbiology Group

# **Other New Phage-Related Sites**

- Bacteriophage (an overview)
- Bacteriophage (an overview)
- Bacteriophage (an overview)

- Bacteriophage (an overview)
- Bacteriophage (Xrefer)
- Bacteriophage Lecture Notes
- Completed Genomes: Phages (EMBL-European Bioinformatics Institute)
- Development of Second Generation Bacteriophage Resistant Starter Cultures for Cheddar and Mozzarella Cheese Manufacture
- Electron Micrograph Libarary Virus & Bacteriophage
- Encyclopedia.Com Bacteriophage
- FastPlaqueTB
- Frank's Complete Public Bacteriophage Genomes List
- Genomes Phages (this is an annotated list of phage genomes; very cool!) (
- International Bacteriophage Genomics Groups
- Isolation and characterization of bacteriophage resistance mechanisms in Lactococcus lactis and use of them for construction of more phage resistant starter cultures
- Mycobacteriophage Replication Technology
- Phage-Tech Interest Group (PhTIG)
- [Phage] Virus [a computer program that "creates a feminist map of the machine through its non-hierarchical organization and its divorce of creative control (and reproductive control) from the user to the machine"!] (no, I haven't tested it on my own computer)
- Test for Phage Contamination of IMAGE Clones
- The T-Phage Page
- Viruses (overview with lots of phage stuff)
- www.bacteriophage.com (a.k.a., MAGS.net)
- www.bacteriophage.net (a.k.a., D & D Pharma)
- www.desiere.com (a.k.a., Frank Desiere's Homepage)
- www.desiere.net (ditto)
- www.phage.com (a.k.a., Phage ISP Business Solutions)
- www.phage.net (a.k.a., Exponential Biotherapies, Inc.)
- www.phagebase.org (a site devoted to bacteriophage genomics)
- www.phages.org (a.k.a., The Phage Phorum)
- www.phagetherapy.com
- Yahoo! Phage Group
- Your Home Bacteriophage Lab

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# **New Features**

In this section I 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.

#### No entry.

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# **Meetings**

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

Editor's note: Please send photos, etc. from meetings for inclusion in this section.

**Below:** Scene from the Saint Lawrence Seaway final-night banquet at the June, 2000, Montreal Phage meeting (Dwight Hall (far left), ?, ?, Sarah Larson (center-left), ?, Chelsea Thomas (center-right) Lindsay Black (far right)):





Below: The 2001 Microbial Population Biology Gordon Conference (use your browser zoom-in option for a clearer view of the participants):



Lin Chao, Chair & Siv G.E. Andersson, Vice Chair

Gordon Research Conferences Microbial Population Biology Williams College, Williamstown, Massachusetts July 29-August 3, 2001 Chair: Lin Chao

#### Left to Right

Row 1: C. Wilke, J. Eisen, M. Blot, J. Smith, C. Frank, M. Winfield, S. Sarkar, E. Ostrowski, J. Bacher, L. Chao, S. Andersson, L. Cowen, M. Achtman, B. Levin, R. Redfield, C. Devine, L. Ancel, R. Zappala, A. Platt

Row 2: C. Adami, K. Stredwick, C. Ofria, P. Turner, R. Milkman, L. Raleigh, D. Rozen, J. Strassmann, D. Queller, M. DuBois, T. Cooper, L. Wahl, I. Matsumura, S. Remold, V. Souza, L. Eguinte, M. Riehle, J. Yin, L. Pagie, B. Kerr

Row 3: C. Davis, C. Jessup, D. Stoebel, C. Istock, J. Bell, M. Roosnick, E. Domingo, P. Reens, D. Gordon, J. Baum, S. Wain-Hobson, D. Schneider, R. Woods, S. Brisse, H. Ochman, D. Dyer, C. Schlotterer, D. Brisoon

Row 4: M. Liu, I. Wang, S. Patin, F. Fiegna, O. Tenaillon, O. Silander, P. Young, P. Fuerst, A. Poon, B. Kirkup, J. Wertz, F. Cohan, T. Dean, K. O'Keefe, F. Stewart, C. Burch, N. Balaban

Row 5: D. Guttman, B. Bohannan, J. Lawrence, F. Dionisio, I. Matic, M. Travisano, M.

Poss, S. Abeden, G. Velicer, F. Attan, D. Weinreich, T. Berngruber, V. Cooper, S. Bonoeffer, M. Ackermann, C. Strompl, K. Lythgoe

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### Jobs

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

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### **Submissions**

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

#### No entry.

### **Submissions Archive**

- On an Invisible Microbe Antagonistic to the Dysentery Bacillus by Felix d'Herelle
- Obituary: Hansjürgen Raettig Collector of Bacteriophage References (October 12, 1911 December 1, 1997)
- Some Quotations
- Bacteriophages: A Model System for Human Viruses
- How Big is 10<sup>30</sup>?
- Selling Phage Candy
- A List of Phage Names

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### Letters & Questions

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., Mansfield, 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 by clicking the authors name. Please note that these questions have not been edited for grammar, spelling, or clarity.

#### 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. No copyrighted material without permission, please!





### **Phage Image Archive**

- BEG Phage Images Page
- The Face of the Phage
- Bacteriophage T2 by H.-W. Ackermann
- SSV1-Type Phage
- Saline Lake Bacteriophage David Bird
- Coliphage LG1 Larry Goodridge
- Bacteriophage HK97 Bob Duda
- Phage T4 (art) Francis S. Lin
- Phage T4 on the pedestal outside of Barker Hall at Berkeley
- Electron micrograph of phage P22
- Thin section of T4 phages hitting a microcolony of E. coli K-12 John Wertz

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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*.

Editors Note: In the following list I've included a number of publications that deal with the evolutionary biology of eukaryote viruses growing in tissue culture (see When Grown In Vitro, do Parasites of Multicellular Organisms (MOPs) become Unicellular in the October 1, 1999, edition of BEG News). I've also incorporated into the BEG bibliography all such studies (that I know of) authored by Santiago Elena and Isabel Novella. If you know of any additional studies of this type, please let me know.

- 1. Bacteriophage latent-period evolution as a response to resource availability. Abedon, S. T., Herschler, T. D., Stopar, D. (2001). Applied and Environmental Microbiology 67:4233-4241. [PRESS FOR ABSTRACT]
- 2. Le matin des bactériophages. Ackermann, H.-W. (2001). Virologie 5:35-43. [PRESS FOR ABSTRACT]
- 3. Frequency of morphological phage descriptions in the year 2000. Brief Review. Ackermann, H.-W. (2001). Archives of Virology 146:843-857. [PRESS FOR ABSTRACT]

- Optimisation and standardisation of a method for detecting and enumerating bacteriophages infecting Bacteroides fragilis. Araujo, R., Muniesa, M., Mendez, J., Puig, A., Queralt, N., Lucena, F., Jofre, J. (2001). Journal of Virological Methods 93:127-136. [PRESS FOR ABSTRACT]
- 5. The use of bacteriophages for treatment and prevention of bacterial disease in animals and animal models of human infection. Barrow, P. A. (2001). *Journal of Chemical Technology and Biotechnology*. 76:677-682. [PRESS FOR ABSTRACT]
- 6. Phage treatment: can we utilise it for certain infective diseases in India? Bhatia, R. S. (2001). Journal of the Association of Physicians of India 49:590. [NO ABSTRACT]
- 7. Phages of Lactococcus lactis: an ecological and economical equilibrium. Boucher, I, Moineau, S. (2001). Recent Research Developments in Virology 3:243-256. [PRESS FOR ABSTRACT]
- 8. Generalized transduction in *Streptomyces coelicolor*. Burke, J., Schneider, D., Westpheling, J. (2001). *Proceedings of the National Academy of Sciences of the United States of America* 98:6289-6294. [PRESS FOR ABSTRACT]
- 9. The origins and evolution of viruses. Campbell, A. (2001). Trends in Microbiology 9:61-61. [NO ABSTRACT]
- 10. Phages and their application against drug-resistant bacteria. Chanishvili, N., Chanishvili, T., Tediashvili, M., Barrow, P. A. (2001). *Journal of Chemical Technology and Biotechnology*. 76:689-699. [PRESS FOR ABSTRACT]
- Application of digital image analysis and flow cytometry to enumerate marine viruses stained with SYBR gold. Chen, F., Lu, J. R., Binder, B. J., Liu, Y. C., Hodson, R. E. (2001). *Applied and Environmental Microbiology* 67:539-545. [PRESS FOR ABSTRACT]
- 12. Estimation of the average burst size of Phix174 am3, cs70 for use in mutation assays with transgenic mice. Delongchamp, R. R., Valentine, C. R., Malling, H. V. (2001). *Environmental and Molecular Mutagenesis* 37:356-360. [PRESS FOR ABSTRACT]
- 13. Transduction by phiBB-1, a bacteriophage of *Borrelia burgdorferi*. Eggers, C. H., Kimmel, B. J., Bono, J. L., Elias, A. F., Rosa, P., Samuels, D. S. (2001). *Journal of Bacteriology* 183:4771-4778. [PRESS FOR ABSTRACT]
- 14. Development and optimization of a novel immunomagnetic separation- bacteriophage assay for detection of Salmonella enterica serovar enteritidis in broth. Favrin, S. J., Jassim, S. A., Griffiths, M. W. (2001). Applied and Environmental Microbiology 67:217-224. [PRESS FOR ABSTRACT]
- 15. Reviewing efficacy of alternative water treatment techniques. Hambidge, A. (2001). *Health Estate* 55:23-25. [PRESS FOR ABSTRACT]
- Reduction in exopolysaccharide viscosity as an aid to bacteriophage penetration through *Pseudomonas aeruginosa* biofilms. Hanlon, G. W., Denyer, S. P., Olliff, C. J., Ibrahim, L. J. (2001). *Applied and Environmental Microbiology* 67:2746-2753. [PRESS FOR ABSTRACT]
- 17. Filamentous phage associated with recent pandemic strains of *Vibrio parahaemolyticus*. lida, T., Hattori, A., Tagomori, K., Nasu, H., Naim, R., Honda, T. (2001). *Emerging Infectious Diseases* 7:477-478. [PRESS FOR ABSTRACT]
- 18. Human adenoviruses and coliphages in urban runoff-impacted coastal waters of Southern California. Jiang, S., Noble, R., Chu, W. (2001). *Applied and Environmental Microbiology* 67:179-184. [PRESS FOR ABSTRACT]
- 19. Comparative study of vaginal *Lactobacillus* phages isolated from women in the United States and Turkey: prevalence, morphology, host range, and DNA homology. Kilic, A. O., Pavlova, S. I., Alpay, S., Kilic, S. S., Tao, L. (2001). *Clinical and Diagnostic Laboratory Immunology* 8:31-39. [PRESS FOR ABSTRACT]
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- 39. Flow cytometric detection of viruses. Brussaard, C. P. D., Marie, D., Bratbak, G. (2000). Journal of Virological Methods 85:175-182. [PRESS FOR ABSTRACT]
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- 58. [Isolation and comparative study of a group of temperate bacteriophages of rhizospheric pseudomonads *Pseudomonas putida*]. Shaburova, O. V., Burkal'tseva, M. V., Pleteneva, E. A., Krylov, V. N. (2000). *Genetika* 36:915-919. [PRESS FOR ABSTRACT]
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- 60. Phage conversion of exfoliative toxin A production in *Staphylococcus aureus*. Yamaguchi, T., Hayashi, T., Takami, H., Nakasone, K., Ohnishi, M., Nakayama, K., Yamada, S., Komatsuzawa, H., Sugai, M. (2000). *Molecular Microbiology* 38:694-705. [PRESS FOR ABSTRACT]
- 61. Rate of deleterious mutation and the distribution of its effects on fitness in vesicular stomatitis virus. Elena, S. F., Moya, A. (1999). *Journal of Evolutionary Biology* 12:1078-1088. [PRESS FOR ABSTRACT]
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- 64. Effect of population patchiness and migration rates on the adaptation and divergence of vesicular stomatitis virus quasispecies populations. Miralles, R., Moya, A., Elena, S. F. (1999). *Journal of General Virology* 80:2051-2059. [PRESS FOR ABSTRACT]
- 65. Lack of evolutionary stasis during alternating replication of an arbovirus in insect and mammalian cells. Novella, I. S., Hershey, C. L., Escarmis, C., Domingo, E., Holland, J. J. (1999). *Journal of Molecular Biology* 287:459-465. [PRESS FOR ABSTRACT]
- 66. Exponential fitness gains of RNA virus populations are limited by bottleneck effects. Novella, I. S., Quer, J., Domingo, E., Holland, J. J. (1999). *Journal of Virology* 73:1668-1671. [PRESS FOR ABSTRACT]
- 67. Evolutionary dynamics of fitness recovery from the debilitating effects of Muller's ratchet. Elena, S. F., Davila, M., Novella, I. S., Holland, J. J., Domingo, E., Moya, A. (1998). *Evolution* 52:309-314. [PRESS FOR ABSTRACT]
- 68. Bacteriophage: tools toward a cell-targeted delivery. Paillard, F. (1998). Human Gene Therapy 9:2307-2308. [NO ABSTRACT]
- 69. Population dynamics of phytoplankton and viruses in a phosphate-limited mesocosm and their effect on DMSP and DMS production. Wilson, W. H., Turner, S., Mann, N. H. (1998). *Estuarine, Coastal and Shelf Science* 46 (Supplement a):49-59. [PRESS FOR ABSTRACT]

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### **New Publications with Abstracts**

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

1. Bacteriophage latent-period evolution as a response to resource availability. Abedon, S. T., Herschler, T. D., Stopar, D. (2001). Applied and Environmental Microbiology 67:4233-4241. Bacteriophages (phages) modify microbial communities by lysing hosts, transferring genetic material, and effecting lysogenic conversion. To understand how natural communities are affected it is important to develop predictive models. Here we consider how variation between models in eclipse period, latent period, adsorption constant, burst size, the handling of differences in host quantity and host quality, and in modeling strategy can affect predictions. First we compare two published models of phage growth, which differ primarily in terms of how they model the kinetics of phage adsorption; one is a computer simulation and the other is an explicit calculation. At higher host quantities (~10<sup>8</sup> cells/ml), both models closely predict experimentally determined phage population growth rates. At lower host quantities (10<sup>7</sup> cells/ml), the computer simulation continues to closely predict phage growth rates, but the explicit model does not. Next we concentrate on predictions of latent-period optima. A latent-period optimum is the latent period that maximizes the population growth of a specific phage growing in the presence of a specific quantity and quality of host cells. Both models predict similar

latent-period optima at higher host densities (e.g., 17 min at 10<sup>8</sup> cells/ml). At lower host densities, however, the computer simulation predicts latent-period optima that are much shorter than those suggested by explicit calculations (e.g., 90 versus 1,250 min at 10<sup>5</sup> cells/ml). Finally, we consider the impact of host quality on phage latent-period evolution. By taking care to differentiate latent-period phenotypic plasticity from latent-period evolution, we argue that the impact of host quality on phage latent-period evolution may be relatively small.

- 2. Le matin des bactériophages. Ackermann, H.-W. (2001). Virologie 5:35-43. With about 5 150 electron microscopic observations, phages contstitute the larges of all viral groups. The International Committee on Taxonomy of Viruses (ICTV) persently recognized one order, 13 families, and 30 genera. The order *Caudovirales* includes three families of tailed phages and about 5 000 members (96.4%). The 10 families of icosahedral, filamentous, or pleomorphic phages totalize 186 viruses. Phages are found in all the bacterial world and, with up to 10<sup>10</sup> particles/ml in seawater, seem to be the most frequent microbes of Earth. Phages are polyphyletic in origin. Tailed phages appear to be the msot ancient viruses and recombination with exchange of genes or gene blocs (modular evolution) seems to be their preferred way of evolution. Tailed phages and herpesviruses present multiple analogies. Harmful phages can create havoc in bacterial fermentations, especially in the dairy industry. By contrast, phages are very useful in general bacteriology, therapy of infectious diseases is making a come-back and phages are likely to have a brilliant future in research.
- 3. Frequency of morphological phage descriptions in the year 2000. Brief Review. Ackermann, H.-W. (2001). Archives of Virology 146:843-857. Over 5100 bacteria viruses have been examined in the electron microscope since 1959. About 4950 phages (96%) are tailed and only 186 phages (3.6%), are cubic, filamentous, or pleomorphic. Phages belong to 13 virus families and occur in over 140 bacterial genera. Phages are listed by morphotypes and host genera. Siphoviridae or phages with long, noncontractile tails compromise 61% of tailed phages. The distribution of phages in different bacterial phylogenetic divisions is shown.
- 4. Optimisation and standardisation of a method for detecting and enumerating bacteriophages infecting Bacteroides fragilis. Araujo, R., Muniesa, M., Mendez, J., Puig, A., Queralt, N., Lucena, F., Jofre, J. (2001). Journal of Virological Methods 93:127-136. A method for the detection and enumeration of bacteriophages infecting Bacteroides fragilis has been standardised. The recommended host strain is RYC2056 (ATCC 700786) because of the relatively high counts (10(4)-10(5) PFU/100ml) that it recovers in sewage from very different geographical areas. The addition of 0.25% bile to the culture and assay media and the manipulation of the host strain under strict anaerobic conditions resulted in a significant increase (more than 100%) in the number of phages detected. No other changes in the media and culture conditions resulted in changes in the phage counts detected. However, these increases do not justify changing the culture conditions and media described, taking into consideration that bile renders the media cloudy making it difficult to follow the host growth and that most laboratories do not have the facilities to work under strict anaerobic conditions. Nalidixic acid (100 microg/ml) and kanamycin (100 microg/ml) in the assay medium significantly reduce the background flora from polluted samples without affecting the phage counts. Freezing cultures just before the end of the log-phage growth at (-70+/-10) degrees C with BSA-sucrose as cryoprotector, storing of 1-2 ml in glass vials at (-70+/-10) degrees C and using them directly to inoculate fresh broth allows the obtention of cultures ready for phage enumeration in about 2.5 h. All these developments have been incorporated into a procedure that makes the method for detecting phages infecting *B. fragilis* as workable as the standardised methods available for the detection of coliphages
- 5. The use of bacteriophages for treatment and prevention of bacterial disease in animals and animal models of human infection. Barrow, P. A. (2001). Journal of Chemical Technology and Biotechnology. 76:677-682. A brief history of the use of lytic bacteriophages in bacterial disease therapy is presented. After early disillusionment with the idea following poor experimental work, control of phages and field trials, studies were set up in the 1980's in the UK to study their use in farm animal infections. Work with E. coli septicaemia and diarrhoea has shown that phages can be highly effective prophylactically and therapeutically and more effective than antibiotics. There is considerable potential for their use in a limited number of infection types in both man and animals.
- 6. Phage treatment: can we utilise it for certain infective diseases in India? Bhatia, R. S. (2001). Journal of the Association of Physicians of India 49:590.
- 7. Phages of Lactococcus lactis: an ecological and economical equilibrium. Boucher, I, Moineau, S. (2001). Recent Research Developments in Virology 3:243-256. Lactic acid bacteria (LAB) are a group of organisms widely used in food fermentation. Interests in these microorganisms have increased sharply in the last decade; these organisms have even been dubbed the bugs of the new millennium. One distinctive fact about LAB-fermented foods is that they are produced in non-sterile conditions. Thus, LAB are susceptible to infection by lytic bacteriophages naturally present in these environments. Recent developments (by our group and others) in the field of bacteriophages of Lactococcus, the most studied LAB, are investigated and presented in the review.
- 8. Generalized transduction in *Streptomyces coelicolor*. Burke, J., Schneider, D., Westpheling, J. (2001). *Proceedings of the National Academy of Sciences of the United States of America* 98:6289-6294. We report the isolation of generalized transducing phages for *Streptomyces* species able to transduce chromosomal markers or plasmids between derivatives of *Streptomyces coelicolor*, the principal genetic model system for this important bacterial genus. We describe four apparently distinct phages (DAH2, DAH4, DAH5, and DAH6) that are capable of transducing multiple chromosomal markers at frequencies ranging from 10<sup>-5</sup> to 10<sup>-9</sup> per plaque-forming unit. The phages contain DNA ranging in size from 93 to 121 kb and mediate linked transfer of genetic loci at neighboring chromosomal sites sufficiently close to be packaged within the same phage particle. The key to our ability to demonstrate transduction by these phages was the establishment of conditions expected to severely reduce superinfection killing during the selection of transductants. The host range of these phages, as measured by the ability to form plaques, extends to species as distantly related as *Streptomyces avermitilis* and *Streptomyces verticillus*, which are among the most commercially important species of this genus. Transduction of plasmid DNA between *S. coelicolor* and *S. verticillus* was observed at frequencies of approximately 10<sup>-4</sup> transductants per colony-forming unit
- 9. The origins and evolution of viruses. Campbell, A. (2001). *Trends in Microbiology* 9:61-61.
- 10. Phages and their application against drug-resistant bacteria. Chanishvili, N., Chanishvili, T., Tediashvili, M., Barrow, P. A. (2001). Journal of Chemical Technology and Biotechnology. 76:689-699. At the beginning of the 20th century the

phenomenon of spontaneous bacterial lysis was discovered independently by Twort and d'Herelle. Despite the suggestion at that time by d'Herelle that these agents might be applied to the control of bacterial diseases in the west this idea was explored in a desultory fashion only and was eventually discarded largely due to the advent of extensive antibiotic usage. However, interest was maintained in countries of the former Soviet Union where bacteriophage therapy has been applied extensively since that time. Central to this work was the Eliava Institute of Bacteriophage, Microbiology and Virology in Tbilisi, Georgia, which was founded in 1923 through the joint efforts of d'Herelle and the Georgian George Eliava. Ironically, given his contributions to public health in the Soviet Union, Eliava was branded as an enemy of the people in 1937 and executed. d'Herelle never again returned to Georgia. In spite of these tragic events this institute remained the focus for phage therapy in the world and despite being continuously active in this field for 75 years, now struggles for its financial life. In the Eliava Institute, phages were sought for bacterial pathogens implicated in disease outbreaks in different parts of the Soviet Union and were dispatched for use in hospitals throughout the country. Although infections caused by a wide variety of bacterial pathogens have been treated, much of this has been published in Russian and is not readily available in the west. Work has also been carried out in Poland over many years and this has only recently been published in English. By contrast, interest in the west has been limited to a small number of enthusiasts and academics and until very recently little interest has been shown. The main reason that the medical and scientific communities are now beginning to take notice, is the continuing world-wide rise in the incidence of multiply-antibiotic-resistant bacterial pathogens and the absence of effective means for their control. Recent publicity over the work of the Eliava Institute has concentrated the minds of the western world on the potential for infectious disease control that bacteriophage offer, a procedure that is biologically more acceptable than antibiotic use and which has been in use for several decades already.

- 11. Application of digital image analysis and flow cytometry to enumerate marine viruses stained with SYBR gold. Chen, F., Lu, J. R., Binder, B. J., Liu, Y. C., Hodson, R. E. (2001). Applied and Environmental Microbiology 67:539-545. A novel nucleic acid stain, SYBR Gold, was used to stain marine viral particles in various types of samples. Viral particles stained with SYBR Gold yielded bright and stable fluorescent signals that could be detected by a cooled charge-coupled device camera or by flow cytometry. The fluorescent signal strength of SYBR Gold-stained viruses was about twice that of SYBR Green I-stained viruses. Digital images of SYBR Gold-stained viral particles were processed to enumerate the concentration of viral particles by using digital image analysis software. Estimates of viral concentration based on digitized images were 1.3 times higher than those based on direct counting by epifluorescence microscopy. Direct epifluorescence counts of SYBR Gold-stained viral particles were in turn about 1.34 times higher than those estimated by the transmission electron microscope method. Bacteriophage lysates stained with SYBR Gold formed a distinct population in flow cytometric signatures. Flow cytometric analysis revealed at least four viral subpopulations for a Lake Erie sample and two subpopulations for a Georgia coastal sample. Flow cytometry-based viral counts for various types of samples averaged 1.1 times higher than direct epifluorescence microscopic counts. The potential application of digital image analysis and flow cytometry for rapid and accurate measurement of viral abundance in aquatic environments is discussed
- 12. Estimation of the average burst size of Phix174 am3, cs70 for use in mutation assays with transgenic mice. Delongchamp, R. R., Valentine, C. R., Malling, H. V. (2001). Environmental and Molecular Mutagenesis 37:356-360. In mutation assays using transgenic mice, with recoverable vectors such as PhiX174 am3, cs70, mutations originate from two sources: (1) in vivo mutations, that is, mutations that were fixed in the mouse, or (2) ex vivo mutations, that is, mutations that were fixed during recovery or plating. When a bacteriophage infects a bacterium, it multiplies and bursts the cell, releasing a number of phages referred to as the burst size. Our method for distinguishing between in vivo mutations and ex vivo mutations estimates the average number of bursts, the denominator of in vivo mutant frequencies, by dividing the total plaque-forming units (PFU) by the average number of phages in a burst. Herein, we outline a probability model relating observed plaque counts to the burst size and present the statistical method used to estimate the burst size. The average size of a single burst from nonrevertant phages was estimated in eight studies under the conditions of our mutation assay. The average burst size was stable across studies at 182.5 plaques per burst (standard error, 14.25). The probability that a burst is a specific size was approximated by a negative binomial distribution, which implies a Poisson-Pascal distribution for the observed plaque counts. The observed plaque counts were adequately fit by this approximation.
- 13. Transduction by phiBB-1, a bacteriophage of Borrelia burgdorferi. Eggers, C. H., Kimmel, B. J., Bono, J. L., Elias, A. F., Rosa, P., Samuels, D. S. (2001). Journal of Bacteriology 183:4771-4778. We previously described a bacteriophage of the Lyme disease agent Borrelia burgdorferi designated phiBB-1. This phage packages the host complement of the 32-kb circular plasmids (cp32s), a group of homologous molecules found throughout the genus Borrelia. To demonstrate the ability of phiBB-1 to package and transduce DNA, a kanamycin resistance cassette was inserted into a cloned fragment of phage DNA, and the resulting construct was transformed into B. burgdorferi CA-11.2A cells. The kan cassette recombined into a resident cp32 and was stably maintained. The cp32 containing the kan cassette was packaged by phiBB-1 released from this B. burgdorferi strain. phiBB-1 has been used to transduce this antibiotic resistance marker into naive CA-11.2A cells, as well as two other strains of B. burgdorferi. This is the first direct evidence of a mechanism for lateral gene transfer in B. burgdorferi
- 14. Development and optimization of a novel immunomagnetic separation- bacteriophage assay for detection of Salmonella enterica serovar enteritidis in broth. Favrin, S. J., Jassim, S. A., Griffiths, M. W. (2001). Applied and Environmental Microbiology 67:217-224. Salmonella is the second-leading cause of food-borne illness in most developed countries, causing diarrhea, cramps, vomiting, and often fever. Many rapid methods are available for detection of Salmonella in foods, but these methods are often insensitive or expensive or require a high degree of technical ability to perform. In this paper we describe development and characterization of a novel assay that utilizes the normal infection cycle of bacteriophage SJ2 for detection of Salmonella enterica serovar Enteritidis in broth. The assay consists of four main stages: (i) capture and concentration of target cells by using immunomagnetic separation (IMS); (ii) infection of the target bacterium with phage; (iii) amplification and recovery of progeny phage; and (iv) assay of progeny phage on the basis of their effect on a healthy population of host cells (signal-amplifying cells). The end point of the assay can be determined by using either fluorescence or optical density measurements. The detection limit of the assay in broth is less than 10<sup>4</sup> CFU/ml, and the assay can be performed in 4 to 5 h. The results of this study demonstrate that the IMS-bacteriophage assay is a rapid, simple, and sensitive technique for detection of Salmonella serovar Enteritidis in broth cultures which can be applied to preenriched food samples
- 15. Reviewing efficacy of alternative water treatment techniques. Hambidge, A. (2001). *Health Estate* 55:23-25. This section is designed to provide a brief summary of some of the findings. A good deal of work has been conducted by Mr N. L. Pavey and the team at BSRIA, Bracknell. The BSRIA publications are an excellent source of further information. Ultraviolet radiation: UV radiation of wavelength 254 nm destroys bacteria by a mechanism of damaging nucleic acids by producing thymine dimers which disrupt DNA replication [Gavdy and Gavdy, 1980]. *L. pneumophila* has been reported as sensitive to UV dosages of 2,500-7,000 uW.s/cm2 [Antopol & Ellner, 1979; Knudson, 1985]. Antopol and Ellner [1979] examined the susceptibility of *L*.

pneumophila to UV dosage. Their results indicated that 50% of the organisms were killed by 380 uWs/cm2 and 90% were killed by 920 uWs/cm2. Kills of 99 and 99.9% were obtained using 1,840 and 2,760 uWs/cm2 respectively. Muraca et al [1987] showed that continuous UV irradiation resulted in a 5 logarithm decrease in waterborne L. pneumophila in a circulating system. Gilpin [1984] reported that in laboratory buffer solutions, exposure to 1 uW of UV radiation per cm2 achieved a 50% kill of L longbeachae in 5 minutes, L. gormanii in 2-30 minutes and L pneumophila in 17 minutes. Exposure times for 99% kills for L. longbeachae, L pneumophila and L. Gormanii were 33, 48 and 63 minutes respectively. The same research worker conducted experiments using a 3 litre circulating water system, connected to a stainless steel housing containing a UV source. The UV lamp output was 7 ergs/mm2 per second per 100 cm at 254 nm. L. pneumophila was killed within 15 seconds, that is within their first pass through the system. Continuous disinfection with UV has the advantages of imparting no taste, odour or harmful chemical by-products and requires minimal operation and maintenance [Muraca et al 1988]. Keevil et al [1989] state that UV irradiation fails to clear systems of biofilm because of poor penetration into microflocs of the micro-organisms. Copper/silver ionisation: A recent study of full scale hot water test rigs incorporating copper-silver ionisation systems has been reported by Pavey, 1996. Copper and silver ions were introduced into the water by electrolysis. One of the principal mechanisms of biocidal action of these ions is thought to be cell penetration. The positively charged copper ions form electrostatic bonds with negatively charged sites on the cell wall. The cell membrane is thus distorted, allowing ingress of silver ions which attack the cell by binding at specific sites to DNA, RNA, respiratory enzymes and cellular protein, causing catastrophic failure of the life support systems of the cell. Silver and copper ion concentrations of 40 and 400 ug/L respectively were effective against planktonic Legionellae in cold water systems and hot water systems containing soft water. In hard water, the ionisation was ineffective due to the inability to control silver ion concentrations. This was caused by scaling of the electrodes and silver ion complexation by the high concentration of dissolved solids. Bosch et al [1993] had earlier extended the application of copper-silver disinfection to human enteric viruses in water, such as adenovirus, rotavirus, hepatitis A virus, and poliovirus. Their work showed that copper and silver ions in the presence of reduced levels of free chlorine did not ensure the total elimination of viral pathogens from water. In the case of an amoeba, Naegleria fowleria [responsible for primary amoebic meningoencephalitis], Cassells et al [1995] have demonstrated that a combination of silver and copper ions were ineffective at inactivating the amoebae at 80 and 800 ug/L respectively. However addition of 1.0 mg/L free chlorine produced a synergistic effect, with superior inactivation relative to either chlorine or silver-copper in isolation. A similar synergy was reported by Yahya et al [1989] in their study of Staphylococcus sp. and Pseudomonas aeruginosa. Yahya et al [1992] also suggested an additive or synergistic effect in the inactivation of coliphage MS-2 and poliovirus. Other techniques: There are a number of other techniques. We have conducted trials of most of these in the control of Legionella sp., but these fall out of the scope of this article, and as such less emphasis has been placed on them here. Ozonation: Ozone [O3] is an oxidising gas, generated electrically from oxygen [O2]. L. pneumophila can be killed at < 1 mg/L of ozone [Edelstien et al 1982]. Muraca et al [1987] found that 1-2 mg/L of continuous ozone over a six hour contact time, produced a 5 logarithm decrease of L. pneumophila. The effectiveness of ozone treatment against a range of bacteria and coliphages has been studied Botzenhart et al [1993]. E. coli was least resistant to ozone, followed by MS 2-coliphage and PhiX 174-coliphage, with L. pneumophila and Bacillus subtilis spores being the most resistant. (ABSTRACT TRUNCATED)

- 16. Reduction in exopolysaccharide viscosity as an aid to bacteriophage penetration through Pseudomonas aeruginosa biofilms. Hanlon, G. W., Denyer, S. P., Olliff, C. J., Ibrahim, L. J. (2001). Applied and Environmental Microbiology 67:2746-2753. To cause an infection, bacteriophages must penetrate the alginate exopolysaccharide of Pseudomonas aeruginosa to reach the bacterial surface. Despite a lack of intrinsic motility, phage were shown to diffuse through alginate gels at alginate concentrations up to 8% (wt/vol) and to bring about a 2-log reduction in the cell numbers in 20-day-old biofilms of P. aeruginosa. The inability of alginate to act as a more effective diffusional barrier suggests that phage may cause a reduction in the viscosity of the exopolysaccharide. Samples (n = 5) of commercial alginate and purified cystic fibrosis (CF) alginate were incubated with 2 x 10<sup>8</sup> purified phage per ml for 24 h at 37 degrees C. After incubation the samples and controls were subjected to rheological analysis with a Carrimed controlled stress rheometer. The viscosities of phage-treated samples were reduced by up to 40% compared to those of controls incubated in the absence of phage. The experiment was repeated by using phage concentrations of 10<sup>10</sup> and 10<sup>12</sup> phage per ml and samples taken for analysis at intervals up to 4 h. The results indicated that there was a time- and concentration-dependent reduction in viscosity of up to 40% compared to the viscosities of the controls. Commercial and purified CF alginate samples, both phage treated and untreated, were subjected to gel filtration chromatography by using Sephacryl High Resolution S-400 medium in order to obtain evidence of degradation. The results demonstrated that alginate treated with phage had a lower molecular weight than untreated alginate. The data suggest that bacteriophage migration through P. aeruginosa biofilms may be facilitated by a reduction in alginate viscosity brought about by enzymic degradation and that the source of the enzyme may be the bacterial host itself
- 17. Filamentous phage associated with recent pandemic strains of Vibrio parahaemolyticus. Iida, T., Hattori, A., Tagomori, K., Nasu, H., Naim, R., Honda, T. (2001). *Emerging Infectious Diseases* 7:477-478. A group of pandemic strains of Vibrio parahaemolyticus has recently appeared in Asia and North America. We demonstrate that a filamentous phage is specifically associated with the pandemic V. parahaemolyticus strains. An open reading frame unique to the phage is a useful genetic marker to identify these strains
- 18. Human adenoviruses and coliphages in urban runoff-impacted coastal waters of Southern California. Jiang, S., Noble, R., Chu, W. (2001). Applied and Environmental Microbiology 67:179-184. A nested-PCR method was used to detect the occurrence of human adenovirus in coastal waters of Southern California. Twenty- to forty-liter water samples were collected from 12 beach locations from Malibu to the border of Mexico between February and March 1999. All sampling sites were located at mouths of major rivers and creeks. Two ultrafiltration concentration methods, tangential flow filtration (TFF) and vortex flow filtration (VFF), were compared using six environmental samples. Human adenoviruses were detected in 4 of the 12 samples tested after nucleic acid extraction of VFF concentrates. The most probable number of adenoviral genomes ranged from 880 to 7,500 per liter of water. Coliphages were detected at all sites, with the concentration varying from 5.3 to 3332 PFU/liter of water. F-specific coliphages were found at 5 of the 12 sites, with the concentration ranging from 5.5 to 300 PFU/liter. The presence of human adenovirus was not significantly correlated with the concentration of coliphage (r = 0.32) but was significantly correlated (r = 0.99) with F-specific coliphage. The bacterial indicators (total coliforms, fecal coliforms, and enterococci) were found to exceed California recreational water quality daily limits at 5 of the 12 sites. However, this excess of bacterial indicators did not correlate with the presence of human adenoviruses in coastal waters. The results of this study call for both a reevaluation of our current recreational water quality standards to reflect the viral quality of recreational waters and monitoring of recreational waters for human viruses on a regular basis
- 19. Comparative study of vaginal *Lactobacillus* phages isolated from women in the United States and Turkey: prevalence, morphology, host range, and DNA homology. Kilic, A. O., Pavlova, S. I., Alpay, S., Kilic, S. S., Tao, L. (2001). *Clinical and Diagnostic Laboratory Immunology* 8:31-39. Lactobacilli play an important role in maintaining vaginal

health. However, during bacterial vaginosis lactobacilli decrease for unknown reasons. Our preliminary study showed that phages could infect vaginal lactobacilli. Therefore, the aim of this study was to analyze the distribution, virulence, and types of vaginal Lactobacillus phages isolated from women of two countries: the United States and Turkey. A total of 209 vaginal lactobacilli were isolated from reproductive-aged women in the United States (n = 107) and Turkey (n = 102). By analysis of 16S rRNA gene sequence and by comparison of protein profiles, most lactobacilli were identified as L. crispatus, L. gasseri, and L. jensenii. After mitomycin C induction, 28% of American lactobacilli and 36% of Turkish lactobacilli released phages. A total of 67 phages were isolated and further characterized by their host range, electron microscopy, and DNA homology. All 67 phages were infective against lactobacilli from both collections. The host ranges of most phages were broad, including multiple Lactobacillus species. Even though the phages were all temperate, they were able to cause lytic infection in various strains. The electron micrographs of these phages showed a hexagon-shaped head and a long tail with or without a contractile tail sheath. Based on their morphology, these phages belonged to Bradley's phage groups A and B, and could be further classified into four morphotypes. All four types were found among American phages, but only three were found among Turkish isolates. DNA hybridization with labeled probes of the four types of phages revealed that additional genetic types existed within each morphotype among these phages. The phage genomic sizes ranged between 34 and 55 kb. Many of the lysogenic Lactobacillus strains released phages spontaneously at a high frequency of 10<sup>-3</sup> to 10<sup>-4</sup> PFU/cell. In conclusion, lysogeny in vaginal lactobacilli is widely spread. Some lysogenic lactobacilli spontaneously release phages with a broad host range

- 20. Use of bacteriophage therapy in surgical practice. Lakhno, V. M., Bordunovskii, V. N. (2001). Vestnik Khirurgii Imeni I. I. Grekova 160:122-125.
- 21. Bacteriophage WO and virus-like particles in Wolbachia, an endosymbiont of arthropods. Masui, S., Kuroiwa, H., Sasaki, T., Inui, M., Kuroiwa, T., Ishikawa, H. (2001). Biochemical & Biophysical Research Communications 283:1099-1104. Wolbachia are intracellular symbionts mainly found in arthropods, causing various sexual alterations on their hosts by unknown mechanisms. Here we report the results that strongly suggest that Wolbachia have virus-like particles of phage WO, which was previously identified as a prophage-like element in the Wolbachia genome. Wolbachia (strain wTai) infection in an insect was detected with the antibody against Wsp, an outer surface protein of Wolbachia, by fluorescence microscopy and immunoelectron-microscopy for the first time. Virus-like particles in Wolbachia were observed by electron-microscopy. The 0.22-&mgr;m filtrate of insect ovary contained DAPI-positive particles, and PCR analysis demonstrated that a phage WO DNA passed through the filter while Wolbachia DNA were eliminated, suggesting that the DAPI-positive particles were phage WO.
- 22. Phenotype characterization of genetically defined microorganisms and growth of bacteriophage in biofilms. McLean, R. J., Corbin, B. D., Balzer, G. J., Aron, G. M. (2001). *Methods in Enzymology* 336:163-174. Phenotypic characterization will be a pivotal aspect of future research in understanding the biofilm mode of growth. We hope that the concepts and techniques presented in this chapter will benefit other investigators in this field. Although initial studies will necessarily involve monocultures, eventually mixed culture work will have to be performed to understand biofilm growth in the natural environment. As the study of biofilm-phage interactions is new, there is considerable fundamental work that needs to be addressed. Here, we anticipate that some phage are better adapted to growth in biofilms, some are adept in growing in mixed culture biofilms, and others are better adapted to infecting planktonic organisms. Whereas biofilms are now widely accepted as a fundamental aspect of microbial growth in nature, the field of phage ecology is quite new and an exciting challenge for the future
- 23. Phi29 family of phages. Meijer, W. J., Horcajadas, J. A., Salas, M. (2001). Microbiology and Molecular Biology Reviews 65:261-287. Continuous research spanning more than three decades has made the Bacillus bacteriophage phi29 a paradigm for several molecular mechanisms of general biological processes, such as DNA replication, regulation of transcription, phage morphogenesis, and phage DNA packaging. The genome of bacteriophage phi29 consists of a linear double-stranded DNA (dsDNA), which has a terminal protein (TP) covalently linked to its 5' ends. Initiation of DNA replication, carried out by a proteinprimed mechanism, has been studied in detail and is considered to be a model system for the protein-primed DNA replication that is also used by most other linear genomes with a TP linked to their DNA ends, such as other phages, linear plasmids, and adenoviruses. In addition to a continuing progress in unraveling the initiation of DNA replication mechanism and the role of various proteins involved in this process, major advances have been made during the last few years, especially in our understanding of transcription regulation, the head-tail connector protein, and DNA packaging. Recent progress in all these topics is reviewed. In addition to phi29, the genomes of several other Bacillus phages consist of a linear dsDNA with a TP molecule attached to their 5' ends. These phi29-like phages can be divided into three groups. The first group includes, in addition to phi29, phages PZA, phi15, and BS32. The second group comprises B103, Nf, and M2Y, and the third group contains GA-1 as its sole member. Whereas the DNA sequences of the complete genomes of phi29 (group I) and B103 (group II) are known, only parts of the genome of GA-1 (group III) were sequenced. We have determined the complete DNA sequence of the GA-1 genome, which allowed analysis of differences and homologies between the three groups of phi29-like phages, which is included in this review
- 24. Multiple infection dynamics has pronounced effects on the fitness of RNA viruses. Miralles, R., Ferrer, R., Sole, R. V., Moya, A., Elena, S. F. (2001). Journal of Evolutionary Biology 14:654-662. Several factors play a role during the replication and transmission of RNA viruses. First, as a consequence of their enormous mutation rate, complex mixtures of genomes are generated immediately after infection of a new host. Secondly, differences in growth and competition rates drive the selection of certain genetic variants within an infected host. Thirdly, but not less important, a random sampling occurs at the moment of viral infectious passage from an infected to a healthy host. In addition, the availability of hosts also influences the fate of a given viral genotype. When new hosts are scarce, different viral genotypes might infect the same host, adding an extra complexity to the competition among genetic variants. We have employed a two-fold approach to analyse the role played by each of these factors in the evolution of RNA viruses. First, we have derived a model that takes into account all the preceding factors. This model employs the classic Lotka-Volterra competition equations but it also incorporates the effect of mutation during RNA replication, the effect of the stochastic sampling at the moment of infectious passage among hosts and, the effect of the type of infection (single, coinfection or superinfection). Secondly, the predictions of the model have been tested in an in vitro evolution experiment. Both theoretical and experimental results show that in infection passages with coinfection viral fitness increased more than in single infections. In contrast, infection passages with superinfection did not differ from the single infection. The coinfection frequency also affected the outcome: the larger the proportion of viruses coinfecting a host, the larger increase in fitness observed.
- 25. Characterization of VPI pathogenicity island and CTXphi prophage in environmental strains of *Vibrio cholerae*. Mukhopadhyay, A. K., Chakraborty, S., Takeda, Y., Nair, G. B., Berg, D. E. (2001). *Journal of Bacteriology* 183:4737-4746. Environmental isolates of *Vibrio cholerae* of eight randomly amplified polymorphic DNA (RAPD) fingerprint types from

Calcutta, India, that were unusual in containing toxin-coregulated pilus or cholera toxin genes but not O1 or O139 antigens of epidemic strains were studied by PCR and sequencing to gain insights into *V. cholerae* evolution. We found that each isolate contained a variant form of the VPI pathogenicity island. Distinguishing features included (i) four new alleles of tcpF (which encodes secreted virulence protein; its exact function is unknown), 20 to 70% divergent (at the protein level) from each other and canonical tcpF; (ii) a new allele of toxT (virulence regulatory gene), 36% divergent (at the protein level) in its 5' half and nearly identical in its 3' half to canonical toxT; (iii) a new tcpA (pilin) gene; and (iv) four variant forms of a regulatory sequence upstream of toxT. Also found were transpositions of an IS903-related element and function-unknown genes to sites in VPI. Cholera toxin (ctx) genes were found in isolates of two RAPD types, in each case embedded in CTXphi-like prophages. Fragments that are inferred to contain only putative repressor, replication, and integration genes were present in two other RAPD types. New possible prophage repressor and replication genes were also identified. Our results show marked genetic diversity in the virulence-associated gene clusters found in some nonepidemic *V. cholerae* strains, suggest that some of these genes contribute to fitness in nature, and emphasize the potential importance of interstrain gene exchange in the evolution of this species

- 26. Naturally occurring lactococcal plasmid pAH90 links bacteriophage resistance and mobility functions to a foodgrade selectable marker. O' Sullivan, D., Ross, R. P., Twomey, D. P., Fitzgerald, G. F., Hill, C., Coffey, A. (2001). Applied and Environmental Microbiology 67:929-937. The bacteriophage resistance plasmid pAH90 (26,490 bp) is a natural cointegrate plasmid formed via homologous recombination between the type I restriction-modification specificity determinants (hsdS) of two smaller lactococcal plasmids, pAH33 (6,159 bp) and pAH82 (20,331 bp), giving rise to a bacteriophage-insensitive mutant following phage challenge (D. O'Sullivan, D. P. Twomey, A. Coffey, C. Hill, G. F. Fitzgerald, and R. P. Ross, Mol. Microbiol. 36:866-876; 2000). In this communication we provide evidence that the recombination event is favored by phage infection. The entire nucleotide sequence of plasmid pAH90 was determined and found to contain 24 open reading frames (ORFs) responsible for phenotypes which include restriction-modification, phage adsorption inhibition, plasmid replication, cadmium resistance, cobalt transport, and conjugative mobilization. The cadmium resistance property, encoded by the cadA gene, which has an associated regulatory gene (cadC), is of particular interest, as it facilitated the selection of pAH90 in other phage-sensitive lactococci after electroporation. In addition, we report the identification of a group II self-splicing intron bounded by two exons which have the capacity to encode a relaxase implicated in conjugation in gram-positive bacteria. The functionality of this intron was evident by demonstrating splicing in vivo. Given that pAH90 encodes potent phage defense systems which act at different stages in the phage lytic cycle, the linkage of these with a food-grade selectable marker on a replicon that can be mobilized among lactococci has significant potential for natural strain improvement for industrial dairy fermentations which are susceptible to phage inhibition
- 27. Contingent neutrality in competing viral populations. Quer, J., Hershey, C. L., Domingo, E., Holland, J. J., Novella, I. S. (2001). Journal of Virology 75:7315-7320. The replicative fitness of a genetically marked (MARM-C) population of vesicular stomatitis virus was examined in competition assays in BHK-21 cells. In standard fitness assays involving up to eight competition passages of the mixed populations, MARM-C competes equally with the wild type (wt), but very prolonged competitions always led to the wt gaining dominance over MARM-C in a very slowed, nonlinear manner (J. Quer et al., J. Mol. Biol. 264:465-471, 1996). In the present study we show that a number of quite unrelated environmental perturbations, which decreased virus replication during competitions, all led to an accelerated dominance of the wt over MARM-C. These perturbations were (i) the presence of added (or endogenously generated) defective interfering particles, (ii) the presence of the chemical mutagen 5fluorouracil (5-FU), or (iii) an increase in temperature to 40.5 degreesC. Thus, the "neutral fitness" of the MARM-C population is contingent. We have determined the entire genomic consensus sequence of MARM-C and have identified only six mutations. Clearly, some or all of these mutations allowed the MARM-C quasispecies population to compete equally with wt in a defined constant host environment, but the period of neutrality was shortened when the environment was perturbed during competitions. Interestingly, when four passages of each population were carried out independently in the presence of 5-FU (but in the absence of competition), no significant differences were detected in the fitness changes of wt and MARM-C, nor was there a difference in their subsequent abilities to compete with each other in a standard fitness assay. We propose a model for this contingent neutrality. The conditions employed to generate the MARM-C guasispecies population selected a small number of mutations in the consensus sequence. It appears that the MARM-C quasispecies population has moved into a segment of sequence space in which the average fitness value is neutral but, under environmental stress, beneficial mutations cannot be generated rapidly enough to compete with those being generated concurrently by competing wt virus quasispecies populations.
- 28. Method for host-independent detection of generalized transducing bacteriophages in natural habitats. Sander, M., Schmieger, H. (2001). Applied and Environmental Microbiology 67:1490-1493. Despite an increasing interest in horizontal gene transfer in bacteria, the role of generalized transduction in this process has not been well investigated yet. Certainly one of the reasons is that only a small fraction of general transducing bacteriophages have been characterized, because many bacterial hosts needed for propagation and identification are not culturable or are simply unknown. A method for host-independent detection of transducing bacteriophages was developed. Phage-encapsulated DNA was used as a template for PCR amplification of 16S ribosomal DNA using primers specific for the 16S rRNA genes of most eubacteria. Sequencing of the cloned amplification products permits the identification of the host bacteria. The Salmonella phage P22 was used as an example. Applying this method to a sample of the supernatant of the mixed liquor in the aeration tank of an activated sludge treatment works revealed the presence of transducing phages infecting several bacterial species for which such phages have not yet been described. This method is suitable for estimating the contribution of generalized transduction to horizontal gene transfer in different habitats
- 29. Phage-mediated transfer of virulence genes. Saunders, J. R., Allison, H., James, C. E., McCarthy, A. J., Sharp, R. (2001). Journal of Chemical Technology and Biotechnology. 76:662-666. Bacteriophages as accessory genetic elements play a crucial role in the dissemination of genes and the promotion of genetic diversity within bacterial populations. Such horizontal transfer of DNA is critical in the emergence of new pathogenic organisms, through the dissemination of genes encoding virulence factors such as toxins, adhesins and agressins. Phages can transfer genes that are not necessary for bacteriophage persistence and are generally recognised by their ability to convert their host bacteria to new phenotypes. This phenomenon is known as phage conversion. If such converting genes encode for virulence factors, the consequences of phage infection may include increased virulence of the host bacteria, and the conversion of a non-pathogenic strain to a potentially dangerous pathogen. A number of virulence factors in bacteria causing diseases in plants, animals and humans are encoded by converting phages, the vast majority of which are temperate as opposed to lytic in nature.
- 30. Bacteriophages: biology and history. Sharp, R. (2001). Journal of Chemical Technology and Biotechnology. 76:667-672. Bacteriophages were initially considered to offer the key to the control of bacterial infections; early studies, however, proved largely unsuccessful. In the 1940s and 1950s, pioneering studies into the structure and physiology of host/phage interactions laid the basis for the development of molecular biology and a spectrum of new biotechnologically-based industries. Bacteriophages

able to infect most procaryotic groups of organisms have been isolated, and are readily isolated from soil, water, and sewage and most environments colonised by bacteria. Ecologically, phages are as varied and as versatile as their hosts with some able to survive extremes of temperature (up to 95 °C) and extremes of pH as low as pH 1.

- 31. The social evoltuion of bacterial pathogenesis. Smith, J. (2001). Proceedings of the Royal Society of London Series B: Biological Sciences 268:61-69. Many of the genes responsible for the virulence of bacterial pathogens are carried by mobile genetic elements that can be transferred horizontally between different bacterial lineages. Horizontal transfer of virulence-factor genes has played a profound role in the evolution of bacterial pathogens, but it is poorly understood why these genes are so often mobile. Here, I present a hypothetical selective mechanism maintaining virulence-factor genes on horizontally transmissible genetic elements. For virulence factors that are secreted extracellularly, selection within hosts may favour mutant 'cheater' strains of the pathogen population within a host. Using simple mathematical models, I show that if this occurs then selection for infectious transmission between hosts favours pathogen strains that can reintroduce functional copies of virulence-factor genes into cheaters via horizontal transfer, forcing them to produce the virulence factor. Horizontal gene transfer is thus a novel mechanism for the evolution of cooperation. I discuss predictions of this hypothesis that can be tested empirically and its implications for the evolution of pathogen virulence.
- 32. Evidence for holin function of tcdE gene in the pathogenicity of *Clostridium difficile*. Tan, K. S., Wee, B. Y., Song, K. P. (2001). *Journal of Medical Microbiology* 50:613-619. Toxigenic strains of *Clostridium difficile* produce two large bacterial toxins called toxins A (TcdA) and B (TcdB). tcdA and tcdB genes are located on the pathogenicity locus of *C. difficile*, a unique characteristic of toxigenic strains of this species. Intergenic to the two toxin genes is tcdE, a small 501-bp open reading frame of unknown function. Expression of the tcdE gene in Escherichia coli caused bacterial cell death. Computational analysis of the amino acid sequence of TcdE revealed structural features that are strikingly similar to a class of bacteriophage proteins called holins. Holins are cytolytic proteins that cause lysis of bacterial hosts to effect the release of progeny phages. Further analysis of the recombinant clone expressing TcdE by transmission electron microscopy confirmed that the site of action of TcdE is on the bacterial cell membrane. The results provide evidence that TcdE is structurally and functionally similar to holin proteins. TcdE may function as a lytic protein to facilitate the release of TcdA and TcdB to the extracellular environment, as these toxins lack signal peptide
- 33. Phylogeny of the major head and tail genes of the wide-ranging T4-type bacteriophages. Tétart, F., Desplats, C., Kutateladze, M., Monod, C., Ackermann, H.-W., Krisch, H. M. (2001). Journal of Bacteriology 183:358-366. We examined a number of bacteriophages with M-type morphology that propagate in different genera of enterobacteria, Aeromonas, Burkholderia, and Vibrio. Most of these phages had a prolate icosahedral head, a contractile tail, and a genome size that was similar to that of T4. A few of them had more elongated heads and larger genomes. All these phages are phylogenetically related, since they each had sequences homologous to the capsid gene (gene 23), tail sheath gene (gene 18), and tail tube gene (gene 19) of T4. On the basis of the sequence comparison of their virion genes, the T4-type phages can be classified into three subgroups with increasing divergence from T4: the T-evens, pseudoT-evens, and schizoT-evens. In general, the phages that infect distantly related hosts. However, some of the phages appear to be chimeras, indicating that, at least occasionally, some genetic shuffling has occurred between the different T4-type subgroups. The compilation of a number of gene 23 sequences reveals a pattern of conserved motifs separated by sequences that differ in the M-type subgroups. Such variable patches in the gene 23 sequence analysis provides molecular evidence that phages related to T4 are widespread in the biosphere and diverged from a common ancestor in acquiring the ability to infect different host bacteria and to occupy new ecological niches.
- 34. Induction of hepatitis B virus-specific cytotoxic T lymphocytes response in vivo by filamentous phage display vaccine. Wan, Y., Wu, Y., Bian, J., Wang, X. Z., Zhou, W., Jia, Z. C., Tan, Y., Zhou, L. (2001). Vaccine 19:2918-2923. The ability of inducing MHC class I restricted cytotoxic T lymphocytes response in vivo via recombinant filamentous phage was investigated. The recombinant filamentous phage particles that displayed the Hepatitis B virus epitope S(28--39) were injected into BALB/c (H-2d) mice without adjuvants. A MHC class I restricted HBs specific CTL response was found 8 days after injection. The potentiality of using the recombinant filamentous phage as anti-virus vaccine was discussed
- 35. Regulatory issues for phage-based clinical products. Withington, R. (2001). Journal of Chemical Technology and Biotechnology. 76:673-676. Phage-based therapeutic products are members of a growing and diverse group of products categorised as biologics, biologicals or biotechnological products. They are regulated in much the same way as conventional drugs although in America they have their own division at FDA, the Centre for Biologics Evaluation and Research, CBER (as opposed to CDER, the corresponding drugs division). The distinction is important because there are significant differences between the two divisions in the amount of toxicological characterisation, clinical testing and manufacturing data that must be submitted for approval. Also, there are important differences in the extent to which multiparty manufacturing arrangements are permitted. There are a number of regulatory issues surrounding phage-based clinical products that, if addressed early during product development, will not become blocks to progress later on. The regulatory issues arise in part because of the unique nature of phage-based clinical products and in part because of their intended clinical use.
- 36. **Simulating the growth of viruses. You, L., Yin, J. (2001).** *Pacific Symposium on Biocomputing* **532-543.** To explore how the genome of an organism defines its growth, we have developed a computer simulation for the intracellular growth of phage T7 on its *E. coli* host. Our simulation, which incorporates 30 years of genetic, biochemical, physiological, and biophysical data, is used here to study how the intracellular resources of the host, determined by the specific growth rate of the host, contribute toward phage development. It is also used to probe how changes in the linear organization of genetic elements on the T7 genome can affect T7 development. Further, we show how time-series trajectories of T7 mRNA and protein levels generated by the simulation may be used as raw data to test data-mining strategies, specifically, to identify partners in protein-protein interactions. Finally, we suggest how generalization of this work can lead to a knowledge-driven simulation for the growth of any virus
- 37. Purification of Piscirickettsia salmonis and associated phage particles. Yuksel, S. A., Thompson, K. D., Ellis, A. E., Adams, A. (2001). Diseases of Aquatic Organisms 44:231-235. Piscirickettsia salmonis was isolated from cell culture using differential centrifugation and purified on a 30% Percol gradient. The purity of the preparation was assessed by transmission electron microscopy and phage-like particles were found to be associated with some of the *P. salmonis* isolates examined. This is believed to be the first report of a phage associated with rickettsia from fish

- 38. Microbiological quality of the Catania coastal sea water. Aulicino, F. A., Mauro, L., Marranzano, M., Biondi, M., Ursino, A., Carere, M. (2000). Annali di Igiene 12:533-541. This study was carried out from 1997 to 1998 along a selected coastal area near Catania to ascertain bacteriological and virological quality of marine waters. 44 seawater samples, collected from 4 stations, were assayed for the presence of total and fecal coliforms, fecal streptococci, coliphages, Salmonellae and enteric viruses. Two stations localized at canal outfalls showed high levels of fecal pollution. The other stations were of good microbiological quality and showed a limited number of samples exceeding the standards laid down as guide values for bathing waters by Italian normative during the bathing period. Salmonellae were isolated in 8 out of 44 sea water samples (18%). Their presence was ascertained mainly in samples of the two polluted stations. Enteroviruses were not isolated. Enteric viruses such as Reoviruses were isolated from all stations, in 12 out of 44 samples (27%). The presence of these viruses was ascertained only during autumnal and winter seasons. The results of this study showed that, notwithstanding some stations showed high levels of bacteriological indicators of fecal pollution and presence of Salmonellae, enteroviruses growing on cell cultures were not isolated. Reoviruses confirmed their high diffusion in marine waters
- 39. Flow cytometric detection of viruses. Brussaard, C. P. D., Marie, D., Bratbak, G. (2000). Journal of Virological Methods 85:175-182. Representatives from several different virus families (Baculoviridae, Herpesviridae, Myoviridae, Phycodnaviridae, Picornaviridae, Podoviridae, Retroviridae, and Siphoviridae) were stained using a variety of highly fluorescent nucleic acid specific dyes (SYBR Green I, SYBR Green II, OliGreen, PicoGreen) and examined using a standard flow cytometer equipped with a standard 15 mW argon-ion laser. The highest green fluorescence intensities were obtained using SYBR Green I. DNA viruses with genome sizes between 48.5 and 300 kb could easily be detected. The fluorescence signals of the small genome-sized RNA viruses (7.4–14.5 kb) were found at the limit of detection. No significant linear relationship could be found between genome size and the green fluorescence intensity of the SYBR Green I stained virus preparations. To our knowledge, this is the first report of detecting and discriminating between a wide range of different viruses directly using flow cytometry. This rapid and precise assay represents a new and promising tool in the field of virology.
- 40. Effect of five dietary antimutagens on the genotoxicity of six mutagens in the microscreen prophage-induction assay. Cabrera, G. (2000). Environmental and Molecular Mutagenesis 36:206-220. Dietary antimutagens have been studied extensively in the last two decades, using mainly bacterial and mammalian cells. These studies have shown that certain dietary antimutagens, acting individually or as mixtures, are useful in counteracting the effects of certain mutagens and/or carcinogens to which humans are commonly exposed. However, there are some inconsistencies among publications using different bioassays. The general purpose of the research presented here was to conduct a comparative study of the antigenotoxic activity of five dietary antimutagens against six mutagens, using three rather different short-term tests: the Microscreen prophage-induction assay, the Tradescantia micronucleus test, and the Salmonella/mammalian microsome test. In this study I report the results with the Microscreen prophage-induction assay. The antimutagens selected were chlorophyllin, betacarotene, and vitamins A, C, and E. The mutagens selected were 2-aminoanthracene, benzo[a]pyrene, 2-nitrofluorene, toxaphene, dichlorvos, and nitrofen. The results show that chlorophyllin and beta-carotene inhibited the genotoxicity of all six mutagens; vitamin E inhibited all except dichlorvos; and vitamins C and A inhibited 2-aminoanthracene, benzo[a]pyrene, 2-nitrofluorene, and nitrofen
- 41. CTX prophages in classical biotype Vibrio cholerae: functional phage genes but dysfunctional phage genomes. Davis, B. M., Moyer, K. E., Boyd, E. F., Waldor, M. K. (2000). Journal of Bacteriology 182:6992-6998. CTXphi is a filamentous, lysogenic bacteriophage whose genome encodes cholera toxin, the primary virulence factor produced by Vibrio cholerae. CTX prophages in O1 El Tor and O139 strains of V. cholerae are found within arrays of genetically related elements integrated at a single locus within the V. cholerae large chromosome. The prophages of O1 El Tor and O139 strains generally yield infectious CTXphi. In contrast, O1 classical strains of V. cholerae do not produce CTXphi, although they produce cholera toxin and they contain CTX prophages integrated at two sites. We have identified the second site of CTX prophage integration in O1 classical strains and characterized the classical prophage arrays genetically and functionally. The genes of classical prophages encode functional forms of all of the proteins needed for production of CTXphi. Classical CTX prophages are present either as solitary prophages or as arrays of two truncated, fused prophages. RS1, a genetic element that is closely related to CTXphi and is often interspersed with CTX prophage arrangements in classical strains will not yield extrachromosomal CTX DNA and thus will not yield virions, and our experimental results confirm this prediction. Thus, failure of O1 classical strains of V. cholerae to produce CTXphi is due to overall deficiencies in the structures of the arrays of classical prophages, rather than to mutations affecting individual CTX prophage genes
- 42. Genomic relatedness of Staphylococcus aureus phages of the International Typing Set and detection of serogroup A, B, and F prophages in lysogenic strains. Doskar, J., Pallova, P., Pant, cek, Rosypal, S., R, zickova, Pant, ckova, Kailerova, J., Kleparnik, K., Mala, Z., Bocek, P. (2000). Canadian Journal of Microbiology 46:1066-1076. On the basis of Hindlll-restriction digest analysis of genomic DNAs, the S. aureus bacteriophages of the International Typing Set were divided into five clusters designated as A, F, Ba, Bb, and Bc. The clusters A and F include all the phages of serogroups A and F and correspond to species 3A and 77 proposed by Ackermann and DuBow (1987). On the other hand, the phages of serogroup B were divided into three clusters designated as Ba, Bb, and Bc that differ significantly each from the other in their restriction patterns. The clusters Ba and Bb may represent two separate species, while the cluster Bc may include more than one phage species. For each of the phage serogroups A, B, and F, common HindIII-restriction fragments of phage 3A (1700 bp), of 53 (4060 bp), and of 77 (8300 bp) were used for the preparation of probes specific to the phages of serogroups A, B, and F. These probes were very effective, making it possible to detect up to three different prophages in a given lysogenic strain at the same time. Restriction enzyme maps of phages 3A, 53, and 77, each representing a different serogroup, were constructed. The restriction maps of phage 3A and that of phage 77 are linear, whereas that of phage 53 is circular and exhibits a circular permutation. DNAs of the phages of serogroups A and F have cohesive ends. On each restriction map, the sites corresponding to specific probes are indicated. The size of intact genomic DNA of all phages estimated by PFGE varies within the range of 41.5-46.2 kb
- 43. Bacteriophages of spirochetes. Eggers, C. H., Casjens, S., Hayes, S. F., Garon, C. F., Damman, C. J., Oliver, D. B., Samuels, D. S. (2000). *J Mol Microbiol Biotechnol* 2:365-373. Historically, a number of bacteriophage-like particles have been observed in association with members of the bacterial order Spirochetales, the spirochetes. In the last decade, several spirochete bacteriophages have been isolated and characterized at the molecular level. We have recently characterized a bacteriophage of the Lyme disease agent, *Borrelia burgdorferi*, which we have designated phiBB-1. Here we review the history of the association between the spirochetes and their bacteriophages, with a particular emphasis on phiBB-1 and its prophage, the 32-kb circular plasmid family of *B. burgdorferi*

- 44. The two faces of mutation: Extinction and adaptation in RNA viruses. Elena, S. F., Miralles, R., Cuevas, J. M., Turner, P. E., Moya, A. (2000). *IUBMB Life* 49:5-9. From a population standpoint, two main features characterize the replication of RNA viruses and viruses that use RNA as a replicative intermediate: high genetic variability, and enormous fluctuations in population size. Their genetic variability mainly reflects a lack of the proof-reading and post-replicative error correction mechanisms that operate during cellular DNA replication, but recombination and segment exchange can also play an important role, Viral population size can change tremendously as a consequence of transmission between hosts or between different tissues within an infected host. A new infection can be initiated with very few particles that subsequently expand many trillion-fold. Repeated bottleneck events can lead to drastic fitness losses or even to viral extinction, whereas continuously large population sizes result in fitness gains and adaptation. Here we review experimental evidence for the effects of mutation, selection, and genetic drift on the adaptation and extinction of RNA viruses.
- 45. Titration of infective and noninfective Ff filamentous bacteriophages using a monoclonal antibody against g3p. Frisch, C., Knappik, A., Choidas, M., Tesar, M. (2000). *BioTechniques* 29:26-8, 30.
- 46. Horizontal gene transfer in bacterial and archaeal complete genomes. Garcia-Vallve, S., Romeu, A., Palau, J. (2000). Genome Research 10:1719-1725. There is growing evidence that horizontal gene transfer is a potent evolutionary force in prokaryotes, although exactly how potent is not known. We have developed a statistical procedure for predicting whether genes of a complete genome have been acquired by horizontal gene transfer. It is based on the analysis of G+C contents, codon usage, amino acid usage, and gene position. When we applied this procedure to 17 bacterial complete genomes and seven archaeal ones, we found that the percentage of horizontally transferred genes varied from 1.5% to 14.5%. Archaea and nonpathogenic bacteria had the highest percentages and pathogenic bacteria, except for Mycoplasma genitalium, had the lowest. As reported in the literature, we found that informational genes were less likely to be transferred than operational genes. Most of the horizontally transferred genes were only present in one or two lineages. Some of these transferred genes include genes that form part of prophages, pathogenecity islands, transposases, integrases, recombinases, genes present only in one of the two Helicobacter pylori strains, and regions of genes functionally related. All of these findings support the important role of horizontal gene transfer in the molecular evolution of microorganisms and speciation
- 47. Inducible stx2 phages are lysogenized in the enteroaggregative and other phenotypic *Escherichia coli* O86:HNM isolated from patients. lyoda, S., Tamura, K., Itoh, K., Izumiya, H., Ueno, N., Nagata, K., Togo, M., Terajima, J., Watanabe, H. (2000). *FEMS Microbiology Letters* 191:7-10. We characterized two Shiga toxin-producing *Escherichia coli* (STEC) O86:HNM isolates from a patient with hemolytic uremic syndrome (HUS) or bloody diarrhea. Both of them did not possess the eaeA gene. However, the isolate from a HUS patient carried genetic markers of enteroaggregative *E. coli* (EAEC) and showed aggregative adherence pattern to HEp-2 cells. The other isolate from bloody diarrhea, which was negative with EAEC markers, was diffusely adhered to HEp-2 cells. The stx2 gene in both E. coli O86:HNM strains was encoded in each infectious phage, which was partially homologous to that of strain EDL933, a STEC O157:H7. These results will help to explain the genotypic divergences of STEC
- 48. Isolation of coliphages specific to enterotoxigenic *E. coli* (ETEC). Jothikumar, N., Reddy, C. G., Sundari, R. B., Saigopal, D. V. (2000). Journal of Environmental Monitoring 2:372-374. Bacteriophages specific to Enterotoxigenic *E. coli* (ETEC) are reported for the first time. Out of 15 isolated phages only 10 were specific to strains of ETEC. All ten phages of dsDNA could be grouped into three different genotypes based on their RAPD patterns observed and it is likelly that they belong to only 3 different strains. The three phages yielded clear plaques on 10 strains of ETEC within 4-6 h at 37 degrees C
- 49. Influence of infected cell growth state on bacteriophage reactivation levels. Kadavy, D. R., Shaffer, J. J., Lott, S. E., Wolf, T. A., Bolton, C. E., Gallimore, W. H., Martin, E. L., Nickerson, K. W., Kokjohn, T. A. (2000). Applied and Environmental Microbiology 66:5206-5212. Reactivation of UV-C-inactivated Pseudomonas aeruginosa bacteriophages D3C3, F116, G101, and UNL-1 was quantified in host cells infected during the exponential phase, during the stationary phase, and after starvation (1 day, 1 and 5 weeks) under conditions designed to detect dark repair and photoreactivation. Our experiments revealed that while the photoreactivation capacity of stationary-phase or starved cells remained about the same as that of exponential-phase cells, in some cases their capacity to support dark repair of UV-inactivated bacteriophages increased over 10-fold. This enhanced reactivation capacity was correlated with the ca. 30-fold-greater UV-C resistance of *P. aeruginosa* host cells that were in the stationary phase or exposed to starvation conditions prior to irradiation. The dark repair capacity of *P. aeruginosa* cells that were infected while they were starved for prolonged periods depended on the bacteriophage examined. For bacteriophage D3C3 this dark repair capacity declined with prolonged starvation, while for bacteriophage G101 the dark repair capacity continued to increase when cells were starved for 24 h or 1 week prior to infection. For G101, the reactivation potentials were 16-, 18-, 10-, and 3-fold at starvation intervals of 1 day, 1 week, 5 weeks, and 1. 5 years, respectively. Exclusive use of exponential-phase cells to quantify bacteriophage reactivation should detect only a fraction of the true phage reactivation potential
- 50. Vibrio cholerae O139 bacteriophages. Kudriakova, T. A., Makedonova, L. D., Kachkina, G. V., Saiamov, S. R. (2000). Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii 28-30. Cholera bacteriophages have been isolated from 27 lysogenic cultures of V. cholerae O139. As shown the pages under study belong to two morphological groups A1 and F1 and serological types II and XII. The use of prophage typing and the sensitivity test to specific phage made it possible to differentiate V. cholerae strains, serogroup O139
- 51. Diminishing returns of population size in the rate of RNA virus adaptation. Miralles, R., Moya, A., Elena, S. F. (2000). *Journal of Virology* 74:3566-3571. Whenever an asexual viral population evolves by adapting to new environmental conditions, beneficial mutations, the ultimate cause of adaptation, are randomly produced and then fixed in the population. The larger the population size and the higher the mutation rate, the more beneficial mutations can be produced per unit time, With the usually high mutation rate of RNA viruses and in a large enough population, several beneficial mutations could arise at the same time but in different genetic backgrounds, and if the virus is asexual, they will never be brought together through recombination, Thus, the best of these genotypes must outcompete each other on their way to fixation, This competition among beneficial mutations has the effect of slowing the overall rate of adaptation. This phenomenon is known as clonal interference. Clonal interference predicts a speed limit for adaptation as the population size increases. In the present report, by varying the size of evolving vesicular stomatitis virus populations, we found evidence clearly demonstrating this speed limit and thus indicating that clonal interference might be an important factor modulating the rate of adaptation to an in vitro cell system. Several evolutionary and epidemiological implications of the clonal interference model applied to RNA viruses are discussed.

- 52. Properties of natural interspecific hybrids of transposable phages of *Pseudomonas aeruginosa*: specific characteristics of phage PL24 transposition. Mit'kina, L. N., Krylov, V. N. (2000). Genetika 36:1330-1339. Properties of natural hybrid transposable phages (TP) of *Pseudomonas aeruginosa*, including phage PL24 and lysogens for this phage, were studied. PL24 possesses the properties of TP from two previously described groups, B3 and D3112. Its genome, unlike the genome of D3112, contains many sites susceptible to the SalGI restriction endonuclease and possesses no more than 100 nucleotides of bacterial origin located at the left genome end. However, unlike B3, phage PL24 failed to induce auxotrophic mutants upon integration in the bacterial genome. This phage differed from both B3 and D3112 in sensitivity to chloroform treatment. A more detailed examination of a group containing 25 randomly isolated lysogens for phage PL24 revealed previously unknown processes occurring at early stages of bacterial lysogenization. There are at least two different modes of cell lysogen) at individual sites. In the second case, polylysogenic bacteria appeared, and, after primary integration of a phage genome, replicative transposition occurred at new sites (often accompanied by the appearance of prophage clusters at these sites). The choice of the mode of lysogenization can be determined both by differences in the physiological state of bacteria and by specific features of phage PL24, which possibly affect the time of repressor accumulation to the concentration sufficient for blocking phage growth or the stability of the lysogenic state
- 53. The evolution of RNA viruses: A population genetics view. Moya, A., Elena, S. F., Bracho, A., Miralles, R., Barrio, E. (2000). Proceedings of the National Academy of Sciences of the United States of America 97:6967-6973. RNA viruses are excellent experimental models for studying evolution under the theoretical framework of population genetics. For a proper justification of this thesis we have introduced some properties of RNA viruses that are relevant for studying evolution. On the other hand, population genetics is a reductionistic theory of evolution. It does not consider or make simplistic assumptions on the transformation laws within and between genotypic and phenotypic spaces. However, such laws are minimized in the case of RNA viruses because the phenotypic space maps onto the genotypic space in a much more linear way than on higher DNA-based organisms. Under experimental conditions, we have tested the role of deleterious and beneficial mutations in the degree of adaptation of vesicular stomatitis virus (VsV). a nonsegmented virus of negative strand. We also have studied how effective population size, initial genetic variability in populations, and environmental heterogeneity shapes the impact of mutations in the evolution of vesicular stomatitis virus. Finally, in an integrative attempt, we discuss pros and cons of the quasispecies theory compared with classic population genetics models for haploid organisms to explain the evolution of RNA viruses.
- 54. Phage therapy—advantages over antibiotics? Pirisi, A. (2000). Lancet 356:1418. As antibiotic-resistant bacteria continue to threaten standard therapies against bacterial infections, a new breed of antimicrobials may be on the horizon. Many researchers believe that bacteriophages—viruses that only infect bacteria—are a promising potential therapy for bacterial disease treatment.
- 55. Bacterial monopolists: the bundling and dissemination of antimicrobial resistance genes in gram-positive bacteria. Rice, L. B. (2000). *Clinical Infectious Diseases* 31:762-769. Antibiotic resistance is the unavoidable result of our placing selective pressure on the microbial community. Advances in molecular biology techniques in the past 2 decades have allowed us to greatly improve our understanding of the mechanisms by which resistance emerges and disseminates among human pathogenic bacteria. Gram-positive bacteria employ a diverse array of elements, including plasmids, transposons, insertion sequences, and bacteriophages, to disseminate resistance. An understanding of these mechanisms and their prevalence can improve our ability to treat clinical infections in hospitalized patients, as well as to predict and control the spread of resistant bacteria in the nosocomial environment
- 56. Comparison of methods for detecting genotypes of F-specific RNA bacteriophages and fingerprinting the origin of faecal pollution in water samples. Schaper, M., Jofre, J. (2000). Journal of Virological Methods 89:1-10. The performance of Salmonella typhimurium WG49 and Escherichia coli HS(pFamp)R was compared on detecting the different genotypes of F-specific RNA bacteriophages by plaque hybridisation. The sensitivity of this assay was also compared with the sensitivity of RT-PCR followed by Southern blotting for detecting F-specific RNA bacteriophages belonging to genotype III in water. S. typhimurium WG49 detected slightly higher numbers of F-specific RNA bacteriophages than E. coli HS(pFamp)R both in mixtures of pure culture bacteriophage suspensions and in water samples. There were no differences between the two host strains with regard to detection of the four genotypes of F-specific RNA phages both in mixtures of pure culture bacteriophages. In urban sewage samples, the host strains detected genotypes II and III as the predominant F-RNA bacteriophages. Plaque transfer to a N(+) hybond membrane and posterior hybridisation was easier using S. thyphimurium WG49 as the host strain. The efficiency of detection in sewage of genotype III F-specific RNA bacteriophages by RT-PCR was inferior to that of plaque hybridisation with the assay conditions described below. Hybridisation of plaques obtained on WG49 seems to be the most sensitive method to study the distribution of genotypes of F-specific RNA bacteriophages in water samples.
- 57. Twenty-three years of *Klebsiella* phage typing: a review of phage typing of 12 clusters of nosocomial infections, and a comparison of phage typing with K serotyping. Sechter, I, Mestre, F., Hansen, D. S. (2000). *Clin Microbiol Infect* **6:233-238.** OBJECTIVE: To review phage typing of 12 clusters of nosocomial *Klebsiella* infections which occurred between 1974 and 1997, and to compare phage typing and K serotyping. Materials and methods A total of 489 clinical and laboratory Klebsiella isolates were phage typed using 110 different phage preparations and K typed by counter current immunoelectrophoresis against 77 K antisera. RESULTS: A total of 152 phage types (PT) and 82 K types were found. Thirty-six phage types and 14 K types were represented only by the reference type strains. Of the remaining 68 K types, 60 could be subdivided into from two to 10 phage types. Ten out of 12 clusters of nosocomial Klebsiella infections could be verified as outbreaks by phage typing, whereas two clusters were found to be accumulations of sporadic cases. K typing performed retrospectively confirmed these results. In addition, for a subset of 104 epidemiologically unrelated isolates, O typing and pulsed field gel electrophoresis typing data were available. Based on these results the discriminative power of phage typing was found to be very useful, although it seems less suitable for long-term surveillance purposes
- 58. [Isolation and comparative study of a group of temperate bacteriophages of rhizospheric pseudomonads *Pseudomonas putida*]. Shaburova, O. V., Burkal'tseva, M. V., Pleteneva, E. A., Krylov, V. N. (2000). Genetika 36:915-919. We have isolated several new temperate bacteriophages for rhizosphere pseudomonads *Pseudomonas putida*. Examination of these phages, along with two previously isolated temperate phages PP56 and PP71 of P. putida PpG1 (biovar A), allowed us to classify them into four species on the basis of DNA cross-homology; relative genomic size; and, to a certain extent, the morphology of phage particles. Two of these species are represented by nonidentical variants. No transposable phages were found among these two new species. Three phage species cause various-types of lysogenic conversion

manifested in growth suppression of other phage species. This seems to account for the fact that the temperate phage of rhizosphere pseudomonads are seldom encountered. The new phages described can be used for selection of phage-resistant bacterial forms exhibiting antifungal activity that are commercially produced and used for treatment of seeds of cultivated plants

- 59. Cost of host radiation in an RNA virus. Turner, P. E., Elena, S. F. (2000). Genetics 156:1465-1470. Although host radiation allows a parasite to expand its ecological niche, traits governing the infection of multiple host types can decrease fitness in the original or alternate host environments. Reasons for this reduction in fitness include slower replication due to added genetic material or modifications, fitness trade-offs across host environments, and weaker selection resulting from simultaneous adaptation to multiple habitats. We examined the consequences of host radiation using vesicular stomatitis virus (VSV) and mammalian host cells in tissue culture. Replicate populations of VSV were allowed to evolve for 100 generations on the original host (BHK cells), on either of two novel hosts (HeLa and MDCK cells), or in environments where the availability of novel hosts fluctuated in a predictable or random way. As expected, each experimental population showed a substantial fitness gain in its own environment, but those evolved on new hosts (constant or fluctuating) suffered reduced competitiveness on the original host, However, whereas evolution on one novel host negatively correlated with performance on the unselected novel host, adaptation in fluctuating environments led to fitness improvements in both novel habitats.
- 60. Phage conversion of exfoliative toxin A production in *Staphylococcus aureus*. Yamaguchi, T., Hayashi, T., Takami, H., Nakasone, K., Ohnishi, M., Nakayama, K., Yamada, S., Komatsuzawa, H., Sugai, M. (2000). *Molecular Microbiology* 38:694-705. The staphylococcal exfoliative toxins (ETs) are extracellular proteins that cause splitting of human skin at the epidermal layer during infection in infants. Two antigenically distinct toxins possessing identical activity have been isolated from *Staphylococcus aureus*, ETA and ETB. The gene for ETA (eta) is located on the chromosome, whereas that for ETB is located on a large plasmid. The observation that relatively few clinical isolates produce ETA suggests that the eta gene is acquired by horizontal gene transfer. In this study, we isolated a temperate phage (phiETA) that encodes ETA and determined the complete nucleotide sequence of the phiETA genome. phiETA has a head with a hexagonal outline and a non-contractile and flexible tail. The genome of phiETA is a circularly permuted linear double-stranded DNA, and the genome size is 43 081 bp. Sixty-six open reading frames (ORFs) were identified on the phiETA genome, including eta, which was found to be located very close to a putative attachment site (attP). phiETA converted ETA non-producing strains into ETA producers. Southern blot analysis of chromosomal DNA from clinical isolates suggested that phiETA or related phages are responsible for the acquisition of eta genes in *S. aureus*
- 61. Rate of deleterious mutation and the distribution of its effects on fitness in vesicular stomatitis virus. Elena, S. F., Moya, A. (1999). Journal of Evolutionary Biology 12:1078-1088. Despite their importance, the parameters describing the spontaneous deleterious mutation process have not been well described in many organisms. If mutations are important for the evolution of every living organism, their importance becomes critical in the case of RNA-based viruses, in which the frequency of mutation is orders of magnitude larger than in DNA-based organisms. The present work reports minimum estimates of the deleterious mutation rate, as well as the characterization of the distribution of deleterious mutational effects on the total fitness of the vesicular stomatitis virus (VSV). The estimates are based on mutation-accumulation experiments in which selection against deleterious mutations was minimized by recurrently imposing genetic bottlenecks of size one. The estimated deleterious mutation rate was 1.2 mutations per genome and generation, with a mean fitness effect of -0.39% per generation. At the end of the mutation-accumulation experiment, the average reduction in fitness was 38% and the distribution of accumulated deleterious effects was, on average, left-skewed. The magnitude of the skewness depends on the initial fitness of the clone analysed. The implications of our findings for the evolutionary biology of RNA viruses are discussed.
- 62. Horizontal gene transfer in glycosyl hydrolases inferred from codon usage in *Escherichia coli* and *Bacillus subtilis*. Garcia-Vallve, S., Palau, J., Romeu, A. (1999). *Molecular Biology and Evolution* 16:1125-1134. Glycosyl hydrolase (GH) genes from *Escherichia coli* and *Bacillus subtilis* were used to search for cases of horizontal gene transfer. Such an event was inferred by G + C content, codon usage analysis, and a phylogenetic congruency test. The codon usage analysis used is a procedure based on a distance derived from a Pearson linear correlation coefficient determined from a pairwise codon usage comparison. The distances are then used to generate a distance-based tree with which we can define clusters and rapidly compare codon usage. Three genes (yagH from *E. coli* and xynA and xynB from *B. subtilis*) were determined to have arrived by horizontal gene transfer and were located in *E. coli* CP4-6 prophage, and *B. subtilis* prophages 6 and 5, respectively. In this study, we demonstrate that with codon usage analysis, the proposed horizontally transferred genes can be distinguished from highly expressed genes
- 63. Clonal interference and the evolution of RNA viruses. Miralles, R., Gerrish, P. J., Moya, A., Elena, S. F. (1999). Science 285:1745-1747. In asexual populations, beneficial mutations that occur in different lineages compete with one another. This phenomenon, known as clonal interference, ensures that those beneficial mutations that do achieve fixation are of Large effect. Clonal interference also increases the time between fixations, thereby stowing the adaptation of asexual populations. The effects of clonal interference were measured in the asexual RNA virus vesicular stomatitis virus; rates and average effects of beneficial mutations were quantified.
- 64. Effect of population patchiness and migration rates on the adaptation and divergence of vesicular stomatitis virus quasispecies populations. Miralles, R., Moya, A., Elena, S. F. (1999). *Journal of General Virology* 80:2051-2059. The effect of migration among different isolated virus quasispecies populations on their adaptation and diversity was analysed through experimental evolution. An in vitro cell system was employed to simulate migration of vesicular stomatitis virus between isolated homogeneous host cell populations. The results clearly demonstrated a positive correlation between the migration rate and the magnitude of the mean fitness reached by the virus quasispecies populations. The results also showed, although less clearly, that fitness differences among quasispecies decreased with the magnitude of migration. These results are in close agreement with predictions of standard population genetics theory. These results can be explained in terms of the spread of beneficial mutations, originating in a single isolated quasispecies, through the entire system formed by the different quasispecies populations contained in different host cell populations.
- 65. Lack of evolutionary stasis during alternating replication of an arbovirus in insect and mammalian cells. Novella, I. S., Hershey, C. L., Escarmis, C., Domingo, E., Holland, J. J. (1999). *Journal of Molecular Biology* 287:459-465. The evolution of vesicular stomatitis virus (VSV) in a constant environment, consisting of either mammalian or insect cells, has been compared to the evolution of the same viral population in changing environments consisting in alternating passages in mammalian and insect cells. Fitness increases were observed in all cases. An initial fitness loss of VSV passaged in insect cells was noted when fitness was measured in BHK-21 cells, but this effect could be attributed to a difference of temperature during VSV replication at 37 degrees C in BHK-21 cells. Sequencing of nucleotides 1-4717 at the 3' end of the VSV genome (N, P, M and G genes)

showed that at passage 80 the number of mutations accumulated during alternated passages (seven mutations) is similar or larger than that observed in populations evolving in a constant environment (two to four mutations). Our results indicate that insect and mammalian cells can constitute similar environments for viral replication. Thus, the slow rates of evolution observed in natural populations of arboviruses are not necessarily due to the need for the virus to compromise between adaptation to both arthropod and vertebrate cell types.

- 66. Exponential fitness gains of RNA virus populations are limited by bottleneck effects. Novella, I. S., Quer, J., Domingo, E., Holland, J. J. (1999). Journal of Virology 73:1668-1671. Fitness is a parameter that quantitatively measures adaptation of a virus to a given environment. We have previously reported exponential fitness gains of large populations of vesicular stomatitis virus replicating in a constant environment (I. S. Novella et al., Proc. Natl. Acad. Sci. USA 92:5841-5844, 1995). In this paper, we report that during long-term passage of such large viral populations, fitness values reached a high-fitness plateau during which stochastic fitness variations were observed. This effect appears likely to be due to bottleneck effects on very high fitness populations.
- 67. Evolutionary dynamics of fitness recovery from the debilitating effects of Muller's ratchet. Elena, S. F., Davila, M., Novella, I. S., Holland, J. J., Domingo, E., Moya, A. (1998). Evolution 52:309-314. The great adaptability shown by RNA viruses is a consequence of their high mutation rates. The evolution of fitness in a severely debilitated, clonal population of the nonsegmented ribovirus vesicular stomatitis virus (VSV) has been compared under five different demographic regimes, ranging from severe serial bottleneck passages tone virion) to large population passages (10(6) virions or more) under similar environmental conditions (cell culture type and temperature). No matter how small the bottleneck, the fitness of the evolved populations was always higher than the fitness of the starting population; this result is clearly different from that previously reported for viruses with higher fitness. The reattainment of fitness under a regime of serial population passages showed two main characteristics: (1) the rate of adaptation was higher during early passages; and (2) a maximum fitness value was reached after a large number of passages. The maximum fitness reached by this initially debilitated clone was similar to the fitness of wild-type virus. The practical implications of these findings in the design of vaccines using attenuated viruses are also discussed.
- 68. Bacteriophage: tools toward a cell-targeted delivery. Paillard, F. (1998). Human Gene Therapy 9:2307-2308.
- 69. Population dynamics of phytoplankton and viruses in a phosphate-limited mesocosm and their effect on DMSP and DMS production. Wilson, W. H., Turner, S., Mann, N. H. (1998). Estuarine, Coastal and Shelf Science 46 (Supplement a):49-59. The effect of phosphate limitation on viral abundance, phytoplankton bloom dynamics and production of dimethylsulphoniopropionate (DMSP) and dimethyl sulphide (DMS) was investigated in seawater mesocosm enclosures, in a Norwegian fjord, during June 1995. Daily estimates of viral concentrations, based on transmission electron microscope (TEM) counts, varied on an apparently random basis in each of the enclosures. A large Synechococcus spp. bloom developed in an enclosure which was maintained at a high N:P ratio, simulating phosphate-deplete growth conditions. Following phosphate addition to this enclosure, there was a large increase in estimated virus numbers shortly before an apparent collapse of the Synechococcus bloom. It is tentatively suggested that lysogenic viruses were induced following phosphate addition to the phosphate-limited enclosures, and that these observations add to a growing body of evidence which supports the hypothesis that nutrient availability may be responsible for the switch between lysogeny and lytic production. High DMS concentrations and viral numbers were observed on the demise of the flagellate (predominantly Emiliania huxleyi) and diatom blooms, but overall there was no significant correlation. Highest concentrations of DMSP were associated with blooms of E. huxleyi, for which an intracellular concentration of 0.5 pg cell<sup>-1</sup> (SD, 0.06) was calculated. Good correlation of DMSP with Synechococcus spp. cell numbers was observed, suggesting that these species of picoplankton may be significant producers of DMSP. No effects of phosphate limitation on DMS and/or DMSP production were evident from the data.

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# **Acknowledgements**

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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.