ranging from 0.3 to 2. In the analysis dissimilarity values in the matrix are transformed to distances between stimuli in a spatial configuration because the relationship between dissimilarity and distance is assumed to be monotonic—that is, similar stimuli are close together and very different ones are far apart. MDS searches for the spatial configuration and the monotonic relationship that minimize a criterion function known as "stress."

The two-dimensional configuration for the ten stimuli with Minkowski coefficient 0.5 and the correspondence between the alphabetical symbols and the electrode pairs are shown in Fig. 1. The Minkowski coefficient 0.5 in the range 0.3 to 2 corresponds to minimum stress among the two-dimensional configurations. A two-dimensional configuration was considered suitable because the stress (8.4 percent) was much smaller than that for one dimension (27 percent). The two-dimensional configurations with other Minkowski coefficients followed closely the configuration as depicted in Fig. 1, and the difference in stress values between one and two dimensions was also similar.

The close resemblance between the two configurations of the alphabets in the left and right panels in Fig. 1 indicates that the axes of the two-dimensional configuration can be interpreted as corresponding to the position of the more apical electrode and the more basal electrode of the two-electrode combination. A Minkowski coefficient of 0.5 implies that the dissimilarities between two electrode pairs differing in both apical and basal electrodes is even greater than the sum of the two component dissimilarities. These results suggest that two-electrode stimulation was perceived as a sensation with two components and may therefore be used to present speech information with two components, such as the first and second formants. The two axes for electrical stimulation in Fig. 1 parallel those corresponding to the first and second formant frequencies in the analysis of acoustic vowel confusion by MDS (*11*). If a one-dimensional configuration had been acceptable, this would have indicated that the sensation produced by two-electrode stimulation would not be able to convey information with two components.

The results indicate that, in addition to the presentation of the second formant by activation of only one electrode during a stimulus period in a speech processor, as described by Tong *et al.* (*5*), speech information such as the first formant may be presented to implant patients by two-electrode stimulation. The speech information related to the fundamental frequency of speech, on the other hand, can be encoded in electric repetition rate as in the speech processor. This was shown to be feasible (*9*) because the discriminability of repetition rate for two-electrode stimulation was as good as or better than that for single-electrode stimulation. Finally, we should note that the electric pulse trains used in this study, although adequate in generating two-component sensations, may not be the optimal stimulus configuration for use in a speech processor with two-electrode stimulation. Differences in the perceptual characteristics produced by electric stimulus configurations that differ both in the order of occurrence and the amount of delay between the two apical and basal electrodes are not yet known.

Y. C. TONG
R. C. DOWELL
P. J. BLAMEY
G. M. CLARK
Department of Otolaryngology, University of Melbourne, Parkville, Victoria 3052, Australia

References and Notes

1. For summary of results from various centers see the abstracts and papers in *Proceedings of the International Cochlear Implant Conference*, (New York Academy of Sciences, New York, April 1982).
2. F. B. Simmons, J. M. Epley, R. C. Lummis, N. Guttman, L. S. Frishkopf, L. D. Harmon, E. Zwicker, *Science* **148**, 104 (1965).
3. Y. C. Tong, G. M. Clark, R. C. Dowell, L. F. A. Martin, P. M. Seligman, J. F. Patrick, *Acta Oto-Laryngol.* **92**, 193 (1981).
4. G. M. Clark, Y. C. Tong, L. F. A. Martin, *Laryngoscope* **91**, 628 (1981).
5. Y. C. Tong, G. M. Clark, P. J. Blamey, P. A. Busby, R. C. Dowell, *J. Acoust. Soc. Am.* **71**, 152 (1982).
6. Y. C. Tong, P. J. Blamey, R. C. Dowell, G. M. Clark, paper presented at the 102nd meeting of the Acoustical Society of America, December 1981.
7. R. Plomp, *Aspects of Tone Sensation* (Academic Press, London, 1976), p. 109.
8. R. C. Dowell, Y. C. Tong, P. J. Blamey, G. M. Clark, paper presented at the 102nd meeting of the Acoustical Society of America, December 1981.
9. Y. C. Tong and G. M. Clark, in *Proceedings of the International Cochlear Implant Conference* (New York Academy of Sciences, New York, April 1982).
10. J. B. Kruskal, *Psychometrika* **29**, 1 (1964).
11. R. N. Shepherd, in *Human Communication: A Unified View*, E. E. David and P. B. Denes, Eds. (McGraw-Hill, New York, 1972).
12. Supported by the National Health and Medical Research Council of Australia, the Department of Science and Technology of Australia, the Channel 10 Deafness Appeal, the Deafness Foundation of Victoria, and the Lions International.

13 May 1982; revised 9 August 1982

Virus Infection of Culturable *Chlorella*-Like Algae and Development of a Plaque Assay

Abstract. *Four distinct viruses with double-stranded DNA are known to replicate in* Chlorella*-like algae symbiotic with hydras and paramecia. An attempt was made to infect a number of cultured* Chlorella *strains derived from invertebrate hosts with these viruses. One of the viruses, PBCV-1, replicated in two of the algal strains. Restriction endonuclease analysis of the viral DNA showed that the infectious progeny virus was identical to the input virus; thus, Koch's postulates were fulfilled. Viral infection of the two* Chlorella *strains has allowed the large-scale production of a eukaryotic algal virus and the development of a plaque assay for the virus.*

Although viruses that infect cyanobacteria (blue-green algae) have been studied extensively (*1*), little is known about viruses of eukaryotic algae (*1, 2*). Most viruses or virus-like particles in eukaryotic algae have been detected by ultrastructural studies, and only a few attempts (*3*) have been made to characterize these particles because the viruses are difficult to obtain in sufficient quantity. Several factors contribute to this lack of material: (i) usually only a few algal cells contain particles, (ii) usually the cells contain particles in only one stage of the life cycle, (iii) the cells that have particles may not lyse, and (iv) the particles may not be infectious. Consequently, finding a culturable eukaryotic alga that can be synchronously and efficiently infected with a virus would provide an opportunity to study a new type of virus-host relationship.

We recently identified and partially characterized four distinct viruses with double-stranded DNA that replicate in *Chlorella*-like green algae symbiotic with hydras or paramecia (*4, 5*). These viruses, designated HVCV-1, HVCV-2, HVCV-3 (from *Hydra viridis Chlorella*), and PBCV-1 (from *Paramecium bursaria Chlorella*), first appear in the algae a few hours after the algae are isolated from their hosts. Within 24 hours most of the algae are lysed. The source of the viruses is unknown, but we have found them in all symbiotic algae from hydras and paramecia that we have examined. The viruses may be latent in some or all of the algae as long as the latter remain in a symbiotic relationship with their host (*4, 5*). Removing the algae from their host may induce the viruses to enter a lytic phase. Regardless of the source of the viruses, their appearance usually pre-

cludes the successful isolation and culturing of symbiotic algae. A few investigators have reported success in culturing *Chlorella*-like algae from *Paramecium bursaria* (*6*) and some *Hydra viridis* strains (*7*). We now describe attempts to use the four viruses to infect several culturable *Chlorella* strains derived from invertebrate hosts and two free-living *Chlorella* strains.

Chlorella isolates (Table 1) were grown in liquid culture (*8*) and inoculated with virus (*9*). Algal growth was monitored by light scattering at A_{640} (absorption at 640 nm) for 72 hours. Two of the *Chlorella* strains (NC64A and ATCC-30562), originally isolated from *Paramecium bursaria*, were lysed within 24 hours of their inoculation with PBCV-1. Growth of the other *Chlorella* isolates was not affected by any of the viruses. To determine whether PBCV-1 infected and replicated in *Chlorella* strains NC64A and ATCC-30562, 48-hour-old lysates were subjected to the scheme used to purify PBCV-1 (*5*). A single sharp zone of absorption in the ultraviolet at 254 nm was obtained in sucrose density gradients that sedimented at the same rate as PBCV-1 (about 2300*S*) (*5*). The amount of virus recovered was 50 to 100 times greater than the input virus for both *Chlorella* strains. Examination of this material with an electron microscope indicated viral particles similar to PBCV-1 in size and morphology (*5*).

The virus produced in culture was compared to PBCV-1 by restriction endonuclease analysis (BamH I, Sma I, and Hind III) of isolated DNA (*5*). The restriction patterns of the progeny virus DNA were identical to those of PBCV-1 DNA (Fig. 1). The progeny virus particles also infected fresh cultures of both *Chlorella* strains. Thus, Koch's postulates were fulfilled, and PBCV-1 is a true virus and not simply a virus-like particle.

We attempted to develop a biological (plaque) assay for PBCV-1 analogous to that used for bacteriophages. *Chlorella* strains NC64A and ATCC-30562 were grown to a density of 2×10^7 to 3×10^7 algae per milliliter, concentrated by centrifugation, and resuspended in MBBM (*8*) at 38×10^7 algae per milliliter. Two hundred microliters of algae (7.6×10^7 algae) plus 100 μl of appropriate dilutions of PBCV-1 were added to 2.5 ml of 0.7 percent agar in MBBM (48° to 50°C) and immediately overlaid on petri plates containing 15 ml of MBBM plus 1.5 percent agar. The plates were then incubated at 25°C in continuous light (*8*). Plaques were visible after 36 to 48 hours for NC64A and 3 to 4 days for ATCC-30562. It was easiest to count the NC64A plaques with a plaque counter after 4 days (Fig. 2). There was a linear relation between viral concentration, as measured by light scattering at A_{260}, and number of plaques. Typically, 1.5 to 3×10^{10} plaque-forming units of PBCV-1 were obtained per unit of absorption at 260 nm (uncorrected for light scatter).

Heretofore there have been only a few reports of an infectious virus for any eukaryotic algae (*10*); to our knowledge those findings were not investigated further. The PBCV-1–*Chlorella* system described in this report has allowed the production of milligram quantities of virus. The development of a biological assay for PBCV-1 will allow one-step growth studies, genetic studies, and characterization of the virus. Thus PBCV-1 should serve as a prototype for studies of viruses of eukaryotic algae. In addition, to our knowledge the PBCV-1–*Chlorella* system represents the first vi-

Table 1. *Chlorella* isolates inoculated with viruses HVCV-1, HVCV-2, HVCV-3, and PBCV-1.

Chlorella isolate	Host	Source
CF-FC	Hydra, Florida strain	D. Chapman*
CF-FD	Hydra, Florida strain	D. Chapman
CF-FF	Hydra, Florida strain	D. Chapman
CF-FG	Hydra, Florida strain	D. Chapman
CF-77/K3	Hydra, Florida strain	D. Chapman
CF-77/K5	Hydra, Florida strain	D. Chapman
CF-75	Hydra, Florida strain	D. Chapman
CAI-2	Hydra, California strain	D. Chapman
JHA-1	Hydra, Jubelee strain	D. Weis†
EHA-4	Hydra, European strain	D. Weis
3N813-1	Paramecium	D. Weis
1	Paramecium	D. Weis
NC64A	Paramecium	L. Muscatine*
ATCC-30562	Paramecium	American Type Culture Collection
Spi SES-255	Stentor	D. Weis
UTEX 838	Sponge	D. Weis
Chlorella vulgaris, UTEX-251	Free-living	D. Chapman
Chlorella pyrenoidosa, UTEX-397	Free-living	D. Chapman

*University of California, Los Angeles. †Cleveland State University.

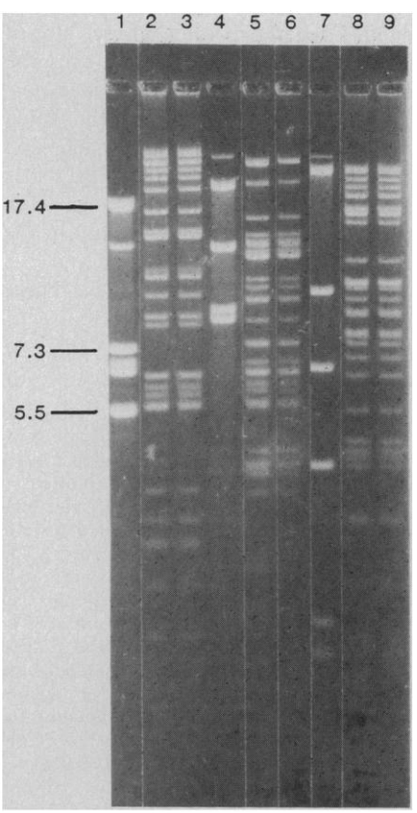
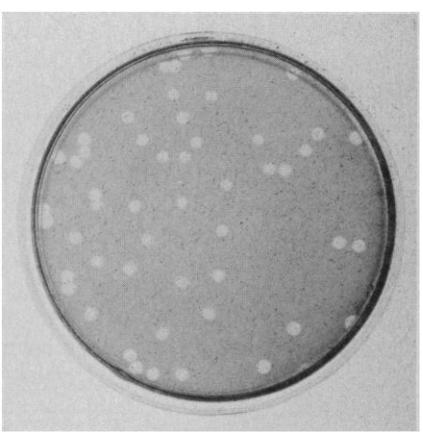


Fig. 1 (left). Electrophoresis of algal virus DNA after treatment with BamH I (lanes 1 to 3), Sma I (lanes 4 to 6), and Hind III (lanes 7 to 9). Lanes 1, 4, and 7 contain λ DNA; lanes 2, 5, and 8, PBCV-1 DNA; and lanes 3, 6, and 9 DNA from viral particles produced by PBCV-1 infection of *Chlorella* strain NC64A. The size of some of the λ DNA fragments (kilobase pairs) is given on the left. Patterns identical to those of PBCV-1 were also observed after PBCV-1 infection of *Chlorella* ATCC-30562. Fig. 2 (right). Plaques formed in *Chlorella* NC64A lawns by PBCV-1.



ral plaque assay on a plant host. In contrast to viral infection of higher plants, PBCV-1 infection of *Chlorella* can be synchronized. This should expedite studies of viral replication and gene expression.

JAMES L. VAN ETTEN
DWIGHT E. BURBANK
Department of Plant Pathology, University of Nebraska, Lincoln 68583
DANIEL KUCZMARSKI
RUSSEL H. MEINTS
School of Life Sciences, University of Nebraska, Lincoln

References and Notes

1. L. A. Sherman and R. M. Brown, in *Comprehensive Virology*, H. Frankel-Conrat and R. R. Wagner, Eds. (Plenum, New York, 1978), vol. 12, p. 145.
2. P. A. Lemke, *Annu. Rev. Microbiol.* **30**, 105 (1976); J. A. Dodds, *Experientia* **35**, 440 (1979).
3. A. Skotnicki, A. J. Gibbs, N. G. Wrigley, *Virology* **75**, 457 (1976); J. A. Dodds and A. Cole, *ibid.* **100**, 156 (1980); A. Cole, J. A. Dodds, R. I. Hamilton, *ibid.*, p. 166; L. H. Stanker, L. R. Hoffman, R. MacLeod, *ibid.* **114**, 357 (1981).
4. R. H. Meints, J. L. Van Etten, D. Kuczmarski, K. Lee, B. Ang, *ibid.* **113**, 698 (1981); J. L. Van Etten, R. H. Meints, D. E. Burbank, D. Kuczmarski, D. A. Cuppels, L. C. Lane, *ibid.*, p. 704.
5. J. L. Van Etten *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3867 (1982).
6. S. J. Karakashian and M. W. Karakashian, *Evolution* **19**, 368 (1965); D. S. Weis, *J. Protozool.* **25**, 366 (1978).
7. E. Jolley and D. C. Smith, *New Phytol.* **81**, 637 (1978).
8. The algae were grown on Bold's basal medium [H. W. Nicols and M. C. Bold, *J. Phycol.* **1**, 34 (1965)] modified by the addition of 0.5 percent sucrose and 0.1 percent proteose peptone (MBBM). Fifty-milliliter cultures were inoculated with algae (1×10^6 per milliliter) and grown at 25°C on a rotary shaker (150 rev/min) in continuous light (~ 30 $\mu E/m^2$ per second).
9. The *Chlorella* cultures were grown to a density of 5×10^6 cells per milliliter and inoculated with virus sterilized with a 0.4-μm Nuclepore filter at a multiplicity of infection of 5 to 10.
10. A. Gibbs, A. H. Skotnicki, J. E. Gardiner, E. S. Walker, M. Hollings, *Virology* **64**, 571 (1975); L. Oliveira and T. Bisalputra, *Ann. Bot.* **42**, 439 (1978); J. A. Mayer and F. J. R. Taylor, *Nature (London)* **281**, 299 (1979); B. V. Gromov and K. A. Mamkaeva, *Arch. Hydrobiol. Suppl.* **60**, 252 (1981).
11. We thank D. Chapman, T. Hohman, D. Weis, and L. Muscatine for providing *Chlorella* cultures and M. Brakke and L. Lane for helpful comments. This research was supported in part by the Research Council of the University of Nebraska, Lincoln, and by NIH biomedical research support grant RR-07055. This report is published with the approval of the director as paper 6962, journal series, Nebraska Agricultural Experiment Station.

16 August 1982; revised 30 September 1982

Global Mean Sea Level: Indicator of Climate Change?

Etkins and Epstein (*1*) have combined surface air temperature and sea level time series to draw erroneous conclusions concerning the discharge of polar ice sheets. They used records of Northern Hemisphere land-surface air temperature (*2, 3*) that are unrepresentative of global sea-surface temperature, which should be used for comparison with global sea level records. In the climate model experiment they cited (*4*), surface air temperatures over land increased by 0.43°C in January and 0.48°C in July in response to a doubling in the atmospheric CO_2 concentration when sea-surface temperatures are fixed at their climatological values, thus completely negating their assertion that this experiment shows that land-based surface air temperature records indicate changes in ocean temperature.

Actually, a record of the global surface temperature, incorporating sea-surface temperatures, measured with buckets from ships, does exist (*5*) and is plotted in Fig. 1 together with a correct plot of sea level change (*6*); this plot uses the correct scale and omits the dashed portion on the right in figure 1B of (*1*), which was added by Etkins and Epstein and does not appear in (*6*). From Fig. 1 it is evident that the sea level change from 1910 to 1960 is, given the quality of the data, due to thermal expansion and it is not necessary to consider the discharge of polar ice sheets.

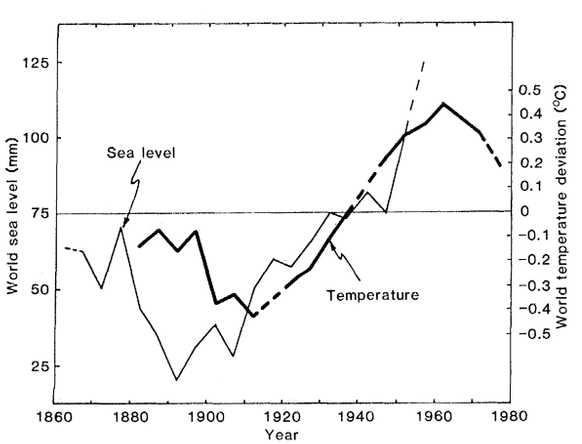


Fig. 1. Five-year averages of global average surface temperature (*5*) and global average sea level (*6*), plotted so that 80 mm of sea level change is equivalent to 1°C of temperature change. The dashed portions at the ends of both curves signify that the end point is only a 3- or 4-year average. The dashed portions in the middle of the temperature curve signify one missing data point for each portion, due to World War I and World War II.

Emery, whose data (*7*) were used by Etkins and Epstein (*1*) to give sea level changes for the past 40 years, arbitrarily excluded stations with no sea level trend significant at the 80 percent level and also excluded all stations with a downward sea level trend. My recalculation, based on the use of all his stations with significant trends, gives a sea level rise of 1.7 mm per year for the period, not 3 mm, and this is an overestimate because all stations with zero trend have been excluded. Thus the 45-mm rise from 1940 to 1960 (Fig. 1) accounts for most, if not all, of the total sea level rise since 1940, and it is not necessary to postulate any cause other than thermal expansion.

The claim (*1*) of 0.4°C as the externally imposed change in mean surface temperature from 1890 to 1980 is based on one study (*8*) of the effects of CO_2 and completely neglects volcanic dust, which has been shown in both observational (*2, 9*) and modeling (*10*) studies to have been the major external forcing of climate during the past 90 years.

Externally imposed volcanic dust and CO_2 forcings can adequately account for the observed temperature changes of the last 100 years. Global sea level has changed in passive response to climate change as a result of thermal expansion. Discharges of polar ice need not be invoked to explain the records, have not been observed (*11*), and indeed could not have taken place without substantially increasing sea level faster than has been observed.

ALAN ROBOCK
Department of Meteorology, University of Maryland, College Park 20742

References and Notes

1. R. Etkins and E. S. Epstein, *Science* **215**, 287 (1982).
2. J. M. Mitchell, Jr., *Ann. N.Y. Acad. Sci.* **95**, 235 (1961).
3. M. I. Budyko, *Tellus* **21**, 611 (1969); R. Yamamoto, "Change of global climate during recent 100 years" (available from the author at the Geophysical Institute, Kyoto University, Kyoto, 606, Japan).
4. W. L. Gates, K. H. Cook, M. E. Schlesinger, *J. Geophys. Res.* **86**, 6385 (1981).
5. G. Paltridge and S. Woodruff, *Mon. Weather Rev.*, **109**, 2427 (1981).
6. R. W. Fairbridge and O. A. Krebs, Jr., *Geophys. J.* **6**, 532 (1962).
7. K. O. Emery, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6968 (1980).
8. R. D. Cess and S. D. Goldenberg, *J. Geophys. Res.* **86**, 498 (1981).
9. C. Mass and S. H. Schneider, *J. Atmos. Sci.* **34**, 1995 (1977); B. L. Taylor, T. Gal-Chen, S. H. Schneider, *Q. J. R. Meteorol. Soc.* **106**, 175 (1980).
10. A. Robock, *J. Atmos. Sci.* **35**, 1111 (1978); *Science* **206**, 1402 (1979); *J. Volcanol. Geotherm. Res.* **11**, 67 (1981); J. Hansen, D. Johnson, A. Lacis, S. Lebedeff, P. Lee, D. Rind, G. Russell, *Science* **213**, 957 (1981).
11. E. Epstein, personal communication.
12. I thank R. Etkins, E. Epstein, and K. O. Emery for discussing with me the material in their papers and C. Villanti for drafting Fig. 1. This work was supported by NSF grant ATM-7918215.

16 February 1982