

- book 231 (1962); J. R. E. Jones, *Fish and River Pollution* (Butterworths, London, 1964), p. 138.
9. Fish toxicity data from O. B. Cope, *U.S. Fish Wildlife Serv. Circ. 226* (1965); —, *U.S. Fish Wildlife Serv. Circ. 167* (1963), p. 26; and J. M. Lawrence, *U.S. Dept. Agr. Agr. Handbook 231* (1962). Rat toxicity data from manufacturer's literature.
 10. D. G. Crosby, E. Leitis, W. L. Winterlin, *J. Agr. Food Chem.* 13, 204 (1965); D. G. Crosby and E. Leitis, unpublished.
 11. Supported in part by PHS (grant EF-00306) and the Chevron Chemical Company. We are grateful to Mitzi Garskis and Robin Lenn for assistance and to D. E. Seaman for advice and samples of herbicides.
- * Present address: U.S. Fish and Wildlife Service, Denver, Colorado.

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Stylet-Borne Virus: Active Probing by Aphids Not Required for Acquisition

Abstract. Anesthetized aphids, whose stylets had been dipped into capillaries containing purified concentrated cucumber mosaic virus, acquired the virus and, after recovery from the anesthetic, were able to transmit it in a low percentage of cases. Although this study does not eliminate active probing as a means of virus acquisition, experimentally, it clearly establishes passive contamination of aphid mouthparts as a method of virus acquisition.

Most plant viruses that are transmitted by aphids can be acquired and transmitted within seconds. Aphids lose the ability to transmit these viruses very rapidly unless they have access to another virus source (1). This type of transmission, originally called nonpersistent (2), has since been termed stylet-borne (3).

The very short times required for ac-

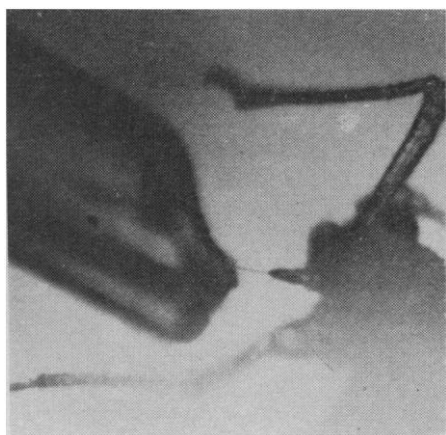


Fig. 1. Photomicrograph showing exposed stylets of an aphid (*Myzus persicae* Sulz.) being dipped into a capillary containing purified cucumber mosaic virus.

quisition and transmission (1), and the inhibition of transmission by treating stylet tips of viruliferous aphids with ultraviolet light or formaldehyde (4), have been interpreted as evidence that these viruses are carried as contaminants of the stylets. On the other hand, a recent attempt to transmit henbane mosaic virus, which is stylet-borne (3), with anesthetized aphids whose stylets had been inserted into cells of infected leaves was unsuccessful (5). This was regarded as evidence that virus is not carried as a contaminant of the stylets, but that it must be acquired by active probing.

We have recently shown (6) that purified preparations of certain stylet-borne viruses can be acquired and transmitted by aphids that are allowed to make 20- to 40-second acquisition probes through a Parafilm membrane. Experiments described in this report were made to determine whether anesthetized aphids could be made viruliferous by dipping their stylets into a suspension of a purified stylet-borne virus.

The C-1, Imperial 78, and Wisconsin 102 strains (7) of cucumber mosaic virus were used. Viruses were propagated in tobacco *Nicotiana tabacum*, L., variety Havana 425) and purified by the method of Scott (8). Aphids (*Myzus persicae* Sulz.) were propagated on Tendergreen mustard (*Brassica perviridis* Bailey) as previously described (6); they were anesthetized while feeding on these plants by being exposed to a stream of CO₂ for 1 minute and then removed from the plants by suction with a fire-polished capillary pipette attached to a vacuum pump. Suction was adjusted so that the insect could be held securely by the dorsal surface of the abdomen without injury. Exposed stylets of aphids were dipped in a concentrated suspension of purified virus in a fine capillary (Fig. 1); the insects were then released and placed in a glass dish for recovery from the anesthetic, which occurred in 4 to 6 minutes after removal from CO₂. After recovery, aphids were placed singly on healthy tobacco seedlings, covered with a cellulose tube to prevent movement to another plant, and allowed a test feeding of 4 to 12 hours. Plants were sprayed with the insecticide mevinphos (2-methoxycarbonyl-1-methyl or vinyl dimethyl phosphate) and removed to the greenhouse where they were observed for development of symptoms.

Table 1. Transmission of strains of cucumber mosaic virus acquired by anesthetized aphids whose stylets had been dipped in purified virus suspensions or by aphids making probes through a Parafilm membrane. A single aphid was placed on each test plant.

Virus strain	Transmission* of viruses acquired by	
	Dipping stylets	Probes through membranes
Wis. 102	3/100	5/24
Imp. 78	2/138	10/128
C-1	13/100	6/24

* Transmission is expressed as the number of plants infected (numerator) out of the number tested (denominator).

To determine the transmissibility of virus preparations acquired by natural probes, aphids were allowed to acquire the virus through a Parafilm membrane, with the use of techniques previously described (6). Test feedings were the same as those described for the anesthetized insects. As controls, an equal number of aphids were removed from healthy mustard plants and placed directly on tobacco seedlings. These seedlings were then treated and maintained in the same way as test plants.

Results (Table 1) show that the three strains of cucumber mosaic virus were acquired by the anesthetized insects, although the rate of transmission by aphids that acquired virus in this manner was somewhat lower than the rate of those that acquired it after probing through the Parafilm membrane. In both cases, rate of transmission was considerably less than that which would be expected if the virus were acquired by aphids from infected leaves (9). None of the control plants became infected.

These results do not necessarily conflict with those obtained when exposed stylets were inserted into cells of infected leaves (5), since, in addition to the fact that a different virus was used, it is possible that the cells into which stylets were inserted did not contain virus. It has also been suggested (10) that aphids acquire stylet-borne viruses intercellularly. Although our study does not eliminate active probing as a means of virus acquisition, experimentally, it clearly establishes passive contamination of aphid mouthparts as a method of virus acquisition.

CHARLES B. BARNETT, JR.

THOMAS P. PIRONE

Department of Plant Pathology,
Louisiana State University,
Baton Rouge

References and Notes

1. K. Maramorosch, *Ann. Rev. Entomol.* **8**, 369 (1963).
2. M. A. Watson and F. M. Roberts, *Proc. Roy. Soc. Ser. B* **127**, 543 (1939).
3. J. S. Kennedy, M. F. Day, V. F. Eastop, *A Conspectus of Aphids as Vectors of Plant Viruses* (Commonwealth Institute of Entomology, London, 1962).
4. R. H. E. Bradley and R. Y. Ganong, *Can. J. Microbiol.* **1**, 775 (1955); *ibid.*, p. 783.
5. M. Watson and R. Gamez, *Virology* **22**, 292 (1964).
6. T. P. Pirone, *ibid.* **23**, 107 (1964); ——— and E. Megahed, *ibid.*, in press.
7. Strains Imperial 78 and Wisconsin 102 obtained from R. Grogan, Univ. of California, Davis; C-1 from A. F. Ross, Cornell Univ.
8. H. A. Scott, *Virology* **20**, 103 (1963).
9. E. Megahed and T. P. Pirone, *Phytopathology*, in press.
10. H. A. van Hoof, *Onderzoekingen over de Biologische Overdracht van een Nonpersistent Virus* (Van Putten and Oortmeijer, Alkmaar, Netherlands, 1958).
11. Supported in part by PHS grant GM-12389. We thank Dorothy Perez for technical assistance.

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Middle Devonian Day and Month

Aveni (1) argues that simple dynamical calculations conflict with conclusions drawn from the study of daily, monthly, and annual increments of coral growth. The number of months in the Middle Devonian year was obtained by Scrutton (2) from Middle Devonian rugose corals; he divided Wells's (3) count of daily-growth ridges in annual bands by his own count of their number in monthly bands. Aveni concludes that either the growth bands are not related to time, and the approximate agreement is fortuitous, or our understanding of the history of the Earth-Moon system is incomplete.

Aveni's conclusions are based on a false assumption—that is, a formula for the Earth-Moon distance (r) at time (t) since Earth's origin given by Jeffreys (4):

$$t \text{ (in } 10^9 \text{ years)} = 4.5 (r/a)^{23/2} \quad (1)$$

where a is the present Earth-Moon distance. Jeffreys assumed the simplest law of lunar friction: that the decelerating torque exerted by Moon on Earth is inversely proportional to the sixth power of the Earth-Moon distance (r). If L is the orbital angular momentum of Moon, this torque equals

$$dL/dt = k/r^6 \quad (2)$$

The assumption that k has been constant may be unfounded. It is known that if tidal friction occurs in the oceans the dissipation of energy must

be wholly in the shallow seas, the extents and positions of which have altered even within the last few thousand years. It could be argued that k may still have remained roughly constant because, if sea level had changed, the places where tidal friction was effective also would have changed, although the magnitude may have remained constant. If the dissipation of energy by tides occurs mainly in nonelastic processes in Earth's mantle, it may be more reasonable to suppose a constant k , although friction may occur between the faulted blocks of the crust rather than bodily throughout the mantle, because an elastic behavior of the mantle over periods as brief as 12 hours 50 minutes has not been found for strains as small as those in the bodily tides (10^{-7}). Tectonics, continental drift, and other crustal processes may then have altered the dissipation of energy over geological time.

By applying Kepler's second and third laws of motion to the Earth-Moon system and neglecting changes in the orbital eccentricity, Runcorn (5) obtains a formula, true for any time in the past, that gives the length of the sidereal month (T):

$$[G^2 (M + m)^2 m^3] T = 2\pi L^3 \quad (3)$$

where G is the gravitational constant and M and m are the masses of Earth and Moon, respectively. Similarly one may also derive

$$[G (M + m) m^2] r = L^2 \quad (4)$$

where r is the Earth-Moon distance.

The eq. 1 quoted by Aveni is simply obtained by integrating Eqs. 2 and 4, with the dubious assumption that k has been constant throughout Earth's life (4.5×10^9 years) and that the constant of integration equals zero—which is equivalent to accepting the entirely speculative hypothesis that r equalled zero at Earth's origin. Aveni's conclusions derive from these assumptions, which he did not state.

I believe that a more profitable way to examine the paleontological counts is to postulate only that, at least since the Devonian, Earth's orbital angular momentum about Sun has been constant. Runcorn (5) shows that the loss of angular momentum by Earth to Moon by tidal friction is given by

$$(L_0 - L) = L_0 \{1 - [13.4/(w/s)]^3\} \quad (5)$$

where L_0 is the present orbital angular momentum of Moon, w is the number of sidereal days in the year (399), and s is the number of sidereal months (28.4) derived from Wells's and Scrutton's counts for the Middle Devonian. Runcorn shows from eq. 4 that the mean lunar tidal frictional torque over the last 370 million years is 3.9×10^{23} dyne cm, which is the same as the value given by Munk and MacDonald (6) from astronomical values of the longitudes of Sun, Moon, Venus, and Mercury for the last 300 years.

By use of Aveni's formula with Eqs. 1 and 4, a value of 1.5×10^{23} dyne cm for the present lunar tidal frictional torque is obtained, which would imply that the value obtained astronomically is anomalously large.

Aveni also seems to state in his last paragraph that, because of the change in r since the Devonian, the lunar tidal frictional torque determined from the paleontological data should be larger than that determined astronomically. This effect is not important, considering the degree of accuracy at present attainable. The paleontological data show that the present sidereal month is 1.048 times the Devonian sidereal month, if one takes the year to be a constant unit of time. Thus Kepler's third law shows that the Earth-Moon distance has increased by 3.2 percent since the Devonian. Now the average value of the torque since the Devonian will be greater by 9.6 percent than the present value if one assumes a constant k .

One should note that Scrutton's and Wells's results prove that the equilibrium tide during the Devonian was 9.6-percent higher than at present. The checking of this prediction by means of sedimentary structures and by study of paleotides seems a desirable next step.

S. K. RUNCORN

School of Physics, University of Newcastle upon Tyne, England, and School of Environmental and Planetary Sciences, University of Miami, Florida

References

1. A. F. Aveni, *Science* **151**, 1221 (1966).
2. C. T. Scrutton, *Paleontology* **7**, 522 (1965).
3. J. W. Wells, *Nature* **197**, 948 (1963).
4. H. Jeffreys, *The Earth* (Cambridge Univ. Press, London, 1924).
5. S. K. Runcorn, *Nature* **204**, 823 (1964).
6. W. H. Munk and G. J. F. MacDonald, *The Rotation of the Earth* (Cambridge Univ. Press, London, 1960).

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