Table 1. Base composition of the RNA of a variant of reovirus type 3. The percentages given are calculated as moles of base per 100 moles of total base in the RNA.

Percentage			
Guanine	Adenine	Cytosine	Uracil
Dearing strain variant			
20.2	29.8	21.0	29.1
Dearing prototype strain (1)			
19.3	29.7	20.5	30.5

temperature range, reacts only slightly with formaldehyde, and is resistant to hydrolysis by pancreatic ribonuclease. Thus, it is likely that reovirus RNA is a double-stranded helix (*1*). Reovirus and wound-tumor virus RNA's (*1*) are the only RNA's in nature known to possess such a secondary structure.

A variant of the original Dearing strain of reovirus type 3 has been isolated in this laboratory (*2*). Dearing strain variant is not significantly different from the original Dearing virus in antigenic constitution, size, fine structure, rate of adsorption to L cells, latent period, or cytopathic effects. However, it is less sensitive to specific antibodies and is released to a lesser extent from L cells. Our results indicate that the base composition of the Dearing strain variant is similar to that of the original prototype strain.

Cultures of L cells were grown for 48 hours in monolayers in phosphate-free reinforced Eagle's medium containing 5 percent fetal bovine serum and 30 μc/l of carrier-free P^{32} orthophosphate per milliliter. They were then inoculated with the Dearing strain variant, and incubation at 37°C in the radioactive growth medium was continued. When the cells had degenerated, the cultures were frozen and thawed three times and the suspension was clarified at 8000*g* for 10 minutes. The supernatant was then centrifuged at 42,000*g* at 10°C for 2 hours, and the sediment was resuspended in a medium containing deoxyribonuclease, 16 μg/ml, ribonuclease, 20 μg/ml, and 0.003*M* $MgCl_2$. The suspension was incubated for 12 hours at 4°C and for 30 minutes at 37°C. Fetal bovine serum was then added to a final concentration of 5 percent; and the suspension was centrifuged at 80,000*g* for 2 hours. The pellet was resuspended in 0.02*M* phosphate buffer, *p*H 7.2, and fractionated on a column of diethylaminoethyl cellulose by increases in the NaCl concentration. The radioactivity, hemagglutinating activity, and infectivity of the various fractions were determined. Virus was first eluted from the column at a salt concentration of 0.3*M*. The elution pattern showed a sharp simultaneous rise of the radioactivity and virus which reached a maximum and then gradually declined. To the eluate showing maximum infectivity and radioactivity were added the eluates from neighboring tubes and the combined eluates were centrifuged at 80,000*g* for 2 hours. The pellet was resuspended in a cesium chloride solution of an average density of 1.34 and centrifuged at 100,000*g* for 24 hours. No bands were visible because relatively small amounts of virus had been used. Successive drops were collected from below, and assayed for radioactivity and infectivity. The peak concentrations of radioactivity and infectivity were coincident, and were located below the center of the centrifugation tube. The contents of the tubes containing virus were pooled and centrifuged at 80,000*g* for 2 hours.

The pellet was resuspended in deionized water. The nucleic acid was extracted from the virus with phenol at 50°C. The RNA was hydrolyzed in 1*N* KOH at 22°C for 22 hours, and then K^+ ions were removed (*3*). Carrier ribonucleotides were added to the hydrolysate and the nucleotides were separated by electrophoresis on Whatman No. 3 paper (*4*, *5*). The ultraviolet absorbing areas were located and eluted; the radioactivity of these eluates was measured. The base composition of the RNA was estimated from the amount of P^{32} incorporated in the nucleotides.

The base composition of the RNA of the Dearing strain variant is similar to that of the original Dearing prototype strain of reovirus 3 (Table 1). The close similarity is specially noteworthy since the analytical procedures used were different; with the original Dearing virus, the bases were measured by direct chemical determination (*1*). In both virus strains, calculated as moles per 100 moles of total base in RNA, the percentage of guanine closely approximates that of cytosine, and that of adenine approximates that of uracil. The combined percentage of guanine plus cytosine is 41.2 percent of the total in the variant, whereas in the original Dearing virus it was 39.8 percent. No unusual bases have been detected in either strain (*6*).

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References and Notes

1. P. J. Gomatos and I. Tamm, *Proc. Natl. Acad. Sci. U.S.* **49**, 707 (1963).
2. P. J. Gomatos, I. Tamm, S. Dales, R. M. Franklin, *Virology* **17**, 441 (1962).
3. J. W. Littlefield and D. B. Dunn, *Biochem. J.* **70**, 642 (1958).
4. J. N. Davidson and R. M. S. Smellie, *Biochem. J.* **52**, 594 (1952).
5. J. D. Smith, in *The Nucleic Acids*, E. Chargaff and J. N. Davidson, Eds. (Academic Press, New York, 1955), vol. 1, p. 267.
6. Supported by research grant E-3445 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

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Plant Morphology: Its Control in Proserpinaca by Photoperiod, Temperature, and Gibberellic Acid

Abstract. *Growth forms controlled by the photoperiod are modified by temperature and gibberellic acid. Gibberellic acid changes leaf orientation and prostrate growth habit of the short-day plant to that typical of the long-day plant and modifies leaf shape in both types of plants. Low temperature resembles short days in its effect on leaf shape and stem orientation of long-day plants. Geotropic responses of the stem appear to be modified by photoperiod, temperature, and gibberellic acid.*

Heterophylly, or change of leaf shape, of the aquatic angiosperm *Proserpinaca palustris* L., is controlled by photoperiod and temperature (*1*). However, little attention has been given to several other morphological characters that are also modified by these stimuli. We wish to draw attention to the many reversible responses of a single plant species to changes in photoperiod, temperature, and the growth regulator, gibberellic acid.

Figure 1 shows that aerial *Proserpinaca* plants grown under short days scarcely resemble those grown under long days. Short-day plants are prostrate and have short internodes and brown or green stems which bear dark-green dissected leaves (Fig. 2*F*). The leaves are inserted helically but are not oriented helically. They are appressed to the stem and oriented in the plane parallel to the stem, which gives the shoot a dorsiventral symmetry. Long-

day plants, on the other hand, are erect and have red stems with longer internodes, axillary flowers, and pale-green serrate lanceolate leaves (Fig. 2*A*). The leaves are oriented helically. Both types of plants bear transitional leaves if a change in photoperiod or temperature occurs (Fig. 2, *B–E*). Long-day plants, when transferred to short days, may become completely prostrate or bent horizontally a short distance below the tip.

The following experiments indicate that leaf shape (Fig. 2) and stem orientation (Fig. 1) are the result of a photoinductive process. Plants were raised in a growth room; the long-day plants received 16 hours of light and 8 hours of darkness and the short-day plants received 8 hours of light and 16 hours of darkness. Fluorescent lights provided illumination (700 lu/ft^2); temperature varied from 70° to 82°F, relative humidity from 60 to 65 percent. Three plants obtained from cuttings were grown in a pot of soil kept moist by partial immersion in a cup of tap water.

In all experiments one pot each of short- and long-day plants was used per treatment; all experiments were repeated. Plants raised in one photoperiod were transferred to the opposite photoperiod for 4, 6, 8, and 10 cycles and then returned to the initial photoperiod. Long-day plants receiving 4 to 6 short-day inductive cycles produced transitional leaves only before reverting to entire leaves. Short-day plants receiving 4 to 6 long-day cycles also produced transitional leaves before reverting to dissected leaves. Long-day plants did not produce completely dissected leaves (Fig. 2*F*) even after 10 short days. One-third of the short-day plants produced entire leaves (Fig. 2*A*) after 8 to 10 long days; prostrate short-day plants became erect with as few as 4 long-day cycles, while erect long-day plants were just slightly bent to the horizontal even when given 10 short-day cycles. These results indicate that a change in photoperiod affects short-day plants more than it affects long-day plants.

Davis reported that continuous low temperatures increased the degree of leaf dissection on plants grown in long days (*1*). He did not show that the effects are thermoinductive or that temperature treatments may cause other responses similar to short days in *Proserpinaca palustris*. The following experiment demonstrates that responses of long-day plants obtained by thermoinductive treatments are similar to those obtained by photoinduction with short days. Long-day plants were given 4 to 10 cycles of 55° to 57°F temperature with illumination of 400 lu/ft^2 from fluorescent lights. The plants were then returned to a temperature of 70° to 84°F with the same level of illumination. Control plants were not given low-temperature treatment. All the plants that received 4 to 10 inductive cycles of cold developed dissected leaves (Fig. 2*D*), and the stems of the plants declined to a horizontal position. The control plants had entire leaves (Fig. 2*A*) and the stems remained erect.

The shoot of the short-day plant resembles a rosette stage with its short internodes. Lang (*2*) used applications of gibberellic acid to obtain bolting in the rosette stage of *Hyoscyamus*, and we therefore tried to obtain an analogous effect on *Proserpinaca*. A 10-μl drop containing 1 μg of gibberellic acid in 0.1-percent Tween-20 was applied to the tips of plants grown under their respective photoperiods in the growth room. Control plants received 0.1-percent Tween-20. By the 5th day after treatment, the internodes on the treated plants were longer than those on the controls. The stems of the treated short-day plants also became erect and developed the color and leaf orientation of the long-day plants. While some of the leaves on the treated short-day plants also increased in mid-blade width, the serrations on some of the leaves on the treated long-day plants also became more extreme. The prostrate habit of short-day plants is thus controlled by photoperiod, and gibberellic acid simulates long days in producing erect habit in these plants.

The prostrate habit of short-day plants appears to be a geotropism, as shown by the following experiments. Plants in a box in the growth room were illuminated through a window from a horizontal or vertical direction by fluorescent light (200 lu/ft^2). Others were illuminated horizontally with incandescent light (700 lu/ft^2) at a temperature of 68° to 77°F. In 3 to 5 days all the horizontally illuminated prostrate plants turned toward the light. The vertically illuminated prostrate plants did not. Therefore, short-day plants are positively phototropic but are unable to grow toward an overhead light source, presumably because of some interfering response. Changes in stem orientation with respect to the horizontal were tested by laying pots of prostrate plants on their sides so that the stems were pointed vertically upward. In 1 to 3 days the stems had bent back to the horizontal with the dorsal side of the stem upward, despite the overhead lights. These same pots were then placed upright so the stems pointed vertically downward. The stems again resumed their horizontal position in 2 to 3 days, with the dorsal side up. Short-day plants left in darkness became more erect in 4 to 5 days, which indicates that short days are necessary for prostrate habit. Long-day plants left in darkness developed stem curvatures and appression of the leaves against the stem. An integral part of all stem bending was the maintenance of the original dorsiventral symmetry. The change from dorsiventral to helical leaf orientation parallels the change from prostrate to erect habit in short-day plants treated with long days, periods of darkness, or gibberellic acid.

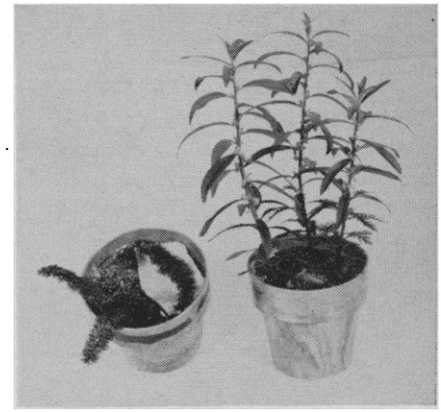

Fig. 1. Changes in morphology of *Proserpinaca* induced by different photoperiods. (Left) Aerial short-day plants. (Right) Aerial long-day plants. (× 0.22)

Our experiments indicate that photoperiod, temperature, and gibberellic acid have related effects on leaf shape and on stem and leaf orientation in

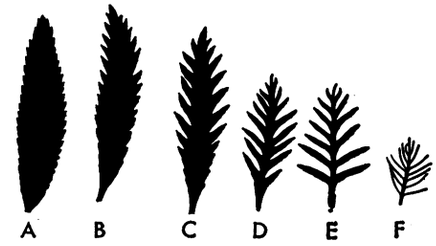

Fig. 2. Variations in leaf shape with photoperiod and temperature. (*A*) entire leaf; (*B–E*) typical transitional leaf types; (*F*) dissected aerial leaf. (× 0.7)

Proserpinaca. Gibberellic acid promotes the type of vegetative growth associated with long days. It promotes adult features typical of long-day-grown *Eucalyptus* (*3*) and juvenile features typical of long-day-grown *Kalanchoë* (*4*). It also induces erect growth of the long-day-plant *Trifolium* (*5*) which has an inherited light-imposed prostrate habit like that of short-day *Proserpinaca.* Low temperature has an effect opposite to that of gibberellic acid on *Proserpinaca.* It induces horizontal habit and formation of dissected leaves in long-day plants. Since photoperiod also affects habit, the response of the plant to gravity appears to be mediated by phytochrome, the pigment system which controls plant responses to photoperiod. The phytochrome system therefore interacts with temperature and externally applied gibberellic acid to control leaf shape, leaf orientation, and the geotropic response of the shoot.

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References and Notes

1. G. J. Davis, thesis, University of North Carolina (1956).
2. A. Lang, *Naturwissenschaften* **43**, 257 (1956).
3. G. Scurfield and G. W. E. Moore, *Nature* **181**, 1276 (1958); G. Scurfield, *Australian J. Botany* **9**, 37 (1961).
4. R. Harder and R. Bünsow, *Planta* **51**, 201 (1958).
5. L. E. Bendixen and M. L. Peterson, *Plant Physiol.* **37**, 245 (1962).

26 March 1963

Ribosomes: A Common Structural Feature

Abstract. *A reflection between 45 and 50 Å has been observed in x-ray diffraction patterns from ribosomes extracted from* Escherichia coli, Drosophila *larvae, rat liver, and rabbit reticulocytes. This spacing appears to correspond to a common substructural feature within the ribosomes. The intensity distribution is consistent with a model in which part of the RNA is in the form of four or five parallel double helices 45 to 50 Å apart.*

Knowledge of the substructure of ribosomes is of obvious importance for an understanding of the mechanism of protein synthesis. Electron-microscope studies of *Escherichia coli* ribosomes (*1*) have outlined the gross features; the published evidence for substructure merely indicates that it appears to be in the size range 25 to 70 Å (*1, 2*). Earlier studies by x-ray diffraction (*3*) were concentrated on the wide-angle part of the diagram and they showed that the structure of the ribonucleic acid (RNA) and protein components are somewhat independent.

Table 1. Reflection in x-ray diffraction patterns of ribosomes. The experimental error is ± 1 Å.

Source and size of ribosomes	Spacing (Å)
E. coli, 50*S*	45.5
E. coli, 70*S*	46.5
E. coli, 100*S*	46.5
Drosophila larvae, 80*S*	48.5
Rat liver, 80*S*	49.5
Rabbit reticulocyte 78*S*	48.5

The hypochromism at 260 mμ of intact *E. coli* ribosomes is the same as that of the extracted RNA (*4*), and since the x-ray diffraction patterns from noncrystalline fibers of ribosomal RNA are identical to those from noncrystalline transfer RNA, which has a DNA-like double helical structure (*5*), it is likely that sections at least of ribosomal RNA have this structure. Low-angle absolute intensity x-ray scattering measurements of RNA of high molecular weight (probably mainly ribosomal) from ascites tumor cells, *E. coli*, and yeast have been interpreted to signify short rigid rods of about 50 to 150 Å in length with a weight per unit length similar to double-stranded helical DNA, joined by small flexible regions (*6*). The observation of a broad reflection at 12.5 Å is consistent with the presence of RNA double helices in the intact ribosome (*7*).

More-detailed low-angle x-ray diffraction patterns of concentrated gels of *E. coli* ribosomes (*7*) indicate a linear aggregation of the particles. Only one reflection, at 45.5 Å, cannot be fitted to the predicted diffraction from such a structure. This reflection is strong and fairly sharp and occurs even in preparations for which the other reflections are diffuse or very weak. It appears to correspond to substructure within the particle (*7*).

Further x-ray diffraction studies have now been made of ribosomes isolated from *E. coli, Drosophila* larvae, rat liver, and rabbit reticulocytes. Specimens were prepared and x-ray diffraction photographs were taken as previously described (*7*). Exposure times ranged from 50 to 150 hours. A reflection between 45 and 50 Å was observed in the diffraction patterns of ribosomes from all the sources studied, implying a common structural feature (Table 1).

The sharpness of the reflection requires at least four repeats of this characteristic distance within the ribosome, and since all ribosomes appear to have a maximum dimension of about 200 to 250 Å, the number of possible repeats is limited to four or five. A second-order reflection at 23 to 25 Å is expected, but this is very weak or absent in all the diffraction patterns; thus the transform of the repeating unit is large near 45 to 50 Å and falls nearly to zero in the 23 to 25 Å region. The equatorial intensity transform of double-helical nucleic acid (*8*) has this distribution, and calculation shows that an array of four or five parallel RNA double helices, 45 to 50 Å apart, gives an intensity distribution in good qualitative agreement with that observed.

Since ribosomal RNA appears to be in one or two large pieces when carefully extracted (*9*), and since RNA double helices are quite rigid, it is probable that the helices are arranged in short lengths connected by regions where the double-helical structure is not preserved. The structure proposed for extracted RNA (*6*) with lengths of 50 to 150 Å for the double-helical regions is in good agreement with this model. The protein component of the ribosome might fulfill a structural function in preserving the 45 to 50 Å spacing in the ordered RNA, perhaps interacting with the RNA in the nonhelical regions (*10*).

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References and Notes

1. C. E. Hall and H. S. Slayter, *J. Mol. Biol.* **1**, 329 (1959); H. E. Huxley and G. Zubay, *ibid.* **2**, 10 (1960).
2. M. Beer, P. J. Highton, B. J. McCarthy, *ibid.* p. 447.
3. G. Zubay and M. H. F. Wilkins, *ibid.* p. 105; A. Klug, K. C. Holmes, J. T. Finch, *ibid.* **3**, 87 (1961).
4. D. Schlessinger, *ibid.* **2**, 92 (1960).
5. M. Spencer, W. Fuller, M. H. F. Wilkins, G. L. Brown, *Nature* **194**, 1014 (1962).
6. S. N. Timasheff, J. Witz, V. Luzzati, *Biophys. J.* **1**, 525 (1961).
7. R. Langridge and K. C. Holmes, *J. Mol. Biol.* **5**, 611 (1962).
8. R. Langridge, D. A. Marvin, W. E. Seeds, H. R. Wilson, C. W. Hooper, M. H. F. Wilkins, L. D. Hamilton, *ibid.* **2**, 38 (1960).
9. B. D. Hall and P. Doty, *ibid.* **1**, 111 (1959); C. G. Kurland, *ibid.* **2**, 83 (1960).
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