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16. At this writing, it is not clear why some of the early observations showed the E ring better on one side of the planet than on the other (*1*, *9*) but more recent data show it equally well on both sides (*9*, *10*, *12*). An illumination effect or uneven distribution of material in the ring could account for this.

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Energy Requirement for Nitrogen Fixation in Actinorhizal and Legume Root Nodules

Abstract. *The ratio of respiration to nitrogenase activity was measured in five species of actinorhizal root nodules and eight species of legume nodules. The two types of nodules could not be distinguished on the basis of this ratio; this evidence thus indicates that the energy cost of nitrogen fixation is similar for both.*

In order to evaluate the benefits of nitrogen fixation to higher plants, it is essential to know the amount of energy consumed in this process. Although there is increasing information about the energy requirement for nitrogen fixation in legume root nodules (*1*, *2*), as far as we know there have been no such studies of actinomycete-induced root nodules, which occur in eight families of dicots; the term "actinorhizal" is now being used for this association (*3*). Actinorhizal nodules are very different from legume nodules not only in the nature of the endophyte but also in other characteristics including anatomy, the lack of leghemoglobin, and higher partial pressures of oxygen in the region of the nodule containing the endophyte (*4*). Thus one would not necessarily expect the two kinds of nodules to be equally efficient in energy usage. Nevertheless, we have found that both the absolute rate of nitrogenase activity and the energy cost of nitrogen fixation are similar in the two types of nodules. This finding should give encouragement to efforts to develop new nitrogen-fixing plants, since it indicates that there is flexibility in the means of obtaining an efficient symbiosis.

We estimated the amount of energy consumed in nodules during nitrogen fixation in terms of the rate of CO_2 evolved from nodule respiration; nitrogenase activity was simultaneously measured in terms of the rate of C_2H_2 reduction (*5*). The ratio of CO_2 evolution to C_2H_2 evolution provides an estimate of the energy required for nitrogen fixation. Field populations of plants were examined so that a wide range of species could be studied and to avoid the abnormalities that are possible in greenhouse-grown plants. The rates of CO_2 and C_2H_2 evolution in these field-collected nodules were quite variable (Table 1), but there was no correlation with the type of endophyte, which confirms earlier reports (*6*, *7*). The ratio of CO_2 evolution to C_2H_4 evolution was much less variable, with values ranging from 3.4 to 5.6 for legume nodules and from 2.8 to 8.7 for actinorhizal nodules. If the higher ratios for *Ceanothus* and *Myrica* are disregarded (*8*), the range for actinorhizal nodules is from 2.8 to 3.8; if the value for *Melilotus* is disregarded, the range for legumes is from 3.4 to 4.5. Additional measurements will undoubtedly reveal lower ratios for the species studied, but the ratios are not likely to be much lower because the lowest values found approach theoretical limits (discussed below).

The values in Table 1 generally agree with earlier results for the energy requirement for nitrogen fixation by legume nodules (*1*). However, Pate and his co-workers have found values equivalent to 1.2 moles of CO_2 per mole of C_2H_2 reduced in both peas and cowpeas (*2*).

Detachment of root nodules, as done in our field measurements, may sometimes cause substantial reductions in nitrogenase activity (*9*). To test whether this could have any effect on our conclusions, we studied the effects of nodule detachment on CO_2 and C_2H_4 evolution by actinorhizal (*Alnus rubra*) and legume (*Glycine max*) nodules. Figures 1 and 2 show that for representative nodules excision has no effect on the ratio of CO_2 evolution to C_2H_4 evolution for the first 3 hours after excision, although there was a modest decrease in the absolute rate of CO_2 and C_2H_4 evolution by nodules of

Table 1. Results of measurements of nitrogenase activity (measured as C_2H_4 evolution) and CO_2 evolution in root nodules. Nodules were collected from the field between 19 and 30 August 1978, except as noted. Sites 1 and 2 were 1 mile apart. All collections were made in central Massachusetts. Nodules that were much larger or smaller than typical were excluded. Detached nodules (25 to 100 mg total) were placed in a 10-ml plastic syringe which contained a moist piece of filter paper, and the syringe was then capped with a rubber serum stopper. The assays were initiated (25 to 60 minutes after collection of the nodules in the field) by flushing the syringes with air and adding 10 percent C_2H_2. After incubating the nodules for 25 to 40 minutes at 22°C, gas samples were taken for analysis of CO_2 and C_2H_4 with a gas chromatograph (Carle model AGC 111) equipped with a thermal conductivity detector. The column (3.1 m by 1.7 mm, inside diameter) was packed with Porapak N, the carrier gas was helium, and the oven temperature was 40°C. Data are means ± standard errors of the mean; *N*, number of assays.

Species	*N*	C_2H_4 [μmole $hour^{-1}$ g^{-1} (fresh weight)]	CO_2 [μmole $hour^{-1}$ g^{-1} (fresh weight)]	CO_2/C_2H_4
		Actinomycete-induced nodules		
Alnus rugosa, site 1	7	12.1 ± 3.2	41.4 ± 7.8	3.4
Alnus rugosa, site 2	5	21.5 ± 1.9	80.9 ± 5.4	3.8
Ceanothus americanus	6	1.8 ± 0.1	15.8 ± 1.9	8.7
Ceanothus americanus, 4 September 1979	6	11.1 ± 1.8	42.0 ± 5.7	3.8
Comptonia peregrina	4	7.1 ± 0.7	21.2 ± 1.8	3.0
Elaeagnus umbellata	5	12.7 ± 0.9	35.9 ± 2.9	2.8
Myrica gale	4	6.1 ± 1.6	46.2 ± 6.0	7.6
Myrica gale, 3 July 1979	3	12.1 ± 2.3	42.4 ± 8.1	3.5
		Rhizobium-induced nodules		
Amphicarpa bracteata	5	9.5 ± 0.4	42.0 ± 3.4	4.4
Apios americana	5	10.4 ± 2.6	47.3 ± 6.9	4.5
Desmodium sp.	5	12.0 ± 0.7	41.4 ± 1.8	3.5
Melilotus sp.	8	5.8 ± 0.7	32.7 ± 4.6	5.6
Phaseolus vulgaris	5	13.1 ± 0.5	51.2 ± 2.7	4.0
Robinia pseudoacacia				
Nodules < 1 year old	4	9.8 ± 1.6	43.9 ± 6.1	4.4
Nodules > 1 year old	4	4.8 ± 0.6	20.9 ± 1.7	4.3
Trifolium pratense	2	25.7 ± 4.4	107.0 ± 19.0	4.2
Vicia sp.	4	23.2 ± 1.6	78.1 ± 5.3	3.4

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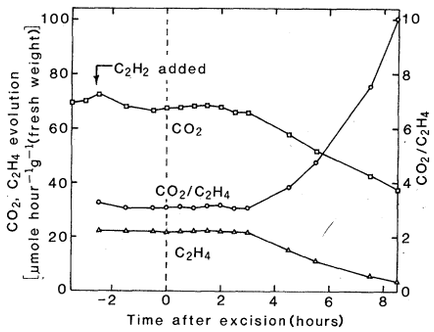

Fig. 1. Evolution of CO_2 and C_2H_4 by a nodule of *Alnus rubra* before and after nodule excision. Seedlings were inoculated with a strain of actinomycete isolated from *Alnus rubra* (*16*) and were grown in a greenhouse with roots in a nutrient mist (*17*). Measurements were made on plants 3 months old in a plant growth chamber with an air and root temperature of 20°C and a light intensity of 500 μE m^{-2} per second of photosynthetically active radiation. The nodule and adjacent 3 to 4 mm of root were enclosed in a gas-tight cuvette, and gas was circulated through a loop containing the cuvette, a pump, and a bag of Saran plastic. Activity was calculated from the difference in the CO_2 and C_2H_4 concentrations at 2 and 22 minutes after the addition of a mixture of 87.5 percent air, 10 percent C_2H_2, and 2.5 percent O_2 to the cuvette. The nodule fresh weight was 0.15 g.

Glycine max. The data also show that the presence of C_2H_2 causes no substantial change in the rate of energy consumption in these nodules, since the CO_2 evolution rate was about the same before and after C_2H_2 addition. We have observed similar results for *Myrica gale*.

From the above data we conclude that the energy cost for C_2H_2 reduction is similar in actinorhizal and legume nodules, since both the range and minimum values for the ratio of CO_2 evolution to C_2H_4 evolution are approximately the same. It is possible that there is a small difference in energy efficiency between the two groups of nodules, but, if so, it is less than the natural variation found within each group under normal field conditions.

Molecular nitrogen and C_2H_2 differ somewhat in their properties as substrate for reduction by nitrogenase. For example, when C_2H_2 is reduced to C_2H_4, there is little simultaneous reduction of H^+ to H_2 by nitrogenase, whereas, when N_2 is reduced to NH_3, there is also a substantial reduction of H^+ to H_2. Thus, further measurements are needed to establish the absolute values for the energy requirement for N_2 reduction in root nodules. However, the limited data available indicate that the ratio of C_2H_2 reduction to N_2 reduction is similar in legume and actinorhizal nodules (*10*).

Moreover, the CO_2 evolution rate may somewhat underestimate the true rate of energy consumption in nodules, since some CO_2 may be fixed in the synthesis of carbon skeletons for amino acids. But in lupin nodules CO_2 fixation consumes only 11 percent of the total CO_2 evolved (*11*). Energy consumption could also be measured from the rate of O_2 uptake instead of from CO_2 evolution. In measurements of legume nodules from seven genera (*12*) and actinorhizal nodules from three genera (*13*), the ratio of CO_2 evolution to O_2 uptake was approximately 1.0 for both kinds of nodules. Since carbohydrate is thought to be the photosynthetic product translocated into both legume and actinorhizal nodules (*14*), CO_2/O_2 ratios of 1.0 suggest that in large part respiration in both kinds of nodules consists of oxidation of carbohydrate to CO_2 and H_2O. Thus both CO_2 evolution and O_2 uptake are probably good measures of energy consumption in actinorhizal and legume root nodules.

It is of interest to consider our results in relation to the minimum energy required for nitrogenase in vitro, which is about 4 ATP (ATP is adenosine 5′-triphosphate) for the transfer of 2 e^- by the purified enzyme (*15*). If one assumes a value of 36 ATP produced per glucose oxidized by the bacterial symbiont and 24 e^- of reductant per glucose, then the reduction of 1 mole of C_2H_2 to C_2H_4 would require 1/9 mole of glucose for ATP plus 1/12 mole of glucose for reductant. This would be equivalent to the evolution of 1.2 moles of CO_2. The minimum value that we have observed is 2.5 moles of CO_2 per mole of C_2H_2 reduced (Fig. 2). Thus, if nitrogenase were functioning at maximum efficiency, about half of the energy used in the most efficient legume and actinorhizal nodules would be consumed directly by nitrogenase. If the enzyme efficiency is less, as might be expected in a nodule, or if ATP production is less efficient than assumed, then even more than half of the energy consumption would be due to the nitrogenase enzyme. A significant amount of energy must also be used in other processes such as growth, cell maintenance, amino acid synthesis, and transport. This it seems unlikely that markedly more efficient nodules of either legume or actinorhizal type will be found.

The similarity in the energy requirement for nitrogen fixation in actinorhizal and legume root nodules is interesting. Actinorhizal root nodules would appear to be more primitive on the basis of characters such as an anatomy that more closely resembles lateral roots, the absence of leghemoglobin, and a wide range of hosts. But the presumably more specialized characters of legume nodules

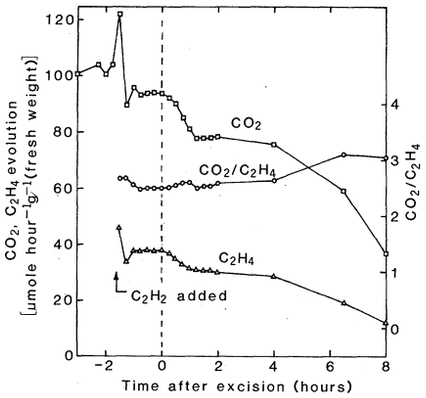

Fig. 2. Evolution of CO_2 and C_2H_4 by nodules of *Glycine max* cv. Wilkin before and after nodule excision. The rhizobium strain was U.S. Department of Agriculture DES 122; plant age at the date of assay was 6 weeks, nodule age was 28 days, and the fresh weight of the three nodules enclosed in the cuvette was 37 mg. The methods used are the same as those described in Fig. 1.

do not seem either to have brought about an increased rate of nitrogen fixation or to have decreased its energy cost. Thus actinorhizal root nodules deserve to be examined in greater detail, since they might be more easily extended to new families of host plants than rhizobial symbioses. They are also of very substantial ecological and economic importance (*7*).

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Patterning and Assembly of Ciliature Are Independent Processes in Hypotrich Ciliates

Abstract. *Mirror-imaged doublets of the hypotrich ciliate* Pleurotricha lanceolata *were induced and analyzed with respect to the overall patterning (structural asymmetry and polarity) of the individual components of the ciliature. The overall pattern is arranged as a mirror image, but the individual components in the two halves of the doublet show the same organizational asymmetry. These data demonstrate the independence of the mechanisms for this kind of large-scale (global) patterning and control of assembly of the individual ciliary components.*

Among unicells, the mechanism of cell patterning has been studied most extensively in the ciliated protozoa in which complex arrays of cilia form the cell pattern. The demonstration by Sonneborn of the lack of genic differences, genic activity differences, or differences in the fluid cytoplasm in cells possessing different cortical phenotypes (*1*, *2*) emphasizes the value of these organisms in studies of cell patterning and intracellular localizations. A question remaining from such studies is whether or not the overall pattern of the ciliature is exclusively a reflection of the assembly processes of the individual components of the ciliature. We report that the overall pattern of the ciliature is determined independently from the detailed structure and assembly of the component ciliature.

The hypotrich ciliates, including *Pleurotricha lanceolata*, are well suited for studies of cell patterning because of the specific localization of ciliary units, structural polarity and asymmetry of each ciliary unit, overall cellular polarity and asymmetry, and developmental flexibility (*3*-*5*). The typical morphostatic cell (Fig. 1a) has an oral apparatus composed of parallel arrays of rectangularly packed cilia (each array is a membranelle) occupying approximately the antero left cell quadrant. Furthermore, clusters of hexagonally packed cilia are located regularly elsewhere on the ventral surface (the ventral ciliature and the marginal rows of ciliature, one on the left side, and two on the right). In addition to this overall polarization and asymmetry of the cell, each component of the ciliature likewise is polarized and asymmetric. Each membranelle is composed of four rows of cilia; the two postero-most the longest, the antero-most composed of only three cilia at the antero right edge of each membranelle (Figs. 1e and 2b).

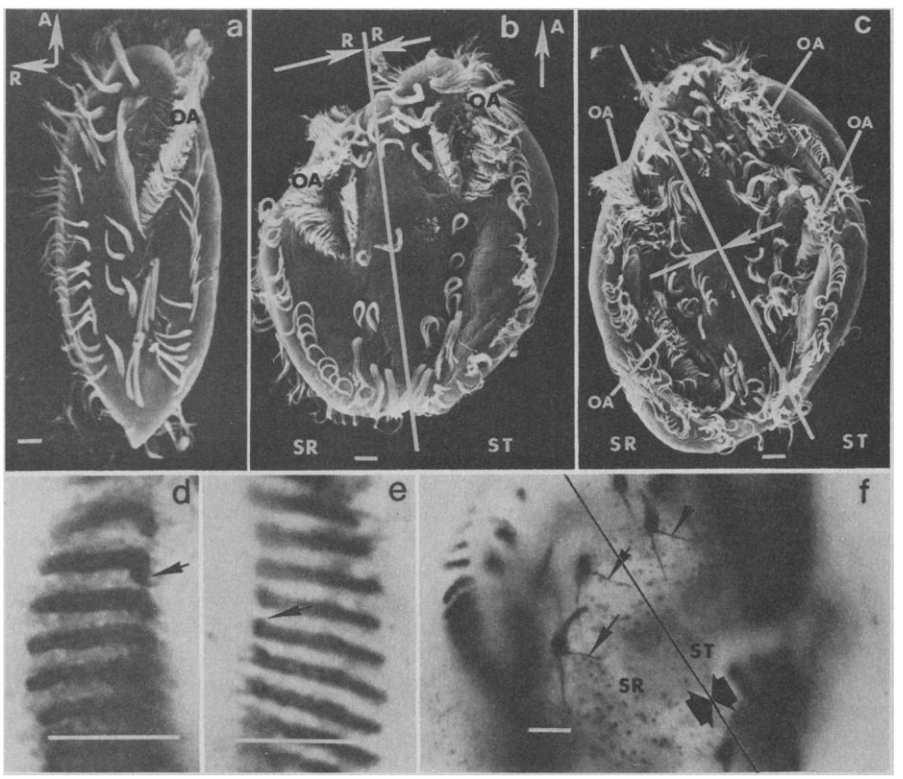

Fig. 1. Micrographs of the ventral surface of *Pleurotricha lanceolata* visualized from outside the cells. (a to c) Scanning electron micrographs; (d to f) light micrographs of silver-stained preparations. A-P, cellular polarity; L-R, axis of asymmetry of the entire cell or halves of the cell; *OA*, oral apparatus. Each bar represents 10 micrometers. (a) Typical morphostatic singlet cell illustrating the standard asymmetric array of ventral ciliature as well as the position and curvature of the oral apparatus (*OA*) in the antero left quadrant. (b) Morphostatic mirror-imaged doublet, the common polarity of both halves (approximate line of bilateral symmetry marked by vertical line) and mirrored patterning of ciliature, including curvature of the oral apparatus are shown. The lateral arrows indicate L-R asymmetry of each half. *ST*, standard symmetry half of the doublet [see (a)]; *SR*, symmetry reversal half of the doublet. (c) Predivision mirror-imaged doublet. Lateral arrows indicate L-R asymmetry of each half as above. There are four oral apparatuses (*OA*), and ventral ciliature is present on both sides of the line of bilateral symmetry (vertical line). (d) Organization of membranelles within the symmetry reversed oral apparatus (*OA* of *SR*) [see (b)]. The short fourth row (at arrow) is on the postero left margin of each membranelle; that is, it is an *inversion* of the standard arrangement [*OA* of *ST* illustrated in (e)] rather than a *mirror image* of the standard arrangement (Fig. 2). (e) Structure of membranelles in the oral apparatus of typical singlet cells and the standard symmetry half of mirror-imaged doublets. The short fourth row (arrow) located on the antero right margin of each membranelle (see also Fig. 2). (f) Morphostatic mirror-imaged doublet illustrating the same position of the lateral fiber bundles (at narrow arrows) on the ventral ciliature of both the standard symmetry half of the cell (*ST*) as well as on the symmetry reversed half of the cell (*SR*).

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