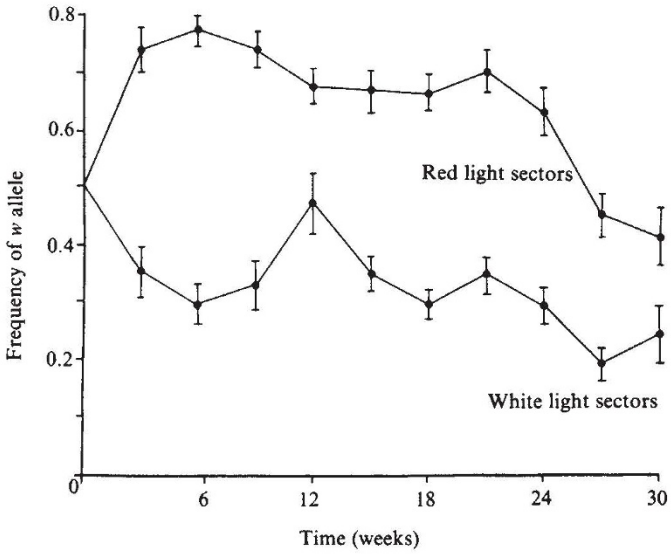

Fig. 2 Divergence in the frequency of the *w* allele between the red light and white light sectors of the experimental cages, summed over all cages.

There is considerable divergence in allele frequency between the two halves of the experimental cages (Fig. 2). To assess gene flow between red and white sectors two movement experiments were carried out. In the first, 12 vials containing *w* larvae were placed in the white light half of an experimental cage, and the same number of vials containing + larvae placed in the red light half. Flies emerged from the vials over the following 7 days and assorted themselves according to their habitat preference. The frequency of each allele among the offspring of the experimental flies was assessed by placing four sampling vials in each sector 1 week later. The frequency of *w* in the flies emerging from the sampling vials was 0.47 in the red light sector and 0.38 in the white light ($x_1^2 = 7.1$; $P < 0.01$). In the second experiment the 12 *w* vials were placed in the red light sector and the 12 + vials in the white light. Their offspring were sampled in the same way as in the first experiment; the frequency of *w* was 0.42 in the red light sector and 0.17 in the white light ($x_1^2 = 31.1$; $P < 0.001$). There is, therefore, the opportunity for substantial habitat selection by each generation between the environmental patches in the experimental cages. This is true whether or not the flies emerge into their preferred microenvironment, and must play a part in maintaining the differences in gene frequency between these patches shown in Fig. 2.

Associations between the extent of genetic polymorphism in natural populations and the ecological diversity of the habitats in which they live have often been reported. Populations of *Drosophila willistoni* living in florally complex environments have more inversion polymorphism than do those from simpler habitats[11] and there may be in some groups of animals a general association between niche breadth and morphological variation[12]. Similarly, *Drosophila* populations exposed to diverse environments in the laboratory retain chromosomal and molecular polymorphism to a greater extent than do those kept in stable conditions[13,14]. Natural and laboratory environments containing a variety of microhabitats offer the opportunity for genotypes to select those which are optimal. Differences in the tendency of individual members of natural populations to select particular microenvironments have indeed been found in *Drosophila*[15,16], and selection of the appropriate niche increases the relative fitness of, for example, melanic moths, cryptically coloured molluscs and lizards, and perhaps also of enzyme polymorphisms in *Asellus* and *Drosophila* exposed to a variety of foods[17–21].

Habitat selection can increase the fitness of an allele which is already advantageous. The experiments described here show that it can also lead to the maintenance of polymorphism for an allele which is otherwise deleterious to its carriers.

We thank Drs Derek Cosens, P. Hedrick, C. A. B. Smith and C. E. Taylor for useful discussions. R. F. P. was supported by a studentship from the SRC.

Received 3 March; accepted 15 August 1980.

1. Levene, H. *Am. Nat.* **87**, 331–333 (1953).
2. Maynard Smith, J. & Hoekstra, R. *Genet. Res., Camb.* **35**, 45–57 (1980).
3. Hedrick, P. W., Ginevan, M. E. & Ewing, E. P. *A. Rev. Ecol. Systematics* **7**, 1–32 (1976).
4. Taylor, C. E. *Genetics* **83**, 887–894 (1976).
5. Waddington, C. H., Woolf, B. & Perry, M. *Evolution* **8**, 89–96 (1954).
6. Markow, T. A. & Scavarda, N. J. *Behav. Genet.* **7**, 139–146 (1977).
7. Rockwell, R. F. & Seiger, M. B. *Am. Scient.* **61**, 339–345 (1973).
8. Chabora, P. C. & Kessler, M. E. *Behav. Genet.* **7**, 281–290 (1977).
9. Reed, S. C. & Reed, E. W. *Evolution* **4**, 34–42 (1950).
10. Grossfield, J. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2669–2673 (1971).
11. Da Cunha, A. B. & Dobzhansky, Th. *Evolution* **8**, 119–134 (1954).
12. Van Valen, L. *Am. Nat.* **99**, 377–390 (1965).
13. McDonald, J. F. & Ayala, F. J. *Nature* **250**, 572–574 (1974).
14. Powell, J. R. *Science* **174**, 1035–1036 (1971).
15. Taylor, C. E. & Powell, J. R. *Genetics* **85**, 681–695 (1977).
16. Stalker, H. D. *Genetics* **82**, 323–347 (1976).
17. Boardman, M., Askew, R. R. & Cook, L. M. *J. zool. Res.* **172**, 343–355 (1974).
18. Giesel, J. T. *Evolution* **24**, 98–119 (1970).
19. Schoener, T. W. & Schoener, A. *Evolution* **30**, 650–658 (1976).
20. Christensen, B. *Hereditas* **87**, 21–26 (1977).
21. Cavener, D. *Behav. Genet.* **9**, 359–365 (1979).

Vesicle formation and acetylene reduction activity in *Frankia* sp. CPI1 cultured in defined nutrient media

John D. Tjepkema, William Ormerod & John G. Torrey

Cabot Foundation for Botanical Research, Harvard University, Petersham, Massachusetts 01366

It has been reported that certain species of free-living *Rhizobium* were capable of producing nitrogenase[1–5], thereby demonstrating conclusively that the *nif* genes reside in the bacterial component of the dinitrogen-fixing symbiosis between *Rhizobium* and leguminous host plants. In the comparable dinitrogen-fixing symbiosis between the actinomycete *Frankia* and the actinorhizal woody dicotyledons that serve as nodulated host plants, the endophytic filamentous bacteria have only recently been brought into culture[6–8]. We report here evidence that free-living *Frankia* sp. grown in appropriate nutrient conditions show nitrogenase activity (acetylene reduction) associated with a distinctive morphological structure formed in effective nodules and also developed in culture.

Frankia sp. CpI1 isolated from root nodules of *Comptonia peregrina* and grown in liquid yeast–extract nutrient medium[6] develops as a finely branching filamentous mat that produces enlarged intrahyphal or terminal sporangia of considerable size (up to 60 μm) and complexity containing numerous 1–2-μm diameter spores[9]. During the development of defined synthetic media for the culture of CpI1, it was observed that growth rates and morphological structures of the filamentous bacterium were affected by preparation procedures and chemical components of the medium. A defined medium was developed that elicited sporangium formation and also extensive formation of small spherical to ovoid structures borne terminally on short side branches, which we term vesicles, in agreement with earlier literature. Similar structures have been observed by others[10,11]. Under Nomarski interference phase optics, the vesicles show thickened walls (Figs 1, 2), in contrast to immature developing sporangia. Thus far, they have proved difficult to preserve and section for transmission electron microscopy.

In the yeast-extract medium[8], used for routine subculture, vesicles are not usually observed. Such cultures show no

0028-0836/80/410633—03$01.00

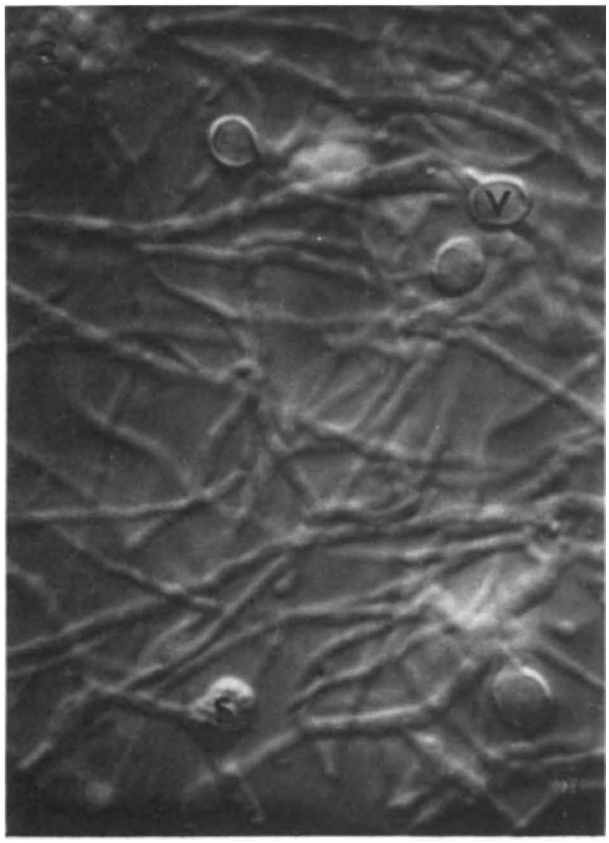

Fig. 1 A 4-week-old culture of *Frankia* sp. CpI1 grown in defined medium photographed with Nomarski phase interference optics. Branched bacterial filaments, enlarged sporangia (S) and a number of thick-walled vesicles (V) are visible. ×2,890.

acetylene reduction activity. The following procedure was used to elicit vesicle formation. The filamentous mat from yeast-extract medium was collected after 4–6 weeks of standing culture in flasks. The mat was homogenized in a Potter–Elvejhem homogenizer and then washed three times with mineral salt solution from the defined medium (see below) and its packed cell volume measured. A sample of known volume was resuspended in mineral salt solution and added to test tubes containing 5 ml of defined medium with the following composition. Mineral salt solution (gm l^{-1}): 1.0 KH_2PO_4, 0.1 $MgSO_4 \cdot 7H_2O$, 0.01 $CaCl_2 \cdot 2H_2O$, 0.01 FeNaEDTA; 1 ml l^{-1} of the following micronutrient stock solution (gm l^{-1}): 2.86 H_3BO_3, 1.81 $MnCl_2 \cdot 2H_2O$, 0.22 $ZnSO_4 \cdot 7H_2O$, 0.08 $CuSO_4 \cdot 5H_2O$, 0.025 $Na_2MoO_4 \cdot 2H_2O$; 1 ml l^{-1} of the following stock solution (mg per 100 ml): 10 thiamine HCl, 50 nicotinic acid, 50 pyridoxine HCl; and 1.2 gm l^{-1} succinic acid, *p*H adjusted to 6.8. Note that the only nitrogen-containing compounds in this medium are EDTA and the vitamins, and that they contain only very small quantities.

Table 1 Time course of nitrogenase activity by *Frankia* sp. CpI1 cultured in defined medium

Time (days)	No. of vesicles per tube ($\times 10^6$)	Acetylene reduction (nmol per tube per day)
0	0	0
5	1.67 ± 0.35	7.3 ± 0.4
7	1.85 ± 0.26	11.9 ± 3.0
14	2.73 ± 0.41	2.9 ± 0.7
	Defined medium plus 100 mg l^{-1} glutamic acid	
7	1.17 ± 0.41	6.6 ± 0.7

Values are ±s.e.m., $n = 5$.

In a typical experiment 0.8 ml packed cell volume of *Frankia* was used to inoculate 100 tubes (an inoculum of 8 μl packed cell volume per tube). Tubes were stoppered with cotton plugs and incubated at 25°C in the dark without agitation. For each sample used in time-course studies, five tubes were sampled for nitrogenase activity, using the acetylene reduction assay[12] with gentle agitation of the tubes. Vesicle counts were made by sonicating the contents of each tube for 30 s at 60 W and then counting in a Petroff-Hausser chamber under phase illumination.

Representative data are given in Table 1 showing the relationship between vesicle formation and acetylene-reducing activity over a culture period of 2 weeks. Cultures at the outset lacked vesicles and showed no acetylene reduction. Vesicle formation was first observed at 5 days and the number of vesicles per tube increased with time. Acetylene reduction occurred only in cultures with vesicles and this activity increased roughly in proportion with the number of vesicles formed between days 5 and 7. Nitrogenase activity decreased at 14 days, even though vesicle numbers continued to increase. The cause of this decrease is not known but may have been due to accumulation of inhibitory substances. The maximum activity recorded in any of these experiments was 44 nmol per tube per day. One experiment was conducted with *Frankia* sp. Ar13 from *Alnus rubra*[7] and this also resulted in vesicle formation and nitrogenase activity.

The nitrogenase activity observed is consistent with that of intact nodules, which is ~10 μmol C_2H_4 h^{-1} cm^{-3} of nodule volume. We estimate that vesicles make up about 1% of nodule volume; assuming that the vesicles are the site of nitrogenase, their activity would be 1,000 μmol C_2H_4 h^{-1} cm^{-3} of vesicle volume. Using a vesicle diameter of 2.5 μm, the highest activity we have observed (Table 2) is equivalent to 180 μmol h^{-1} cm^{-3}, or 18% of the value estimated for vesicles in nodules. For comparison, we calculate that bacteroids in legume nodules have a nitrogenase activity of ~100 μmol C_2H_4 h^{-1} cm^{-3} of bacteroid volume.

It is very unlikely that the nitrogenase activity of our cultures was due to contaminating organisms as activity was observed only in cultures with vesicles and was correlated with the number of vesicles. Further evidence is the lack of growth when CpI1 is inoculated into Difco nutrient broth, absence of turbidity in all cultures, and lack of growth and nitrogenase activity in anaerobic conditions.

Inoculum density was an important variable not easily controlled because of the difficulty in accurately measuring

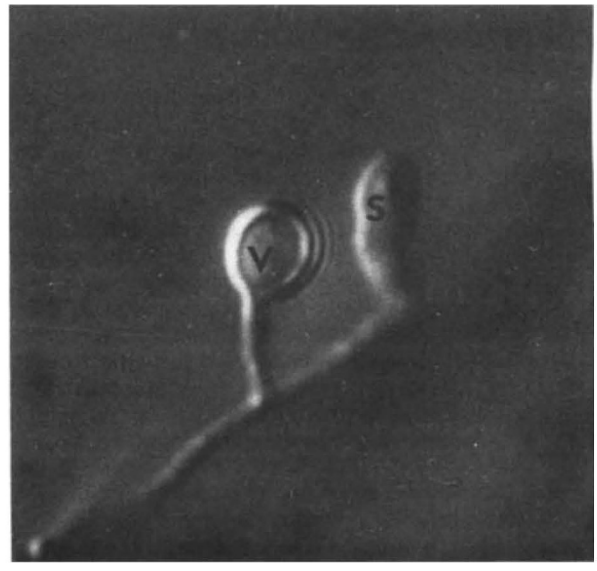

Fig. 2 A young culture of CpI1 showing a bacterial filament with an immature developing sporangium (S) side by side with a mature thick-walled vesicle (V). Nomarski optics. ×4,375.

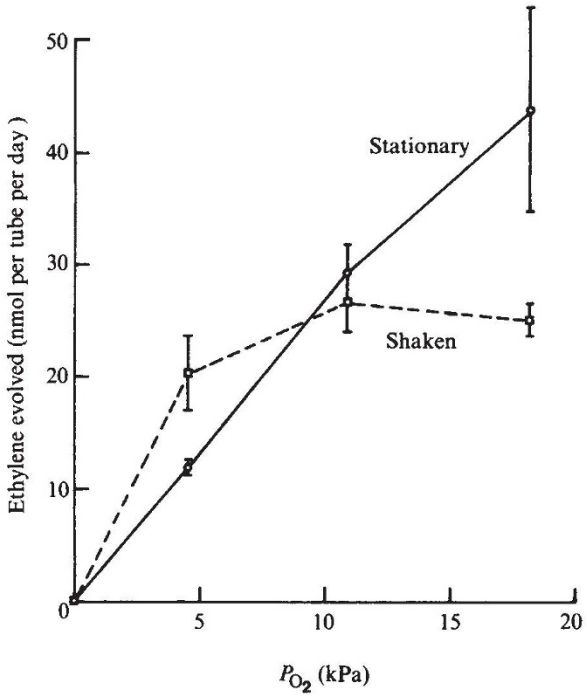

Fig. 3 Acetylene reduction by cultures of *Frankia* as a function of P_{O_2}. One set of tubes was shaken throughout the experiment and the other was stationary. From 0 to 19 days after inoculation, the tubes were incubated in air. They were then gassed with mixtures of air, N_2, and 10% C_2H_2. Vertical bars represent ±s.e.m.; $n = 5$.

actinomycete mass and growth. Positive correlations were found between inoculum density, vesicle number, and acetylene reduction activity (Table 2). Experimental differences in similar tests were probably most often attributable to variation in initial inoculum density.

In several series of experiments we tested the effect of additions of inorganic or organic components to the defined medium on acetylene reduction activity. Cellobiose added at 1% (w/v) completely inhibited vesicle formation and acetylene reduction over a period of 3 weeks. Glutamic acid added at 100 mg l^{-1} reduced the number of vesicles and nitrogenase activity by about half (Table 1). Ammonium chloride (54 mg l^{-1}) completely suppressed vesicle formation and acetylene reducing activity. From the experiments performed to date, medium additions which inhibit acetylene reduction activity do so by suppressing vesicle formation. We have no information to suggest that the ammonium chloride and glutamate inhibited nitrogenase activity *per se*.

Finally, in view of the known instability of the nitrogenase enzyme to molecular oxygen[13], we were interested in studying the effects of P_{O_2} on acetylene reduction activity in cultures of CpI1 in defined medium. As shown in Fig. 3, acetylene reduction by shaken cultures was little affected by the P_{O_2} values examined, while stationary cultures were oxygen limited. Apparently, the enzyme within the vesicle is protected from oxygen in some way, either by special metabolism within the vesicle or by the relative impermeability of the thickened wall. This suggests analogy with heterocyst formation and function in the blue-green algae.

These experiments establish several new and interesting facts. They give the first published evidence that free living *Frankia* sp. are capable of nitrogenase activity in appropriate nutrient conditions. The positive correlation between vesicle formation and acetylene reduction activity *in vitro* gives strong support to the already expressed view[8,13–17] that vesicles are the site of nitrogenase activity in actinorhizal plants. Our experiments further show that vesicle formation is elicited or made possible by the physico-chemical conditions which surround the micro-organism *in vitro*, a fact which raises interesting questions about the lack of vesicle formation in certain incompatible cross inoculations[16,18]. These observations open up a new approach to the problems of structure and function in these important symbiotic nitrogen fixing relationships.

This research was supported in part by the Maria Moors Cabot Foundation for Botanical Research of Harvard University, by NSF grant DEB 77-2249 and USDA grant 5901-0410-8-0055-0.

Received 5 March; accepted 24 July 1980.

Table 2 Effect of inoculum density on nitrogenase activity by *Frankia* sp. CpI1 cultured in defined medium

Time (days)	Inoculum density (%)	No. of vesicles per tube ($\times 10^6$)	Acetylene reduction (nmol per tube per day)
0	100	0	0
7	100	—	1.4±0.3
14	100	0.95±0.09	25.0±3.1
14	50	0.56±0.04	20.4±3.9
14	25	0.23±0.04	9.1±2.7
14	10	0.05±0.01	1.0±0.7

Values are ±s.e.m., $n = 5$.

1. Keister, D. L. *J. Bacteriol* **123,** 1265–1268 (1975).
2. Kurz, W. G. W. & LaRue, T. A. *Nature* **256,** 407–408 (1975).
3. McComb, J. A., Elliot, J. & Dilworth, M. J. *Nature* **256,** 409–410 (1975).
4. Pagan, J. D., Child, J. J., Scowcroft, W. & Gibson, A. H. *Nature* **256,** 406–407 (1975).
5. Tjepkema, J. & Evans, H. J. *Biochem. biophys. Res. Commun.* **65,** 625–628 (1975).
6. Callaham, D., Del Tredici, P. & Torrey, J. G. *Science* **199,** 899–902 (1978).
7. Berry, A. & Torrey, J. G. in *Symbiotic Nitrogen Fixation in the Management of Temperate Forests* (eds Gordon, J. C., Wheeler, C. T. & Perry, D. E.) 69–83 (Oregon State University Press, 1979).
8. Baker, D., Torrey, J. G. & Kidd, G. H. *Nature* **281,** 76–78 (1979).
9. Newcomb, W., Callaham, D., Torrey, J. G. & Peterson, R. L. *Bot. Gaz.* **140,** Suppl. S22–S34 (1979).
10. Pommer, E. *Ber. dt. bot. Ges.* **72,** 138–150 (1959).
11. Lalonde, M. & Calvert, H. E. in *Symbiotic Nitrogen Fixation in the Management of Temperate Forests* (eds Gordon, J. C., Wheeler, C. T. & Perry, D. E.) 95–110 (Oregon State University Press, 1979).
12. Burris, R. H. in *The Biology of Nitrogen Fixation* (ed. Quispel. A.) 9–33 (North-Holland, Amsterdam, 1974).
13. Benson, D. R., Arp, D. J. & Burris, R. H. *Science* **205,** 688–689 (1979).
14. Akkermans, A. D. L. thesis, University of Leiden (1971).
15. Becking, J. H. in *Recent Developments in Nitrogen Fixation* (eds Newton, W., Postgate, J. R. & Rodriguez-Barrueco, C.) 551–567 (Academic, London, 1977).
16. Mian, S., Bond, G. & Rodriguez-Barrueco, C. *Proc. R. Soc.* **B194,** 285–293 (1976).
17. Mian, S. & Bond, G. *New Phytol.* **80,** 187–192 (1979).
18. Baker, D., Newcomb, W. & Torrey, J. G. *Can. J. Microbiol.* (in the press).

Complete reversal of antero–posterior polarity in a centrifuged insect embryo

Klaus-Gerhard Rau & Klaus Kalthoff

Department of Zoology, University of Texas at Austin, Austin, Texas 78712

Spatial pattern formation during embryogenesis is ascribed to differential gene expression, which in turn is thought to result in part from interactions of nuclei with cytoplasmic determinants[1]. In the chironomid midge *Smittia*, and probably in other dipterans as well, blastoderm cells seem to make an early decision as to whether they contribute to cephalic and thoracic or to abdominal (and possibly thoracic) structures[2]. Inactivation or translocation of cytoplasmic components involved in this antero–posterior decision could conceivably lead to duplications of head and thorax, or abdomen, or to complete but inverted embryos forming the head posteriorly and the abdomen anteriorly in the egg. Whereas the former two malformations have been described[3,4], completely inverted embryos are reported for the first time, to our knowledge, in this letter. Reversal of partial germ bands has previously been observed following combined ligation and cytoplasmic translocation in eggs of the leaf hopper, *Euscelis plebejus*[5].

0028–0836/80/410635—03$01.00