



Figure 2 Freiberg *et al.*¹ have sequenced a 536-kb plasmid (pNGR234a), which is not required for free-living bacterial growth, from a strain of *Rhizobium*. Included among the genes that they have identified are the *nod*, *nif* and *fix* genes, involved in the establishment of nitrogen-fixing nodules on the roots of legumes. This *Rhizobium* strain contains a second plasmid, which may also carry genes that are necessary for an effective symbiosis.

nitrogen-fixation genes. Surprisingly, some of these sequences are upstream of genes that are probably involved in polysaccharide biosynthesis or modification. Although surface polysaccharides are unlikely to be directly involved in nitrogen fixation, there are clearly considerable changes to the bacterial cell surface during the maturation of bacteroids⁴ (bacterium-like cells found in root nodules). Within root nodules, nitrogen-fixing bacteroids are in intimate contact with the 'peribacteroid membrane' — a differentiated and modified form of the plant plasma membrane that controls the flow of metabolites between plant cytoplasm and nitrogen-fixing bacteroids. Perhaps alterations to the bacterial cell surface are necessary to maintain this crucial interface.

An advantage of having symbiosis genes on a plasmid is that, in principle, they can be transferred into previously non-symbiotic bacteria that may be better adapted to the particular soil conditions. Such transfer, which, for example, has been observed in New Zealand soils⁵, enables the plasmid genes to survive, albeit in another bacterial host. Freiberg *et al.* found a cluster of plasmid-transfer genes on pNGR234a. These are probably induced by acyl homoserine lactones in a cell-density-dependent way, analogous to that seen in *Agrobacterium* spp.^{6,7}. But surprisingly, although pNGR234a has been studied genetically for over 15 years, no one noticed that it is self-transmissible — and sequencing a 536-kb plasmid seems to be a roundabout way to find this out!

Several genes that would be expected to be involved in rhizobial symbiotic nitrogen fixation are absent from pNGR234a. Examples include the oxygen-responsive regulatory genes *fixLJ*; the regulator *fixK*; *fixNOQP*, which encode the bacteroid high-affinity cytochrome oxidase complex; the *fixABC* genes that are required for nitrogen activity; and the *fixGHIS* genes, which are thought to encode a redox-process-coupled ion pump. These genes are all conserved among diverse rhizobia⁸ and, although they

are not found in pNGR234a, they are expected to be present elsewhere. Have they moved to the chromosome or are they on the second rhizobial mega-plasmid⁹, which is thought to be half as big again? It is a pity that Freiberg *et al.* do not refer to this second plasmid, as it may also carry genes that are required for an effective symbiosis (Fig. 2).

Almost one-fifth of the total pNGR234a sequence is made up of insertion elements and mosaic sequences, and what we see can only be a snapshot of what must be a constantly evolving replicon. The insertion elements are clustered, and they tend to flank functionally important regions. So can the nitrogen-fixation genes move as a single, huge, mobile element, using flanking insertion sequences as a means of transposition? Such an event might explain how in some rhizobia (such as *Bradyrhizobium japonicum*) the symbiosis genes come to be chromosomal. The scattered distribution of the nodulation genes might reflect the relatively recent acquisition of genes that promote nodulation. The insertion elements could stimulate gene capture, either by transposition or by providing homologous regions that allow genes to be acquired from other replicons through recombination. The differences in the amounts of guanine/cytosine in the nodulation and nitrogen-fixation genes implies that they have evolved separately and, presumably, been captured from different sources.

As expected for a non-essential plasmid, pNGR234a does not contain any crucial genes that encode enzymes involved in central metabolism or DNA replication and repair. But there are 139 open reading frames (about one-third of the total sequence) with no homologies to database entries. Defining the functions of such genes could be difficult, and may have to await new database entries derived from more directed genetic or function-based approaches, possibly by others working with different bacteria. This illustrates a weakness of plasmid-genome sequencing, and highlights the point that, although genome sequencing projects reveal much information, in many ways they are a beginning — and there is still a lot of



100 YEARS AGO

Cambridge. — The Committee for promoting the admission of women to titular degrees appears to be disintegrating. On May 17 a fly-sheet was circulated by the President of Queens' (Dr. Ryle), the Registry (Mr. J. W. Clark), Mr. E. S. Roberts (tutor of Caius), and Mr. W. L. Mollison (tutor of Clare), expressing their decision to withdraw their support from the proposals before the Senate. They are now convinced the removal of the alleged grievance, felt by a comparatively small number of women, would be bought at too high a price, when considerably more than half of the resident members of the Senate are bitterly opposed to the measure, and would view it, if carried, as a grave betrayal of trust.

From *Nature* 20 May 1897.

50 YEARS AGO

The increase of milk production is one of the desiderata that is commonly accepted as necessary, and one of the equally commonly accepted ways of securing an increase is by the use of a proven sire. ... It is to this end that the Mount Hope Farm was established. The work with dairy cattle started in 1916 when the first were bought, but contagious abortion delayed the start of real work on breeding until the herd had been purged of this disease, by which time (1924) a young Guernsey herd had been acquired which was thought likely to be good. For various causes a satisfactory bull did not appear, and in 1925 a bull was bought because he ranked well according to the system of valuing dairy bulls developed at the Maine Agricultural Experiment Station. ... Misfortune followed the choice, for the bull became impotent and a new bull was selected on the Turner measure for proven sires; but this was found unsatisfactory, and this particular system is no longer used "because it has been found that it over-estimated the inheritance which the sire contributed to its off-spring"; and Mr. Parmalee Prentice and his co-workers found it conceivable that the daughters of this bull were good "not only because his influence was good but also because their dams had contributed as much as the sire" — which seems not unreasonable.

From *Nature* 24 May 1947.