The Evolution of Nodulation in Rhizobium: Altruism in the Rhizosphere

I. Olivieri and S. A. Frank

In this note, we suggest that kin-selection could be the main selective force maintaining nodulation and nitrogen fixation in *Rhizobium* bacteria. Our model stresses how the self-sacrificing individuals that form nodules gain by increasing the flow of root exudates to their relatives in a more or less continuous rhizospheric population.

It has been suggested (Jimenez and Casadesus 1989) that there is no obvious direct selective force maintaining the ability of *Rhizobium* to form nodules and fix nitrogen with legumes. We propose that these bacteria are favored to differentiate into nodules and thereby sacrifice their own reproduction in order to aid their kin living in nearby soil.

After infecting a suitable host, rhizobia multiply and then differentiate into bacteroids, which are specialized for nitrogen fixation. These bacteroids are very unlikely to be able to dedifferentiate and reproduce again (references in Jimenez and Casadesus 1989). Thus, although there is

a multiplication phase of the bacteria within a nodule prior to differentiation, forming a nodule is likely to be a reproductive deadend for those "nod+" bacteria.

The host plant does not feed nodules that contain only nonfixing bacteria, so that very little multiplication occurs in "fix−" nodules (Denariè et al. 1976). Moreover, each nodule is usually initiated by a single bacterium. Thus, as shown by Jimenez and Casadesus (1989), fix+ genotypes (able to fix nitrogen) are selected for within nod+ bacteria. The reason for the maintenance of nod+ bacteria is less clear.

Jimenez and Casadesus (1989) argued that nod+/fix+ genotypes are maintained because they provide protection to clonal relatives that are undifferentiated within the nodule. These undifferentiated bacteria are released to the rhizosphere at the end of the plant lifecycle, at the time of nodule senescence. The idea is that when the plant dies, the soil population crashes because the population is then lacking the root exudates it uses for food and also because of drought. The bacteria that are released from nodules could then represent a high proportion of the population, thus favoring the spread of altruistic, nodulating bacteria. Whether nodules maintain a viable bacterial population remains an open question. Quispel's (1988) summary of available data suggests that only very recently-infected cells contain viable bacteria.

There is another reason why nod+/fix+ bacteria may gain from their own suicide when fixing nitrogen for the plant. The exudates produced by the plant roots provide the main source of food for the soil bacterial population (Jimenez and Casadesus 1989; Soberon and Shapiro 1989; Sprent and Sprent 1990). Thus, an alternative to Jimenez and Casadesus's (1989) explanation for the maintenance of nod+/fix+ bacteria is that the apparent "suicide" of a nodule-forming bacteria actually results in the increase of the local rhizospheric population because of increased root exudates. This population level advantage is not sufficient, however, because it only specifies how the group benefits from individual sacrifice and does not explicitly consider the fitness of the nod+/fix+ genotype.

Altruism in a biological context is defined as sacrifice of individual reproduction in order to benefit the reproduction of others. Altruism can evolve—according to the well-known theory of kin selection (Hamilton 1964)—when the benefits are conferred disproportionately to genetic relatives. The approximate criterion for the evolutionary success of an altruistic phenotype is $rb − c > 0$, where c is the reproductive cost to the donor for performing the altruistic act, b is the benefit to the recipients, and r is the kin selection coefficient of genetic relatedness between donor and recipients. The parameter r is a measure of genetic similarity between the donor and the recipients. When r is 1, the donor and recipients are genetically identical, and altruism is favored by clonal selection. Altruism can also spread when r is <1, in which case altruistic behavior extends beyond benefits given to genetically identical individuals.

In the case of nodulation, the cost, c, is the average number of descendants that an individual would have had if it had not terminally differentiated. The term rb is the weighted gain in number of descendants for all individuals in the local subpopulation as the result of a single bacterium forming a nodule, where the gain for each individual is weighted by the coefficient of relatedness from the genotype forming the nodule to the individual receiving the benefit. A local subpopulation is the set of individuals that are affected by additional benefits (root exudates) caused by the nodulation event.

The simple kin selection criterion, $rb − c > 0$, suggests which factors must be measured in natural systems to understand the suicidal behavior of nodulating bacteria. Only a few relevant data are available at present. For the benefit parameter, b, it has been shown that the presence of the legume host may increase the bacterial population by several orders of magnitude (Lowendorf 1980; Phillips et al. 1990). The relatedness parameter, r, is mostly determined by the dispersal pattern of the bacteria. Nothing is known about dispersal of rhizobia, and thus about the degree of mixing of various strains around a plant. Note that the criterion $rb − c > 0$ can be satisfied even when r is very low if the benefits reach many individuals, so that the sum of rb over individuals is high. This seems likely to be the case, so that only minimal population structure and a low value of r may explain the maintenance of nod+.

Our model emphasizes the benefits returned through root exudates to genetically related soil-borne neighbors, where the relatedness between the nodule-forming individual and the neighbors may be quite low. By contrast, Jimenez and Casadesus (1989) emphasize the benefits of protection to clonal neighbors within the nodule. Another difference between Jimenez and Casadesus's model and ours concerns the population dynamics of the rhizosphere. In Jimenez and Casadesus's model, long-term survival of a lineage requires alternating periods spent within the protection of nodules and survival in the rhizosphere after nodule senescence. By contrast, our model stresses how the self-sacrificing individuals that form nodules gain by increasing the flow of root exudates to their relatives in a more or less continuous rhizospheric population. The rhizospheric population may fluctuate in our model; the key difference is that we do not require a lineage to alternate life phases between nodules and the soil for each generation of the host plant.

References

Denarié J, Truchet G, and Bergeron B, 1976. Effects of some mutations on symbiotic properties of *Rhizobium*. In: Symbiotic nitrogen fixation in plants (Nutman PS, ed). Cambridge: Cambridge University Press; 47–63.

Hamilton WD, 1964. The genetical evolution of social behavior. J Theor Biol 7:1–16.

Jimenez J and Casadesus J, 1989. An altruistic model of the *Rhizobium*-legume association. J Hered 804: 335–337.

Lowendorf HS, 1980. Factors affecting survival of *Rhizobium* in soil. In: Advances in microbial ecology, vol. 4 (Alexander M, ed). New York: Plenum Press; 87–124.

Phillips DA, Harwig UA, Maxwell CA, Joseph CM, Wery J, Hungria M, and Tsai SM, 1990. Host legume control of nodulation by flavonoids. In: Nitrogen fixation: achievements and objectives (Gresshoff PM, Roth LE, Stacey G, and Newton WE, eds). New York/London: Chapman and Hall; 331–338.

Quispel A, 1988. Bacteria–plant interactions in symbiotic nitrogen fixation. Physiol Plant 74:783–790.

Soberon J and Shapiro E, 1989. Population dynamics of a *Rhizobium*-legume interaction. A mathematical model. J Theor Biol 140:305–316.

Sprent JI and Sprent P, 1990. Nitrogen fixing organisms. Pure and applied aspects. London: Chapman and Hall.