Sanctions and mutualism stability: when should less beneficial mutualists be tolerated?

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Abstract

Why do mutualists perform costly behaviours that benefit individuals of a different species? One of the factors that may stabilize mutualistic interactions is when individuals preferentially reward more mutualistic (beneficial) behaviour and/or punish less mutualistic (more parasitic) behaviour. We develop a model that shows how such sanctions provide a fitness benefit to the individuals that carry them out. Although this approach could be applied to a number of symbioses, we focus on how it could be applied to the legume-rhizobia interaction. Specifically, we demonstrate how plants can be selected to supply preferentially more resources to (or be less likely to senesce) nodules that are fixing more N_2 (termed plant sanctions). We have previously argued that appreciable levels of N_2 fixation by rhizobia are only likely to be selected for in response to plant sanctions. Therefore, by showing that plant sanctions can also be favoured by natural selection, we are able to provide an explanation for the stability of the plant-legume mutualism.

Introduction

The widespread occurrence of mutualisms (mutually beneficial relationships between members of different species) poses a problem for evolutionary theory (Leigh & Rowell, 1995; Herre *et al.*, 1999). Why should an organism perform a behaviour (usually with some short-term cost) that provides a benefit for an individual of a different species? Reciprocal altruism has been the classical explanation for such cooperation between different species (or nonrelatives in general; Trivers, 1971), and has usually been studied with the Prisoner's Dilemma (PD). If the same partners interact repeatedly (iterated PD), then the best strategy can be to cooperate with other individuals who cooperate (tit-for-tat; Axelrod & Hamilton, 1981).

Although this idea is intuitively appealing, extensions of the PD model to conditions appropriate for mutualisms (distinct species which compete for different resources and intermediate levels of cooperation) found that cooperation is not generally selected for [Doebeli & Knowlton, 1998; see Clutton-Brock (2002) for a recent critique of the PD applied to cooperation within species]. Specifically, cooperation is only selected for when there are: (a) spatial correlations between species in the tendency to cooperate (arising through processes such as limited dispersal by both species), and (b) high relatedness between symbionts within a host (Hamilton, 1972; Leigh, 1983; Frank, 1994; Doebeli & Knowlton, 1998; West *et al.*, 2002b). Although this could apply to some interactions, it is clear that there are also many to which it will not (e.g. legumes and rhizobia, mycorrhizae and plants, figs and fig wasps can all have multiple symbiont genotypes per host plant; Herre, 1989; Hagen & Hamrick, 1996a,b; Souza *et al.*, 1997; Silva *et al.*, 1999).

An alternative to limited dispersal and high symbiont relatedness is that 'good behaviour' could be enforced by the immediate fitness consequences of actively rewarding cooperation and/or punishing less mutualistic behaviour. An example of this can be seen by considering the mutualism between legumes and the rhizobia bacteria that fix atmospheric N_2 inside root nodules. We have argued that appreciable levels of N_2 fixation are only favoured when plants preferentially supply more

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resources to (or are less likely to senesce) nodules containing rhizobia that are fixing more N_2 (termed plant sanctions; Denison, 2000; West *et al.*, 2002b). If we are to understand the stability of mutualisms in such cases it is therefore fundamental that we understand the evolution of such sanctions. Specifically, are the kinds of sanctions that would make symbiotic cooperation an evolutionary stable strategy (ESS) for rhizobia also an ESS for legumes?

In some cases the evolution of sanctions against less mutualistic partners are clearly favoured. For example, yuccas preferentially abort flowers in which all the seeds are likely to be eaten, preventing the investment of resources into something (moths) that is likely to produce no fitness return (Pellmyr & Huth, 1994; see also Murray, 1985; Bull & Rice, 1991). However, in less extreme cases where the less mutualistic partners may still provide some benefit, sanctions may incur a cost and so their usefulness is less clear. For example, considering rhizobia, sanctions would waste plant resources because nodules are produced and then senesced, therein decreasing the total benefit (fixed N_2) obtained from mutualists. Although this may provide a long-term increase in the benefit provided by mutualists (evolution of greater effectiveness), it will not necessarily benefit the individual that carried out the sanction, or their relatives (depending upon factors such as form and timing of dispersal). Consequently, we should ask whether there are short-term individual fitness benefits for carrying out sanctions against less mutualistic partners?

Here we develop a model to show how sanctions can provide a fitness benefit at the individual level. We develop our model with the legume-rhizobia interaction in mind, but use a simple and general framework that could, in principle, be applied easily to any symbiosis in which multiple lineages infect an individual host. Specifically, our model could be applied, with little alteration, to any case in which individuals are capable of monitoring the performance of different mutualistic partners and applying sanctions or rewards based on this (e.g. plants and mycorrhizae). A crucial aspect (and complicating factor) of this model is that we consider the simultaneous evolution of sanctions (what fraction of nodules are senesced) and the initial investment into the mutualistic interaction (number of nodules produced). These factors must be studied simultaneously because their favoured levels will interact, and so nonintuitive predictions can arise. For example, a plant that initially produces more nodules may senesce a larger fraction of them (setting a higher standard for continuing to support a given nodule), while still retaining enough active nodules to meet its N needs. Similarly, a plant that imposes more stringent sanctions may need to initially produce more nodules.

Materials and methods

Natural history of the legume-rhizobium mutualism

Our discussion of sanctions is based on the legume-rhizobia mutualism, and so before proceeding further we briefly describe the relevant biology; for further details see Denison (2000) and West *et al.* (2002b). Rhizobia (*Rhizobium, Bradyrhizobium, Mesorhizobium, Sinorhizobium,* or *Azorhizobium* spp.) are bacteria that, to varying extents, fix atmospheric N_2 inside the root nodules of leguminous plants. Nodules and the rhizobia they contain consume about 20% of net photosynthate production (in two representative legume species, *Lupinus albus* and *Vigna unguiculata*), but that is often still a better investment for the plant than the alternative of growing and supporting a larger root system (Pate, 1986), especially

Table 1 Definitions of notation used throughout the paper, and key equations. Symbols are listed in the order in which they appear. The key equations, which define the assumptions of our model, are given after the relevant symbols have been defined.

Symbol	Definition
w	Fitness of a focal plant.
R	Resources that the plant puts into growth.
N	Rate at which the plant acquires Nitrogen.
	$w = R * N$
r	Fraction of resources allocated to the production and maintenance of nodules.
s	Fraction of nodules that are senesced through plant sanctions.
α	Relative importance of the resources saved by sanctioning nodules.
	$R = (1\text{-}r+rs\alpha)$
p	Rate at which Nitrogen is acquired by the plant roots directly from the soil (reflecting the level of soil N).
$\overline{m}$	Relative mean rate of N_2 fixation after sanctions have been applied by the plant
	$N = P+r(1\text{-}s)\overline{m}$
r^*	ESS value of r.
s^*	ESS value of s.
c	Mean rate of N_2 fixing in the rhizobia taken from the soil.
b	Rate at which the mean rate of N_2 fixing increases with the level of sanctions.
	$\overline{m} = bs + c$

if neighbouring plants have already taken much of the N available in the soil. Legumes typically reduce nodulation when more soil N is available (Streeter, 1988). Legumes appear to be unable to distinguish between more mutualistic and more parasitic (which fix little or no N_2, but still consume plant resources) rhizobia before they are established inside root nodules. However, there is some evidence that a nodule which fails to fix N_2 within a week or two after infection may be subject to sanctions, either early senescence of the whole nodule or against individual bacteroids (Denison, 2000). These sanctions presumably reduce rhizobial fitness; the evidence for their occurrence, and physiological mechanisms are discussed in detail by Denison (2000). Our focus here is on the fitness consequences for a legume of imposing sanctions – in a previous paper (West *et al.*, 2002b) we examined the consequences of sanctions for rhizobia behaviour.

A sanctions model – basic assumptions

We assume the following life history. An annual plant has a pool of resources, which it divides between growth (shoots and roots) and nodule production / maintenance. A proportion r is initially put into nodule production and maintenance (i.e. a higher r means more nodules are produced), and the rest into growth (Table 1). Plants are infected by rhizobia which have a variable rate of N_2 fixation. After a short period of time (such that N_2 fixation before senescence is negligible to that after), the plant carries out sanctions and senesces a proportion s of the nodules (note that because we assume sanctions are applied at the nodule level our model applies equally to species with determinate or indeterminate nodules). Sanctions are applied against rhizobia with lower N_2 fixing rates, and so the average N_2 fixing rate of rhizobia in a plant is higher after sanctions. We assume that, once a nodule is sanctioned / senesced by a plant, it has no further costs or benefits, and so allows the resources that would have been put into nodule maintenance to be used for plant growth. After sanctions are applied, the plant then grows for the rest of the season, at which point it reproduces, and its fitness is proportional to its size and resources at the end of the season.

The fitness of a plant (w) is assumed to be positively related to its (i) resources put into growth, and (ii) the rate at which it acquires N (from the soil and nodules). One way to encapsulate this is:

$$w \propto R * N, \tag{1}$$

where R is the resources that the plant puts into growth, which will be reduced by resources put into nodule production and maintenance (e.g. it has been estimated that as much as one-third of net photosynthate can be diverted to nodules in young pea plants; Pate, 1977) and N is the rate at which the plant acquires Nitrogen (a higher Nitrogen content in the leaves means a higher photosynthesis rate; Bethlenfalvay *et al.*, 1978; Field & Mooney, 1986). We use a product of these two functions because total fitness should be zero for a minimum amount (e.g. zero) of resources into growth or N acquired, and this relationship is appropriate for the total plant photosynthesis rate (Bethlenfalvay *et al.*, 1978; Field & Mooney, 1986).

Equation 1 illustrates the trade-offs that are fundamental to this model. Putting more resources into nodule production can decrease fitness because it leaves less resources for growth, but can increase fitness because it raises the rate at which N is acquired. Increasing the fraction of nodules sanctioned can increase plant fitness because it decreases the resources put into nodule maintenance, but can decrease fitness because it lowers the total rate of N acquisition (although it will generally increase the rate of N_2 fixing per nodule, as less 'effective' nodules are sanctioned). This model could be generalized with other organisms by assuming that R is a function of the resources that the host puts into growth, and N is a function of the resources supplied by the mutualist partner (although in some cases it might be more appropriate to combine these terms additively, rather then multiplicatively).

Specific functions

To proceed further we need to assume specific forms for the two functions given above. We have assumed as simple forms as possible, and the predictions that we give are qualitative and not quantitative (as with most ESS models; Parker & Maynard Smith, 1990; Frank, 1998), a point that we return to in the discussion.

We assume that the resources put into growth (R) are given by the equation

$$R = (1 - r + rs\alpha), \tag{2}$$

where R represents the resources available for growth, relative to what would be available if the plant formed no nodules at all. This equation encapsulates two effects. First, the more resources allocated into the production and maintenance of nodules (r) the less are available for growth. Secondly, if more nodules are senesced (higher s), the costs of nodule maintenance are reduced, freeing more resources for growth. The parameter α (which must be <1) determines the relative importance of the resources saved by sanctioning nodules – lower values of α implying that the resources put into nodule maintenance are small compared with those put into initial production of nodules and / or that resources put into early growth are more important (e.g. more growth early on means the potential for more growth later on, especially when plants are competing and there are large advantages to early growth that shades competitors; Black, 1958).

We assume that the amount of N obtained (N) is given by the equation

$$N = p + r(1 - s)\overline{m} \tag{3}$$

This is the rate at which N is acquired by plant roots, directly from the soil (p) plus the total rate at which N_2 is fixed by rhizobia in the nodules that were not senesced ($r(1 - s)\overline{m}$). This total rate of N_2 fixation depends upon the number of nodules initially produced (which is directly proportional to r), the proportion of nodules that are not senesced ($1 - s$), and the relative mean rate of N_2 fixation across the individual nodules ($\overline{m}$). The parameter $\overline{m}$ is the relative mean rate of N_2 fixation after sanctions have been applied by the plant; this is scaled so that if all resources are devoted to N_2 fixation ($r = 1$), and no sanctions are applied ($s = 0$), then the total rate of N_2 fixation would be $\overline{m}$. This is a function of s because the plant selectively senesces nodules containing rhizobia with lower N_2 fixing rates (less effective rhizobia) and so $\overline{m}$ is positively correlated to s – a plant that senesces a larger fraction of nodules retains only the best, leading to a higher mean N_2 fixation rate. At this point we assume no specific form for $\overline{m}$.

If we put eqns 2 and 3 into eqn 1 we obtain:

$$w \propto (1 - r + rs\alpha)(p + r(1 - s)\overline{m}) \tag{4}$$

where w is plant fitness.

Results

We are interested in the ESS (strategy that cannot be beaten by a mutant playing any other strategy, denoted by *; Maynard Smith, 1982) value of two parameters: (1) The number of nodules produced (proportional to r^*); (2) The severity of sanctions (s^*). The ESS value for the two parameters depend upon each other, and can be found by inspecting the selection differentials $\partial w/\partial r$ and $\partial w/\partial s$:

$$\frac{\partial w}{\partial r} = \overline{m}(1 - s) - p(1 - s\alpha) - 2\overline{m}(1 - s)r(1 - s\alpha) \tag{5a}$$

$$\begin{aligned}\frac{\partial w}{\partial s} &= r[\alpha p + \overline{m}(\alpha r(1 - s) - (1 - r) - rs\alpha) \\ &\quad + (1 - r + rs\alpha)(1 - s)\overline{m}'],\end{aligned} \tag{5b}$$

where $\overline{m}'$ denotes the derivative of $\overline{m}$ with respect to s.

For positive values of r, the right-hand side of (5a) is nonnegative only if $\overline{m}(1 - s) > p(1 - s\alpha)$, which implies $\overline{m} \geq p$. In other words:

Result 1

The production of nodules is favoured by selection ($r^* > 0$) when rhizobia fix N_2 at least as efficiently as the plant can obtain N directly from the soil ($\overline{m} \geq p$).

Suppose plants can sanction rhizobia such that investment is terminated in all nodules with a N_2 fixation rate below a certain threshold value. Let μ denote the lowest fixation rate among all nodules. In the appendix we prove the following result:

Result 2

Selection favours sanctions ($s^* > 0$) against rhizobia with low fixation rates if the minimum fixation rate μ satisfies

$$\mu < \alpha\overline{m} \tag{6}$$

When μ is zero this condition is always satisfied, hence if there are parasitic rhizobia that do not fix N_2 then sanctions are always favoured. More generally, sanctions are favoured whenever the distribution of fixation rates among nodules has a sufficiently large variance and/or when the cost of maintaining nodules is high relative to their initial production cost (higher α). Note that the right-hand side of (6) is independent of the level p of soil N_2. This implies that sanctions may be favoured even if all rhizobia have a fixation rate higher than p.

In order to proceed further it is necessary to assume a relationship between the mean rate of N_2 fixation by rhizobia ($\overline{m}$), and the level of plant sanctions (s). For simplicity, we assume the relationship $\overline{m} = bs + c$, where c is the mean rate of N_2 fixing in the rhizobia taken from the soil (i.e. that observed in the absence of sanctions), and b scales how this increases with the level of sanctions ($b \leq c$ otherwise total N_2 fixing would initially increase as some nodules are sanctioned). Given this assumption, the ESS strategy is found by simultaneously solving $\partial w/\partial r = 0$ and $\partial w/\partial s = 0$ ($\partial^2 w/\partial r^2 < 0$ and $\partial^2 w/\partial s^2 < 0$), and values for r^* and s^* obtained numerically. This leads to the following results:

Result 3

Production of more nodules (higher r^*) is favoured by: (i) a lower level of soil N (p; Fig. 1); (ii) a higher mean rate of N_2 fixing in the rhizobia taken from the soil (c; Fig. 1); (iii) a higher proportion of resources saved by sanctioning nodules (higher α), and (iv) a higher rate at which sanctions increase the mean N_2 fixing rate (higher b).

A lower level of soil N (p) favours the production of more nodules because it means a greater gain from any fixed N_2. The ESS investment into nodules (r^*) is further increased as only more efficient and beneficial nodules are maintained (i.e. as sanctions reduce the cost of maintaining nodules, and increase the average rate of N_2 fixation per nodule), which occurs when sanctions are applied and as they provide a greater fitness benefit, either by saving more resources (higher α) or leading to greater N_2 fixing (higher b).

Result 4

The ESS severity of sanctions, i.e. the optimal fraction of nodules senesced (s^*): (i) decreases with a higher mean rate of N_2 fixing in the rhizobia taken from the soil (c; Fig. 2); (ii) increases as a higher proportion of resources is saved by sanctioning nodules (higher α; Fig. 2), and (iii) shows no change (invariant) with variation in either the

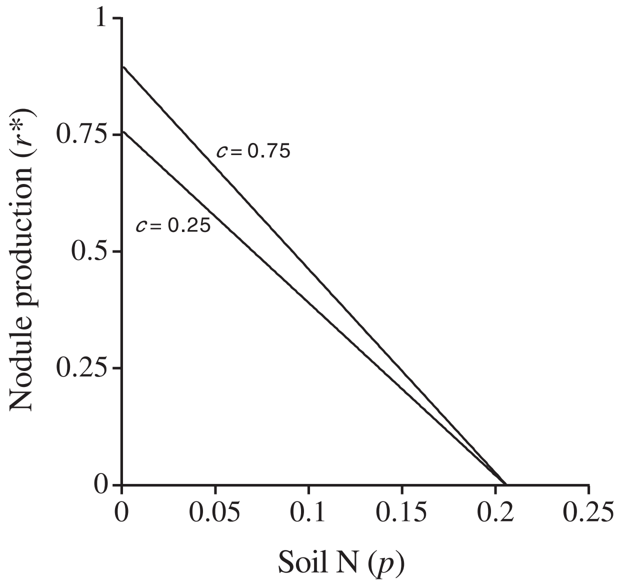

Fig. 1 The evolutionary stable strategy level of investment into the production of nodules (r^*) plotted against the rate at which the plant can acquire N directly from the soil (p). The different lines show the predictions for different mean N_2 fixing rates of the rhizobia that infect the plant, before sanctions occur (c). In both cases $\alpha = 0.5$, $b = 0.25$. Results were obtained numerically.

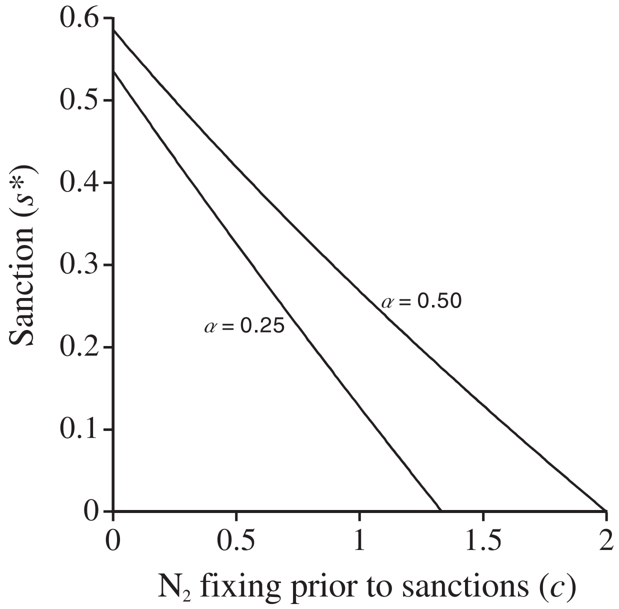

Fig. 2 The evolutionary stable strategy severity of sanctions (s^*) plotted against the mean N_2 fixing rates of the rhizobia that infect the plant (i.e. before sanctions occur, c). The different lines show the predictions for different relative importance of the resources saved by sanctioning nodules (α). Results were obtained numerically.

level of soil N (p) or the rate at which sanctions increase the mean N_2 fixing rate (b).

A higher rate of N_2 fixation in the rhizobia from the soil (c) means that each nodule provides a greater fitness benefit, favouring the sanctioning of fewer nodules, and so a lower ESS value of s^* (conversely, a lower rate of N_2 fixing, with more parasitic rhizobia would reduce the fitness benefit from each nodule and favour more severe sanctions, a higher ESS value of s^*). An increase in the proportion of resources saved by sanctioning nodules (α) favours greater sanctions because it increases the advantage to be gained from senescing nodules with low N_2 fixing rates.

The ESS severity of sanctions (s^*) does not depend upon the level of soil N (p), and so with respect to this variable it is an invariant (see Charnov, 1993 for a detailed discussion of life history invariants). Why is this so? A higher level of soil N (higher p) means that the N_2 fixed by each additional nodule makes less contribution to the overall N supply in the plant, favouring the sanctioning (senescing) of more nodules (higher s^*). However, this is exactly cancelled by the influence of changing nodule production – a higher level of soil N favours the production of fewer nodules (smaller r^*; Fig. 1), which favours less severe sanctions (lower s^*), because it decreases the resources that may be saved by sanctioning nodules (i.e. there are fewer nodules and so the plant cannot afford to sanction as many). We found that s was invariant with respect to p regardless of the function for $\overline{m}$ that we used (because we assumed that fitness is log-linear in both N and R; see eqns 2 and 3), although different forms of equations 1–3 can predict slight increases in s^* for higher p.

Discussion

When sanction?

Our model shows that plant (legume) sanctions against rhizobia that fix relatively little N_2 (whilst inside nodules) can provide a direct fitness benefit to plants at the individual level. The crucial point here is that sanctions are favoured when the benefit of making mutualist partners more efficient (i.e. increasing the rate of N_2 fixation per rhizobium or nodule and hence per unit of plant C expended) is greater than the cost of reducing the total benefit from the mutualist partners (i.e. reducing the total amount of N_2 fixing). Although phrased in terms of the legume-rhizobia interaction this model could be applied easily to other mutualisms/symbioses in which one partner is able to selectively reduce interactions with less mutualistic partners (by putting appropriate functions into eqn 1). Such sanctions could be of widespread importance because they can stabilize mutualistic interactions in conditions that are normally thought to be hard to explain – for example, a high number of symbiont strains per host, and without limited dispersal.

Future directions

Our model suggests several lines of empirical work. (1) Do mutualists apply sanctions against less beneficial and less mutualistic partners? Although there is suggestive evidence for sanctions by legumes (Denison, 2000; Simms & Taylor, in press; see natural history section above), further experiments are required to demonstrate clearly that they do occur and that they lead to a resultant fitness decrease for rhizobia. (2) Our model predicts the evolution of mean nodule production and sanction severity, as well as facultative variation in response to fluctuations in soil N (p) or the mean level of N_2 fixing (m) (i.e. conditional outcomes that are context dependent; Bronstein, 1994). For example, does the amount of soil N influence the production of nodules and severity of sanctions as predicted? Although the form of sanctions has not been examined, there is already considerable evidence for our prediction that nodule production is lower when levels of soil N are higher (Streeter, 1988; Rubio Arias *et al.*, 1999; Vargas *et al.*, 2000; Kiers *et al.*, in press). This prediction can be complicated by the fact that the amount of soil N can influence the ESS N_2 fixation rate by rhizobia, but this effect is predicted to be very small when plant sanctions occur (West *et al.*, 2002b). (3) How do sanctions influence the diversity of rhizobia, or the mean and variance in N_2 fixing rates ($\overline{m}$)? One important consideration here is the extent to which individual nodules contain more than one lineage of rhizobia (e.g. Diatloff & Brockwell, 1976; Moawad & Schmidt, 1987). For example, if nodules contain more than one rhizobia lineage, the efficiency of sanctions could be reduced (i.e. lower b) if they are applied at the nodule level, but less so if spatial segregation of lineages within mixed nodules (Hahn & Studer, 1986) allowed selective targeting of bacteria. Further theoretical work in this area would profit greatly from a detailed knowledge of rhizobia diversity within nodules and the mechanisms involved in sanctions.

We have made several simplifying assumptions with our models, and there are a number of ways in which it could be expanded. (1) We have assumed a static model, without different stages of plant growth, or stage (time) dependent variation in the N_2 fixation rate. Although this simplification provides an approximation, more subtle and state-dependent sanctions could be predicted by dynamic models (Mangel & Clark, 1988). For example, if rhizobia initially fixed N_2 at a high rate to avoid sanctions, but then reduced N_2 fixation rates, sanctions might be applied more gradually as N_2 fixation is reduced or stopped in different nodules. This might produce particularly interesting results if the N_2 fixed in nodules prior to the decision point for nodule senescence is not of negligible importance (as we assumed). (2) We suggest that perennials could have time-dependent strategies. If there is a chance that a perennial will be infected in future years by rhizobia that infected its own nodules in a previous year, then more severe sanctions might be expected in earlier years in order to increase the mean N_2 fixing rate (m) of the bacteria with which they will interact in later years. Similarly, more severe sanctions might be favoured in cases where the rhizobia emerging from a plant may infect mainly the offspring or relatives of that plant (although this could be partially negated by competition between related plants; Queller, 1994; Frank, 1998; Taylor & Irwin, 2000; West *et al.*, 2001, 2002a).

Mutualism stability

How does our sanction model relate to previous work? There is an enormous body of theory on the evolution of cooperation between nonrelatives and mutualisms, and a full discussion of this work is beyond the scope of this paper. However, we note five points. (1) In discussing the evolution of cooperation and mutualisms, Bull & Rice (1991) distinguished between 'partner-fidelity' models such as the iterated PD, and 'partner-choice' models. Our model is similar to 'partner-choice' models in that it involves an asymmetrical interaction and simultaneous interactions with multiple partners, but it differs because their definition of partner choice assumes the choice can be made before paying any cost (possible exploitation), which is not the case for rhizobia as there is a cost of nodule production. Furthermore, by assuming that N_2 fixation prior to any sanctions is a negligible fraction of the total, we have implicitly assumed that less-mutualistic rhizobia fix less N_2 from the start. If some rhizobia fix at high rates initially, but then reduce N_2 fixation as they begin hoarding plant resources (as discussed above), the situation would be even more different from that envisioned by Bull and Rice. Perhaps 'ongoing partner choice' would cover this situation. (2) Frank (1994, 1998) developed a very general model, in which the cooperation between species is favoured by a positive correlation in space between altruistic individuals of different species. Sanctions provide a way for producing this correlation. (3) Related policing/punishment models have also been developed by Frank (1995, 1996), and Clutton-Brock & Parker (1995), showing how policing or punishment of individuals can favour cooperative behaviours – a fundamental difference is that their models focus on interactions between members of the same species, who are in direct competition with each other for the same resources, whereas ours involves interactions between individuals of different species. (4) Murray (1985) developed a model showing how more mutualistic behaviour (reproductive constraint) could be favoured in fig wasps if fig trees preferentially aborted figs which contained few seeds. Although Murray's model was developed for the fig system, it is conceptually very similar to ours – for example, his parameter *l* corresponds to our α. (5) More generally, providing a single unifying theoretical framework for the stability of mutualisms, as has been done for other areas of social evolution (Frank, 1998), remains a major task (Herre *et al.*, 1999; van Baalen & Jansen, 2001).

We conclude with two general points. First, we have considered the evolution of legume (plant sanctions; this paper) and rhizobia (N_2 fixing; West *et al.*, 2002b) strategies separately, but these models suggest how they could coevolve. Here we have shown that less mutualistic rhizobia (lower $\overline{m}$) select for more severe sanctions (higher *s*). We have previously shown that more severe sanctions select for more mutualistic rhizobia (West *et al.*, 2002b). Together these two factors have the capacity to stabilize mutualism, providing an alternative mechanism to the requirements for limited dispersal and high symbiont relatedness. More general coevolutionary models that examined both partners simultaneously would be very useful, although this could be considerably more complicated. Secondly, our models show how the influence of a single factor can have multiple, possibly conflicting, influences on the evolution of cooperation and mutualisms. For example, high numbers of rhizobia lineages infecting each plant can: (a) favour less mutualistic rhizobia and (b) make sanctions more effective (in the extreme, if only one lineage infects each plant then sanctions could have no influence on the mean rate of N_2 fixation, $\overline{m}$). The relative importance of these influences may vary, and play crucial roles at different stages of evolution and maintenance of mutualism. For example, we speculate that the mutualism initially evolved in conditions where low numbers of rhizobia lineages infected each plant, and so N_2 fixing was favoured because it increased plant growth and therefore the resources available to rhizobia (kin selection between rhizobia in the plant – Model I in West *et al.*, 2002b). Then as mutualism evolved, the number of lineages infecting each plant increased, favouring lower levels of N_2 fixing, but also opening up greater selection for plant sanctions, which can favour high levels of N_2 fixing even with high rhizobia lineage diversity per plant (Model II of West *et al.*, 2002). Analogous suggestions have been made for the role of different mechanisms at different stages of evolution and maintenance of cooperative breeding (i.e. mutualism within species; Clutton-Brock *et al.*, 2001; Kokko *et al.*, 2001; Griffin & West, 2002).

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Appendix

Proof of result 2

We assume that selection favours a certain investment r^* into nodules. In other words, the right-hand side of (5a) equals zero. Then

$$r^* = \frac{1}{2}\frac{\overline{m}(1-s) - p(1-\alpha s)}{\overline{m}(1-s)(1-\alpha s)}. \qquad (A1)$$

Selection favours sanctions if the right-hand side of (5b) is larger than zero when s equals zero. This gives us

$$\alpha p + \alpha r^* \overline{m} + (1-r^*)(\overline{m}' - \overline{m}) > 0. \qquad (A2)$$

To evaluate this, we need an expression for the derivative $\overline{m}'$. Let rhizobial fixation rates x be distributed according to an arbitrary smooth distribution $q(x)$ with minimum value μ, i.e. $\int_\mu^\infty q(x)dx = 1$. Plants have a sanction rule y such that the proportion $s = s(y)$ of nodules terminated is given by

$$s(y) = 1 - \int_{\mu+y}^{\infty} q(x)dx. \qquad (A3)$$

Given y, the mean fixation rate is

$$\overline{m} = \frac{\int_{\mu+y}^{\infty} xq(x)dx}{\int_{\mu+y}^{\infty} q(x)dx}. \qquad (A4)$$

Now, $d\overline{m}/ds = (d\overline{m}/dy)/(ds/dy)$, which gives, applying the fundamental theorem of calculus several times,

$$\frac{d\overline{m}}{ds}\Big|_{s=y=0} = \overline{m} - \mu. \qquad (A5)$$

Plugging the right-hand side of this equation together with (A1) into inequality (A2) yields the inequality of Result 2.