Thanks to mutualisms, many species acquire new biological functions, extend their ecological range, and generally increase their fitness. However, each partner has to allocate resources towards its own reproduction and the service it provides to the other. Therefore, even within mutualistic interactions, conflicts of interest can arise from resource allocation trade-offs and lead to mutualism breakdown (Sachs & Simms, 2006). Stabilizing mechanisms are required to prevent such breakdown (Herre et al., 1999). In this issue of *New Phytologist*, Friesen (pp. 1096–1111) focuses on the symbiosis involving leguminous plants and nitrogen-fixing bacteria called rhizobia that develop in their root nodules. This symbiosis implies large costs for both partners (nitrogen fixation for rhizobia and nodule organogenesis for legumes) but also large benefits: rhizobia can expect a large fitness output (Denison & Kiers, 2011) and legumes gain access to an exclusive source of nitrogen. Friesen took up the challenge of investigating the quantitative relationships between these costs and benefits and questioned the existence of conflicts of interest in this mutualism.

‘Undoubtedly, estimating fitness is critical for addressing conflicts of interest but fitness is intrinsically difficult to estimate.’

Conflicts of interests

Some properties of the legume–rhizobium symbiosis point to the existence of conflicts. Rhizobia are horizontally transmitted, preventing stabilization of the mutualism through partner fidelity feedback (Sachs et al., 2004). In vertically-transmitted mutualisms, the offspring of both partners is correlated, causing a direct advantage for the symbiont to increase its host’s fitness, a mechanism that cannot apply to legumes and rhizobia. Besides, empirical data show variation in effectiveness in nitrogen fixation among rhizobial strains (Burdon et al., 1999). For example, farmers can be confronted with the issue of highly competitive but poorly effective strains that replace inoculated strains in their crop’s nodules (Kosslak et al., 1983). Furthermore, cheaters are hypothesized rhizobial genotypes taking advantage of the mutualism while failing to fix nitrogen at a sufficient rate and therefore maximizing their own fitness at the expense of their host – and at the expense of cooperative rhizobia.

Friesen observed that conflicts of interest imply a negative correlation between host and symbiont fitness and noted that agronomical and ecological literature abounds with studies investigating the performance of different strains in competition assays or cross-inoculation experiments. The aims of these studies differ: functional genetics using mutants (e.g. Lagares et al., 1992), co-evolutionary mechanisms (e.g. Heath, 2010), or agronomical management (e.g. Rodriguez-Navarro et al., 1999). Taken together, they offer an opportunity for a meta-analysis spanning decades of research on the legume–rhizobium interaction and covering many different host–symbiont couples.

Estimating legume and rhizobium fitness

Undoubtedly, estimating fitness is critical for addressing conflicts of interest but fitness is intrinsically difficult to estimate. Plants such as legumes, at least in the case of annual species, exhibit convenient characters that can be used as fitness proxies, such as seed yield and above-ground biomass. By contrast, rhizobia are much less convenient organisms when evaluating fitness. The number of cells emerging from nodules is difficult to measure, subject to errors and not available on a large scale. Furthermore, rhizobia could extend their inclusive fitness through free-living kin feeding on root exudates outside nodules (Oliviéri & Frank, 1994; Bever & Simms, 2000). Usual proxies for rhizobial fitness are nodule number, total nodule biomass, and competitive success such as relative nodule occupancy – measures that are all indirect and might not reflect directly the actual number of rhizobia that will emerge from nodules. Real rhizobial success also depends on differentiation and reproductive strategy within nodules (Ono et al., 2009) and relative investment towards energy storage rather than nitrogen fixation, leading to a potential discrepancy between nodule occupancy and contribution to the next generation (Ratcliff et al., 2011). The challenge for the meta-analysis of Friesen was to overcome the diversity and limitations of fitness estimates that can blur the signal of fitness correlations.

Alignment of legume and rhizobial fitness

Of course, Friesen found much variation among studies, but the overall picture is of a fitness alignment: the more rhizobia fix nitrogen, the more they are competitive and derive fitness output. This observation suggests that mechanisms coupling legume and
rhizobial fitness dominate over the pressure of selection for withholding resources invested in symbiosis. Friesen highlighted genetic variance for rhizobial effectiveness and competitiveness, pointing to an evolutionary potential for improving the performance of the mutualism, to the benefit of both partners.

How does coupling work

Friesen proposes several molecular mechanisms to explain why legume and rhizobial fitness are correlated, but I will focus on the hypothesis of host sanctions (Denison, 2000) and show that it provides an explanation for fitness alignment but also for the evolution of specificity in the legume–rhizobium symbiosis, while still allowing for the expression of conflicts.

Fig. 1 presents a model for fitness alignment that can be viewed as a simplified version of Fig. 1 in Friesen’s article, and is based on a schematic representation of processes contributing to legume and rhizobial fitness. Naturally, carbon fixation (i.e. photosynthesis) fuels plant metabolism. However, rhizobium-provided nitrogen provides a major contribution whenever symbiosis is successful. In exchange, the plant provides energy to rhizobia (sugar transfer) but also by supporting nodule growth. For rhizobia the only benefits of nitrogen fixation come from this reward. However, rhizobia can adopt a saprophytic behavior and grow as free-living organisms in the soil or feed on root exudates.

Legumes must pay first: the growth of the nodule and the rhizobial population that it contains is at the host’s expense. Few rhizobial cells (potentially, one) infect nodules, and they do not bring enough resources to initiate nitrogen fixation. However, the system of host sanctions ensures that rhizobia cannot escape from nodules and take advantage of plant-provided sugar without providing nitrogen in return. Therefore, there is a closed loop that forces both partners to cooperate in order to harvest benefits.

Does it mean that there is no conflict of interest?

Natural situations are more complex than conceptual models and laboratory experiments. Conflicts of interest can be resolved by mechanisms such as sanctions, but still occur in the field. First, the sanction system is applied at the nodule level, allowing less profitable rhizobia to hitchhike good cooperators through co-infections. Second, nodule senescence does not guarantee that the most frequent rhizobial strain in nodules will contribute most to the next generation. Less cooperative strains could stop fixing nitrogen and divide or store sugar earlier. These sources of fitness might not be easy to detect using widely available fitness estimates.

The results presented in this issue by Friesen show that fitness-alignment mechanisms dominate conflicts of interest at a broad scale. But consider co-adaptation. Symbiotic nitrogen fixation is a physiologically complex process and implies an intimate interaction between partners, coordinated gene expression on both sides, and co-adaptation. Focusing on a couple of co-adapted partners would remove the variation caused by non-optimal combinations and allow remaining conflicts of interest to be revealed. Furthermore, it is reasonable to expect that cheaters are adapted to a given host. The negative correlation between host and symbiont fitness observed in the couple *Medicago truncatula*–*Sinorhizobium meliloti* (Heath, 2010) supports this hypothesis.

Evolutionary consequences of fitness alignment

Friesen notes that fitness alignment mechanically selects for an improvement of the performance of symbiosis as a whole, to the benefit of both partners. The legume–rhizobium symbiosis is indeed known for its high yields of nitrogen fixation. As mentioned in the previous paragraph, this performance improvement is likely associated with co-adaptation. Interestingly, this could also explain the high levels of specificity between legumes and rhizobia. Both partners express multiple signaling systems to enforce specificity and partner choice (Wang et al., 2012). Furthermore, both partners release signals and receptors, showing that they both have an interest in specificity. If hosts and symbionts are co-adapted, and incompatible interactions are detrimental to both, then it is logical to expect that strict specificity will be selected on both sides.

These two mechanisms – functional co-adaptation and specificity – can also reinforce dynamic evolution caused by biogeographic or climatic factors, by competition of different rhizobia for a given host or by host/symbiont captures. In addition, both legumes and rhizobia could switch between generalist and specialist strategies over time. Finally, there can be a selective pressure on both hosts and cooperative symbionts to evade cheaters and potential parasites of the mutualism hijacking signaling mechanisms in order to enter nodules, causing a runaway process promoting fast evolution and diversification.
Conclusion

Fitness-coupling mechanisms play an essential role in shaping the legume–rhizobia symbiosis and its patterns of evolution. These mechanisms contribute to stabilizing the symbiosis as a whole by preventing the success of cheaters and the ultimate breakdown of the mutualism. Furthermore, they promote evolution towards a more co-adapted, more efficient and possibly more dynamically-evolving symbiosis.

Acknowledgements

Thanks to Mathieu Siol for his comments on the draft and useful suggestions.

Stéphane De Mita
INRA Nancy, 54280 Champenoux, France (tel +33 383394052; email sdemita@nancy.inra.fr)

References


Key words: co-evolution, conflicts, cooperation, legume, rhizobium, sanctions, specificity.