

NEWS AND VIEWS**Perspective**

A window into the transcriptomic basis of genotype-by-genotype interactions in the legume–rhizobia mutualism

Corlett W. Wood¹  | John R. Stinchcombe^{1,2} ¹Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada²Koffler Scientific Reserve, University of Toronto, Toronto, ON, Canada**Correspondence**

Corlett W. Wood, Department of Ecology & Evolutionary Biology, University of Toronto, Toronto, ON, Canada.

Email: corlett.wood@utoronto.ca

The maintenance of genetic variation in the benefits provided by mutualists is an evolutionary puzzle (Heath & Stinchcombe, 2014). Over time, natural selection should favour the benefit strategy that confers the highest fitness, eroding genetic variation in partner quality. Yet abundant genetic variation in partner quality exists in many systems (Heath & Stinchcombe, 2014). One possible resolution to this puzzle is that the genetic identity of both a host and its partner affects the benefits each mutualist provides to the other, a pattern known as a genotype-by-genotype interaction (Figure 1). Mounting evidence suggests that genotype-by-genotype interactions between partners are pervasive at the phenotypic level (Barrett, Zee, Bever, Miller, & Thrall, 2016; Heath, 2010; Hoeksema & Thompson, 2007). Ultimately, however, to link these phenotypic patterns to the maintenance of genetic variation in mutualisms we need to answer two questions: How much variation in mutualism phenotypes is attributable to genotype-by-genotype interactions, and what mutualistic functions are influenced by each partner and by the interaction between their genomes? In this issue of *Molecular Ecology*, Burghardt et al. (2017) use transcriptomics to address both questions in the legume–rhizobia mutualism.

KEYWORDS

adaptation, coevolution, ecological genetics, species interactions

In the legume–rhizobia mutualism, bacteria (rhizobia) provide their plant host with nitrogen in exchange for the products of photosynthesis. There is substantial variation in partner quality, some of which is due to genotype-by-genotype interactions between plants and rhizobia (Heath, 2010). To explore the mechanisms underlying plant genotype-specific responses to rhizobia, Burghardt et al. (2017) grew four genotypes of the model legume *Medicago truncatula* (Figure 2a) with two rhizobia species and sequenced the transcriptomes of root nodules (the symbiotic organ in which plants house rhizobia; Figure 2b) for all eight plant–rhizobia combinations. Their study substantively extends previous work in the same system (Heath, Burke, & Stinchcombe, 2012) using genetically diverged symbiont strains

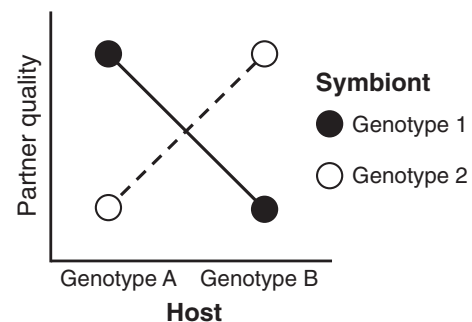


FIGURE 1 A genotype-by-genotype interaction for partner quality between two hypothetical mutualists. In this example, partner quality depends on the specific combination of host and symbiont genotype. Host genotype A provides a higher benefit to symbiont genotype 1 than to symbiont genotype 2, while the opposite is true for host genotype B

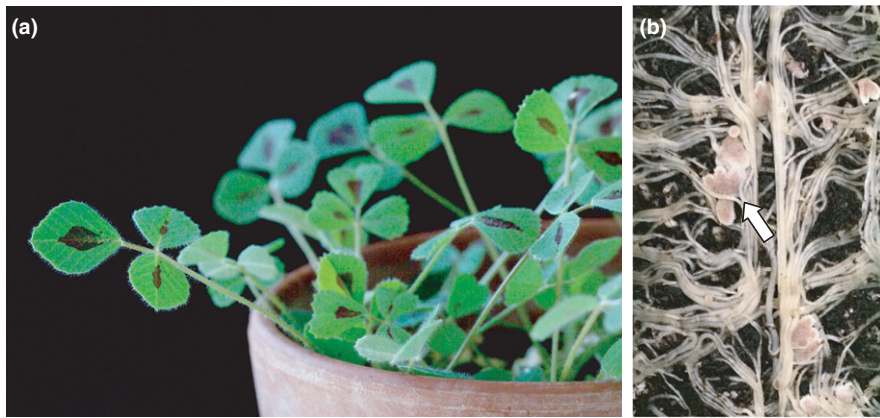


FIGURE 2 (a) The barrelclover *Medicago truncatula*. (b) Nodules housing rhizobia on *M. truncatula* roots. The nodules are the pinkish structures, one of which is indicated by the white arrow

(species) and surveying a substantially larger fraction of the transcriptome.

The decreasing cost of next-generation sequencing offers the potential to study the molecular phenotypes associated with hard-to-observe but ecologically and potentially economically important interactions. Especially in microbial symbioses like the legume–rhizobia mutualism, gene expression offers a wealth of functional information about an interaction that is microscopic, hard to observe and challenging to quantify with traditionally measured phenotypes (e.g., biomass, phenology). For example, Burghardt et al. (2017) showed that the bacterial strain that formed more nodules consistently lead to lower vegetative biomass, a potential correlate of fitness. What are the biological mechanisms that might underlie such a pattern? Is this pattern due to the rhizobia (increased nodulation), the plant (decreased regulation of nodule numbers) or the interaction between the two?

Burghardt et al. (2017) showed that genetic context is an important source of variation in this mutualism. Nearly 70% of *Medicago* genes expressed in nodules differed in expression among plant genotypes, and 27% differed in expression between rhizobia species. Strikingly, plant-by-rhizobia interactions accounted for a substantial fraction of variation in gene expression as well. The effect of plant genotype on gene expression depended on the identity of its rhizobia partner for 21% of the genes expressed in nodules. (The three categories do not sum to 100% because both plants and rhizobia additively affected the expression of some genes). The extensive plant-by-rhizobia interactions for gene expression suggest that genotype-by-genotype interactions may play a major role in maintaining genetic variation in the legume–rhizobia mutualism.

One fascinating aspect of this study was the authors' functional enrichment analysis of each category of differentially expressed genes (i.e., genes whose expression was affected by plant genotype, rhizobia species or a plant-by-rhizobia interaction). Burghardt et al. (2017) reasoned that enrichment analyses could reveal the mutualistic functions that depend on each partner because genes whose expression was affected by plant genotype should be enriched for functions that are influenced by the plant, while genes whose expression was affected by the interaction between plant and rhizobia genotypes ($G \times G$ genes) should be enriched for functions that

depend jointly on both partners. Intriguingly, genes with a plant-by-rhizobia interaction for expression were enriched for processes involved in nitrogen and carbohydrate exchange. One gene in this category, glutamine synthetase, catalyses the first step required for plants to assimilate nitrogen. The enrichment of malate synthesis, involved in delivering energy to bacteria in nodules, implicates the main carbon reward as well. Genotype-by-genotype interactions therefore shape the central resource-exchange functions of the legume–rhizobia mutualism.

Another aspect of Burghardt et al.'s (2017) functional enrichment analysis is particularly noteworthy. They performed this analysis in a clever way: they calculated functional enrichment relative to genes expressed in the nodules, rather than relative to all genes in the genome. Using gene expression in this symbiotic structure as a baseline, they extracted an informative set of candidate genes and pathways that are directly relevant to the biological questions they asked. The mutualism between legumes and rhizobia is well suited to this approach because plant–rhizobia interactions localize to discrete organs. Future studies in other systems should adopt Burghardt et al.'s (2017) approach whenever possible, especially where the relevant ecological interactions localize to a specific organ or tissue.

Burghardt et al.'s (2017) study joins a growing body of literature illustrating how transcriptomics advance our understanding of the evolutionary ecology and evolutionary genetics of species interactions (Afkhani & Stinchcombe, 2016; Klinger, Lau, Heath, & Heath, 2016). The pervasive plant, rhizobia and plant-by-rhizobia interactions for gene expression make a strong argument for sampling naturally occurring genetic variation rather than focusing on a single model genotype. It is clearly important to integrate across genetic contexts to understand patterns of gene expression in ecologically relevant systems. The functional enrichment analyses were a clever extension of their study. These analyses yielded a window into the physiological underpinnings of the mutualism, shedding light on the complementary roles each partner played in the interaction.

Finally, Burghardt et al.'s (2017) results suggest that a broader perspective on mutualism might fundamentally change our understanding of its evolutionary dynamics. Two of their many intriguing results stood out to us as raising new questions about the evolution of mutualisms. First, their functional enrichment analyses revealed

that genotype-by-genotype interactions shape the primary function of the legume–rhizobia mutualism. Is this a general feature of all mutualisms? Are genes with genotype-by-genotype interactions for expression functionally enriched for resource exchange in resource mutualisms, but enriched for genes that mediate interactions with defensive symbionts in defensive mutualisms? Can functional enrichment analyses illuminate whether mutualism genes also regulate other biological functions, and if so, what auxiliary functions they perform? Characterizing the pleiotropic effects of mutualism genes could provide a window into the direct and indirect selection pressures shaping mutualisms. Burghardt et al.'s (2017) study and future ones like it offer a way forward using transcriptomes to study the underlying biology of interspecific interactions, beyond generating a list of GO categories.

Second, Burghardt et al. (2017) found that genes that differed in expression among plant genotypes were enriched for genes involved in pathogenesis and defence. One explanation for this pattern is many genes were originally annotated in plants that do not form rhizobial mutualism (e.g., *Arabidopsis*). Burghardt et al.'s (2017) results are consistent with past work showing that these defence genes function more broadly than their original annotations suggest, and also play a role in mediating positive species interactions (Zamioudis & Pieterse, 2012). The enrichment of defence genes raises the exciting possibility that the mechanisms mediating plant responses to mutualists overlap with the defence response in *Medicago* and other mutualistic species. Overlap between the genetic pathways that regulate mutualistic and pathogenic interactions could create fitness trade-offs if genotypes that are effective at establishing mutualisms are more vulnerable to infection. Any fitness trade-offs due to mediating multiple interspecific interactions could, of course, contribute to maintaining genetic variation in mutualist quality. A multispecies perspective that integrates host responses to multiple species that span the continuum from mutualism to parasitism may therefore be crucial to understanding the processes maintaining genetic variation in mutualisms.

ORCID

Corlett W. Wood  <http://orcid.org/0000-0002-7083-062X>

John R. Stinchcombe  <http://orcid.org/0000-0003-3349-2964>

REFERENCES

- Afkhami, M. E., & Stinchcombe, J. R. (2016). Multiple mutualist effects on genomewide expression in the tripartite association between *Medicago truncatula*, nitrogen-fixing bacteria and mycorrhizal fungi. *Molecular Ecology*, 25, 4946–4962.
- Barrett, L. G., Zee, P. C., Bever, J. D., Miller, J. T., & Thrall, P. H. (2016). Evolutionary history shapes patterns of mutualistic benefit in Acacia–rhizobial interactions. *Evolution*, 70, 1473–1485.
- Heath, K. D. (2010). Intergenomic epistasis and coevolutionary constraint in plants and rhizobia. *Evolution*, 64, 1446–1458.
- Heath, K. D., Burke, P. V., & Stinchcombe, J. R. (2012). Coevolutionary genetic variation in the legume–rhizobium transcriptome. *Molecular Ecology*, 21, 4735–4747.
- Heath, K. D., & Stinchcombe, J. R. (2014). Explaining mutualism variation: A new evolutionary paradox? *Evolution*, 68, 309–317.
- Hoeksema, J. D., & Thompson, J. N. (2007). Geographic structure in a widespread plant–mycorrhizal interaction: Pines and false truffles. *Journal of Evolutionary Biology*, 20, 1148–1163.
- Klinger, C. R., Lau, J. A., Heath, K. D., & Heath, K. D. (2016). Ecological genomics of mutualism decline in nitrogen-fixing bacteria. *Proceedings of the Royal Society*, 283, 20152563.
- Zamioudis, C., & Pieterse, C. M. J. (2012). Modulation of host immunity by beneficial microbes. *Molecular Plant–Microbe Interactions*, 25, 139–150.

How to cite this article: Wood CW, Stinchcombe JR.

A window into the transcriptomic basis of genotype-by-genotype interactions in the legume–rhizobia mutualism. *Mol Ecol*. 2017;26:5869–5871. <https://doi.org/10.1111/mec.14370>