

## ON THE NATURE OF THINGS: ESSAYS

*New Ideas and Directions in Botany*

# Here come the commensals<sup>1</sup>

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Metagenomic approaches to understanding microbial symbiont communities, the microbiome, have provided an unprecedented window into the diversity of microbial organisms associated with virtually all eukaryotic hosts. However, our ignorance of what these symbionts *do* has been illuminated as well. Nowhere is the contrast between what we know, and what we do not know, more starkly realized than in the Kingdom Fungi with descriptions of less than 5% of the estimated 1.5 to 5 million taxa (Blackwell, 2011). While there is delight in discovery, and fortitude in the face of the cryptic nature of fungal symbioses, understanding the diversity of ecological functions for fungal symbionts remains a formidable challenge. Here, I use endophytic fungi to draw attention to the particular conundrum of commensal symbionts, symbionts that seem to do little but likely make up most of the microbial communities occupying plant and animal hosts. Endophytic fungi are those symbionts living within healthy living tissues of plants (Wilson, 1995) and thus, in terms of their interactions with the host, are best considered commensals. H. Anton de Bary in 1878 put commensals squarely in the middle of the continuum of symbioses between beneficial mutualists and detrimental parasites because commensals have few apparent direct *effects on their hosts* (translation by Oulhen et al., 2016). Others have argued against typecasting commensal symbioses as those in which nothing happens (Hirsh and Fujishige, 2008) or those for which we do not know what happens (Zapalski, 2011). Here, I advocate for the perspective that the commensal symbiotic habit is critical to study because commensalism enables the evolution and maintenance of tremendous diversity in ecological functions.

## THEORY AND CONCEPT

The pervasive assumption that microbiome symbionts, including endophytic fungi, must benefit the host echoes the “balance of nature” perspectives found in early theoretical and conceptual models

for parasites and for microbiomes. For parasites, virulence was thought maladaptive because the host on which the microbe depends is damaged and evolution toward low virulence states was predicted (reviewed by Ewald, 1983). For the human microbiome, Henderson and Wilson (1998, p. 1680) posited that diverse microbial communities “evolved cytokine-modulating molecules to live in harmony with host mucosal surfaces” and envisioned the microbiome as a communistic collective. In contrast, modern theory predicts that competition for host resources among multiple infecting symbionts should drive the evolution of greater virulence toward the host (reviewed by Alizon et al., 2013). Limits to virulence arise from a trade-off between parasite reproduction and parasite-induced host mortality (Anderson and May, 1982). Still, with the explosion of research on the microbiome, the concept that the “holobiont” (host plus microbes) evolves as a superorganism has been strongly forwarded (Bordenstein and Theis, 2015) and actively debated (Moran and Sloan, 2015). One way out of this conceptual mess may come from results that suggest a very dynamic nature of microbiome communities (Burns et al., 2016) with relatively few taxa comprising a “core microbiome” (Douglas and Werren, 2016). Employing evolutionary models that bring in “third party” interactions, we might then predict that the ever-changing landscape of microbial symbionts drives the evolution of traits such as antagonism against microbial competitors. Further, if ecological outcomes are conditional on population history and the identity of interacting species, as seems to be the case for endophytic fungi (Busby et al., 2016b), tremendous variation in traits that affect these interactions can be maintained species-wide (Bronstein, 1994; Gomulkiewicz et al., 2003). For now, we might best assume that most symbiotic species of the microbiome muddle along, pulled in different directions by the ever-changing ecological context set up by the nature of the commensal habit. Occasionally, but importantly, commensals “escape” and evolve more extreme symbiotic modes toward the host—mutualism and parasitism.

## WHY COMMENSAL SYMBIONTS ARE INTERESTING TO STUDY

Although somewhat hampered by an apparent publication bias toward reporting beneficial effects, a recent review on endophytic fungi suggests that these symbionts harbor extensive variation in

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ecological functions, including facilitation, antagonism, and neutral effects on plant parasites (Busby et al., 2016b). To think about how such variation evolves while the commensal state is stable, consider first the results of a key paper that used using ancestral state reconstruction to trace the evolution of trophic modes in ascomycete fungi (Arnold et al., 2009). One of the most interesting results of this work is the stability and prevalence of the endophytic and endolichenic (occupying lichens) trophic modes across the phylogeny. Of the relatively few transitions accounted, shifts between parasitic and endophytic/endolichenic states are most frequent, results echoing the early predictions of Carroll (1988). What then of the mutualistic functions for endophytes so often assumed in the literature? When we focus on traits for interactions with the enemies of plants, we find several lines of evidence suggesting that the endophytic fungi actually vary considerably in their effects on plant parasites and that these effects depend on the ecological context. For example, results for well-studied taxa such as *Fusarium* species have demonstrated variation within species and among closely related species in their effects on plant hosts, including commensalism, parasitism, defensive mutualism, and parasite facilitation (e.g., Kaldau and Yates, 2000). Recent experimental ecological studies reveal variation within and between endophytic taxa for antagonistic and facilitative effects on a pathogen, and surprisingly, many endophytic strains have no statistically discernable phenotype (Fig. 1; Busby et al., 2016a). While endophytic fungi provide novel sources of disease control for agriculture (Ledford, 2015), the emerging picture is that endophytic fungi are commensal with respect to direct effects on their host, while harboring tremendous genetic variation for ecological functions such as interactions with other symbionts.

## FUTURE STUDIES AND APPROACHES

To understand the evolution and maintenance of variation for symbiont interaction traits, we might productively consider an axis of symbiont function that is distinct from the traditional continuum from parasite to mutualist. Perhaps Henderson and Wilson

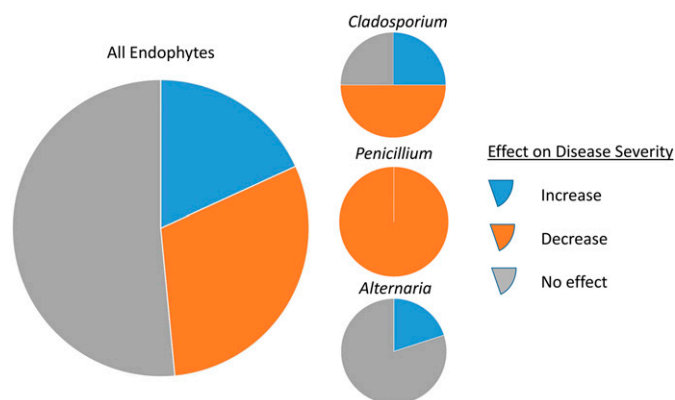
(1998) had it right in their emphasis on the importance of community ecological context—the direct and indirect interactions among the ever-changing members of the symbiont community are a strong force in the maintenance of the commensal state and of genetic variation within populations for interactions among symbionts (May and Nelson, 2014). How might we move forward to address these questions? The dizzying array of -omic tools has allowed extraordinary assessment of what is there, and of their genomic functions, but also raised a thicket of data that may actually obscure our view of the evolutionary process. For example, while the number of sequence reads on tiny bits of the genome is broadly acknowledged as a poor estimator of a species' abundance, no other measures using metagenomics data alone are available. Rather than being consumed with the task of building bioinformatics pipelines to nowhere, we join with Peay (2014) in calling for research addressing the basic biology of organisms. In particular, reproduction is fundamental to both ecological and evolutionary models. However, sometimes we do not even know *where* symbiotic organisms reproduce and for cryptic microorganisms, life history traits can be difficult, but not impossible to estimate (Bruns et al., 2014). There is hope—many fungi are culturable with effort, and there is increasing interest in maintaining microbial culture collections as a basis for experimentation and taxonomic description (Bai et al., 2015). Consequently, the door is opening for experimental study of interactions among diverse organisms that can provide quantitative results for phenotypic variation in ecological and symbiotic functions (Busby et al., 2016a). Together with demographic and population genetic studies (Oono et al., 2014), such studies will illuminate evolutionary processes generating and maintaining variation for interactions among symbionts and between symbionts and their hosts.

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**FIGURE 1** Fungi isolated as endophytes demonstrate varying effects on the disease severity caused by a fungal rust pathogen in poplar. Approximately half of the endophytes have no apparent effect (gray), a quarter increase disease severity (facilitation, blue), and a quarter decrease severity (antagonism, orange). Isolates within individual genera vary in their effects as illustrated here for *Cladosporium*, *Alternaria*, and *Penicillium* (adapted from results in fig. 2 of Busby et al., 2016a).

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