

## Searching for Evidence against the Mutualistic Nature of Hereditary Symbioses: A Comment on Faeth

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### Introduction

Hereditary symbioses occur when symbionts are transmitted vertically from hosts to their offspring. Given their strong ecological effects and their roles in major evolutionary transitions (Rudgers et al. 2007; Douglas 2008; Haine 2008), understanding their dynamics has attracted substantial interest. Here we critique a recent publication in the *American Naturalist* (Faeth 2009) that raises general issues in the study of symbiosis. Specifically, we address three questions: (1) What are the appropriate measures of the fitness consequences of symbiosis for the host? (2) How should benefits to hosts be assessed in symbioses that confer protection against natural enemies? (3) What are the appropriate measures of the fitness consequences of symbiosis for the symbiont?

Associations between class 1 fungal endophytes (sensu Rodriguez et al. 2009) and grasses have served as particularly useful systems for exploring the nature and significance of hereditary symbioses. Class 1 endophytes comprise a group of related fungal species in the family Clavicipitaceae that grow intercellularly in aboveground plant tissues. Clay (1988) proposed that many associations between grasses and fungal endophytes represent protection mutualisms in which the endophytes produce physiologically active alkaloids that defend host plants against herbivory. Since then, substantial evidence has accumulated that shows endophyte-mediated increases in host plant growth, fecundity, and resistance to herbivores and other stresses, most notably in agronomic grass species

such as tall fescue and perennial ryegrass (Clay and Schardl 2002; Müller and Krauss 2005 and references therein).

However, there is a long-standing debate over whether endophyte benefits in agronomic hosts are the consequences of artificial selection or similar benefits accrue in the wild (Saikkonen et al. 2006). A cornerstone of this controversy has been comparisons between agronomic grasses (particularly tall fescue, *Lolium arundinaceum*) and a single wild host, Arizona fescue (*Festuca arizonica*), for which the benefits of endophytes have not been obvious and costs are sometimes reported (e.g., Saikkonen et al. 1999; Faeth et al. 2004). A recent contribution (Faeth 2009) prompted us to consider how evidence on host-symbiont dynamics should be interpreted. In particular, Faeth (2009) proposed that endophyte symbiosis in Arizona fescue represents a case of reproductive parasitism, whereby the endophyte gains fitness benefits by inducing early host plant reproduction and thereby imposes a potential cost on lifetime host fitness. Here we present an alternative, testable framework for interpreting recent data and informing future research.

Hereditary symbiosis can lead to mutualism through partner fidelity feedback, which is created when an extended series of exchanges directly links the fitness of two species (Ewald 1987; Sachs et al. 2004). Partner fidelity feedback can be an important mechanism to generate and stabilize mutualism because there are direct, negative fitness consequences for species that fail to cooperate (Foster and Wenseleers 2006). In hereditary symbioses that are exclusively vertically transmitted from parent to offspring, detrimental symbionts should be quickly lost from host populations because of the lack of other mechanisms for symbiont transmission (i.e., there is no contagious spread). Gundel et al. (2008) showed that a net positive effect of symbiosis is required for the local persistence of a symbiont but that this effect can be very small (and difficult to measure) if rates of vertical transmission are high. Saikkonen et al. (2002) proposed that a symbiont with a net

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negative effect on its host can persist locally, provided that there is migration from surrounding populations in which the symbiont is beneficial. Thus, the persistence of strictly vertically transmitted symbionts, such as some class 1 endophytes, seems paradoxical if the symbionts reduce host fitness.

However, within hereditary symbioses, partner fidelity feedbacks can be decoupled, leading to conflict between partners. Most notably, uniparental transmission of the symbiont (typically, maternal inheritance) can lead to reproductive parasitism, in which the symbiont alters the host sex ratio through feminization, cytoplasmic incompatibility, male killing, or parthenogenesis. Uniparental transmission occurs commonly in hereditary symbioses, and reproductive parasitism has been especially well documented for *Wolbachia*, bacterial symbionts in many invertebrates (Werren et al. 2008).

Faeth (2009) proposed another type of decoupling of host and symbiont fitness. He found significantly higher reproduction and growth of symbiotic Arizona fescue plants relative to experimentally disinfected controls. Reproductive effort (seeds/aboveground mass) was greater for symbiotic than for symbiont-free plants in the first two years but not significantly different in the final year. The seed output of symbiotic plants was also higher under ambient herbivory than under reduced herbivory (insecticide treatment), but only during the first year. From these results, Faeth concluded that endophytes shifted plant resource allocation toward earlier reproduction, generating benefits to the endophyte but potentially reducing lifetime plant fitness because of the costs of reproduction. Faeth's hypothesis has two key requirements: (1) there is a trade-off between host reproduction and host growth or survival, and (2) symbiont fitness increases independently of host fitness via earlier host reproduction. Neither was tested by Faeth (2009). We argue that in the absence of information on life-history trade-offs or symbiont fitness, proposing that the symbiosis is parasitic is premature and potentially misleading.

We appreciate the importance of Faeth's study for documentation of the ecological effects of hereditary symbiosis for a native grass. However, his conclusions overlook the most parsimonious explanation of the data, which is that the endophyte had a positive effect on plant performance during his study. In evaluating Faeth (2009), we raise three general issues broadly relevant to symbiosis research. First, we consider the fitness effects of symbionts on their hosts and ask what evidence would substantiate a symbiont-triggered shift to earlier host reproduction, consistent with Faeth's hypothesis of "reproductive parasitism." Second, we critique Faeth's methods for studying the mechanisms underlying symbioses that confer protection to the host. Finally, we ask what factors influence symbiont fitness, to

identify conditions under which a symbiont could benefit from shifting the timing of host reproduction.

#### What Are the Appropriate Measures of the Fitness Consequences of Symbiosis for the Host?

Understanding mechanisms that decouple host and symbiont fitness requires assessing the stage- (and sex-) specific effects of symbiosis on the host and connecting these measures to the rate of host population growth. Put simply, in a demographic context, the costs of symbiosis at one host life-history stage could be outweighed by strong benefits at a different stage. Furthermore, all stages do not contribute equally to the rate of host population growth. Thus, determining whether a symbiosis is mutualistic or parasitic cannot be accomplished with data on only a subset of host life-history stages. An accurate assessment of the fitness consequences of symbiosis requires combining experimental manipulation of symbiont presence with lifetime host demographic data.

Assessments of the performance of host stage classes must also separate host ontogeny from temporal variation in the environment. For example, a difference between seedlings measured in year 1 and year-old plants measured the following year could reflect host age or year-to-year variation in the environment. To evaluate Faeth's reproductive-parasitism hypothesis, multiple host stages must be compared during the same year (e.g., Roach 2003). Finally, care must be taken to record host performance under natural conditions and to avoid methods that could disrupt demographic pathways.

*Faeth (2009) results.* Differences in growth and seed production between symbiotic and symbiont-free Arizona fescue plants declined over three years. This temporal pattern was interpreted as evidence that the symbiotic endophyte had shifted plant allocation toward early reproduction, potentially at a cost to lifetime plant fitness: "Infection by the asexual *Neotyphodium* endophyte profoundly alters resource allocation in their native grass hosts over time. ... Infected plants also shift allocation to increased reproductive effort in early life stages" (Faeth 2009, p. 561). "If *Neotyphodium*-mediated shifts to early allocation of growth and reproduction increase endophyte fitness at the expense of reduced lifetime fitness of the host via trade-offs in other life-history parameters, then asexual endophytes may be reproductive parasites" (p. 563).

*Alternative hypotheses.* We suggest that there is more evidence for a mutualistic role for the endophyte in Arizona fescue than for a role as a reproductive parasite. Despite a decline in the magnitude of endophyte benefits through time, symbiosis never reduced plant growth or net reproduction, although Faeth gave no data on seed viability or germination, which are essential for quanti-

fyng host reproductive fitness. There was also no evidence for a trade-off between plant reproduction and plant survival. In fact, Faeth and Hamilton (2006) found no significant differences in the survival of symbiotic versus experimentally symbiont-disinfected seeds, seedlings, or adults or in the survival of symbiotic versus naturally symbiont-free adults surveyed over a 5–7-year period at two sites. Thus, there is no reason to assume that an endophyte-mediated increase in reproduction must come at a cost to plant survival, particularly when the growth of Arizona fescue was also increased by symbiosis. Even if early flowering reduced long-term plant survival, seed production by early progeny could more than make up for later reproduction by longer-lived parental plants (Charnov and Schaffer 1973; Bazzaz and Grace 1997). Furthermore, experimental methods, such as Faeth's harvest of all aboveground biomass at the end of each growing season, could introduce artifacts that disrupt the demographic transitions of perennial hosts and alter the effects of experimental treatments. In summary, while neither mutualism nor parasitism can be confirmed without lifetime demographic data, the evidence presented by Faeth (2009) is most consistent with the alternative hypothesis that the endophyte is a mutualist (3 years of positive effects on plant reproduction and growth; see fig. A1 in Faeth 2009).

In addition, host age and temporal environment were confounded in Faeth (2009); therefore, temporal changes in the benefits of endophyte symbiosis could have resulted from variation in environmental conditions rather than from variation in host ontogeny (see also Olejniczak and Lembicz 2007). Faeth (2009) documented considerable year-to-year variation in both herbivore abundance and water availability, two stresses against which endophytes have been shown to be protective. For example, insect herbivores were 8–10 times as abundant in the first two years as in the final year, and experimental water addition significantly improved plant performance only in the final year. Because biotic and abiotic factors alter the benefits of symbiosis (e.g., Müller and Krauss 2005), it is important to consider environmental changes, in addition to developmental changes, as an explanation for shifting costs and benefits. This alternative hypothesis was not considered by Faeth (2009). Whether weakened benefits of the endophyte later in the study indicate complex shifts in plant allocation (such as earlier flowering) or simple variation in the environment cannot be resolved with the data at hand. Ultimately, measuring different host stage (or age) classes during the same year and collecting data on stage-specific endophyte frequencies will better inform the prediction of life-history trade-offs. For example, in natural populations, are the oldest plants more likely to be symbiont-free than symbiotic?

### How Should Benefits to Hosts Be Assessed in Protection Symbioses?

Protection symbioses are context dependent because a third species (the host's enemy) or an environmental condition (e.g., drought stress) is required for the interaction to be mutualistic (see also Bronstein 1998; Rodriguez et al. 2008). A complete assessment of the symbiosis requires identifying the mechanisms through which benefits are conferred. For symbiont-mediated protection against host enemies, two potential mechanisms may contribute: symbiont-mediated resistance to host enemies (e.g., production of toxic metabolites) and symbiont-mediated tolerance to host enemies (e.g., enhanced host regrowth after damage). Resistance-based mechanisms have garnered the most attention (Haine 2008). In either case, inferring symbiont-mediated protection without data on damage caused by the enemy could produce misleading conclusions. Specifically, we argue that positive (or negative) correlations between enemy abundance and host performance are not sufficient evidence to demonstrate improved host tolerance (or resistance; see also Karban and Baldwin 1997). While quantifying damage to some host taxa, such as grasses, can be challenging, we think that it is a critical step toward documenting the mechanisms underlying protection symbioses.

*Faeth (2009) results.* In Arizona fescue, the magnitude of endophyte benefit to plant reproduction was five times as great under ambient arthropod herbivory as under experimentally reduced herbivory. However, the total abundance (and biomass) of herbivores was higher on symbiotic plants than on symbiont-free plants. Faeth's (2009) interpretation was that endophytes "increase reproductive effort and seed production under increased herbivore loads in early host life stages, which suggests endophyte-mediated tolerance or overcompensation of hosts to herbivory" (p. 561). He also inferred ontogenetic variation in resistance: "endophyte-mediated resistance to herbivores is also dynamic, similar to plant-based changes in resistance to herbivory ..., with decreased resistance during the early ontogenetic stages of rapid growth ... and then equivalent resistance in more mature plants" (p. 563).

*Alternative hypotheses.* An alternative hypothesis is that the enhanced performance of symbiotic plants under ambient herbivory (relative to that under reduced herbivory) reflected endophyte-mediated protection, as has been widely reported in other endophyte symbioses (Clay and Schardl 2002). Faeth's (2009) conclusion made the implicit assumption that herbivore abundance and biomass accurately measured herbivore damage. The evidence showed only a positive correlation between herbivore abundance and seed production: symbiotic plants had both more herbivores per plant volume and higher seed

production than symbiont-free plants. This pattern could arise if herbivorous taxa were attracted to vigorous, highly reproductive plants (e.g., thrips increasing with abundant pollen); it does not verify that endophytes increased herbivore damage, because net damage may not scale directly with herbivore biomass or abundance. For example, while thrips, mirids, and plant hoppers strongly increased on symbiotic plants, herbivore taxa with more significant effects on plant fitness (e.g., grasshoppers) may have declined. Large and highly mobile taxa, such as grasshoppers, can be undersampled by the vacuum collection methods used by Faeth (2009), and food webs could be altered—such that an increase in one group results from the decline of other groups, such as parasites or predators. In short, Faeth's (2009) data were insufficient to conclude that the endophyte conferred tolerance, overcompensation, or reduced resistance to herbivory.

#### What Are the Appropriate Measures of the Fitness Consequences of Symbiosis for the Symbiont?

Faeth's (2009) general hypothesis for reproductive parasitism relies on a decoupling of host and symbiont fitness, such that symbiont fitness increases if hosts reproduce earlier and die younger. Here, we evaluate what data would be required to assess symbiont fitness. Exclusively vertically transmitted symbionts reproduce by colonizing host offspring. Therefore, quantifying symbiont fitness requires measuring not only lifetime host reproduction but also the rate of symbiont transmission to the next generation. Symbiont transmission is an often-overlooked but essential component of symbiont fitness. A breakdown of partner fidelity feedback could occur if the rate of symbiont transmission varies during host ontogeny, for example, with a higher rate of transmission during younger host life stages relative to older stages. Thus, evaluating symbiont fitness necessitates measurements of transmission rates associated with each stage in the host life cycle (e.g., for plants: germination, establishment, growth, and reproduction) as well as host performance at each stage. Symbiont losses during transmission have long been recognized, and recent studies have documented significant variation in vertical transmission for class 1 endophytes in grasses (Afkhami and Rudgers 2008; Gundel et al. 2009). The most significant losses appear to occur during the seed stage rather than as adult plants age. However, data on ontogenetic variation in vertical transmission rates are sparse. Ultimately, models that incorporate empirically derived symbiont transmission rates will be useful for predicting symbiont fitness and symbiont frequency within host populations (e.g., Gundel et al. 2008).

*Faeth (2009) results.* Faeth presented no data on endophyte fitness. However, he concluded that endophyte-

mediated shifts in plant allocation were an adaptive strategy to increase endophyte fitness, without consideration of alternative hypotheses. "Increased reproductive effort in early host ontogeny should benefit the symbiont by increasing transmission" (Faeth 2009, p. 554). "Asexual and nonhybrid endophytes ... may indirectly promote herbivory that shifts resources to seed production, thus enhancing transmission of the endophyte by producing more seeds early in the life span of the host" (p. 563).

*Alternative hypotheses.* The hypothesis that endophyte fitness increases with early host reproduction remains unsupported, and alternatives are equally plausible. First, endophyte fitness could rise with delayed, rather than early, host reproduction if seeds produced by older plants were better provisioned or more highly infected. Second, both endophyte and host fitness could increase with early host reproduction (no decoupling of fitness). Third, endophyte fitness depends on transmission rate as well as on offspring quantity and quality. Transmission of the endophyte could vary with host plant age and a suite of biotic and abiotic factors, but this has not been documented for any endophyte system. Thus, we are a long way from having support for the assertion that endophyte fitness will increase with shifts to earlier host reproduction.

#### Conclusion

We posit that the most parsimonious conclusions from Faeth (2009) are (1) that endophyte symbiosis increases short-term plant growth and seed production in Arizona fescue and (2) that these benefits are stronger when herbivores are abundant. These results are consistent with data from other grasses whose mutualistic endophytes provide defense against herbivory (Clay and Schardl 2002; Müller and Krauss 2005). Asserting instead that the endophyte is a reproductive parasite requires implicit assumptions that remain unsupported by current data. More broadly, understanding the dynamic consequences of symbiosis for both the host and the symbiont will require longer-term demographic studies that incorporate correlations among life-history stages as well as symbiont transmission rates. Such work has the potential to yield greater insight into the ecology and evolution of hereditary symbiosis.

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### Literature Cited

- Afkhami, M. E., and J. A. Rudgers. 2008. Symbiosis lost: imperfect vertical transmission of fungal endophytes in grasses. *American Naturalist* 172:405–416.
- Bazzaz, F. A., and J. Grace, eds. 1997. *Plant resource allocation*. Academic Press, San Diego, CA.
- Bronstein, J. L. 1998. The contribution of ant-plant protection studies to our understanding of mutualism. *Biotropica* 30:150–161.
- Charnov, E. L., and W. M. Schaffer. 1973. Life-history consequences of natural selection: Cole's result revisited. *American Naturalist* 107:791–793.
- Clay, K. 1988. Fungal endophytes of grasses: a defensive mutualism between plants and fungi. *Ecology* 69:10–16.
- Clay, K., and C. Schardl. 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *American Naturalist* 160(suppl.):S99–S127.
- Douglas, A. E. 2008. Conflict, cheats and the persistence of symbioses. *New Phytologist* 177:849–858.
- Ewald, P. 1987. Transmission modes and evolution of the parasitism-mutualism continuum. *Annals of the New York Academy of Sciences* 503:295–306.
- Faeth, S. H. 2009. Asexual fungal symbionts alter reproductive allocation and herbivory over time in their native perennial grass hosts. *American Naturalist* 173:554–565.
- Faeth, S. H., and C. E. Hamilton. 2006. Does an asexual endophyte symbiont alter life stage and long-term survival in a perennial host grass? *Microbial Ecology* 52:748–755.
- Faeth, S. H., M. L. Helander, and K. T. Saikkonen. 2004. Asexual *Neotyphodium* endophytes in a native grass reduce competitive abilities. *Ecology Letters* 7:304–313.
- Foster, K. R., and T. Wenseleers. 2006. A general model for the evolution of mutualisms. *Journal of Evolutionary Biology* 19:1283–1293.
- Gundel, P. E., W. B. Batista, M. Teixeira, M. A. Martínez-Ghersa, M. Omacini, and C. M. Ghersa. 2008. *Neotyphodium* endophyte infection frequency in annual grass populations: relative importance of mutualism and transmission efficiency. *Proceedings of the Royal Society B: Biological Sciences* 275:897–905.
- Gundel, P. E., L. A. Garibaldi, P. M. Tognetti, R. Aragon, C. M. Ghersa, and M. Omacini. 2009. Imperfect vertical transmission of the endophyte *Neotyphodium* in exotic grasses in grasslands of the flooding pampa. *Microbial Ecology* 57:740–748.
- Haine, E. R. 2008. Symbiont-mediated protection. *Proceedings of the Royal Society B: Biological Sciences* 275:353–361.
- Karban, R., and I. T. Baldwin. 1997. *Induced responses to herbivory*. University of Chicago Press, Chicago.
- Müller, C. B., and J. Krauss. 2005. Symbiosis between grasses and asexual fungal endophytes. *Current Opinion in Plant Biology* 8:450–456.
- Olejniczak, P., and M. Lembic. 2007. Age-specific response of the grass *Puccinellia distans* to the presence of a fungal endophyte. *Oecologia* (Berlin) 152:485–494.
- Roach, D. A. 2003. Age-specific demography in *Plantago*: variation among cohorts in a natural plant population. *Ecology* 84:749–756.
- Rodriguez, R. J., J. Henson, E. Van Volkenburgh, M. Hoy, L. Wright, F. Beckwith, Y.-O. Kim, and R. S. Redman. 2008. Stress tolerance in plants via habitat-adapted symbiosis. *ISME Journal* 2:404–416.
- Rodriguez, R. J., J. F. White, A. E. Arnold, and R. S. Redman. 2009. Fungal endophytes: diversity and functional roles. *New Phytologist* 182:314–330.
- Rudgers, J. A., J. Holah, S. P. Orr, and K. Clay. 2007. Forest succession suppressed by an introduced plant-fungal symbiosis. *Ecology* 88:18–25.
- Sachs, J. L., U. G. Mueller, T. P. Wilcox, and J. J. Bull. 2004. The evolution of cooperation. *Quarterly Review of Biology* 79:135–160.
- Saikkonen, K., M. Helander, S. H. Faeth, F. Schulthess, and D. Wilson. 1999. Endophyte-grass-herbivore interactions: the case of *Neotyphodium* endophytes in Arizona fescue populations. *Oecologia* (Berlin) 121:411–420.
- Saikkonen, K., D. Ion, and M. Gyllenberg. 2002. The persistence of vertically transmitted fungi in grass metapopulations. *Proceedings of the Royal Society B: Biological Sciences* 269:1397–1403.
- Saikkonen, K., P. Lehtonen, M. Helander, J. Koricheva, and S. H. Faeth. 2006. Model systems in ecology: dissecting the endophyte-grass literature. *Trends in Plant Science* 11:428–433.
- Werren, J. H., L. Baldo, and M. E. Clark. 2008. *Wolbachia*: master manipulators of invertebrate biology. *Nature Reviews Microbiology* 6:741–751.

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