Incorporating the process of vertical transmission into understanding of host–symbiont dynamics

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Variation exists in the frequency of obligate, vertically transmitted symbiotic organisms within and among host populations; however, these patterns have not been adequately explained by variable fitness effects of symbionts on their hosts. In this forum, we call attention to another equally important, but overlooked mechanism to maintain variation in the frequency of symbioses in nature: the rate of vertical transmission. On ecological time scales, vertical transmission can affect the equilibrium frequencies of symbionts in host populations, with potential consequences for population and community dynamics. In addition, vertical transmission has the potential to influence the evolution of symbiosis, by affecting the probability of fixation of symbiosis (and therefore the evolution of complexity) and by allowing hosts to sanction against costly symbionts. Here we use grass–epichloae symbioses as a model system to explore the causes and consequences of variation in vertical transmission rates. We identify critical points for symbiont transmission that emerge from considering the host growth cycle devoted to reproduction (asexual vs sexual) and the host capability to maintain homeostasis. We also use information on the process of transmission to predict the environmental factors that would most likely affect transmission rates. Altogether, we aim to highlight the vertical transmission rate as an important process for understanding the ecology and evolution of symbiosis, using grass–epichloae interactions as a case study.

Major evolutionary transitions have reorganized independent, but strongly interacting, organisms into new units of adaptation or integrated entities (Sadras and Denison 2009). For example, organelles in both plants and animals have evolved from the fixation of vertically transmitted symbionts (Douglas 2008). Many interspecific interactions are thought to be intermediate stages in the evolution toward more complex organisms (Herre et al. 1999, Selosse and Schardl 2007, Tikhonovich and Provorov 2009). Examples include the dependency of macro-organisms (e.g. aphids, wasps, grasses) on intimate relationship with symbiotic bacteria or fungi to obtain essential nutrients or protection against enemies (Selosse and Schardl 2007, Douglas 2008, Werren et al. 2008). Here we propose that understanding how interspecific interactions lead to the fixation (or loss) of symbiosis, and therefore to the evolution of complexity, will depend on knowledge not only of the costs and benefits of the interaction, but also of the rate with which organisms are transmitted between hosts in both space and time (Herre et al. 1999, Bright and Bulgheresi 2010).

The rate of vertical transmission can influence the equilibrium frequency of symbiosis in host populations (Gundel et al. 2008a) and thereby affect the probability that a symbiont becomes a 'fixed' component of the host genome. However, independently of the system, explanations for the fixation of symbiosis have traditionally focused attention on documenting the benefits of symbiosis to host fitness. Among others, most studies on the interaction between arthropods and their associated bacteria (e.g. *Arsenophonus*, *Buchnera*, *Wolbachia*, *Hamiltonella*, *Cardinium*, *Rickettsia*) have been aimed at understanding the effects that the symbiosis has on host fitness (Douglas 2008, Werren et al. 2008, Engelstädter and Hurst 2009); and many studies focusing on the interaction between symbiotic fungi and their host species similarly have targeted effects on plant biomass allocation or reproduction (reviewed by Clay and Schardl 2002, Cheplick and Faeth 2009). Nonetheless, as the impact of a symbiont depends on its incidence in the host population (proportion of symbiotic individuals), the mode and rate of symbiont transmission should be considered equally important to symbiont effects on host fitness in affecting host–symbiont dynamics (Clay and Schardl 2002, Darby and Douglas 2003, Rudgers et al. 2010).

The process of vertical transmission also provides a mechanism for host species to sanction against costly symbionts (Douglas 2008). Mutualistic symbioses can breakdown in the presence of cheater' symbionts which gain rewards without providing benefits (Sachs and Simms 2006). By restricting

the rate of vertical transmission, hosts gain the capacity to limit the reproduction of costly symbionts. After an interaction between two partners has been established, sanctions represent an important mechanism to maintain mutualism despite cheating (Kiers et al. 2003, Douglas 2008, Kiers and Denison 2008), and may be particularly valuable when the benefits and costs of symbiosis are context-dependent. For example, in symbioses between cool season grasses and epichloid fungi, the production of bioprotective alkaloids by the fungi may become costly under nutrient limitation (Cheplick and Faeth 2009). Host plants with the ability to restrict the vertical transmission under resource-limiting conditions could gain a fitness advantage. To our knowledge, host control of vertical transmission in response to costly symbionts, has not been experimentally tested in any system. Quantitative data on the relative contributions of the different modes of transmission as well as the rate of each process is critical to understanding the underlying mechanisms for symbiont persistence and frequency in host populations (Clay and Schardl 2002, Darby and Douglas 2003, Bright and Bulgheresi 2010); however, this information is strikingly scarce.

In this article we call attention to the importance of the process of vertical transmission in the ecology and evolution of host–symbiont associations, using the grass–epichloae system as a model for exploring the causes and consequences of variation in vertical transmission rates. In grasses, epichloae endophytes grow in aboveground tissues, often providing a defense against host enemies, such as herbivores. Within the epichloae, *Epichloë* spp. may be transmitted both vertically and horizontally, while *Neotyphodium* endophytes are only known to be transmitted vertically from the host to offspring via vegetative growth of fungal hyphae (Clay and Schardl 2002, Rudgers et al. 2009, but see Tadych et al. 2007). Theory based on modeling of the interacting effect of mechanisms and processes involved in the symbiosis dynamics in populations and evidence from recent field surveys have suggested that the rate of vertical transmission for epichloae species may be a key process underlying both the frequency of symbiotic associations and the evolution of the grass–epichloae symbiosis (Ravel et al. 1997, Saikkonen et al. 2002, Afkhami and Rudgers 2008, Canals et al. 2008, Gundel et al. 2008a, 2009a).

We propose that recognition of the importance of vertical transmission rates will improve the ability to predict the dynamics of host–symbiont interactions and the frequency of symbioses in nature, both in the specific case of grass–*Neotyphodium* interactions, and in hereditary symbioses more generally. First, we critically review existing evidence documenting variation in the frequency of endophyte symbiosis in grass populations and outline the current hypotheses to explain this variation. Second, we review the existing, albeit sparse, evidence for variation in the rate of vertical transmission of the endophyte, and suggest a framework to fill current gaps in the empirical data. Finally, by exploring those factors that are known to affect and modulate the dynamics of symbiont growth in host grasses, we suggest factors that may significantly influence the process of symbiont transmission. We emphasize the importance of transmission in mediating the interaction between endophytes and grasses, but also recognize that transmission rates may be additionally sensitive to external, environmental variation. When possible, we suggest general mechanisms to explain variation in the rate of vertical endophyte transmission.

Variation in the frequency of symbiosis

Essentially, primary symbionts such as *Buchnera*, usually exhibit the highest infection frequencies in host populations (i.e. all the individuals in the host population are symbiotic); while secondary symbionts, which are not essential for host survival, may show more variable infection frequencies (Darby and Douglas 2003, Douglas 2008). The interaction with endophytic fungi is apparently not essential for host grass populations and, in accordance with this asymmetry, the infection frequency is usually found to be variable (Lewis et al. 1997, Clay and Schardl 2002, Rudgers et al. 2009). In this section, we highlight attempts to explain this natural variation in endophyte frequency. Importantly, these attempts have focused on the relative fitness of symbiotic and non-symbiotic hosts (e.g. spatiotemporal variation in symbiont benefits), to the exclusion of alternative hypotheses.

Assessing the frequency of endophyte-symbiotic plants across grass populations has been a common approach to gain knowledge about possible controls on the outcome of grass–epichloae interactions. Several studies have proposed that variation in endophyte frequency is due to environmental factors, such as climate. For example, early studies, such as Lewis and Clements (1986) and White and Baldwin (1992), reported that *Lolium perenne* in England were free of endophyte symbiosis, while populations of this species were highly symbiotic in parts of New Zealand and the United States. Prevailing climatic conditions in each site (cold and wet in England vs hot, dry summers in the other sites) were proposed as driving forces. In support of this hypothesis, manipulative experiments confirmed that the endophyte in *L. perenne* (as well as and endophytes in other grass hosts) can enhance plant tolerance to drought (Malinowski and Belesky 2000). This foundational analysis has been essentially maintained until today. For example, Lewis et al. (1997) and more recently Malinowski and Belesky (2006) found positive correlations between the frequency of endophyte-symbiotic plants and hot, dry climatic conditions in Europe. While the first study only included wild populations of *Lolium* spp., the second also evaluated tall fescue *Schedonorus phoenix* = / *Festuca arundinacea* and meadow fescue *Festuca pratensis*. These latest results as well as other individual studies (Oldenburg 1997, Saikkonen et al. 2000, but see Jensen and Roulund 2004) largely support a latitudinal gradient in Europe with a high frequency of symbiotic plants at low latitudes (Mediterranean) and a low frequency of symbiotic plants at high latitudes. However, current data represent a mixture of studies in natural and agricultural system, and it would be useful to explore natural systems independently because they have not been directly subjected to artificial selection.

Following similar approaches, several recent works have correlated variation in the frequency of endophyte-symbiotic plants with other environmental variables, including anthropogenic factors such as the degree of herbicide application, grazing by domestic cattle or wild herbivores, and the intensity of public use. For example,

endophyte frequency in populations of annual ryegrasses (*Lolium multiflorum* from Argentina and *L. rigidum* from Australia) was positively correlated with selection pressure by the herbicide diclofop-methyl (Vila-Aiub et al. 2003). In addition, the frequency of endophyte-symbiotic plants in *F. altaica* and *L. perenne* was higher in areas of heavier grazing (Jensen and Roulund 2004, Koh and Hik 2007) and under more extensive public use (Jensen and Roulund 2004). Similarly, the frequency of symbiotic *Festuca rubra* plants with the vertically transmitted *Epichloë festucae* was positively associated with grazing by reindeer at low altitudes (Bazely et al. 2007, Granath et al. 2007).

Despite these patterns in the frequency of symbiotic plants across host populations, many data suggest little consistency in correlations between endophyte infection frequency and environmental variables. For example, the frequency of endophyte-symbiotic *Bromus setifolius* plants was found to be positively correlated with mean annual precipitation in southern Patagonian region (Argentina; Novas et al. 2007), in direct contrast to the drought amelioration hypothesis. Similarly, in a study of three *Festuca* species (*F. rubra*, *F. ovina* and *F. vivipara*) in Sweden, one showed a positive correlation between endophyte frequency and elevation, another showed a negative correlation with elevation, and a third showed no significant correlation with elevation (Bazely et al. 2007, Granath et al. 2007). Similarly, infection frequency of *Neotyphodium starrii* in *Festuca arizonica* populations native to southwestern USA showed no association with either grazing pressure or soil nitrogen (Schulthess and Faeth 1998).

In general, all studies have adopted a similar line of reasoning to explain endophyte frequency: where the frequency of symbiotic plants is high the endophyte confers tolerance/resistance to a given biotic or abiotic stress, but where the frequency of symbiotic plants is low, the endophyte is less beneficial for alleviating stress or the stress is a weaker selective force. However, none of these correlation-based approaches were designed to estimate the relative fitness between symbiotic and non-symbiotic plants (or variation in the cost/benefit of the symbiosis) and therefore, they cannot determine what mechanisms (i.e. relative fitness or transmission rate) actually drive variation in the frequency of endophyte-symbiotic plants. For example, the effect of diclofop-methyl herbicide on either relative fitness or transmission rate in annual ryegrasses remains unknown, and either mechanism could explain the observed positive correlation. Furthermore, endophyte-mediated improvements to relative host plant fitness have not been consistent across studies or species. For example, in some cases, most notably in *Festuca arizonica*, it has been difficult to find the expected fitness enhancement of endophytes on plants from populations with high frequencies of symbiotic plants (Faeth and Sullivan 2003, Lewis 2004, Faeth and Hamilton 2006). In sum, current hypotheses may not be sufficient to account for natural variation in symbiont frequency (Ravel et al. 1997, Gundel et al. 2008a), suggesting a need for alternative mechanisms.

Two important mechanisms missing in these studies are the rate of vertical transmission and seed migration among host populations. Vertical transmission and migration could have effects at both local and regional (metapopulation) scales, but most attention has focused on local scales, with

one exception. Saikkonen et al. (2002) showed by means of modeling that under metapopulation dynamics, the endophyte can persist in local populations where it is not mutualistic even with failures in the endophyte transmission if there is sufficient migration from populations where the endophyte is beneficial. However, no data exist indicating that endophyte-symbiotic seed migrations may affect local population dynamics. In contrast, relatively more information is available on local rates of vertical transmission.

The importance of the rate of endophyte vertical transmission as a mechanism underlying variation in the local frequency of symbiotic plants was pointed out by several authors that used modeling approaches (Ravel et al. 1997, Saikkonen et al. 2002, Gundel et al. 2008a), but transmission process has usually been neglected in both experiments and their interpretations (with one exception – Granath et al. 2007; see critique by Gundel et al. 2008b). In particular, models show that symbiont frequencies in local host populations can be highly sensitive to small variations in the rate of vertical transmission, which may be equally as important, if not more important, than variation in the relative fitness of symbiotic and non-symbiotic hosts. In other words, imperfect vertical transmission rate always limits the equilibrium level of symbiotic hosts, even for symbionts with a strong mutualistic effect (Gundel et al. 2008a). This raises the possibility that the rate of vertical transmission is a primary determinant of the frequency of symbioses in host populations (Ravel et al. 1997, Gundel et al. 2008a).

In fact, variation in vertical transmission rates has been documented. For the best studied host species (tall fescue and perennial ryegrass), vertical transmission is usually high (>90%); however, even in these hosts, there is evidence for variation in the proportion of endophyte-free seeds produced by symbiotic plants (Welty et al. 1994, Hill et al. 2005, Gundel et al. 2009a). Notably, large variation in the transmission rate among individual plants within a tall fescue population was shown (Welty et al. 1994), although this variation could not be directly ascribed to plant genotype versus micro-environment. Annual ryegrasses appear to be more variable in transmission rate than either tall fescue or perennial ryegrass, and in some introduced populations the transmission rate was as low as 60% (Canals et al. 2008, Gundel et al. 2009a). Natural populations of native grass species can also be highly variable in the endophyte transmission rate. For example, significant variability in the frequency of endophyte-symbiotic tillers per plant was observed in the native host, *Festuca arizonica* (Schulthess and Faeth 1998). More recently, Afkhami and Rudgers (2008) documented variation in the process of vertical transmission for several native species from North America (*Elymus hystrix*, *E. riparius*, *E. virginicus*, *Festuca subverticillata*, *Poa alsodes*, *P. sylvestris* and *Sphenopholis nitida*). In this multi-species survey, both the type and degree of endophyte losses differed among host species, host populations and probably among environments. This is important since a direct association has been found between the frequency of endophyte-symbiotic plants and the rate of vertical transmission (Afkhami and Rudgers 2008, Canals et al. 2008, Gundel et al. 2009a).

Tying all of these elements together – the inconsistency of correlations between symbiont frequency and environmental gradients, the existence of cases where relative fitness

benefits are puzzlingly absent despite high endophyte frequencies, models highlighting the importance of the transmission process, and recent documentation of imperfect transmission in nature – suggests the possibility for an important role of the transmission process in explaining the persistence and frequency of heritable symbioses.

Endophyte variation in transmission rate and growth dynamics

Quantitative evaluation of transmission rates was recognized as a crucial component for understanding the ecology and coevolutionary dynamics in early studies of bacteria-arthropod associations (Darby and Douglas 2003, Douglas 2008, Oliver et al. 2008). Within each particular pair of interacting species, there can be great variation in the complexity of transmission mechanisms (Bright and Bulgheresi 2010). Whereas primary symbionts present perfect transmission, secondary symbionts, which often play a protective role, have complex patterns of transmission (e.g. transmission may be associated with female lineages and even within a lineage, which lead to imperfect transmission process) (Darby and Douglas 2003, Werren et al. 2008, Bright and Bulgheresi 2010). Epichloae endophyte symbionts can be characterized as 'protectors' that are not vital for host plants (Clay and Schardl 2002, Saikkonen et al. 2004), hence, imperfect transmission may be an important mechanism mediating the interaction. Across symbiotic systems, the density of the symbiont within the host may influence the rate of vertical transmission. In grass–epichloae symbioses, a few studies have addressed endophyte growth dynamics and suggested impacts on the transmission process. We argue that documenting how symbiont concentration varies within the host is the first step toward understanding the transmission process.

While limited data preclude the ability to make general conclusions about the relative importance of factors affecting endophyte concentrations, it is clear that external environmental factors can influence endophyte growth. Techniques such as counting mycelium per unit of plant tissue under light microscopy and real-time PCR have made it possible to describe endophyte growth dynamics and thereby to identify factors that can affect concentrations within host plants. Furthermore, even though it is possible that higher fungal biomass in host tissues will result in more effective vertical transmission to seeds and seedlings, variation in hyphal concentration has not yet been directly linked to rates of vertical transmission; this is an important goal for future studies. Current research has shown that growth dynamics of endophytic fungi follow the seasonality of host plant growth and development, with minimal hyphae in winter (when host growth rate is lowest) and higher densities in spring through early summer (during the peak of host reproduction; di Menna and Waller 1986, Ball et al. 1995). Although the amount of hyphae is usually positively associated with plant biomass (Groppe et al. 1999, Mack and Rudgers 2008), environmental factors such as water, temperature and nutrients can also affect hyphal concentrations. For example, it has been proposed that low temperatures may decouple the synchrony of growth between the two partners because base temperatures for growth were higher for the endophyte than

for host plants (Ju et al. 2006). Similarly, nitrogen fertilization reduced hyphal concentration in perennial ryegrass, apparently by promoting host growth relatively more than endophyte growth (i.e. dilution effect; Rasmussen et al. 2007). However, fertilization increased hyphal densities in leaves of tall fescue (Mack and Rudgers 2008). More work is clearly needed to resolve the key environmental influences on hyphal concentration within plants.

Dissecting the process of transmission across host life history stages can be useful for modeling the dynamics of symbiosis and serves as a general approach for studying vertically transmitted symbioses. For example, besides the female parthenogenesis, vertical transmission of bacterial secondary symbionts occurs through seminal material in female and male aphids (Darby and Douglas 2003, Oliver et al. 2008, Bright and Bulgheresi 2010). In a symbiotic plant, endophyte hyphae colonize developing ovaries before anthesis and pollen fertilization (Philipson and Christey 1986, Majewska-Sawka and Nakashimab 2004, Sugawara et al. 2004). Therefore, the rate of symbiont vertical transmission can be categorized into two broad phases, pre-zygotic and post-zygotic.

In plant hosts, these divisions represent the transmission from the plant to seeds (pre-zygotic) and from seeds to seedlings (post-zygotic), respectively (Gundel et al. 2008a, Fig. 1). To flesh this out using a specific grass–epichloae example, within the pre-zygotic phase for an annual host grass, there are three key life history stages: seedling, vegetative tiller and reproductive tiller (or inflorescence). Vital rates connect each successive stage. During the pre-zygotic phase these rates are 'Tillering' (between seedling and vegetative tiller), 'Flowering' (between vegetative tiller and inflorescence) and 'Fecundity' (between inflorescence and seed) (Fig. 1). The post-zygotic phase includes only the seed stage, and the vital rate that mediates the transition between seed and seedling is 'Germination'. Linked to each vital rate, there is a partial transmission rate reflecting the proportion of host organs that remain symbiotic during the transition from the previous stage. For example, the associated partial transmission rate to 'Germination' establishes the proportion of symbiotic seedlings relative to symbiotic seeds (Fig. 1).

Factors that may influence endophyte loss during the pre-zygotic phase remain largely uninvestigated. As we suggest above, factors that affect hyphal concentrations in leaves and tillers may ultimately alter pre-zygotic transmission. Also, it has been suggested that transmission rate from plant to seeds could depend on host species identity, host or endophyte population (or genotype), and environmental conditions (do Valle Ribeiro 1993, Afkhami and Rudgers 2008), but we are not aware of any direct tests of these possible influences.

More is known about the controls on endophyte transmission at the post-zygotic phase than at the pre-zygotic phase. Most of this evidence has come from seed storage studies due to the practical need to predict the longevity of endophyte symbiosis in agronomic seed stores. For most of the environmental conditions tested, the rate of viability loss was typically higher for the endophyte fungus than for the seed, with particularly strong negative effects of combined high heat and humidity on the viability of the fungus (Welty et al. 1987, do Valle Ribeiro, 1993, Gundel et al. 2009b). However, both the endophyte and seed
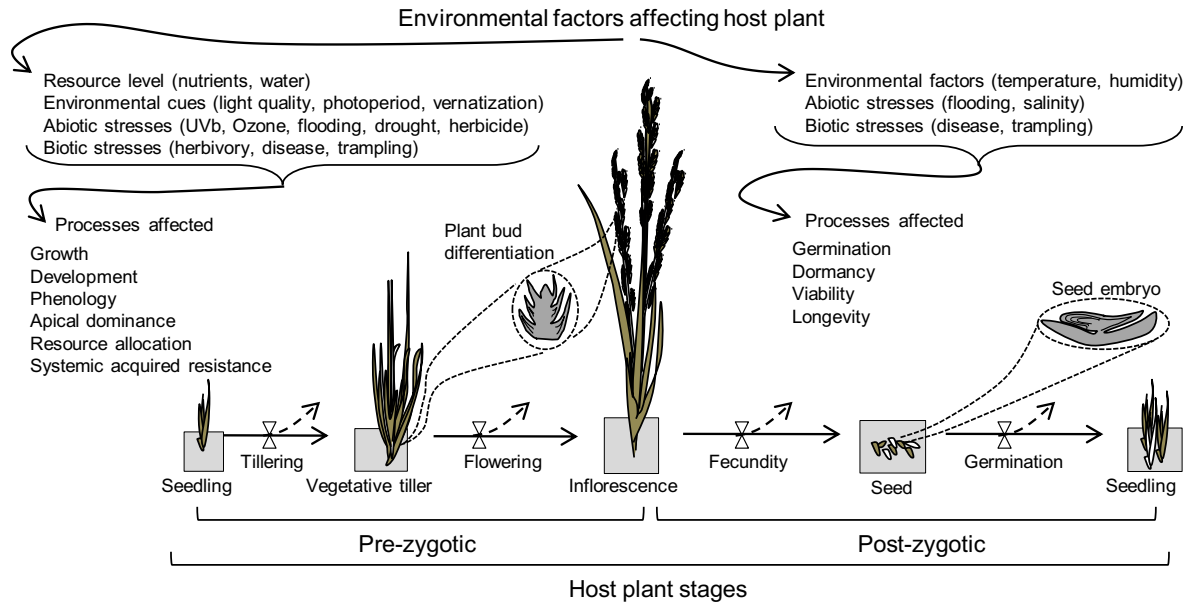
Figure 1. Schematic diagram depicting the grass species symbiotic with the endophytic fungus *Neotyphodium* and the annual life cycle consisting of four stages (Seedling, Vegetative tiller, Inflorescence and Seed) and vital rates between each successive stage (*Tillering, Flowering, Fecundity and Germination*). Control flow keys (⋈) indicate those points in the host life cycle in which failures in vertical transmission of the endophyte can occur (those points critical for the endophyte transmission to each structure within a life history stage are enclosed in dotted lines). To illustrate variation in vertical transmission, the structures of a symbiotic individual are shown in grey, and those organs that have escaped from fungal colonization are shown in white. Below the host life cycle are indicated those stages that are comprised in the pre-zygotic (Seedling, Vegetative tiller, Inflorescence) and post-zygotic (Seed) phases of the vertical transmission process.

remain viable longer if at least one of these two environmental variables is low enough (Welty et al. 1987, Gundel et al. 2009b). Unfortunately, most of the work has been conducted in artificial laboratory environments, and the impacts of natural environmental conditions are mostly unresolved. Exceptions include seed burial studies, which found that when seeds were buried in soil (imitating burial by plow in croplands), the endophyte did not lose viability faster than the seed (Hume and Barker 2005, Canals et al. 2008). However, we have recently found that moving seeds from the air to the soil surface, and thereby simulating seed dispersal under natural conditions, reduced the post-zygotic transmission rate of the endophyte in annual ryegrass (Gundel et al. 2010). Clearly, more work is needed to extend our understanding of post-zygotic endophyte dynamics to more natural conditions.

Identifying the mechanisms underlying variation in rates of vertical transmission

In this section, we explore the potential impact of factors that may affect the rate of vertical transmission, based on current knowledge of *Neotyphodium* endophyte growth dynamics within host grasses. We focus on environmental and genetic factors that are known to affect plant growth dynamics and the relative costs and benefits of endophyte symbiosis. We propose that the impacts of environmental influences on endophyte transmission will largely depend on the host's capability to regulate the apoplast (i.e. the environment where the endophyte grows). In addition, we posit that these impacts will depend not only on the nature and intensity of the environmental factor but also on the life history stage during which the impact occurs. Specifically, we

propose that the influence of environmental and genetic factors on the endophyte would be mostly mediated by the host during the pre-zygotic phase, and especially during bud differentiation and at anthesis (Fig. 1). In contrast, during the post-zygotic phase, the endophyte would be exposed more directly to environmental stresses, especially at the mature seed stage, and the host may have a smaller role in modulating the impact of the environment (Fig. 1).

First, resource availability, through its effects on host growth, may influence rates of vertical transmission. Resource limitation could make symbiosis more costly. If hosts reduced transmission rate when resources were limiting, this would control cheating/costs of symbiosis and promote the maintenance of mutualism (i.e. allow for host sanctions). In plants, for example, growth is dynamic and strongly influenced by the level of resources and other environmental factors (Fig. 1). Allometric relationships predict that a minimum plant biomass is required to produce reproductive organs (seeds), and there is a positive relationship between vegetative and reproductive biomass (Weiner et al. 2009). Under low resource availability, endophyte symbiosis can become costly due to the fungal requirements for carbon from the plant (Cheplick 2004), resulting in symbiotic plants with lower biomass and/or reproduction than symbiont-free plants. It is not known whether this cost to the plant also affects the transmission rate of the endophyte, although it seems plausible that low resource availability could reduce rates of transmission. Indeed, the positive relationship that has been documented between plant biomass and endophyte hyphal concentration (Groppe et al. 1999, Mack and Rudgers 2008) suggests that higher rates of endophyte transmission could be expected to occur in plants with higher biomass and lower rates in plants with lower biomass.

Second, several types of stress are known to affect the balance of costs and benefits of symbiosis, generally, and could also affect the rate of vertical transmission. The cost/benefit balance and the relative contribution and effectiveness of the modes of transmission of the secondary symbionts that are known to confer tolerance to heat and parasitoids in aphids are highly dependent on the environments (Darby and Douglas 2003, Oliver et al. 2008). In grass–epichloae symbioses, drought stress and herbivory are known to increase the relative fitness benefits of endophyte symbiosis (Malinowski and Belesky 2000, Clay and Schardl 2002) and could additionally affect transmission rates. For example, endophyte hyphal concentration within tall fescue *Schenodorus phoenix* plants was found to increase after drought (Cheplick 2004), and such changes in concentration within the host could result in increased fungal transmission to seeds. Similarly, grazing is usually found to promote endophyte-symbiotic plants in grasslands (Jensen and Roulund 2004, Granath et al. 2007, Koh and Hik 2007), although nothing is yet known about the possible effects of this factor on endophyte transmission. Stress is most likely to affect endophyte transmission through changes in the host plant. Plants under stress often flower earlier and may show profound changes in morphology or allometric relationships, apical dominance (affecting the number of tillers per plant), and seed production (Weiner et al. 2009); these effects could alter the life history dynamics of the endophyte, particularly in the pre-zygotic phase. In addition, because stress can reduce plant biomass, it could indirectly affect transmission success, for example, if host biomass is positively correlated with endophyte concentration. Under such a scenario, one might even find opposing forces acting on endophyte frequency: stress may increase the relative fitness benefit of symbiosis (as has been shown for herbivory and drought), but reduce the vertical transmission rate relative to unstressed, symbiotic plants.

Stress can also alter host physiology in ways that could impact symbiont transmission. Under stress, a set of host responses is triggered at the molecular, biochemical and physiological levels, including hormone signals and reactive oxygen species (ROS) (Fig. 1; e.g. for plants, Fujita et al. 2006, Ballaré 2009). ROS are apoplastic products of the oxidative burst and hyper-sensitive response that also mediate the systemic acquired resistance response to pathogens (Fig. 1; Fujita et al. 2006, Tikhonovich and Provorov 2009). Recently, it was proposed that the growing endophyte in the apoplast induces the production of ROS and, in response to this effect, a higher level of antioxidants enhances the ability of symbiotic plants to overcome external stressors (White and Torres 2010). Similarly, Tanaka et al. (2006) proposed that the fungus can change from a mutualist to a parasite by losing fungal ROS accumulation. Whether or not ROS level is part of a fine-tuned mechanism of control of fungal growth and activity within the plant, any stress factor that alters this level could also affect the endophyte growth and transmission.

Third, the timing of stress relative to the transmission of symbionts could be critical to predicting its effects on transmission. For example, stresses occurring at tillering and flowering in plants that are related to physiological differentiation of buds (Fig. 1) may have a much stronger effect than stress during vegetative growth (Sadras and Denison 2009). For example, if the host undergoes stress during flowering, when endophytes are growing into the developing ovaries, the transmission process could be more strongly influenced than stress occurring at another host life history stage. These changes are likely to alter the proportion of endophyte-symbiotic seeds produced by a host plant. Similarly, since the internal environment of the mature seed is poorly regulated, stress during seed germination and early seedling growth could be also important (Fig. 1). Variation in seed temperature and water potential are driven by changes in the external environment. Therefore, during the seed and seedling stages, endophytes may have a high risk of being directly affected by abiotic stress factors (Hill et al. 2005, Gundel et al. 2010). Seed hydration or changes in osmotic potential and ion concentrations (i.e. Na) may strongly impact endophyte viability or the ability to grow into and colonize the seedling. Currently, there is abundant information on how these factors affect seed germination and seedling growth and survival, but no information on how these factors may affect transmission efficiency.

Fourth, individual host or symbiont genotypes are likely to affect rate of vertical transmission. Although this variation has not yet been well documented for any symbiosis, populations of the same host–endophyte species combination have been shown to vary in transmission rate (Afkhami and Rudgers 2008, Gundel et al. 2009a). Perhaps more interesting is variation that arises due to the compatibility of particular host–symbiont genotype pairs. Trait matching in phenotypic and genetic characteristics of endophytes and their corresponding host grass populations has been suggested to indicate local coevolution, and differential gene flow among populations could generate genetic mismatches between the traits of the host and the traits of the symbiont (Arroyo García et al. 2002, Faeth and Sullivan 2003, Saikkonen et al. 2004). Evidence for variation in compatibility comes from cross inoculation studies that put endophyte hyphae into new hosts; unsuccessful inoculations, transmission failures, and stunted host growth have been all interpreted as evidence of lack of compatibility (Latch and Christensen 1985, Tanaka et al. 2006). For example, recent work involving a large number of artificial inoculations into host genotypes found that artificial combinations of the host and endophyte had a lower proportion of tillers bearing the endophyte compared to natural endophyte–host combinations; however, this effect on the endophyte frequency in tillers did not generate altered endophyte frequencies in the seeds or seedlings (Saikkonen et al. 2010).

The importance of genetic mismatching in the success of vertical transmission may depend on the reproductive strategy of the host organism. When hosts are self-incompatible and freely outcrossing (e.g. wind pollinated grasses) every maternal ovary could be fertilized by a genetically unique lineage, allowing the maintenance of high intra-population genetic variability (Sadras and Denison 2009). In such host species, symbionts are constantly subjected to genetically novel host genotypes due to reproduction and recombination (Fig. 1). Given the vegetative and strict vertical transmission of *Neotyphodium*, and the high gene flow and the intrinsic high genetic variability in host populations (particularly in grass species with high rates of hybridization), we

might expect strong selection for endophytes to be generalists under such conditions (Herre et al. 1999). In contrast, for host species with high self-compatibility and high rates of selfing, pairs of host and symbiont genotypes may be maintained for long time periods in nature due to low outcrossing rates. In these systems, artificial inoculations of symbionts into hosts would be more likely to create maladapted genetic mismatches between host and symbiont genotypes than in species that are obligately outcrossing. These systems may prove good models for investigating how genetic mismatching affects the rate of vertical transmission.

Conclusions

Heritable symbioses are likely to be the intermediate states along a coevolutionary path toward the fixation of a symbiosis into an integrated and superior biological unit, underlying the evolution of complexity. However, in the general study of symbioses has long been centered on the host organisms, rather than on the symbiont. This host-centric perspective has led researchers to overlook the importance of the rate of symbiont vertical transmission at the scale of individuals, populations and metapopulations. Using the grass–epichloae symbiosis as a model for hereditary symbioses, we proposed: 1) the existing variation in symbiont frequency may be caused by the variation in the vertical transmission rate; and 2) understanding the mechanisms vertical transmission will require a demographic approach, separating effects at the pre-zygotic and post-zygotic stages. Finally, cost/benefit analysis should not only consider that symbiont presence can be costly to the host organism, but it should acknowledge that the transmission process may also be costly. Therefore, we want to encourage further study of the process of transmission and its sensitivity to ecological factors (e.g. environmental variation) and (co-)evolutionary processes (e.g. genetic compatibilities). We believe that incorporation of the vertical transmission rate into a more general framework will enhance our understanding not only of the ecology and evolution of the symbioses between cool-season grasses and epichloae endophytes, but also of host–symbiont interactions more generally.

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