

BRIEF COMMUNICATION

Vertically transmitted symbionts as mechanisms of transgenerational effects¹

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PREMISE OF THE STUDY: A transgenerational effect occurs when a biotic or abiotic environmental factor acts on a parental individual and thereby affects the phenotype of progeny. Due to the importance of transgenerational effects for understanding plant ecology and evolution, their underlying mechanisms are of general interest. Here, we introduce the concept that inherited symbiotic microorganisms could act as mechanisms of transgenerational effects in plants.

METHODS: We define the criteria required to demonstrate that transgenerational effects are microbially mediated and review evidence from the well-studied, vertically transmitted plant–fungal symbiosis (grass–*Epichloë* spp.) in support of such effects. We also propose a basic experimental design to test for the presence of adaptive transgenerational effects mediated by plant symbionts.

KEY RESULTS: An increasingly large body of literature shows that vertically transmitted microorganisms are common in plants, with potential to affect the phenotypes and fitness of progeny. Transgenerational effects could occur via parental modification of symbiont presence/absence, symbiont load, symbiont products, symbiont genotype or species composition, or symbiont priming. Several of these mechanisms appear likely in the grass–*Epichloë* endophytic symbiosis, as there is variation in the proportion of the progeny that carries the fungus, as well as variation in concentrations of mycelia and secondary compounds (alkaloids and osmolytes) in the seed.

CONCLUSIONS: Symbiont-mediated transgenerational effects could be common in plants and could play large roles in plant adaptation to changing environments, but definitive tests are needed. We hope our contribution will spark new lines of research on the transgenerational effects of vertically transmitted symbionts in plants.

KEY WORDS inherited symbionts; maternal effects; epigenetics; *Epichloë* fungal endophytes; microorganisms

A transgenerational effect (TGE) occurs when a biotic or abiotic environmental factor acts on a parental individual and thereby affects the phenotype of progeny. A TGE can be part of a suite of fine-tuned mechanisms, selected over evolutionary time, which allow parent organisms to plastically match the traits of progeny to the prevailing local ecological conditions by transferring information and/or resources to the next generation (Herman and Sultan, 2011). However, TGEs may also be nonadaptive. Although the adaptive value of TGEs cannot be assumed a priori, there is substantial

evidence that TGEs influence the ecological interactions and performance of progeny in many plant and animal species (Mousseau and Fox, 1998; Agrawal et al., 1999; Marshall and Uller, 2007; Bischoff and Müller-Schärer, 2010; Agrawal, 2001; Herman and Sultan, 2011).

The study of mechanisms underlying TGEs is an increasingly active field of research (Mousseau and Fox, 1998; Herman and Sultan, 2011; Pieterse, 2012; Dall et al., 2015). Here, we introduce the concept that symbiotic microorganisms that are inherited across host generations could act as mechanisms of TGEs. We focus on *vertically* transmitted symbionts because they have high potential for transmitting information from parental plants to offspring and because vertically transmitted symbionts have been shown to mediate TGEs in animals (e.g., Freitak et al., 2014). Furthermore, the roles of *horizontally* acquired microorganisms in TGEs of induced resistance in plants have recently received some attention (Pieterse et al., 2014; Balmer et al., 2015; Vivas et al., 2015). First, we briefly define and review mechanisms of TGEs. We then delineate the criteria required

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to demonstrate that TGEs are mediated by microbes, providing a basic experimental design to do so. Next, we explore general routes through which symbiotic microbes could modulate TGEs in plants. Finally, we provide a case study to illustrate the largely unexplored potential for vertically transmitted symbionts to mediate TGEs in plants, by reviewing evidence from the relatively well-studied symbioses between fungal endophytes (*Epichloë* spp.) and grasses.

Transgenerational effects: General mechanisms in plants—

Maternal plants are typically larger contributors to TGEs than are paternal plants because progeny directly inherit multiple maternal components in addition to their genetic material. Maternal plants contribute plastids (mitochondria and chloroplasts), the endosperm and the seed coat, and mediate the effect of the environment by controlling nutrition (e.g., seed size and nutrient content), informational molecules (e.g., quantity and state of photoreceptors), hormones, and secondary metabolites involved in defense (Roach and Wulff, 1987; Donohue, 2009; Herman and Sultan, 2011; Latzel, 2015). In addition, epigenetic mechanisms can also be involved; these include changes in DNA methylation, histone modifications, chromatin-remodeling proteins, and DNA silencing by noncoding RNAs (Kaeppeler et al., 2000; Molinier et al., 2006; Bruce et al., 2007; Chinnusamy and Zhu, 2009; Latzel, 2015). Although non-epigenetic maternal effects are assumed to have short-term duration, epigenetic changes can be transmitted by both parental plants and can have long-lasting consequences for several generations (Molinier et al., 2006; Bruce et al., 2007; Iwasaki and Paszkowski, 2014).

Microbial symbionts as mediators of transgenerational effects—

All plants harbor symbiotic microbes, which can be found in roots, shoots, leaves, and/or seeds (Bacon and White, 2000). These endophytic microorganisms include systemic and nonsystemic bacterial and fungal endophytes, as well as viruses (Arnold, 2007; Romo et al., 2007; Zabalgoceazcoa et al., 2013; Hodgson et al., 2014; Truyens et al., 2015). Thus, the plant is most appropriately considered a holobiont, with its fitness a consequence of both intrinsic plant traits as well as microbial phenotypes (Bordenstein and Theis, 2015; Vandenkoornhuysen et al., 2015). The diverse symbiotic microbiota in plants likely differ in the nature and magnitude of their roles in mediating TGEs. When, and how much, microbes influence TGEs are open questions. Understanding the mechanisms of microbial benefits to host plants and their progeny (Pieterse et al., 2014; Balmer et al., 2015; Vivas et al., 2015) could improve the ability to harness microbes to increase yields in agriculture (Gundel et al., 2013; Johnson et al., 2013) and to manage plant–microbe symbioses under a changing climate (Kivlin et al., 2013).

Both the mode and efficiency of symbiont transmission may influence TGEs. For example, symbionts that regularly use a vertical mode of transmission from parent to offspring likely have higher potential for mediating TGEs than horizontally transmitted symbionts, which are reacquired from the environment in each generation. Symbionts with vertical transmission are also the most natural candidates for *adaptive* TGEs because their benefits to hosts typically return advantages to the symbiont via partner fidelity feedback (Sachs et al., 2004). Although a role for horizontally transmitted microorganisms in mediating transgenerational effects has been recognized (Roberts, 1983; Lammerink et al., 1984; Luna et al., 2012; Slaughter et al., 2012; Pieterse et al., 2014; Balmer et al.,

2015; Vivas et al., 2015), the influence of vertically transmitted microorganisms has been largely overlooked, despite the fact that they may have more potential to mediate TGEs than horizontally transmitted microorganisms. We propose that symbiosis with vertically transmitted microorganisms constitutes an effective route to transmit TGEs.

Several plant families form associations with vertically transmitted symbionts. In some cases, these associations show evidence of long coevolutionary histories (e.g., Schardl, 2010). For example, many species in the Fabaceae (e.g., in *Astragalus* and *Oxytropis*) host the vertically transmitted fungus *Undifilum oxytropis*, which is responsible for the locoweed toxins that deter herbivores (Ralphs et al., 2011). Similarly, species in the Convolvulaceae (*Ipomoea* spp.) host vertically transmitted *Periglandula* spp. fungi that produce alkaloids and also protect hosts against herbivores (Beaulieu et al., 2013; Panaccione et al., 2014). Other fungal species isolated from dicot herbs have the capacity for both seed and pollen transmission (Hodgson et al., 2014). Many fungal endophytes (*Epichloë* spp.) of grasses (subfamily Poöideae) are vertically transmitted and can endow hosts with alkaloid-based resistance against herbivores, increased drought tolerance, or increased nutrient content (Clay and Schardl, 2002; Schardl, 2010; see case study below). In addition to housing fungi, seeds can also transmit bacteria from one generation to the next (reviewed by Truyens et al., 2015). Several plant species, including *Arabidopsis thaliana*, *Brassica napus*, *Eucalyptus* spp., *Panicum virgatum*, *Triticum aestivum*, and *Zea mays*, harbor bacterial strains that are consistently found in plant tissues, seeds, and/or seedlings, a pattern that is suggestive of vertical transmission (Ferreira et al., 2008; Johnston-Monje and Raizada, 2011; Liu et al., 2012; Ringelberg et al., 2012; Croes et al., 2013; Gagne-Bourgue et al., 2013; Truyens et al., 2013). These bacterial endophytes can be sources of secondary compounds that provide defensive (antibiotic) or growth-promoting (e.g., hormonal) properties to plants (reviewed by Truyens et al., 2015), and the presence/absence of certain bacteria in the seed can be associated with the success/failure of emerging seedlings (e.g., White et al., 2015). If certain bacterial communities are transmitted from mother plants to offspring, as available evidence suggests, there is strong potential for seed-borne bacterial symbionts to mediate TGEs in plants.

We suggest that several criteria must be satisfied to demonstrate that vertically transmitted microorganisms can mediate TGEs in host plants, and to show that these effects are adaptive (Box 1; Fig. 1). To our knowledge, these criteria have not yet been demonstrated for any vertically transmitted symbiont, nor has a full test even been attempted.

Potential mechanisms of microbially mediated TGEs—

Here, we report general avenues for how transgenerational effects could be caused by vertically transmitted symbionts. Then, in the next section, we describe specific evidence from symbioses between grasses and vertically transmitted *Epichloë* spp. that is consistent with one or more of these avenues.

(1) *Parental modification of symbiont presence/absence*—Following a biotic or abiotic stimulus, the vertical transmission of the microorganism—i.e., the proportion of the progeny that carries the symbiont/s—could increase or decrease. For an individual offspring, this would translate to either presence or absence of the symbiont and thus different phenotypic states.

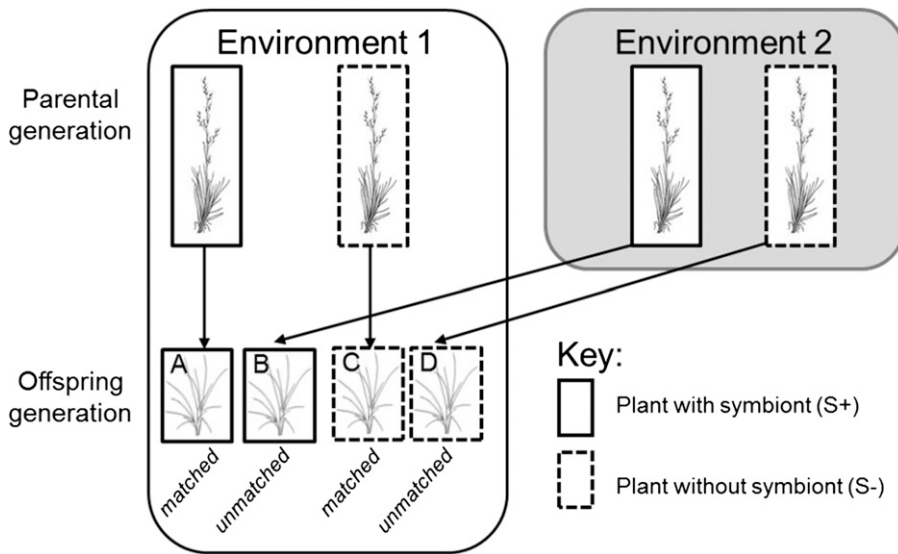


FIGURE 1 Basic experimental design for detecting microbially mediated transgenerational effects. Symbiont-hosting and symbiont-free plants (S+ and S–, respectively) are grown in at least two environments (e.g., stress and control). For symbiont effects to be isolated, host individuals vegetatively propagated from the same plant (clones) should be used so that each host genotype is represented in S+ and S– forms. In the case that a plant species cannot be cloned, full-sib or half-sib families could be used as an alternative, which would provide some control over plant genotype. Depending on the symbiosis, symbionts can be either be removed from hosts via cleansing treatments (e.g., antibiotic, fungicides, or high temperature) or they can be added to hosts via inoculation. Offspring of each maternal plant are then grown in at least one of the environments and their phenotypes and fitness measured. Offspring are considered “matched” or “unmatched” based on whether they are growing in the environment to which their parents were exposed. Comparisons of the phenotypes/fitness of the four types of progeny (A–D) test the criteria for microbially mediated transgenerational effects (see Box 1).

(2) *Parental modification of symbiont abundance*—The overall symbiont load (e.g., bacterial abundance, mass of fungal mycelium) per progeny unit (seed or fruit) could be modified by the maternal plant in response to an environmental stimulus.

(3) *Parental modification of symbiont products*—The parental plant could act as a filter on symbiont secondary products, selectively increasing or reducing their concentration in seeds or pollen. The possibility also exists that the parental symbiont, rather than the parental plant, responds to the environmental stimulus; thus, changes in the plant offspring could occur through microbial, rather than plant-based, TGEs. For example, if the symbiont can sense the environment (e.g., drought, herbivory), it may increase production of secondary metabolites (e.g., hormones, osmolytes, antioxidants, alkaloids) and transmit these compounds directly to the progeny (e.g., increasing the concentration of antioxidants in the seed). Microbial sensing could occur directly or could be mediated through changes in the host plant that signal environmental change.

(4) *Parental modification of symbiont genotype/species composition*—In cases of bacteria or nonsystemic fungal endophytes, where diverse strains or species coinhabit single plants, the environmental stimulus experienced by the parent could promote the vertical transmission of certain microbial taxa or genotypes over others. This mechanism would include parental modification of the relative

abundance of symbiont taxa in cases where multiple species are vertically transmitted. In the case of vertically transmitted fungi that have many hyphal tips spreading in seeds, the possibility also exists for parents to preferentially transmit to their offspring subsets of hyphae that have acquired specific DNA mutations.

(5) *Parental modification of symbiont priming*—If the symbiont can sense the environment, then it may pass information to the next symbiont generation via priming and/or acclimation signals that occur through the accumulation of transcription factors or epigenetic mechanisms (e.g., methylation of symbiont DNA). This mechanism may occur in symbionts, like *Epichloë*, that reproduce clonally into the next generation.

Case study: Plant symbiosis with fungal endophytes (*Epichloë* spp.)—Foliar fungal endophytes in the genus *Epichloë* are present in ~25% of grass species (Poaceae), concentrated in the diverse Pooideae clade of C₃ grasses, that is common in temperate regions (Leuchtmann, 1993; Rudgers et al., 2009; Semmartin et al., 2015). Grass–*Epichloë* symbioses are well known to modulate plant interactions with the environment. For example, many grass–*Epichloë* symbioses can be defensive mutualisms, in which the fungus confers resistance to herbivory by synthesizing bioactive alkaloids (Clay, 1988; Schardl et al., 2004;

Saikkonen et al., 2013). Thus, in addition to the plant’s own defensive mechanisms, grasses are often endowed with symbiont-mediated defensive mechanisms (see Simons et al., 2008). Other benefits include enhanced plant performance under abiotic stressors, such as drought, heavy metals, or herbicides (reviewed by Malinowski and Belesky, 2000; Hamilton et al., 2012). The *Epichloë* spp. are mostly asexual, and a majority of described taxa are vertically transmitted from mother plants to seeds, although they are not known to transmit through pollen (Schardl et al., 2004; Gundel et al., 2011b; Leuchtmann et al., 2014). Fungal biomass in the host apoplast tracks plant growth (intercalary hyphal growth, Christensen et al., 2008) and can correlate positively with levels of alkaloids and other fungal secondary compounds (e.g., mannitol; Rasmussen et al., 2007). The symbiosis is obligate for the fungus, which lacks a free-living stage, but is facultative for the grass.

Vertically transmitted *Epichloë* spp. could cause TGEs in grasses through several possible routes. First, parents could alter how frequently the symbiont is transmitted to individual offspring (Mechanism 1, above). There is ample evidence that symbiotic parents vary in the proportion of seeds produced that receive the endophyte (Afkhani and Rudgers, 2008; Gundel et al., 2009, 2011a). However, it is unknown whether the frequency of vertical transmission can be modulated by the level of damage caused by herbivores or other environmental stimuli experienced by the parental generation (Box 1, Criterion 1). One of the most suggestive cases showed that the prevalence of endophytic tillers in field populations of tall fescue grass

To demonstrate that vertically transmitted microorganisms can generate TGEs in host plants, the following criteria need to be satisfied:

(1) An environmental stimulus to a parent plant must alter the phenotype of the offspring. The most basic experimental design involves growing parental plants in two environments, then growing their progeny in one of the environments and assessing phenotype (Fig. 1). This criterion is satisfied if $P_A \neq P_B$ and/or $P_C \neq P_D$, where P_X represents the mean value of a phenotypic trait in offspring of group X.

(2) The alteration of offspring phenotype caused by the stimulus must differ between symbiotic and nonsymbiotic plants. This criterion is satisfied if $(P_A - P_B) \neq (P_C - P_D)$. Note that the right side of the equation represents the TGEs mediated by the host plant itself, while the left side represents the combined TGEs mediated by host and symbiont (and any interaction).

To demonstrate that symbiont-mediated TGEs are *adaptive*, two additional criteria must be satisfied.

(3) The fitness of matched offspring must be higher than the fitness of unmatched offspring. This criterion is satisfied if $(\omega_A > \omega_B)$ and/or $(\omega_C > \omega_D)$, where ω_X represents the mean fitness (e.g., seed number, growth rate) in offspring of group X.

(4) The fitness benefit from having parents in a matched environment must be greater for symbiotic vs. nonsymbiotic offspring. This criterion is satisfied if $(\omega_A - \omega_B) > (\omega_C - \omega_D)$.

Box 1. Criteria for demonstrating microbially mediated transgenerational effects

(*Schedonorus arundinaceus*) increased more rapidly under higher herbivore pressure than when herbivores were experimentally excluded (Clay et al., 2005). While this could simply indicate a fitness benefit of symbiosis (Gundel et al., 2011b), it could also reflect an increased rate of vertical transmission from symbiotic plants to their progeny. In the tall fescue case, the fitness benefits of symbiosis are clear, but whether increased symbiont prevalence occurs via TGEs (Box 1, Criteria 3, 4) is not known.

Second, the grass-*Epichloë* symbiotum may respond to herbivore or pathogen damage by altering the abundance of hyphae in seeds (Mechanism 2) or the concentration of fungal secondary metabolites (Mechanism 3). In parental plants, fungal alkaloid production can increase when plants are challenged with herbivory (Boning and Bultman, 1996; Bultman and Bell, 2003; Bultman et al., 2004); results that parallel the upregulation of fungal genes encoding alkaloids (Sullivan et al., 2007; Zhang et al., 2009). Induced responses to damage that are not microbially mediated appear to be a common pathway for TGEs in plants (Agrawal et al., 1999; Agrawal, 2001; Rasmann et al., 2012; Luna et al., 2012; Slaughter et al., 2012). However, such effects have not yet been fully investigated for defensive mechanisms facilitated by symbioses. Host seeds can be loaded with fungal alkaloids (see Vazquez-de-Aldana et al., 2001), which may be the cause of observed endophyte-mediated deterrence of seed predators, seed pathogens (Madej and Clay, 1991; Uchitel et al., 2011; Pérez et al., 2016), and even herbivores of seedlings. For

example, *Lolium perenne* seedlings with the endophyte *Epichloë festucae* var. *lolii* were less attacked by the Argentine stem weevil (*Listronotus bonariensis*) than endophyte-free counterparts (Stewart, 1985); thus, a direct protective role of accumulated alkaloids in seeds could be transferred to seedlings. However, the protection conferred by alkaloids stored in the seeds vs. synthesized during the seedling growth phase have not yet been parsed, so in most cases, it remains unclear whether the effect is transgenerational (Box 1, Criterion 1) or simply an environmental response of the seedling. One exception may be the association of *Ipomoea* spp. and *Periglandula* fungi in which very high levels of ergot alkaloids have been quantified in both seeds and germinating seedlings suggesting these compounds are maternally transmitted (Beaulieu et al., 2013). More studies are required to reveal the ecological role of the seed-accumulated alkaloids on the fitness of emerging seedlings of grasses.

Besides herbivore deterrence, the *Epichloë* symbiosis may produce secondary metabolites that improve plant tolerance to abiotic stress (Malinowski and Belesky, 2000; Hamilton et al., 2012). For example, both the accumulation of osmoprotectants (such as the sugar alcohol mannitol, Rasmussen et al., 2008) and increased antioxidant capacity (Hamilton et al., 2012) have been suggested as mechanisms of endophyte-mediated tolerance of abiotic stressors. Symbiosis with *Epichloë* spp. can alter the content of antioxidants in seeds of both *Festuca rubra* and *L. multiflorum* (Gundel et al., 2012, 2015). However, while it is clear that this mechanism would be microbial in nature (Box 1, Criterion 2), it is not yet known whether levels of microbial products in offspring are caused by a response of the parental plant to the environment (Box 1, Criterion 1) or whether the altered phenotype increases plant fitness (Box 1, Criteria 3, 4).

Third, because the fungus reproduces clonally and is vertically transmitted, it may carry information from one environment to the next via its own priming or acclimation response (Mechanism 5, above). For example, one reported case of resistance to an herbicide (diclofop-methyl) in *L. multiflorum* was ascribed to symbiosis with an *Epichloë* sp. (Vila-Aiub et al., 2003). Plant tolerance was built up by recurrently exposing plant to sublethal doses, and the clonally generated offspring of parents exposed to the herbicide had higher tolerance than those from non-exposed plants (Vila-Aiub and Ghersa, 2005). Since the endophyte was experimentally removed from mother plants, the sexually produced progeny did not inherit the increased tolerance to herbicide (Vila-Aiub and Ghersa, 2005). This result suggests that the vertically transmitted endophyte, which is known to increase herbicide tolerance in the plant, may also modulate the acclimation response in the progeny. Further work is needed to test whether increased herbicide tolerance translates to fitness benefits, as expected if TGEs are adaptive (Box 1, Criteria 3, 4).

CONCLUSIONS

Investigating the role of vertically transmitted microorganisms in TGEs opens a wide window for new and exciting research on plant adaptations. We envision a fertile field of research that brings together the accumulating evidence that many symbiotic microbes are present in seeds with our increasing understanding of the genetic underpinnings of phenotypic variation. We hope this approach will improve understanding of symbionts' roles in plant adaptation, especially in the context of global change.

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