

Maternal effects on tree phenotypes: considering the microbiome

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Abstract

The biotic and abiotic environmental experience of plants can influence the offspring without any changes in DNA sequence. These effects can modulate the development of the progeny and its interaction with micro-organisms. This interaction includes fungal endophytic communities which have significant effects on trees and their associated ecosystems. We highlight potential maternal mechanisms through which endophytes could influence the progeny. We argue that a better understanding of these interactions might help predict the response of trees to stress conditions and to enhance the efficiency of tree breeding programs.

The tree's phenotype and its endophytes

Genotype and environmental conditions during development are the strongest determinants of an individual tree phenotype. Although genetic and environmental conditions are dominant, in recent years, there has been accumulating evidence indicating that the biotic and abiotic environmental experience of the parents can modulate the development and pathogen resistance of their progeny [1,2]. Specifically, maternal plants are supposed to have significantly higher impact on offspring phenotype and fitness than paternal plants, because they directly provide seedlings with a large amount of substances [3,4].

An aspect of the biotic maternal environment that might have important consequences on the phenotype of the plant, but which has hardly been explored, is the impact of associated microbial communities. There is an increasing realization that these microbial communities are an important part of the extended genotype and phenotype of the plant [5]. They can affect various aspects of the physiology, metabolism and ecological interaction of the plants.

The importance of associations between plants and the foliar microbiome has been highlighted previously [6], however the mechanisms and effects of the interaction between these entities remain poorly understood. In this opinion article, we explore possible influences of endophytic fungi on trees within the context of maternal effects (Figure 1). Despite being a big challenge, the research to identify the mechanisms involved in the transmission of maternal environmental effects on the progeny holds much promise to contribute to understanding plant ecology. Such information could also offer the possibility to influence the development and resistance of plantation trees. However, before mastering this technique, a number of challenges remain to fully understand these processes.

Maternal environmental effects on trees

Environmental maternal effects can influence the offspring without any changes in DNA sequence [7]. In plants, these effects have been reported on seed traits, germination, seedling performance, plant-pathogen, and plant-insect interactions [7-10]. Maternal effects have also been shown to attenuate negative consequences related to climate change [2]. There are broad advances in understanding plant offspring responses to abiotic and biotic maternal environmental factors, especially within the first generation (see [8] for review). However, most studies of transgenerational maternal effects on plants over several generations have focused on short-lived annuals. For example, *Arabidopsis thaliana* (*Arabidopsis*) has increased seed production under heat stress in the F₃ generation if the F₀ and F₁ experienced the same stress, even when F₂ was grown without stress [11]. Progeny of *Arabidopsis* plants exposed to herbivores are also more resistant to subsequent attack in the next two generations, compared to progeny from unthreatened parents [12]. Available information for long-lived woody

species across generations is much more limited, because they do not reach reproductive maturity for decades [8,13,14].

The work of Borgman *et al.* [15] is, to our knowledge, the only study on transgenerational maternal effects in long-lived woody plant species over generations. By using twig clippings and seeds of *Pinus aristata* and *Pinus flexilis* from the same maternal plant, this study showed that the effect of the local annual weather conditions in the maternal environment over 2 years affected maternal tree twig growth, seed provisioning and progeny performance. All these traits were positively affected by the warm dry year and negatively affected by the cold wet year. The differences in seed traits corresponded to differences in early seedling growth. However, inter-annual variation in mother tree twig traits did not predict seed mass.

Seed provisioning and epigenetic modifications are the two mechanisms through which these maternal effects are transmitted to the next generation. Seed provisioning, a mechanism in which the mother plants influences the resources allocated to seeds, is thought to affect one offspring generation and becoming less relevant in maturity [3]. By contrast, epigenetic modifications are a set of molecular processes transmitted via the seeds that modulate the phenotype by modifying gene expression; these include for example, DNA methylation, histone modification and small RNA interference [8], and may influence the progeny for a longer time or even several generations [16].

Endophytic communities of trees

Plants comprise one of the biggest terrestrial ecosystems for microbial organisms [6], and microbial communities have colonized all plant organs. Fungi and bacteria are the most prominent members of these communities, but other phylogenetic lineages can be found (e.g., lichens [17], invertebrates [18], viruses [19]). Bacteria and fungi grow

either as phyllosphere [20,21] and mycorrhizal endophytes [22] in plant tissues or as epiphytes [23] on plant surfaces without causing any harmful change in the plant phenotype.

Fungal phyllosphere endophytes can, as far as has been tested, be found in all biomes and an enormous diversity of fungal species has been documented [24-26]. A multitude of studies have shown that endophytic communities play important roles to enhance nutrient uptake, increase environmental stress tolerance, and protect the tree host from pathogens and pests (see [27] for review). Importantly, by faster population and species turnover, endophyte communities most likely respond faster to environmental changes than host plants. Actually, studies have shown that fungal endophytes may mitigate negative consequences of environmental stresses related to climate change in agricultural and natural plant communities [28]. Although our understanding of the exact role of different members of the endophyte community is still limited, their ubiquity, number and diversity suggest that they are an important part of the extended genotype and phenotype of the plant [5]. As a result, the endophytic communities can have important consequences for plant fitness and therefore on plant communities and also ecosystem function.

Of equal importance is to understand how the host plant influences the associated microbiome. The effect of biotic and abiotic environmental stress and the effect of different plant genotypes on fungal endophytic communities have been studied mostly in *Arabidopsis*. The frequency and composition of endophytes in this annual plant depends on the time of the year and the phenological state of the plant [29], but also strongly correlates with the genotype of the plants [30]. Additionally, it has been shown that endophyte variation is most likely related to plant loci that are involved in

defense and cell wall integrity [30] and strongly suggests that resistance to pathogens will influence the endophytic community.

In trees, only few examples about the influence of resistance towards pathogens on endophytic communities are known. One study showed that needles susceptible to *Cyclaneusma* needle-cast contained more endophytes than trees that did not show any symptoms, but it was not clear how this related to the genotype of the tree [31]. A more recent study demonstrated that *Ulmus* species susceptible to Dutch elm disease had a higher frequency and diversity of endophytic fungi in their xylem tissues than resistant elms [32]. The difference in xylem endophyte diversity in this study correlated with differences in the constitutive phenolic profiles of the xylem [23]. This is most likely related to differences in the syringyl/guaiacyl lignin ratios in susceptible and resistant trees [33] and may have triggered diverse tree-endophytes associations.

Endophytes and the maternal effect: possible mechanisms

The reciprocal influence of the endophytic communities and their host plants pointed out above, will also influence both entities in the next generations through maternal effects. This can happen through multiple mechanisms. For example, direct influence occurs where endophytes are transferred vertically from the mother to the offspring through the seed. This transmission of maternal endophytes can enhance seedlings performance and resistance to biotic or abiotic stress, conferring a competitive ability to the offspring [34-37], but has in some cases been shown to be negative [38]. Although the norm in obligatory grass endophytes [36,37], vertical transmission of leaf endophytes in forbs [39] and trees [24] has been recorded infrequently though.

Maternal endophytes could affect seed provisioning, whereby the mother can differentially allocate nutrients to the offspring. The influence that fungal communities

can have on transgenerational effects on plants in this way is well known from mycorrhizal communities. Studies in many mycorrhizal systems have time after time shown the effects of this mutualistic symbiosis on plant performance in a given generation [22]. By providing increased nutrient levels for the production of flowers, fruits and seeds, mycorrhizal symbioses can have significant influence on the performance of subsequent generations [40-42]. Analogous, many phyllosphere endophytes exhibit developmental and physiological effects on trees (see above). These influences will likely also change the resource investment of the mother in the seed.

Epigenetic modifications in plants by interacting fungi could alter the phenotype of the offspring. Such mechanisms have been demonstrated for pathogens, where some fungal toxins alter histone acetylation, thereby interfering with host gene regulation of jasmonic acid and ethylene signal pathway related genes [43,44]. Both of these pathways are known to have critical roles on the development of the seed, even regulating the transfer of nutrients to the seed, with the subsequent influence in the development and resistance of the offspring [45-47].

Open questions and opportunities

Increasing growth and resistance of trees is important for survival under stress conditions and for increasing the production in tree plantations. Selection of tree genotypes less susceptible to pests and diseases is one of the measures to increase plant survival in stress environments. However, as we have pointed out, studies have shown that plant maternal environment, including endophyte communities, additionally influence the offspring, including germination, seedling development and resistance. The understanding of the mechanisms of interaction among maternal environment and their progenies to expose mother plants to appropriate environmental cues can thus be

potentially used as tools to enhance the efficiency of breeding programs. When doing so, it would be important to also consider the composition and possible influence of endophytic communities.

It is not impossible to envision exploiting information about maternal effects for an integrated approach to reducing pest and disease impacts on plantation trees, as well as to predict how plants will respond to stress conditions in a changing environment. When this is done, we argue that the contribution of endophytes to altering the material environment should also be considered. Much work is needed in this regard. Key questions that remain are: (i) how much of the endophytic community is actually inherited from the mother, (ii) is co-evolution a strong factor in plant-endophyte interactions, (iii) what is the influence of the maternal endophytic community on the development and microbe interaction of the progeny, (iv) what is the relative contribution of the maternal endophyte community compared to the genotype and biotic and abiotic environment to the phenotype of the progeny, and (v) what is the extent of the influence of the paternal effect.

The growth in use of next generation sequencing to characterize microbial communities is making it possible for the first time the development of studies to address these questions. Previous tools, including culturing and cloning of amplified barcodes, were too time consuming and labor intensive, and did not allow for deep enough coverage of extreme diverse communities such as foliar fungal endophytes, or the various other microbial communities that interact with all plants. Similarly, these and other high throughput technologies also provide unprecedented opportunities to characterize the functional role of endophytic microbial communities in plants, through parallel transcriptomics and proteomics [48,49]. Experimental set-ups where these tools are applied to microbial inoculation experiments on sterile mother plants [50] or in

experiments where clonal mother plants are replicated across sites with different environmental and microbial compositions [51], holds particular promise to address specific hypotheses linked to the questions we raise above. Addressing these questions might start improving prediction of ecological and evolutionary outcomes of plant-microbe-environmental interactions of progeny phenotypes.

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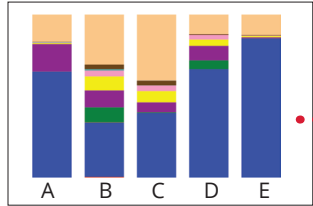
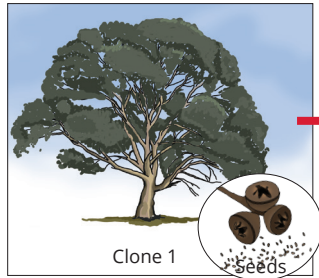
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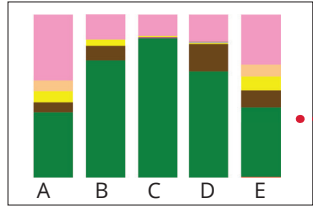
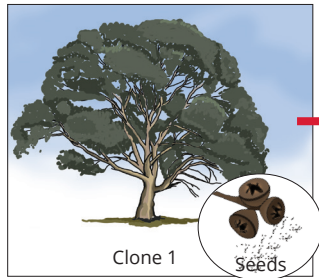
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Figure 1. Hypothetical relationships between environment, fungal communities, genotype and phenotype across two generations of trees. This picture illustrates a single mother clone in two different environments characterized by contrasting biotic and abiotic conditions. The vertical bars are the fungal endophytic community within each clone, with taxonomic diversity in the fungal endophytic communities indicated by different colors. Maternal environmental effects at a particular clone may result from different phenotypic plasticity in the seeds and even in the phenotype and resistance of seedlings growing in a common environment (continuous arrow); Seed size from maternal environment A was bigger and produced taller and more resistant seedlings than seeds from maternal environment B. The influence of the maternal environment on the fungal endophytic community of the progeny (vertical bars without colors) is, however, unknown. Differences in the maternal fungal endophytic community should be considered as biotic maternal environmental effects. It is also important to understand to what extent these maternal fungal endophytic communities could be transferred vertically through the seeds across next generation (discontinuous arrow). If these maternal environmental or fungal community effects translate into changes in the development, resistance and/or fungal endophytic community of the progeny, it could have important impacts on ecosystems and for the management of trees in production.

Environment A



Environment B



Common environment

