

## Article Addendum

# Within-population genetic variability in mycorrhizal interactions

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The geographic mosaic theory of coevolution hypothesizes that natural selection on species interactions varies among ecosystems, partly because the genes involved in species interactions differ in their fitness effects among environments. This selection mosaic may be expressed, at the extreme, as ecological outcomes ranging from mutualism to parasitism among environments. In a recent laboratory experiment on the interaction between a plant, bishop pine (*Pinus muricata*), and a root-symbiotic ectomycorrhizal fungus, *Rhizopogon occidentalis*, we demonstrated the potential for selection mosaics in that interaction, and the existence of substantial within-population genetic variation for symbiotic compatibility in the interaction. Here, we present the results from a second experiment on the interaction between the same ectomycorrhizal fungus and a different plant, shore pine (*Pinus contorta* var. *contorta*), designed to test for the presence of genetic variation for symbiotic compatibility in another similar system, and also to test whether such variation might be generated in part by adaptation of fungal lineages to individual trees. In this experiment, we found no genetic variation among plant lineages for compatibility with the fungal symbiont, and no evidence for adaptation of fungal lineages to individual plants, but the two fungal genotypes differed greatly in their compatibility with the plant hosts. Specifically, one of the two fungal genotypes not only colonized host plants less intensively than the other, but also had a negative effect on plant growth. Altogether, these results suggest the potential for ongoing natural selection on the ectomycorrhizal fungus, *R. occidentalis*, for different levels of symbiotic compatibility with particular pine hosts, but the mechanisms generating and maintaining genetic variation for symbiotic compatibility remain unclear. Such results will aid in efforts to develop realistic models of how plants and their symbionts coevolve over broad geographic ranges in which they co-occur.

One of the fundamental processes generating and maintaining biodiversity is coevolution—the reciprocal evolutionary effects that interacting species have on each other's traits. Because the ecological interactions between two species often differ among environments, e.g., ranging from mutualism to parasitism, natural selection that species exert on each other's traits may vary among environments. This spatial variation in selection has been described as a geographic selection mosaic, and can be viewed either broadly or more directly as a genotype by genotype by environment interaction.<sup>1,2</sup> Selection by species on each other may also vary in its degree of reciprocity among populations, generating coevolutionary hotspots in some locations and coldspots in others.<sup>3,4</sup> When combined with dispersal, gene flow, which influence the distribution of traits among populations and even the rates of coevolution, these processes result in a geographic mosaic of coevolution.<sup>1,2</sup> Mathematical models and microcosm experiments of the geographic mosaic theory of coevolution have shown that such spatially-structured coevolution can generate and maintain genetic and ecological diversity within and among populations of species.<sup>5-9</sup>

Some field studies also now provide evidence of geographic selection mosaics in natural populations,<sup>10-16</sup> but most empirical studies have not controlled for genotypes of both interacting species across multiple environments to explicitly assess whether the fitness of one species is influenced by genetic variation in another interacting species, and whether this effect varies across different biotic and abiotic environmental variables. That is, these studies assess whether selection on coevolving species appears to differ among environments, but not whether those differences are due to variation in how selection acts on the same genotypes in different environments. We did so in a recent laboratory study of the ectomycorrhizal symbiosis between bishop pine (*Pinus muricata*) and a *Pinus*-specific Basidiomycete fungal false truffle (*Rhizopogon occidentalis*), in which the host plant typically provides photosynthetically derived carbohydrates to the root-symbiotic fungi, and which in turn provides the host plant enhanced access to mineral nutrients in the soil. By measuring plant and fungal performance of multiple symbiotic combinations of plant and fungal genotypes (2 half-sib families of each) across two soil types and in the presence or absence of other non-mycorrhizal soil microbes,<sup>17</sup> we showed the potential for geographic selection mosaics on plant and fungal fitness. For example, fungal colonization intensity on host roots, which should correlate positively with fungal fitness, depended strongly on an interaction between plant genotype

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and soil type. In addition, the two plant lineages differed substantially in their overall growth responses to fungal inoculation, and the two fungal lineages differed significantly in their overall effects on root growth of the plants.

One striking aspect of these results is that despite limited sampling of the genetic diversity of hosts and symbionts from a single location—a single pine forest stand in Point Reyes National Seashore in Marin County, California, USA—we found substantial heritable genetic variation within the host and symbiont for their responses to each other and to other environmental factors. This observation raises two important questions. First, is such substantial extant genetic variability for response of host and symbiont to each other the norm in ectomycorrhizal symbioses? These symbioses are prevalent in dominant managed and natural ecosystems worldwide, including the extensive forests dominated by plants in the families Pinaceae, Fagaceae, Myrtaceae and Dipterocarpaceae, but we know very little about geographic patterns of genetic variability in traits of the ectomycorrhizal symbiosis, such as compatibility between particular pairs of plant and fungal species. Second, how is this genetic variation maintained and structured within populations? For example, in our system, such variation could be driven by gene flow among populations, and/or by local coevolutionary selection between pines and false truffles. In fact, our study of bishop pines and false truffles from Point Reyes National Seashore was inspired by a previous experiment in another pine-false truffle interaction—the ectomycorrhizal symbiosis between shore pine (*Pinus contorta* var. *contorta*) and the same false truffle *R. occidentalis* from the coastal dunes of Oregon, USA.

The results of that experiment, presented here for the first time, not only allow us to test for genetic variability in the ectomycorrhizal symbiosis in an additional study system, but more importantly to begin to address the question of how variation is maintained and structured within populations. The details of the shore pine experiment, including experimental set-up and data collection and analysis, were identical in most respects to the previously published bishop pine experiment,<sup>17</sup> except for three important differences: the only two factors manipulated were plant and fungal genotype, we examined additional plant genotypes (4 half-sib families), and the fungal spore families were collected explicitly from the roots of two of the same maternal trees whose seeds were included in the experiment (rather than haphazardly from nearby trees). We compared plant growth (relative growth rate of height over 19 weeks) and fungal colonization (# root tips colonized after 19 weeks) among eight different combinations of plant and fungal lineages (replicated six times each), two of which represented “home” combinations in which fungal spore families were paired with the offspring of the same maternal tree from which the fungi were collected, and six of which were “away” combinations of fungi with seedlings from maternal trees located at least 50 meters away from the home tree of the fungi. We also included six non-inoculated replicates of each plant family, so that we could calculate plant growth responses with and without fungal colonization, i.e., responses of plants to fungal colonization. Overall, this experimental design allowed us to test not only for within-population genetic variation in compatibility between the plants and the fungi, but also to test whether such variation might be generated by adaptation of fungal lineages to individual trees.

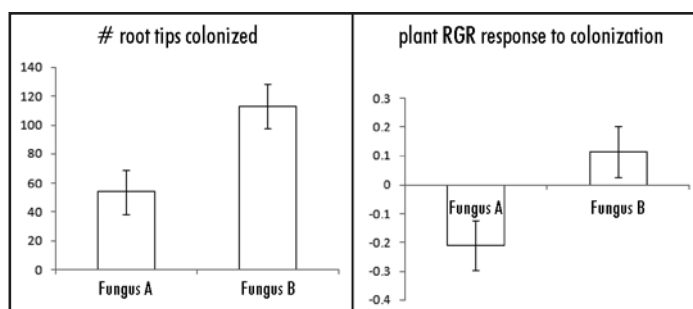


Figure 1. Differences between two fungal genotypes (A and B) in mean ( $\pm$  SE) root tip colonization and relative growth rate (RGR) response to colonization of shore pine (*Pinus contorta* var. *contorta*). Plant RGR response to mycorrhizal colonization was calculated for each mycorrhizal replicate as  $\ln(X_m/X_n)$  where  $X_m$  is RGR of mycorrhizal plants and  $X_n$  is the mean RGR of non-mycorrhizal plants. RGR was calculated as  $[\ln(Ht2) - \ln(Ht1)]/\text{days}$  where  $Ht1$  and  $Ht2$  are plant heights before and after inoculation, respectively, and  $\text{days}$  is the number of growth days after inoculation. Error bars represent standard errors calculated as variation among  $n = 24$  replicates each for Fungus A and Fungus B.

In contrast to the bishop pine experiment, the four plant genotypes did not differ in their relative growth rate responses to fungal colonization ( $F_{3,40} = 0.26$ ,  $p = 0.851$ ) or in fungal colonization intensity ( $F_{3,40} = 1.21$ ,  $p = 0.318$ ), nor did plant genotype interact with fungal genotype to influence those same responses ( $F_{3,40} = 1.53$ ,  $p = 0.222$ ,  $F_{3,40} = 1.24$ ,  $p = 0.307$ ). The two fungal genotypes differed strongly, however, in their effects on plant growth ( $F_{1,40} = 7.09$ ,  $p = 0.011$ ) and in their colonization intensity on plant roots ( $F_{1,40} = 7.33$ ,  $p = 0.0099$ ). Specifically, one fungal genotype had a significantly negative effect on plant growth rate, and also exhibited lower root colonization intensity than the other fungal genotype (Fig. 1). Overall, these results suggest that plant genetic variability for symbiotic compatibility with the ectomycorrhizal symbiont *R. occidentalis* may be lower in the Oregon Dunes shore pines compared to the Pt. Reyes bishop pines. The fungal symbiont, however, exhibited striking genetic variability in its compatibility with its pine hosts at both sites, despite the inclusion of only two different fungal genotypes from each site, and we found no evidence of adaptation by fungi to individual trees.

Previous studies have found genetic variation for compatibility in the mycorrhizal symbiosis among lineages or isolates of plant or fungal species.<sup>18</sup> In most cases, however, genetic variation was not usually sampled from particular populations, and/or the experiments did not control genetic variation in both the host and the symbiont. Our previous study of within-population variability<sup>17</sup> and the study presented here, however, by explicitly controlling for both plant and fungal genetic variation within particular populations, were designed to lend insight into the potential coevolutionary dynamics within those populations. Both of those studies found substantial variation within fungal populations, and inconsistent levels of variation within plant populations. A third study of ours, designed to explore variability in pines and false truffles among populations on the west coast of North America, rather than within populations, found consistent variation among fungal populations for compatibility with plants, and no larger scale geographic pattern of variation among plant populations for compatibility with fungi.<sup>13</sup>

Altogether, these results suggest the existence of the raw material (genetic variation) for ongoing natural selection on the

ectomycorrhizal fungus *R. occidentalis*, for different levels of symbiotic compatibility with particular pine hosts, both within and among fungal populations. What forces may be generating and maintaining this observed genetic variability are still unclear, although we found no evidence that adaptation of fungi to individual pine genotypes in the Oregon Dunes is responsible. Furthermore, we need to know more about how commonly measured fungal performance variables, such as root colonization, as well as less often measured variables, such as the mass of extra-radical mycelium in the soil, relate to fungal fitness in different environments. Pines, on the other hand, may harbor some heritable genetic variation for compatibility with *R. occidentalis* within some populations, such as the bishop pines at Point Reyes, and very little additional variation among populations leading to larger scale geographic patterns in compatibility. Such empirical results may aid efforts to develop realistic models of how plants and their potentially mutualistic root symbionts coevolve over the broad geographic range in which the same taxa co-occur.

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