A new dawn – the ecological genetics of mycorrhizal fungi

Many human activities, such as ore mining and smeltering, sewage sludge treatment and fossil fuel consumption, result in toxic soil concentrations of 'heavy metals' (Al, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Ti, Zn and others) (Gadd, 1993). There are also natural soils, such as serpentine, with levels of heavy metals that inhibit or preclude the growth of many plants and soil microorganisms. However, certain plants and microorganisms do grow in these metalliferous sites. Understanding the physiology, ecology and evolution of tolerance to elevated soil metal concentrations is important in an applied setting, and is also of interest in theoretical biology. Applied importance relates to the improvement of forest health in areas subject to increasing pollution, rehabilitation of severely polluted sites by phytostabilization of metals, and metal removal using hyperaccumulating plants (Krämer, 2000; Ernst, 2000). Areas of theoretical interest include the evolution of local adaptation (Sork et al., 1993) and how it is shaped by the combined influences of natural selection, gene flow and genetic architecture, as well as metal influences on various species interactions (Pollard, 2000). A paper appears on pages 367–379 in this issue by Jan Colpaert and coworkers which adroitly combines the disparate fields of physiology, genetics and ecology to answer several outstanding questions concerning heavy metal tolerance in mycorrhizal fungi.

Mycorrhizal fungi, which interact mutualistically with the majority of plant species, are well known for improving the P status of their hosts (Smith & Read, 1997). Some mycorrhizal fungi are also able to mobilize N and P from organic substrates and to provide plants with improved micronutrient and water acquisition, pathogen resistance, and a variety of other benefits (Smith & Read, 1997). One of these additional benefits is the amelioration of toxicity in metalliferous soils.

Surprisingly widespread tolerance

Several negative impacts of heavy metal pollution on mycorrhizal communities are well documented. Extremely polluted sites typically have lower rates of mycorrhizal colonization, fewer fungal propagules, and lower fungal species diversity (Gadd, 1993; Hartley et al., 1997; Leyval et al., 1997). Evolutionary impacts of metalliferous soils on plants have received much more attention than impacts on fungi. Some of the steepest selection gradients yet recorded in plants occur across the transitions from clean to heavily polluted sites (Antonovics et al., 1971; McNeilly, 1979). Evolutionary adaptation of these plants to the polluted conditions can be accepted based on numerous lines of evidence, including genetic variation in tolerance, heritability of tolerance, higher fitness of tolerant individuals on polluted sites, and higher fitness of non-tolerant individuals on unpolluted sites. One might expect such sites to exert similarly strong selection on mycorrhizal fungi, and that it might result in genetic differentiation among fungal populations. Indeed, there are numerous reports of the isolation of metal tolerant strains of both arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi from polluted sites (Hartley et al., 1997, Leyval et al., 1997). However, very few studies have undertaken proper comparisons that might demonstrate a statistical difference in tolerance between communities or populations of fungi occurring in polluted soils versus those in similar, but unpolluted soils. Furthermore, there has also been some disagreement over the existence of novel, adaptive tolerance (as opposed to widespread, constitutive tolerance) because fungal strains or species from unpolluted sites often have remarkably high heavy metal tolerance.

In the study presented in this issue, Colpaert et al. demonstrate a clear statistical difference in tolerance between strains of the ECM basidiomycete *Suillus luteus* from a polluted and a nearby unpolluted site. The two sites are former industrial lands, only one of which has elevated heavy metals, and which were planted with nursery saplings by the same forester about 25 years ago. Colpaert et al. obtained *Suillus* isolates from randomly selected fruitbodies occurring at each site. They found that the Cd and Zn tolerances of strains from the polluted site were much higher than the tolerances of strains from the unpolluted site, while strains from the two sites did not differ in Cu or Ni tolerance. Cd and Zn are drastically elevated at the polluted site, Cu is only somewhat elevated, and Ni levels are equivalent at the two sites. Although there was significant variation in tolerance among strains from the unpolluted site, suggesting considerable standing genetic variation, the tolerances achieved in the polluted site were far higher than those seen in any strain from the unpolluted site.

Since the two sites are separated by only 20 km, and the trees were brought to the site as unsterile containerized saplings, it is conceivable that the *Suillus luteus* populations at the two sites are descended from an identical source population. If this is true, it means that from an evolutionary perspective the phenotypic differences in tolerance arose extremely rapidly. However, because only a single site of each habitat type (polluted versus unpolluted) was studied, the higher tolerance of one population could be due to a historical artifact. Similar studies of multiple sites will be required to demonstrate a statistical correlation between tolerance and edaphic factors, and to suggest a causal relationship. Nevertheless, a *Suillus luteus* isolate from another polluted site had similarly high tolerances, and similar patterns were seen in single isolates of *S. bovinus* from polluted and unpolluted sites (Colpaert & Van Assche, 1992), all of which indicates that patterns reported in the present study are probably due to soil pollution.

The likelihood of rapid evolution of tolerance in this fungus suggests that a large selection coefficient may be operating. Of course, the demonstration of natural selection and evolutionary change would require several additional tests, including ruling out developmental plasticity as an explanation for the phenotypic differences in metal tolerance. While the unequivocal demonstration of natural selection is a prodigious undertaking, there are essentially no data on this important subject in mycorrhizal fungi. The extensive compilation of studies demonstrating natural selection in the wild by Endler (1986) does not include a single fungal example (Endler, 1986). Heavy metal tolerance may offer a productive system for pursuing studies of natural selection in fungi.

Input from population genetics

In a recent Commentary (Read, 2000), Read points out several dangers of inferring ecological function solely from DNA data. The Colpaert et al. paper in this issue answers Read's imperative by combining direct functional

measures, in this case metal tolerance, with analysis of genetic diversity at the DNA level.

Data were collected on polymorphisms across numerous putative loci among the strains from both populations using the anonymous PCR technique, inter-simple sequence repeat (ISSR) amplification. This approach is akin to the RAPD technique, and is directed at amplifying highly variable microsatellite regions. By scoring the presence and absence of bands across strains as dominant markers, the authors could distinguish individuals (genets) and analyze population genetic structure. Similar techniques have recently been used to measure the clone sizes of ECM fungi in several studies (Bonello *et al*., 1998; Gherbi *et al*., 1999; Jumpponen, 1999). However, the present study is one of the first to relate genetic structure to an ecological influence such as habitat variation. Colpaert *et al*. found that the population from the polluted site had much lower genetic variation than the population from the unpolluted site. If these patterns are replicated in other sites, it may be possible to make a strong case for reduced genetic variation, in addition to reduced species diversity, in polluted sites. They also found large fungal genets, spanning 10–12 m, at each site, demonstrating that some genotypes were quite vigorous at the polluted site.

The molecular data also showed that the two populations are genetically distinct. If the two populations shared the same inoculum source, the rapid genetic divergence is striking. Another interesting genetic aspect of their data is that the most Cd tolerant strains were the least tolerant of Zn, and *vice versa*. It would be interesting to know whether this result is due to the limited time period over which the site has been occupied (implying few recombination events) or is due to a constraint arising from the underlying genetic architecture of metal tolerance (in the simplest case, alternative alleles at a single locus).

Impacts on mycorrhizal plants

The impacts of fungi on metal tolerance in their host plants appear to be complex, as some studies have demonstrated reduced metal concentrations in mycorrhizal plants as opposed to non-mycorrhizal plants, while others have not found such an effect (Hartley *et al*., 1997; Leyval *et al*., 1997). In addition, the most tolerant fungi *in vitro* are not necessarily the most tolerant in the symbiotic condition, nor the most beneficial to their hosts (Hartley *et al*., 1997; Leyval *et al*., 1997). Regardless of metal concentrations in host plants, nearly all studies agree that plants inoculated with ECM fungi grow and perform better than non-mycorrhizal plants under low N and P conditions, both in polluted and unpolluted soil. Furthermore, Colpaert *et al*. (1993) have previously shown that an *S. luteus* isolate from the same polluted site was more effective than an isolate from an unpolluted site in reducing shoot Zn concentration in a high Zn treatment.

In the present paper, Colpaert *et al*. show that pine needles collected at the polluted site had normal Zn concentrations but had elevated Cd concentrations, suggesting that the plant–fungal combination was quite effective in excluding Zn, but not Cd. It is important to note that *Suillus luteus* was probably not the only mycobiont at these sites. Analysis of the community structure of ectomycorrhizal fungi on and off polluted sites would repay further study. In highly mycotrophic trees such as oak, pine or fir, mycorrhizal associations are essential and these trees are unlikely to colonize a site that is too toxic to support ECM fungi. Therefore, fungal tolerance of heavy metals such as Cd may be of great value to the plant even if Cd uptake is not reduced. Now that well characterized isolates and field sites are available, a number of interesting field manipulations could be performed to ascertain the relationships between fungal tolerance, plant tolerance, plant–fungal interactions, and plant and fungal fitnesses across pollution gradients.

Local adaptation and co-evolution

Matching allele models predict that mutualistic interactions will favor a single, optimal genotype in both host and symbiont (positive frequency-dependent selection) and will thus lead to evolutionary stasis and reduced genetic variation in both host and symbiont (Law & Koptur, 1986). In contrast, these models predict an 'arms race' between antagonistic interactants, in agreement with the vast genetic variation within populations that conditions interactions between hosts and parasites (Burdon & Thrall, 1999). However, to the degree to which a mutualistic symbiont must also adapt to its external environment, or the quality of the interaction changes across habitats, the prediction of stasis and little genetic variation breaks down (Nuismer *et al*., 1999). Indeed, conditional outcomes of mutualistic interactions appear to be the norm (Bronstein, 1994), suggesting that genetic variation and ongoing coevolution (in the sense of reciprocal genetic change) may be widespread, even in mutualisms.

Coevolution in the mycorrhizal symbiosis is likely to be important (Bever *et al*., 1997), but has received little attention. Similarly, local genetic adaptation, such as to metalliferous soils, may well be widespread in mycorrhizal fungi. Perhaps coevolution has been considered unimportant to mycorrhizal interactions because plants display little mycorrhizal specialization and can associate with hundreds or thousands of fungal species (but see Taylor & Bruns, 1997, for an important exception). However, 'diffuse' selection, mediated by interactions with a guild comprising many species, can be a powerful force for evolutionary change (Juenger & Bergelson, 1998). Furthermore, 'pairwise' coevolution is possible even in the midst of interactions with other members of the same guild, although the conditions under which this can occur are restricted (Hougen-Eitzman & Rausher, 1994).

Local adaptation in fungi may well contribute to determining the costs and benefits of particular plant–fungus interactions. For example, Zn tolerant *Suillus* genotypes probably increase plant fitness in polluted, P-deficient sites, while the same genotype could theoretically decrease plant fitness in an unpolluted, P-rich site, due to the carbon drain of the extensive extramatrical mycelium. These ideas suggest a conceptual framework – the geographic mosaic (Thompson, 1999). The geographic mosaic perspective is based on the assumption that species interactions vary in outcome in a spatially explicit manner due to habitat influences, gene flow, historical contingency and selection. A speculative application of the geographic mosaic concept to mycorrhizas and metal tolerance is presented in Fig. 1. There are several predictions concerning species interactions and co-evolution that arise from the geographic mosaic concept (Thompson, 1999). These predictions have been little tested in any group of organisms, but mycorrhizal interactions and metal tolerance may offer a system in which to do so.

Summary

Mycorrhizal interactions have not served as model systems in the fields of ecology and evolution. This is probably because the most basic ecological variables – such as the distribution, abundance, survival and fecundity of indi-
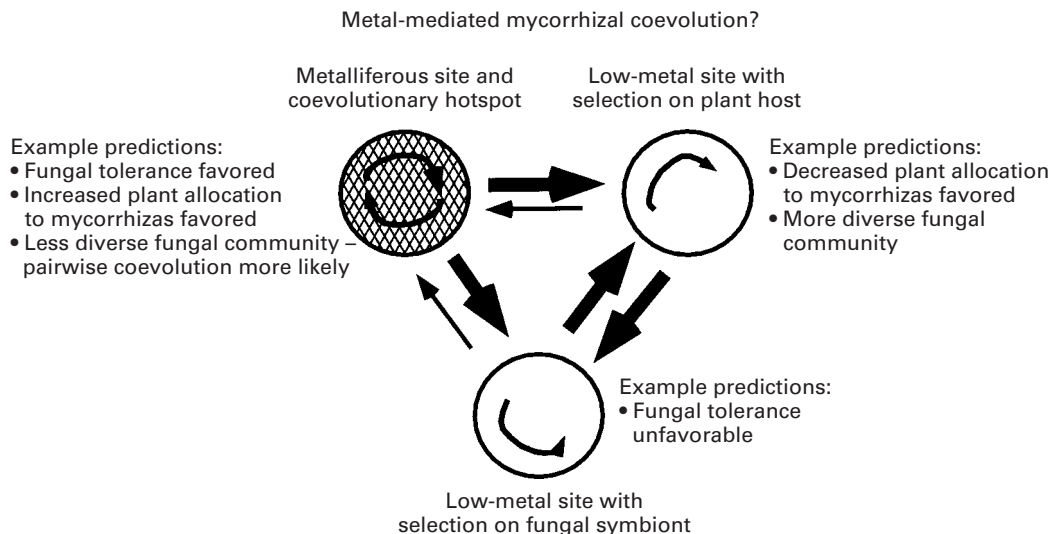
Metal-mediated mycorrhizal coevolution?

Metalliferous site and coevolutionary hotspot

Low-metal site with selection on plant host

Example predictions:
• Fungal tolerance favored
• Increased plant allocation to mycorrhizas favored
• Less diverse fungal community – pairwise coevolution more likely

Example predictions:
• Decreased plant allocation to mycorrhizas favored
• More diverse fungal community

Example predictions:
• Fungal tolerance unfavorable

Low-metal site with selection on fungal symbiont

Fig. 1. Circles represent populations – one on a high metal (metalliferous) site and two on low metal sites. Arrows inside circles, and their angles, represent the trajectories of selection on the plant or fungus. Arrows outside the populations represent gene flow, and depict restricted gene flow into the metalliferous site. All conditions and predictions are hypothetical, and many versions of such a mosaic can be envisioned. *Adapted from Thompson (1999).*

viduals – are exceedingly difficult to measure in mycorrhizal fungi. Estimating fitness is therefore problematic. However, the role of mycorrhizas in plant ecology can no longer be ignored, because of their proven impacts on plant community structure (van der Heijden *et al.*, 1998), diversity–productivity relationships (Klironomos *et al.*, 2000) and biogeochemical cycling (Northup *et al.*, 1995). As the Colpaert paper illustrates, molecular tools for the identification of fungal individuals and the measurement of genetic structure are now available, and can be applied to ecological questions. Hence, the new dawn of molecular ecological genetics in mycorrhizal fungi lights the path to a cornucopia of rewarding studies in evolutionary ecology.

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