Editorial

Unwrapping the *Laccaria* genome

Without the presence of ectomycorrhizal fungi, the forests of the world as we see them today would probably not exist. The majority of tree species form mycorrhizal interactions and these provide essential nutrients to trees, such as nitrogen and phosphate (Read & Perez-Moreno, 2003). The relationship between ectomycorrhizal fungi and the roots of their hosts is an intimate one, involving specialized development by the fungus in the root cortex and extensive communication between plant and fungus (Martin *et al.*, 2001). The mycorrhizal fungus acquires plant-derived carbohydrates in return for its provision of nutrients to the plant and the interactions are both long-lived and fundamental to the operation of forest ecosystems. The special feature in this issue of *New Phytologist* is devoted to analysis of the first genome sequence from an ectomycorrhizal fungus *Laccaria bicolor* (Martin *et al.*, 2008).

What have we learned so far from analysing the *Laccaria* genome? That question is addressed directly in a comprehensive Tansley review from Francis Martin and Marc-André Selosse (Martin & Selosse, this issue, pp. 296–310) and then in a series of new papers, each describing an additional insight that has been gained from access to the genome sequence (Deveau *et al.*, pp. 379–390; Fajardo López *et al.*, pp. 365–378; Labbé *et al.*, pp. 316–328; Lucic *et al.*, pp. 343–364; Morel *et al.*, pp. 391–407; Niculita-Hirzel *et al.*, pp. 329–342). From these studies, we have learned that *L. bicolor* is a complex organism with an estimated 19 102 protein-encoding genes, which form part of a dynamic genome sharing little synteny to related fungal species and possessing a plethora of transposable elements and repeated DNA sequences. *L. bicolor* has also undergone extensive gene family expansion compared with other basidiomycete fungi and these genetic innovations have often been associated with genes that encode proteins involved in symbiotic interactions. Perhaps most significantly, we now know that *L. bicolor* possesses a battery of up to 300 small secreted proteins (SSPs), some of which are specifically produced during symbiotic growth and may be secreted from the Hartig net – a fungal network of hyphae within the root peripheral tissues – during establishment of the ectomycorrhiza. Determining whether these SSPs have effector-like functions, suppressing host defence mechanisms or communicating directly with plant cell signalling pathways to allow fungal invasion and establishment of the symbiotic interaction, will be an important and exciting challenge for the future. Indeed, this challenge will be tackled head on at the 22nd New Phytologist Symposium (Effectors in plant–microbe interactions: http://www.newphytologist.org/effectors/default.htm). There have been further revelations, too, such as the lack of plant cell wall-degrading enzymes, highlighting that this fungus is a true mutualist and apparently even lacks the capacity to break down the most abundant plant polymers, cellulose and lignin. The absence of a gene encoding invertase from the *Laccaria* genome is also reported in this issue and is another surprise. It shows the dependence of the fungus on the host plant's invertase activity within the root to supply monosaccharides to the fungus, and underlines again the mutual dependence of both partners. The nutritional relations and interplay between fungus and plant are fascinating, and research in this area has been propelled forward dramatically by access to the *L. bicolor* genome. The use of transcriptional profiling to study the patterns of gene expression during mycorrhiza development, which has arisen from the genome project, is also tremendously exciting and, when partnered with biochemical analysis, provides a powerful means of determining the metabolic changes that accompany ectomycorrhiza formation.

When considered together, it is clear from the wealth of new information presented in this issue of *New Phytologist* that having access to both the genome sequence of *L. bicolor* and one of its hosts, the poplar, *Populus trichocarpa* (Tuskan *et al.*, 2006), has provided an unprecedented opportunity to identify the plant and fungal genes necessary for establishing ectomycorrhizal interactions and the regulatory networks that allow sequestration and movement of nutrients between the mutualistic partners and the formation of a balanced symbiotic association. As outlined in the pages that follow, the challenges ahead are to carry out gene functional analysis to test the predictions made from genomic analysis and identify the key determinants of ectomycorrhiza development. The deployment of next generation DNA sequencing technologies will also allow the study of ectomycorrhiza evolution and the extent of polymorphism in the *L. bicolor* population, allowing recognition of key fungal genes that have been selected for their role in host recognition. A number of other ectomycorrhizal fungi are also now being sequenced, which should allow recognition of the key gene sets associated with this lifestyle and comprehensive comparative analysis with free-living saprotrophs, plant pathogens and lichen-forming fungi. In this way it should be possible in future to determine how fungi have evolved such a wide range of interactions with plants.

Sequencing the *Laccaria* genome is therefore an important landmark in the study of ectomycorrhiza and will lead to a new degree of understanding of these fascinating plant–microbe interactions that are so important to the ecology and success of forests on this planet. We hope you enjoy this *New Phytologist* special feature and the wealth of information that is already flowing from the unwrapping of this genome sequence.

Nicholas J. Talbot
Editor

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Key words: ectomycorrhizal fungi, forest ecosystems, genomics, *Laccaria bicolor*, poplar (*Populus trichocarpa*), small secreted proteins (SSPs), symbiosis, transcription profiling.

Commentary

Sex and truffles: first evidence of Perigord black truffle outcrosses

The Perigord truffle (*Tuber melanosporum* Vittad.) is a 'cult-food', one of the worldwide recognized icons of European gastronomy and culture, for which genomic and genetic information could act as a knowledge platform to improve its production and environmental persistence. The fruiting body of *T. melanosporum* is an edible truffle (= hypogeous ascocarp), which is a delicacy highly appreciated for its delicate organoleptic properties (i.e. taste and perfumes). This fungus belongs to the Ascomycota (Pezizales; Tuberaceae). It is endemic to calcareous soils in southern Europe and found in symbiotic association with roots of deciduous trees, mostly oaks and hazelnut trees, but also poplars. In this symbiotic relationship – the ectomycorrhizal association – long, branching fungal filaments known as hyphae ramify between cells of the root's outer layers, form a sheath around the root and radiate outwards into the surrounding soil and litter. In late Summer, extramatrical hyphae aggregate to form fruit body initials, from which the fruiting bodies then develop during Fall and early Winter. In truffles, the fruit body (or ascocarp) is formed by sterile hyphae (gleba) and fertile hyphae in which are found the ascospores. The spores released from mature truffles germinate in the following Spring, producing a homokaryotic vegetative mycelium (Paolocci *et al.*, 2006), which results in colonization of tree root tips and further development of the symbiosis completing the truffle life cycle.

> *'Even if direct evidence is still lacking, outcrossing is probably a common strategy used in all (or almost all)* Tuber *sp.'*

Within ascomycetous fungi, sexually reproducing species usually follow one of the three basic sexual reproductive strategies: homothallism, pseudohomothallism (secondary homothallism) and heterothallism (Pöggeler, 2001). Sexual reproduction in filamentous ascomycetes is controlled by idiomorphic mating-type alleles, MAT1-1 and MAT1-2 (Pöggeler, 2001). Homothallic species contain both mating types, while for heterothallic species the two mating types carry one of the two idiomorphs.

It is of primary importance to characterize the reproductive mode of fungal species because the mating-type genes play a

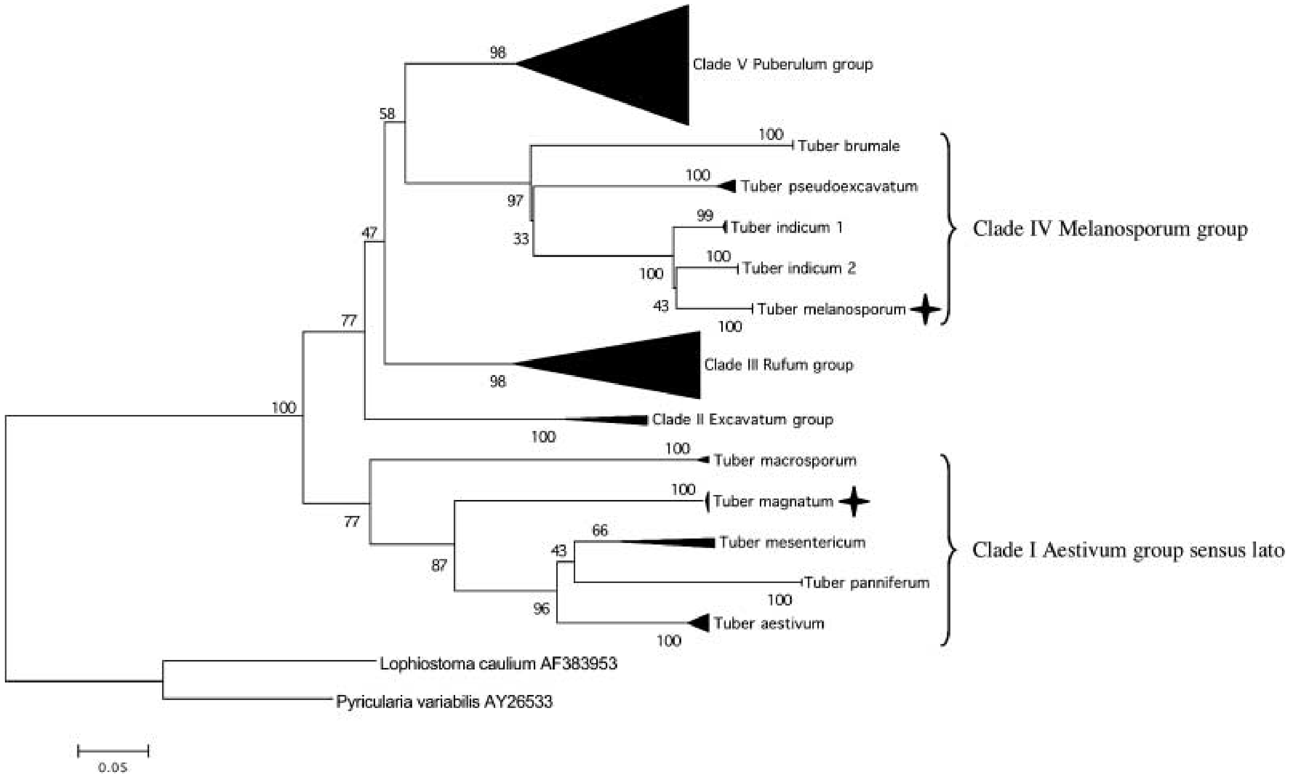

Fig. 1 Neighbour-Joining phylogenetic tree generated from the 5.8S-ITS2 rRNA region of 108 *Tuber* specimens. The tree was created with MEGA 4.0 using Kimura-2-parameter distances, pairwise deletion and bootstrap values corresponding to 500 replicates. Some clades have been concatenated according to Jeandroz *et al*. (2008). A star highlights the two species for which outcrossing has been demonstrated.

role in virulence (Mandel *et al*., 2007), survival (Houbraken *et al*., 2008) and fruiting body formation (Nolting & Pöggeler, 2006). Characterizing the reproduction mode of truffle species will therefore help to further our understanding of their biology and ecology.

As yet, experimental procedures to follow the whole life cycle of *Tuber* sp. in the laboratory are not available and hence this precludes any comprehensive study of *Tuber* genetics. Based on the available molecular markers, *T. melanosporum* was thought to be homothallic or even exclusively selfing (Bertault *et al*., 1998; Murat *et al*., 2004). Recently, genetic analyses using simple sequence repeats (SSR) carried out on the white truffle, *Tuber magnatum*, showed that outcrossing occurs for this species (Paolocci *et al*., 2006). In this issue of *New Phytologist*, Riccioni *et al*. (pp. 466–478) elegantly demonstrated that the Perigord black truffle also outcrosses. They identified additional alleles in the asci beside those present in the surrounding uniparental gleba. In the same study, Riccioni and colleagues, using multi-loci and single loci molecular markers, highlighted a genetic structure among *T. melanosporum* populations and found that the southern-most populations have the highest allele richness. Similar results have been found for oaks, the main hosts of truffles, suggesting that the evolutionary history of ectomycorrhizal fungi and their host trees is linked (Petit *et al*., 2003; Murat *et al*., 2004).

In 2007, in their *New Phytologist* Letter, Rubini and colleagues raised some key questions about the biology of truffles:

(1) Can all *Tuber* sp. outcross?
(2) Are truffles prevalently outcrossing or heterothallic species?
(3) What is the morphology of the mating structures in these fungi?

So far, evidence for outcrossing in the genus *Tuber* has been obtained for *T. magnatum* and *T. melanosporum* (Paolocci *et al*., 2006; Ricionni *et al*., 2008). Both species are phylogenetically divergent (Fig. 1) and their split probably occurred more than 180 million years ago (Jeandroz *et al*., 2008). Even if direct evidence is still lacking, outcrossing is probably a common strategy used in all (or almost all) *Tuber* sp. We recently pointed out that the Chinese black truffle (*Tuber indicum* Cook and Massee) is a potentially invasive species threatening *T. melanosporum* in Europe (Murat *et al*., 2008). Indeed, *T. indicum* is more competitive than *T. melanosporum* and both species are phylogenetically closely related (Fig. 1). It remains to be demonstrated whether the two are able to breed. The paper by Riccioni *et al*. confirms that we cannot exclude breeding between both species, especially if outcrossing occurs for both truffles.

The demonstration that *T. melanosporum* outcrosses is an important finding that has several consequences for species management, such as its introduction through inoculated seedlings and its conservation. Indeed, since the 1970s, seedlings have been inoculated with *T. melanosporum* spore suspensions and implanted worldwide to generate artificial truffle grounds (Chevalier & Grente, 1979). The results presented by Riccioni *et al.* call for a better control of genotypes used as inoculum. Traditionally, spore suspensions are prepared from a handful of ascocarps, allowing different mating types to be present. Currently, new techniques are being developed to inoculate seedlings based on mycelia produced in pure culture (Zambonelli & Iotti, 2004). In this case, it is recommended that the mating type of the different mycelia is characterized in order to inoculate seedlings with compatible strains.

It is known that *T. melanosporum* prefers open forest ecosystems and that canopy closure leads to a rapid decline in the production of ascocarps. Using amplified length fragment polymorphism (AFLP), Riccioni *et al.* identified nine genets out of 11 samples and seven genets out of seven samples in two truffle grounds located at Capodacqua (Italy) and Cerreto di Spoleto (Italy), respectively. This unexpected genetic diversity revealed that *T. melanosporum* is able to form numerous genets and, consequently, *T. melanosporum* can be considered as an 'early stage fungus' favouring the sexual reproduction. This contention needs to be verified by population surveys of various natural truffle grounds over several years.

Truffles: homothallic or heterothallic species? The genome of the Perigord black truffles has recently been sequenced (*Tuber* Genome Consortium) and will soon be available. In this genome, the mating type genes have been identified, confirming that *T. melanosporum* is heterothallic (A. Rubini & F. Paolocci, pers. comm.). The analysis of these genes will allow a better understanding of the *T. melanosporum* lifecycle and the formation of ascocarps. On the other hand, the mating-type genes will be used as molecular markers to investigate the population genetics of this species, as already carried out for *Coccidioides* spp. (Mandel *et al.*, 2007).

The second important finding of Riccioni and colleagues concerns the genetic structure of *T. melanosporum* populations. Analysing population genetics of a species provides information about (1) its history, (2) the factors that have generated the genetic differentiation among populations and (3) the occurrence of hot spots of genetic diversity. In 1998, Bertault and colleagues claimed that there was no genetic structure in *T. melanosporum* populations. In contrast, a strong geographic pattern for *T. melanosporum* has been identified more recently (Murat *et al.*, 2004) using single nucleotide polymorphisms (SNPs) of the nuclear rRNA internal transcribed spacer (ITS). In their paper, Riccioni and colleagues confirmed that there is a significant genetic structure among *T. melanosporum* populations. Moreover, they analysed a higher number of samples from southern Europe than any previous studies and they found that the southern-most populations have a higher allelic richness. As already shown for oaks, the main host of truffles, the populations with the highest allele richness are indicative of the potential species refuge during the last glaciation (Petit *et al.*, 2003). This is the first experimental evidence which indicates that *T. melanosporum* probably took refuge in southern Europe during the last glaciation. Interestingly, the two genotyped Spanish populations showed the highest values of allele richness. A more extensive sampling of the Spanish populations is required for confirming whether the Iberic peninsula represented another potential species refuge during the last glaciations.

Truffles provide an important source of income for truffle hunters and traders in different regions (the selling price of truffles can be as high as €1000 per kg). Consequently, local administrations aim to promote and market their local truffle populations using specific appellations, for example 'Truffe du Tricastin', 'Tartufo nero pregiato di Norcia'. However, it is currently impossible to differentiate ascocarps harvested in different regions or countries by genetic fingerprinting. The genetic differentiation, highlighted by Murat *et al.* (2004) and Riccioni *et al.*, among *T. melanosporum* populations suggested that the characterization of molecular markers to identify the regional origin of ascocarps is within reach. In the fungal genome several thousand SSR motifs can be identified (Lim *et al.*, 2004) given a large set of polymorphic markers (Kim *et al.*, 2008). A *T. melanosporum* genome survey identified a large set of polymorphic SSR (C. Murat *et al.*, unpublished) thus providing new molecular markers to analyse the natural populations of this truffle.

Sex will save truffles? The study by Riccioni *et al.*, together with the first data from the *T. melanosporum* genome, are providing new and important data about the life cycle and population genetics of this species; however, we can be sure that *T. melanosporum* has not finished delivering all its secrets.

Claude Murat* and Francis Martin

UMR INRA-Nancy Université 1136 'Interactions Arbres/
Microorganismes', INRA-Nancy, 54280 Champenoux,
France (*Author for correspondence:
tel +33 (0)3 83 39 40 40; fax +33 (0)3 83 39 40 69;
email claude.murat@nancy.inra.fr)

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Key words: amplified length fragment polymorphism (AFLP), ectomycorrhizal fungi, genetic structure, life cycle, microsatellites, outcrosses, ribosomal DNA, truffle.

Towards a global view of ectomycorrhizal ecology

Research on ectomycorrhizal ecology has failed to reflect the diversity and distribution of ectomycorrhizal plants, with most (> 60%) published research concerning a single, northern-hemisphere plant family (the Pinaceae; Table 1) and a geographic range largely restricted to Europe and North America. In this issue of *New Phytologist*, Tedersoo *et al.* (pp. 479–490) make a large step towards addressing this imbalance in the literature by providing a detailed analysis of ectomycorrhizal communities and host preference for three plant hosts in Tasmania: *Eucalyptus*, *Nothofagus* and *Pomaderris.* Other than a passing reference by Warcup (1991) this is the first below-ground analysis of fungal associates in the Pomaderrideae (an Australasian subfamily of the otherwise arbuscular mycorrhizal Rhamnaceae), and one of only a limited number of studies of *Nothofagus* fungal associates.

> *'The cosmopolitan distribution of ectomycorrhizal fungi differs markedly from the distribution of ectomycorrhizal plants.'*

A remarkable unremarkability

Despite representing a major expansion in the geographic and phylogenetic spread of ectomycorrhizal knowledge, one of the most remarkable outcomes of the community analysis carried out by Tedersoo *et al.* is the sheer unremarkablity of fungal community composition at the level of genera. Of the dominant genera (*Lactarius*, *Laccaria*, *Descolea*, *Russula*, *Tomentella*, *Tulasnella*, *Cortinarius*, *Clavulina*), only one (*Descolea*) is not found in the northern hemisphere. Many of these genera also occur as important components of Palaeotropical ectomycorrhizal

Table 1 Survey of ectomycorrhizal research in Web of Science shows a disproportionate focus on a single northern hemisphere plant family (the Pinaceae), with other ectomycorrhizal groups much less well represented

Ectomycorrhizal clade	Ectomycorrhizal citations (*n*)	Percentage of literature
Pinaceae	1536	62
Fagales	553	22
(*Nothofagus*)	(28)	(1.1)
Myrtaceae	288	12
(*Eucalyptus*)	(272)	(11)
Salicaceae (*sensu stricto*)	182	7.3
Ectomycorrhizal Leguminosae	130	5.2
Basal Malvales (Cistaceae, Sarcolenaceae, Dipterocarpaceae)	89	3.6
Tilia	36	1.4
ECM Rosaceae	25	1.0
Uapaca	15	0.60
Nyctaginaceae (*Neea/Pisonia/Guapira*)	8	0.32
Polygonum	7	0.28
Kobresia	7	0.28
Coccoloba	3	0.12
Gnetum	2	0.08
Asteropeiaceae	1	0.04
Pomaderridae	1	0.04
Total	2495	

Citations were downloaded on August 18 2008 from Web of Science using the search term TS = (ectomycorr*). For each ectomycorrhizal plant clade all genera and plant families were searched, including known synonyms based on the Kew Gardens listing of plant families and genera (www.kew.org/data/genlist.html). Of 6675 citations, 2495 cited the name of a particular ectomycorrhizal plant genus in the abstract. Tedersoo *et al*. double the available information on *Pomaderris* ectomycorrhiza, and significantly increase our knowledge of *Nothofagus* and *Eucalyptus* associates.

communities, and the same pattern is observed in the Neotropics: *Russula*, *Lactarius* and *Tomentella*/*Thelephora* occur in Ecuador on Nyctaginaceae hosts (Haug *et al.*, 2005); *Clavulina*, *Cortinarius*, *Russula* and *Lactarius* are among the most common species in Guyana on roots of Caesalpinaceous hosts (K. L. McGuire, pers. comm.); and *Clavulina*, *Cortinarius*, *Tomentella* and *Russula* are common associates of the dipterocarp endemic, *Pakaraimaea*, from Venezuelan Guayana (Moyersoen, 2006; B. Moyersoen, unpublished). The cosmopolitan distribution of ectomycorrhizal fungi differs markedly from the distribution of ectomycorrhizal plants: no ectomycorrhizal plant genera are shared among Tasmania, the tropics and the northern hemisphere.

Does the cosmopolitan distribution of fungal genera inform our understanding of fungal biogeography and ecology? At the most trivial level, this clearly indicates some combination of vicariance (Halling *et al.*, 2008) and the ability to disperse over long distances (Moyersoen *et al.*, 2003), although the importance and scale of dispersal and vicariance remain unclear for most genera (Lumbsch *et al.*, 2008). However, there may also be more general lessons to be learned.

Outside the fungal kingdom, there are a number of other genera shared between the northern hemisphere and Tasmania. Examples include the genera *Tyto* (barn owls), *Circus* (harriers), *Poa* (grasses), *Carex* (sedges) and *Gaultheria* (snow berry). These nonfungal cosmopolitan genera all have distinct ecological niches that they fill across their distributions. That is to say, at least in a general sense, there is an 'owl niche' filled by the genus *Tyto* that is not filled by other avian genera, while the genus *Tyto* is not likely to occupy fundamentally different niches across its wide distribution.

Can we extend these concepts to fungi by analogy? If so, this implies that individual genera such as *Cortinarius*, *Russula*, *Lactarius*, or *Laccaria* have functional niches that are unique to these genera. We have only the most rudimentary knowledge of what these niches might be. *Laccaria*, for example, are frequently nitrophilic species that respond positively to disturbance. In some cases, clues as to the niches of species may come from morphology – the extensive hyphae and rhizomorphs common in *Cortinarius* imply a different ecological role from that of the nearly smooth mycorrhiza common in many *Russula* species (Agerer, 2001). Further elucidating the niches of these fungi will require deliberate efforts to achieve cross-continental comparisons in fungal community ecology. We also recognize that there will be considerable variation within genera and that genera may not be the best taxonomic level at which to describe niches. Nonetheless, to the extent that there are distinct fungal niches at the level of genus, elucidating these niches provides a conceptual framework for understanding ectomycorrhizal ecology despite the overwhelming diversity of ectomycorrhizal fungal communities.

Dissimilarities as well as similarities

While the cosmopolitan nature of ectomycorrhizal genera argues for broad-scale generic niches, Tedersoo *et al.* and others (e.g. Ishida *et al.*, 2007) have shown clear evidence for host-preference in fungal communities, suggesting that host specificity may be more the rule than the exception (Bruns *et al.*, 2002). Previously, the most widely recognized cases of host preference have been where preference occurs at the level of the fungal genus. For example, the strong host-preference of the *Suillus–Rhizopogon* group on Pinaceae and *Alnicola* on *Alnus* explains the absence of these fungal genera from Tasmania. The results of Tedersoo *et al.* suggest that host-preference is also common at the level of species within fungal genera with cosmopolitan distributions. This suggests that while there may be environmental 'niches' at the level of genus, host-preference may, in some cases, control community composition at the level of species. Host-preference has important environmental implications because the success of invasion by foreign ectomycorrhizal fungal species will depend on matching both niche and plant host in the native vegetation.

The occurrence of widespread host-preference among ectomycorrhizal fungi raises important questions as to the evolutionary pressures and physiological controls over specificity (Hoeksema & Bruna, 2000; Bruns *et al.*, 2002; Egger & Hibbett, 2004). Tedersoo *et al.* find that specificity is more strongly driven by root identity than by tree proximity, which suggests that host-preference is not driven by litter chemistry but rather by interactions at the scale of individual roots. Further work is needed on the mechanisms driving this host-preference, particularly exploring both fungal (e.g. competition) and plant (e.g. active selection) pathways favouring host-preference. Mechanisms for reproductive isolation caused by host-preference also need to be further explored. We know that ectomycorrhizal fungi can influence host phenology (Dickie *et al.*, 2007 and citations therein); if the reverse is true, could plant hosts be favouring reproductive isolation of fungi by shifting the phenology of sporocarp production?

From molecules to a global integration

The 'black box' of ectomycorrhizal communities is opening increasingly wider. Researchers are identifying individual species in communities using sporocarp matching via restriction fragment length polymorphism (RFLP) or terminal-restriction fragment length polymorphism (T-RFLP), or sequencing and BLAST comparisons, with upcoming techniques such as pyrosequencing likely to further this progress (Martin & Slater, 2007; Bruns *et al.*, 2008). Combined with molecular phylogenies, these advances make the possibility of a global perspective on ectomycorrhizal community ecology possible. To achieve this, however, there needs to be a much greater emphasis on the under-studied ectomycorrhizal plant taxa and regions of the world. Without these cross-continental comparisons, patterns observed only in northern hemisphere pines have to be seen as merely local phenomena.

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I. A. Dickie[1]* and B. Moyersoen[2]

[1]Landcare Research, PO Box 40, Lincoln 7640, New Zealand; [2]Université de Liège, B22, algologie, mycologie et systématique expérimentale, Bd du Rectorat 27, 4000 Liège, Belgium
(*Author for correspondence: tel +64 3 321 9646; fax +64 3 321 9998; email dickiei@landcareresearch.co.nz)

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Key words: biogeography, dispersal, ectomycorrhizal fungi, host-preference, niche.

Local adaptation and the consequences of being dislocated from coevolved enemies

Plants are being moved across the globe at an increasing rate both within and outside their current distributional range. Research in the past few decades has focused intensely on invasive exotic plant species that are moved outside their distributional range. Such invaders can have dramatic impacts on indigenous communities through altered biotic interactions with competitors, mutualists and antagonists (Mitchell *et al.*, 2006). But likewise, translocation within the distributional range exposes nonnative plant genotypes to interactions with nonlocally coevolved competitors, mutualists and antagonists. A major concern in restoration ecology is that translocation of seeds from foreign seed sources introduces plant genotypes that are maladapted to local conditions and subsequently hybridize with locally adapted conspecifics, decreasing mean population fitness (Hufford & Mazer, 2003). Interestingly, while local adaptation of plants to their *abiotic* environment is well documented, warranting the concern, studies of plant local adaptation to their *biotic* environment have shown less unequivocal results, ranging from local adaptation to local maladaptation for interactions with antagonists (Greischar & Koskella, 2007). A large reciprocal transplant field study by Crémieux *et al.*, reported in this issue of *New Phytologist* (pp. 524–533), offers a prime example of the diversity of outcomes of altered biotic interactions that we can observe following translocation of plants within their current distributional range even within a single community. In one plant species, local plant genotypes were more resistant to the local demes of a specialist antagonist than nonlocal plant genotypes, suggesting plant local adaptation to important aspects of the biotic environment. However, in another plant species, the reverse pattern was observed. The take-home message from this study is a teeth-grinder for restoration ecologists: it is hard to make a general prediction as to whether foreign seed provenances pose a risk of introducing alleles causing low biotic resistance in the restoration area or not.

Plant local adaptation to the abiotic vs biotic environment

What do we know about the relative contributions of adaptation to abiotic versus biotic aspects of the environment to patterns of plant local adaptation? The surprising answer is: very little. Plant local adaptation can be defined as the higher fitness of local plants at their home site compared with that of nonlocal plants. Performance at the home site is governed by

'It is curious to note that research on plant local adaptation has seldom followed an integrated approach ....'

the ability to cope with local abiotic as well as biotic conditions. Moreover, responses to abiotic and biotic aspects of the environment may be contingent upon each other (Fig. 1). First, host condition often affects susceptibility to antagonists, so differentiation at loci involved in coping with important aspects of the abiotic environment may result in local plants attaining larger size, having different chemistry, or capturing more resources for defense than nonlocal plants, affecting their interactions with competitors, mutualists, and antagonists (Fig. 1, bold arrows). Secondly, abiotic conditions can affect the expression of genetic differences in resistance among plants and host exploitation between antagonists (Fig. 1, dashed arrow) even to the extent of altering the direction of local adaptation in plant–antagonist interactions (Laine, 2008). Plant evolutionary responses to environmental factors that do not coevolve with the plant, such as abiotic conditions, are expected to result in patterns ranging from neutral to plant local adaptation. Indeed, studies emphasizing abiotic aspects of the environment have generally shown plants to be locally adapted, although this pattern is not universal (Hufford & Mazer, 2003). By contrast, plant evolutionary responses to biotic factors that continuously coevolve to maximize plant exploitation could conceivably result in patterns ranging from local adaptation to local maladaptation, and indeed, this whole range is observed for interactions with antagonists (Greischar & Koskella, 2007). It is therefore curious to note that research on plant local adaptation has seldom followed an integrated approach of assessing the relative contributions of abiotic and biotic adaptation to overall local adaptation. On the one hand, studies have addressed local adaptation at large, typically focusing on local adaptation along abiotic environmental gradients without explicitly considering the contribution of adaptation to the biotic environment. On the other hand, local adaptation in plant–antagonist studies is often studied from the antagonist's perspective (Kawecki & Ebert, 2004), in work inspired by questions about the evolution of parasites in host–parasite interactions, not surprisingly without considering its contribution to host local adaptation at large. Indeed, an integrated approach would require extensions of traditional reciprocal transplant studies to include treatments that manipulate, control or otherwise factor out particular aspects of the biotic environment, for instance using exclosures or fungicide/insecticide treatments (Fine *et al*., 2004; Abdala-Roberts & Marquis, 2007). Such studies can offer important insights but are still rare.

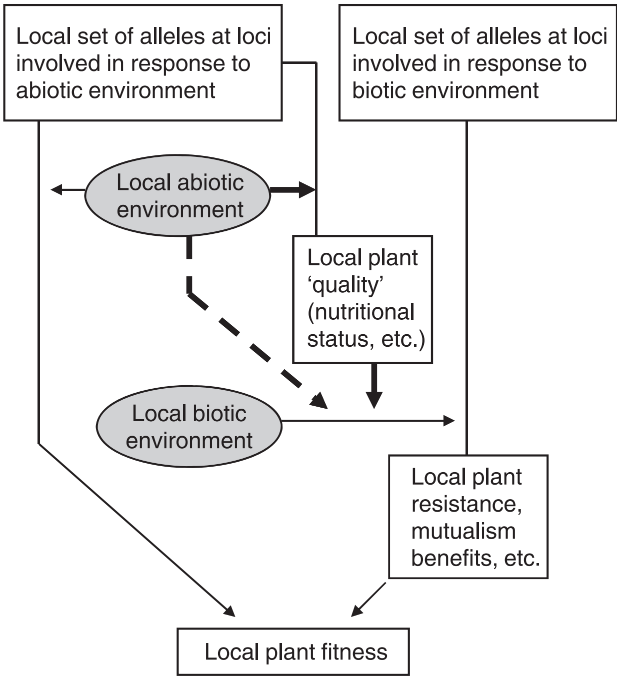

Fig. 1 Simplified diagram of interactions between the abiotic and biotic environments in determining local plant fitness (for details, see text).

Plant local (mal)adaptation to the biotic environment: what should we expect?

The study by Crémieux *et al.* shows plant local adaptation in a plant–insect herbivore interaction and plant local maladaptation in a plant–fungal pathogen interaction. Should we expect such widely divergent outcomes? And what do we know about mechanisms causing adaptation versus maladaptation in biotic interactions? Divergent outcomes are not unexpected. Most host–parasite models assume that parasites specialize on locally common host genotypes, creating a fitness advantage for rare, resistant, genotypes, leading to time-lagged cycles of host resistance and parasite infectivity alleles. A crucial assumption is that parasites have an evolutionary advantage over their hosts by virtue of their shorter generation times, higher mutation/recombination rates, and/or higher relative migration rates. As a consequence, they can closely track their locally common host genotypes, and are expected to be better at infecting local than nonlocal hosts which are likely to be in a different phase of the coevolutionary cycle. Thus, depending on where in the cycle we sample hosts and parasites, we could observe the whole spectrum from host local adaptation to host local maladaptation, but the latter should prevail (Kaltz & Shykoff, 1998). This is indeed what experimental studies show (Greischar & Koskella, 2007). As predicted by theory (Lively, 1999), the rare cases of host local adaptation are more frequently observed for interactions with parasites that have low migration rates relative to their hosts (Greischar & Koskella, 2007; Hoeksema & Forde, 2008). It would be

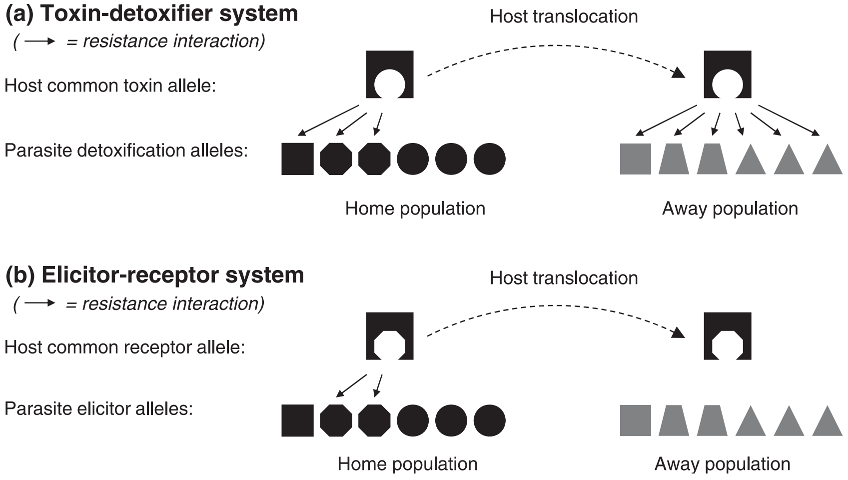

Fig. 2. Predictions of local adaptation in host–parasite interactions depend on the underlying interaction mechanisms. (a) In toxin-based defenses, hosts benefit from translocation (= more resistance interactions; arrows) because the locally evolved set of parasite detoxification alleles mismatches the most common toxin allele from the invading host (hosts are locally maladapted). (b) In recognition-based defenses, hosts suffer from translocation (= fewer resistance interactions; arrows) because their most common receptor fails to recognize the locally evolved set of elicitors of parasites in the invaded population (hosts are locally adapted). Based on a verbal model by Kniskern & Rausher (2001).

interesting to see whether the fungal pathogen used by Crémieux *et al.* is characterized by a high and the insect herbivore by a low relative migration rate, as this could be one of the explanations for the patterns of parasite local adaptation and maladaptation that they observed in these cases, respectively. Opposite patterns for insect herbivores and fungal pathogens may also follow from differences in underlying interaction mechanisms. Kniskern & Rausher (2001) contrasted two basic interaction mechanisms, toxin–detoxifier and elicitor–receptor systems. Toxin–detoxifier systems (Fig. 2a) give rise to a true arms race: plants produce toxins and local antagonists evolve ways to detoxify them, prompting plants to evolve modified toxins, and so on. By contrast, elicitor–receptor systems (Fig. 2b) are based on recognition of antagonists by the plant, required to mount defenses. This gives rise to an information race: antagonists produce molecules (elicitors) and local plants evolve receptors that can recognize them, prompting antagonists to evolve modified elicitors evading recognition, and so on. As Kniskern and Rausher point out, if local populations follow different coevolutionary trajectories, the two systems lead to opposite predictions for local adaptation. Plants translocated to nonlocal populations will fail to recognize elicitors of many novel antagonists (Fig. 2a) but will possess toxins that many novel antagonists cannot deal with (Fig. 2b). Thus, toxin–detoxifier systems, considered as an important mechanism in plant–insect interactions, predict an overall pattern of plant local maladaptation whilst elicitor–receptor systems, considered as an important mechanism in plant–pathogen interactions, predict an overall pattern of plant local adaptation to antagonists. Unfortunately, these predictions are exactly opposite to the patterns observed by Crémieux *et al.*, who showed plant local adaptation in the plant–herbivore and plant local maladaptation in the plant–pathogen system. Clearly, while underlying interaction mechanisms may be important determinants of patterns of local adaptation, studies clarifying their actual involvement in specific plant–herbivore or plant–pathogen interactions are needed to test such ideas.

Outlook

Understanding patterns of plant local adaptation necessitates a better integration of studies on plant adaptation to their abiotic environment with studies on reciprocal adaptation of plants and their biotic environment. The study by Crémieux *et al.* is illustrative in this respect. While velvet grass (*Holcus lanatus*) is locally maladapted to one of its important fungal antagonists, in a related paper (Bischoff *et al.*, 2006) the authors show that local plants of this species nevertheless attain higher fitness than nonlocal conspecifics. Thus, even when plants are locally maladapted to important antagonists, this maladaptation may or may not be strong enough to override local adaptation to the abiotic environment, and an overall pattern of plant local adaptation can still be found. One of the current challenges is to understand the varied patterns observed in such biotic interactions, ranging from local adapation to local maladaptation. While theoretical studies are increasingly providing directions for focusing research on potentially important underlying mechanisms (the impact of relative migration rates, transmission modes, interaction mechanisms, etc.) recent meta-analyses make clear that a major bottleneck in detecting the impact of such factors on patterns of local adaptation is the number of empirical studies available for such analyses.

Arjen Biere* and Koen J. F. Verhoeven

Department of Multitrophic Interactions,
Netherlands Institute of Ecology, NIOO-KNAW,
P.O. Box 40, 6666 ZG Heteren, The Netherlands
(*Author for correspondence:
tel +31 26 47 91212; fax +31 26 47 23227; email
a.biere@nioo.knaw.nl)

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Key words: biotic interaction, host–parasite interaction, local adaptation, plant–herbivore interaction, plant–pathogen interaction, reciprocal transplant, restoration ecology.

Meetings

The coevolutionary genetics of plant–microbe interactions

Joint annual meetings of the Society for the Study of Evolution (SSE), Society of Systematic Biologists (SSB), and American Society of Naturalists (ASN), Minneapolis, MN, USA, June 2008

The tightly coevolved interactions between plants and their microbial symbionts play key roles in the functioning of terrestrial communities and ecosystems. Plant–pathogen interactions, for example, influence the assembly of plant communities (Augspurger, 1988), and the evolution of mycorrhizal interactions has been linked with plant adaptation to life on land and subsequent ecological diversification (Pirozynski & Malloch, 1975; Selosse & Le Tacon, 1998). Evolution 2008, the joint annual meetings of the Society for the Study of Evolution (SSE), the Society of Systematic Biologists (SSB), and the American Society of Naturalists (ASN), took place recently at the University of Minnesota. Coevolution was one theme of this year's meeting, and many talks focused on the interactions between plants and their microbial symbionts. The authors highlighted a wide range of coevolutionary research, including the role of intergenomic epistasis in dynamic coevolution, community effects on the coevolutionary process, and the integration of genetic information into evolutionary research.

> *'... stepping beyond the pairwise interaction and incorporating a community perspective can illuminate the forces shaping genetic variation and coevolutionary stability of a mutualism.'*

Intergenomic epistasis and partner fitness

Genetic variation for the benefits exchanged in plant–microbe interactions has long been appreciated, for example for nitrogen fixation (Fred *et al.*, 1932) and pathogen resistance (Flor, 1942). Of course, in microbial populations and communities, much about the very nature of genotypes, as well as how we define the individual, remains controversial (Gould, 1992). For example, Caroline Angelard (Université de Lausanne, Switzerland) presented evidence that different nuclei within even a single individual of an arbuscular mycorrhizal fungus (AMF) can differ dramatically in their effects on host plant fitness. Even in simpler genetic systems, however, the interactions *between* plant and symbiotic microbial genomes (i.e. intergenomic epistasis, or genotype (G) × genotype (G) interactions) can have important effects on the rate or even direction of coevolutionary selection (Wade, 2007). Lucie Salvaudon (Université Paris Sud, France) showed that the transmission success of the powdery mildew pathogen (*Hyaloperonospora parasitica*), and the effects on its *Arabidopsis thaliana* host plants, were dependent on the interaction between pathogen and host genotypes (G × G interactions). Similarly, G × G interactions also influence the fitness outcomes of interactions between the legume *Medicago truncatula* and its nitrogen (N_2)-fixing mutualist *Sinorhizobium meliloti* (Katy Heath, University of Toronto, Canada) and can even shift the symbiosis from mutualism to parasitism.

Additionally, both talks (one focused on a parasitism and the other on a mutualism) highlighted the point that variation in overall 'compatibility' between partners may result in the lack of a negative correlation between host and symbiont fitnesses. Instead of consistent trade-offs between host and symbiont fitnesses, both studies found that increases in symbiont fitness do not necessarily result in decreased host fitness. Additionally, using a novel experimental coevolution approach, Maren Friesen (University of California, Davis, USA) presented data showing that N_2-fixing rhizobium strains evolved increased competition for symbiosis in the laboratory, but that these more competitive strains had no detrimental effect on host plants – again suggesting that increased symbiont fitness does not necessarily come at a cost to host fitness. Because the trade-off between host and symbiont fitnesses is a fundamental assumption of many coevolutionary models, violations of this assumption have the potential to alter predictions about such coevolved traits as pathogen virulence and mutualism benefits.

Plant–microbe interactions in a community context

Species interactions are typically studied in a pairwise context (Stanton, 2003). However, the community context in which these interactions evolve can have profound effects on the ecological (e.g. Thompson & Cunningham, 2002) and evolutionary (e.g. Benkman, 1999) outcomes of species interactions. Natural legume communities at Bodega Marine Reserve in California include *Lotus* and *Lupinus* legume species that share rhizobium symbionts (*Bradyrhizobium* species). Martine Ehinger (University of California Berkeley, USA) presented evidence of strong differentiation at an N_2-fixation locus (*nifD*) between rhizobia isolated from the two hosts, despite ample horizontal transfer across the rest of the genome. This result implies that selection imposed by sympatric host species may maintain allelic diversity within a single rhizobium population. Ellen Simms (University of California Berkeley, USA) also presented work showing that many rhizobia in the population are nonsymbiotic (at least with the hosts tested) but instead inhabit 'rhizofilms' on plant roots. Although these strains have an ecology and phylogeny that are distinct from those of their symbiotic brethren, they nevertheless can have important effects on the coevolution of the symbiosis by serving as a source of 'cheater' rhizobium strains that gain high fitness from the interaction while giving little or no benefits to their hosts. For example, one such *Lotus*-associated cheater, which forms nodules but fixes little nitrogen, appears to be the result of horizontal transfer of the *nifD* gene from *Lupinus*-associated rhizobia into a strain most closely related to rhizofilm rhizobia. These results have important implications for our understanding of mutualism stability (reviewed in Douglas, 2007), because rhizobium cheaters did not arise from within a symbiotic clade, but instead arose from a distinct clade of rhizobia that utilize a nonsymbiotic strategy. Moreover, this work provides an example of how stepping beyond the pairwise interaction and incorporating a community perspective can illuminate the forces shaping genetic variation and coevolutionary stability of a mutualism.

Coevolutionary genomics

Ecological genomics aims to integrate the fast-accumulating molecular genetic information into a meaningful ecological and evolutionary context. While most investigations to date have focused on the evolution of a single species in response to abiotic conditions (reviewed in Stinchcombe & Hoekstra, 2008), an advantage of model species interactions is the potential to understand how the coevolutionary process has affected specific loci in the genome of one or both partners. The flax–flax rust interaction, for example, enjoys a long history of study (Flor, 1942, Burdon & Thrall, 2000). Luke Barrett (University of Chicago, USA) presented evidence suggesting that genes contributing to virulence specificity (pathogen effectors *AvrP123* and *AvrP4*) in the flax rust *Melampsora lini* have diversified in response to geographic variation in resistance in host flax (*Linum marginale*) from natural Australian populations.

Coevolution with multiple host species can also lead to diversification in pathogen *avr* sequences. Joel Kniskern (University of Chicago, USA) showed that allelic variants of the effector gene *AvrPphB* in *Pseudomonas syringae* confer differential performance with *Phaseolus vulgaris* versus *A. thaliana* hosts – suggesting that these sequences have diversified in response to distantly related hosts. These studies contribute an understanding of how sequence variation at specific candidate loci contributes to reciprocal adaptation among species, and how that variation varies among host populations and species.

Outlook

In this year's ASN presidential address, 'The Coevolving Web of Life,' John Thompson (University of California Santa Cruz, USA) highlighted the importance of geographic variation (or 'mosaics') in coevolutionary selection. In particular, G × G × environment (E) interactions, in which the fitness outcomes of particular host–symbiont combinations depend on the abiotic or biotic environment, define a selection mosaic (i.e. the geographic patterns of selection across a species range). Scott Nuismer (University of Idaho, USA) presented a methodological approach for evaluating the contributions of G × G, G × E, and G × G × E effects to local adaptation (Nuismer & Gandon, 2008). Although challenging for many plant–microbe interactions, this technique has the potential to shed light on whether reciprocal genetic changes between two coevolving species, versus the adaptation of each species to the local environment, generate the majority of geographic variation observed in species interactions.

Vijay Panjeti (University of Virginia, USA) presented a model suggesting that the effects of plant pathogens on the demography and genetics at the interior of a species' range can actually facilitate adaptation to marginal habitats at the edge of the range. Few empiricists have addressed the topic of whether biotic versus abiotic factors determine distributional range limits. For example, do plant–microbe interactions facilitate the colonization of novel habitats (e.g. in invasive species) or serve to constrain range expansion, and are antagonistic or mutualistic interactions most important to range limit evolution? The integration of coevolutionary studies with theory on species range limits is likely to be a promising direction for future research.

We still know little about which specific loci contribute to symbiotic variation in nature, but gene expression analysis may prove useful for identifying candidate loci involved in coevolutionary interactions (Ranz & Machado, 2006). For example, Alexandre Colard (Université de Lausanne, Switzerland) used a model mycorrhizal mutualism to show that different genotypes of the AMF *Glomus intraradices* differentially affect plant gene expression at symbiosis-related loci. Indeed, one way to uncover the genetic targets of coevolutionary selection would be to screen plant and symbiont genomes for genes that differ in their expression in response to partner genotypes. Tools are readily available for many model organisms (e.g. *Medicago truncatula*, *A. thaliana* and *Pseudomonas* spp.), yet such work has not been attempted to date. Moreover, as tools such as Solexa (Warren *et al.*, 2007) and 454 (Margulies *et al.*, 2005) sequencing and gene expression analysis become increasingly available, genome-wide screening techniques will become a reality for nonmodel interactions. The majority of plant–microbe talks at this year's meeting focused on a handful of model organisms; however, future coevolutionary research will also benefit from the incorporation of a broader cross-section of plant and microbial diversity.

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Katy D. Heath

Department of Ecology and Evolutionary Biology,
University of Toronto, Toronto, Canada
(email katy.heath@utoronto.ca)

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Key words: coevolution, epistasis, fitness, pathogen, plant–microbe interaction, mutualism, symbiosis.