

Commentary

Poplar genomics comes of age

The value of poplars as model species for molecular tree biology has been steadily growing for the past decade (Bradshaw *et al.*, 2000; Wullschleger *et al.*, 2002; Brunner *et al.*, 2004). Starting with the creation of DNA marker maps (Bradshaw *et al.*, 1994; Wu *et al.*, 2000; Cervera *et al.*, 2001; Yin *et al.*, 2004) and transgenic trees (Jouanin *et al.*, 1993; Peña & Seguin 2001; Strauss *et al.*, 2001), it has more recently accelerated with the production of a large number of expressed sequence tags (ESTs) (Sterky *et al.*, 1998; Bhalerao *et al.*, 2003; Kohler *et al.*, 2003; Déjardin *et al.*, 2004; Ranjan *et al.*, 2004) and analysis of thousands of genes on DNA microarrays (Hertzberg *et al.*, 2001; Kohler *et al.*, 2003; Andersson *et al.*, 2004). The imminent release of the genome sequence (Joint Genome Institute, USA Department of Energy: <http://www.jgi.doe.gov/>) is taking poplar genomics one large step further by providing a catalog of all genes and, most importantly, of their regulatory environments. Indeed, many biologists expect that variation in gene regulation, whether natural or induced, will be more important for controlling developmental, adaptive and economic traits than variation in protein coding sequences (Doebley & Lukwens, 1998; Carroll, 2000; Frary *et al.*, 2000).

A tree species is being dissected with a level of genetic precision that seemed a distant dream just a few years ago

Most of the papers published to date have been 'first looks' at problems – first maps, first descriptions of EST expression patterns, first transgenic populations. But several of the papers in this issue show that the field has begun to move beyond the exploratory, descriptive phase, into detailed studies of diverse biological phenomena and technologies. Studies range across the field, covering topics as diverse as heavy metal tolerance and metallothionein genes (Kohler *et al.*, pp. 83–93) and cryopreservation as an effective means of preserving large numbers of mutant lines (Tsai & Hubscher, pp. 73–81). Perhaps most excitingly, several of the papers make use of the preliminary (unannotated, unorganized) poplar genome sequence that has been available on the internet for several months (Kohler *et al.*; Yin *et al.*, pp. 95–105; Brunner & Nilsson, pp. 43–51; Joshi *et al.*, pp. 53–61), showing that the age of genome sequence-based poplar genomics has truly begun.

Developmental processes

Wood is a complex and highly variable tissue, whose formation is developmentally and environmentally regulated. Although cellulose and lignin biosynthesis have been intensely investigated in poplar over the last decade, the precise molecular mechanism of their biosynthetic process in poplars is still not well understood (Boerjan *et al.*, 2003; Rogers & Campbell, pp. 17–30; Joshi *et al.*). Tension wood can frequently be observed on one side of stems or branches that have been displaced by wind or a load of some kind. Its formation is associated with increased growth rate and the development of fibers with high cellulose content (Pilate *et al.*, pp. 63–72). Fifteen poplar cDNA encoding fasciclin-like arabinogalactan proteins (PopFLAs) were characterized by Lafarguette *et al.* (pp. 107–121). Ten PopFLAs were specifically expressed in tension wood.

Brunner & Nilsson discuss new genomic approaches for understanding the control of flowering and its onset in poplars. They pointed out how many aspects of the control of flowering are different in large perennial plants from that in annuals such as *Arabidopsis*. Competence for flowering, and timing of initiation, has long-term temporal (years) and complex spatial (crown and shoot position) dimensions that are very poorly understood. Numerous genes have been identified in the large MADS-box gene family that participate in control of the timing of flowering, as well as determine floral organ structures. In a large phylogenetic analysis of poplar MADS-box genes, they found that the much studied *FLC* (flowering locus C) clade of MADS-box genes appears to be entirely absent in poplar. This gene, whose expression delays flowering, takes part in epigenetic control of flowering time in *Arabidopsis*. Stratification, a cold treatment that accelerates flowering, represses *FLC* expression. Overexpression of a poplar form of *FLC* was therefore expected to be a powerful means for engineering sterility for gene containment in poplar via postponement of reproductive maturity. However, it looks as though poplar has other things in mind; it has taken a different evolutionary path in the regulation of its flowering than has *Arabidopsis* and the rest of the Brassicaceae. Poplar bioengineers may therefore also need to chart a different path.

Adaptation to biotic and abiotic stresses

Infection by rust fungi has a devastating impact on poplar plantations worldwide. Although some resistance loci have been genetically identified, no disease resistance genes have yet been cloned despite the significant advances in this

field overall (Talbot, 2003). A number of genes have likely evolved in the host to defend against pathogens, and many of these resistance genes may be clustered together on chromosomes. In an attempt to elucidate the molecular mechanisms of *Melampsora* rust resistance in *P. trichocarpa*, Lescot *et al.* (2004) and Yin *et al.* have mapped two resistance loci, *MXC3* and *MER*, and intensively characterized the flanking genomic sequence for the *MXC3* locus and the level of linkage disequilibrium in natural populations. The *MER* gene appears to be embedded in such a cluster, making it difficult to identify the particular resistance gene of interest from among the many closely linked candidates. On the other hand, the *MXC3* gene may well exist outside of such clusters, thereby facilitating functional characterization.

Poplars are also challenged by many other biotic stresses, including herbivores (Arnold *et al.*, pp. 157–164) and viruses. Smith *et al.* (pp. 123–136) provide a detailed microarray analysis of changes in the poplar transcriptome in response to abrasion and inoculation with the poplar mosaic virus. Making use of the microarray capabilities of the poplar molecular biology group in Umeå, Sweden, they hybridized arrays with more than 10 000 gene targets represented. When abrasion or virus inoculation was considered, more than 2000 targets were elevated in expression and 740 were decreased in expression. When virus effects were considered independently of inoculation, 600 genes showed an increase of expression. Of the genes that exhibited the largest increase in transcript abundance in response to viral infection, seven were predicted to encode metallothioneins. They used cluster analysis to identify sets of genes with similar expression patterns and found many groups with distinct expression spectra. For example, 25 genes were identified that were important to lignification and cell wall development, key means for plant defense.

In an effort to understand processes which are related to heavy metal sequestration, Kohler *et al.* identified six genes and their flanking regulatory sequences in the poplar genome sequence that were similar to known metallothionein genes. Metallothioneins are thought to be important to heavy metal sequestration, but are also likely to have additional roles in development and stress tolerance (see for example Guo *et al.*, 2003). They used reverse transcription PCR to show that the genes had widely varying tissue-level patterns of expression, supporting the hypothesis that metallothionein genes have a diversity of physiological functions. Yeast has been a useful tool for modelling likely plant responses in heavy metal tolerance (Clemens & Simm, 2003). Kohler *et al.* found that one gene, when overexpressed in transgenic yeast, markedly elevated the ability of yeast to tolerate toxic concentrations of cadmium. This gene may therefore be a useful tool for identification of natural variants, or genetic engineering, to increase heavy metal tolerance and bioremediation capacity in poplars.

Metabolism

Jing *et al.* (pp. 137–145) produced eight independent transgenic events with an overexpressed glutamate synthase gene from pine. They tested the transgenic poplars in a field trial in Spain for three years and found that the transgenics were an average of 41% taller than controls, had normal lignin and polysaccharide contents, but had higher nitrogen concentrations in their stems. They suggested that the results were promising as a means to bolster productivity of poplar plantations, but that more studies were needed to understand productivity–nutrition relationships, and impacts on final plantation yield. This kind of work might also provide a new way to engineer trees to elevate nitrogen uptake from the environment for aid in biofiltration. Poplars are often used near to farms, along streams in cities and rural areas, and for treatment of municipal wastes.

Defence against herbivory requires complex metabolic adjustments (Gatehouse, 2002). It is approached in this issue by Arnold *et al.* (2004) through examination of the influence of source-to-sink carbohydrate flow on the development of constitutive and inducible levels of phenylpropanoids in hybrid poplar foliage. They wished to determine whether secondary metabolic processes in plant modules can be inhibited in a predictable manner by events such as herbivory and the development of new leaves or reproductive structures which alter the path of phloem-borne resources. They found that high and inducible sink strength in developing poplar leaves provides resources for phenolic biosynthesis and, as a result, argue that restriction or re-direction of carbohydrates can affect foliar quality for herbivores. Sink strength and the vascular architecture of plants, which confers upon them a modular nature, can determine the direction and magnitude of defense responses in trees.

Carbon fluxes in trees are strikingly altered by the development of ectomycorrhizal symbioses (Nehls *et al.*, 2001; Nehls, 2003). Grunze *et al.* (pp. 147–155) studied five putative monosaccharide transporter genes isolated from ectomycorrhizas between poplar and the fungus *Amanita muscaria* that may provide new means for dissecting carbon transfer. Three highly expressed genes were studied in depth: two had reduced expression upon infection; and one had markedly increased expression upon inoculation. The latter gene had a very high basal expression level in non-inoculated poplar roots. They argue that its expression pattern suggests that plants actively compete with fungi for hexoses even while infected, and this gene may provide an important part of the mechanism whereby sugar export in response to nutrient supply by the fungal partner is regulated.

Perspectives

The poplar research highlighted in this issue gives substance to the promise of poplar genomics that has been touted by

many, including ourselves. It is not a hoax. It is not a scam. A tree species is being dissected with a level of genetic precision that seemed a distant dream just a few years ago. And unlike studies of *Arabidopsis*, because of the extensive wild populations and diverse uses of poplars, the implications of research for ecology, conservation, breeding, and biotechnology will often be direct.

Steven H. Strauss^{1*} and Francis M. Martin²

¹Department of Forest Science, Oregon State University, Corvallis, OR 97331–5751, USA. ²UMR INRA/UHP 1136 Interactions Arbres/Micro-organismes centre INRA de Nancy, 54 280 Champenoux, France.

(*Author for correspondence: tel +1541 7376578; fax +1541 7371393; email Steve.Strauss@oregonstate.edu)

References

- Andersson A, Keskitalo J, Sjinin A, Bhalerao R, Sterky F, Wissel K, Tandré K, Aspeberg H, Moyle R, Ohmiya Y, Bhalerao R, Brunner A, Gustafsson P, Karlsson J, Lundeberg J, Nilsson O, Sandberg G, Strauss S, Sundberg B, Uhlen M, Jansson S, Nilsson P. 2004. A transcriptional timetable of autumn senescence. *Genome Biology* 5: R24.
- Arnold TM, Appel H, Patel V, Stocum E, Kavalier A, Schultz J. 2004. Carbohydrate translocation determines the phenolic content of *Populus* foliage: a test of the sink-source model of plant defense. *New Phytologist* 164: 157–164.
- Bhalerao R, Keskitalo J, Sterky F, Erlandsson R, Björkbacka H, Birve SJ, Karlsson J, Gardeström P, Gustafsson P, Lundeberg J, Jansson S. 2003. Gene expression in autumn leaves. *Plant Physiology* 131: 430–442.
- Boerjan W, Ralph J, Baucher M. 2003. Lignin biosynthesis. *Annual Review of Plant Biology* 54: 519–546.
- Bradshaw HD, Ceulemans R, Davis J, Stettler R. 2000. Emerging model systems in plant biology: poplar (*Populus*) as a model forest tree. *Journal of Plant Growth Regulation* 19: 306–313.
- Bradshaw HD Jr, Villar M, Watson BD, Otto KG, Stewart S, Stettler RF. 1994. Molecular genetics of growth and development in *Populus*. III. A genetic linkage map of a hybrid poplar composed of RFLP, STS, and RAPD markers. *Theoretical Applied Genetics* 89: 167–178.
- Brunner AM, Busov VB, Strauss SH. 2004. Poplar genome sequence: functional genomics in an ecologically dominant plant species. *Trends in Plant Sciences* 9: 49–56.
- Brunner AM, Nilsson O. 2004. Revisiting tree maturation and floral initiation in the poplar functional genomics era. *New Phytologist* 164: 43–51.
- Carroll SB. 2000. Endless forms: The evolution of gene regulation and morphological diversity. *Cell* 101: 577–580.
- Cervera MT, Storme V, Ivens B, Gusmao J, Liu BH, Hostyn V, Van Slycken J, Van Montagu M, Boerjan W. 2001. Dense genetic maps of three *Populus* species (*Populus deltoides*, *P. nigra*, and *P. trichocarpa*) based on AFLP and microsatellite markers. *Genetics* 158: 787–809.
- Clemens S, Simm C. 2003. *Schizosaccharomyces pombe* as a model for metal homeostasis in plant cells: the phytochelatin-dependent pathway is the main cadmium detoxification mechanism. *New Phytologist* 159: 323–330.
- Déjardin A, Leple JC, Lesage-Descauses MC, Costa G, Pilate G. 2004. Expressed sequence tags from poplar wood tissues – A comparative analysis from multiple libraries. *Plant Biology* 6: 55–64.
- Doebley J, Lukens L. 1998. Transcriptional regulation and the evolution of plant form. *Plant Cell* 10: 1075–1082.
- Frary A, Nesbitt TC, Frary A, Grandillo S, van der Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert K, Tanksley SD. 2000. *fw2.2*: a quantitative trait locus key to the evolution of tomato fruit. *Science* 289: 85–87.
- Gatehouse JA. 2002. Plant resistance towards insect herbivores: a dynamic interaction. *New Phytologist* 156: 145–169.
- Grunze N, Willmann M, Nehls U. 2004. The impact of ectomycorrhiza formation on monosaccharide transporter gene expression in poplar roots. *New Phytologist* 164: 147–155.
- Guo W-J, Bundithya W, Goldsbrough PB. 2003. Characterization of the *Arabidopsis* metallothionein gene family: tissue-specific expression and induction during senescence and in response to copper. *New Phytologist* 159: 369–381.
- Hertzberg M, Aspeberg H, Schrader J, Andersson A, Erlandsson R, Blomqvist K, Bhalerao R, Uhlen M, Teeri TT, Lundeberg J, Sundberg B, Nilsson P, Sandberg G. 2001. A transcriptional roadmap to wood formation. *Proceedings of the National Academy of Sciences, USA* 98: 14732–14737.
- Jing ZP, Gallardo F, Pascual MB, Sampalo R, Romero J, Torres de Navarra A, Cánovas FM. 2004. Improved growth in a field trial of transgenic hybrid poplar overexpressing glutamine synthetase. *New Phytologist* 164: 137–145.
- Joshi CP, Bhandari S, Ranjan P, Kalluri UC, Liang X, Fujino T, Samuga A. 2004. Genomics of cellulose biosynthesis in poplars. *New Phytologist* 164: 53–61.
- Jouanin L, Brasileiro ACM, Leplé JC, Pilate G, Cornu D. 1993. Genetic transformation: a short review of methods and their applications, results and perspectives for forest trees. *Annals of Forest Sciences* 50: 325–336.
- Kohler A, Blaudez D, Chalot M, Martin F. 2004. Cloning and expression of multiple metallothioneins from hybrid poplar. *New Phytologist* 164: 83–93.
- Kohler A, Delaruelle C, Martin D, Encelot N, Martin F. 2003. The poplar root transcriptome: analysis of 7000 expressed sequence tags. *FEBS Letters* 542: 37–41.
- Lafarguette F, Leplé J-C, Déjardin A, Laurans F, Costa G, Lesage-Descauses M-C, Pilate G. 2004. Poplar genes encoding fasciclin-like arabinogalactan proteins are highly expressed in tension wood. *New Phytologist* 164: 107–121.
- Lescot M, Rombauts S, Zhang J, Aubourg S, Mathé C, Jansson S, Rouzé P, Boerjan W. 2004. Annotation of a 95-kb *Populus deltoides* genomic sequence reveals a disease resistance gene 2 cluster and novel class-I and class-II transposable elements. *Theoretical Applied Genetics* 109: 310–322.
- Nehls U. 2003. Ectomycorrhizal development and function – transcriptome analysis. *New Phytologist* 159: 5–7.
- Nehls U, Mikolajewski S, Magel E, Hampp R. 2001. Carbohydrate metabolism in ectomycorrhizas: gene expression, monosaccharide transport and metabolic control. *New Phytologist* 150: 533–541.
- Peña L, Seguin A. 2001. Recent advances in the genetic transformation of trees. *Trends in Biotechnology* 19: 500–506.
- Pilate G, Déjardin A, Laurans F, Leplé J-C. 2004. Tension wood as a model for functional genomics of wood formation. *New Phytologist* 164: 63–72.
- Ranjan P, Kao YY, Jiang H, Joshi CP, Harding SA, Tsai CJ. 2004. Suppression subtractive hybridization – mediated transcriptome analysis from multiple tissues of aspen (*Populus tremuloides*) altered in phenylpropanoid metabolism. *Planta* doi: 10.1007/s00425-004-1291-9.
- Rogers LA, Campbell MM. 2004. The genetic control of lignin deposition during plant growth and development. *New Phytologist* 164: 17–30.
- Smith CM, Rodriguez-Buey M, Karlsson J, Campbell MM. 2004. The response of the poplar transcriptome to wounding and subsequent infection by a viral pathogen. *New Phytologist* 164: 123–136.

- Sterky F, Regan S, Karlsson J, Hertzberg M, Rohde A, Holmberg A, Amini B, Bhalerao R, Larsson M, Villarroel R, Van Montagu M, Sandberg G, Olsson O, Teeri TT, Boerjan W, Gustafsson P, Uhlen M, Sundberg B, Lundeberg J. 1998. Gene discovery in the wood-forming tissues of poplar: analysis of 5692 expressed sequence tags. *Proceedings of the National Academy of Sciences, USA* 95: 3330–13335.
- Strauss SH, DiFazio SP, Meilan R. 2001. Genetically modified poplars in context. *Forest Chronicle* 77: 1–9.
- Talbot NJ. 2003. Functional genomics of plant–pathogen interactions. *New Phytologist* 159: 1–4.
- Tsai C-J, Hubscher SL. 2004. Cryopreservation in *Populus* functional genomics. *New Phytologist* 164: 73–81.
- Wu RL, Han HF, Fang JJ, Li L, Li ML, Zeng ZB. 2000. An integrated genetic map of *Populus deltoides* based on amplified fragment length polymorphisms. *Theoretical Applied Genetics* 100: 1249–1256.
- Wullschlegel SD, Jansson S, Taylor G. 2002. Genomics and forest biology: *Populus* emerges as the perennial favorite. *Plant Cell* 14: 2651–2655.
- Yin T-M, DiFazio SP, Gunter LE, Jawdy SS, Boerjan W, Tuskan GA. 2004. Genetic and physical mapping of *Melampsora* rust resistance genes in *Populus* and characterization of linkage disequilibrium and flanking genomic sequence. *New Phytologist* 164: 95–105.
- Yin TM, DiFazio SP, Gunter LE, Riemenschneider D, Tuskan GA. 2004. Large-scale heterospecific segregation distortion in *Populus* revealed by a dense genetic map. *Theoretical Applied Genetics* doi: 10.1007/500122-004-1653-5.

Key words: DNA marker maps, DNA microarrays, ESTs, functional genomics, molecular tree biology, *Populus* (poplar), transgenic trees.

Hyphal fusion to plant species connections – giant mycelia and community nutrient flow

Hyphal fusion (anastomosis) is a ubiquitous phenomenon in filamentous fungi and it is widely assumed that vegetative hyphal fusion is important for intrahyphal communication, translocation of water and nutrients and general homeostasis within a colony (Glass *et al.*, 2004). In arbuscular mycorrhizal (AM) fungi, H-type hyphal fusions in root-internal mycelia were documented by Gallaud in 1905 (Smith & Smith, 1997) and frequent hyphal anastomoses in the presymbiotic growth phase were reported by Mosse (1959). These early observations have been confirmed and extended by Manuela Giovannetti and coworkers in Pisa and it appears that AM fungi resemble other filamentous fungi in the ability of hyphae to fuse. In this issue (pp. 175–181), Giovannetti *et al.* present beautiful micrographs of anastomosing root-external mycelia of a *Glomus mosseae* isolate, revealing that anastomoses can also form between individual mycelia originating from different plant species. This could potentially result in the formation of large mycelia and thereby have significant consequences for nutrient foraging by the mycotroph and for nutrient cycling in plant communities.

Fusions between individual mycelia

Giovannetti *et al.* show that anastomoses form readily in two-dimensional membrane ‘sandwiches’ with 30–80 cm hyphae cm⁻² where the chance for interhyphal contact must be high. However, it seems likely that they could also form in soil, which typically contains 1500 cm cm⁻³ (see Olsson *et al.*, 2002). An important prerequisite for fusion of individual mycelia in soil is that they intermingle and that they originate from propagules of the same AM fungal isolate (Giovannetti *et al.*, 2003). The community structure of AM fungi in different ecosystems is not well understood, but is likely to be characterised by the dominance of sporulating and nonsporulating genotypes in disturbed and undisturbed systems, respectively. Accordingly, the conditions for fusion to occur will be different in these contrasting systems. In disturbed systems, fusion of individual mycelia originating from dispersed propagules could result in rapid built-up of large mycelia. The probability for mycelia meeting will depend on the initial density of infective propagules and on the distance of spread of the root-external mycelium. Such spread can be considerable in experiments with semisterile soil (Jakobsen *et al.*, 1992; Jansa *et al.*, 2003) while recent work by Rosendahl and Stukenbrock (2004) indicates that mycelia of, for example, *Glomus mosseae* may be rather small in undisturbed soil. Such limited mycelium sizes were also indicated in a field study of P uptake into pea plants, which grew equally well in symbiosis with the native AM fungi in untreated soil and with an inoculant *G. caledonium* in fumigated soil (I. Jakobsen, unpublished). Surprisingly, uptake of ³²P from hyphal in-growth cores by the native community was only 10% of the uptake by the inoculant fungus. This suggests that fungal spread was considerably depressed by components present in untreated, but not fumigated, soil. Occurrence and importance of anastomosis in soil could be investigated by simultaneous labelling with ³²P and ³³P of neighbouring mycorrhizal plants separated by a root-free zone. The experiment should use one AM fungal isolate and a root-free zone containing natural soil at different zone width.

The widespread occurrence of an unidentified AM fungus along 30 m transects in undisturbed plots was suggested to reflect either individual mycelia or large mycelia of at least 10 m width (Rosendahl & Stukenbrock, 2004). An attractive hypothesis is that anastomosis could have played a role in the formation of such large mycelia. Host specificity would probably be an interactive component in the formation of large mycelia from the fusion of individual ones. Specificity in root colonisation is not usually obvious in single host–fungus pairs, but appears to be common in the field situation resulting in a selection pressure of plants on AM fungal communities (Vandenkoornhuysen *et al.*, 2003; Gollotte *et al.*, 2004). The latter reported from a field study that monocultures of two grass species shared only one of the 10 AM fungal genotypes detected in the roots in total. This implies

that only one of nine AM fungi could potentially form interspecific links between these grasses when grown in mixture. Similarly, in the study by Rosendahl and Stukenbrock (2004), a widespread nonsporulating fungus, forming a potentially large mycelium, was the fungus that also had the least host specificity (S. Rosendahl, pers. comm.).

Fungal foraging and resource allocation

Although Giovannetti *et al.* show that the frequency of anastomoses between individual mycelia is somewhat modulated by the host plant species involved, this will certainly play a minor role as compared to the requirement of genome similarity between the AM fungi involved. A mycelium with anastomosing hyphae represents a web-like structure with improved fitness because hyphal bridging creates alternative routes for efficient translocation and transmigration of nutrients and organelles under localised exposure to stress. Any increase in size of an AM fungal mycelium resulting from fusion with other individual mycelia is expected to influence foraging for carbon and mineral nutrients and resource allocation in the mycelium.

Carbon is the primary limiting nutrient for growth of the biotrophic AM fungi and an increased mycelium size would also increase the probability of encountering new carbon sources in the form of roots. Linkage of the mycelium with plants in different growth phases would improve the continuity in carbon supply to the fungus. The longest possible distance for resource allocation in AM fungal mycelia is unknown as yet but could well be longer than the 10–15 cm observed for phosphate and zinc by Jansa *et al.* (2003). The potential importance of large mycelia in efficient foraging for mineral nutrients is supported by observations of increased growth of AM fungal hyphae in nutrient patches (Hodge *et al.*, 2001; Gavito & Olsson, 2003). Similarly, one of three AM fungi tested showed plasticity in P uptake, which was enhanced from a P-enriched patch at the expense of uptake from a nonenriched patch (I. Jakobsen, unpublished data). Anastomosing mycelial webs could be important for such plasticity in AM fungi and for the allocation of nutrients to sites of expansion of intra- and extraradical mycelium.

Will plants benefit from anastomosing mycelia?

While the potential advantage of anastomosis to integrity and function of the fungus itself is obvious, the importance for the associated host plants is less clear. The following discussion is particularly relevant to undisturbed systems where large mycelia are most likely to occur, where anastomosis has a potential impact on their formation (see above) and where several individuals and species of plants may be connected into a common mycelium. Importantly, the functional aspects discussed will be generally valid for large interconnecting mycelia irrespective of the possible role of anastomosis for their formation.

Interplant transfer of carbon via a connecting AM fungal mycelium has been reported by several authors, but in most cases the transferred carbon remained in the roots of the receiver plant (Fitter *et al.*, 1998). It is most likely that this carbon is localised in the intraradical mycelium, as biochemical studies have failed to detect any fungus–plant transfer mechanism for carbon in mycorrhizal roots (Pfeffer *et al.*, 2004). Recent results do indicate AM fungal mediation of carbon parasitism between some neighbouring plants (Carey *et al.*, 2004). This may represent a transition to the possible importance of AM fungi in carbon nutrition of nonphotosynthetic hosts (Bidartondo *et al.*, 2002) and deserves further investigation. Assuming that fungus-to-plant carbon transfer does not normally occur, it is still possible that plants linked into a common mycelia network might differ in the amount of carbon they transfer to the mycelium. A ‘cheater’ plant providing relatively little carbon to the mycelium would obtain its mineral nutrients at a lower expense than other interlinked plants and thereby seem to achieve a competitive advantage.

A large root-external mycelium will effectively absorb nutrients from soil and from dying roots and thereby represent a readily available pool of nutrients for the interconnected plants. However, it is largely unknown whether the fungal nutrient pool is equally accessible to the plant individuals differing in species and growth phase. Net transport might be towards sites of highest sink strength, such as the root-internal mycelium in rapidly growing plants. Several authors have investigated the importance of mycelial networks for seedling establishment (Kytoviita *et al.*, 2003; van der Heijden, 2004), but unequivocal conclusions cannot be drawn. The apparent regulatory role of a neighbour plants representing a strong sink is supported by unpublished data showing that tomato seedlings were P deficient and grew poorly in root-free soil containing a well-established mycelium from a neighbouring cucumber plant (Fig. 1). In a parallel treatment, cucumber shoots had been removed before planting of tomato, which in this case grew much larger. A ^{32}P tracer added to the cucumber root compartment just after excision of cucumber shoots in one treatment could be detected only in tomato seedlings linked with mycelium from the shoot-free cucumber. A ‘carbon-cheating’ seedling linking into a well-established mycelium may thus be a poor competitor for nutrients pools in the fungus. This problem may be amplified by the fact that the AM fungal P uptake pathway appears to dominate over direct root uptake, at least under low P conditions (Smith *et al.*, 2004). Anastomosis could influence the time required for rendering a nutrient patch available to the mycelium and to the interlinked plants. Importantly, it is unlikely that mineral nutrients will move from tissues of one living plant to another. All studies of interplant transfer of mineral nutrients show that transfer of nutrients occurs in significant amounts only from donor plants with dying root systems (Newman, 1988; Johansen & Jensen, 1996).

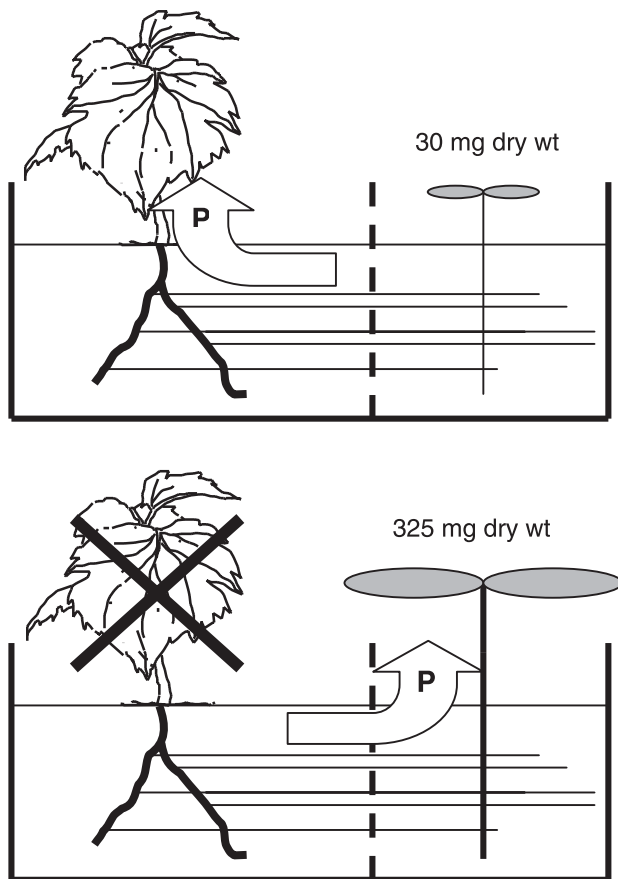


Fig. 1 Dry weights of tomato seedlings growing into a pre-established mycelium of *Glomus claroideum* produced in association with cucumber donor plants. After 6 weeks, cucumber shoots were removed in the lower treatment, ^{32}P tracer was supplied to the donor roots and germinated tomato seeds were planted. Radioactivity in tomato plants was monitored and plants were harvested 40 d after sowing.

Perspectives

Future research on anastomosis in AM fungi should clarify three questions. (1) Is linkage of individual mycelia common in disturbed systems, which are dominated by sporulating genotypes, presumably forming rather small mycelia? (2) What is the importance of anastomosis in the formation of large mycelia (if they exist)? (3) Which genetic distance is required to prohibit the formation of anastomosis between two individual AM fungi? The third question is of particular interest since hyphal fusion appears to be the most likely mechanism for generation of new species of these clonal organisms. Already, back in 1959, Barbara Mosse observed that anastomoses were equally common between hyphae from the same or from different parent spores and pointed out: 'In a coenocytic fungus like the *Endogone*, such anastomoses must provide endless opportunities for exchange of genetic material by asexual means' (Mosse, 1959). In order to predict the effect of such exchange of nuclei, it is important to clarify whether

the nuclei in individual genotypes are heterokaryotic (Kuhn *et al.*, 2001) or homokaryotic (Pawlowska & Taylor, 2004).

Iver Jakobsen

Risø National Laboratory, Plant Research Department,
DK-4000 Roskilde, Denmark (tel +45 46774154;
fax +45 46774102; email iver.jakobsen@risoe.dk)

References

- Bidartondo MI, Redecker D, Hijri I, Wiemken A, Bruns TD, Dominguez L, Sersic A, Leake JR, Read DJ. 2002. Epiparasitic plants specialized on arbuscular mycorrhizal fungi. *Nature* 419: 389–392.
- Carey EV, Marler MJ, Callaway RM. 2004. Mycorrhizae transfer carbon from a native grass to an invasive weed: evidence from stable isotopes and physiology. *Plant Ecology* 172: 133–141.
- Fitter AH, Graves JD, Watkins NK, Robinson D, Scrimgeour C. 1998. Carbon transfer between plants and its control in networks of arbuscular mycorrhizas. *Functional Ecology* 12: 406–412.
- Gavito ME, Olsson PA. 2003. Allocation of plant carbon to foraging and storage in arbuscular mycorrhizal fungi. *FEMS Microbiology Ecology* 45: 181–187.
- Giovannetti M, Sbrana C, Avio L, Strani P. 2004. Patterns of below-ground plant interconnections established by means of arbuscular mycorrhizal networks. *New Phytologist* 164: 175–181.
- Giovannetti M, Sbrana C, Strani P, Agnolucci M, Rinaudo V, Avio L. 2003. Genetic diversity of isolates of *Glomus mosseae* from different geographic areas detected by vegetative compatibility testing and biochemical and molecular analysis. *Applied and Environmental Microbiology* 69: 616–624.
- Glass NL, Rasmussen C, Roca MG, Read ND. 2004. Hyphal homing, fusion and mycelial interconnectedness. *Trends in Microbiology* 12: 135–141.
- Golotte A, Van Tuinen D, Atkinson D. 2004. Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. *Mycorrhiza* 14: 111–117.
- van der Heijden MGA. 2004. Arbuscular mycorrhizal fungi as support systems for seedling establishment in grassland. *Ecology Letters* 7: 293–303.
- Hodge A, Campbell CD, Fitter AH. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* 413: 297–299.
- Jakobsen I, Abbott LK, Robson AD. 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum*. 1: Spread of hyphae and phosphorus inflow into roots. *New Phytologist* 120: 371–380.
- Jansa J, Mozafar A, Frossard E. 2003. Long-distance transport of P and Zn through the hyphae of an arbuscular mycorrhizal fungus in symbiosis with maize. *Agronomie* 23: 481–488.
- Johansen A, Jensen ES. 1996. Transfer of n and, p. from intact or decomposing roots of pea to barley interconnected by an arbuscular mycorrhizal fungus. *Soil Biology and Biochemistry* 28: 73–81.
- Kuhn G, Hijri M, Sanders IR. 2001. Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. *Nature* 414: 745–748.
- Kytoviita MM, Vestberg M, Tuom J. 2003. A test of mutual aid in common mycorrhizal networks: Established vegetation negates benefit in seedlings. *Ecology* 84: 898–906.
- Mosse B. 1959. The regular germination of resting spores and some observations on the growth requirements of an *Endogone* sp. causing vesicular-arbuscular mycorrhiza. *Transactions of the British Mycological Society* 42: 273–286.
- Newman EI. 1988. Mycorrhizal links between plants – their functioning and ecological significance. *Advances in Ecological Research* 18: 243–270.

- Olsson PA, Jakobsen I, Wallander H. 2002. Foraging and resource allocation strategies of mycorrhizal fungi in a patchy environment. In: van der Heijden, MGA, Sanders, IR, eds. *Mycorrhizal ecology*. Berlin, Germany: Springer, 93–116.
- Pawlowska TE, Taylor JW. 2004. Organization of genetic variation in individuals of arbuscular mycorrhizal fungi. *Nature* 427: 733–737.
- Pfeffer P, Douds DD, Bucking H, Schwartz DP, Shachar-Hill Y. 2004. The fungus does not transfer carbon to or between roots in an arbuscular mycorrhizal symbiosis. *New Phytologist* 163: 617–627.
- Rosendahl S, Stukenbrock EH. 2004. Community structure of arbuscular mycorrhizal fungi in undisturbed vegetation revealed by analyses of LSU rDNA sequences. *Molecular Ecology* (In press).
- Smith FA, Smith SE. 1997. Structural diversity in (vesicular)-arbuscular mycorrhizal symbioses. *New Phytologist* 137: 373–388.
- Smith SE, Smith FA, Jakobsen I. 2004. Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. *New Phytologist* 162: 511–524.
- Vandenkoornhuysen P, Ridgway KP, Watson IJ, Fitter AH, Young JPW. 2003. Co-existing grass species have distinctive arbuscular mycorrhizal communities. *Molecular Ecology* 12: 3085–3095.

Key words: anastomosis, arbuscular mycorrhizal (AM) fungi, filamentous fungi, hyphal fusion, nutrient foraging.

Are ericoid and ectomycorrhizal fungi part of a common guild?

The ectomycorrhizal (ECM) and ericoid mycorrhizal (ERM) symbioses have commonly been thought to involve different sets of fungi (Smith & Read, 1997). While ECM fungi have been studied and recognized mainly as basidiomycetes, ERM fungi have predominantly been identified as a restricted group of ascomycetes. In the latter group, *Hymenoscyphus ericae* represents the most extensively studied model taxon for the ecology and function of the ericoid mycorrhizal symbiosis (Smith & Read, 1997; Read & Perez-Moreno, 2003). Molecular studies have blurred the well established distinction between ECM and ERM fungi, partly because *H. ericae* has been recognized as being part of a larger fungal complex (cf. the *H. ericae* aggregate; Vrålstad *et al.*, 2000), with a steadily growing range of closely related genotypes detected in ERM and ECM partnerships worldwide (e.g. Vrålstad *et al.*, 2000, 2002b; Allen *et al.*, 2003; Cairney & Meharg, 2003; Rosling *et al.*, 2003; Haug *et al.*, 2004). The hypothesis that ERM and ECM plants may share common mycorrhizal partners (Vrålstad *et al.*, 2000) has been supported by observations of identical fungal genotypes in roots of coexisting ERM and ECM hosts (Bergero *et al.*, 2000; Vrålstad *et al.*, 2002b), but conclusive evidence has, until now, been missing. In pages 183–192 of this issue, Villarreal-Ruiz *et al.* demonstrate for the first time that a single fungal mycelium can develop ERM and ECM simultaneously. This raises a serious question mark over the ‘accepted wisdom’ that ericoid and

ectomycorrhizal fungi represent separate fungal guilds (see also Cairney & Meharg, 2003).

Focal differences in ERM and ECM research

Read (1991) recognized that major global gradients and general climatic and edaphic conditions in nature have led to a selection of distinctive types of mycorrhiza that predominate in different biomes. It is, therefore, easy to understand the differences in the ERM and ECM research fields. ERM research has largely focused on heathlands and extreme ecosystems where ericaceous hosts predominate, and ECM research has for the same reasons focused on woodlands. In ERM research, isolates of *H. ericae* and *Oidiodendron* spp. have become the most extensively used model fungi, while a limited group of readily cultivable ECM-forming basidiomycetes have served as ECM models (Read & Perez-Moreno, 2003). The ERM models exhibit enzymatic capabilities comparable to brown-rot fungi, and also possess an impressive resistance to heavy metals. They are therefore thought to play a key role in host nutrient acquisition and protection in nutrient poor heathlands and other stressed and toxic habitats dominated by ericaceous plants (Cairney & Meharg, 2003; Read & Perez-Moreno, 2003). In ECM research, some of the model fungi also exhibit saprotrophic capabilities, but commonly not to the extent observed in the ERM model fungi. A large number of studies have uncovered an enormous below-ground diversity of ECM fungi (cf. Horton & Bruns, 2001) and brought new understanding to the functional role of ECM relationships in temperate and boreal forests (Lindahl *et al.*, 2002; Read & Perez-Moreno, 2003). However, there is a major deficiency among mycorrhizal studies in acknowledging that ERM plants and associated fungi predominate in the woodland understorey of several characteristic temperate and boreal forest habitats.

Identification of ECM and ERM fungi

Formation of ECM has traditionally been associated with a number of sporocarp-producing basidiomycetes (Molina *et al.*, 1992), and earlier studies of ECM communities were described on the basis of above-ground sporocarp formation. The past eight years of molecular diversity studies have demonstrated a considerable lack of correspondence between the above-ground community of ECM sporocarps and the below-ground community of fungi involved in ECM root tips (cf. Horton & Bruns, 2001). Molecular identification approaches have also added a significant number of fungal taxa to the list of ECM-formers, including representatives of the ascomycete lineage previously thought exclusively to include ERM fungi (cf. the *H. ericae* aggregate; Vrålstad *et al.*, 2000, 2002b).

In ERM research, isolation of fungal cultures from hair roots has served as the method for identification of fungal

partners. Numerous molecular studies have detected a much larger genetic diversity of the ERM fungal isolates than previously envisaged (cf. Cairney & Meharg, 2003), still predominantly within the Helotiales (Ascomycota). However, identification based on fungal cultivation only yields cultivable fungi. Consequently, through apparent circular reasoning the common view has been established that ERM fungi merely comprise readily cultivable ascomycetes (Smith & Read, 1997; Read & Perez-Moreno, 2003). Only a fraction of the thousands of known ECM-forming fungi are readily cultivable, and these possess according to Read and Perez-Moreno (2003) probably more 'decomposer' capabilities than the numerous non-cultivable ECM mutualists. Could this also apply for the ERM mutualists? Allen *et al.* (2003) recently demonstrated that the ericoid culture identification concept probably maintains a serious identification bias. They showed that conventional culturing from hair roots of the North American ericoid shrub *Gaultheria shallon* predominantly detected the ascomycetes *H. ericae* and *Capronia* spp., while direct DNA extraction revealed that *Sebacina*-like genotypes dominated the fungal community of the hair roots. Combined with earlier reports and observations, this strongly suggested that basidiomycetes of the *Sebacina* complex must be included in the group of ERM-forming fungi. As a parallel story to the *H. ericae* aggregate of the Ascomycota, the *Sebacina*-complex of the Basidiomycota apparently includes a broad range of closely related genotypes that are involved in ERM (Allen *et al.*, 2003), ECM (Selosse *et al.*, 2002a; Urban *et al.*, 2003) as well as orchid mycorrhizal partnerships (Selosse *et al.*, 2002b).

***Cadophora finlandia* – a fungal bridge between ERM and ECM**

Phylogenetic analysis based on nuclear ribosomal internal transcribed spacer (ITS) sequences recognize the *H. ericae* aggregate (Vrålstad *et al.*, 2000) as a monophyletic unit consisting of some well supported clades with numerous closely related genotypes. The clade including *Cadophora finlandia* (previously *Phialophora finlandia*) is dominated by ECM-forming isolates. Similarly, ERM isolates are concentrated in the *H. ericae* clade (Vrålstad *et al.*, 2002a, 2002b). *Cadophora finlandia* has earlier been reported to form ECM, ectendomycorrhiza (ECEM) and intracellular hyphal coils resembling ERM with respective hosts (see Villarreal-Ruiz *et al.*). Identical *C. finlandia*-like genotypes were also isolated from co-occurring ECM and ERM roots (Vrålstad *et al.*, 2002b), and the ERM isolate was later confirmed to form ECM with *Pinus sylvestris* *in vitro* (T. Vrålstad, unpublished). All these reports strongly suggest that *C. finlandia*-like fungi may act as ERM and ECM partners simultaneously. Conclusive evidence for this assumption is presented for the first time by Villarreal-Ruiz *et al.* They demonstrate that a single isolate from the *C. finlandia* clade, simultaneously in

time and from a continuous mycelium, develops structurally and apparently complete ERM (intracellular coils) with *Vaccinium myrtillus* and ECM (Hartig net and mantle) with *P. sylvestris*. The fungal colonization induced a substantial growth response in *V. myrtillus* roots, and no sign of pathogenic reactions were observed in any of the host plants. The functional aspects of this intriguing triangular relationship are beyond the scope of their study, and important questions about possible mutual benefit to all involved partners (cf. Read, 2000) still remain unanswered. However, demonstrating that a single mycelium is compatible with ERM and ECM roots (i.e. developing the characteristic ERM and ECM organs) is a breakthrough in the ongoing debate and an important encouragement for initiating further research on the ecological, evolutionary and functional relevance of this fungal bridge between ERM and ECM hosts.

Judging from the divergent groups of fungi involved in ERM symbioses (cf. Allen *et al.*, 2003), the ERM strategy has probably evolved independently in several fungal lineages. However, the *H. ericae* aggregate and particularly the *C. finlandia* clade represent one of the evolutionary links between ECM and ERM strategies. The predominantly ectomycorrhizal *C. finlandia* clade may be ancestral to the *H. ericae* clade, which seems more specialized on ERM. This aggregate may provide the key to a better understanding of the evolution of ERM, and potential switches between ECM and ERM. However, the results reported by Villarreal-Ruiz *et al.* emphasize that from the fungal point of view distinguishing between these strategies may not always be justified. More studies are required before we can predict whether host-specific and/or habitat-specific lineages have evolved within the *H. ericae* aggregate, or if these fungi are to a major extent compatible with a large range of hosts and habitats worldwide.

ERM in woodlands

By referring to how spruce planted into heathland ecosystems commonly show pathogenic reactions, Read (2000) argued that even though sharing of fungal symbionts between ECM trees and ERM shrubs may occur, it is open to considerable doubt as to what extent this is to the benefit of both host plants. There are probably several complex reasons why out-planted spruce seedlings may face severe problems in heathland soils. However, studies on the functional relevance of a shared fungus and the potential mutual benefit to all involved partners should select habitats where ECM and ERM hosts and fungi naturally co-occur. Interestingly, and of significant relevance to the discussion, the *C. finlandia*-like isolate used by Villarreal-Ruiz and colleagues had been isolated from an ECM root tip of *P. sylvestris* in a 160-year-old woodland where ECM and ERM plants (including *V. myrtillus*) naturally co-occur. In this respect, their study is a legitimate reminder of the highly unexplored role of ERM in the boreal woodland understorey.

Fig. 1 (a–d): Norway spruce dominated bilberry woodland represents a common natural North boreal forest habitat of intimately coexisting ectomycorrhizal trees and ericoid mycorrhizal shrubs. This woodland is characterized by an overstorey of *Picea abies* (Norway spruce) and a persistent understorey predominated by *Vaccinium myrtillus* (bilberry) (a–c). Bilberry is a low-growing deciduous shrub of the family Ericaceae that is native to northern Europe. Ripe bilberry fruits (d) are among the most valuable, tasty and appreciated boreal forest berries. Photos: Trude Vrålstad (a–c) and Leif Ryvarden (d).



Boreal and temperate woodlands are commonly characterized by an overstorey of coniferous trees with an understorey of ericaceous hosts. In Mediterranean mature *Quercus ilex* woodland devoid of ericaceous hosts, Bergero *et al.* (2003) used *Erica arborea* as a 'bait' plant (a native ericaceous host dominating in earlier successional stages). They found that the Mediterranean woodland soil contained an effective and diverse inoculum of fungi forming ERM with *E. arborea*. Interestingly, none of the ERM-forming fungi represented the taxa that we typically perceive as ERM fungi, such as *H. ericae* and *Oidiiodendron* spp. Instead, the ERM woodland inoculum represented unknown genotypes from different classes of ascomycetes (Leotiomycetes and Dothideomycetes). One possibility is that the ERM fungi persist in soils as saprotrophs, but another alternative is that the ericoid hosts take advantage of the community of ECM fungi that is present (Bergero *et al.*, 2000, 2003). This study suggests that the fungi predominating in ERM relationships may vary significantly between different biomes.

Boreal forests cover *c.* 17% of the global land surface and represent a biome where organic matter accumulates due to low temperatures and recalcitrant conifer and ericaceous litter rich in polyphenolic compounds (cf. Lindahl *et al.*, 2002). Nutrient and carbon cycling in these systems depend heavily on saprotrophic and mycorrhizal fungi (Lindahl *et al.*, 2002; Read & Perez-Moreno, 2003). While the diversity and density of ECM fungi in boreal forests is huge and relatively well studied (Taylor *et al.*, 2000; Horton & Bruns, 2001), we currently know very little about the fungi involved in woodland ERM relationships despite the significant abundance of ericaceous hosts. For example, Norway spruce-dominated bilberry woodland (Fig. 1) characterized by *Picea abies* (Norway spruce) and a persistent understorey predominated by *V. myrtillus* (bilberry) is by far the most common forest type in Norway, covering 35% of the pro-

ductive forest area (Larsson *et al.*, 1994). Bilberry, in terms of plant individuals and biomass (above- and below-ground) evidently possesses a significant role here (cf. Figure 1). It was from a similar boreal habitat that Villarreal-Ruiz *et al.* captured their ECM- and ERM-forming *C. finlandia* isolate. In a biome where light might be a limiting factor for understorey vegetation, the possibility that ectomycorrhizal trees and ericoid shrubs may be physically interconnected via common mycorrhizal networks (CMN) is intriguing. A fungal link would provide a theoretical canal for transfer of carbon, which is possible between different ECM species in the field (Simard *et al.*, 1997), as well as between ECM species and specialized mycoheterotrophic plants (Leake, 1994).

Cross-communication in the ERM and ECM partnerships

For the development of a mycorrhizal organ, temporally and spatially controlled activity of genes and proteins participating in morphogenetic processes are required (Martin *et al.*, 2001). For successful mycorrhizal development, the plant and fungal partner have to recognize each other and communicate. Understanding the molecular basis for the development of mycorrhizas requires studies of gene expression and cell-to-cell communication between fungi and roots (Tagu *et al.*, 2002). A fungus possessing the ability to communicate both with ERM and ECM hosts is a highly interesting study system. Eliminating the problem with fungal taxon-related bias, a model fungus of the caliber found by Villarreal-Ruiz and colleagues should allow studies on the gene-regulated differences and similarities that are involved in the development of the structurally different ERM and ECM organs. Clearly, the results presented by Villarreal-Ruiz *et al.* should encourage creative studies and experiments across several sectors of the mycorrhizal research field.

Trude Vrålstad

Department of Biology, University of Oslo,
PO Box 1045 Blindern, N-0316 Oslo, Norway
(tel +4722854663; fax +4722857655;
email trudev@ulrik.uio.no)

References

- Allen TR, Millar T, Berch S, Berbee ML. 2003. Culturing and direct DNA extraction find different fungi from the same ericoid mycorrhizal roots. *New Phytologist* **160**: 255–272.
- Bergero R, Girlanda M, Bello F, Luppi AM, Perotto S. 2003. Soil persistence and biodiversity of ericoid mycorrhizal fungi in the absence of the host plant in a Mediterranean ecosystem. *Mycorrhiza* **13**: 69–75.
- Bergero R, Perotto S, Girlanda M, Vidano G, Luppi AM. 2000. Ericoid mycorrhizal fungi are common root associates of a Mediterranean ectomycorrhizal plant (*Quercus ilex*). *Molecular Ecology* **9**: 1639–1649.
- Cairney JWG, Meharg AA. 2003. Ericoid mycorrhiza: a partnership that exploits harsh edaphic conditions. *European Journal of Soil Science* **54**: 735–740.
- Haug I, Lempe J, Homeier J, Weiss M, Setaro S, Oberwinkler F, Kottke I. 2004. *Griffithsia emarginata* (Melastomataceae) forms mycorrhizas with Glomeromycota and with a member of the *Hymenoscyphus ericae* aggregate in the organic soil of a neotropical mountain rain forest. *Canadian Journal of Botany* **82**: 340–356.
- Horton TR, Bruns TD. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology* **10**: 1855–1871.
- Larsson JY, Kielland-Lund J, Søgne SM. 1994. *Barskogens vegetasjonstyper*. Oslo, Norway: Landbruksforlaget.
- Leake JR. 1994. The biology of myco-heterotrophic (saprophytic) plants. *New Phytologist* **127**: 171–216.
- Lindahl BL, Taylor AFS, Finlay RD. 2002. Defining nutritional constraints on carbon cycling in boreal forests – towards a less ‘phytocentric’ perspective. *Plant and Soil* **242**: 123–135.
- Martin F, Duplessis S, Ditengou F, Lagrange H, Voiblet C, Lapeyrie F. 2001. Developmental cross talking in the ectomycorrhizal symbiosis: signals and communication genes. *New Phytologist* **151**: 145–154.
- Molina R, Massicotte H, Trappe JM. 1992. Specificity phenomena in mycorrhizal symbioses: Community-ecological consequences and practical implications. In: Allen, MJ, ed. *Mycorrhizal functioning – an integrated plant-fungal process*. London, UK: Chapman & Hall, 357–423.
- Read DJ. 1991. Mycorrhizas in ecosystems. *Experientia* **47**: 376–391.
- Read DJ. 2000. Links between genetic and functional diversity – a bridge too far? *New Phytologist* **145**: 363–365.
- Read DJ, Perez-Moreno J. 2003. Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? *New Phytologist* **157**: 475–492.
- Rosling A, Landeweert R, Lindahl BD, Larsson KH, Kuyper TW, Taylor AFS, Finlay RD. 2003. Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. *New Phytologist* **159**: 775–783.
- Selosse MA, Bauer R, Moyersoen B. 2002a. Basal hymenomycetes belonging to the Sebacinaceae are ectomycorrhizal on temperate deciduous trees. *New Phytologist* **155**: 183–195.
- Selosse MA, Weiss M, Jany JL, Tillier A. 2002b. Communities and populations of sebacinoïd basidiomycetes associated with the achlorophyllous orchid *Neottia nidus-avis* (L.) LCM Rich. & neighbouring tree ectomycorrhizae. *Molecular Ecology* **11**: 1831–1844.
- Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R. 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* **388**: 579–582.
- Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis*. London, UK: Academic Press.
- Tagu D, Lapeyrie F, Martin F. 2002. The ectomycorrhizal symbiosis: genetics and development. *Plant and Soil* **244**: 97–105.
- Taylor AFS, Martin F, Read DJ. 2000. Fungal diversity in ectomycorrhizal communities of Norway spruce (*Picea abies* [L.] Karst.) and Beech (*Fagus sylvatica* L.) along North-South transects in Europe. In: Schulze, ED, ed. *Carbon and nitrogen cycling in European forest ecosystems. Ecological studies series, Vol. 142*. Heidelberg, Germany: Springer-Verlag, 344–365.
- Urban A, Weiss M, Bauer R. 2003. Ectomycorrhizas involving sebacinoïd mycobionts. *Mycological Research* **107**: 3–14.
- Villarreal-Ruiz L, Anderson IC, Alexander IJ. 2004. Interaction between an isolate from the *Hymenoscyphus ericae* aggregate and roots of *Pinus* and *Vaccinium*. *New Phytologist* **164**: 183–192.
- Vrålstad T, Fosheim T, Schumacher T. 2000. *Piceirhiza bicolorata* – The ectomycorrhizal expression of the *Hymenoscyphus ericae* aggregate? *New Phytologist* **142**: 549–563.
- Vrålstad T, Myhre E, Schumacher T. 2002b. Molecular diversity and phylogenetic affinities of symbiotic root-associated ascomycetes of the Helotiales in burnt and metal polluted habitats. *New Phytologist* **155**: 131–148.
- Vrålstad T, Schumacher T, Taylor AFS. 2002a. Mycorrhizal synthesis between fungal strains of the *Hymenoscyphus ericae* aggregate and potential ectomycorrhizal and ericoid hosts. *New Phytologist* **153**: 143–152.

Key words: Boreal forest, *Cadophora finlandia*, Common mycorrhizal network (CMN), Ectomycorrhiza (ECM), Ericoid mycorrhiza (ERM), *Hymenoscyphus ericae*, *Picea abies*, *Pinus sylvestris*, *Vaccinium myrtillus*.

Meetings

The Mediterranean region – a hotspot for plant biogeographic research

Plant evolution in Mediterranean climate zones – IXth Meeting of the International Organization of Plant Biosystematics (IOPB), Valencia, Spain, May 2004

The Mediterranean region has been recognized as one of the 18 world hotspots where exceptional levels of biodiversity occur (cf. Blondel & Aronson, 1999). Representing only 1.6% of Earth's dry land, this region harbours more than 25 000 known vascular plant species, or about 10% of the world total – and more species continue to be described (e.g. Lihová *et al.*, 2004). In fact, more than half the plant species are endemic, and 80% of all European plant endemics are Mediterranean. Such high levels of plant diversity and endemism are generally thought to result from four major factors: biogeography, paleogeological/-climatic history, ecogeographical heterogeneity, and human influence (Blondel & Aronson, 1999). Accordingly, a comprehensive understanding of plant differentiation and speciation in the Mediterranean – though still elusive and lacking a book-length treatment – requires consideration of patterns and processes at a variety of spatio-temporal scales and taxonomic levels, thus fostering discussions among various subdisciplines within plant evolutionary research.

'Located at the crossroads between Europe, Asia, and Africa, the Mediterranean Basin has been considered as a huge "tension zone" for plant lineages of various biogeographic origins.'

The IXth IOPB Meeting (May 2004, <http://www.jardibotanic.org/iopb.html>) did just that, and provided broad-based and up-to-date views of the subject, including: discussions of phylogenetic origins, character evolution, and times of diver-

sification of Mediterranean plant lineages; their response to the climatic vicissitudes of the Pleistocene as evidenced by molecular phylogeographic reconstructions; and detailed discussions about the roles of ecology (Debussche *et al.*, 2004), hybridisation/reticulation (Gutiérrez Larena *et al.*, 2002), and reproductive isolation (Pérez *et al.*, 2003) in the origin of Mediterranean plant species. Genetic diversity, gene dispersal, and metapopulation dynamics of rare and endemic species were also covered (Hardy *et al.*, 2004), with their direct relevance to conservation biology and resource management. While the focus of the symposium was largely on the Mediterranean region (including Macaronesia, and with many studies restricted to the western and central portions of the Basin), all of the topics addressed hold promising lessons for other parts of the world, especially where Mediterranean-type climates (with a predominantly winter rainfall regime) exist, namely parts of California, the Cape Province of South Africa, central Chile, and two disjunct regions in southern and south-western Australia. Here I will focus on some emerging biogeographic themes from this symposium with an emphasis on Macaronesia and the Mediterranean Basin.

Macaronesia

The Macaronesian Islands comprise the Azores, Madeira, Canary Islands, Selvagens, and the Cape Verdes. Inferring the origin of the flora of these Atlantic archipelagos has been the focus of many molecular phylogenetic studies during recent years (for review see Vargas, in press). From a modern biogeographic perspective, there is hardly any other subregion within the Mediterranean realm better explored. At least in part, this reflects the archipelagos' long history of taxonomic research and the relative ease of access to comprehensive sources of plant material, both factors of which form the foundation of molecular phylogenetic studies. Based on those, there is compelling evidence now that: (i) the majority of species endemic to Macaronesia are sister to species/clades that have a predominantly western or, more rarely, eastern Mediterranean distribution (e.g. *Convolvulus*; Mark A. Carine, NHM, London, UK); (ii) many of the taxa studied occupy derived rather than basal positions in phylogenetic trees; and (iii) woodiness is often a novelty in these plants, derived from herbaceous ancestors (Javier Francisco-Ortega, Florida International University, Miami, USA).

Despite the large number of molecular phylogenies available that include Macaronesian taxa, participants in the symposium emphasized that further research is needed, especially with respect to a broader taxon (and gene) sampling

on the continent, and the possibility of incorporating new and advancing approaches to molecular dating analysis (e.g. Sanderson, 2002). Still, the picture appears incomplete since I am not aware of studies that have included taxa most relevant to the long-standing 'Tertiary relict hypothesis' (Vargas, in press). Arriving at an answer will require the production of good species-level phylogenies (and calibrated molecular clocks) of those representatives of the Macaronesian laurel-forest vegetation whose closest living relatives today are restricted to (neo)tropical regions, but which are well-documented in the European Late-Tertiary fossil record, such as members of the laurel family (*Laurus*, *Apollonia*, *Ocotea*, *Persea*) and the olive family (*Picconia*).

The Mediterranean Basin

Compared with Macaronesia, far less attention has been devoted to the origin of the complex admixture of plant taxa found in the Mediterranean Basin itself, and whose biogeographic origins, respective age, and evolutionary histories are thought to vary enormously. Located at the crossroads between Europe, Asia, and Africa, this region has been considered as a huge 'tension zone' for plant lineages of various biogeographic origins (i.e. northern/central Eurasia, south-west/central Asia, Saharo-Arabia, and tropical Africa), plus an indigenous component, corresponding to species that supposedly differentiated *in situ* within the limits of the Basin (Blondel & Aronson, 1999). Traditionally, designation of these five main 'elements' dominating the Basin's extant flora followed from fossil data or the historical interpretation of extant distribution patterns of taxonomic and/or phylogeographical entities (e.g. Quézel, 1985).

Aided by molecular phylogenetic reconstructions and estimates of absolute divergence times, researchers have now the opportunity to confirm long-held hypotheses about single or multiple biogeographic origins as well as the possible paths and periods of migration of plant lineages invading the Mediterranean Basin. For example, Caujapé-Castells *et al.* (2001) employed cpDNA restriction-site analysis to demonstrate that Mediterranean species of *Androcymbium* (Colchicaceae) are of Afro-tropical origin, and that the northward migration routes followed by this genus were at times restricted to the mountain ranges of eastern Africa, which probably acted either as stepping stones or as refugia. Interestingly, the genus likely arrived and started to diversify in the area under review in the Middle Miocene (*c.* 12 million years ago, Mya), hence well before the establishment of the seasonal Mediterranean-type climate, about 3.2–2.8 Mya (Suc, 1984). This suggests that the geophytic life-form of *Androcymbium* species in the Mediterranean did not specifically evolve as adaptation to present-day Mediterranean conditions but rather represents an ancient, plesiomorphic trait supposedly maintained by natural selection. Overall, results obtained for *Androcymbium* are consistent

with traditional biogeographic views about ancient contacts between southern/eastern Africa and the Mediterranean. This is further illustrated by recent phylogenetic work, e.g. in *Senecio* (Asteraceae; Coleman *et al.*, 2003), as well as a symposium presentation dealing with cpDNA haplotype variation in the *Olea europaea* complex, supporting an ancestral origin of eastern Mediterranean variants in subtropical Africa (Guillaume Besnard, University of Lausanne, Switzerland).

As to the temperate Eurasian element, relevant molecular phylogenetic data from Mediterranean buttercups were presented (Elvira Hörandl, University of Vienna, Austria). Both nuclear (ITS) and chloroplast (*matK*) sequence data suggest that Mediterranean members of *Ranunculus* sect. *Ranunculastrum* derive from two distinct lineages in the western and eastern parts of the Basin, respectively, and therefore must have colonized the region independently. Such parallel evolution of independent lineages sets the stage for further inquiry into multiple-trait parallelisms promoted by natural selection (Levin, 2001). If, for example, such lineages evolved after the establishment of the Mediterranean climate, one may ask whether they share the same set of morphologies and life-history traits in response to the similar environmental conditions in which they both occur now. Clearly, if common, such parallel changes may frequently result in faulty estimates of morphology-based classifications but, from an evolutionary perspective, will contribute to an increasing support for determinism in evolution (Wood *et al.*, in press).

However, it is the south-west Asian ('Irano-Turanian') stock that most likely donated the greatest number of derivatives to the Mediterranean Basin and even more remote regions. Here, the genus *Hordeum* (barley) is of particular interest. Based on dated phylogenies derived from three nuclear gene regions, the genus is thought to have originated about 12 Mya in the eastern Mediterranean and adjacent Mesopotamian regions (Frank Blattner, IPK Gatersleben, Germany). From there, it spread not only to Europe and Central Asia but also to the Mediterranean climate zones of South Africa and the Americas, often involving colonization of novel habitats at high elevations and/or latitudes. This startling range expansion now poses the challenge to identify the genetic, genomic and ecological changes associated with it, as well as the primary isolating barriers involved in the adaptive divergence of species in this system (see also Ramsey *et al.*, 2003).

It is certainly premature to make general statements about the patterns, processes, and times of arrival and settlement of the present-day flora of the Mediterranean Basin. This will require coordinated efforts of generating dated phylogenies for a wide diversity of evolutionarily independent plant taxa, perhaps similar to recent molecular studies of the comparative biogeography of European high mountain plants (Comes & Kadereit, 2003).

Illuminating large-scale biogeographic trends is also leading to more detailed intraspecific studies at the regional level, for example, involving populations north and south of

the Gibraltar Strait (separating the Iberian Peninsula from North Africa, 14 km) to test for genetic discontinuities as they may have been impacted since the Messinian salinity crisis (c. 6.0–5.3 Mya) when most of the present-day Mediterranean Sea dried-up. However, in contrast to what has been found in animal systems (Castella *et al.*, 2000), the Strait is probably less likely to act as a severe barrier to historical or current gene flow for plants, as supported by a recent AFLP survey in *Hypochaeris salzmanniana* (Asteraceae; Tod F. Stuessy, University of Vienna, Austria).

Paleoendemism, radiations, and Quaternary climate change

One noteworthy feature of the Basin's extant flora concerns patterns of very limited or disjunct geographical distribution, and low species-genus ratios, with many primarily long-lived taxa restricted to island or mountain habitats (Blondel & Aronson, 1999). In numerous instances, this has fuelled speculations about their status as 'paleoendemics' of likely Tertiary origin. One rare endemic from the Maritime Alps, *Saxifraga florulenta*, does serve as a good example. Here, molecular evidence indicates that the species evolved in the Late Miocene, most likely between the Tortonian (c. 11.6–7.2 Mya) and the Messinian (Elena Conti & Frank Rutschmann, University of Zürich, Switzerland). Precariously, if current trends of global warming continue, the strict ecological adaptation of this saxifrage to siliceous substrates at the highest altitudes of the Maritime Alps might represent an extinction risk. In general, barring the presence of unrecognised sibling/descendant species, such Mediterranean paleoendemics should be of considerable interest to evolutionists as they imply long periods without speciation. In turn, identifying factors that prevent these plants from diversifying (e.g. low dispersal ability) may provide clues to what is required for speciation (Coyne & Orr, 2004).

Nevertheless, there are many primarily short-lived plant groups that have produced large numbers of ecogeographically differentiated species in the Basin and there is increasing evidence that adaptive radiation has taken place relatively recently. This is particular so for a Mediterranean clade of *Senecio* where a molecular phylogeny indicates a near simultaneous and relatively recent diversification of c. 10 species (of presently wide or restricted distribution) within the Late Quaternary (< 1.0 Mya; Coleman *et al.*, 2003). Still, the number of such studies is not great, but is likely to grow in the near future as several basin-wide phylogenies presented during the conference are equally suggestive of relatively recent and 'explosive' speciation events, e.g. within Compositae-Anthemideae (Christoph Oberprieler, University of Regensburg, Germany) or *Centaureum* (Gentianaceae; Guilhem Mansion, University of Neuchâtel, Switzerland).

Especially for short-lived taxa, perhaps this newly emerging picture of recent and rapid radiations is not surprising

given the recent establishment of a Mediterranean-type climate at the Plio–Pleistocene boundary, which may have allowed the invasion of new and unoccupied habitats following the decimation of a Tertiary mesophytic flora (Coleman *et al.*, 2003). Arguably, however, as more information from paleoenvironmental and genetic studies is accumulating (Willis *et al.*, 2004), the question also arises of whether the Quaternary climatic oscillations (≤ 1.8 Mya) have exerted a major influence of patterns of geographic diversification and increased rates of speciation in the Basin, as suggested for Mediterranean warblers of the genus *Sylvia* (Blondel *et al.*, 1996) or Anatolian mountain frogs (Veith *et al.*, 2003).

Unfortunately, we have very few phylogenetic data of Mediterranean plant taxa that have been used to test hypotheses about differential rates of diversification within (or among) clades that may coincide with Quaternary climatic change or associated movements of sea level. This last factor, which in particular provides opportunities for allopatry, is likely responsible for accelerated speciation rates in *Nigella* (Ranunculaceae) from the Aegean archipelago (Christiane Bittkau, University of Mainz, Germany).

Finally, it is against this paleoclimatic background that the rapidly growing discipline of molecular phylogeography (Avice, 2000) comes into full swing, and several participants in the symposium drew attention to the importance of the Quaternary climatic changes in having shaped the present-day spatial genetic structure of a variety of plant species in the Mediterranean Basin, including ferns (*Asplenium*), conifers (*Pinus*), evergreen/deciduous oaks, and various coastal herbs (e.g. *Cakile maritima*). However, it is often difficult to draw firm inferences on the past history of populations entirely from genetic data when paleoecological data are non-informative or lacking. On the other hand, the power of the phylogeographic approach is illustrated by recent mtDNA studies in *Pinus sylvestris*, indicating the existence of refugia in the northern Iberian Peninsula that allowed the species to survive Pleistocene glaciations (Rachid Cheddadi, University of Montpellier, France). Significantly, this invalidates a common misapprehension that Iberian glacial refugia of woodland species were entirely confined to the southernmost parts of the peninsula.

Conclusions

In spite of having witnessed past periods of flourishing plant taxonomic and biosystematic research (e.g. Strid, 1970), it is only recently that the Mediterranean region, and the Basin in particular, have regained interest from evolutionists. As noted above, the area abounds in strikingly varying patterns of species distributions and species-genus ratios. This requires not only consideration of the temporal origins of a wide diversity of plant lineages but also their rates of species formation over time, as well as attempts to correlate these rates with various paleogeological, paleoclimatic or ecological factors,

or inherent biological features of the organisms (key characters) that might promote speciation (Coyne & Orr, 2004).

Although these topics received relatively little attention during the symposium, theoretical and practical advances have been outlined for investigating the general causes and rates of speciation within (and among) clades based on phylogenies derived from molecular data (Barraclough & Nee, 2001; Kadereit *et al.*, 2004). In addition, phylogeny-based methods now exist for inferring the geographic mode of speciation (allopatric, sympatric), e.g. by plotting the degree of range overlap of sister taxa against their age of divergence. This last approach, while criticised on grounds of past range shifts that may eradicate the signature of the biogeography of speciation, has been successfully applied to a diverse range of animals (see Coyne & Orr, 2004), suggesting that sympatric speciation is infrequent in nature.

Evidently, results obtained from all the above approaches must be qualified in view of their underlying assumptions, including the issue of (in)complete taxon sampling, the resolution and robustness of the underlying phylogenies (which too often are confounded by hybridisation, incomplete lineage sorting, or both), or the difficulties to calibrate branch lengths in real time. If reliable, these new analytical methods will provide an effective approach toward settling some long-standing questions about the origin and temporal course of plant diversification in the Mediterranean region and the forces involved.

Acknowledgements

The author apologizes for not having considered all research areas addressed during the Valencia conference in a more balanced way, emphasizing instead biogeographic topics. Joachim W. Kadereit (Mainz University) made valuable comments on an earlier draft of this article. Travelling support by a grant from the Deutsche Forschungsgemeinschaft (DFG Co 254/3-1) is also gratefully acknowledged.

Hans Peter Comes

Institut für Spezielle Botanik, Johannes
Gutenberg-Universität Mainz, D-55099 Mainz, Germany
(tel +49 6131 39-2-3169; fax +49 6131 39-2-3524;
email comes@uni-mainz.de)

References

- Avise JC. 2000. *Phylogeography: the history and formation of species*. Cambridge, MA, USA: Harvard University Press.
- Barraclough TG, Nee S. 2001. Phylogenetics and speciation. *Trends in Ecology and Evolution* 16: 391–399.
- Blondel J, Aronson J. 1999. *Biology and wildlife of the Mediterranean region*. Oxford, UK: Oxford University Press.
- Blondel J, Catzeflis F, Perret P. 1996. Molecular phylogeny and the historical biogeography of the warblers of the genus *Sylvia* (Aves). *Journal of Evolutionary Biology* 9: 871–891.
- Castella V, Ruedi M, Excoffier L, Ibáñez C, Arlettaz R, Hausser J. 2000.

- Is the Gibraltar Strait a barrier to gene flow for the bat *Myotis myotis* (Chiroptera: Vespertilionidae)? *Molecular Ecology* 9: 1761–1772.
- Caujapé-Castells J, Jansen RK, Membrives N, Pedrola-Monfort J, Montserrat JP, Ardanuy A. 2001. Historical biogeography of *Androcymbium* Willd. (Colchicaceae) in Africa: evidence from cpDNA RFLPs. *Botanical Journal of the Linnean Society* 136: 379–392.
- Coleman M, Liston A, Kadereit JW, Abbott RJ. 2003. Repeat intercontinental dispersal and Pleistocene speciation in disjunct Mediterranean and desert *Senecio* (Asteraceae). *American Journal of Botany* 90: 1446–1454.
- Comes HP, Kadereit JW. 2003. Spatial and temporal patterns in the evolution of the flora of the European Alpine System. *Taxon* 52: 451–462.
- Coyne JA, Orr HA. 2004. *Speciation*. Sunderland, MA, USA: Sinauer Associates.
- Debussche M, Garnier E, Thompson JD. 2004. Exploring the causes of variation in phenology and morphology in Mediterranean geophytes: a genus-wide study of *Cyclamen* L. *Botanical Journal of the Linnean Society* 145: 469–484.
- Gutiérrez Larena B, Fuertes Aguilar J, Nieto Feliner G. 2002. Glacial-induced altitudinal migrations in *Armeria* (Plumbaginaceae) inferred from patterns of chloroplast DNA haplotype sharing. *Molecular Ecology* 11: 1965–1974.
- Hardy OJ, Gonzáles-Martínez SC, Fréville H, Boquien G, Mignot A, Colas B, Olivieri I. 2004. Fine scale structure and gene dispersal in *Centaurea corymbosa* (Asteraceae) I. Pattern of pollen dispersal. *Journal of Evolutionary Biology* 17: 795–806.
- Kadereit JW, Griebeler E, Comes HP. 2004. Quaternary diversification in European alpine plants: pattern and process. *Philosophical Transactions of the Royal Society London B* 359: 265–274.
- Levin D. 2001. *The origin, expansion, and demise of plant species*. New York, NY, USA: Oxford University Press.
- Lihová J, Tribsch A, Stuessy TF. 2004. *Cardamine apennina*: a new endemic diploid species of the *C. pratensis* group (Brassicaceae) from Italy. *Plant Systematics and Evolution* 245: 69–92.
- Pérez R, Vargas P, Arroyo J. 2003. Convergent evolution of flower polymorphism in *Narcissus* (Amaryllidaceae). *New Phytologist* 161: 235–252.
- Quézel P. 1985. Definition of the Mediterranean region and origin of its flora. In: Gomez-Campo, C, ed. *Plant conservation in the Mediterranean area*. Dordrecht, The Netherlands: Dr W Junk, 9–24.
- Ramsey J, Bradshaw HD Jr, Schemske DW. 2003. Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution* 57: 1520–1534.
- Sanderson MJ. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution* 19: 101–109.
- Strid A. 1970. Studies in the Aegean Flora. XVI. Biosystematics of the *Nigella arvensis* complex with special reference to the problem of non-adaptive radiation. *Opera Botanica* 28: 1–169.
- Suc J-P. 1984. Origin and evolution of the Mediterranean vegetation and climate in Europe. *Nature* 307: 429–428.
- Vargas P. In press. Are Macaronesian islands refugia of relict plant lineages? A molecular survey. In: Weiss, SJ, Ferrand, N, eds. *Phylogeography in southern European refugia: evolutionary perspectives on the origins and conservation of European biodiversity*. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Veith M, Schmidler JF, Kosuch J, Baran I, Seitz A. 2003. Palaeoclimatic changes explain Anatolian mountain frog evolution: a test for alternating vicariance and dispersal events. *Molecular Ecology* 12: 185–199.
- Willis KJ, Bennett KD, Walker D, eds. 2004. The evolutionary legacy of the ice ages. *Philosophical Transactions of the Royal Society London B* 359.
- Wood TE, Burke JM, Rieseberg LH. In press. Parallel genotypic adaptation: when evolution repeats itself. *Genetica*.

Key words: biogeography, Macaronesia, Mediterranean, Quaternary, speciation.

Timelines

Magnolioid roots – hairs, architecture and mycorrhizal dependency

1913–2003: Geoffrey T.S. Baylis

The importance of root system architecture in determining the degree of benefit available to plants from forming mycorrhizal associations, especially arbuscular mycorrhizas (AM), is now a commonplace idea. The Glomeromycota clearly coevolved with the land plants and they may even have been the decisive enabling element in the colonisation of land (Nicolson, 1967; Nicolson, 1975; Pirozynski & Malloch, 1975). The earliest land plants had no root systems and acquisition of phosphate by their unbranched axes must have been minimal once plants moved from an aquatic environment to the land. The most ancient plants today (e.g. lycopods) have root systems with simple, often dichotomous branching, whereas recently evolved taxa such as grasses have intensely branched root systems. Mycorrhizal benefit tends to map neatly onto this pattern of root branching (Hetrick, 1991; Newsham *et al.*, 1995).

Most mycorrhizal researchers, if asked to trace the ancestry of these ideas, would cite Geoff Baylis. The recent sad news of Geoff's death at the age of 90 may prompt a re-examination of his remarkably insightful contributions. His two best-known papers (Baylis, 1967; Baylis *et al.*, 1975) have been influential, and his ideas were outlined even earlier (Baylis, 1959) but their key message has often been misunderstood, including by myself.

Magnolioid roots

In his 1975 paper at the memorable Leeds symposium on Endomycorrhizas organised by Francis Sanders and Bernard Tinker, Geoff discussed in detail the evolutionary trends in root system architecture and coined the phrase 'magnolioid root' to describe the coarse, little-branched, hairless roots of the Magnoliales, among the more ancestral orders in the flowering plants, and he clearly linked architecture to dependence on mycorrhizas. However, he also recognised that 'in most advanced families, root evolution has been plastic enough to provide a range of types', citing *Coprosma* and *Coffea* in the Rubiaceae.

I have made the mistake in print, and, worse, in a lecture when Geoff was in the audience, of crediting him with the idea that root system architecture determined mycorrhizal dependency. He took me aside afterwards and gently pointed out that this was not his view: try instead, 'the length and frequency of the root hairs is clearly the best single index of a plant's capacity for non-mycotrophic growth' (Baylis, 1975, p. 381).

There is other evidence to support this statement. Schweiger *et al.* (1995) showed a good relationship between the length of hairs and the P concentration needed for maximum growth in non-mycorrhizal plants, but surprisingly few comparative studies have included consideration of root hairs.

Root hairs and root system architecture – correlations and questions

Of course, there is a correlation between root hair density and length on the one hand and root system architecture on the other, which makes it hard to disentangle the two effects, but itself poses important questions: why should such a correlation exist? One might imagine that the benefit of long, dense root hairs would be greatest on coarse, unbranched roots, and that they would add little to an already efficiently foraging grass root system. Probably the answer lies in root longevity: coarse roots are generally long-lived, fine roots short-lived. Root hairs cannot be long-lived and would serve no purpose if they were, at least in terms of phosphate uptake, since they would persist in long-depleted soil. A greater mystery is the explanation for variation in root hair length. Why should root hair length vary from < 0.1 mm to > 2 mm (Baylis, 1975)? Such variations can easily be found within a family of plants, and possibly within a genus. Why, indeed, are root hairs not much longer still – what limits their elongation?

Geoff Baylis' legacy to science remains to be fully explored. The biology and ecology of root hairs is a rich field of study awaiting the marriage of molecular genetics with ecological and evolutionary theory.

Alastair H. Fitter

Department of Biology, University of York, PO Box 373,
York, YO10 5YW, UK (tel +44 1904328 555;
fax +44 1904328 510; email ahf1@york.ac.uk)

References

- Baylis GTS. 1959. Effect of vesicular-arbuscular mycorrhizas on growth of *Griselinia littoralis* (Cornaceae). *New Phytologist* **58**: 274–280.
- Baylis GTS. 1967. Experiments on the ecological significance of phycomycetous mycorrhizas. *New Phytologist*. **66**: 231–243.
- Baylis GTS. 1975. The magnolioid mycorrhiza and mycotrophy in root systems derived from it. In: Sanders FE, Mosse B, Tinker PB, eds. *Endomycorrhizas*. London, UK: Academic Press, 373–389.
- Hetrick BAD. 1991. Mycorrhizas and root architecture. *Experientia* **47**: 355–362.
- Newsham KK, Fitter AH, Watkinson AR. 1995. Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology and Evolution* **10**: 407–411.
- Nicolson TH. 1967. Vesicular-arbuscular mycorrhiza – a universal plant symbiosis. *Science Progress, Oxford* **55**: 561–581.
- Nicolson TH. 1975. Evolution of vesicular-arbuscular mycorrhizas. Sanders FE, Mosse B, Tinker PB, eds. *Endomycorrhizas*. London, UK: Academic Press, 25–34.
- Pirozynski KA, Malloch DW. 1975. The origin of land plants; a matter of mycotrophism. *Biosystems* **6**: 153–164.
- Schweiger PF, Robson AD, Barrow NJ. 1995. Root hair length determines beneficial effect of a *Glomus* species on shoot growth of some pasture species. *New Phytologist* **131**: 247–254.

Key words: arbuscular mycorrhiza, ecology, evolution, mycorrhizal dependency, mycotrophy, phosphate, root hairs, root system architecture.



About *New Phytologist*

- *New Phytologist* is owned by a non-profit-making **charitable trust** dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at www.newphytologist.org
- Regular papers, Letters, Research reviews, Rapid reports and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as-ready' via *OnlineEarly* – the 2003 average submission to decision time was just 35 days. Online-only colour is **free**, and essential print colour costs will be met if necessary. We also provide 25 offprints as well as a PDF for each article.
- For online summaries and ToC alerts, go to the website and click on 'Journal online'. You can take out a **personal subscription** to the journal for a fraction of the institutional price. Rates start at £108 in Europe/\$193 in the USA & Canada for the online edition (click on 'Subscribe' at the website)
- If you have any questions, do get in touch with Central Office (newphytol@lancaster.ac.uk; tel +44 1524 592918) or, for a local contact in North America, the USA Office (newphytol@ornl.gov; tel 865 576 5261)