Commentary

Ectomycorrhizas – out of Africa?

Ectomycorrhizas are the most frequent and widespread mycorrhizal type in the forests and woodlands of cool-temperate and boreal latitudes. Tree species from all the major plant families that provide the dominant trees of these regions (e.g. Pinaceae, Fagaceae, Betulaceae, Salicaceae) habitually form ectomycorrhizas under natural conditions. The ectomycorrhizal (ECM) habit shows particular adaptations for nutrient capture in temperate and boreal forests (Read & Perez-Moreno, 2003). On soils of low pH, where litter decomposition, for reasons of climate or litter quality, is slow, ectomycorrhizas are produced abundantly in soil-surface organic layers, and ECM fungi mobilize nitrogen and phosphorus from organic residues. There are strong links between plant mycorrhizal status and ecosystem carbon dynamics, because the foliage of temperate and boreal ECM tree species has significantly lower mean litter decomposition rates than that of comparable arbuscular mycorrhizal species (Cornelissen *et al.*, 2001). For these reasons, and also (one suspects) because the overwhelming majority of active researchers live and work in temperate regions, ectomycorrhizas have long been thought of as primarily a feature of temperate and boreal forests. However, it has been known for at least 50 yr (Peyronel & Fassi, 1956) that ECM trees also occur in tropical forest. It is true that most tropical tree taxa form arbuscular mycorrhizas (Alexander, 1989a), but an ecologically important minority of taxa form ectomycorrhizas. This includes, *inter alia*, the Dipterocarpaceae (Lee, 1998) and Fagaceae (Corner, 1972), many legumes in Caesalpinioideae (Alexander, 1989b), and Myrtaceae in the subfamily Leptospermoideae (Moyersoen *et al.*, 2001). A glance at this list of plant families is, in itself, enough to emphasize the importance of ectomycorrhizas in tropical ecosystems. Dipterocarps extend from East Africa and Madagascar, through India, Bangladesh and Sri Lanka, to Southeast Asia, ranging from south China in the north to Papua New Guinea in the south. There are two genera in South America. Dipterocarps make up 80% of the canopy trees and up to 40% of the understorey in Southeast Asian lowland and montane rainforest, and dominate the dry monsoonal forests of north India, Burma and Thailand. ECM legumes form groves or extensive monodominant stands in the rainforests of the Guineo–Congolian basin (Newbery *et al.*, 1988), while others cover vast areas in the Zambezian *miombo* woodlands of East and South–Central Africa, and the Sudanian savannah woodlands of the sub-Sahara. One of this group of ECM legumes (*Intsia*) extends into the dipterocarp forests of Southeast Asia, while another (*Dicymbe*) forms monodominant stands on poor soils throughout the Guyana shield of South America. The ecology and selective advantage of the ECM habit for host trees in the tropics is less well researched and well understood than in temperate and boreal forest. However, ECM trees in tropical forests occur in 'monodominant' stands, or stands where they make up a high percentage of basal area, or they attain 'family' dominance over very large areas. They appear to function in concert with one or more of a suite of other characters (large seeds, shade tolerance, mast fruiting, litter quality) to promote the success of their hosts (Alexander & Lee, 2005).

'Such a date places the evolution of angiosperm ectomycorrhizas close to the origin and early radiation of angiosperms, and well before the oldest fossils attributable to angiosperm lineages that nowadays form ectomycorrhizas.'

When and where did the ECM habit evolve?

Did the ECM habit arise in boreal and temperate forest, where ectomycorrhizas are most widespread today and where their selective advantage is clear, only later moving into tropical latitudes? Or might it have arisen independently, conceivably originally, in the tropics?

Modern plant phylogenies show that the ECM habit has arisen independently over the course of evolution in Pinaceae and several disparate lineages of angiosperms (Fitter & Moyersoen, 1996; Bruns & Shefferson, 2004). Ectomycorrhizas are clearly 'younger' than the ancient arbuscular mycorrhizal symbiosis, which has been around since the earliest stages of land-plant evolution (Remy *et al.*, 1994). The oldest known fossil ectomycorrhizas date from 50 million yr ago (mya) (LePage *et al.*, 1997), but it seems certain that the symbiosis predates this by some time, because the Pinaceae and many of the angiosperm families whose members now form ectomycorrhizas were extant well

before 50 mya (Wing & Boucher, 1998; Wang *et al.*, 2000), along with the major fungal lineages with modern ECM representatives (Berbee & Taylor, 2001). The discovery (Ducousso *et al.*, 2004) that the Sarcolaenaceae (an endemic family from Madagascar, sharing a common ancestor with Dipterocarpaceae) are ECM has been seen as evidence that ectomycorrhizas evolved in that lineage before dipterocarps drifted away from Madagascar/East Africa with the India/Seychelles land mass around 88 mya. In this issue, Moyersoen (pp. 753–762) argues that the evolution of ectomycorrhizas can be pushed even further back, to before 135 mya. This argument is based on his confirmation that the South American dipterocarp *Pakaraimaea dipterocarpacea*, endemic to Guayana and basal in the dipterocarp clade, is ECM. Because dipterocarps originated in Gondwana (Dayanandan *et al.*, 1999; Ducousso *et al.*, 2004), Moyersoen reasons that the ancestors of Dipterocarpaceae must have already had the capacity to form ectomycorrhizas prior to the separation of South America from Africa by the Atlantic around 135 mya.

Such a date places the evolution of angiosperm ectomycorrhizas close to the origin and early radiation of angiosperms, and well before the oldest fossils attributable to angiosperm lineages that nowadays form ectomycorrhizas (Willis & McElwain, 2002). Nevertheless, Moyersoen's hypothesis is very attractive and, as he points out, it gains support from an apparently parallel situation in the tribe Amherstieae of Caesalpiniaceae. This is a primarily African clade whose members have long been known to be ECM (Alexander, 1989b), and where the isolated South American genus *Dicymbe* has also recently been shown to form ectomycorrhizas (Henkel *et al.*, 2002). Assuming a common Gondwanaland ancestor for African and South American Amherstieae, a similar argument can be made for that ancestor to have been ECM prior to the continental break-up. One might also point out that the gymnosperm *Gnetum*, whose pantropical distribution could reflect a Gondwanan origin (Markgraf, 1929), is also ECM (Fassi, 1957).

What alternative explanations might there be?

First, one could argue that the ancestors of the ECM dipterocarps and legumes were not themselves ECM prior to the break-up of Gondwanaland; they merely carried in their genome the information that would allow their descendents independently to become ECM at some later time on either side of the Atlantic. Second, an increasing number of dated molecular phylogenies suggest that long-distance transoceanic dispersal may be more important in explaining angiosperm disjunctions, such as those between Africa and South America, than previously supposed, and that this can obscure any effect of tectonic history, previously assumed to have been the major cause of their biogeographical patterns (Pennington *et al.*, 2004). If this is the case, then the date of 135 mya for the evolution of ectomycorrhizas becomes suspect. With particular reference to the taxa mentioned here, Wikström *et al.* (2001) obtained a date for the origin of the Dipterocarpaceae, between 14 and 28 mya, precluding continental drift as an explanation for the observed distribution, although this date has been disputed (Givnish & Renner, 2004). Lavin *et al.* (2004) concluded that the age of most legume clades, including those with present-day distributions on either side of the Atlantic, precluded a large influence of continental vicariance on distribution patterns, and that long-distance dispersal was likely to be the primary explanation. Similarly, molecular data suggest that *Gnetum* probably reached its disjunct pantropical range either through transoceanic dispersal or through a Laurasian expansion followed by southwards spread well after the break-up of Gondwanaland (Won & Renner, 2006). So, while Moyersoen's reasoning is sound, his conclusion about the date of ECM evolution should be treated with caution until the debate over the role of vicariance vs long-distance dispersal in these key host taxa is resolved.

What is missing from the above discussion is any consideration of fungi. This is often the case in mycorrhizal matters, where a decidedly phytocentric approach prevails, but it is good to remember that the evolution of ectomycorrhizas is likely to have been at least as important for the fungi involved as for the hosts. Just as in plants, the ECM symbiosis has evolved independently among multiple lineages of fungi (Bruns & Shefferson, 2004). The major radiations of the ECM basidiomycete lineages took place certainly by the Eocene–Oligocene (Bruns *et al.*, 1998) and probably earlier in the Cretaceous (Berbee & Taylor, 2001). As Moyersoen points out, if ectomycorrhizas were present on the ancestors of dipterocarps and caesalpinioid legumes prior to the rifting of the South Atlantic, then one would expect that comigration of symbionts would result in at least some of their modern-day fungal associates showing similar disjunct distributions. It may be significant that ectomycorrhizas formed by Sebacinales were recorded both by Moyersoen on *Pakaraimaea*, and by Henkel *et al.* (2004) on *Dicymbe*. Sebacinales are the most basal group of basidiomycetes with known ECM members (Weiss *et al.*, 2004), and a dated phylogeny of this group – including material from South America, Africa and Asia – could be very informative. The Russulaceae are another group that would repay attention. Although Moyersoen, in his necessarily restricted sampling, did not record Russulaceae in association with *Pakaraimaea*, Henkel *et al.* (2002) found Russulaceae in association with *Dicymbe*, and they show high abundance and diversity as ECM associates of dipterocarps and legumes in both Africa and Asia (Buyck *et al.*, 1996; Lee *et al.*, 2003). Many years ago, Pirozynski (1983) suggested that Russulaceae were warm-adapted ECM fungi that had their origins in Africa, and recent work seems to support this view (Buyck, 1997). Here again, if a dated phylogeny were possible, it might reveal whether South American Russulaceae

 © The Authors (2006). Journal compilation © *New Phytologist* (2006)

have a Gondwanan origin, and shed further light on the question of where and when the ECM habit first arose.

Ian J. Alexander

School of Biological Sciences,
University of Aberdeen, AB24 3UU, UK
(tel +44 1224 272697; fax: +44 1224 272703;
email i.alexander@abdn.ac.uk)

Acknowledgements

I thank Ursula Eberhardt for useful discussions.

References

Alexander IJ. 1989a. Mycorrhizas in tropical forests. In: Proctor J, ed. *Mineral nutrients in tropical forest and savanna ecosystems*. Blackwell Scientific Publications, Oxford, pp. 169–188.

Alexander IJ. 1989b. Systematics and ecology of ectomycorrhizal legumes. In: Stirton CH, Zarucchi JL, eds. *Advances in legume biology*. Monographs in Systematic Botany No. 29. St Louis, MO, USA: Missouri Botanical Garden, pp. 607–624.

Alexander IJ, Lee SS. 2005. Mycorrhizas and ecosystem processes in tropical rain forest: implications for diversity. In: Burslem D, Pinard M, Hartley S, eds. *Biotic interactions in the tropics*. Cambridge, UK: Cambridge University Press, pp. 165–203.

Berbee ML, Taylor JW. 2001. Fungal molecular evolution: gene trees and geologic time. In: McLaughlin DJ, McLaughlin EG, Lemke PA, eds. *The Mycota: a comprehensive treatise on fungi as experimental systems for basic and applied research systematics and evolution, Part B*. Berlin: Springer-Verlag, pp. 229–245.

Bruns TD, Shefferson RP. 2004. Evolutionary studies of ectomycorrhizal fungi: recent advances and future directions. *Canadian Journal of Botany* **82**: 1122–1132.

Bruns TD, Szaro TM, Gardes M, Cullings KW, Pan JJ, Taylor DL, Horton TR, Kretzer A, Garbelotto M, Li Y. 1998. A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. *Molecular Ecology* **7**: 257–272.

Buyck B. 1997. *Flore Illustrée des Champignons d'Afrique Centrale – 15–17: Russula (Russulaceae) III*. Meise, Belgium: National Botanic Gardens of Belgium.

Buyck B, Thoen D, Watling R. 1996. Ectomycorrhizal fungi of the Guinea–Congo region. *Proceedings of the Royal Society of Edinburgh, B* **104**: 313–333.

Cornelissen JHC, Aerts R, Cerabolini B, Werger MJA, Van Der Heijden MGA. 2001. Carbon cycling traits of plant species are linked with mycorrhizal strategy. *Oecologia* **129**: 611–619.

Corner EJH. 1972. *Boletus in Malaysia*. Singapore: Government Printing Office.

Dayanandan S, Ashton PS, Williams SM, Primack RB. 1999. Phylogeny of the tropical tree family Dipterocarpaceae based on nucleotide sequences of the chloroplast *rbc*L gene. *American Journal of Botany* **86**: 1182–1190.

Ducousso M, Béna G, Bourgeois C, Buyck B, Eyssartier G, Vincelette M, Rabevohitra R, Randrihasipara L, Dreyfus B, Prin Y. 2004. The last common ancestor of Sarcolaenaceae and Asian dipterocarp trees was ectomycorrhizal before the India–Madagascar separation, about 88 million years ago. *Molecular Ecology* **13**: 231–236.

Fassi B. 1957. Ectomycorrhizes chez le *Gnetum africanum* Welw. due à *Scleroderma* sp. *Bulletin of the de la Societé Mycologique Francaise* **73**: 280–286.

Fitter AH, Moyersoen B. 1996. Evolutionary trends in root–microbe symbioses. *Philosophical Transactions of the Royal Society of London B* **351**: 1367–1375.

Givnish TJ, Renner SS. 2004. Tropical intercontinental disjunctions: Gondwana breakup, immigration from the boreotropics, and transoceanic dispersal. *International Journal of Plant Science* **165**: S1–S6.

Henkel TW, Terborgh J, Vilgalys RJ. 2002. Ectomycorrhizal fungi and their leguminous hosts in the Pakaraima Mountains of Guyana. *Mycological Research* **106**: 515–531.

Henkel TW, Roberts P, Aime MC. 2004. Sebacinoid species from the Pakaraima Mountains of Guyana. *Mycotaxon* **89**: 433–439.

Lavin M, Schrire B, Lewis G, Pennington RT, Delgado-Salinas A, Thulin M, Hughes C, Beyra Matos A, Wojciechowski MF. 2004. Metacommunity process rather than continental tectonic history better explains geographically structured phylogenies in legumes. *Philosophical Transactions of the Royal Society of London B* **359**: 1509–1522.

Lee SS. 1998. Root symbiosis and nutrition. In: Appanah S, Turnbull JM, eds. *A Review of Dipterocarps: taxonomy, ecology and silviculture*. Bogor, Indonesia: Center for International Forestry Research, pp. 99–114.

Lee SS, Watling R, Turnbull E. 2003. Diversity of putative ectomycorrhizal fungi in Pasoh Forest Reserve. In: Okuda T, Manokaran N, Matsumoto Y, Niiyama K, Thomas SC, Ashton PS, eds. *Pasoh: Ecology of a lowland rain forest in Southeast Asia*. Tokyo: Springer, pp. 149–159.

LePage BA, Currah RS, Stockey RA, Rothwell GW. 1997. Fossil ectomycorrhizae from the middle Eocene. *American Journal of Botany* **84**: 410–412.

Markgraf F. 1929. Monographie der Gattung *Gnetum*. *Bulletin du Jardin Botanique de Buitenzorg Series* **3**: 407–511.

Moyersoen B. 2006. *Pakaraimaea dipterocarpacea* is ectomycorrhizal, indicating an ancient Gondwanaland origin for the ectomycorrhizal habit in Dipterocarpaceae. *New Phytologist* **172**: 753–762.

Moyersoen B, Becker P, Alexander IJ. 2001. Are ectomycorrhizas more abundant than arbuscular mycorrhizas in tropical heath forests? *New Phytologist* **150**: 591–599.

Newbery DM, Alexander IJ, Thomas DW, Gartlan JS. 1988. Ectomycorrhizal rainforest legumes and soil phosphorus in Korup National Park, Cameroon. *New Phytologist* **109**: 433–450.

Pennington RT, Cronk QCB, Richardson JA. 2004. Introduction and synthesis: plant phylogeny and the origin of major biomes. *Philosophical Transactions of the Royal Society of London B* **359**: 1455–1464.

Peyronel B, Fassi B. 1956. Micorrize ectotrofiche in una Cesalpiniacea del Congo Belga. *Atti della Accademia delle Scienze Torino* **91**: 1–8.

Pirozynski KA. 1983. Pacific mycogeography: an appraisal. *Australian Journal of Botany Supplementary Series* **10**: 137–159.

Read DJ, Perez-Moreno J. 2003. Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? *New Phytologist* **157**: 475–492.

Remy W, Taylor TN, Haas H, Kerp H. 1994. Four hundred-million-year-old vesicular–arbuscular mycorrhizae. *Proceedings of the National Academy of Sciences, USA* **91**: 11841–11843.

Wang XQ, Tank DC, Sang T. 2000. Phylogeny and divergence times in Pinaceae: evidence from three genomes. *Molecular Biology and Evolution* **17**: 773–781.

Weiss M, Selosse M-A, Rexer K-H, Urban A, Oberwinkler F. 2004. Sebacinales: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. *Mycological Research* **108**: 1003–1010.

Wikström N, Savolainen V, Chase MW. 2001. Evolution of the angiosperms: calibrating the family tree. *Philosophical Transactions of the Royal Society of London B* **268**: 2211–2220.

Willis KJ, McElwain JC. 2002. *The evolution of plants*. Oxford, UK: Oxford University Press.

Wing SL, Boucher LD. 1998. Ecological aspects of the Cretaceous flowering plant radiation. *Annual Review of Earth and Planetary Sciences* **26**: 379–421.

Won H, Renner SS. 2006. Dating dispersal and radiation in the gymnosperm *Gnetum* (Gnetales) – clock calibration when outgroup relationships are uncertain. *Systematic Biology* **55**: 610–622.

Key words: breakup of Gondwanaland, caesalpinioideae, Dipterocarpaceae, evolution of ectomycorrhiza.

New Phytologist

Letters

From crop to model to crop: identifying the genetic basis of the staygreen mutation in the *Lolium/Festuca* forage and amenity grasses

Lolium/Festuca staygreen in context

The practical application of model systems to crop species is a topic of much current debate in the plant science community, and in this Letter we describe the progress made in determining the genetic control of staygreen in the *Lolium/Festuca* grasses, using a combination of experimental genetic analysis and publicly available genomic and transcriptomic resources. The analysis of *Lolium/Festuca* staygreen can be considered to be a useful model in this regard, as staygreen is an economically significant (and genetically recessive) trait for which molecular markers are required for efficient breeding purposes, and the gene determining staygreen plays a fundamental but, as yet, unspecified role in chlorophyll degradation. Additionally, as *Lolium/Festuca* grasses have been well characterized in terms of synteny with rice (*Oryza sativa*) (Armstead *et al.*, 2002; Jones *et al.*, 2002; Alm *et al.*, 2003; Sim *et al.*, 2005), the system is sufficiently developed to allow advantage to be taken of the available comparative genomics resources. In this Letter we will refer to the wild-type (normal yellowing) and mutant (staygreen) loci as *Y* and *y*, respectively.

Mutation of a gene regulating the pathway of chlorophyll degradation has been shown to result in the indefinite retention of greenness in senescing leaves of the temperate grass *Festuca pratensis* (Thomas & Stoddart, 1975; Thomas, 1987; Vicentini *et al.*, 1995), a phenotype that has been incorporated into *Lolium/Festuca* amenity grasses in order to enhance their year-round utility (e.g. AberNile; Department for Environment, Food and Rural Affairs, 2006, p. 6). Staygreen in *Lolium/Festuca* and other species is an example of one of a number of distinct genetic variants that interfere with the normal expression of senescence (Thomas & Smart, 1993; Thomas & Howarth, 2000) and advanced varieties of some of the world's major crops, for example maize (*Zea mays*) and *Sorghum* (Thomas & Howarth, 2000; Morgan *et al.*, 2002; Valentinuz & Tollenaar, 2004) owe their productivity and stress tolerance, at least in part, to the staygreen trait. The detailed biochemistry, cell biology, physiology, genetics and introgressive gene transfer of the staygreen phenotype from *F. pratensis* have been extensively described (Hauck *et al.*, 1997; Kingston-Smith *et al.*, 1997; Thomas *et al.*, 2002; Moore *et al.*, 2005).

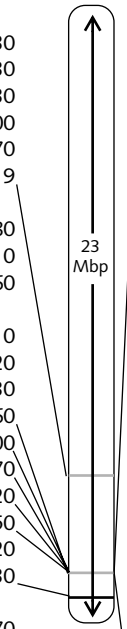
Crops and models: the experimental approach

The development of the initial *Lolium/Festuca* mapping population ($n = 100$) segregating for *F. pratensis*-derived *y* was reported previously (Moore *et al.*, 2005) and this population was used to assign *y* to *Lolium/Festuca* chromosome (C) 5 using anchored comparative mapping markers (Table 1a). Earlier studies (Armstead *et al.*, 2002; Jones *et al.*, 2002; Alm *et al.*, 2003; Sim *et al.*, 2005) had indicated that this region of *Lolium/Festuca* C5 shows a degree of synteny with rice C9, a rice chromosome known to contain a quantitative trait locus (QTL) for staygreen (Cha *et al.*, 2002). Using rice sequences taken from the The Institute for Genome Research (TIGR) rice C9 pseudomolecule (http://www.tigr.org/tdb/e2k1/osa1/), which flanked the position of this QTL as templates for primer design, it was possible to develop comparative mapping markers which demonstrated that *F. pratensis*-derived and rice staygreen phenotypes were determined from syntenically equivalent genomic regions. From an extended mapping family of 1627 individuals, 60 genotypes were identified which showed recombination in a 10-cM interval around *y*. Further implementation of the comparative mapping strategy based on these 60 recombinant genotypes allowed refinement of the relationship between *Lolium/Festuca* C5 and rice C9 to the extent that *F. pratensis*-derived staygreen could be localized to an equivalent region of rice C9 consisting of *c.* 200 kb, which contained 30 rice gene models (Table 1a,b). The annotations of these gene models in the TIGR database indicated that one of these, LOC_Os09g36200, was predicted to be a senescence-inducible chloroplast stay-green protein (Table 1c), although no direct evidence for this function was available in the published literature. To provide further validation for LOC_Os09g36200 as a candidate for *y*, the 30 implicated rice gene models were analysed for the presence of putative chloroplast transit peptides, using ChloroP 1.1 (http://genome.cbs.dtu.dk/services/ChloroP/; Emanuelsson *et al.*, 1999). Additionally, the temporal and organ-specific expression patterns of their most similar Arabidopsis gene models (Table 1d) were obtained through microarray data available in the Genevestigator® Meta-Analyzer database (https://www.genevestigator.ethz.ch/; Zimmerman *et al.*, 2004). Along with seven other gene models from this region, LOC_Os09g36200 was predicted to contain a chloroplast

 © Institute of Grassland & Environmental Research (2006). Journal compilation © *New Phytologist* (2006)

Table 1 Syntenic relationship between *Lolium/Festuca* C5 and rice C9. (a) Genetic markers mapped in rice (*Oryza sativa*) and *Lolium/Festuca* and their equivalent positions on the TIGR rice 9 pseudomolecule. (b) Diagrammatic representation of the physical position of mapped markers and genes on the TIGR rice 9 pseudomolecule. (c) TIGR gene models and predicted functions for candidate sequences for the *Festuca pratensis* staygreen gene. (d) Most similar Arabidopsis sequence

(a)	Genetic distance of markers on *L. perenne* C5 and rice C9			Genetic distance from* or no. recombinants with† *y* on rice C9 or *Lm/Fp* C5			(b)	(c)		(d) AGI proteins (TAIR)[b]	
Marker	*Lp* C5	riceA C9	riceB C9	riceC *y**	*Lm/Fp yA**	*Lm/Fp yB†*	TIGR C9 gene model	TIGR gene model	TIGR putative annotation	Arabidopsis gene model	*P*
								Os09g36060	Endoglucanase 1 precursor	AT4G39010	2.4e-161
E51174S	3.1	–	–	–	49.7	–	–	Os09g36070	Cytochrome P450	AT3G26310	4.4e-70
C1176.2	8.2	–	35.2	–	–	–	Os09g17830	Os09g36080	Cytochrome P450	AT1G13080	1.0e-72
BCD1087	8.2	–	38.5	–	–	–	Os09g17730	Os09g36090	Plus-3 domain	AT3G51120	3.8e-148
RZ206	9.2	–	38.5	–	–	–	Os09g19730	Os09g36100[c]	Expressed protein	AT3G51100	1.2e-24
R1751	15.2	49.3	–	–	–	–	Os09g24200	Os09g36110	Subtilisin N-terminal region	AT4G10550	6.1e-170
PSR574	12	62.4	–	–	–	–	Os09g27870	Os09g36120[c]	Expressed protein	AT5G17280	4.8e-12
CDO412	19	–	61.9	–	10.7	–	Os09g28019	Os09g36130[c]	Expressed protein	AT2G21385	2.5e-102
RM160	–	–	–	9.7	–	–	–	Os09g36140	RNA recognition motif 2 family protein	AT3G26120	2.5e-24
RG570	–		80.3	7.2	–	–	Os09g33780	Os09g36150	Retrotransposon protein	AT2G38950	0.060
S2655	–	77.7	–	–	3.4	–	Os09g33810	Os09g36160	Expressed protein	AT5G66350	2.1e-40
C1263	–	78.5	–	4.2	–	–	Os09g34960	Os09g36170	Hypothetical protein	AT1G17980	0.26
RG662	–	–	–	1.8	–	–	–	Os09g36180[c]	Glycosyl transferase family 8 protein	AT3G61130	1.1e-221
R3330	–	79.1	–	–	–	4	Os09g35710	Os09g36190[c]	Glycosyltransferase QUASIMODO1	AT3G61130	8.9e-249
9g35920	–	–	–	–	–	2	Os09g35920	Os09g36200[c]	Senescence-inducible chloroplast stay-green protein	AT4G22920	1.5e-73
9g36030	–	–	–	–	–	4	Os09g36030	Os09g36210	Harpin-induced protein 1	AT4G01410	6.6e-15
9g36060	–	–	–	–	–	1	Os09g36060	Os09g36220	Two-component response regulator-like PRR95	AT5G24470	2.9e-79
9g36200	–	–	–	–	–	0	Os09g36200	Os09g36230[c]	Hypothetical protein	AT5G46100	5.0e-33
9g36270	–	–	–	–	–	0	Os09g36270	Os09g36240	Deoxyribodipyrimidine photolyase	AT5G46100	2.7e-104
9g36320	–	–	–	–	–	0	Os09g36320	Os09g36250	ODORANT1 protein	AT4G22680	3.1e-65
9g36350	–	–	–	–	–	2	Os09g36350	Os09g36260	Hypothetical protein	AT3G57900	0.16
C985[a]	–	82.1	–	2.1	–	–	Os09g36420	Os09g36270	Pantothenate kinase 2	AT4G32180	6.8e-297
R2710	65	–	–	–	3.4	–	Os09g38030	Os09g36280	Glycosyl hydrolases family 17	AT3G57270	3.4e-50
T4	–	90.1	–	10.8	–	–	–	Os09g36290	Serine/threonine protein phosphatase	AT5G63140	5.7e-119
RM189	–	–	–	16.3	–	–	Os09g38370	Os09g36300	Lon protease homologue 1	AT5G47040	0.
RZ404	78	94.2	–	–	–	–	Os09g38580	Os09g36310	Hypothetical protein	AT5G28560	0.21
								Os09g36320[c]	Serine/threonine-protein kinase NAK	AT5G47070	4.5e-103
								Os09g36330	Hypothetical protein	**NONE**	–
								Os09g36340	HVA22-like protein i	AT5G42560	1.5e-38
								Os09g36350	Glycosyl hydrolase family 9	AT1G75680	5.0e-168



riceA, RGP 2003 high-density rice genetic map; riceB, Cornell RFLP 2001 rice genetic map (data obtained from Gramene database; http://www.gramene.org/); riceC, Cha *et al*. (2002) *sgr* (*y*) population.
Lm/Fp yA, original *Lolium/Festuca* introgression *y* screening population (*n* = 100); *Lm/Fp yB*, recombinant genotypes (*n* = 60) identified in expanded *Lolium/Festuca* introgression *y* screening population (*n* = 1627).
[a]Alternative position to that in Gramene database consistent with relative genetic map position. Identified by BLAST search of GenBank accession number D22707 against rice pseudomolecules database at http://www.tigr.org/tdb/e2kl/osal/.
[b]Most significant alignment present in Genevestigator® chipset database as determined by WU-BLAST2 searches at The Arabidopsis Information Resource (TAIR; https://www.arabidopsis.org/wublast/index2.jsp). AGI, Arabidopsis Genome Initiative.
[c]Chloroplast transit peptide predicted by ChloroP 1.1.

© Institute of Grassland & Environmental Research (2006). Journal compilation © *New Phytologist* (2006)

New Phytologist

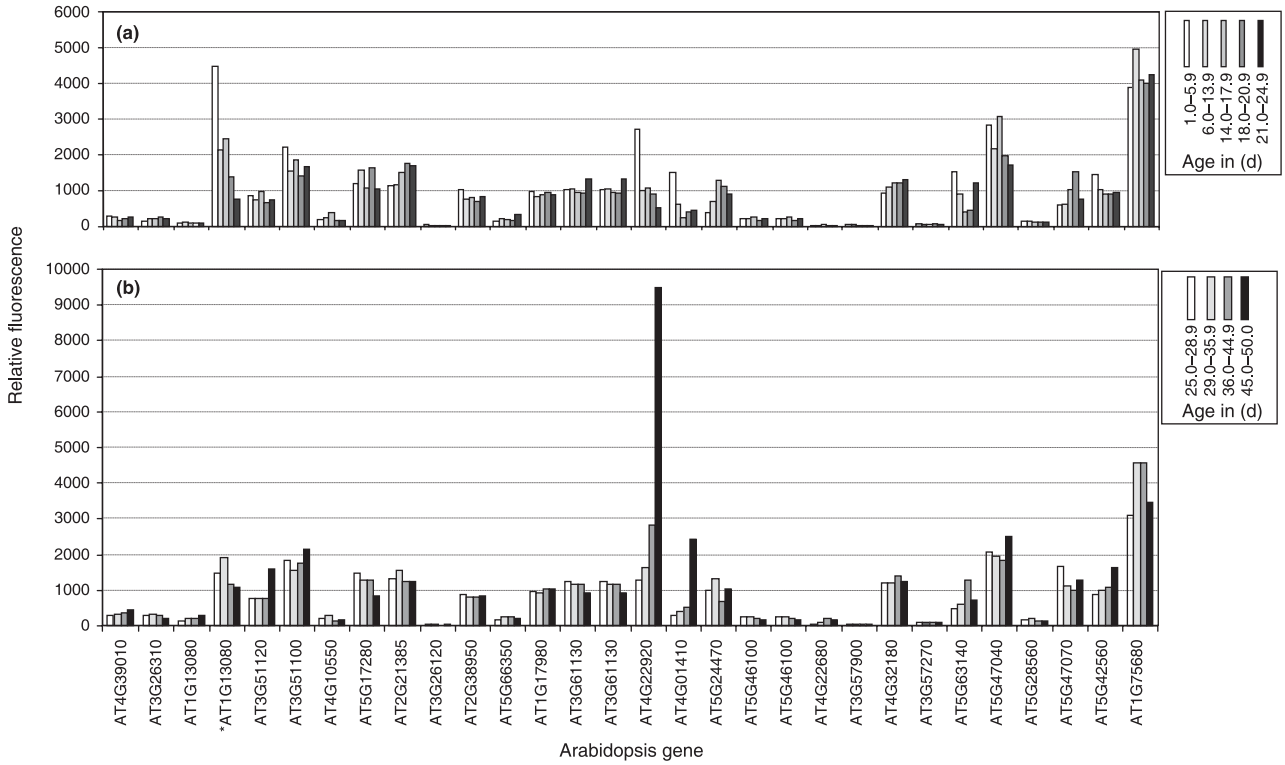


Fig. 1 Relative fluorescence values from microarray analysis of selected Arabidopsis genes; each Arabidopsis gene is an orthologue of a TIGR rice (*Oryza sativa*) gene model predicted to be nonrecombinant with *Festuca pratensis*-derived *y* on the basis of synteny (Table 1). Results are expressed according to Arabidopsis whole-plant growth stage: (a) 1–24.9 d and (b) 25–50 d (see https://www.genevestigator.ethz.ch/ for growth stage definitions). Values were obtained from the Genevestigator® Meta-Analyzer database. *Data from two different probe sets.

transit peptide (Table 1c). In terms of the expression profiles, the relative fluorescence of At4g22920 (the most similar Arabidopsis protein to LOC_Os09g36200) was clearly up-regulated in days 45–50, coinciding with maximal senescence in the Arabidopsis life-cycle (Fig. 1b). At4g01410 (the most similar Arabidopsis protein to LOC_Os09g36210) also showed a similar expression pattern, although of smaller relative magnitude (Fig. 1a). None of the remainder of the candidate genes indicated obvious specific induction in days 45–50. Examination of the tissue-specific pattern of expression of At4g01410 and At4g22920 indicated that the former was most strongly expressed in the seed and showed no specific association with the senescent leaf (Fig. 2a). The latter was also strongly expressed in the seed as well as in the petals and sepals. Notably, however, it was associated most strongly with the senescent leaf (Fig. 2b). Subsequent northern analysis of this gene in *Lolium temulentum* and staygreen *F. pratensis* confirmed senescence-associated expression in *Lolium*/*Festuca* leaves. Therefore, on the basis of genetic association, comparative genomics, putative gene function and expression profile, a recessive mutation in the *F. pratensis*-derived homologue of LOC_Os09g36200 was considered to be a strong candidate for *y*.

In order to develop an allele-specific molecular marker for staygreen, the genomic sequence of the *L. perenne* homologue of LOC_Os09g36200 was obtained by polymerase chain reaction (PCR) screening of an *L. perenne* bacterial artificial chromosome (BAC) library (Farrar *et al.*, 2006) and this was used to develop primers for the amplification of regions of *y*. Allele-specific primers are often most efficiently developed by amplifying intronic sequences; however, in this case a 4-bp ATAT insertion was identified in the second predicted exon of the candidate gene (Fig. 3). This allowed the development of both a size-specific molecular marker suitable for high-throughput screening and a cleaved amplified polymorphic sequence (CAPS) marker (the ATAT insertion removed an existing *Bst*F5I restriction enzyme site present in the wild-type sequence) suitable for analysis on agarose gels (Fig. 3a). To date, assays of these markers on experimental mapping populations and application in existing breeding lines have identified no recombination between *y* and the molecular markers.

Beyond the development of a molecular marker, the presence of a 4-bp insertion in an exon represents a translational frameshift, and comparison of the rice and *Lolium* candidate genes with homologous sequences from other species indicates that this 4-bp insertion is peculiar to staygreen lines containing the *F. pratensis*-derived *y* locus

 © Institute of Grassland & Environmental Research (2006). Journal compilation © *New Phytologist* (2006)

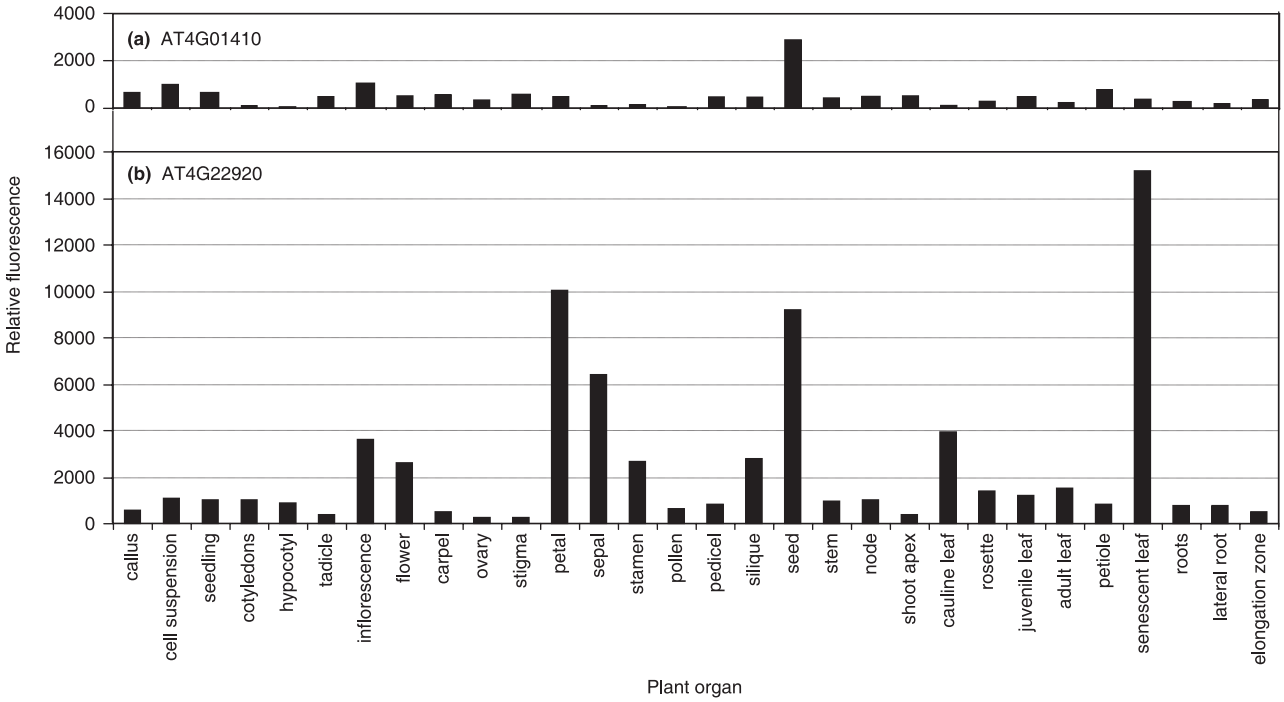


Fig. 2 Relative fluorescence values from microarray analysis of Arabidopsis genes (a) AT4g01040 and (b) AT4G22920, orthologues of TIGR rice (*Oryza sativa*) gene models LOC_Os09g36210 and LOC_Os09g36200, respectively. Results are expressed according to plant organ specificity. Values were obtained from the Genevestigator® Meta-Analyzer database.

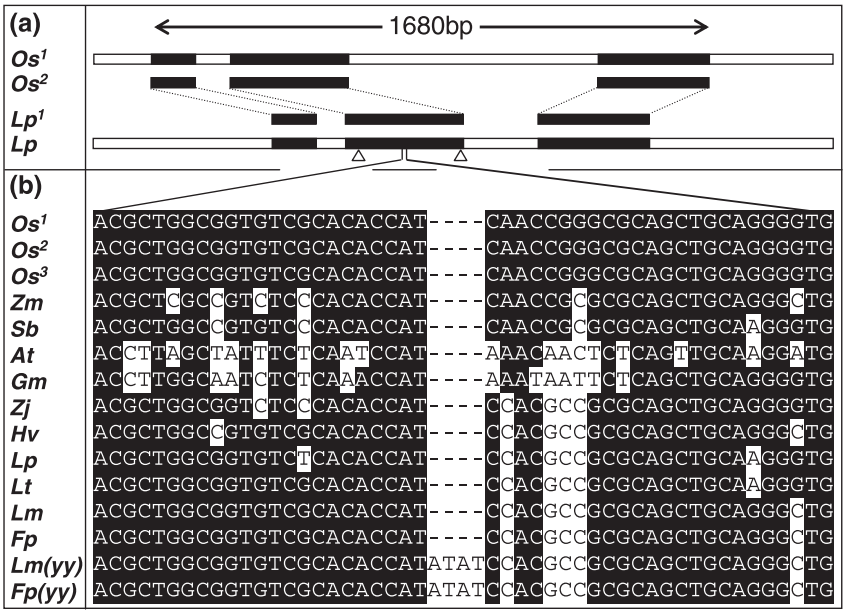


Fig. 3 (a) Diagrammatic representation of the physical distribution of exons in rice (*Oryza sativa*) LOC_Os09g36200 and its homologue from *Lolium perenne*: black horizontal bars, exons; white horizontal bars, noncoding genomic sequence; open triangles, priming sites for marker used in the detection of the ATAT insertion. Dotted lines connect equivalent exons. (b) Partial sequence alignment of exon 2 from LOC_Os09g36200 and its homologues from a number of plant species, illustrating the ATAT insertion in *Lolium/Festuca yy* genotypes. *Os*[1], LOC_Os09g36200 gene sequence; *Os*[2], LOC_Os09g36200 CDS; *Os*[3], *O. sativa* (AY850134); *Lp*, *L. perenne* bacterial artificial chromosome (BAC) sequence; *Lp*[1], *L. perenne* BAC sequence predicted coding sequences (CDS); *Hv*, *Hordeum vulgare* (AY850135); *Zj*, *Zoysia japonica* (AY850154); *Zm*, *Zea mays* (AY850138); *Sb*, *Sorghum bicolor* (AY850140); *Gm*, *Glycine max* (AY850141); *At*, *Arabidopsis thaliana* (AY850161); *Lt*, *Lm* and *Fp*, *Lolium temulentum*, Lolium multiflorum and *Festuca pratensis* genomic polymerase chain reaction (PCR) products covering the second predicted exon; (*yy*), mutant genotype expressing staygreen. GenBank accession numbers are indicated in parentheses.

© Institute of Grassland & Environmental Research (2006). www.newphytologist.org
Journal compilation © *New Phytologist* (2006)

(Fig. 3b). The mechanism by which this mutation was produced is unknown, but short insertion sequences of this type have been shown to arise as footprints following mobilization of a transposable element (Pooma *et al.*, 2002), although active transposition has not been characterized directly in *Lolium*/*Festuca*. Approximately 1250 bp of the *F. pratensis y* genomic sequence has been isolated so far, spanning the complete first and second exons and, up to the ATAT insertion, the peptide predicted from this is identical to the wild-type *L. perenne* protein. Use of the complete *L. perenne* gene, with and without the ATAT insertion, as a model for the comparison of mutant and wild-type proteins shows that the insertion radically changes the amino acid sequence of the mutant protein from position 100 and gives a final protein consisting of 232 residues, as compared to 279 in the wild type. The region of the protein derived from the second exon is highly conserved across a number of different species (Fig. 3b), indicating that one or more active sites may be affected by the frameshift mutation; this could give rise to a functional knockout mutation, as seen for *y*. Work is under way, using immunochemical approaches, to understand and localize the effect of mutation in *Y* on protein expression in both crop and model species

Staygreen and chlorophyll catabolism

It is established that *Y* is a key gene involved in chlorophyll catabolism, and it is interesting to speculate upon its function. The biochemical lesion represented by *y* in staygreen *F. pratensis* has been shown to be located in the pathway of chlorophyll catabolism at the step where the chlorin macrocycle is opened by oxygenolytic cleavage (Vicentini *et al.*, 1995). Measured *in vitro*, activity of the enzyme responsible (phaeophorbide a oxygenase, PaO) is abnormally low in staygreen lines. There is, however, good genetic and biochemical evidence to show that PaO is expressed, although inactive, in staygreen *Festuca* and *Lolium* (Vicentini *et al.*, 1995; Roca *et al.*, 2004): it is therefore likely that the product of the *Y* gene modulates the activity of PaO. Chlorophyll degradation is organizationally complicated, as the pigment substrates for the catabolic enzymes are complexed with thylakoid membrane proteolipids (Thomas, 1987). Disassembly of these complexes must take place in a regulated fashion if the photodynamic tendencies of chlorophyll catabolites are to be kept in check (Thomas, 1987; Eckhardt *et al.*, 2004; Hörtensteiner, 2004). Recently, a protease (FtsH6) has been described that specifically breaks down chlorophyll-binding proteins in senescence (Zelisko *et al.*, 2005). We propose that disassembly and degradation of the pigment-proteolipids of thylakoids is mediated by a complex of enzymes, including PaO (which in turn interacts with a second enzyme, red chlorophyll catabolite reductase (RCCR) reductase (Hörtensteiner *et al.*, 2000; Eckhardt *et al.*, 2004)), *Y* (Hörtensteiner, 2006) and FtsH6 (Zelisko *et al.*, 2005) – and possibly other activities too, such as acyl hydrolase (He & Gan, 2002). A search for a multienzyme complex that functions as a machine for dismantling chloroplast membranes is justifiable. Identification of *Y* and other recently reported genes as the putative components of such a machine is a significant step towards an understanding of the mechanism and control of this central event in senescence.

Conclusion

This report has described how classical genetics and detailed biochemical analysis paved the way for the identification of a strong candidate for a key gene in the chlorophyll catabolic pathway using the tools of modern comparative genomics and transcriptomics. It has also shown how, by identifying this candidate and determining its protein product, it has now become possible to formulate and test new hypotheses concerning the biochemical regulation of chlorophyll catabolism. The practical application of this work has also been significant, as it has led to the development of an allele-specific marker which, to date, has shown no recombination with *y* and which is currently being employed in breeding programmes to transfer this recessive phenotype.

Acknowledgements

This work was supported by the BBSRC (UK), the Swiss National Science Foundation (3100A0-105389) and USDA/CSREES for Hatch Project MONB00235.

Ian Armstead[1]*, Iain Donnison[1], Sylvain Aubry[2], John Harper[1], Stefan Hörtensteiner[2], Caron James[1], Jan Mani[2], Matt Moffet[3], Helen Ougham[1], Luned Roberts[1], Ann Thomas[1], Norman Weeden[3], Howard Thomas[1] and Ian King[1]

[1]Institute of Grassland and Environmental Research, Aberystwyth SY23 3EB, UK; [2]Institute of Plant Sciences, University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland; [3]Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT 59717, USA.
(*Author for correspondence:
tel +1970 823108; fax +1970 823242;
email ian.armstead@bbsrc.ac.uk)

References

Alm V, Fang C, Busso CS, Devos KM, Vollan K, Grieg Z, Rognli OA. 2003. A linkage map of meadow fescue (*Festuca pratensis* Huds) and comparative mapping with other Poaceae species. *Theoretical and Applied Genetics* **108**: 5–40.

 © Institute of Grassland & Environmental Research (2006). Journal compilation © *New Phytologist* (2006)

Armstead IP, Turner LB, King IP, Cairns AJ, Humphreys MO. 2002. Comparison and integration of genetic maps generated from F2 and BC1-type mapping populations in perennial ryegrass. *Plant Breeding* **121**: 501–507.

Cha KW, Lee YJ, Koh HJ, Lee BM, Nam YW, Paek NC. 2002. Isolation, characterization, and mapping of the stay green mutant in rice. *Theoretical and Applied Genetics* **104**: 526–532.

Department for Environment, Food and Rural Affairs. 2006. *Plant varieties and seeds gazette.* Special edition number 501. Cambridge, UK: The Plant Variety Rights Office.

Eckhardt U, Grimm B, Hörtensteiner S. 2004. Recent advances in chlorophyll biosynthesis and breakdown in higher plants. *Plant Molecular Biology* **56**: 1–14.

Emanuelsson O, Nielsen H, von Heijne G. 1999. ChloroP, a neural network-based method for predicting chloroplast transit peptides and their cleavage sites. *Protein Science* **8**: 978–984.

Farrar K, Asp T, Lubberstedt T, Xu M, Thomas A, Christiansen C, Humphreys MW, Donnison IS. 2006. Construction of two *Lolium perenne* BAC libraries and identification of BACs containing candidate genes for disease resistance and forage quality. *Molecular Breeding.* (In press.)

Hauck B, Gay AP, Macduff J, Griffiths CM, Thomas H. 1997. Leaf senescence in a non-yellowing mutant of *Festuca pratensis*, implications of the stay-green mutation for photosynthesis, growth and nitrogen nutrition. *Plant, Cell & Environment* **20**: 1007–1018.

He Y, Gan S. 2002. A gene encoding an acyl hydrolase is involved in leaf senescence in *Arabidopsis*. *Plant Cell* **14**: 805–815.

Hörtensteiner S. 2004. The loss of green color during chlorophyll degradation – a prerequisite to prevent cell death? *Planta* **219**: 191–194.

Hörtensteiner S. 2006. Chlorophyll degradation during senescence. *Annual Review of Plant Biology* **57**: 55–77.

Hörtensteiner S, Rodoni S, Schellenberg M, Vicentini F, Nandi OI, Qui YL, Matile P. 2000. Evolution of chlorophyll degradation, the significance of RCC reductase. *Plant Biology* **2**: 63–67.

Jones ES, Mahoney NL, Hayward MD, Armstead IP, Jones JG, Humphreys MO, King IP, Kishida T, Yamada T, Balfourier F, Charmet G, Forster JW. 2002. An enhanced molecular marker-based map of perennial ryegrass (*Lolium perenne* L.) reveals comparative relationships with other Poaceae species. *Genome* **45**: 282–295.

Kingston-Smith AH, Thomas H, Foyer CH. 1997. Chlorophyll a fluorescence, enzyme and antioxidant analysis provide evidence for the operation of an alternative electron sink during leaf senescence in a stay-green mutant of *Festuca pratensis*. *Plant, Cell & Environment* **20**: 1323–1337.

Moore BJ, Donnison IS, Harper JA, Armstead IP, King J, Thomas H, Jones RN, Jones TH, Thomas HM, Morgan WG, Thomas A, Ougham HJ, Huang L, Fentem T, Roberts LA, King IP. 2005. Molecular tagging of a senescence gene by introgression mapping of a mutant stay-green locus from *Festuca pratensis*. *New Phytologist* **165**: 801–806.

Morgan PW, Finlayson SA, Childs KL, Mullet JE, Rooney WL. 2002. Opportunities to improve adaptability and yield in grasses, lessons from sorghum. *Crop Science* **42**: 1791–1799.

Pooma W, Gersos C, Grotewold E. 2002. Transposon insertions in the promoter of the *Zea mays* a1 gene differentially affect transcription by the myb factors P and C1. *Genetics* **161**: 793–801.

Roca M, James C, Pruzinska A, Hortensteiner S, Thomas H, Ougham H. 2004. Analysis of the chlorophyll catabolism pathway in leaves of an introgression senescence mutant of *Lolium temulentum*. *Phytochemistry* **65**: 1231–1238.

Sim S, Chang T, Curley J, Warnke SE, Barker RE, Jung G. 2005. Chromosomal rearrangements differentiating the ryegrass genome from the Triticeae, oat, and rice genomes using common heterologous RFLP probes. *Theoretical and Applied Genetics* **110**: 1011–1019.

Thomas H. 1987. Sid, a Mendelian locus controlling thylakoid membrane disassembly in senescing leaves of *Festuca pratensis*. *Theoretical and Applied Genetics* **73**: 551–555.

Thomas H, Howarth CJ. 2000. Five ways to stay green. *Journal of Experimental Botany* **51**: 329–337.

Thomas H, Ougham H, Canter P, Donnison I. 2002. What stay-green mutants tell us about nitrogen remobilisation in leaf senescence. *Journal of Experimental Botany* **53**: 801–808.

Thomas H, Smart CM. 1993. Crops that stay green. *Annals of Applied Biology* **123**: 193–219.

Thomas H, Stoddart JL. 1975. Separation of chlorophyll degradation from other senescence processes in leaves of a mutant genotype of meadow fescue (*Festuca pratensis*). *Plant Physiology* **56**: 438–441.

Valentinuz OR, Tollenaar M. 2004. Vertical profile of leaf senescence during the grain-filling period in older and newer maize hybrids. *Crop Science* **44**: 827–834.

Vicentini F, Schellenberg M, Thomas H, Matile P. 1995. Chlorophyll breakdown in senescent leaves, identification of the biochemical lesion in a stay-green genotype of *Festuca pratensis* Huds. *New Phytologist* **129**: 247–252.

Zelisko A, Garcia-Lorenzo M, Jackowski G, Jansson S, Funk C. 2005. AtFtsH6 is involved in the degradation of the light-harvesting complex II during high-light acclimation and senescence. *Proceedings of the National Academy of Sciences, USA* **102**: 13699–13704.

Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W. 2004. GENEVESTIGATOR *Arabidopsis* microarray database and analysis toolbox. *Plant Physiology* **136**: 2621–2632.

Key words: Arabidopsis, chlorophyll catabolism, comparative genomics, *Festuca*, *Lolium*, rice, senescence, staygreen.

© Institute of Grassland & Environmental Research (2006).
Journal compilation © *New Phytologist* (2006)

New Phytologist

Meetings

Mucking through multifactor experiments; design and analysis of multifactor studies in global change research

Ecological Society of America (ESA), 91st Annual meeting, Memphis, TN, USA, August 2006

The composition of the atmosphere and global climate have changed significantly since the start of the industrial revolution and these changes are impacting ecosystem structure and function (Prather *et al.*, 2001). Experimental studies that manipulate atmospheric concentrations of CO_2 and O_3, nitrogen deposition, or temperature and precipitation regimes, provide evidence that these factors can have significant and sustained impacts on net primary productivity (NPP) (Weltzin *et al.*, 2000; Zavaleta *et al.*, 2003; Hanson *et al.*, 2005; Morgan *et al.*, 2005; Norby & Iversen, 2006). Because of the interactive effects and feedbacks that emerge when factors are applied together, experiments in which only single factors are manipulated may be inadequate to fully predict the impacts of future atmospheric change on ecosystems. With this in mind, many experiments, both empirical and theoretical, have been initiated that examine the effect of multiple global change factors on ecosystems. Despite the knowledge gained from these experiments, many questions remain unresolved and the broad goal of multifactor experiments – to understand the interactions between multiple global change factors and their impacts on ecosystem structure and function – remains elusive. To aid in reaching this goal, the symposium 'Mucking through multifactor experiments; design and analysis of multifactor studies in global change research' was organized by Aimée Classen (Oak Ridge National Laboratory) in cooperation with the NSF Research Coordination Network 'Terrestrial Ecosystem Response to Atmospheric and Climatic Change' (TERACC) and the *New Phytologist* Trust at the 91st annual meeting of the Ecological Society of America in Memphis, Tennessee.

'… although a single factor may have striking effects on ecosystem function when applied alone, when global change factors are mixed, the physiological and growth adjustments within the ecosystem may dampen the overall response.

Using empirical data

A large body of empirical data already exists on the response of ecosystems to global change factors, such as elevated CO_2 and O_3 applied in isolation (Ainsworth & Long, 2005; Jablonski *et al.*, 2002). By incorporating process-based information from experimental data into multifactor models, Paul Hanson (Oak Ridge National Laboratory, USA) showed how inferences can be made on the response of ecosystems to more realistic global change scenarios. Using empirical data from many single factor experiments, Hanson *et al.* (2005) simulated the interactive effects of elevated CO_2 and O_3, and changes in precipitation and warming on the growth of upland oak forests in south-eastern United States. By generating alternative climate scenarios and using key physiological and growth responses in a probabilistic framework, the authors were able to generate predictions of forest response to a variety of conditions in 2100. One of the key findings was that, with physiological adjustments to elevated CO_2 and temperature, a growth response of 20% may be maintained for south-eastern forests in 2100. Additionally, they were able to rank the global change factors in order of importance, e.g. temperature, O_3 and precipitation. This exercise also highlighted the fact that although a single factor may have striking effects on ecosystem function when applied alone, when global change factors are mixed in, the physiological and growth adjustments within the ecosystem may dampen the overall response. Jeff Dukes (University of Massachusetts, Boston, USA) also presented a similar result for California grasslands (Shaw *et al.*, 2002).

Empirical experiments are limited by their duration, but many of the important effects of global change will become evident only over large spatial and temporal scales. By assembling short-term data from a variety of ecosystem types, Steve Del Grosso (Colorado State University, USA) was able to simulate the effects of multifactor global change on large scale and long-term processes such as decomposition, carbon storage and nitrogen leaching. Using DAYCENT, the daily time-step version of CENTURY (Parton *et al.*, 1987,

 © The Authors (2006). Journal compilation © *New Phytologist* (2006)

1998), a gradual increase of 3°C in nightly minimum temperatures and a more conservative increase of 1°C in daily temperatures were simulated over 100 yr, for shortgrass steppe, tallgrass prairie, boreal forest, temperate coniferous and deciduous forest. The authors found that NPP and soil organic carbon increased in most systems, but in the boreal forest, soil carbon stocks decreased after 100 yr. Focusing on the temperate deciduous *Liquidambar styraciflua* forest at Oak Ridge, Tennessee, they found a good agreement between measured and simulated NPP. However, their simulation of root NPP was less reliable and underestimated NPP in elevated CO_2. Ross McMurtrie (University of New South Wales, Australia) offered a simpler model that could also be applied across large spatial and temporal scales. To answer 'Why the plant-growth response to rising CO_2 is amplified when water is limiting, but diminished when nitrogen is limiting?' he showed that optima exist for leaf N, stomatal conductance and leaf area index in a variety of global change scenarios. Like the model of Del Grosso *et al.*, McMurtrie's model confirmed much of the experimental work that has gone before, e.g. larger relative response to CO_2 in drier conditions. Both of these models are widely applicable and highlighted significant knowledge gaps and testable hypotheses for future empirical studies.

Multifactor experiments

Although they are expensive and often difficult to implement, experiments that expose ecosystems to a range of global change factors are necessary to understand the mechanisms and interactions that drive responses to global change. At the OCCAM (Old-Field Community Climate and Atmospheric Manipulation) experiment in eastern Tennessee, an old field ecosystem was exposed to elevated CO_2, warming and altered precipitation. Aimée Classen (Oak Ridge National Laboratory, USA) showed that water availability, but not elevated CO_2 or warming, was the major driver of aboveground biomass in this system. However, this effect varied across years, and in 2005, the effects of soil moisture on biomass were reduced by elevated CO_2 and increased by warming. Dukes *et al.* also found a minimal role for elevated CO_2 at a grassland in Jasper Ridge, Northern California; there, the major driver of ecosystem biomass was an increase in nitrogen deposition. Teis Mikkelsen (Risø National Laboratory, Denmark) described a new experiment in which CO_2, temperature, and precipitation are being manipulated in a semi-natural grass and shrub ecosystem.

Many multifactor experiments have been set up to take advantage of a traditional ANOVA framework and often deal inadequately with interannual variation and 'surprises'. By focusing on the probabilities of seedling recruitment in forest gaps, Brian Beckage (University of Vermont, Vermont, USA) showed how abrupt ecological responses to climate change may be modelled using hierarchical Bayesian analysis. Classen *et al.* are exploring how path analysis may offer a more complete analysis than the traditional ANOVA framework. By employing non-parametric approaches, it may be possible to model abrupt ecological responses to global change and to take full advantage of data from empirical and observational studies.

Applying our knowledge

Ultimately, experimental data should aid policymakers and other stakeholders in predicting the response of ecosystems to future climate scenarios. The extensive datasets that exist from long running global change experiments may aid in this goal. The aspen-FACE experiment in northern Wisconsin has been operating for nine years, and exposes aspen and birch to elevated CO_2 and O_3 in a fully factorial design. At levels already occurring across much of the globe, elevated O_3 offsets the gains in productivity seen in elevated CO_2 in *Populus tremuloides* (King *et al.* 2005). Given the ecological and economical importance of *P. tremuloides*, there is an urgent need to determine the regional implications of this result. As well as accumulating data on the physiological and growth responses of the ecosystem to CO_2 and O_3, Kevin Percy (Canadian Forest Service, New Brunswick, Canada) has also compiled extensive data on many environmental parameters coupled over space and time. By regressing growth against environmental and expected dose parameters for O_3, Percy *et al.* determined that the O_3, wind speed and growing degree days predicted about 88% of the variation in tree growth across the duration of the experiment. This analysis was extended to show the predicted growth change in *P. tremuloides* across a wide range of sensitivity to O_3 exposure. Thus, empirical data from a multifactor experiment provided predictor variables extending important results into the public domain, and contributing to the scientific literacy of stakeholders.

Perspectives

Ecosystems across the globe are already being exposed to changing atmospheric composition, temperature and precipitation. Many gaps remain in our knowledge of the impacts of these changes on ecosystems, e.g. the role of below-ground processes, disturbance, and invasive species. To address these gaps, one single approach will not be sufficient. Multi-factor modelling must be utilized in conjunction with factorial experiments, to generate hypotheses and expand empirical findings, particularly across broader spatial and temporal scales. At this symposium there was a strong bias towards temperate natural systems; more information is required from under-represented ecosystems, especially those in the tropics and northern latitudes. Factorial experiments are costly and complicated: to maximize their relevance, sound statistical procedures must be applied,

© The Authors (2006). Journal compilation © *New Phytologist* (2006)

and non-parametric statistics may offer new insight into feedbacks and interactions between processes within ecosystems. By continuously measuring ancillary variables such as O_3 and precipitation, it may be possible to retrospectively understand and extrapolate results from simple experiments to more complex scenarios. To this end, it is encouraging that these issues were addressed by an international team, who no doubt will continue to expand and improve on the information presented at this symposium.

Orla Dermody

University of Tennessee, Knoxville, Tennessee, USA
(tel +1 865 974 4226;
fax +1 865 974 3067;
email odermody@utk.edu)

References

Ainsworth EA, Long SP. 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist* **165**: 351–371.

Hanson PJ, Wullschleger SD, Norby RJ, Tschaplinski TJ, Gunderson CA. 2005. Importance of changing CO_2, temperature, precipitation, and ozone on carbon and water cycles of an upland-oak forest: incorporating experimental results into model simulations. *Global Change Biology* **11**: 1402–1423.

Jablonski LM, Wang XZ, Curtis PS. 2002. Plant reproduction under elevated CO_2 conditions: a meta-analysis of reports on 79 crop and wild species. *New Phytologist* **156**: 9–26.

King JS, Kubiske ME, Pregitzer KS, Hendrey GR, McDonald EP, Giardina CP, Quinn VS, Karnosky DF. 2005. Tropospheric O_3 compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric CO_2. *New Phytologist* **168**: 623–635.

Morgan PB, Bollero GA, Nelson RL, Dohleman FG, Long SP. 2005. Increase in above-ground net primary production and yield of soybean is less when [CO_2] is elevated in the open. *Global Change Biology* **11**: 1–10.

Norby RJ, Iversen CM. 2006. Nitrogen uptake, distribution, turnover, and efficiency of use in a CO_2 enriched sweetgum forest. *Ecology* **87**: 5–14.

Parton WJ, Hartman M, Ojima D, Schimel D. 1998. DAYCENT and its land surface submodel: description and testing. *Global and Planetary Change* **19**: 35–48.

Parton WJ, Schimel DS, Cole CV, Ojima DS. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. *Soil Science Society of America Journal* **51**: 1173–1179.

Prather M, Ehhalt D, Dentener F *et al.* 2001. Atmospheric Chemistry and Greenhouse Gases. In: Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linder PJ, Dai X, Maskell K Johnson CA, eds. *Climate Change 2001: the Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK: Cambridge University Press, 183–240.

Shaw MR, Zavaleta ES, Chiariello NR, Cleland EE, Mooney HA, Field CB. 2002. Grassland responses to global environmental changes suppressed by elevated CO_2. *Science* **298**: 1987–1990.

Weltzin JF, Pastor J, Harth C, Bridgham SD, Updegraff K, Chapin CT. 2000. Response of bog and fen plant communities to warming and water-table manipulations. *Ecology* **81**: 3464–3478.

Zavaleta ES, Shaw MR, Chiariello NR, Thomas BD, Cleland EE, Field CB, Mooney HA. 2003. Grassland responses to three years of elevated temperature, CO_2, precipitation, and N deposition. *Ecological Monographs* **73**: 585–604.

Key words: empirical, interactions, multifactorial, theoretical.

More complexity in the mycorrhizal world

5th International Conference on Mycorrhiza, Granada, Spain, July, 2006

The 5th International Conference on Mycorrhiza (ICOM5) was held in Granada (Andalucia, Spain), a multicultural city with a heritage that is both Arabic and European. Over 650 mycorrhizal scientists from more than 50 countries attended the meeting and took part in the varied social events, such as the night visit to the Alhambra (Fig. 1) and the traditional 'Wines of the World' contest. All of which contributed towards making this an open and friendly meeting, enhancing exchange and discussion of the current progress in this fast-moving field. This ICOM edition was thus very successful.

'The Laccaria *genome is thus strikingly multifaceted, with Martin comparing it to the two-headed roman god Janus'*

As the conference opened, the Joint Genome Institute (JGI, Walnut Creek, CA, USA; US Department of Energy, USA) launched the genome sequence portal (http://genome.jgi-psf.org/Lacbi1/Lacbi1.home.html) for the mycorrhizal fungus, *Laccaria bicolor*. This being the official release of the first complete genome for a mycorrhizal fungus made it one of the major breakthroughs widely discussed during this meeting. Ecology and evolution dominated among the posters as well as in the sessions and workshops and, as usual, most talks focused on arbuscular mycorrhizas (AMs, involving Glomeromycetes that form arbuscular haustoria in host cells) and ectomycorrhizas (ECMs, associating Ascomycetes and Basidiomycetes with trees and shrubs). ECMs only half as popular as AMs among talks on cellular, molecular and genomic aspects of the symbiosis, but both were equally represented among talks on ecology and evolution. Such an overrepresentation of ECMs, as compared with their relatively smaller host

 © The Authors (2006). Journal compilation © *New Phytologist* (2006)

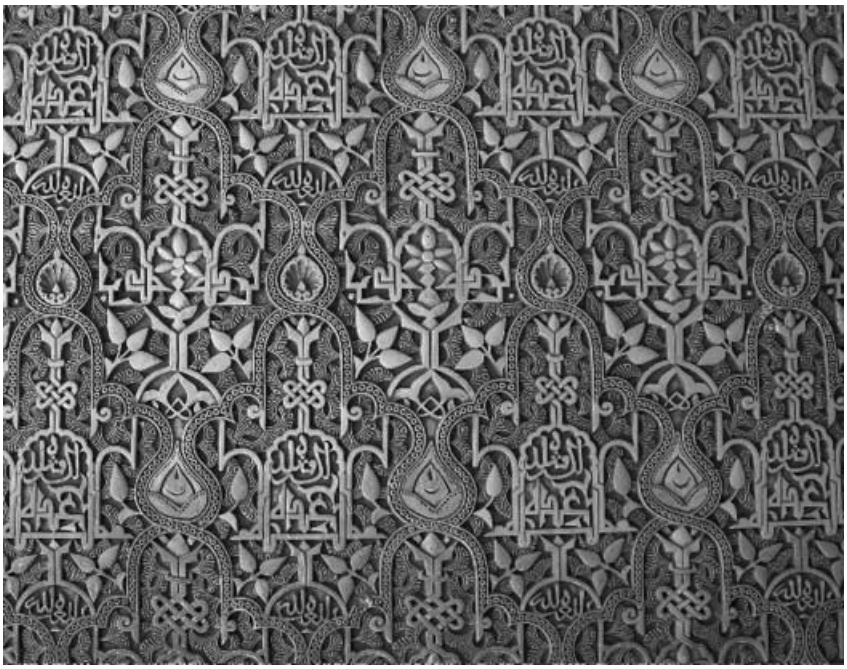


Fig. 1 ICOM5 participants visited the Alhambra at night. This series of palaces and gardens, built under the Nazari dynasty in the 14th century, introduced mycorrhizologists to complexity (here, wall plasterwork ornaments), a complexity reminiscent of that of mycorrhizal networks, fungal speciation and genes networks. (Photograph by M.-A. Selosse.)

spectrum and geographic breadth, probably arises because they are easily tractable and because most research groups work in temperate regions, where they dominate. It was noteworthy, with a few exceptions, that tropical mycorrhizal models were also underrepresented; however, given that ICOM6 will be held in Brazil in 2009 it is hopeful that tropical models will have a much larger prominence there!

The dawn of genomics in the mycorrhizal world

Availability of genome sequences from ecologically and taxonomically diverse fungi will allow ongoing research into these species and enhance the value of other sequences through comparative studies of gene and genome evolution, metabolic and regulatory pathways, as well as symbiosis and pathogenesis. Francis Martin (INRA Nancy, France) summarized the major features of the 65 megabase *L. bicolor* genome obtained by a whole-shotgun sequencing strategy in 2005. The International Ectomycorrhizal Genome Consortium (http://mycor.nancy.inra.fr/IMGC/) had brought together experts from various research areas to define the contours of the most important gene families. From > 20 000 putative genes identified so far, Martin pinpointed: (i) the important number of signalling pathway genes; (ii) the numerous genes coding short polypeptides (about 3000 genes encoding proteins of < 100 amino acids, including several secreted proteins); and (iii) the so-called (by Martin) 'saprotrophic' machinery, as *Laccaria* possesses most functions necessary for a saprotrophic lifestyle, as compared with *Coprinus cinereus* or *Phanerochaete crysosporium*. This last point is particularly interesting with regard to the hypothesis that ECM fungal clades experienced reversals to the saprotrophic lifestyle during their evolution (Hibbet *et al.*, 2000). Besides, several genes falling into the signalling and secretome categories have homologues involved in pathogenesis in plant pathogenic basidiomycetes. The *Laccaria* genome is thus strikingly multifaceted, with Martin comparing it to the two-headed roman god Janus. A major challenge will be to determine the role of such genes in symbiosis, using genetic transformation techniques now available for *L. bicolor* (Kemppainen *et al.*, 2005).

Peter Lammers (New Mexico State University, NM, USA) presented the sequencing project of the AM *Glomus intraradices*, sequenced by the JGI along with *Laccaria* in a project focusing on microbes interacting with *Populus trichocarpa* (Martin *et al.*, 2004; Tuskan *et al.*, 2006). Because of its obligate biotrophy, obtaining sufficient amounts of uncontaminated high-molecular-weight DNA had been a major problem. Low content in GC nucleotides (*c.* 30%) also biased routine cloning and delayed sequencing but the JGI was finally able to obtain a draft of the genome sequence (4 × coverage). Analysis of this second genome will help towards understanding what allows a fungus to build a long-lasting, beneficial interaction with plants. Surprisingly, Lammers reported the expression of a homologue of *Dmc1* (*disruption of meiotic control1*), a gene involved in meiotic recombination, in *G. intraradices* germinating spores. The finding of homologues for two more meiotic genes, *Msh4* and *Msh5*, and mating type genes by Hélène Niculita and Alexandre Colard from Ian Sanders' group (University of Lausanne, Switzerland) raised the question of the role of such genes in fungi considered as asexual.

© The Authors (2006). Journal compilation © *New Phytologist* (2006)

ECM nutrition and transport in a genomic perspective

Uwe Nehls (University of Tübingen, Germany) addressed the role of the plant nutritional status on development of the ECM symbiosis. He has identified families of sugar transporters in poplar and ECM fungi, and described the regulation of their expression during ECM formation regarding different nutrient supplies. His results supported an interesting hypothesis that emphasizes the permissive role of carbohydrate flux in mycorrhizal development, and this can be brought together with the hypothesis for a link between nutrient fluxes and the development of the AM symbiosis (Fitter, 2006). Michel Chalot (University of Nancy, France) presented the annotation and characterization of nitrogen mobilization and transport enzymes families of *L. bicolor* and *P. trichocarpa*, reporting on the expansion of some gene families in *Laccaria* as compared with other fungi. Both talks highlighted the strength of genomics that allows gene fishing in genome sequences (Martin *et al.*, 2004; Tuskan *et al.*, 2006). Access to the genomes of both AM and ECM fungi and a common partner tree will open up greater possibilities for the study of mycorrhizal biology in future years and the next ICOM should be particularly exciting in this regard. A synergy can be expected with the parallel development of new techniques announced by several groups, such as the use of laser microdissection techniques to get closer to the molecular events in the different plant or fungal compartments, or the refinement of microscopy fluorescent techniques (Lohse *et al.*, 2006).

Early signalling events in the AM symbiosis

Another breakthrough since the previous ICOM was the end of the 'Branching Factor' (BF) race. Kokhi Akiyama (Osaka University, Japan) and Nathalie Séjalon-Delmas (CNRS, Toulouse University, France) presented independent works that led to identification of such molecules in AM plants (Akiyama *et al.*, 2005; Besserer *et al.*, 2006). BF consists of strigolactones that are recognized by AM fungi at the presymbiotic stage and trigger hyphal branching. These groups now work on the identification of the Myc factor (MF), the fungal signal molecule triggering plant responses for a successful fungal colonization, and Akiyama expressed his hope to identify MF by the next ICOM. Séjalon-Delmas showed the effect of sorgolactone (Sorghum BF) and two chemical analogues from the AM *Gigaspora rosea* (Besserer *et al.*, 2006). A transcriptome analysis conducted on fungal spores subjected to BF reveals specific effects on mitochondrial biogenesis and lipid catabolism after signal perception at the presymbiotic stage. Other presentations on different sides of the AM symbiosis showed promising progress in the molecular dissection of the early events of symbiosis development, although large gaps still remain.

Cryptic species and fungal ecology and evolution

Classical species concepts in ECM fungi are mostly based on fruitbody (or vegetative) morphology. Molecular tools now allow delineation of biological species, that is groups of individuals among which molecular markers are shared and recombine. The fingerprint of recombination is that phylogenies of individuals within the same biological species are not congruent for different loci. In contrast to this, phylogenetic trees of different loci are expected to be congruent between species. The point at which congruence between phylogenetic trees of different loci arises therefore delineates biological species (Douhan & Rizzo, 2005). This was successfully used to show that the widespread ECM *Cenococcum geophilum* encompasses cryptic species, that is biological species undistinguished by ECM or sclerotia morphology (Douhan & Rizzo, 2005). Additional molecular dissections of ECM fungal species were reported at ICOM5, for example in '*Tricholoma scalpturatum*' (Gryta *et al.*, 2006) and among *Xerocomus* spp., for which Andy Taylor (Department of Forest Mycology, Uppsala, Sweden) showed an underestimated cryptic species diversity, together with an overestimated species overlap between continents. However, mechanisms for evolutionary rise of cryptic species remain unclear.

Studies on mycorrhizal fungal diversity now face a problem familiar to marine microbiologists, the so-called Hutchinson's 'paradox of the plankton': why are there so many species, apparently sharing the same niche, when competitive exclusion predicts extinction of the least competitive ones? Should we imagine that so many different niches exist, for example because of host diversity? Indeed, *Xerocomus* cryptic species vary in specificity (Taylor *et al.*, 2006), making host specialization a possible motor for their emergence – but evidence for such specificity is lacking in other cases, and host-poor ecosystems still harbour considerable fungal diversity. Soil heterogeneities might favour large niche diversity. For niche delimitation, as well as a better understanding of the functional implication of ECM species diversity, enzymatic profiling is also a promising tool (Courty *et al.*, 2005). In this approach, microplate tests measure several enzymatic activities on excised ECM root tips, offering clever insights into fungal resource utilization over time and space. Alternatively, fluctuations or unpredictability in ecosystems might allow coexistence of species with redundant niches, just by limiting population below any competition. Peter Kennedy (University of California, Berkeley, CA, USA) reported new laboratory and field-based evidence of competitive priority effects as well as hierarchies among a suite of closely related ECM *Rhizopogon* species (Kennedy & Bruns, 2005), so biotic interactions also contribute towards shaping different communities. Data on competition between mycorrhizal fungi are still rare, and the 'paradox of mycorrhizal diversity' remains intriguing.

 © The Authors (2006). Journal compilation © *New Phytologist* (2006)

Also noteworthy is that spatial scaling of mycorrhizal community richness might differ from that of free-living microbes. Investigating islands of ECM trees separated by non-ECM plant communities, Kabir Peay (University of California, Berkley) reported that the slope of the relationship between species number and island area is more similar to plants and other macro-organisms than the slopes usually associated with microbes (approx. three to six times lower). Symbiosis might shape ECM communities, and thus their evolution. Indeed, James Bever (Indiana University, Bloomington, IN, USA) provided the first convincing evidence that plants can choose their AM partners under some conditions: in a split root system separately inoculated with two AM strains (one mutualistic, the other less beneficial), the mutualistic fungus received more carbon and had increased fitness, as measured by spore production. Interestingly, however, the better strain was the worse competitor, so the spatial dynamics of root colonization may also be important in determining mycorrhizal species richness at small spatial scales. Besides stabilization of the mutualism in mycorrhizal interaction (Kiers & van der Heijden, 2006), such plant sanctions on less rewarding mycorrhizal partners might strongly contribute to fungal evolution.

Mycorrhizal networks: linking plants and shaping communities?

An individual fungal mycelium can associate with two or more plants, which are not conspecific, resulting in a common mycorrhizal network (CMN) linking plants (Selosse *et al.*, 2006). Several descriptive works currently focus on extraradical mycelium, the 'linking part' of CMNs. Ian Anderson (The Macaulay Institute, Aberdeen, Scotland) reported on the fine-scale distribution and repartition of extraradical mycelia and ECM root tips by ITS analysis. This work revealed some discrepancies (Genney *et al.*, 2006); for example, extraradical mycelia can be found in soil layers different from those containing ECM root tips and seven times as many species were found among extraradical mycelia than on root tips. At the functional level, Jonathan Leake (University of Sheffield, UK) reported that, in a grassland ecosystem, AM fungi represent one-third of soil microbiota and respire 5–9% of the carbon fixed by host plants within 34 h.

Leanne Philip (University of British Columbia, Vancouver, BC, Canada) re-investigated carbon transfers through CMNs previously reported by Simard *et al.* (1997). Where *Pseudotsuga menziesii* (Douglas-fir) and *Betula papyrifera* (paper birch) seedlings were grown together in pot systems or root chambers, transfer intensity was greater, (i) in the presence of shared ECM fungi (vs noninoculated cultures), and (ii) when hyphal connections were intact (vs severed). In the field, CMNs comprising three ECM species (paper birch, Douglas-fir, *Larix occidentalis*) showed reversion of the net flux over the year (to *B. papyrifera* in spring and autumn, when *P. menziesii* photosynthesis was higher, and to Douglas-fir in summer when paper birch photosynthesis was higher), suggesting that such transfers could be more cooperative than previously thought. Stable isotopes are also major tools for demonstrating carbon transfers: since ^{13}C content of ECM fungi differs from that of autotrophic plants, the contribution of fungal carbon to plant biomass can be quantified without artificial labelling. Gerhard Gebauer (University of Bayreuth, Germany) reported that several orchid species (e.g. Julou *et al.*, 2005) and other understorey green plants from ECM temperate forests partly rely on fungal carbon in addition to photosynthesis, a strategy called 'mixotrophy' (Selosse *et al.*, 2006). Since their mycorrhizal fungi form ECM on surrounding trees, a CMN is likely to transfer nutrients from overstorey trees to the mixotrophs. However, carbon transfers were received with more scepticism by AM ecologists. Although, as Jonathan Leake recalled, some purely heterotrophic AM plants exclusively use this carbon source, AM mixotrophs are hitherto unknown. Carbon transferred from fungi to the host plant could arise from another source; Jean Garbaye (INRA Nancy, France) suggested that carbon heterotrophically obtained by *Lactarius quietus* could contribute to the unbalanced spring carbon budget in *Quercus* spp. Indeed, *L. quietus* ECM tips undergo a flush of enzymes degrading complex substrates, at a time where carbon budget of the tree seems in deficit. As a dramatic example, an achlorophyllous Japanese orchid was reported to be mycorrhizal with saprophytic Coprinaceae (Yamato *et al.*, 2005). So, how often is dead soil carbon available to plants through mycorrhizal fungi?

Common mycorrhizal networks also act by providing an inoculum to seedlings. Michael Booth (University of Alaska at Yale, CT, USA) reported that ECMs limit the effect of competition of overstorey trees on seedlings (Booth, 2004). Although nutrient transfer is unlikely to be involved (as shown by analysis of ^{13}C content), CMNs provide a diverse ECM inoculum. Kazuhide Nara (University of Tokyo, Japan) showed experimental and circumstantial evidence that, in primary successions on volcanic soils, ECM fungi recruited by *Salix reinii* facilitate settlement of conspecifics (Nara, 2006) and other species associated with ECM fungi, such as *Betula*, *Larix* or *Pyrola* species. CMNs therefore drive ecological successions, among other soil factors (Kardol *et al.*, 2006).

Finally, mycorrhizas integrate into even larger networks of interactions: Wim van der Putten (Netherlands Institute of Ecology, Heteren, the Netherlands) showed the importance of looking at multitrophic interactions, including above- and below-ground biota linked by plants. For example, plant defence above ground is strongly influenced by below-ground interactions (Bezemer *et al.*, 2005), among which mycorrhizas play a significant role. Stefan Reidinger

(University of London, UK) showed how mycorrhizas can lead to changes in the structure of herbivore insect communities (Gange *et al.*, 1999). Most multitrophic interactions reported at ICOM5 focused on mycorrhizospheric bacteria, making frequent use of analysis of phospholipid fatty acids to describe the fungal and bacterial diversity at high systematic level.

Enlarging the spectrum of interacting organisms and using the genomes of model symbioses will probably enhance the complexity, but also the physiological and ecological relevance, of mycorrhizal research. More species from different fungal clades are currently on the way to being sequenced. Perhaps in the future, increased overlap of once disparate fields within mycorrhizal research will be possible, for example the fusion of ecological and genomic research. Bringing field and *in silico* investigations together in common 'ecogenomics' sessions may be what we should expect at ICOM6!

M.-A. Selosse[1]* and S. Duplessis[2]

[1]CEFE-CNRS, UMR 5175, Equipe Interactions Biotiques, 1919 Route de Mende, F-34 293 Montpellier cedex 5, France; [2]UMR 1136 INRA/UHP-Nancy 1, Interactions Arbres Micro-organismes, Centre INRA de Nancy, F-54280 Champenoux, France
(*Author for correspondence:
tel +33(0)4 67 61 32 31; fax +33(0)4 67 41 21 38;
email ma.selosse@wanadoo.fr)

References

Akiyama K, Matsuzaki K, Hayashi H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* **435**: 824–827.

Besserer A, Puech-Pagès V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais J-C, Roux C, Bécard G, Séjalon-Delmas N. 2006. Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. *PLoS Biology* **4**: e226.

Bezemer TM, De Deyn GB, Bossinga TM, Van Dam NM, Harvey JA, Van der Putten WH. 2005. Soil community composition drives aboveground plant–herbivore–parasitoid interactions. *Ecology Letters* **8**: 652–666.

Booth MG. 2004. Mycorrhizal networks mediate overstorey-understorey competition in a temperate forest. *Ecology Letters* **7**: 538–546.

Courty P-E, Pritsch K, Schloter M, Hartmann A, Garbaye J. 2005. Activity profiling of ectomycorrhiza communities in two forest soils using multiple enzymatic tests. *New Phytologist* **167**: 309–319.

Douhan GW, Rizzo DM. 2005. Phylogenetic divergence in a local population of the ectomycorrhizal fungus *Cenococcum geophilum*. *New Phytologist* **166**: 263–271.

Fitter AH. 2006. What is the link between carbon and phosphorus fluxes in arbuscular mycorrhizas? A null hypothesis for symbiotic function. *New Phytologist* **172**: 3–6.

Gange AC, Bower E, Brown VK. 1999. Positive effects of mycorrhizal fungi on aphid life history traits. *Oecologia* **142**: 123–132.

Genney DR, Anderson IC, Alexander IJ. 2006. Fine scale distribution of pine ectomycorrhizas and their extramatrical mycelium. *New Phytologist* **170**: 381–390.

Gryta H, Carriconde F, Charcosset J-Y, Jargeat P, Gardes M. 2006. Population dynamics of the ectomycorrhizal fungal species *Tricholoma populinum* and *Tricholoma scalpturatum* associated with black poplar under differing environmental conditions. *Environmental Microbiology* **8**: 773–778.

Hibbett DS, Gilbert L-B, Donoghue MJ. 2000. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. *Nature* **407**: 506–508.

Julou T, Burghardt B, Gebauer G, Berveiller D, Damesin C, Selosse M-A. 2005. Evolution of mixotrophy in orchids: insight from a comparative study of green and achlorophyllous *Cephalanthera damasonium*. *New Phytologist* **166**: 639–653.

Kardol P, Bezemer TM, van der Putten WH. 2006. Temporal variations in plant-soil feedback controls successions. *Ecology Letters* **9**: 1080–1088.

Kemppainen M, Circosta A, Tagu D, Martin F, Pardo AG. 2005. Agrobacterium-mediated transformation of the ectomycorrhizal symbiont *Laccaria bicolor* S238N. *Mycorrhiza* **16**: 19–22.

Kennedy PG, Bruns TD. 2005. Priority effects determine the outcome of ectomycorrhizal competition between two *Rhizopogon* species colonizing *Pinus muricata* seedlings. *New Phytologist* **166**: 631–638.

Kiers ET, van der Heijden GA. 2006. Mutualistic stability in the arbuscular mycorrhizal symbiosis: exploring hypotheses of evolutionary cooperation. *Ecology* **87**: 1627–1636.

Lohse S, Hause B, Hause G, Fester T. 2006. FtsZ characterization and immunolocalization in the two phases of plastid reorganization in arbuscular mycorrhizal roots of *Medicago truncatula*. *Plant and Cell Physiology* **47**: 1124–1134.

Martin F, Tuskan GA, DiFazio SP, Lammers P, Newcombe G, Podila GK. 2004. Symbiotic sequencing for the *Populus* mesocosm. *New Phytologist* **161**: 330–335.

Nara K. 2006. Ectomycorrhizal networks and seedling establishment during early primary succession. *New Phytologist* **169**: 169–178.

Selosse M-A, Richard F, He X, Simard S. 2006. Mycorrhizal networks: *les liaisons dangereuses*. *Trends in Ecology and Evolution* **11**: 621–628.

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.

Taylor AFS, Hills A, Simonini G, Both EE, Eberhardt U. 2006. Detection of species within the *Xerocomus subtomentosus* complex in Europe using rDNA-ITS sequences. *Mycological Research* **110**: 276–287.

Tuskan G, DiFazio S, Bohlmann J, Grigoriev I, Hellsten U, Jansson S, Putnam N, Ralph S, Rombauts S, Salamov A, Schein J, Sterck L, Aerts A, Bhalerao RR, Bhalerao RP, Blaudez D, Boerjan W, Brun A, Brunner A, Busov V *et al.* 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray ex Brayshaw). *Science* **313**: 1596–1604.

Yamato M, Yagame T, Suzuki A, Iwase K. 2005. Isolation and identification of mycorrhizal fungi associating with an achlorophyllous plant, *Epipogium roseum* (Orchidaceae). *Mycoscience* **46**: 73–77.

Key words: cryptic species, ecology, evolution, genomics, mycorrhiza, mycorrhizal networks, plant nutrition, signalling.

 © The Authors (2006). Journal compilation © *New Phytologist* (2006)