

# Horizontal Gene Transfer (HGT) from Fungi is the basis for plant pathogenicity in oomycetes

It always seemed rather odd that some oomycetes (*Oomycota*) were so fungal-like in their behaviour as plant pathogens. Now whole-genome comparisons are starting to reveal just why. Richards *et al.* (2011) have undertaken a painstaking gene-by-gene analysis of the proteomes of *Hyaloperonospora parasitica* and three species of *Phytophthora* (*P. infestans*, *P. ramorum*, and *P. sojae*) which reveals an extensive pattern of cross-kingdom horizontal gene transfer (HGT) from *Fungi*. In the case of *P. ramorum*, an amazing 7.6% of the secreted proteome appears to have been acquired from true fungi. In all, 34 cases of HGT were identified, of which the case for 21 was strongly supported, most

of which seem to have occurred close to the shift from phagotrophy to osmotrophy and the evolution of the fungal cell wall at the base of the monophyletic *Fungi* clade. Amongst the genes evidently transferred, are one related to features such as the ability to break down plant cell walls and take up sugars, nitrogen and phosphates, and further ones implicated in overcoming plant defence mechanisms and attacking plant cells. A schematic diagram of the functional proteome of oomycetes derived from *Fungi* is provided (their Fig. 2) at which one can only marvel at the complexity. The phenomenon was already recognized some years ago by Richards *et al.* (2006), but only five HGT gene transfers were reported at

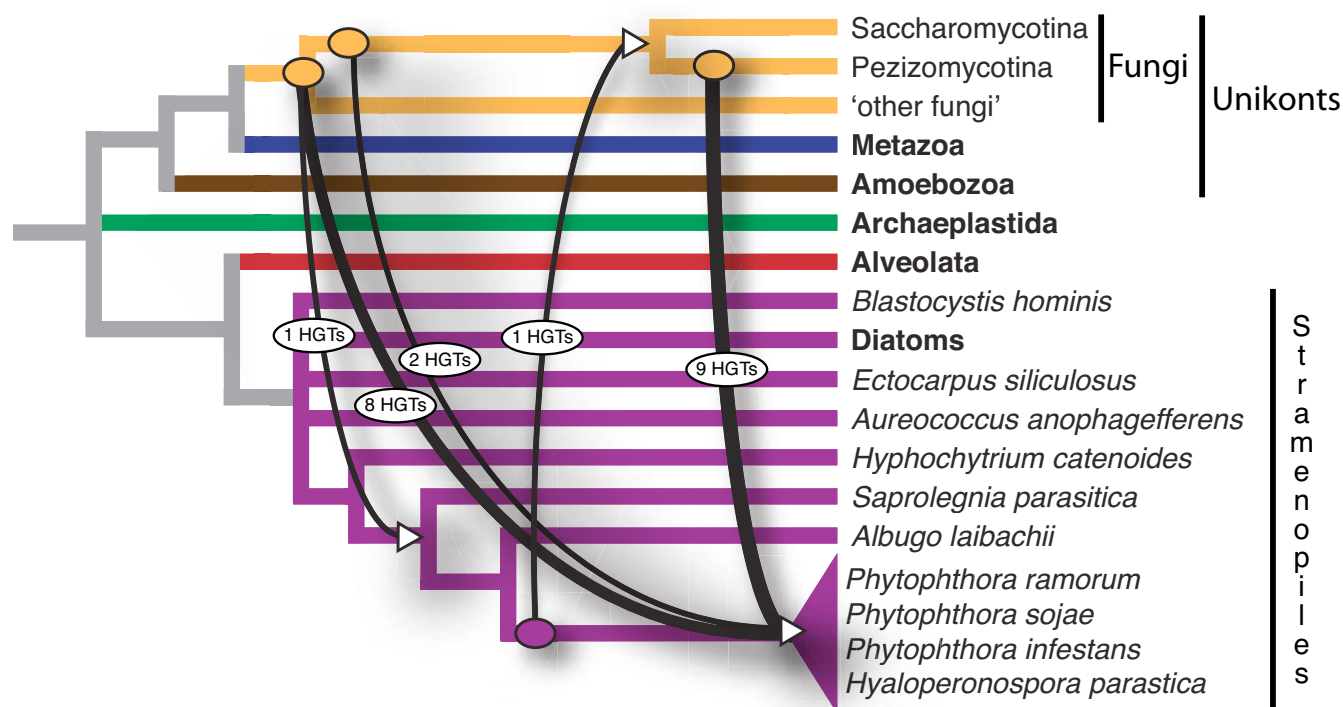
that time. This more detailed study, made possible by increasingly available genome sequences, reveals that this phenomenon is much more extensive than might have been imagined. Thus, it is not a matter of oomycetes merely being 'fungal analogues' or 'pseudofungi', they are actually partly *Fungi*.

It should be noted that HGT is not only unidirectional from *Fungi* into to other organisms. Indeed, in another paper published this summer, this same group of researchers report identifying 323 examples of HGT into *Fungi* from prokaryotes (principally bacteria) and also other *Fungi* (Richards *et al.* 2011b).

Richards TA, Dacks JB, Jenkinson JM, Thornton CR, Talbot NJ (2006) Evolution of filamentous plant pathogens: gene exchange across eukaryotic kingdoms. *Current Biology* **16**: 1857–1864.

Richards TA, Leonard G, Soanes DM, Talbot NJ (2011b) Gene transfer into fungi. *Fungal Biology Reviews* **25**: 98–110.

Richards TA, Soanes DM, Jones MDM, Vasieva O, Leonard G, Paszkiewicz K, Foster PG, Hall N, Talbot NJ (2011a) Horizontal gene transfer facilitated the evolution of plant parasitic mechanisms in the oomycetes. *Proceedings of the National Academy of Sciences, USA* **108**: 15258–15263.



Schematic figure showing the pattern of horizontal gene transfer (HGT) between fungi and oomycetes, adapted from Richards *et al.* (2011a: fig. 1). In total Richards *et al.* (2011) provide evidence of 34 gene transfer events. Using phylogenetic methods combined with alternative topology tests they polarised the ancestry of 21 transfer events and are illustrated in this figure. The figure shows the transfers were generally concordant with the diversification of plant parasitic oomycetes, consistent with putative annotation of many of these genes which suggest that they are important for plant parasitism. Figure courtesy of Tom A. Richards.

## Fungal pathogens as a driver of tree species diversity in tropical forests

It has been postulated that at least one of the factors promoting the maintenance of diversity in tropical forests is the action of host-specific parasites and pathogens which are more likely to kill seedlings near the parent tree species, the so-called Janzen-Connell (J-C) model. In order to test whether this held for plant pathogens, Konno *et al.* (2011) isolated *Colletotrichum anthrisci* from seedlings of four trees growing below the same tree species where they had been killed by damping-off beneath: *Cornus controversa*, *Fraxinus*

*lanuginosa*, *Magnolia obovata*, and *Prunus grayana*. The isolates from all four species were confirmed as identical by 99–100 % similarity in ITS sequences (5.8SrDNA, ITS1 and ITS2), and inoculated into seedlings of *F. lanuginosa* and *Prunus grayana*. In all cases some damage to the seedlings occurred, but this was most severe in seedlings inoculated with isolates from the conspecific host tree. This suggests a degree of specialization whereby seedlings from the same tree species are more likely to be eliminated than those from

different hosts – consequently reducing the probability of seedlings of the same tree species surviving when growing near examples of the same species. Konno *et al.* postulate that if this situation is common within several pathogens in a mixed tree forest, then diversity will tend to be maintained as proposed in the J-C model. The study also shows that fungi with identical ITS sequences obtained from native forest trees can differ in their degree of pathogenicity to other tree species.

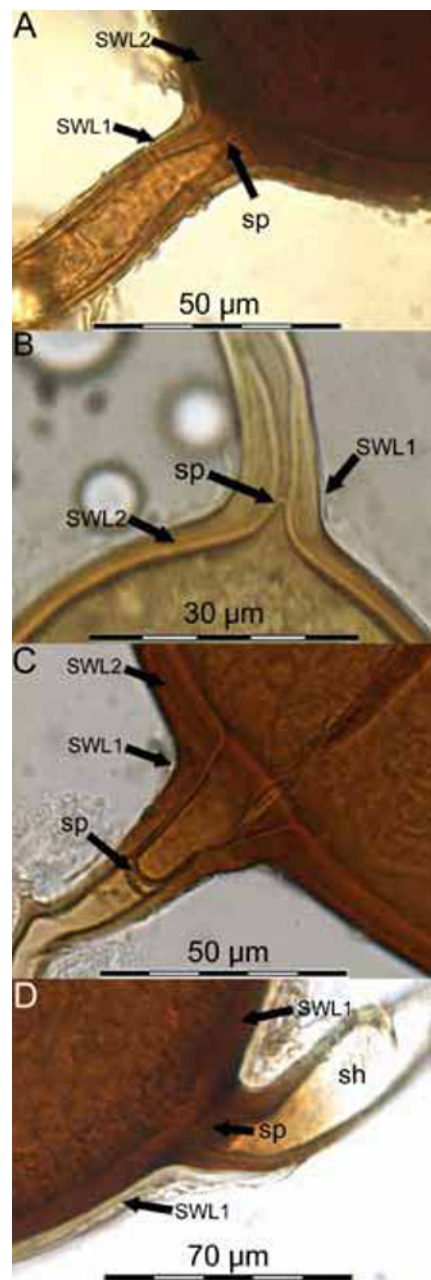
Konno M, Iwamoto S, Seiwa K (2011) Specialization of a fungal pathogen on host tree species in a cross-inoculation experiment. *Journal of Ecology* 99: 1394–1401.



*Colletotrichum anthrisci* (from ex-type strain CBS 125334). a–b. acervuli; c. tip of a seta; d–e. conidiophores; f. conidiophores and setae; g–i. appressoria; j–k. conidia; all from ex-type culture CBS 125334. a, c–e, j: from *Anthriscus* stem; b, f, g, k: from SNA. a–b: DM; c–k: DIC. — Scale bars: a = 200  $\mu$ m; e = 10  $\mu$ m; a applies to a–b; e applies to c–k. Photos courtesy Ulrike Damm.

# A new system for the arbuscular mycorrhizal fungi (*Glomeromycota*)

In the course of the last ten years, and especially during the last five, immense progress has been made in understanding the molecular phylogenetic relationships



of arbuscular mycorrhizal fungi, members of the phylum *Glomeromycota*. As might have been expected for a group which was already represented by modern-looking representatives in the Devonian, and which forms mutualistic associations with some 80–85 % of land plants around today, there was much diversity to be detected. In 2011 a succession of key papers describing new classes, families, and genera has appeared, mainly prepared by Fritz Oehl (Zürich, Switzerland), Gladstone Alves da Silva (Recife, Brazil), and Javier Palenzuela (Granada, Spain), with various colleagues, has appeared (e.g. Oehl *et al.* 2011a–d). Building on the pioneering work of Christopher Walker, Arthur Schüßler, and James B. Morton in particular, these researchers have established robust correlations between microscopic features and the major groupings emerging from molecular studies. An elegant consumer-friendly digest and synthesis of the new system for the phylum has now been

prepared, which we are proud to include in the current issue of *IMA Fungus* (Oehl *et al.* 2011e).

In the new system, three classes (*Archaeosporomycetes*, *Glomeromycetes*, and *Paraglomeromycetes*), five orders (*Archaeosporales*, *Diversisporales*, *Gigasporales*, *Glomerales*, and *Paraglomerales*), 14 families, and 29 genera are recognized. Key anatomical and morphological features characterizing the molecularly supported taxa are spore formation, the number of spore walls, germination type and structure, and mycorrhizal structures (stained in Trypan blue). These characters are illustrated and tabulated down to genus level in the synthesis paper, and using this many genera will now be separable using light microscopy alone. This paper is set to become the key reference work on this remarkable fungal phylum for ecologists and others investigating or utilizing endomycorrhizal fungi.

Oehl F, Silva GA, Goto BT, Sieverding E (2011a) *Glomeromycetes*: three new genera and glomoid species reorganized. *Mycotaxon* 116: 75–120.

Oehl F, Silva DKA, Maia LC, Sousa NMF de, Vieira HEE, Silva GA (2011b) *Orbispora* gen. nov., ancestral in the *Scutellosporaceae* (*Glomeromycetes*). *Mycotaxon* 116: 161–169.

Oehl F, Silva GA, Goto BT, Maia LC, Sieverding E (2011c) *Glomeromycota*: two new classes and a new order. *Mycotaxon* 116: 365–379.

Oehl F, Silva GA, Sánchez-Castro I, Goto BT, Maia LC, Vieira HEE, Barea JM, Sieverding E, Palenzuela J (2011d) Revision of *Glomeromycetes* with entrophosporoid and glomoid spore formation with three new genera. *Mycotaxon* 117: 297–316.

Oehl F, Sieverding E, Palenzuela J, Ineichen K, Silva GA (2011e) Advances in *Glomeromycota* taxonomy and classification. *IMA Fungus* 2:191–199.

Examples of characteristics of spore bases and subtending hyphae in *Glomeromycota*. A. *Glomus ambisporum*. B. *G. aureum*. C. *Funneliformis coronatus*. D. *Septoglomus constrictum*. sw = spore wall layers; sp = bridging septum; sh = subtending hypha. See Oehl *et al.* (*IMA Fungus* 2: 191–199, 2011) for further explanation. Photos courtesy Fritz Oehl.

## New insights into global fungal species numbers?

Blackwell (2011) has revisited the issue of how many fungi exist on Earth, and the impact that molecular studies, and especially high-throughput environmental sequencing has had on our understanding of the extent

of that diversity. She draws attention to the state of knowledge of the fungi in particular habitats, and the issue of phylogenetic species not or hardly separable by other features. Attention is drawn to the increased

number of flowering plants suggested to exist beyond the 270 000 used in the extrapolations of Hawksworth (1991): for example, Paton *et al.* (2008) provide a figure of 352 000 for known species, and Joppa





*et al.* (2010) expect that to grow by 10–20% – i.e. to 387 200–422 400 species. If these larger figures are used, then the 6 : 1 ratio of fungi : plants used by Hawksworth (1991) would yield a figure of 2.3–2.5 M species of fungi.

Blackwell also emphasises the results of the study of O'Brien *et al.* (2005) from environmental sequences from soil which suggested a total number of 3.5–5.1 M fungus species. However, results from high-throughput sequencing studies have to be

approached with some caution as critical studies with bacterial populations suggest that they may overestimate the number of taxa actually present by a factor of about six (Quince *et al.* 2009). If it were justified to apply that factor in the O'Brien *et al.* study, their figure would not have been so far ahead of other estimates.

A different approach to estimating global species numbers of all organisms was taken by Mora *et al.* (2011), who found that the description of taxa according to rank followed a predictable pattern across different groups of organisms. In the case of the fungi, the validity of the approach might be questioned as our knowledge is so poor, and also as they worked with a figure of just 43 271 species of fungi – the number in the *Catalogue of Life 2010 Annual Checklist* (Bisby *et al.* 2010) rather than the 100 000 figure currently in general use (e.g. Kirk *et al.* 2008). However, what was intriguing is that based on that data set, they predicted 611 000 ( $\pm$  297 000) fungal species which implies that only around 7% (range

4.5–13.5%) are now known, perhaps not so different from the 5% previously proposed (Hawksworth 1991).

While it now seems that the 1.5 M estimate may indeed be conservative, as stated when it was proposed (Hawksworth 1991), the jury remains out as to how much by. For that reason I am inclined to still work with “at least 1.5 M” as the additional factor is so uncertain, as I concluded a decade ago (Hawksworth 2001). Much more field-truthed data are needed to improve the current estimates, and as Blackwell points out, this “can be speeded by enlisting more biologists to accomplish the goal” (p. 434). But how can that be done? The answer may lie in an increasing recognition within the scientific community of the scale of the problem, the excitement of discovering novel taxa, and a heightened appreciation of the crucial role of fungi in so many aspects of human concern from health to food security and climate change. A paradigm shift in the foci of biological research may be required.

Bisby FA, Ropkov YR, Orrell TM, Nicolson D, Paglinawan LE, *et al.* (2010) *Species 2000 and ITIS Catalogue of Life: 2010 annual checklist*. Reading: Species 2000.

Blackwell M (2011) The *Fungi*: 1, 2, 3... 5.1 million species. *American Journal of Botany* **98**: 426–438.

Hawksworth DL (1991) The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycological Research* **95**: 641–655.

Hawksworth DL (2001) The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological Research* **105**: 1422–1432.

Joppa LN, Roberts DL, Pimm SL (2010) How many species of flowering plants are there? *Proceedings of the Royal Society of London, B*, **278**: 554–559.

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) *Ainsworth & Bisby's Dictionary of the Fungi*. 10<sup>th</sup> edn. Wallingford: CAB International.

Mora C, Tittensor DP, Adl S, Simpson AGB, Worm B (2011) How many species are there on Earth and in the Ocean? *PLoS Biology* **9**(8): e1001127.

O'Brien BL, Parrent JL, Jackson JA, Moncalvo JM, Vilgalys R (2005) Fungal community analysis by large-scale sequencing of environmental samples. *Applied and Environmental Microbiology* **71**: 5544–5550.

Paton AJ, Brummitt N, Govaerts R, Harman K, Hinchcliffe S, Allkin B, Lughadha EM (2008) Towards Target 1 of the Global Strategy for Plant Conservation: a working list of all known plant species – progress and prospects. *Taxon* **57**: 602–611.

Quince C, Lanzén A, Curtis TP, Davenport RJ, Hall N, Head IM, Read IF, Sloan WT (2009) accurate determination of microbial diversity from 454 pyrosequencing data. *Nature Methods* **6**: 639–641.

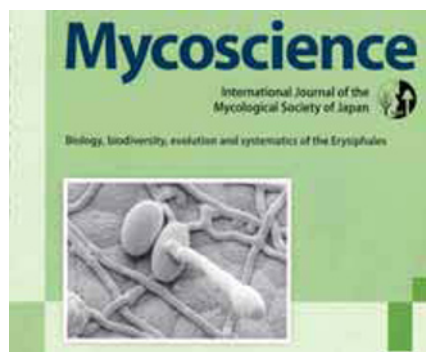
## Powdery mildews under scrutiny

The Special Interest Group (SIG) meetings held during IMC9 in August 2010 were exceedingly popular. Mini-reviews based on the SIG events have already been published in previous issues of IMA FUNGUS, and a longer review on molecular diagnostic methods is included in this issue (pp. 177–189). In the case of the SIG on powdery mildew fungi (*Erysiphales*), convened by Uwe Braun, Levente Kiss and Susumu Takamatsu, the papers presented are published as an issue of *Mycoscience* **52** (3) (May 2011) under the title ‘Biology,

biodiversity, evolution and systematics of the *Erysiphales*’.

The focus on the papers is on the biology and evolution of pathogenesis and variation at the molecular level in particular species or complexes. Aleš Lebeda *et al.* (pp. 59–164) review the pathotype and race designations and tests used in two powdery mildews attacking cucurbits, *Golvinomyces cichoracearum* and *Podospaera xanthii*. The situation has become confusing as a result of the use of different *Cucumis* species and cultivars in challenge testing,

with, for example, three systems being used by different research groups for race denomination; the adoption of two sets of host genotypes and a concise designation system are commended. *Quercus* is host to more powdery mildews than any other genus, with over 50 species recognized on members of the genus. Those present on oak in Europe, which had all been considered as introduced, are reviewed by Marie-Laure Desprez-Loustau *et al.* (pp. 165–173). Molecular phylogenetic studies confirm that within *Erysiphe* three species are



involved; *E. alphitoides* (syn. *Microsphaera alphitoides*) which is the most pathogenic, *E. hypophylla*, and *E. quercicola* recently discovered in France and only known from a single ITS sequence. Affinities with powdery mildews known from tropical hosts discovered were a surprise, and host-jumping and specialization on *Q. robur* is postulated to have occurred in the evolution of *E. alphitoides*. Molecular phylogenetic studies on *Erysiphe* species on *Ligustrum* and *Syringia* (both *Oleaceae*) by Yusuke Seko *et al.* (pp. 174–182), revealed two groups which could also be distinguished by the pigmentation of the appendages to the ascomata; *E. syringiae* occurred only on *Syringia* and probably evolved in

North America, and *E. ligustri* and *E. syringae-japonicae* on *Ligustrum* and *Syringia* respectively, with the last species having arisen in eastern Asia.

Three contributions are on biological and development aspects. Roger Cook *et al.* (pp. 183–197) report on appressorium formation on the germ tubes of 36 *Erysiphe* species. Unlobed appressoria (“alobatus-type”) occurred in three species, while in others they were lobed. Viewed from below in the plane in contact with the host, five-lobed appressoria were formed by 120° dichotomous branchings; species of *Neoerysiphe* and *Phyllactina* branched at the same angle. I found the light and scanning electron micrographs and explanatory drawings particularly illuminating. In *Oidium neolycopersici*, Yoshihiro Takikawa *et al.* (pp. 198–203) endeavoured to find whether the point of initiation of germ tubes was triggered by contact with the host leaves or if it was predetermined; in their experiments using electrostatic spore collectors and different substrates, they showed germination was always stimulated by contact but that they almost exclusively arose subterminally regardless of the actual

point of contact. Takikawa *et al.* (pp. 204–209) also studied the behaviour of conidia in *E. trifoliorum*. In that species, on host and non-host leaves as well as several artificial surfaces; they discovered that the so-called “two-step” germination process was actually the result of a first unsuccessful attempt at host penetration. Microcyclic conidiogenesis, the formation of conidia directly from a conidium without little or no hyphal growth, is documented by Alexandra Pintye *et al.* (pp. 213–216) from species of four genera based on light and low-temperature scanning electron microscopy.

Finally, Uwe Braun (pp. 210–212) provides a brief overview of the current state of systematics in these fungi, which includes a synopsis of the tribe and subtribe system to be used in the forthcoming *Manual of the Erysiphales (Powdery Mildews)* he has prepared with Roger Cook and which is expected to be published in April 2012; that work will evidently recognize about 820 species, a marked increase from the 515 accepted in Uwe’s 1987 monograph – it will be a “must have” for both field mycologists and plant pathologists worldwide.