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Remodelling growth and survival in response to the host by *Penicillium marneffei*

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Penicillium marneffei is an important fungal pathogen of humans, in particular those who are immunocompromised. *P. marneffei* has the capacity to alternate between a hyphal and a yeast growth form, a process known as dimorphic switching, in response to temperature. *P. marneffei* grows in the hyphal form at 25°C and in the yeast form at 37°C. The hyphal form produces conidia which are likely to be the infectious agent while the yeast growth form is the pathogenic form found in infected patients. These yeast cells exist intracellularly in the mononuclear phagocyte system of the host. The molecular events which establish and maintain the developmental states and control of the dimorphic switching process in *P. marneffei* are poorly understood.

P. marneffei is the only true pathogen in a genus comprising a large number of species and is also the only dimorphic fungus in this group. As an intracellular pathogen, *P. marneffei* must be able to utilise the available nutrient sources in order to grow while evading or tolerating the host's defence systems. The genes required for tyrosine catabolism are located in a conserved gene cluster and are induced specifically at 37ºC and during infection in fungal pathogens. Tyrosine can provide both carbon and nitrogen for growth and also results in the formation of protective melanin. The expression of tyrosine catabolic genes is under the complex control of a number of systems which respond to substrate, temperature and host signals and it is postulated that these have evolved to control the balance between growth and survival.

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Gene expression in 3D: Hidden genomic functionality is revealed in the maturing yeast colony

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Human development is a clear example of how multicellular communities of genetically identical cells can generate an astounding level of complexity. We recently showed that the individual cells of the simple model eukaryote, Saccharomyces cerevisiae, display different gene-expression patterns depending on their position within a mature colony. Whereas cells of the colony exterior are metabolically active, the cells of the colony interior are starving. To our surprise, a disproportionately large number of genes that were differentially expressed between the inner and outer layers of the colony have no known function, encoding either uncharacterized proteins or being non-coding RNAs. To better understand how the growth environment of yeast shapes gene-expression, we used a custom RNA-seq approach to transcriptionally profile yeast cells growing in either the standard laboratory conditions (liquid culture with high glucose and high aeration) and compared these to maturing (2, 3 and 4 day) yeast colonies grown on nutritionally identical solid media. These experiments reveal that the mature yeast colony expresses a large proportion of its genome that is silent under standard conditions. Among genes specifically unregulated in the colony are, non-coding RNA, transcription factors, regulatory RNA-binding proteins, and a suite of enzymes with catabolic functions that are normally expressed at low levels or in only a few cells in the liquid culture population. Of particular interest to us is Ngl3, a poorly characterized deadenylase whose expression is unregulated ~50 fold in cells growing as colonies and whose expression is limited to the colony interior. We will present data showing that Ngl3 is a homolog of the metazoan protein Nocturnin, a deadenylase essential for normal fat metabolism. We will report on our current work in search of the targets of deadenylation by Ngl3 in the mature yeast colony.

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A two-component transposon system for generating tagged mutants in the dimorphic pathogen *Penicillium* (*Talaromyces*) *marneffei*

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Penicillium marneffei (recently renamed *Talaromyces marneffei*) is well placed as a model experimental system to investigate processes controlling fungal growth and pathogenicity. An opportunistic pathogen of humans, *P. marneffei* is a dimorphic fungus that displays multicellular hyphal growth and asexual development (conidiation) at 25°C and unicellular fission yeast

growth at 37°C and in host cells. We have adopted a forward genetic approach to discover novel genes contributing to the dimorphic switch and pathogenic potential of this fungus. While traditional chemical mutagenesis screens involve extensive genetic mapping and large-scale sequencing or cloning by complementation with whole-genome libraries, insertional mutagenesis systems facilitate isolation of the affected gene since the genetic lesion is tagged with the insertional marker. Transposon-based insertional mutagenesis systems take advantage of mobile genetic elements (DNA transposons) that are able to excise and re-insert in the genome *in vivo*.

We have developed a two-component transposon mutagenesis system for *P. marneffei* using the *piggyBac* transposon of *Trichoplusia ni*. The key features of this system include: the ability to control the activation of the transposon by induction/repression of transposase gene expression; the capacity to detect transposon movement by positive and negative selection; and the rapid identification of transposon insertion sites using splinkerette PCR/sequencing. Here we present the parameters affecting transposition frequency, the capacity to isolate mutants affected for growth *in vitro* and *in vivo*, and high-throughput approaches for identifying transposon insertion events including potential downstream applications.

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Analysis of a novel protein induced in a fluconazole tolerant strain of *Cryptococcus gattii*

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The limited availability of antifungal drugs makes the treatment of fungal infections problematic. However, the development of novel drugs is slow and very expensive. In recent years, increasing attention has been given to the use of combination therapy to treat fungal infection. The use of a secondary compound that exerts stress on the fungal cell can increase the overall efficacy of treatment. Understanding the response of the fungal cell to an antifungal drug is a necessary first step in selecting possible targets for secondary compounds. To this end, we undertook a study of the proteomic response of the basidiomycetous fungal pathogen *Cryptococcus gattii* to the antifungal fluconazole. We identified a previously uncharacterised protein (designated HARP) that was 47.5-fold upregulated in a fluconazole tolerant strain (MIC = 64 ug/mL), 7.8 and 3.9 fold upregulated in two strains with intermediate fluconazole sensitivity (32 ug/mL) and uninduced in a fluconazole sensitive strain (16 ug/mL) when the cells were treated with the drug. BLAST analysis of the HARP protein sequence against the NCBI nr database yielded no significant homologues. However, PFAM and DELTA-BLAST searches identified domain homology with the Shwachman-Bodian-Diamond Syndrome domain. In humans and *Saccharomyces cerevisae*, proteins containing this domain have a wide range of functions including ribosome maturation, telomere capping and response to osmotic stress. Structural modelling of HARP, and comparison to solved structures of known SBDS domain proteins, revealed very similar tertiary structures. This analysis gives us confidence that HARP may be a homologue of the *S. cerevisae* protein RTC3 that has been shown to have a role in stress response. Deletion studies are currently being conducted in *C. gattii* strains. Analysis of the role of this protein may lead to insights into more effective treatment of difficult or antifungal tolerant cryptococcal strains.

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Ectomycorrhizal fungi from New Caledonian rainforests on ultramafic soils, implication for ecological restoration

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Ectomycorrhizal (ECM) fungi are mutualistic root symbionts that play crucial roles in ecological processes such as element cycling, nutrient mobilization and plant community dynamic. Their key roles in ecosystem functioning is of primary interest for ecological restoration of human disturbed land areas. New Caledonia, an archipelago located in the South West Pacific, is well-recognized for its exceptional biodiversity. Mining activities combined with fires and past logging have lead to the drastic reduction and fragmentation of its original rainforest on ultramafic soils. In order to develop future restoration programs in New Caledonia, we aim to characterize the ECM fungal communities within different rainforest formations representing different states of forest dynamics, i.e. mixed rainforests and rainforests dominated by *Nothofagus aequilateralis* (Nothofagaceae) or *Arillastrum gummiferum* (Myrtaceae). ECM communities have been assessed by sequencing the Internal Transcribed Spacer (ITS) from mycorrhizae collected in the soil. Our preliminary results suggest a strong belowground diversity and the dominance of the /cortinarius lineage under *N. aequilateralis*. Based on their abundance, species belonging to this lineage could thus represent suitable strains for further plant inoculation purposes. Furthermore, this work will increase our knowledge about ECM fungal biodiversity in tropics, regions that have been poorly studied to date. This study will also be placed in the broader context of the on-going plant-microorganisms interactions program undertaken within the "Institut Agronomique néo-Calédonien".

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Fungi, Sex, Genes and Images

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Six years retired, I am fortunate to still have a lab that is researching fundamental questions in Biology. Sex must be good as, despite the cost of genetic disasters it often generates, it has been retained by all the major divisions of the biological world. It is the vehicle by which genes are reshuffled and remodelled, powering evolution. Notwithstanding the biological importance of this cycling and rearrangement of genetic information, there are still many questions to be answered about how it is achieved and regulated. To address some of these questions, my lab has been using the ascomycete fungus *Neurospora crassa* which is a particularly good model system for this purpose. We have been investigating genes that control genetic recombination in specific parts of the genome, the structure of individual recombination events, recombination across the whole genome and the roles of some of the gene products that catalyse recombination. In respect of images, Neurospora, being a filamentous fungus, provides limited scope as a subject for photography and so Pam's, my wife's, second career as a mycologist has provided a welcome means of bringing one of my hobbies into a more professional sphere.

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Plant-microbe interactions in agricultural soils

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Arbuscular mycorrhizas (AM), are associations formed between the roots of most terrestrial plant species and a specialized group of soil fungi. The formation of AM can have important impacts upon plant growth, nutrition and ecology. We have undertaken large scale surveys for AM in agricultural soils, with the aim of linking land management to impacts upon AM. We have also used a novel experimental system to study the effects of arbuscular mycorrhizal fungi (AMF) on plant growth and nutrition, soil ecology and nutrient cycling. We grew a mycorrhizal defective tomato mutant (*rmc*) and its mycorrhizal wildtype progenitor (76RMYC+) in both the field and glasshouse to study the biology of AM. This approach allows us to establish non-mycorrhizal controls without the use of non-specific fumigation/fungicide treatments in control plots, or use of constitutively mycorrhizal and non-mycorrhizal plant species. Thus, our approach allows us to study AM with the wider soil biota, and the many ecosystem services they provide, in tact. Results from our recent and ongoing research are discussed in the context of the role of AM in sustainable ecosystems.

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Diversity of AMF in different soils of New Caledonia

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New Caledonia is a recognized hotspot of biodiversity where one third of the island surface is composed of ultramafic soils containing high concentration of nickel, chromium, manganese and cobalt.

Arbuscular mycorrhizal fungi (AMF) colonize nearly all the endemic plant species enabling, among others, to reduce metal toxicity. Mycorrhizal populations can vary according to plant species, and also according to the chemical composition of the soils. However, less is known about the diversity of species encountered on ultramafic or non-ultramafic soils. This question is however of prime importance due to the widely hypothesized role of AMF in the tolerance towards high metal concentrations.

Based on molecular analysis of 18S rDNA, we have analysed the diversity of AMF present in different soils. This has been performed within roots of two plant genera: *Phyllanthus* and *Psychotria* that are highly represented in both soils and exhibit species that have developed alternative strategies towards the nickel. Indeed, *Psychotria gabriellae* and *Phyllanthus favieri* are nickel hyperaccumulators and the comparison of their associated AMF could let us better understand the role of AMF in the tolerance to heavy metals in ultramafic soils.

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PNEUMOCYSTIS JIROVECII: AN EMERGING NOSOCOMIAL PATHOGEN?

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Pneumonia caused by *Pneumocystis jirovecii* (PJP) has substantial morbidity. New risk groups have emerged affecting patients with cancer, organ transplants and other immunosuppression. Case clusters have occurred.

Clinical risk factors, modes of transmission, and strain variation of the fungus remain uncertain with regard to the genesis of outbreaks, as are strategies to limit infection, including infection control measures and secondary drug prophylaxis,

In Australia, solid organ transplants appear to be highest risk for PJP outside of HIV/AIDS. Case clusters have been reported many months to years after transplantation, in all renal units along the eastern seaboard (and Adelaide) from 2010-2012 (n=83 cases). Multi-locus sequence analysis of *P. jirovecii* DNA identified two common major sequence types characteristic of the outbreaks. However, these genotypes are disparate from those causing outbreaks in other countries. Risks for infection include prior CMV infection, underlying lung disease, graft dysfunction and recent anti-rejection treatments. Mortality is 10-30% with

graft loss occurring in additional 10-15% of cases. Blanket prophylaxis with cotrimoxazole limited the outbreak in transplant units. Isolation precautions were instituted.

Contemporary PJP has shifted form one of early infection in high-risk groups to involve cases that occur "late" after initial immunosuppression. Disease is severe and caused by predominant genotype suggesting patient-to-patient transmission. Infection control measures and universal antimicrobial prophylaxis is essential

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Pythium insidiosum mimic fungal infection

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Pythium insidiosum, an Oomycetes member in Kingdom Straminipila, causes a life threatening infection termed pythiosis, in hosts inhabiting tropical, subtropical, and temperate regions. Clinical manifestation in animals are located in subcutaneous site and gastrointestinal tract, whereas in humans are more frequently vascular and ocular sites. Human pythiosis has been reported, mainly, from Thailand. Hosts with ocular involvement were immunocompetent and those with vascular type usually found in lower extremities with ascending blood vessel infection, had hematologic disorders *i.e.*thalassemia. Its severity depends on how far the organism disseminates toward the main arteries. Thus, an earlier diagnosis and treatment are essential to decrease the mortality rate. *P. insidiosum* possesses sparsely septated hyphae in the host tissue and in vitro, making the misdiagnosis a challenge. The majority of pythiosis cases are misdiagnosed as mycotic infections and unsuccessfully treated with antifungal drugs. Laboratory aid such as serology, i.e., immunodiffusion, ELISA have been developed. Diagnosis, collecting and transporting the specimen are important factors because of its low temperature susceptibility. Currently molecular approaches have been helpful for both diagnosis and culture identification. Using *Cox*II DNA sequences, it was possible to separate *P. insidiosum* into clades with better resolution that using the ITS1-2 region. Thermophilic Helicase DNA amplification and its SNPs provides the advantage to differentiate *Pythium* spp. from Entomophthorales, which cause similar subcutaneous infections. To treat these patients, a combination of amputation; itraconazole and terbinafine; and immunotherapy with *P. insidiosum* antigen (PIA) have been used as a practical approach, even though no standard protocol has been ever introduced. The efficacy of using PIA immunotherapy was about 50-60% by the assumption that immune response after therapy shifts the immune status of the host from a Th2 to a Th1. Very few information on the pathogenesis and genomics, proteomics of *P. insidiosum* are available. Thus, more studies are still needed to advance our understanding of this unusual pathogen that addresses as a fungus but it is Protistal microbe.

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Comparative genomics and transcriptomics of the wine spoilage yeast *Dekkera bruxellensis*

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The yeast species Dekkera bruxellensis shows up in many fermentation processes, playing a strong role in shaping the style of beverages such as wine, beer and cider; and impacting on the efficiency of biofuel production. In wine, growth of *D. bruxellensis* post-alcoholic fermentation is associated with production of volatile phenols that impart 'medicinal' and 'barnyard' aromas. These aromas are known colloquially by the industry as 'Brett' character, and 'Brett' is generally regarded as a negative. To further our understanding of this species, we sequenced and *de-novo* assembled the genome of the predominant spoilage strain in Australia, finding a complex and highly heterozygous triploid genome enriched in membrane transport proteins and oxidoreductase enzymes. Subsequent re-sequencing and transcriptomic studies have been undertaken to gain insight into evolution of the species, and the adaptations that enable its growth and survival for long periods in wine.

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Biodiversity and specificity of fungal endophytes in semi-evergreen vine thickets

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Endophytes are fungi that colonise plant tissues without immediately causing disease. Endophytes are particularly abundant in rainforests as these ecosystems have high rainfall and contain a diversity of host plant species. Few studies have focussed on Australian rainforest endophytes and much is to be learned regarding the fungal taxa present and the basic ecology of their associations eg. specificity patterns. Semi evergreen vine thickets are a type of dry rainforest principally occurring in inland southern Queensland and northern NSW. These ecosystems are nationally endangered and are threatened by expanding agriculture. Few ecological studies exist on SEVT and the fungal endophytes of these ecosystems have not been described. In a basic endophyte biodiversity study, leaves were sampled from each of 21 different SEVT plant species at multiple sites in the Darling Downs region of south-east Queensland. To assess fungal specificity patterns, leaves were also sampled from 22 plants of the SEVT marker species, *Geijera salicifolia*, at 5 sites. Endophytes were grown from leaf samples and were identified using molecular and morphological methods. A total of 228 different fungal taxa were isolated from the 21 different plant species in the biodiversity study, averaging 10 endophytes per plant (std=5.4). Common species included *Nigrospora* spp., *Preussia* spp., Pezizales spp., *Cladosporium* spp., *Xylaria* sp. *Epicoccum* spp., *Pestalotiopsis* spp. and *Phomopsis* spp. Many of these are cosmopolitan endophytes, with the exception of the *Preussia* spp. and members of the Pezizales; the former which are more commonly known as dung dwelling species and the latter not typically recorded as endophytes. *Guignardia* spp. were

the most common endophytes in *G. salicifolia* constituting 49 out of the 188 fungi isolated. These results suggest a level of fungal specificity in this host plant species. The study has therefore provided an overview of the fungal endophytes of a previously unexplored Australian ecosystem and assessed some of the ecology of these associations.

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Mycorrhizal associations in *Sarcochilus* orchids in south-east Queensland Australia

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Orchids require mycorrhizal fungi for seed germination and nutrient uptake throughout their life cycles. Most studies on orchid mycorrhizal biology have focussed on terrestrial orchids despite epiphytic orchids comprising the global majority of species. *Sarcochilus* or "butterfly"orchids are small epiphytic species found typically in rainforests in coastal eastern Australia. These orchids are highly sought by orchid fanciers and at present, 6 of the 18 of known species are considered vulnerable or endangered. Isolation and identification of the mycorrhizal partners of these orchids is important from a conservation perspective as it can enhance *ex situ* horticultural growth and be used to guide suitable reintroduction sites. In our research we have investigated the mycorrhizal biology of two vulnerable *Sarcochilus* species, namely *Sarcochilis weinthallii* (Blotched butterfly orchid) and *S. hartmannii* (Large boulder orchid). With regards *S. weinthallii*, we procured roots from three sites in south-east Queensland and identified the mycorrhizal fungal partner of the species through extraction and sequencing of fungal DNA from colonised orchid roots as well as pure fungal cultures grown out from orchid roots. A single species of *Ceratobasidium* predominated in the orchid suggesting that the orchid displays narrow fungal specificity. We have also sampled roots from *S. hartmannii* at multiple sites in south-east Queensland and north-east NSW. Sequencing of DNA from pure fungal cultures grown from roots suggests that the orchid is also colonised by a *Ceratobasidium* species but a taxon different from that associating with *S. weinthalii.* This information will add to basic understanding of the biology of epiphytic orchids as well as inform conservation efforts on these species.

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A novel branch of phospholipase C-mediated signalling involving inositol polyphosphate synthesis is essential for fungal virulence

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Phospholipase C (PLC) is crucial for the viability of Saccharomyces cerevisiae and Candida albicans, growth and development of filamentous fungi, and virulence of Cryptococcus neoformans (Cn). To investigate the mechanism of PLC-mediated signalling in *C. neoformans*, we established that, similar to the PLCδ isoform in mammalian cells, CnPlc1 hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP_2) to produce inositol trisphosphate (IP_3). However, CnPlc1 does not contribute significantly to calcineurin activation which, in mammalian cells, is triggered by IP_3-induced calcium release. Rather, CnPlc1-derived IP_3 serves as a precursor for inositol polyphosphate (IP) conversion to more complex IPs (IP_{4-8}). Using molecular and biochemical approaches, we characterized two IP kinases, CnArg1 and CnKcs1, and showed that they phosphorylate IP_3 and IP_6 respectively. We also showed that similar to CnPlc1, CnArg1 and CnKcs1 are essential for thermotolerance, cell wall integrity, mating, expression of virulence traits (melanization and secretion of phospholipase B) and virulence in the Galleria mellonella infection model. Our recent findings indicate that IPs derived from Arg1 and Kcs1 affect virulence-related proteins at the transcriptional and post-translational level, and the mechanism of this regulation will be discussed. In summary, our findings show that a key biochemical function of CnPlc1 is production of IP_3 for synthesis of complex IPs and that the inositol polyphosphate anabolic pathway is essential for homeostasis and virulence of *C. neoformans*.

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Diagnosing invasive fungal diseases in the 21st Century: do we have the right tools?

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Twenty years ago managing invasive fungal diseases (IFD) of the immunocompromised host such as those being treatment for acute leukemia and recipients of an allogeneic haematopoietic stem cell transplant was straightforward, simple but inadequate poor. The diagnostic tools were limited to direct microscopic examination, culture and histology assuming there was a proper specimen. Moreover, more often than not such material was only obtained after the fact namely at autopsy. In the intervening years several things have changed for the better. We have better means of imaging to detect lesions consistent with invasive fungal diseases, techniques such as bronchoscopy to obtain lavage samples of the lung, indirect means for detecting fungal components such as beta-D-glucan and galactomannan, and PCR for DNA detection and better drugs now belonging to 4 classes. We also have a better understanding of the epidemiology and evolution of invasive fungal disease, have an agreed framework for defining invasive fungal disease and have gathered evidence through clinical trials to allow guidelines to be proposed.Using invasive aspergillosis as a model we can delineate several patterns of the disease, apply the EORTC/MSG definitions more readily and identify more clearly opportunities for intervention. This allows us to explore different strategies for managing IFD which, in turn, can be used to set up integrated care pathways that optimize management such that

effectiveness can be balanced against costs allowing for practical management of IFD. There are still challenges but we are now in a much better position to meet them.

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Putting PCR for *Aspergillus* through its paces

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Aspergillus PCR has been around since the mid 1980's but the techniques was left out of the EORTC/MSG definitions in 2002 and again in 2008 as the technique was neither standardized nor validated. This galvanized a group a group of scientists to convene a working group of the International Society of Human and Animal Mycology (ISHAM) "The European Aspergillus PCR Initiative" at its congress of 2006 in Paris to remedy this (www.eapcri.eu). The group comprised many of those who had developed their own PCR and had published results on its use. A steering committee was established together with a laboratory and clinical working party and the EAPCRI soon had 86 participants from 69 centres of 24 countries, including Australia. The goal was to develop a standard for *Aspergillus* PCR methodology and to validate this in clinical trials so that PCR could be incorporated into future consensus definitions for diagnosing IFD. The EAPCRI has achieved its aim of developing standards for Aspergillus PCR and published protocols for whole blood and serum and work on plasma is in progress. The group has also identified the critical stages Aspergillus DNA extraction and published a protocol to ensure optimal performance. There is also a specific collaboration with The University of Texas Health Science Center at San Antonio and the Invasive Aspergillosis Animal Models (IAAM) to evaluate the proposed standard in rodents infection models. A calibrator is also being developed that will aid in an External Quality Control programme to enable all laboratories to evaluate performance in using PCR to detect Aspergillus. To this end EAPCRI is working closely with Quality Control for Molecular Diagnostics (QCMD) through an External Quality Assessment (EQA), Proficiency Testing (PT) and other quality initiatives (www.qcmd.org). The EAPCRI is also repeating a systematic review of Aspergillus PCR within the Cochrane Collaboration to assess the impact of the standardized Aspergillus PCR protocols on diagnostic utility. Diagnostic validity is being explored in two ongoing clinical trials, one of the European Organization for Research and Treatment of Cancer (www.EORTC.org), the other the AmBiguard prophylactic study undertaken by Gilead Sciences. The achievements and activities if successful should lead to a standard for Aspergillus PCR methodology and allow it to be incorporated into future EORTC/MSG consensus definitions for diagnosing IFD.

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Investigating a relationship between *Cryptococcus gattii* genotypes and virulence related phenotypes

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Cryptococcus gattii is a primary fungal pathogen of humans capable of causing the disease cryptococcosis. Symptoms of cryptococcosis range from asymptomatic pulmonary colonisation, to potentially fatal dissemination to the central nervous system. Molecular techniques divide *C. gattii* into four genotypes: VGI, VGII, VGIII and VGIV. All four genotypes are capable of causing disease in humans but have some differences in clinical presentation, fertility, epidemiology and response to antifungal drugs. This suggests differences in virulence-related phenotypes may exist among the genotypes. Here, we have collected 88 largely clinical *C. gattii* isolates from a broad geographic range across the four *C. gattii* genotypes. Virulence associated phenotypes that we are investigating include thermal tolerance, extracellular phospholipase production, response to oxidative stress, induction of polysaccharide capsule and host immune response. Preliminary data suggest that the genotypes differ in growth and in their response to elevated temperature. These findings underscore the importance of understanding fungal diversity and genotype for understanding and controlling cryptococcal disease.

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My Top 10 Fungi

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The study of mycology undergoes constant change. Mycologists have always dreamed of having a natural classification of fungi that reflected true taxonomic relationships and when I started out 40 years ago, the electron microscope was the new tool to study fungal taxonomy. However today we now have an array of new molecular tools that will allow younger mycologists to finally achieve this aim (many past mycologists would be very envious). This presentation will highlight the 10 most influential fungi of my career in mycology. Many have also had a profound effect on human and animal health. It all started in 1971, in the Botany Department at La Trobe University, where I studied the ecology and ultrastructure of fungi belonging to the genera, *Phomopsis, Epicoccum* and *Torula*. This lead to a major interest in thermophilic mould genera, such as *Thermomyces, Thermoascus, Aspergillus, Rhizopus*, and *Chaetomium;* many of which were recognised human pathogens. In 1978, I was employed by the Adelaide Children's Hospital where I meet Geraldine Kaminski an eminent medical mycologist. This was my introduction to dermatology and the dermatophytes, like *Microsporum canis* and *Trichophyton rubrum*. The arrival of AIDS, fluconazole, *Candida* and *Cryptococcus* in the early nineties transformed mycology into clinical medicine (overnight mycology became important). The advent of antiretroviral therapy for AIDS, heralded the era of invasive mould infections caused by *Aspergillus, Scedosporium, Fusarium* and the zygomycetes, primarily in high risk haematology, bone marrow transplant and solid organ transplant patients. The past decade has also seen the introduction of standardised antifungal susceptibility testing, new antifungal agents, rapid non-culture diagnostics and molecular typing and identification methods. It's truly an exciting time

to be in mycology. I would also like to acknowledge and thank the numerous colleagues that I have had the privilege to work with both in Australia and overseas.

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Microevolution of the *Cryptococcus* genome during human infection

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The opportunistic fungal pathogen *Cryptococcus neoformans* is a leading cause of mortality among the AIDS population and is known for frequently causing life-threatening relapses. To investigate the potential contribution of in-host microevolution to persistence and relapse, we have analyzed several sets of serial isolates obtained from patients with acquired immunodeficiency syndrome who suffered initial and relapse episodes of cryptococcal meningoencephalitis. Despite being identical by multilocus sequence typing, these isolates differ phenotypically, exhibiting changes in key virulence factors such as nutrient acquisition, metabolic profiles, or the ability to disseminate in an animal model. Whole-genome sequencing has begun to provide insight into the nature of these strains, revealing that those characterized so far exhibit a clonal relationship, but with a few key differences. In one series we have shown the relapse isolate to have lost a predicted AT-rich interaction domain protein due to a frameshift mutation. Gene deletion of the predicted transcriptional regulator produced changes in melanin, capsule, carbon source use, and dissemination in the host, consistent with the phenotype of the relapse isolate. In addition, the deletion mutant displayed altered virulence in a murine model of infection. The observed differences suggest the relapse isolate evolved subsequent to penetration of the central nervous system and may have gained dominance following the administration of antifungal therapy. Remarkably, we have also shown this same gene to have been lost in a relapse isolate from a second patient. These data reveal the first molecular insights into how the *Cryptococcus neoformans* genome changes during infection of humans and the manner in which microevolution progresses in this deadly fungal pathogen.

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The Mycology of Wine

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Wine is a fungal product derived from grapes that has been with us since the dawn of civilisation. Apart from the Noble Rot form of *Botrytis cinerea*, which produces the Sauternes style of wine, filamentous fungi are principally grape spoilage microorganisms. Unicellular fungi, in particular the fermentative yeasts, are the principal fungi in winemaking. In natural, spontaneous, fermentations the microflora is complex with many, diverse, species impacting on the fermentation and wine quality. Members of the *Saccharomyces* sensu stricto clade, especially the species *S. cerevisiae* and *S. bayanus*, predominate during fermentation due to their special adaptation to the fermenting grape must environment, despite the fact they do not colonize the surface of intact grape berries. In recent decades it has become common practice to inoculate grape juice with a single, robust wine strain of *S. cerevisiae*; this takes some of the risks out of relying on the natural microflora to complete fermentation and it minimises the risk of spoilage. However, the more we learn about the beneficial effects of a diverse microflora on wine quality the more we are looking to natural mircroflora of a wine fermentation to develop improved inoculation and fermentation management strategies.

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Interactive effects of climate change factors on mycorrhizal fungi

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Fungi play a key role in carbon and nutrient cycling in Australian sclerophyll forest soils, but little is known about how they will respond to future global change. In particular, the interactive effects of climate change factors (elevated atmospheric CO_2 concentration and elevated temperature) on eucalypt soil fungal diversity and how effects may depend on soil characteristics are poorly understood. In this study we aim to determine the interactive effect of these factors on fungal communities under different soils during early stages of *Eucalyptus tereticornis* establishment. To do so, we collected soil monoliths from three forest sites in New South Wales (Australia) having different soil properties and we planted one *E. tereticornis* seedling in each monolith. Six plants in each soil type were grown for 9 months in controlled environment growth chambers under each of four different climate treatments, *i.e.* ambient conditions, elevated CO_2 only, elevated temperature only or both elevated CO_2 and temperature. At the end of the experiment, soil properties (nutrient contents, pH), plant growth (height, above and belowground biomass, leaf number, leaf area, branch number) and mycorrhizal status were characterized. Soil samples were also collected for the characterisation of bacterial and fungal community composition. Hyphal length and percentage of colonization of *E. tereticornis* roots by both ectomycorrizal and arbuscular fungi are being measured in the different climate treatments and soils. The identity of the different ectomycorrhizal morphotypes is being established by molecular analysis (sequencing of the rDNA ITS region). In this talk I will show preliminary results of this experiment focusing on the responses of mycorrhizal fungi. Preliminary results show differences in percentage of ectomycorrhizal colonization among the three soils. Regardless of the soil, results show an increased percentage of colonization under elevated CO_2 plus elevated temperature conditions with no changes on diversity. Possible implications of observed changes under a global change scenario will be discussed.

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Sequence, Assemble, Annotate, Align, Species Tree .. next?

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The reduction in costs of High Throughput Sequencing (HTS) means it is now tractable for many academic groups to de novo sequence entire genomes. Transforming that data into a useful form for addressing questions concerning evolutionary or functional genomics is not trivial. We are developing software tools aimed at extracting useful evolutionary information from whole genome data. I will focus on software suites that address (1) the de novo assembly / annotation workflow and (2) the challenge of drawing statistically robust phylogenomic inferences from the resulting data. I will particularly expand on (2). This integrated suite is customised for utilising whole genomic sequences to estimate a species tree and to identify sequences evolving in a distinctive manner. The tools are centred on a maximum-likelihood phylogenetic framework that can simultaneously apply codon and nucleotide substitution models to mixed protein coding and non-protein coding DNA sequences. For illustrative purposes, we applied the suite to analyse published whole genome data from Cryptococcus strains. Our results are highly concordant with those obtained from MLST sequences. Additional capabilities of the software, including identifying genes exhibiting the signature of clade specific natural selection, will also be discussed.

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New Fungal Names: What Should Diagnostic Labs Report?

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The recent removal of Article 59 from the International Code of Nomenclature for algae, fungi, and plants (ICN) means that there is no longer a provision for fungi to have different names for their sexual and asexual forms. Consequently, up to 10,000 fungal species names are being (or will soon be) reviewed and rationalised, and changes to some species names will occur. In many cases, well-established and clinically familiar species names will not change. However, some names will or have already changed, with potential for confusion both among laboratory staff and for the clinicians interpreting laboratory reports. For example, *Penicillium marneffei* is now called *Talaromyces marneffei.*[1]

In addition species complexes (e.g. the *Aspergillus fumigatus* complex) are increasingly being recognised, representing groups of closely related and morphologically similar species that can only be reliably distinguished by DNA sequencing.

In this presentation I will describe some recent taxonomic changes among clinically important fungi and discuss how these and future changes can best be managed by diagnostic laboratories.

1. Samson RA, Yilmaz N, Houbraken J, Spierenburg H, Seifert KA, Peterson SW, Varga J, Frisvad JC. Phylogeny and nomenclature of the genus Talaromyces and taxa accommodated in Penicillium subgenus Biverticillium. Stud Mycol. 2011;70(1):159-83.

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The Ecology of Fungal Bio-control agents

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In any natural ecosystem fungi can be found parasitising many different types of organisms including other fungi, insects, nematodes and plants. In the past these fungi might have been regarded as either a scourge (e.g. the plant pathogens) or just curiosities (e.g. nematode trapping fungi) if they were noticed at all. Today we recognise that these fungi play a significant ecological role in regulating populations of their host organisms across natural environments, and some of them can be utilised as bio-control control agents of major pest species. Interest in fungi as bio-control agents has been fuelled by the recognition that chemical based pest and pathogen control strategies are limited by problems such as toxic residues in the environment and food chain and pest resistance to chemicals. Today there are a number of fungal bio-control agents commercially available in Australia for controlling agricultural pests such as locusts and grasshoppers and plant pathogens such as grey mould in grapes and strawberries and white rot in onions. In addition fungi are being deployed to control invasive weeds such as lantana, water hyacinth and wild blackberry in different Australian states. However the selection and successful development of fungi as new bio-control agents requires an appreciation for and understanding of the ecology of these fungi in both their natural environment and the environments in which they are to be deployed. This presentation will describe the pathway from natural environment to commercial bio-control agent.

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Composition of the *Cryptococcus neoformans* secretome and post-mitotic cell separation are affected by the lipid transfer protein Sec14

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The opportunistic fungal pathogen, *Cryptococcus neoformans,* secretes a diverse array of proteins that contribute to its virulence within the mammalian host. We established that the phosphatidylinositol/phosphatidylcholine transfer protein Sec14 regulates secretion of the *C. neoformans* invasin, phospholipase B1 (Plb1), and is essential for virulence. To identify other secreted proteins regulated by CnSec14 that potentially contribute to the virulence of *C. neoformans*, we analyzed the secretomes of WT (strain H99) and a *SEC14* deletion mutant (*Δsec14*) using mass spectrometry. We identified 124 proteins in WT secretions; 32 contained a signal peptide, implying that they are canonically secreted via the ER/Golgi. The abundance of 25 proteins was reduced in *Δsec14* secretions, 11 of which were either, canonically secreted enzymes or cell wall associated proteins/enzymes. In addition to Plb1, secretion of other virulence-related proteins, including acid phosphatase (Aph1) and laccase, was reduced in *Δsec14* mutant. Fluorescent labelling of Aph1 and Plb1 demonstrated that these proteins are transported to the cell periphery via endosome-dependent and independent routes respectively, with both proteins enriched in bud necks. Phenotypic analysis of *Δsec14* revealed cell growth and post-mitotic cell separation defects: *Δsec14* cells were enlarged and formed multicellular aggregates containing nucleated cells connected by septa. Defective septum dissolution in *Δsec14* correlates with reduced secretion of cell wall modifying enzymes. In summary we report the most comprehensive *C. neoformans var grubii* secretome to date and have demonstrated the importance of Sec14 in cell separation and secretion of proteins via the canonical route, particularly those involved in cell wall homeostasis.

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Oscillations in growth of fungi

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Many organisms have been shown to have periodic behaviours. In fungi, sporulation and transport of cytoplasmic contents have been shown to have periodic behaviour that is genetically determined. We examined growth of the fungus *Mucor mucedo* as part of a project comparing the response of the fungus to differing ratios of carbon to nitrogen. We expected a smooth exponential initial growth phase which would plateau as nutrients were depleted. The data better fitted an exponential curve with a sinusoidal overlay of 28 hours duration. The possibility that growth and other physiological processes were out of phase, or offset, thus preserving a linear energy outlay was next tested. The formation of melanin by a different isolate of *Mucor* was in phase with the growth of the organism: both followed the same sinusoidal pattern overlying an exponential curve. This pattern has been noted in the growth of single members of the Ascomycontina and Basidiomycotina, and may be a generalised phenomenon.

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DNA barcoding of human pathogenic fungi

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With the constant increase in invasive fungal infections, the insufficiency of the current identification techniques (morphology/physiology), the limited available therapies and the emergence of resistant fungal strains there is an urgent need to improve fungal identification to enable a substantial improvement in clincial diseases outcome. Molecular based identification allows for an early identifictation of the fungal disease agent directly form clinical specimens or pure culture. The Internal Transcribed Spacer (ITS) regions have been used extensivelly in medical mycology for fungal ID. However, there are no ITS sequences of human pathogenic fungi deposited at BOLD. In 2010 a new ISHAM working goup was established, (1) to stet up a medical barcode database as part of BOLD by incorporating the different existing fungal group specific databases, (2) to extend the number of quality controlled ITS sequences to cover all medical important fungi, and (3) to achieve a special status as quality controlled reference sequences for those sequences within Ganbank. Currently sequence based ID is based on a cut-off of 98-99% similarity with the type culture of the species in question. Population based studies have shown that the sequence variation in clinical samples is much higher. Fungi have species dependent variable rates of polymorphisms in their ITS1/2 regions. Intra-species variation varies from 0-8.35% (*C. parapsilosis* 0% and *C. tropicalis* having 8.35%). Our findings lead to a redefinition of the recommended cut off values to 92% sequence similarity for the ITS1/2 region depending on the fungal species under investigation. As a result of a global collaboration the quality-controlled sequences of more then 2000

fungal strains are now available at www.mycologylab.org. The identified variation raises the question if the ITS region is the most appropriate locus for fungal barcoding. Whole genome sequence comparisions are currently underway to find either alternative genetic loci, better reflecting phylogenetic relationships amoung fungi, enabling a higher discrimination between fungal species and resulting in a more accurate ID.

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Impact of the Changes on Fungal Nomenclature to Ascomycetous Yeasts

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Advances in molecular biology lead to enormous improvements in fungal taxonomy. Multigene phylogenies have been instrumental in the revision of the classification of ascosporic (telemorph) yeasts resulting in a natural system based on lines of decent. This has not yet been done for the anascoporic genus *Candida*, where it is increasingly disappointing to use the same generic name for yeast that have their nearest teleomorphic relatives classified in different families, discrediting all members of the genus *Candida* as potential human pathogens and concealing already known biological information. Generic names should communicate key-knowledge about related species groups. The problem is compounded by the new nomenclature rules reflecting the principle of one fungus = one name. To achieve this principle the current meaning of the name *Candida* = missing of ascospores could be replaced by information on metabolism, lifestyle and phylogenetic relationships if such criteria were integrated in a phylogenetic genus circumscription. Reclassification of *Candida* species by multigene phylogeny can be easily done in well-circumscribed sizable phylogenetic clades allow for the assignment of certain *Candida* species to existing teleomorphic genera with high confidence by multigene phylogeny. *Candida* species that form well-circumscribed sizable multigene phylogenetic clades without any teleomorphic members can become the base for new genera. Phylogenetically isolated species forming long branches in multigene phylogenies, could either be reclassified in small new genera or assigned to the most closely related teleomorph genus. Both options are unsatisfactory as they would results either in many small genera or the addition of distantly related species to homogeneous genera. As such, they should be maintained in the genus *Candida* until neighboring species are described to allow them being integrated in multigene analysis and the resulting groups have gained real biological meaning. When adapting the new nomenclature rules two principles should be followed: 1) Name stability should be honored to the largest possible extend, and 2) Great care should be taken not to create unnecessary names.

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In vitro interactions of *Pseudomonas aeruginosa* and *Scedosporium* spp.

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Pseudomonas aeruginosa and filamentous fungi colonize airways of cystic fibrosis (CF) patients. *P. aeruginosa* has direct/indirect inhibitory effects on fungal growth *in vitro.* Here we assessed *P. aeruginosa* inhibitory effects on *Scedosporium* growth. Twenty-five *P. aeruginosa* strains (isolate PA14, 9 mucoid and 15 non-mucoid isolates) and 1 strain each of *S .aurantiacum* and *S. prolificans* were studied. Strains were co-cultured on Saboraud's dextrose agar. The zone of inhibition index (Zx) was calculated. The fluorescent stain FUN-1 was used to qualitate degree of hyphal damage after co-culturing with selected *P. aeruginosa* strains. Growth inhibition of *Scedosporium* in liquid media was studied using the XTT metabolic assay.

Nineteen (76%) and 20 (80%) *P. aeruginosa* strains demonstrated Zx values of <1 for *S. aurantiacum* and *S. prolificans,* respectively. 5/9 (55.5%) mucoid strains had a Zx <1 for both *Scedosporium* species with 14/16(87.5%) and 15/16 (93.7%) non-mucoid strains showing similar Zx for *S. aurantiacum* and *S. prolificans,* respectively. Overall, similar mean inhibition (Zx=0.76) was observed for both *Scedosporium* species. However, mean Zx was 0.89-0.88 for mucoid strains and 0.68-0.69 for non-mucoid strains respectively. On FUN-1 staining, *S. aurantiacum* and *S. prolificans* co-cultured with live bacteria appeared stunted in growth, damaged or dead. Co-cultivation with dead bacteria revealed actively growing fungi. XTT experiments showed a 55-60% decrease in metabolic activity at 24 hours for both *Scedosporium* species. When co-cultivated with strain PA14, the OD readings were 0.87 for *S. aurantiacum*, and 0.95 for *S. prolificans* (vs. ODs of >2 without PA14 present). Similar results were obtained using a mucoid *Pseudomonas* clinical strain SP-01. We found that *P. aeruginosa* inhibited the growth of *S. aurantiacum* and *S. prolificans* on solid media, in liquid media and by fluorescent microscopy staining. Moderate differences in degrees of inhibition were seen with mucoid vs. non-mucoid strains.

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Understanding the causes of virulence in Scedosporium fungi

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Scedosporium aurantiacum is a fungal pathogen of humans and other animals that is highly prevalent in urban Sydney. The variability of virulence between isolates is putatively a result of genetic differences between them. In an effort to establish the genetic factors underpinning virulence we have generated whole genome sequence for 2 high and 2 low virulence *S. aurantiacum* strains.

We performed *de novo* assembly and whole genome multiple alignments that included a well annotated relative. Utilising PyCogent and a new tool we have developed, we predict one-to-one orthologs via alignment based projection from the annotated relative.
Our analyses reveal that the high and low virulence strains are more closely related within a group than between groups. It is possible this relationship reflects a single gain/loss of virulence since the common ancestor of the different phenotypes. However, it is also possible that the greater genetic similarity within phenotype is just a consequence of our small sample size. In other words, the within phenotype genetic similarities may not causally relate to virulence and the evolution of virulence may not be unique.

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Implications of the new Melbourne Code for naming foodborne fungi

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The Melbourne Botanical Congress of July, 2011 made a number of changes to the way nomenclature of biological species other than animals will be regulated in the future. To start with, the "International Code for Botanical Nomenclature (ICBN)" will henceforth be the "International Code for Nomenclature of Algae, Fungi and Plants (ICN)". To the surprise of most mycologists, including many of those who supported the change, the Melbourne Congress decreed that henceforth each fungus would have only one legitimate name – bringing to an abrupt end 40 years of dual nomenclature for fungi with a pleomorphic life cycle. Food mycologists have been among the strongest supporters of the system, as it provided a means of ready identification to genera such as Penicillium, Aspergillus and Fusarium, while using the differences in sexual states to emphasise spoilage by xerophiles, heat and chemical resistance, plant pathogenicity, etc. Other users of the taxonomies of ommon genera, including curators of art and artefacts, textile and leathergood manufacturers and shippers, and indoor air specialists, came to rely on the use of names of sexual species as an indicator of characteristics of importance to them. This must all now change, but in some genera the way forward is murky. In some common genera, such as Alternaria, the sexual state is rarely seen in culture, so use of the asexual name for all species will cause little difficulty. Phylogenetic differences between subgenera in Penicillium supported splitting of that genus, keeping it for the majority of species and synonymising the sexual genus Eupenicillium with it. At the same time, species in subgenus Biverticillium were synonymised with the sexual genus associated with many of them, Talaromyces. However, the way forward in Aspergillus, which has 11 sexual genera associated with it, and Fusarium, with six, is not at all clear. This paper will describe some of the problems surrounding the naming of common foodborne genera under the Melbourne Code.

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An evolutionarily-informed approach for clustering fungal DNA sequences produces ecologically-relevant operational taxa

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Ecological studies of fungal communities increasingly rely on large scale sequencing of environmental DNA, followed by clustering of sequences into operational taxonomic units (OTUs). To date there is no objective method of clustering sequences into groups that is grounded in an evolutionary theory of what constitutes a biological lineage. Here I describe an approach developed by insect systematists that distinguishes population-level processes within lineages from processes associated with speciation and extinction, thus identifying a distinct point where extant lineages became independent, and use this approach to estimate diversity of fungal endophytes from surveys of environmental DNA. Compared to OTU-based approaches defined by fixed levels of sequence similarity, groups delineated by the evolutionarily-informed approach better explained variation in the distribution of fungi in relation to putative niche-based variables associated with host species identity, environmental variables, and aspects of how the sampled ecosystems were managed. These results suggest the evolutionarily-informed approach successfully groups environmental sequences of fungi into clusters that are ecologically more meaningful than more arbitrary approaches for estimating fungal diversity.

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Difficulties associated with identification of a mystery "yeast" from blood cultures

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Moulds are only rarely recovered from blood cultures and when seen, may have an atypical appearance. However in this setting misidentification can have particularly important clinical consequences, potentially leading to inappropriate treatment. We describe a positive blood culture from an immune-compromised patient whose initial Gram stain showed fungal hyphae. After 24-hours incubation the organism was growing on Sabdex and Candida CHROM media. Examination of the organism showed an elongated yeast cell whose appearance on the chromogenic agar was typical of *C. albicans*. Initially this isolate was reported as a probable *Trichosporon* species as arthroconidia were present by day 3 and urease activity had been demonstrated. Upon further workup an ID32C panel gave no identification and the biochemical pattern seen was not consistent with a *Trichosporon* species.

18S ribosomal gene sequencing gave an identification of *Debaryomyces hansenii* which was also inconsistent with the phenotypic findings. The identification remained elusive until 3 weeks later when Sabdex plates left at room temperature were observed to have a powdery, cream-tan mould-like isolate with mycelia and branching chains of arthroconidia typical of *Arthrographis kalrae*.

This case illustrates some of the difficulties of identifying even common moulds when recovered from blood cultures. Even molecular techniques may not provide sufficient information to provide a definitive genus level identification.

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Elucidating the response of wheat to the exposure of *Stagonospora nodorum* effectors

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The dothideomycete *Stagonospora nodorum* is a necrotrophic fungal pathogen of wheat and is the causal agent of Stagonospora nodorum blotch (SNB)[1]. This disease is responsible for over $100 million of yield losses in Australia annually. Recent studies have shown that this fungus produces a number of effector proteins that are internalised into host cells of susceptible wheat cultivars. The mechanism by which these effectors induce tissue necrosis in susceptible hosts is yet to be fully elucidated. We have applied a functional genomics approaches to elucidate the cellular processes leading to disease and provide insight into the mode-of-action of these effectors. Gas chromatography-mass spectrometry analysis of primary polar metabolites has been undertaken on tissue extracts and apoplastic fluid from SnToxA-infiltrated wheat. Results illustrate widespread perturbations in primary metabolism and reveal the first direct evidence of an increase in energy production in response to a pathogen effector. To further understand the host response to SnToxA at the secondary metabolism level, samples were also analysed using liquid chromatography-mass spectrometry. Our data indicate SnToxA causes an increase in defence-related secondary metabolites. These studies have also revealed the identity of a novel phytoalexin molecule that strongly inhibits the ability of *Stagonospora nodorum* to be able to sporulate. These complementary approaches have provided novel insight into the contribution of the SnToxA effector protein to SNB in wheat.

1. Oliver, R. P. & Solomon, P. S. New developments in pathogenicity and virulence of necrotrophs. *Current Opinion in Plant Biology* (2010).

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Understanding the structure and functional role of the hydrophobin proteins RodA and RodB from *Aspergillus fumigatus*

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Hydrophobins are small proteins produced by filamentous fungi which assemble into amphipathic monolayers at hydrophobic:hydrophilic interfaces. They play a number of different functional roles, including reducing surface tension, facilitating attachment to surfaces and mediating fungal-host interactions. The genome of *Aspergillus fumigatus* encodes six different hydrophobins, RodA-RodF. RodA has been shown to form a protein monolayer on the surface of the conidia (Aimanianda et al. 2009). This layer shields the PAMPS within the cell wall from recognition by the host immune system. RodB is expressed in the mycelium when *A. fumigatus* is grown under aerial static conditions, such as those in vivo under conditions of invasive aspergillosis (Beauvais et al. 2007). RodA and RodB both form filamentous structures known as rodlets, which share many structural characteristics with amyloid fibrils. We have determined the monomeric structures of these two proteins and have carried out a program of mutagenesis to identify residues in the proteins which are critical for assembly into rodlets and formation of the amphipathic monolayers. A clear picture of the structure of the monomeric and polymeric forms of these hydrophobins will lead to an understanding of the specific functional roles played by the different hydrophobin proteins in A. fumigatus during normal growth and host infection.

1. Aimainianda et al. (2009) Nature 460, 1117-1121
2. Beauvais et al.(2007) Cellular Microbiology 9, 1588-1600

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A molecular approach to identification of species causing canine sino-nasal aspergillosis

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Background - Sino-nasal aspergillosis (SNA) is an important cause of chronic nasal disease in dogs. *A. fumigatus* is the most common aetiological agent based on phenotypic identification. However some *Aspergillus* spp. can only be reliably identified using molecular methods.

Hypothesis – *A. fumigatus* is the most common cause of canine SNA.

Animals - Eighty fungal isolates from 79 dogs with previously confirmed SNA, including 53 isolates from the USA (44 from CA;2 each from FL, MO and NJ; 1 each from MA, TX, and TN) and 27 from Australia (21 from NSW, 6 from QLD). Clinical records of all Australian and Californian cases were available for review.

Methods - Fungal genomic DNA was extracted and sequencing of the ITS1-5.8S-ITS2 ribosomal DNA and partial β-tubulin regions were performed. Species identification was determined using the BLAST algorithm against GenBank and Centraalbureau voor Schimmelcultures databases.

Results – Seventy-eight of 80 isolates were identified as *A. fumigatus* and 2 from USA as *A. tubingensis* (*Aspergillus* section *Nigri*). Breeds commonly affected were Golden Retriever (13%) and Miniature Schnauzer (10%). There were 39 (75%) male dogs (4 entire, 35 neutered), and 13 (25%) females (2 entire, 11 neutered). The mean and median age at diagnosis was 6 y (range 1-14 y).

Conclusions – *A. fumigatus* is the most common cause of canine SNA in both Australia and the USA. In contrast to feline SNA there appears to be little heterogeneity in aetiological agents. This is the first report to document a pathogenic role for *A. tubingensis* in dogs.

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New insights into *Candida*-macrophage interactions

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The human fungal pathogen *Candida albicans* is able to drastically change cell morphology to evade mammalian immune responses. The oval yeast form is mostly associated with the commensal (benign) state, whereas the elongated hyphal form is linked to pathogenesis. Host factors also determine disease outcome, as the distinct fungal forms are differentially recognized by the innate immune system. In particular, macrophages are able to induce several inflammatory responses that are thought to protect hosts from fungal infections. However, the role of the different fungal morphologies and innate immunity during pathology are not fully understood at the mechanistic level. We established live cell imaging to monitor *C. albicans* infection of bone marrow derived macrophages, and used fungal mutants with distinct morphogenesis defects, as well as macrophages derived from mice mutant in immune response pathways, to understand how *C. albicans* causes macrophage cell death, and how it escapes. We show that *C. albicans* kills macrophages with a biphasic profile, and that an interplay between host pathways and fungal cell morphology and cell surface architecture controls this process. The transcriptional regulator Mediator is a central factor orchestrating morphogenesis upon phagocytosis of *C. albicans* by macrophages, as well as modulating hyphal cell surface organisation, with individual subunits playing distinct roles. Our data provides new insight into the complex, but carefully regulated interaction between immune cells and fungal pathogens that ultimately determines infections in hosts.

Abstracts from Poster Presentations

(numbered according to order in meeting abstract booklet)

id #6905

The effects of human visitation (tourism) on cave microflora in Naracoorte Caves, Australia

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Cave environments such as those found in UNESCO World Heritage listed Naracoorte Caves in South Australia are at risk of damage by uncontrolled human visitations (tourism). Damages to cave features such as speleothems, rock art and finger flutings can occur because of alterations in the caves' microclimatic conditions and indigenous microbial community. These damages may discourage further human visitation to caves with adverse socio-economic impacts. Therefore in this study, we have selected caves in Naracoorte with high numbers of human visitations (> 15000 annually) and different configurations; Stick-Tomato (open), Alexandria (closed) and Strawhaven (control, no tourist access) in order to assess the effects of human visitation on cave microflora. Assessments were carried out by culture dependent and independent analyses of sediments obtained from tourist accessible and inaccessible cave areas. Tourist accessible areas had significantly higher bacterial counts (5.31-5.38 Log cfu g^{-1} soil) than those from tourist inaccessible areas (4.71-4.93 Log cfu g^{-1} soil) and control cave (1.52 Log cfu g^{-1} soil) with fungal viable counts being variable. However bacterial and fungal diversity as assessed by 16S rRNA and ITS based fingerprints was substantially higher in tourist accessible cave areas than in inaccessible areas. While cluster analyses of the microbial community profiles showed differences between the caves, the microflora in tourist accessible and inaccessible areas was only different in the open access Stick-Tomato Cave suggesting that cave configuration can play important roles in microbial distribution in caves. Therefore, this study has shown differences in microbial counts, microbial diversity and cluster patterns in show caves compared to control cave and between tourist accessible and inaccessible areas in some cases. Different factors such human access, cave use and configurations could have been responsible for these differences and further work is required for effective quantification of tourism effects in these caves.

id #7177

Association between virulence and the major molecular types of the emerging pathogen *Cryptococcus gattii*

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Cryptococcosis is a life-threatening disease caused mostly by *Cryptococcus neoformans*, although the number of cases due to *C. gattii* has increased recently, affecting more frequently immunocompetent hosts. *C. gattii* strains are sub-divided into four major molecular types, VGI to VGIV, which differ in their host range, epidemiology, antifungal susceptibility and geographic distribution. From studies on the Vancouver outbreak strains, it is known that the sub-genotype VGIIa is highly virulent compared to the sub-genotype VGIIb. However, not much is known about the virulence of VGI, VGIII and VGIV strains. In order to evaluate the virulence of all genotypes of this emerging pathogen, 5 female Balb/C mice were inoculated intranasally with 106 yeast cells from 8 VGI, 10 VGIIa, 12 VGIIb, 17 VGIII and 8 VGIV strains. The mice were checked daily and weigh twice weekly to identify signs of disease and weight loss. By comparing the number of survival days after inoculation between the studied strains, the VGIV strains showed the highest virulence followed by the VGIIa, VGI, some VGIII and some VGIIb strains. The VGIII strains showed a wide range of virulence. After 60 days of inoculation, the mice inoculated with some of the low virulent VGIIb strains, including the strain CDCR272, as well as the majority of the VGIII strains did not present signs of disease or weight loss. Granulomas were observed in the lungs independent of the infecting strain. India ink stains made directly from lung tissue showed that both cellular and capsular size of all strains increased drastically in comparison with the cellular and capsular size of the same strains before inoculation ($p<0.0001$), emphasizing the importance of the capsule as a major cryptococcal virulence factor. Culture of heart blood showed the presence of cryptococcal cells in the blood system. Posterior analysis was carried out to determine tissue burden, brain invasion and histological findings. The obtained results correlated with those subsequently obtained with the *Galleria mellonella* larvae model. The results obtained so far indicate that all the *C. gattii* major molecular types show a range of virulence among the strains, with some sub-types of them showing a higher virulence than others, indicating the necessity to sub-type isolates in order to chose an appropriate public health response in an outbreak setting. Overall the molecular type VGIV showed the highest virulence amongst *C. gattii*.

id #6675

Investigating the role of inositol polyphosphate kinases that function downstream of phospholipase C in the virulence and drug tolerance of *Cryptococcus neoformans*

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We previously demonstrated that fungal phospholipase C1 (Plc1) is essential for homeostasis and virulence of *Cryptococcus neoformans* (*Cn*). Our subsequent study established that *Cn*Plc1 provides inositol trisphosphate (IP_3) as a substrate for conversion to more complex inositol polyphosphates (IPs) by the IP_3 kinase, *Cn*Arg1. IP_3 content was reduced in *CnΔplc1* and markedly increased in *CnΔarg1*, confirming an epistatic relationship between *PLC1* and *ARG1*. In the present study the *KCS1* gene, predicted to encode an IP_6 kinase functioning downstream of Arg1, was deleted and the virulence profile of the resulting mutant (*CnΔkcs1*) was compared to that of *CnΔplc1* and *CnΔarg1*. Absence of $IP_{7\text{-}8}$ in the *CnΔkcs1*, as determined by inositol radiolabeling and HPLC, is consistent with Kcs1 functioning as an IP_6 kinase within the linear pathway Plc1-Arg1-Kcs1. Phenotypic analysis of all three mutants revealed that, similar to *CnΔplc1* and *CnΔarg1, CnΔkcs1* is compromised in thermotolerance, cell wall integrity, urease and acid phosphatase activity, tolerance to antifungal drugs, melanisation, and virulence in the *Galleria mellonella* infection model. Secretion of the fungal invasin, phospholipase B (Plb1), was also blocked in all three mutants. However, the size of the capsule, which is also a virulence determinant, was increased in *CnΔkcs1* and decreased in *CnΔplc1* and *CnΔarg1*. The suppressing effect of *KCS1* and *ARG1* deletion on melanisation and Plb1 secretion was further investigated, and demonstrated to occur at the transcriptional (laccase) and post-transcriptional (Plb1) level. In conclusion, these findings demonstrate that Plc1-Arg1-Kcs1 constitute a novel signalling pathway that contributes to the regulation of virulence and drug tolerance in *C. neoformans*. Studies into how IPKs and their IP products contribute to cellular function and the virulence profile are ongoing.

id #6652

Eumycetoma due to *Madurella fahalii*: Case Report and Review of the Literature

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Background: Eumycetoma is a chronic subcutaneous infection endemic to tropical and subtropical countries, but there is limited experience in managing this condition outside of these regions.

Methods: This study reviews the clinical features, radiology, mycology and treatment of a patient with eumycetoma managed by St Vincent's Health, Melbourne.

Results: A 40-year-old Somali-born male presented to our institution with the third recurrence of a subcutaneous mass on the plantar aspect of his 4th metatarsal. The lesion was slow growing, painless and intermittently discharged "small black seeds". The lesion was removed surgically following three months of empiric anti-fungal therapy. Prolonged culture was required to grow the causative organism which was able to be identified as Madurella fahalii via molecular techniques. The patient's therapy was altered to voriconazole 200mg twice daily, and he currently remains on treatment without any evidence of disease recurrence 6 months post-surgery.

Conclusion: For this case of recurrent eumycetoma, combined medical and surgical therapy has resulted in a successful therapeutic outcome. Further research is required to establish the optimal choice of antifungal agent and duration of therapy.

id #7804

Identification of New Antifungal Drugs for Cryptococcosis using a Repositioning Approach

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Cryptococcal meningitis is the most common form of meningitis in human immunodeficiency virus (HIV) infected persons, responsible for over 1 million cases each year resulting in approximately 625, 000 deaths worldwide. Current optimal treatments still result in high mortality rates and more effective antifungal agents are needed urgently. Despite the threat posed by fungal pathogens, there is a shortage of new drugs successfully reaching the market, emphasising the need for more effective drug development strategies. Phenotypic screening using drug repositioning (or repurposing), is now becoming an attractive approach for antimicrobial discovery programmes, with apparent benefits compared to target-based strategies. This was demonstrated in previous work in the Charles Laboratory at the ithree Institute, with the discovery of novel compounds with antimicrobial activity.

Drug repositioning has been explored for *Cryptococcus neoformans* but not for *Cryptococcus gattii*, which is responsible for the majority of cryptococcal infections in immunocompetent individuals and has caused outbreaks with a high level of mortality and morbidity. This project aims to: 1) develop a screening protocol to test the FDA-approved Enzo library against *C. gattii;* 2) identify compounds in the library with significant antifungal activity; and 3) investigate the spectrum of activity of hit compounds against other pathogenic strains and genotypes of *Cryptococcus.*