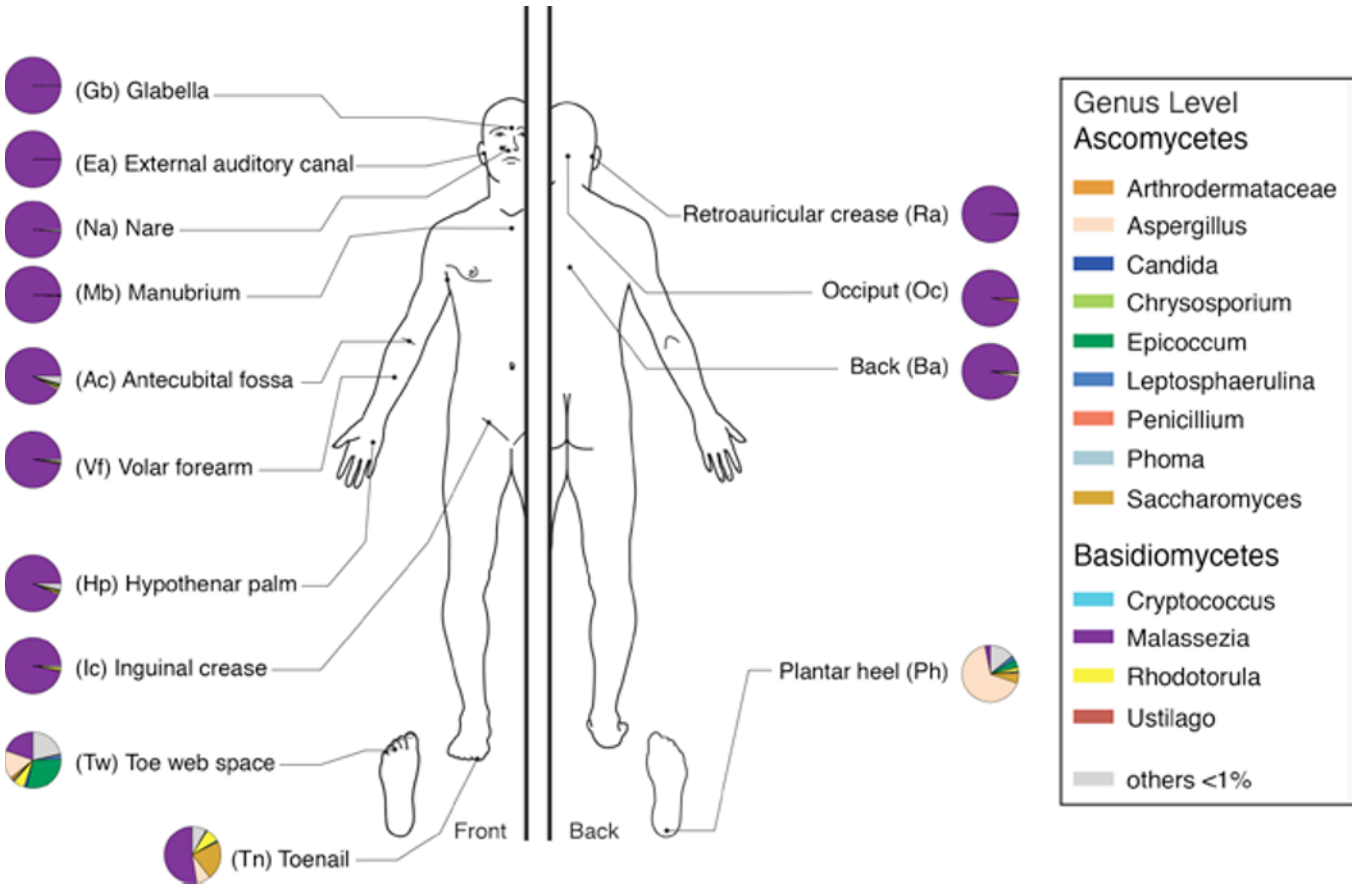
The Human Microbiome Project: fungi on human skin

Surely every mycologist, aware of the universe of spores and mycelium surrounding our bodies and engulfing every living thing, has wondered what is really hiding in the nooks and crannies of our bodies. The considerable attention paid to the human microbiome in the popular press over the past year, emphasizing that we are composed more of bacterial cells than human cells, that our bacterial profiles may define our individuality and influence our health as much as our own genes, must have left the average mycologist wondering, "What about the human Mycobiome?" The latest on-line issue of *Nature* includes an article that begins to address this question.

Keisha Findley *et al.* (2013), of the US National Institutes of Health, studied the skin mycobiota of ten healthy Americans, six men and four women. The subjects were prohibited from using antibacterial or antifungal soaps for seven days and strictly forbidden from showering for 24 hours beforehand before they had skin scraped off. The researchers sampled fourteen parts of these healthy bodies (see figure), including moderately embarrassing regions such as armpits, nostrils and the inguinal crease, and subjected the resulting DNA to 18S cloning to identify fungal genera, ITS pyrosequencing to identify species, and culturing on standard medical mycology media supplemented with olive oil to enhance recovery of the dandruff yeasts, *Malassezia* spp.

They enumerated about 80 fungal genera, including the potentially medically significant *Candida*, *Chrysosporium*, *Cryptococcus* and otherwise unnamed dermatophytes assigned to the *Arthrodermataceae*. Common saprobic genera such as *Aspergillus, Cladosporium, Epicoccum, Leptosphaerulina, Penicillium, Phoma,* and *Rhodotorula* were also frequently detected or isolated. In common with bacterial microbiomes reported in other studies, the mycobiomes differed remarkably among the people sampled, making statistical comparisons difficult. It is tempting to speculate on how these differences come about. In general, feet yielded the greatest fungal diversity, the bottom of the heel, the toenails, the webs between the toes. One can perhaps look at the mycobiomes of these feet and identify the study participants who throw off their shoes and run through meadows, or wallow in mud, or judging by the *Saccharomyces* populations of some, stomp on wine grapes. The authors, of course, are more serious, and consider the past use of antifungal drugs by their subjects, noting that their sampling sites are those most frequently associated with fungal infections.

The big winner in all of this is *Malassezia,* the genus of lipophilic basidiomycetes that apparently coats our bodies with an invisible yeasty slime, especially those parts not encased in shoes and socks. The authors detected three species, *M. globosa, M. restricta,* and *M. sympodialis,* in great profusion on hands, backs, arms and in ears, and a few apparently undescribed species besides. Most mycologists know about these fungi and



Human skin fungal diversity. Figure courtesy of Darryl Leja and Julia Fekecs (National Human Genome Institute, NIH).

their involvement in dandruff, but really, what is going on here? Could it be that we are controlled by these yeasts, that our social customs such as hugging and kissing evolved as mechanisms for exchanging populations of *Malassezia*? Could those aspects of our behaviour that have evolved to promote genetic exchange among our own personal genomes, also enable mating of *Malassezia*? It would probably be best *not* to speculate about this too much in grant proposals, but nevertheless, perhaps mycologists shaking hands at conferences now can take hidden pleasure at the idea that they are facilitating the continuing genomic dance of these little cells that call our bodies home.

Findley K, Oh J, Yang J, Conian S, Deming C, *et al.* (2013) Topographic diversity of fungal and bacterial communities in human skin. *Nature* doi:10.1038/nature12171f (online 22 May 2013).

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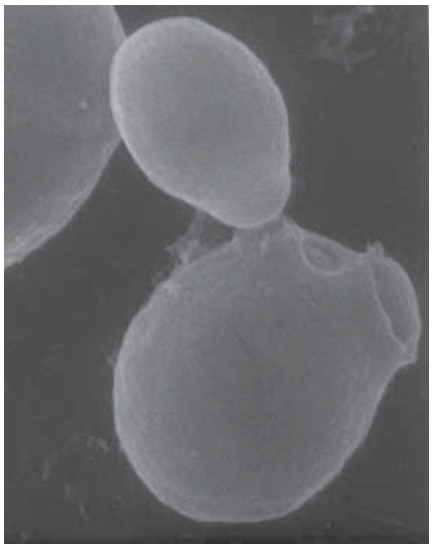
Selecting the "right" genes for phylogenetic reconstruction

It is now the normal practice in preparing phylogenetic trees of fungal lineages to use sequences of several different genes, but testing whether the selected genes are the most appropriate is necessarily somewhat subjective. This is especially so as incongruent trees can be produced. As more complete fungal genomes become available, the possibility of testing the efficacy of particular gene sequences is becoming a reality. In the case of the ascomycetous yeasts, 23 whole genome sequences are now available, across six genera. Salichos & Rokas (2013) have investigated these to examine phylogenomic practices where there is incongruence from conflicting gene trees.

These researchers, from Vanderbilt University (Nashville, TN) analysed a staggering 1 070 orthologous genes from across these genomes. They found that concatenation, the compilation and analysis of numerous genes as a single data set, resolved the species phylogeny, 20 internodes having 100 % bootstrap support, identical to trees recovered from Bayesian and one type of maximum-likelihood analysis tested. However, the tree recovered disagreed with each of the single gene trees. An extended majority-rule consensus (eMRC) phylogeny of the 1 070 separate gene trees gave a tree with a similar topology, but about half of the internodes were only weakly supported. The position of *Candida glabrata* was particularly anomalous, appearing as a sister to *Saccharomyces castellii* and other species of the latter genus – even though only 214 of the 1 070 gene trees favoured that topology.

A novel measure to take incongruence into account is proposed, that of "internode certainty" based on the frequency of that node as opposed to the most conflicting alternatives in the same set of trees. This measure was found to be more informative than that of gene-support frequency (GSF). The problem of incongruence was greatest deepest in the phylogeny, and the authors conclude that inferences in ancient times are dependent on the selection of markers with strong phylogenetic signals. They consider that a fundamental change in current practices is required: (1) bootstrap support should not be used for concatenation analyses of large data sets; (2) the signal in individual genes and trees derived from them should be carefully examined; and (3) internodes that are poorly supported should be identified explicitly. Attention needs to be focussed on the development of new phylogenomic approaches and markers for the resolution of ancient branches in the genealogy of Life. This paper merits careful scrutiny by all mycologists, and other phylogeneticists, exploring early divergences.

Salichos L, Rokas A (2013) Inferring ancient divergences requires genes with strong phylogenetic signals. *Nature* **497**: 327–331.



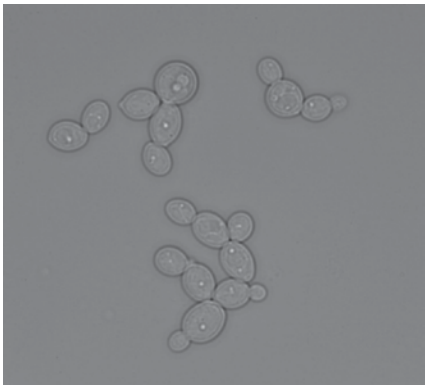
Candida albicans budding cell, CBS562 (scanning electron micrograph).

Haploid *Candida albicans* strains and their significance

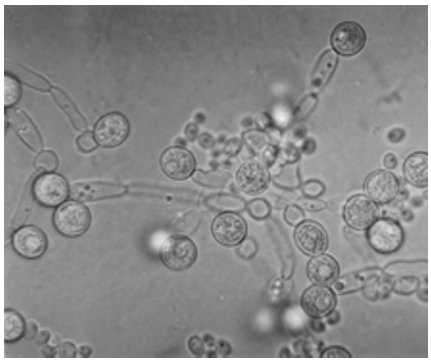
The diverse mechanisms of reproduction and reproductive strategies that evolved in fungi have fascinated not only mycologists but geneticists for generations as more and more intriguing devices come to light. In view of the intense attention that *Candida albicans* has received in recent years from fungal biologists, medical mycologists, and genomics specialists, one might have thought there was nothing basic yet to be uncovered. Not so; there was one secret of success that had remained hidden until now. The yeast cells from clinical cases are invariably diploid (Gow 2013), but now Hickman *et al.* (2013) have found that a halploid state serendipitously in the course of *in vitro* experiments on the loss of heterozygosity. They then used flow cytometry, which enables the amount of DNA in individual cells to be measured, to screen isolates from a wide range of *in vivo* and *in vitro* sources. They found another haploid that had been growing in the halo of the antifungal drug flucanizole, and occurred *in vivo* at the rate of 1–3 haploid cells in every 100 000 cells – no wonder they had not been picked up before!

This finding is of especial significance as it means that some genes that might otherwise have arisen by mutation, and

not been expressed in a diploid because of suppression by genes on the other chromosome set, may be. When a haploid cell forms, that can continue to divide mitotically, and then either form a diploid by mating with a haploid with a different chromosome set to form a regular diploid, or with an identical haploid to form an auto-diploid. In the auto-diploid, genes not normally expressed can potentially be perpetuated and spread in a population. In the particular auto-diploids studied, growth was less rapid than in normal diploid strains, which may partly have caused it to be overlooked by previous workers.



Candida albicans clusters of conidia, CBS562.



Candida albicans producing thick-walled clamydospores, CBS562.

This newly discovered strategy for generating diversity in this most versatile yeast can now be added to those of tetraploid formation with subsequent chromosome loss back to diploid, the production of aneuploids by duplicating some chromosomes, and the mating of compatible diploids (Gow 2013). I wonder what other secrets this fungus still hides . . . . .

Gow NAR (2013) Multiple mating strategies. *Nature* **494**: 45–46.

Hickman MA, Zeng G, Forche A, Hirakawa MP, Abbey D, Harrison BD, Wang Y-M, Su C-H, Bennett RJ, Wang Y, Berman J (2013) The 'obligate diploid' *Candida albicans* forms mating-competent halploids. *Nature* **494**: 55–59.