PROJECT SUMMARY

The majority of biomass in terrestrial ecosystems takes the form of wood and other plant tissues. The task of recycling the carbon and other nutrients sequestered in wood falls largely to the homobasidiomycetes (mushroom-forming fungi). To gain access to cellulose, homobasidiomycetes must degrade or circumvent lignin, which is a highly refractive, heterogeneous polymer that permeates plant cell walls. To accomplish this, "white rot" homobasidiomycetes produce lignin-degrading extracellular enzymes called class II fungal peroxidases, which include manganese-dependent peroxidase (MnP), lignin peroxidase (LiP) and others. Another group of wood-decaying homobasidiomycetes, the "brown rot" fungi, do not degrade lignin appreciably and it is not clear to what extent they also produce class II fungal peroxidases. Mycorrhizal homobasidiomycetes, which form nutritional symbioses with forest trees, have not yet been convincingly demonstrated to possess LiP or MnP.

We propose to study the diversity and evolution of class II fungal peroxidases in approximately 90 species of homobasidiomycetes. We seek to resolve the phylogenetic distribution of these enzymes, reconstruct patterns of gene duplication and loss, and examine possible correlations between shifts in gene copy number with transitions between decayer and mycorrhizal lifestyles, and between white rot and brown rot decay chemistries. We will make predictions regarding functional attributes of proteins by performing homology modelling with reference to published crystal structures, and by surveying sequences for key residues involved in substrate binding and long-range electron transfer pathways.

The proposed research will take advantage of major advances that have occurred recently, including: 1) completion of the genome sequence of *Phanerochaete chrysosporium*, a model white rot fungus, which has been shown to possess five MnP loci, ten LiP loci, and a "hybrid peroxidase"; and 2) improved resolution of the homobasidiomycete phylogeny, including intensive sampling of polypores and "corticioid" fungi, which include the major concentrations of wood-decayers. The *P. chrysosporium* genome will enable us to design primers for PCR amplification and sequencing of class II fungal peroxidase genes, while the homobasidiomycete phylogeny will allow us to develop an appropriate organismal sampling regime.

The proposed research will proceed in two phases, at two phylogenetic scales:
In <u>Phase I</u> we will concentrate on species in the "polyporoid clade", closely related to *P. chrysosporium*. By studying a group of closely related species, we will reconstruct patterns of protein and gene family evolution over relatively short evolutionary timescales. A particular focus will be to compare closely related white rot and brown rot fungi. In <u>Phase II</u> we will sample broadly across all major groups of homobasidiomycetes, including mycorrhizal taxa. A specific goal of Phase II will be to localize the origin of the LiP gene family.

Significance

<u>Intellectual merit</u>. The evolution of wood-decay mechanisms in homobasidiomycetes has had a profound impact on the functioning of terrestrial ecosystems. The proposed research will reconstruct patterns of evolution in class II fungal peroxidases, which are key enzymes in wood decay, and will address whether they are present in mycorrhizal fungi and brown rot fungi. This research has the potential to address the role of gene duplication and functional divergence in ecological adaptation in fungi. This project will synthesize recent and emerging advances from fungal genomics, phylogenetics, and biochemistry.

<u>Broader impacts</u>. Class II fungal peroxidases have potential applications in bioremediation, biopulping, and other green technologies. The proposed research will provide basic information about the diversity and distribution of class II fungal peroxidases, which could be of value to these and other applied areas. Information derived from this project will be made available through a publicly-accessible web site as well as professional print publications. This project will provide training to two graduate students, undergraduates, and high school students from the Clark-affiliated University Park Campus School, who will receive support as summer interns.