II. Sex and the evolution of microbial pathogens

How sexual reproduction contributes to evolution of microbial pathogens is of central importance, and recent studies reveal pathogenic fungi and parasites retain all of the machinery to undergo sexual reproduction but limit sex and thereby generate clonal population structures with limited recombination. We are addressing this question with *Cryptococcus*, a pathogenic fungus distributed worldwide and a common cause of infection in both immunocompetent and immunocompromised hosts that infects most prominently the CNS. The route of infection is via inhalation, and spores are known to be more infectious than yeast cells and hypothesized to represent the infectious propagule. Spores can be produced by mating between two haploid cells of opposite mating-type, **a** and α. While this laboratory defined sexual cycle has been known for >30 years, if, how and where *Cryptococcus* mates in nature has not been established. We find that *Cryptococcus* can complete its sexual cycle on pigeon guano medium, or when co-cultured with plants, and both represent common environmental niches in which sexual reproduction could occur. In collaboration with Tom Mitchell and Ana Litvintseva, we have also contributed to define a unique sub-population in sub-Saharan Africa that is fertile and appears to be undergoing active sexual reproduction. Recent studies provide evidence that α strains preferentially cross the blood brain barrier during co-infection with **a** strains, and we are exploring the hypothesis that this represents an example of intercellular communication, similar to related processes in bacteria and other pathogenic fungi known as quorum sensing.

A central conundrum is that most *Cryptococcus* isolates worldwide are α mating-type, and thus precluded from the traditional **a**-α sexual cycle. α isolates undergo an alternative form of sexual reproduction, known as monokaryotic fruiting involving diploidization (via endoreplication or cell-cell fusion), filamentous growth, meiosis, and sporulation. This unisexual form of mating is a quantitative trait promoted by the mating-type locus α allele. A key question is to what extent either form of mating occurs in nature to contribute to population diversity. The analysis of naturally occurring hybrid strains generated by the fusion of two diverse lineages provides evidence both opposite sex (**a**ADα and αAD**a**) and unisexual mating (αADα) occur in nature. These isolates exhibit hybrid vigor, and studies together with Tom Mitchell and Ana Litvintseva implicate mating in the generation of a robust hybrid lineage that escaped from sub-Saharan Africa. While genetic divergence precludes the AD hybrids from undergoing meiosis, population studies reveal ~8% of clinical and environmental isolates are αAAα diploids. The roles of these isolates as intermediates in the unisexual mating cycle, in the generation of genetic diversity, and in naturally occurring recombinant populations (in collaboration with Dee Carter, University of Sydney) are under active investigation.