Deadly Recombination: Investigation into the Secret Sex Life of the Pathogenic Fungus, *Coccidioides posadasii*

Laux, Klaire, M.S.; Teixeira, Marcus, Ph.D.; Barker, Bridget, Ph.D.

Abstract

Valley fever is a disease caused by inhalation of spores produced by fungi of the genus *Coccidioides*. Infections with these fungi can range from asymptomatic to lethal pneumonia. One potential explanation for the wide range in symptoms may be due to genetic variation generated by sexual recombination. While genetic and microscopic evidence supports the potential for sexual recombination in these fungi, previously, no sexual stage has ever been visualized under laboratory conditions. After screening our isolate library for compatible mating types, mating crosses were set up on a medium without a carbon source and observed using light microscopy. Using this technique, I was able to produce and visualize the structures affiliated with sexual recombination in one of the deadliest strains of *Coccidioides posadasii*, Silveira, for the first time in history. Using this newly developed protocol for the production of sexual spores in *Coccidioides posadasii* strain Silveira, we hope to elucidate the possible strategies these fungi employ to colonize new territories, enhance virulence factors, and generate novel patterns of infection and pathogenesis.

Background

Sex is expensive and dangerous	For many fungi, asexual recombination—or a love the one you're with mating strategy--is a convenient and safe alternative. In the case of *Coccidioides* spp.—the causative agent of Valley fever—it has long been established that asexual spores (arthroconidia) are capable of causing disease when inhaled.[1]
Sexual recombination may provide fitness advantages to fungi in environmentally stressed conditions[2,3]	*Coccidioides* spp. is believed to cause 350,000 coccidioidomycosis (Valley fever) infections in the United States alone [4] Its environmental niche is both expanding and shifting as a consequence of climate change and rates of infection are on the rise [5] If occurring, sexual recombination in this fungus may lead to changes in virulence, geographical location, host range, infectivity, or transmission rates
Sexual recombination in *Coccidioides* spp. to date has yet to be identified	Sex may be occurring in the saprobic or parasitic portions of the fungal life cycle (Figure 1) **The purpose of this work was to catch these fungi in the act of sex**

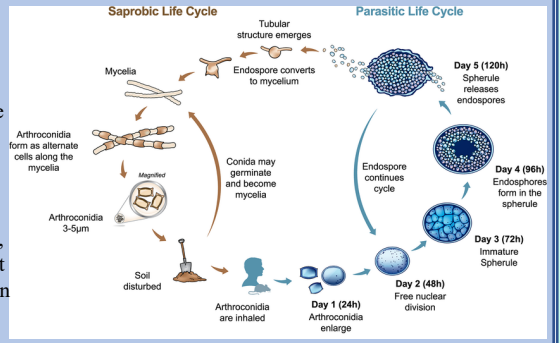

Figure 1. Schematic Representation of the Known Life Cycle of *Coccidioides*. (Image borrowed from reference 6) The known life cycle of *Coccidioides* consists of the formation of two distinct asexual cycles. While never before seen, the possibility of a secret sexual cycle exists within both saprobic and parasitic life cycles.

Results

MAT1-1 isolates fanned out over the surface of mating media until meeting hyphae from the MAT1-2 to generate macroscopic structures called gymnothecia that were so large, they were actually visible to the naked eye (Figure 2). Under the microscope, gymnothecia revealed themselves to be comprised of dense coils (Figure 4) in which fungal cells fused together to generate sexual spores called ascospores encased in and expelled from vase-like structures called asci (Figure 3).

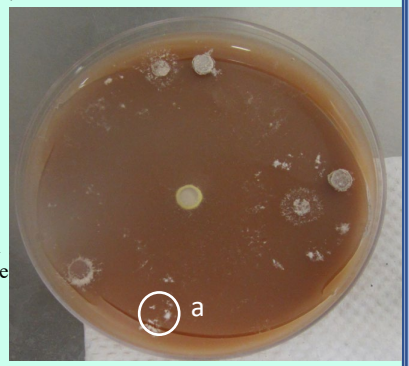

Figure 2. Harem Mating in *Coccidioides posadasii*. In harem mating, a single plug of *Coccidioides posadasii* strain Silveira is surrounded by plugs of the compatible mating type. Gymnothecia (mating structures where compatible fungi meet) appear as white tufts on the plate expanding from the original soil isolate (a).

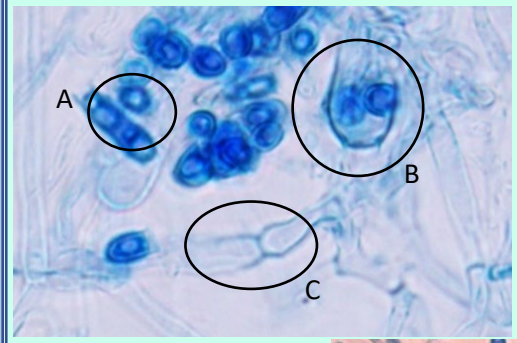

Figure 3. *Coccidioides posadasii* ascospores and asci. Circular blue cells (A) are ascospores that are presumably the product of sexual recombination. Two blue spherical cells seen inside the sexual structure called the "ascus" (B). Fungal cells touch tips (C) in a process called anastomosis which allows for gene exchange. Cells stained with lactophenol blue. Image taken at 630X magnification with Leica DMI3000 B compound microscope

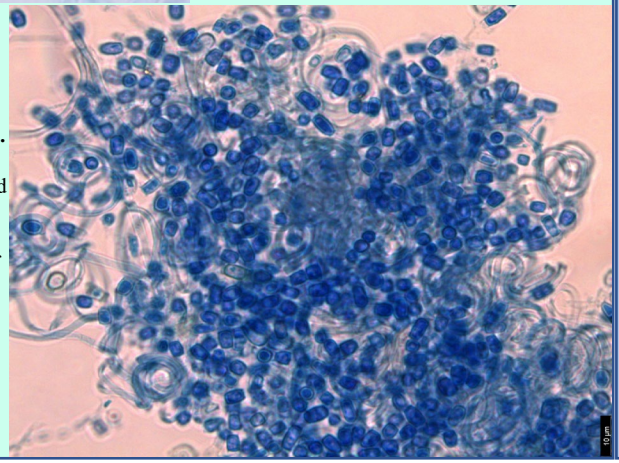

Figure 4. *Coccidioides posadasii* mating structure (gymnothecium). Mating structure of sexual and asexual spores enmeshed with coiled hyphae are visible. Ascospores (result of sexual recombination) are round cells. Arthroconidia (result of asexual reproduction) are barrel shaped cells. Image taken at 630X magnification with Leica DMI3000 B compound microscope

Materials and Methods

Mating Media
- Soil collected from areas conducive to fungal growth
- Autoclaved, filtered, agar, and Hutner's trace elements added
- Poured into deep-welled petri dishes

Mating Crosses
- Compatible fungi estimated based on *MAT* idiomorph determined through sequencing
- Fungi plated on mating media and stored at 30°C for "hot" sex

Visualization
- Gymnothecia (tufts on plate) and fuzzy white growth were selected 3X a week for 6 weeks
- Growth was emulsified in lactophenol blue and sealed in a coverslip with nail polish
- Slides were imaged with a Leica DMI3000 B at 630X

Discussion

The coiled hyphae intertwining to undergo hyphal fusion followed by the formation of vase-like vessels within which circular spores are encased is highly indicative of sexual recombination(Figure 4).

The fact that *Coccidioides* spp. are having sex in response to nutrient limitation provides insight into our fundamental understanding not only of the life cycle of this particular fungus but into the evolution of sex itself.

These insights may prove useful to understanding the potential role of sex in other dimorphic fungal pathogens which collectively account for millions of infections worldwide[7].

References

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