HIGHLY VARIABLE MITOCHONDRIAL INHERITANCE IN INTRA- AND INTER-LINEAGE CROSSES OF *CRYPTOCOCCUS GATTII*

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ABSTRACT

The inheritance of mitochondria and their genomes is predominantly uniparental in plants and animals. However, in fungi, the patterns of inheritance can become complex. Interestingly, in *Cryptococcus neoformans*, the inheritance pattern is predominantly uniparental. Similar to *C. neoformans*, closely related *Cryptococcus gattii* is also a pathogenic fungus, except that it is capable of infecting healthy individuals. In this research, PCR-RFLP was employed on progeny of 14 crosses in order to determine if the mtDNA inheritance pattern is the same in *Cryptococcus gattii* as was found in *C. neoformans.* Additionally, the effect of genetic distance between mating partners on mitochondrial inheritance was investigated by using a range of strains across three of the four distinct lineages in *C. gattii*. My results identified highly variable patterns of inheritance among strains and strain combinations, both within and between the analyzed lineages in *C. gattii*.

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THESIS FORMAT

This thesis is organized into three chapters. The first chapter provides a general introduction of the background information and a general overview of the study. The second chapter presents my project as prepared for submission to a scientific journal for publication. Chapter 3 gives an overall conclusion of the study and suggests further research that could be done to expand upon the research presented here.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Mitochondrial Inheritance in Eukaryotes

Among the organelles found in eukaryotic cells, the mitochondria have a very important function for successful operation of a cell, the production of adenosine triphosphate (ATP) from chemical energy across the mitochondrial membrane through oxidative phosphorylation. One additional characteristic of mitochondria (that is unique along with chloroplasts in plants) is that they contain their own genetic material, which is inherited independently of the nuclear genome. This is also referred to as Non-Mendelian inheritance or cytoplasmic inheritance. This inheritance of mitochondria can differ between species. Details of the inheritance patterns observed are included in the following sections including examples and proven or proposed mechanisms of action. Throughout many years of research the main patterns of mitochondrial inheritance have been determined within animals, plants, protists and fungi (reviewed in Xu 2005).

1.1.1 Uniparental Mitochondrial Inheritance

One very prevalent pattern of mitochondrial inheritance is that it is uniparental, which implies that all of the mitochondrial (mt) DNA in all progeny comes entirely from one of the two parents. This pattern is seen nearly ubiquitously across the animal kingdom and in many plants as well as protists and fungi (Reviewed in Birky 1995, 2001). This kind of inheritance can be either dominant or biased; exclusively one parent's mitochondria or mostly one parent's mitochondria. In the animal kingdom uniparental inheritance is almost entirely dominant with most cases of non-uniparental inheritance occurring in hybridizations of closely related species, which will be discussed in a later section. Since

uniparental mitochondrial inheritance is so widespread, biologists have sought to determine what mechanism(s) is (are) causing the phenomenon. There are many stages at which control mechanisms can be in place in order to ensure only one parent's mitochondria persists in the offspring. These include mechanisms during the production of gametes, during fertilization or fusion, and after zygote formation.

1.1.1.1 Mechanisms during gametogenesis

In plants and animals where gametes are produced for sexual reproduction, events before fertilization can dictate the mitochondrial inheritance pattern. The main event that can cause this pattern is the exclusion of those mitochondria in the gametes of one of the sexes, therefore having no possibility of contribution from that sex. Moses (1961) noticed in red crayfish (*Procambarus clarkii*) that there were no mitochondria present in the final stages of spermatogenesis. This was done through microscopy and showed that the mitochondria had been segregated into peripheral cells that were not to become sperm. This is a startling discovery as it is generally assumed that sperm require energy, and therefore mitochondria, in order to find and fertilize eggs. In this species, however, the sperm lack flagella and are kept in a cavity in the female after copulation in which they fertilize eggs as they develop and pass through the cavity. This lack of requirement to move to find eggs and further lack of flagella can explain the lack of mitochondria in sperm of this species. In addition to this observation in crayfish, some members of the plant Orchidaceae family have also exhibited a lack of mitochondria in the generative cell (the cell formed in the first haploid mitosis of pollen formation that will become the sperm cells) as shown by transmission electron microscopy (Yu & Russell 1992). It is suggested

that this happens due to biochemical gradients and is mediated by actin, a component of the cytoskeleton (reviewed in Mogensen 1996).

Another hypothesized factor contributing to maternal inheritance of organelles that can occur during gametogenesis is the production of anisogamous gametes. While the production of small sperm and large eggs can help to moderate uniparental inheritance by only providing a small amount of mitochondria from the paternal parent and a much larger amount from the maternal parent, it cannot be the only mechanism in place as there will still be some contribution of organelles from the paternal parent. Interestingly, in gymnosperms, where mitochondria are inherited paternally, there is evidence for sperm cells with many mitochondria that are much larger in size than sperm cells of other plant species and are therefore more comparable in size to the egg cells in other plants, as in *Biota orientalis* (Chesnoy 1977). This comparable mitochondrial composition along with other factors associated with the steps that occur during and after fertilization can help to explain how only paternal mitochondria are inherited in a small subset of gymnosperms.

While these mechanisms can help to explain the exclusion of mitochondria in sexual crosses in plants and animals, they cannot explain what is happening in single celled eukaryotes which exhibit uniparental inheritance as these species are isogamous, i.e. gametes of the two parents are morphologically indistinguishable. In addition, during the formation of sexual offspring, the isogamous cells often fuse to form a larger zygote. As a result, there are likely other processes taking place in these organisms without anisogamous gametes that occur during or after fusion.

1.1.1.2 Fertilization mechanisms

At the time of fertilization in plants and animals there are some mechanisms that have been proposed in order to explain uniparental inheritance of mitochondria. In some species, the paternal mitochondria are lost before entering the egg, in others the paternal cytoplasm is excluded from the egg upon fertilization.

In the tunicate *Ascidia nigra* the mature sperm only contains one mitochondrion, which it loses just before entering the perivitelline space and fertilizing the egg (Ursrpung & Schabtach 1965). This was determined by a microscopic study of the fertilization and shows that the paternal mitochondrion does not contribute to the offspring in *A. nigra.*

Another method in which the paternal mitochondria are excluded is that the sperm cytoplasm remains outside of the egg upon fertilization. Enucleated cytoplasmic bodies, slightly smaller than sperm cells, have been observed outside of recently fertilized embryos in cotton (Jensen & Fisher 1967) and confirmed to be of sperm origin. These same types of cytoplasmic bodies have also been reported in *Populus deltoids* (Russell *et al.* 1990) and in tobacco (Huang *et al.* 1993).

Although these mechanisms occurring at fertilization can further help to explain the process by which uniparental mitochondrial inheritance occurs, there is much still unknown about the genetic controls of these processes as they have mostly only been characterized by cytological studies before and after fertilization. These mechanisms also cannot explain what is happening in organisms that undergo fusion of cells to create sexual progeny as well as species that contain many plastids and/or mitochondria upon fertilization and do not exclude cytoplasm of organelles from the fertilization.

1.1.1.3 Post-fertilization mechanisms

If the pre- and during-fertilization mechanisms do not account for uniparental mitochondrial inheritance, i.e. mitochondria of both parents are present in the zygote or embryo after fertilization there are certain post-fertilization mechanisms that can result in uniparental mitochondrial inheritance. These mechanisms can act either by sequestering organelles away from the portion of the egg that will become the individual or by selectively degrading the mitochondria. Sequestering of organelles has been studied primarily in plants using cytological techniques. Selective degradation has two proposed pathways that could occur in order to degrade mitochondria. The first of these two mechanisms involves ubiquitination of the sperm mitochondria leading to degradation. Ubiquitination of sperm mitochondria has been observed in mammals, but not in other eukaryotes. The second mechanism involves methylation protection of maternal organelles followed by degradation of the unprotected paternal mitochondria. This mechanism has been found in algae and fungi.

Owens and Morris (1991) performed a cytological study on douglas fir, *Pseudotsuga menziesii* of the Pinaceae family, in which they hoped to explain the mechanisms behind the pattern of mitochondrial inheritance in this family. This family is part of the gymnosperms which inherit mitochondria from the female parent. Owens and Morris showed that at the time of fertilization sperm cells have many mitochondria, as do egg cells, and all of the cytoplasm from the sperm enters the egg upon fertilization. Shortly after fertilization the female mitochondria form a dense mass around the egg nucleus as the nucleus moves to one end of the cell. As the sperm nucleus moves towards the egg nucleus

to fuse, the male mitochondria trail behind the male nucleus. At the time that fusion of the nuclei occurs the male nucleus is surrounded by female mitochondria. Although this mechanism has been described, it is not fully understood how it occurs, and there is a possibility for male mitochondria leakage, which may require a further mechanism to ensure uniparental inheritance. Furthermore, cytological evidence of a variant of this process has been shown in *Biota orientalis* where mitochondria are paternally inherited (Chesnoy 1977). In this species there is no mass of female mitochondria around the nucleus after fertilization; rather the male cytoplasm (containing many mitochondria) remains tightly associated with the sperm nucleus which migrates towards the egg nucleus at one end of the zygote. Upon fusion of the nuclei, the egg mitochondria have migrated away from the nuclei and begin to be degraded while the sperm mitochondria remain closely associated with the nucleus. Again, while this mechanism has been observed cytologically, the specifics of how it occurs are not understood. Additionally there is still a possibility for leakage which may, again, require another mechanism to be sure of uniparental inheritance.

In animals there has been much evidence for ubiquitination of sperm mitochondria followed by selective proteolysis in the zygotes of bovine, rhesus monkey, mice and humans (Sutovsky *et al.* 1999; 2000). These researchers showed (by use of immuno-fluorescence microscopy) that when bull mitochondria enter cow's eggs ubiquitin is added to the membrane of sperm mitochondria. Later, when the egg is undergoing pre-implantation (4 to 8 cell stage) the fluorescently dyed sperm mitochondria disappeared under the microscope. They initially suggested that this was due to proteosome-mediated proteolysis although none of the 17 proteosome-specific antibodies that they used could

detect proteosomes in association with the ubiquitinated sperm mitochondria. Later they suggested that the mitochondria were degraded in lysosomes first by evidence from electron microscopy and then by the prevention of degradation of sperm mitochondria through using a lysomotrophic agent.

Although this next example is not on mitochondria, but instead on chloroplasts, it is still an excellent example of a mechanism proposed to mediate uniparental organelle inheritance and its principles may be applicable to mitochondrial inheritance. The first evidence for protective methylation of organelles was shown in the green alga *Chlamydomonas reinhardtii*. In this species the mating types are mt+ and mt-, with mt+ being the parent that transmits chloroplasts to the offspring of sexual matings. Two gametes of opposite mating types fuse, with each parent contributing equal amounts of organelles to the zygote, but all progeny have only the mt+ chloroplasts. It has been shown by Nishiyama *et al.* (2002) that mt+ chloroplasts are far more methylated than those of mt- cells. There has been a methyltransferase detected that is targeted to the chloroplast which is expressed much more during gametogenesis of the mt+ cells than that of mt- cells. Additionally if this methyltranferase is overexpressed in mt- cells chloroplasts are paternally transmitted during matings. The authors suggest that this methylation protects the mt+ chloroplast DNA from degradation by nucleases after fusion of the cells. However, how this process in controlled is still unknown.

In *Ustilago maydis*, a fungal plant pathogen, mitochondria are inherited maternally, from the *a2* strains. By using various knockouts of genes present only at the *a2* mating type locus, *lga2* and *rga2*, Fedler *et al.* (2008) showed that when Lga2 is not present in the

a2 strain mitochondria are inherited from both parents while if Rga2 is not present in the *a2* strain mitochondria are inherited from the *a1* strains. Furthermore, if *rga2* is transformed into *a1* strains, mitochondria are transmitted from both parents. They suggested that Rga2 protects the mitochondria from degradation by Lga2 in *a2* strains of *U. maydis* but they do not provide an explanation for why mitochondria are inherited from the *a1* parent when Rga2 is not present in the *a2* strains. This mechanism maybe similar to that of protective methylation as the Rga2 gene may methylate *a2* mtDNA, or may act as a transcription factor for a methytransferase, while Lga2 may act as a mitochondrial nuclease that degrades any unprotected mitochondrial DNA.

Of most relevance to research to be presented in Chapter 2 is the observation of uniparental mitochondrial inheritance in *Cryptococcus neoformans*. In this basidiomycete yeast, it has been shown that mitochondrial inheritance is uniparental from the *MATa* parent regardless of any parameters exhibited by the progeny itself (Xu *et al.* 2000; Yan & Xu 2003). This is the only fungal species known to have this kind of mitochondrial inheritance pattern. It was originally hypothesized that two cells of opposite mating types in *C. neoformans* would fuse together at the onset of mating and some mechanism afterwards would selectively degrade the *MATalpha* mitochondria. Later cytological observations showed that unilateral migration of nuclei plays a large role in the inheritance of mitochondria of *C. neoformans.* McClelland *et al.* (2004) stained two different parental strains and observed the *MATalpha* nucleus migrate into the *MATa* parent via a conjugation tube-like structure. After this occurred hyphae were only seen growing from the *MATa* cell of the pair. However, this mechanism couldn't explain the uniparental mitochondria

inheritance observed for fusion products (i.e. without going through the hyphal stage) in matings in *C. neoformans* (Yan et al. 2004; 2007).

Overall, there are many different proposed mechanisms, some well understood and others not so well understood, to explain uniparental mitochondrial inheritance in animals, plants and other eukaryotes. These mechanisms work before during and after fertilization or fusion of the gametes. They may work alone or in conjunction with other mechanisms.

1.1.2 Other Modes of Mitochondrial Inheritance

Aside from uniparental mitochondrial inheritance there can be biparental inheritance in which the progeny inherit mitochondria from both mating contributors creating offspring with either or both parental mitochondrial haplotypes. When discussing biparental mitochondrial inheritance it is important to decide what exactly the progeny, especially in fungi, is. In cases where biparental mitochondrial inheritance can occur, the presence of both mitochondrial genotypes (or mitotypes) in individual cells is often dependent upon where the cells are sampled from in many fungi.

In Basidiomycota mating occurs by the fusion of two haploid monokaryons creating a dikaryon. At the end of a dikaryon, production of basidiospores occurs after meiosis of the fused haploid nuclei. In *Coprinus cinereus* the mitochondrial inheritance at an individual dikaryon level is uniparental, but at a whole colony level is clearly biparental. This pattern occurs through the reciprocal migration of haploid nuclei, unaccompanied by mitochondria, into the recipient monokaryon. Since the mitochondria are not migrating, there is only the presence of the resident mitochondria in the individual dikaryon causing uniparental inheritance to be observed in most samples of mycelium, especially at a distance from the

junction point. However within the whole colony of mated dikaryotic cells, two or more mitotypes can be observed (Baptista-Ferreira *et al.* 1983). Soon after this, a discovery was further confirmed by May and Taylor (1988) who also extended to show that in some compatible crosses in *C. cinereus* only one nucleus migrates into the other monokaryon, creating dikaryon with only one mitotype in the dikaryotic population. In addition, May and Taylor (1988) showed that dikaryon could be created in which on either side of the mating junction, the mitochondria were from the resident monokaryon, while at the junction, the dikaryotic mycelia were heteroplasmic, containing mitochondria from both mating partners. This result shows that within a mated super-colony, both the individual mitotypes of the mating partners as well as a mix or the recombinant of the two parents can be observed depending upon where samples were taken for analyses. This sample location-dependant mitochondrial inheritance has also been observed in several other filamentous basidiomycetes *Pleurotus ostreatus* (Matsumoto & Fukumasa-Nakai 1996), *Agrocybe aegerita* (Barroso *et al.* 1997) and *Agaricus bitorquis* (Hintz *et al.* 1988). A similar phenomenon has also been observed in ascomycetes *Aspergillus nidulans* (Coenen *et al.* 1996), *Neurospora crassa* (Mitchell and Mitchell 1952) and *Podospora anserina* (Belcour & Begel 1977). This pattern of mitochondrial inheritance is the most common among fungi.

Aside from this sample location dependant mitochondrial inheritance, one can observe yeast-like inheritance as seen in ascomycetes *Saccharomyces cerevisiae* (Thomas & Wilkie 1968), *S. castellii* (Peterson *et al.* 2002) and *Schizosaccharomyces pombe* (Thraikill & Birky 1980). This pattern is somewhat similar to that seen in most other fungi in that the pattern of inheritance depends upon where progeny samples are taken. However, there are

some major differences. For example, in these yeasts, mating begins with the fusion of two compatible cells and ends with a much larger cell. As a result, there is a large proportion of heteroplasmic or recombinant mitochondrial genomes in the progeny. The progeny mitochondrial genotype depends on the proximity of the sampling relative to the conjunction zone where the two parental cells fuse. Specifically, when (buds of zygotes) are taken from either pole of the zygote, it would exhibit uniparental inheritance of mitochondria from the parent of that end. In contrast, if sampling was done near the junction of the mated zygote, those zygote buds would show heteroplasmic inheritance of mitochondria (Strausberg & Pelman 1978, Zinn *et al.* 1987).

The final type of non-uniparental mitochondrial inheritance is called doubly uniparental or progeny sex dependent mitochondrial inheritance. This was first observed in blue mussels species and is unique in that the female progeny inherit only female parental mitochondria, but the male progeny inherit both male and female parental mitochondria (Zouros *et al.* 1994). A similar pattern of inheritance has also been observed in basidiomycete *Ustilago violacea* where progeny with the a_1 mating type allele inherit mitochondria from either parent equally while the a_2 possessing progeny predominantly inherit mitochondria from the a_2 parent (Wilch *et al.* 1992).

1.2 Mitochondrial Inheritance and Hybrid Mating

Although for many species (especially in the animal kingdom) their patterns of organelle inheritance have been determined, these patterns can vary across kingdoms and even across genera and species. Matings between two individuals of the same species are classed as intraspecific crosses whereas matings between two individuals that are of a

different species are called interspecific or hybrid crosses. Often the organelle inheritance patterns are first determined in intraspecific crosses then expanded to test whether or not they apply to interspecific crosses (Gyllensten *et al.* 1985; 1991; Zouros *et al.* 1994).

In cases of hybridization between species or subspecies, the intraspecific uniparental mitochondrial inheritance pattern can be altered due to the breakdown of mechanisms governing uniparental inheritance and result in the presence of organelle DNA from the parent that usually does not contribute. This process is known as leakage and can result in recombination of organelle genomes. Sometimes it is possible to obtain direct evidence for leakage of organelle DNA after hybridization either by lab crosses like in fruit flies (Kondo *et al.* 1990), pine trees (Wagner *et al.* 1991), mice (Gyllensten *et al.* 1991; Shitara *et al.* 1998), mussels (Zouros *et al.* 1998), and periodical cicadas (Fontaine *et al.* 2007), or by analysis of individuals from natural hybrid zones as in mussels (Rawson *et al.* 2006). In other cases, leakage can be inferred from evidence for recombination such as in the great tit, spruce trees, silk moths, and *Cryptococcus* (Kvist *et al.* 2003; Jaramillo-Correa & Bousquet 2005; Arunkmar *et al.* 2006; Xu *et al.* 2009)). Recombination in organelle genomes suggests the presence of heteroplasmy, a state where two different types of DNA, at some point, co-exist in the same cell, which can infer either leakage or genetic mutation. When further analysis of the recombinant organelle genomes reveals that there are significant genomic similarities to the genotypes of the population it would indicate a recombinant of genomes from two individuals rather than a mutant of an existing dominant genotype. It is important to have reference alleles from the population (either putative or known parents) in order to be able to infer mutation vs. recombination. Evidence of

recombination of organelle DNA between two alleles from the population supports the idea that DNA must have leaked.

1.2.1 Animals

In animals mitochondria are always present and aside from the nucleus, are the only organelles that contain their own DNA. The first case in which it was directly proven that mitochondrial DNA (mtDNA) is inherited maternally in animals was in a hybrid cross between two species of frogs, *Xenopus laevis* and *X. mulleri* (Dawid & Blackler 1972). In this study the ratio of DNA hybridization of labelled known mitochondrial cRNA from the mother (^{32}P) and father (H_3) to the unknown samples of offspring mtDNA was used to determine the presence of paternal mtDNA in the embryos and tadpoles from reciprocal crosses. They also measured the ratio of labelled cRNA hybridization to maternal mtDNA. Using this method they determined that since the ratio of hybridization to the offspring mtDNA was similar to that of the maternal mtDNA to itself that there was no presence of paternal mtDNA in the offspring. It is now widely accepted, but still a subject of continuing research, that animal mitochondria follow a maternally uniparental pattern after being shown in a wide array of intraspecific crosses in animals (reviewed in White *et al.* 2008). Although this is the general consensus, not long after it was accepted that mitochondrial inheritance was maternally uniparental in intraspecific crosses biologists started testing whether this held true in interspecific crosses.

The first direct evidence for contribution of paternal mtDNA in hybrid crosses was found in fruit flies, which exhibited a maternally uniparental mtDNA inheritance pattern in intraspecific crosses. In 1990 Kondo *et al.* found indication of paternal mtDNA in

offspring of a hybrid cross between *Drosophila simulans* and *D. mauritiana.* After analyzing 191 lines of 10 backcrosses, paternal mtDNA was found in 4 lines by Southern hybridization to known paternal mtDNA. Most interestingly, in this study 3 of those 4 lines that showed paternal mtDNA did not show presence of maternal mtDNA in the offspring of the 10^{th} backcross. This was also determined by Southern hybridization to known maternal mtDNA. This indicates that there was at least 0.2% leakage of paternal mtDNA in each round of fertilization since in 2% of the final progeny of 10 rounds of backcrosses there was evidence of paternal mtDNA.

Soon after there were experiments in mice looking for paternal contribution of mtDNA in hybrid crosses. The mtDNA inheritance pattern is again maternally uniparental in intraspecific crosses of mice. Gyllensten *et al*. (1985) claimed that there was no evidence of paternal mtDNA in backcrosses between *Mus domesticus* and *M. spretus*. They determined this by analysis of restriction fragment length polymorphisms (RFLPs) of the mtDNA of individuals after 6-8 generations of backcrossing. Soon after with the invention of polymerase chain reaction (PCR) and the detection of paternal mtDNA in *Drosophila* (Kondo *et al.* 1990), Gyllensten *et al.* repeated these backcrosses in mice and extended them further to 23-26 generations. They found that paternal mtDNA did in fact exist in individuals of the final generation of backcrosses. Paternal mtDNA was detected by using species-specific primers for the father of the crosses to amplify a fragment of the mtDNA that was known to be different from the maternal species by sequence comparison. In this study they determined that paternal mtDNA persisted after many generations of backcrossing (Gyllensten *et al.* 1991). Unfortunately Gyllensten *et al.* did not report the frequency with which paternal mtDNA was contributed to offspring.

After this research in *M. domesticus* and *M. spretus,* Kaneda *et al.* (1995) looked at hybrid crosses of *M. musculus* and *M. spretus* and found evidence of sperm mitochondria throughout all stages of development. They did not find paternal mitochondria in intraspecific crosses of *M. spretus* or *M. musculus.* From these results, it was proposed that there is a species specific mechanism that removes paternal mitochondria early in the development of embryos in mice that is impaired in hybridization between the species.

Later Shitara *et al.* (1998) examined how often paternal mtDNA was contributed in mice and whether paternal mtDNA contributed in the initial cross between *M. musculus* and *M. spretus* would be distributed evenly throughout the tissues and whether the paternal DNA would persist in further generations of backcrossing. They looked at the progeny of the first hybrid cross and the ova of these hybrids as well as individuals of a subsequent backcross of a female *M. musculus* mated with a male *M. spretus*. The presence of paternal mtDNA was detected using a nested PCR technique where first a large region of mtDNA was amplified using primers common to both species. Next a second round of PCR using the products of the first PCR as a template is carried out with primers specific to the paternal species (*M. spretus* in these crosses). Shitara *et al.* looked at 12 different tissues in 38 individuals from the initial hybrid cross and found that paternal mtDNA existed in 17 individuals and in these individuals paternal mtDNA was only detected in 1-3 different tissues out of the 12 analysed. One exception was a male that had paternal mtDNA in 6 of the tissues, which was attributed to contamination of tissues with blood that had paternal mtDNA since it was the only individual with paternal mtDNA in the blood. This indicates a percentage of 45% of offspring exhibiting paternal mtDNA leakage. They also determined if leaked paternal mtDNA persisted into the next generation by analysing

ovaries from 91 hybrid females. They detected paternal mtDNA in 6 of these 91 female's ovaries, but none in the ova from any of these 6 individuals that had paternal mtDNA in the ovaries. They analysed 53 embryos from a backcross of the hybrid females with an *M. spretus* male and also did not detect any paternal mtDNA in any of the progeny. These results indicated that although paternal mtDNA contributed often to hybrid crosses of mice, there existed mechanisms to isolate the paternal mtDNA from the gametes and consequentially prevented the leaked paternal mtDNA from being passed on to further generations. This discovery of isolation of leaked paternal mtDNA from the gametes may indicate that the 2% overall leakage found in fruit flies (Kondo *et al.* 1990) that was minimized to be a percentage per fertilization may be entirely from the last fertilization and not a build-up of leakage from each fertilization if a mechanism similar to that in mice exists in fruit flies.

Another animal that has shown paternal mtDNA leakage is the Blue mussels, *Mytilus sp*. Unlike most other animals, the blue mussel has a doubly uniparental or sex-limited mtDNA inheritance pattern. This means that the maternal mtDNA is passed to all progeny but the paternal mtDNA is passed to only the male progeny (Skibinski *et al.* 1994; Zouros *et al.* 1994). After the discovery of this novel mtDNA inheritance pattern in animals much work followed to test the bounds of the model. Zouros *et al.* (1994) showed, at the same time as confirming the doubly uniparental pattern, that when two different species, *M. edulis* and *M. trossulus*, were hybridized in the lab the pattern was not always the same as in intraspecific matings. They revealed anomalies of all kinds in these interspecific crosses including males without male mtDNA and females with male mtDNA as well as one cross that exhibited purely maternally uniparental inheritance. mtDNA type was determined in

this study by preferential or differential PCR amplification of both male and female mtDNA. Female mtDNA was identified using primers to amplify a section of the COII gene that contained an EcoRI restriction enzyme recognition site in female mtDNA that was not present in male mtDNA. After digestion of the amplified fragment with EcoRI it was revealed whether female mtDNA was present or not by whether the fragment was cut. Male mtDNA was identified by first digesting mtDNA with EcoRI then amplifying using the same primers as before, this time selecting for male mtDNA since the female mtDNA would be cut.

Shortly after this observation of anomalies in hybrid crosses of *Mytilus* species, Rawson *et al.* (1996) studied natural *Mytilus sp.* hybrids that were collected from hybrid zones in bays in the US and in the UK. Before doing any analysis of mtDNA types they distinguished hybrid mussels from non-hybrid mussels by confirming that they had alleles specific to the two different species. After identifying hybrids, they detected the presence of male and female specific mtDNA using male and female specific primers to amplify a fragment of the COII gene by PCR and further restriction enzyme digestion was applied since the fragments amplified were of the same length. In the hybrids between *M. galloprovincialis* and *M. trossulus* they found that 28 of 69 hybrid females had paternal mtDNA and 8 of 20 male hybrids had no male mtDNA. This shows a 41% leakage of paternal mtDNA into the females. Their analyses of hybrids of *M. galloprovincialis* and *M. edulis* did not show any differences from the normal doubly uniparental inheritance pattern. What is interesting about this finding is that *M. galloprovincialis* is more closely related to *M. edulis* than *M. trossulus* is suggesting that there is greater leakage in crosses between more distantly related species.

Table 1.1 Observed paternal leakage in hybrid crosses

Organism	Actual Cross	Paternal Leakage*	Reference
Fruit Fly	*Drosophila simulans* x *D. Mauritiana*	0.2% †	Kondo *et al.* (1990)
Pine Trees	*Pinus banksiana* (jack) x *P. contorta* (lodgepole)	7%	Wagner *et al.*(1991)
House Mouse	*Mus domesticus* x *M. Spretus*	Yes (but not quantified)	Gyllensten *et al.* (1991)
	Mus musculus x *M. Spretus*	Yes 45%	Kaneda *et al.* (1995) Shitara *et al.* (1998)
Blue Mussel	*Mytilus edulis* x *M. Trossulus*	Yes (but not quantified)	Zouros *et al.* (1994)
	Mytilus galloprovincialis x *M. Trossulus*	41% **	Rawson *et al.* (1996)
Great Tit	*Parus major major* x *P. major minor*	Yes (but not quantified)	Kvist *et al.* (2003)
Spruce Trees	*Picea mariana (black)* x *P. rubens (red)*	Yes (but not quantified)	Jaramillo-Correa & Bousquet (2005)
Silk Moth	*Antheraea roylei* x *A. Pernyi*	Yes (but not quantified)	Arunkmar *et al.* (2006)
Periodical Cicada	*Magicicadas –decim* x *M. –cassini*	56%	Fontaine *et al.* (2007)
	Magicicadas –decim x M. –decula	26%	Fontaine *et al.* (2007)
Yeast	2 lineages of *Cryptococcus gattii*	Yes (but not quantified)	Xu *et al.* (2009)

*These percentages were calculated using values from the referenced papers by taking the total number of hybrid offspring with paternal mtDNA present and dividing by the total number of hybrid offspring analyzed then multiplying by 100. In cases where only yes is indicated there were not sufficient data in the paper referenced to calculate a percentage of paternal leakage.

†overall leakage was 2% but assuming that there are 10 fertilizations to result in an individual that was analyzed this must be reduced to a value per fertilization (as suggested by Kondo *et al.* 1990)

**this species undergoes doubly uniparental mtDNA inheritance and so paternal leakage can only be expressed in terms of paternal mtDNA in females

Another example of direct evidence for paternal leakage of mtDNA in animals involves the cicada species complex, *Magicicada*. This complex shows maternally uniparental inheritance in intraspecific crosses but has recently been found to show paternal leakage in crosses between different species groups. In the *Magicicada* species complex there are three species groups that each contains two or three different species called -*decim, -cassini* and –*decula.* Fontaine *et al.* (2007) forced crosses between species of different groups by putting a male and an unmated female into an outdoor closed off area. This DNA was extracted from offspring collected at various stages from crosses between different species groups. The mtDNA type was determined using primers specific to the species groups that were crossed i.e. making one a 'paternal' primer set and the other set 'maternal'. In crosses between *M. –decim* with *M. –cassini* 66 progeny were analyzed and of these, 37 contained paternal mtDNA (i.e. paternal leakage of 56%). In a cross between *M. –decim* and *M. decula* 29 offspring were analyzed and 6 had paternal mtDNA (i.e. a 26% paternal leakage of mtDNA). It should be noted that in the inter-species crosses, species pairs showing the least sequence divergence (3-4%) did not mate successfully whereas those with more divergence (7-8%) mated and produced leakage. It would be interesting to know if the sequence divergence alone between *–decim* and *–cassini* is greater than that between *–decim* and *–decula* in order to be able to attribute the greater leakage to a greater genetic distance between the species.

An example of evidence for paternal leakage that is known due to the presence of recombination of mitochondria is the great tit. *Parus major* exists as 4 different subspecies groups, two of which, *P. m. major* and *P. m. minor*, exist sympatrically in the middle Amur valley in far-east Siberia. There is evidence of natural hybridization between these

subspecies in this area, creating a hybrid zone. Kvist *et al.* (2003) analyzed the mitotypes of 27 birds from this area by first amplifying the mitochondrial control region and sequencing this region for all of the birds. They found that the sequence of one of the birds that was phenotypically *P. m. major* consistently contained genetic characteristics of both *major* and *minor* individuals. After discovering this they used *major* or *minor* specific primers and surprisingly amplified both mitotypes. This is clearly evidence of paternal leakage since there is no other way to explain the presence of mitochondrial DNA from both species groups, especially since mtDNA generally comes from the mother in these species.

Another example of paternal leakage that is known due to evidence of recombination is of the synthetically created silk moth, *Antheraea proylei.* This species is known to be a hybrid of *Antheraea roylei* and *A. pernyi.* These moths usually undergo uniparental inheritance of mtDNA from the maternal lineage. *A. proylei* was created by crossing a female *A. roylei* with a male *A. pernyi.* During further analysis of *A. proylei* by Arunkmar *et al.* (2006) while trying to construct a phylogeny of all present day silk moths, an alignment of the mitochondrial control region in all three species was created. This alignment showed that this sequence in the mitochondrial genome of *A. proylei* is far more similar to the paternal parent species of the hybrid cross than to the maternal parent species. This is definite evidence for paternal leakage during this cross.

As has been outlined, there are quite a few examples of paternal mtDNA leakage in hybrid crosses between animal species. More research into the amount of leakage that exists among progeny of hybridizations can help to further understand the extent and

mechanisms behind paternal mtDNA leakage. It has been shown in *Mytilus sp.* that with greater genetic distance between species there is a greater amount of leakage shown. Further research could be done in order to fully understand the amount of distance between species that will cause mechanisms to breakdown allowing for mtDNA leakage. Additionally, understanding the full extent of mitochondrial leakage will allow for this anomaly to be accounted for when using mitochondrial markers in constructing phylogenetic relationships or conducting population genetic studies.

1.2.2 Plants

In plants there was early direct evidence for paternal mtDNA leakage in a study of hybrid crosses in the lab between jack pine (*Pinus banksiana* Lamb.) and lodge pole pine *(P. contorta* Dougl.) (Wagner *et al.* 1991). In all organisms in the *Pinus* genus, which are gymnosperms, the mitochondria from the maternal parent persist in the offspring. In this study 123 seedlings of 11 crosses between different individuals of the species were analysed by RFLP of mitochondrial DNA. Of these 123 seedlings most exhibited maternal restriction fragments but 9 had paternal fragments, giving a paternal leakage percentage of 7%. This was some of the first evidence for paternal leakage of mtDNA in plants with a maternally uniparental inheritance pattern.

Later studies were conducted in spruce trees, which are also gymnosperms where mitochondria are inherited from the female parent (Neale *et al.* 1989). In a study of mitotypes by Jaramillo-Correa and Bousquet (2005) in a hybrid zone of red (*Picea rubens*) and black (*P. mariana*) spruce trees they found that there were only 9 mitotypes in total but only 5 were present in areas of red and black spruce growth where there wasn't any

hybridization. The other 4 mitotypes were shown to be recombinants of the other 5 mitotypes and therefore this case is evidence for paternal leakage in spruce tree hybrids. This is evidence for paternal leakage because recombinant mitotypes can only occur if there are two different types of mtDNA present in a cell which can only happen when paternal leakage has occurred. In this case it is especially clear that there has been paternal leakage since the types that are only in the hybrid zone are a mix of the types found outside of the hybrid zone.

There have only been a few studies that explicitly outline a breakdown of uniparental inheritance of mitochondria in plants likely due to the fact that many of the modes of inheritance in plants have not been well characterized, especially those of gymnosperms aside from conifers as well as non seed plants (Mogensen 1996; Natcheva & Cronberg 2007). Future work should be done to fully understand the patterns and mechanisms of inheritance in plants as well as understanding the prevalence and extent of mitochondrial leakage in uniparental inheritance situations. Further understanding of leakage will help to conduct better population genetic studies as well as to be able to construct more reliable phylogenies once this leakage is taken into account.

1.2.3 Fungi and other single celled organisms

Unicellular eukaryotes include yeast like fungi and protists. These microorganisms, although single celled, have organelles. Organelle inheritance patterns in these organisms are much more variable, yet some organisms follow a uniparental inheritance pattern. One of these organisms is *Cryptococcus neoformans* in which there are two mating types, or sexes, *MATa* and *MATalpha*. In this organism the mtDNA is passed from the *MATa*

individual to all or nearly all of the offspring (Yan & Xu 2003). A study in *C. neoformans* of hybridizations between serotypes A and D indicated that there is no presence of leakage (Xu *et al.* 2000). However, in a very closely related species, *Cryptococcus gattii*, there is evidence of paternal leakage. In a recent study of mitochondrial population genetics of two divergent lineages in *C. gattii* by Xu *et al.* (2009) it was determined that there is evidence for recent recombination and hybridization in the mitochondrial genome. The main evidence for hybridization is that the phylogenies constructed with different genes are not consistent with one another, indicating that there has been recent hybridization. Although there are other eukaryotic microorganisms with uniparental organelle inheritance patterns, as in chloroplasts of *Chlamydomonas reinhardtii* (Sager 1972), there have not been any studies of the organelle inheritance patterns following hybridizations aside from those in *Cryptococcus.* There have been studies looking at recombination in genomes but not many looking at recombination of mitochondrial genomes (reviewed in Barr *et al.* 2005). These types of studies should be done to allow effective use of mitochondrial markers in phylogenetic and population genetic studies of other eukaryotes.

The research presented in Chapter 2 examines crosses within and between lineages of *C. gattii*. Crosses between these lineages are considered hybrid crosses since, although they are within the same species, the strains found in one lineage are quite genetically different from those in another lineage. By examining the mitochondrial distribution after mating between *C. gattii* strains we can determine what the mitochondrial inheritance pattern is within lineages (intralineage) and compare this to the inheritance found in crosses

between lineages (interlineage). These results will allow us to compare the mitochondrial inheritance patterns to those found in the closely related *C. neoformans*, as well as other basidiomycetes. The results obtained will also allow us to determine if there is any difference between intra and interlineage crosses in *C. gattii* and if there is, how does the amount of genetic divergence impact mitochondrial inheritance. We hypothesize that the intralineage crosses will have an inheritance pattern similar to *C. neoformans*, with little to no presence of *MATalpha* mtDNA in the progeny. Furthermore we hypothesize that interlineage crosses representing hybrid mating will exhibit more leakage of *MATalpha* mtDNA than intralineage crosses, as have been shown in hybrid crosses of other eukaryotic species. Finally, we hypothesize that the amount of leakage will increase with increasing genetic distance between mating partners.

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CHAPTER 2

HIGHLY VARIABLE MITOCHONDRIAL INHERITANCE IN INTRA- AND INTER-LINEAGE CROSSES OF *CRYPTOCOCUS GATTII*

PREFACE

This chapter has been prepared for submission to Current Genetics

Author's Contribution

This research was conducted under to supervision of Dr. Jianping Xu. The laboratory work, writing the draft of the manuscript, and table and figure preparation was performed by me. Irina Skosireva (M.Sc.) performed initial testing of mating between various strains available to our lab to find compatible strains before I began my work at McMaster. Irina Skosireva also aided in my learning the techniques required for completing the majority of the project and in doing so assisted in the analysis of one of the crosses. Dr. Jianping Xu provided guidance, when requested, as to the direction the research may go or regarding appropriate methods available to my use to obtain the type of results that were desired. Dr. Jianping Xu also assisted with comments and suggestions for the improvement of the manuscript draft through meticulous examination, editing and proofreading.

Highly variable mitochondrial inheritance in intra- and inter-lineage crosses of *Cryptococcus gattii*

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ABSTRACT

The mtDNA inheritance of *Cryptococcus neoformans*, a close relative of *Cryptococcus gattii*, is uniparental, with progeny inheriting mtDNA almost exclusively from the *MATa* parent. Recently, a study of mtDNA in natural populations of *C. gattii* revealed evidence for recent hybridization and recombination, suggesting mitochondrial inheritance between these two closely related species might be different. Here we examined the mtDNA inheritance of 1145 progeny from 14 crosses between strains from within and between lineages of *C. gattii*. The crosses included 3 between strains of lineages I and III, 4 between lineages II and III, and 7 between strains within lineage III. Significant variations in the percentage of progeny inheriting *MATalpha* mtDNA in crosses were observed for both intra-lineage (3.41% to 92%) and inter-lineage (0% to 93.65%) crosses. The overall leakage is significantly greater than those observed in the closely related species *C. neoformans.* Interestingly, while a negative correlation was found between genetic distance and mtDNA leakage in crosses within lineage III, no such correlation was found for those between lineages. Our results indicate significant variability in mtDNA inheritance among strains and strain combinations in *C. gattii*.

INTRODUCTION

Mitochondrial genes and genomes follow a non-Mendelian pattern of inheritance, unlike those for nuclear genes and genomes. In the majority of animals and plants, progeny typically inherit mitochondrial DNA (mtDNA) from only one of the parents, usually the female (Birky 2001; Xu 2005). Contrary to the relatively uniform pattern of mitochondrial inheritance in plants and animals, fungi exhibit a diversity of patterns. For example, one pattern depends on the location of the progeny relative to the parental strains. In filamentous basidiomycetes, the progeny inherit mtDNA of the resident homokaryon or from the parent in the fused zygote closest to the sampling site. Other patterns of mitochondrial inheritance are sample location independent, where in a given cross, the mitochondria from one parent is preferentially inherited regardless of where the progeny is sampled.

Cryptococcus gattii is an encapsulated basidiomycetous yeast. It is a primary human pathogen that can cause meningoencephalitis in hosts. Within *C. gattii* there are four well established lineages (VGI, VGII, VGIII and VGIV) which are genetically distant from each other. These lineages were established through multi-locus sequence typing. However, they are typically distinguished through the analyses of restriction fragment length polymorphisms (RFLP) of the URA5 gene. Among the four lineages, VGI and VGIII are the most closely related with VGIV being the next closely related to them and VGII being the most distantly related to all other lineages (Bovers *et al.* 2008). The VGII lineage is prevalent in the environments of South America, the Pacific Northwestern United States and Vancouver Island, Canada (Kidd *et al.* 2007). VGII is also the second most prevalent lineage in Australia, but mostly in the northern and western regions (Ellis *et al.*

2000). The VGI lineage is the most prevalent in Australia and India accounting for the majority of environmental and clinical isolates (Ellis *et al.* 2000; Chowdhary *et al.* 2011). VGI has also been found in northwestern United States and Canada (Kidd *et al.* 2007). VGIII is found commonly in Columbia and India as well as southwest United States, while VGIV is rare and found mainly in Africa and Central America (Campbell *et al.* 2005; Meyer *et al.* 2003; Xu *et al.* 2011).

C. gattii has a bipolar mating system with two mating types, *MATa* and *MATalpha* (Hull *et al.* 2005). When in contact with compatible cells of a different mating type and under appropriate conditions, the cells can fuse, produce dikaryotic hyphae, and generate basidiospores through meiosis. In crosses of a closely related species, *C. neoformans,* the mtDNA is inherited predominately from the *MATa* parent where only 0.5% of meiotic progeny inherited mtDNA from the *MATalpha* parent in intra-lineage (serotype D) crosses and 0% in 6 inter-lineage crosses between strains of serotypes A and D (Xu et al. 2000, Yan and Xu 2003). The *MAT* locus, specifically the master regulatory proteins Sxi1alpha and Sxi2a, regulates mitochondrial inheritance in *C. neoformans* such that when either or both genes are deleted, the normally dominant uniparental mitochondrial inheritance pattern is disrupted (Yan *et al.* 2004; Yan *et al.* 2007). Although these proteins are known to be involved in controlling mtDNA inheritance in *C. neoformans,* the precise mechanism by which they work is still unknown.

Based on studies in other organisms such as mammals, several hypotheses have been proposed to explain uniparental mitochondrial inheritance, including sperm ubiquitination and selective proteolysis. This idea is based upon observations in bovine and monkey fertilization where the sperm mitochondria were found more ubiquitinated

than the ovum mitochondria before fertilization and were later targeted for proteolysis in the egg (Thompson *et al*. 2003; Sutovsky *et al*. 1999, 2000, 2003). Additionally, in the model green alga *Chlamydomonas reinhardtii*, a similar mechanism was proposed for controlling chloroplast DNA (cpDNA) inheritance. In *C. reinhardtii*, the uniparental cpDNA inheritance has been attributed to protective methylation of the dominant cpDNA prior to fusion followed by degradation of the non-methylated cpDNA after fusion (Nishiyama *et al.* 2002, 2004). Finally, in *C. neoformans* it has been shown microscopically that during fusion of the two mating partners a conjugation tube-like structure is formed by the *MATalpha* partner which allows the migration of only the MATalpha nucleus into the *MATa* partner, after which the hyphae are only formed out of the *MATa* pole of the zygote (McClelland *et al.* 2004).

In crosses of *Cryptococcus sp.* the proportion of progeny that inherit mtDNA from the *MATalpha* parent is termed mtDNA leakage. Progeny that have heteroplasmic or recombinant mtDNA also belong to the mtDNA leakage group. It has also been shown that changes in environmental conditions can alter mtDNA inheritance in *C. neoformans*. Specifically, UV radiation exposed crosses had increased leakage up to 48% and those crosses performed at high temperatures (28 and 33°C) exhibited higher leakage at 16 and 47% respectively (Yan *et al.* 2007). Additionally, mating between haploid and non-haploid strains of *C. neoformans* can result in leakages ranging from 8 to 40% (Skosireva *et al.* 2010).

Previous studies have shown that mtDNA leakage is greater in hybrid crosses involving genetically divergent parents in laboratory crosses in pine trees (Wagner *et al.*

1991), mice (Gyllensten *et al.* 1991; Shitara *et al.* 1998), mussels (Zouros *et al.* 1998) and periodical cicadas (Fontaine *et al.* 2007) and from natural hybrid zones of mussels (Rawson *et al.* 1996). Interestingly there is also evidence for increased mtDNA leakage with increasing genetic distance in blue mussels where 41% leakage of paternal mtDNA was found in crosses between the two distantly related species *Mytilus galloprovincialis* and *Mytilus trossulus.* In contrast, no leakage was found in crosses between the two more closely related species *M. galloprovincialis* and *Mytilus edulis* (Rawson *et al.* 1996).

In this study, we examined crosses within and between lineages of *C. gattii.* Crosses between these lineages are analogous to hybrid crosses since the strains found in one lineage of *C. gattii* are genetically different from those in another lineage (Litvintseva *et al.* 2011). By performing these matings we can determine what the mitochondrial inheritance pattern is within lineages (intralineage cross) and compare this to the inheritance found in crosses between lineages (interlineage cross). We hypothesize that intralineage crosses should have an inheritance pattern similar to that in the closely related species *C. neoformans*, with little or no *MATalpha* mtDNA in the progeny. Furthermore we hypothesize that interlineage crosses would exhibit more leakage of the *MATalpha* mtDNA as shown in natural strains (Xu et al. 2009) and in hybrid crosses of other eukaryotic species. Finally, we hypothesize that the amount of mtDNA leakage in interlineage crosses would increase with increasing genetic distance between mating partners.

MATERIALS AND METHODS

Strains

All strains except JF101 and JF109 were wild type strains isolated from clinical samples. Strain JF101 is a derivative of a clinical strain NIH312 with the crg1 gene deleted (NIH312 *crg1::NAT*) while strain JF109 is a derivative of another clinical strain B4546 with the crg1 gene deleted (B4546 *crg1::NEO*). The deletion of the crg1 gene allows the strains to mate more efficiently (Fraser et al. 2003). The information about the parental strains used for crosses in this study is shown in Table 1.

Table 2.1 *Cryptococcus gattii* strains used in mitochondrial inheritance analysis

Strain Name	Molecular Type	Serotype	MAT	Wild type or knockout	Strain source
B4544	VGIII	B	MATalpha	Wild type	Clinical, NY State, USA
B4545	VGI	B	MATa	Wild type	Clinical, USA
B4546	VGIII	C	MATa	Wild type	Clinical, NY State, USA
B4495	VGI	B	MATa	Wild type	Clinical, Australia
B4499	VGIII	B	MATalpha	Wild type	Clinical, Australia
LA55n	VGII	B	MATa	Wild type	Clinical, Brazil
LA61n	VGII	B	MATalpha	Wild type	Clinical, Brazil
ATCC32608	VGIII	B	MATa	Wild type	Clinical, California, USA
JF101	VGIII	C	MATalpha	*crg1::NAT*	Lab, Fraser *et al.* 2003
JF109	VGIII	C	MATa	*crg1::NEO*	Lab, Fraser *et al.* 2003

[a] the *nat* gene encodes for nourseothricin resistance while the *neo* gene encodes for G418 resistance

Mating and Progeny Collection

All pairwise mating combinations between MATa and MATalpha strains in Table 1 were attempted on the V8pH7 agar [4.5% V8 vegetable juice (Campbell Soup Co., Etobicoke, Ontario, Canada), 0.5g/L KH_2PO_4, 4% Difco Agar, pH7.0] plates.

For each cross, the two parental strains were first grown on Yeast extract-Peptone-Dextrose agar for 2-3 days at room temperature. Actively growing cells were then re-suspended in sterile distilled water and 20uL of cell suspension (OD approx. 0.5) from each parental strain was plated on the same spot of the mating agar medium. As negative controls, the parental strains were also plated in the vicinity of each other on the same plate as the crosses in order to assess self-filamentation behaviour. Mating spots were left at room temperature in the dark for 7-30 days. Successful mating was indicated by hyphal growth at the periphery of the mating spots that contain yeast cells of the two parents. For those that failed to mate the first time, additional attempts were made, first on V8pH7 agar again and then on V8pH5 agar. For successful crosses, spores and hyphae were collected from the periphery of the mating spot (i.e. away from the parental yeast cells) and suspended in 300uL sterile water for isolation of progeny via one of two methods.

The first was through random spore germination and isolation. In this method, 100uL of this suspension was spread on 3 YPD agar plates and incubated at 30°C for 2 days. Single colonies were then randomly picked and subcultured onto YPD at 30°C for 2 days for DNA extraction. For each successful cross, we picked 96 individual colonies for genotype analyses.

The second method was through microdissection using a micromanipulator (Singer MSM 300, UK). In this method, the spore and hyphae suspension was first vortexed then centrifuged for 1min at 13 000 rpm then 200uL of supernatant was removed and the pellet was resuspended in the remaining liquid. 50uL of this suspension was then placed on a small area of a YPD plate then individual spores were dissected from the source. The plate

was incubated at 30°C for 2 days to allow the dissected cells to form colonies. Progeny obtained through this method allowed the calculation of spore germination rate (number of germinated spores/number of spores microdissected). Similar to the first method, for each successful cross, 96 colonies were subcultured onto YPD at 30°C for 2 days for subsequent DNA extraction and genotyping.

Mitochondrial genotyping of progeny

DNA was extracted from the parental strains as well as 96 progeny from each successful cross following protocol outlined in Xu *et al.* (2000). The extracted DNA, after appropriate dilutions, was used as template to amplify the mitochondrial NADH dehydrogenase subunits 2 (ND2), 4 (ND4), and in some cases 5 (ND5) gene fragments, following the PCR conditions in Table 2. Various restriction endonucleases were used to cut the amplified DNA fragments so as to differentiate parental mtDNA types in the progeny population. The DNA fragment-enzyme combinations used in this study are summarized in Supplemental Table 2. Different from the ND2 and ND4 gene fragments, the ND5 marker was based upon length differences caused by the presence or absence of an intron between certain pairs of parental strains (Supplemental Table 2). Restriction digests followed the manufacturer's recommendations and the digested PCR products were run on a 1.5 % agarose gel in 1x Tris-Acetic-Acid-EDTA buffer. Parental DNA samples were always used as reference controls to allow for the identification of *MATa* parent mtDNA, *MATalpha* parent mtDNA, heteroplasmic mtDNA and recombinant mtDNA (in cases where at least two markers were used).

Progeny were considered heteroplasmic if for any marker we analyzed they exhibited mtDNA genotypes of both parents. Progeny were considered to have a recombinant mtDNA genome if they had inherited one allele from one parent and the other from the other parent. To ensure that heteroplasmic and recombinant mtDNA genotypes had not resulted from partial digests, the mtDNA genotypes of these progeny were reconfirmed by repeated PCR and PCR-RFLP. In addition, the extent to which heteroplasmy could be detected for each cross using the primers and enzymes prescribed was determined by mixing 1:1, 1:10, 1:100 and 1:1000 ratios of *MATa*:*MATalpha* and *MATalpha*:*MATa* DNA prior to PCR amplification, followed by restriction enzyme digestion to reveal at what level all of the bands for heteroplasmy were visible. The results from this pilot experiment are shown in Supplemental Table 2.

MAT-type determination

To determine the mating type of the progeny, the extracted DNA was used as template to amplify the STE20a and STE12α genes (see Table 2 for primers and protocols). This analysis was targeted to crosses with high proportions of the *MATalpha* mtDNA types. A prevalence of progeny with the MATalpha mating type could indicate that the progeny might be derived from self-fruiting of the MATalpha parent, not the result of true mating.

PCR fingerprinting

As a secondary check against self-fruiting in our crosses, progeny with the MATalpha mating type and mtDNA type were further genotyped using DNA fingerprinting. Here, the highly discriminatory M13 primer (5'GAGGGTGGCGGTTCT 3') was employed. In our analyses, any progeny with the exact fingerprinting patterns of

the *MATalpha* parent as well as having the *MATalpha* mtDNA or those with the exact *MATa* fingerprinting pattern and *MATa* mtDNA were considered to be a product of self-fruiting and were excluded from the analyses of leakage.

Sequencing and tree construction

To estimate the genetic divergence among strains, fragments of five protein coding genes were obtained either from the GenBank or through PCR followed by sequencing. The five gene fragments were CAP1, PLB1, GPD1 and TEF1 and LAC1 and the GenBank accession numbers for the strains analyzed here are presented in Supplemental Table 3. The corresponding gene sequences for our strains that were not found in the GenBank were obtained by PCR and sequencing of PCR products. PCR was completed in 1x GoTaq Green Master Mix (Promega, Madison WI) and amplification products were checked on 1.5 % agarose gel electrophoresed in 1x Tris-Acetic-Acid-EDTA buffer. The PCR products were then cleaned and sequenced by MOBIX laboratory at McMaster University (Hamilton, Ontario). The obtained sequences were edited using Geneious (Drummond *et al.* 2010) and aligned using MEGA 5.0 (Tamura *et al.* 2011) before being concatenated to create a NJ tree. Bootstrap values were computed using 1000 replicates. Genetic Distances were also computed using MEGA 5.0. The genetic distances calculated allowed for a Pearson correlation test to be performed in Microsoft Excel between the Genetic Distance between mating partners and the amount of *MATalpha* mtDNA leakage.

Table 2.2 Primers and PCR Protocols

For all sets of primers the following PCR protocol was used: initial denaturation of 4 minutes at 94°C followed by the cycle of 45s at 94°C, 45s at annealing temperature, 1min/1000bp elongation time at 72°C for the prescribed amount of cycles finally followed by one cycle of 7min at 72°C and 4°C until retrieval.

Gene	Sequence 5' → 3'	Annealing temperature	Fragment Size	Cycles
ND2	F: TATGATGGCCGTAGCGCTATC R: TGGTGGTACTCCTGCCATTG	50°C	1145bp*	35
ND4	F: GGGAGAATTTGATTCAAGTGCAAC R: ATGATGTTGCATCTGGCATCATAC	50°C	658bp*	35
ND5	F: CTATTGGTGTTACAGGAGCTCAC R: GAGCCTTCATACCTGCCTTATTTGC	50°C	437bp*, 750bp˜, 1250bp˜	35
STE20a	F: GATCTCTCTCAGCAGGCCAC R: AAATATCAGCTGCCCAGGTGA	60°C	421bp*	32
STE12 alpha	F: CTGAGGAATCTCAAACCAGGGA R: CCAGGGCATCTAGAAACAATCG	55°C	378bp*	35
CAP1	F: CGCCATAGAGAGAGGATGAC R: CCGCCTTACCTTCACAGTCG	59°C	805bp*	45

Fragment sizes *determined from genome published for *C. neoformans* strain H99 or ˜estimated from gel electrophoresis results.

Results and Discussion

Mating attempts and mating success

There were nine crosses attempted among strains within the VGIII lineage, all of these were successful. No crosses were successful between VGI strains (0 out of 2 crosses attempted), and VGII strains (0 out of 1 cross attempted). Since no VGIV strains were available, no crosses involving VGIV were attempted. Three crosses between strains of VGIII and VGII were successful (of 7 crosses attempted) and five crosses were successful between VGIII and VGI strains (out of 9 crosses attempted). No cross was successful between VGI and VGII strains (0 out of 3 crosses attempted). For a complete list of crosses attempted, see Supplemental Table 1.

The mating success rates differed among the different types of crosses. 100% of those crosses within VGIII lineage attempted were successful whereas none of the crosses within both VGII and VGI lineages were successful. This suggests difference in fertility between strains in these lineages. In crosses involving strains of VGIII and VGII, 43% were successful while 56% of crosses between strains of VGIII and VGI were successful. This might be due to the fact that VGIII and VGI strains are more closely related to each other than VGIII and VGII strains.

Lack of a directional effect of crg1 mutation on mtDNA inheritance

We found no directional effect of the *crg1* gene on mtDNA inheritance in *C. gattii*. As JF109 is derived from B4546 crosses JF109 x B4499 and JF109 x B4544 can both be directly compared to crosses B4546 x B4499 and B4546 x B4544 (results in Table 3).

When comparing wild type strain B4546 to *crg1*-mutant strain JF109 mating with B4499 (crosses 1 and 2), the leakage increased from 7.69% to 42.04%. On the other hand, comparing the wild type to *crg1*-mutant mating with B4544 (crosses 3 and 4), the leakage decreases from 17.28% to 3.41%. This shows that while *crg1* is involved in mating, it does not have a directional effect on mitochondrial inheritance. The other crosses with *crg1* mutants corroborate this conclusion. For example, in the VGIII x VGII *crg1*-mutant crosses (9 and 10) more mtDNA leakage occurs than the wild type cross (8). Also, the VGIII x VGI mutant cross (14) has less mtDNA leakage than the wild type crosses (11, 12 and 13). The fact that there was no directional effect of the *crg1* mutation on mtDNA inheritance in *C. gattii* suggested that this gene was not involved in mitochondrial inheritance.

mtDNA inheritance

The results of mtDNA inheritance within and between lineages of *C. gattii* are summarized in Table 3. These results showed extremely varied mtDNA inheritance, with *MATalpha* mtDNA leakage rates ranging from 0% to 93.65%. Contrary to our expectation, the within lineage crosses (crosses 1-7) have similar mtDNA leakage amounts and distribution (average of 49.54% and range from 3.41% to 92.00%) as those observed in inter-lineage crosses (8-14) (average of 48.03% leakage and range from 0% to 93.65%). Comparing the intra-lineage crosses to the inter-lineage crosses in a T-test gives an insignificant value of 0.647 indicating that they are not statistically different from each other with respect to the overall leakage. The exact causes for such varied mtDNA inheritance across the species cannot be pinpointed in this research; however, it is clear that there are overwhelming individual strain variations that are contributing to these results.

The fact that there is much mtDNA leakage from the *MATαlpha* parents seen in *C. gattii* is consistent with the findings of mtDNA recombination in natural populations of *C. gattii* as found by Xu *et al.* (2009).

Table 2.3 Mitochondrial inheritance in intra-lineage crosses

The number of progeny exhibiting each type of mitochondrial inheritance is shown. There were no progeny that showed heteroplasmy of mtDNA. Leakage is calculated as the percentage of progeny exhibiting *MATαlpha* or recombinant mtDNA out of the number of progeny analysed for each cross, excluding those that exhibited *MATαlpha* mtDNA and the same M13 fingerprinting pattern as the *MATαlpha* or *MATa* parent. No progeny had the same M13 fingerprinting pattern as the *MATa* parent. Genetic distance is calculated using the Maximum Composite Likelihood model and are expressed as the number of base substitutions per site from between sequences.

Cross	Number of progeny with				Leakage (%)	Genetic Distance
	***MATa* mtDNA**	***MATα* mtDNA**	**Recombi-nant**	***MATα* mtDNA & M13**		
VGIII x VGIII						
1. B4546 (**Ca-VGIII**) x B4499 (**Bαlpha-VGIII**)	84	7	0*	0	7.69	0.009
2. JF109 (**Ca-VGIII**) x B4499 (**Bαlpha-VGIII**)	51	34	3	0	42.04	0.009
3. B4546 (**Ca-VGIII**) x B4544 (**Bαlpha-VGIII**)	67	1	13	0	17.28	0.009
4. JF109 (**Ca-VGIII**) x B4544 (**Bαlpha-VGIII**)	85	2	1	0	3.41	0.009
5. ATCC32608 (**Ca-VGIII**) x B4499 (**Bαlpha-VGIII**)	17	51	1	0	75.36	0.005
6. B4546 (**Ca-VGIII**) x JF101 (**Cαlpha**)	54	37	0*	0	40.66	0.004
7. ATCC32608 (**Ca-VGIII**) x B4544 (**Bαlpha-VGIII**)	6	69	0	0	92.00	0.005

Table 2.3 Continued

Cross	Number of progeny with				Leakage (%)	Genetic Distance
	***MATa* mtDNA**	***MATα* mtDNA**	**Recombi-nant**	***MATα* mtDNA & M13**		
VGIII x VGIII						
8. LA55n (**Ba-VGII**) x B4544 (**Bαlpha-VGIII**)	91	0	0	0	0.00	0.042
9. JF109 (**Ca-VGIII**) x LA61n (**Bαlpha-VGII**)	36	48	6	0	60.00	0.047
10. LA55n (**Ba-VGII**) x JF101 (**Cαlpha-VGIII**)	38	4	49	0	58.24	0.044
VGIII x VGI						
11. B4495 (**Ba-VGI**) x B4499 (**Bαlpha-VGIII**)	55	19	7	0	32.10	0.039
12. B4495 (**Ba-VGI**) x B4544 (**Bαlpha- VGIII**)	9	38	26	12	87.67	0.039
13. B4545 (**Ba-VGI**) x B4544 (**Bαlpha-VGIII**)	4	52	7	14	93.65	0.040
14. B4545 (**Ba-VGI**) x JF101 (**Cαlpha-VGIII**)	83	4	0	0	4.59	0.041

*These crosses have only been analysed for one marker so a measure of recombinant progeny is impossible.

High mtDNA leakage was not due to self-fruiting

As a control to eliminate any strains with self-fruiting ability, the parental strains were all assessed for filamentation on V8pH5 and V8pH7 agar in vicinity of other mating partners and on plates alone. All strains that were used for mtDNA inheritance analyses did not exhibit any filamentation behaviour alone or in the vicinity of mating partners.

Although these controls were conducted, it is possible that some strains may have exhibited self-filamentation when mixed with a compatible strain instead of undergoing

fusion with that strain. Since there is such a high mtDNA leakage rate, which may be attributed to self-filamentation, in crosses involving strain B4544, the mating type of the progeny was determined using STE12αlpha primers and STE20a primers. The results of these analyses showed that there was a high proportion of *MATαlpha* progeny over *MATa* in the crosses analysed for this (crosses 7, 12 and 13; see Supplemental Tables 4G, 4L and 4M). This indicates that self-fruiting may have occurred.

To further test for self-fruiting, PCR fingerprinting with the highly discriminatory M13 primer was performed on progeny from crosses with high leakages, allowing for exclusion of those progeny that are a product of self fruiting. For the majority of the crosses, there were no progeny that exhibited an M13 fingerprinting pattern exactly the same as either of the parents as shown in the "*MATα* mtDNA & M13" column of Table 3. A few progeny did have M13 fingerprinting matching to the *MATαlpha* parent in crosses12 and 13, which also showed a larger amount of *MATalpha* progeny.

Comparison between C. neoformans and C. gattii

When comparing the results of mtDNA inheritance (Table 3) of *C. gattii* to those found in *C. neoformans* (Xu et al. 2000; Yan & Xu 2003) it is clear that there is much more leakage of *MATαlpha* mtDNA in crosses of *C. gattii* than was found in crosses of *C. neoformans*. Specifically, an average of 43.9% leakage was observed across all crosses between *C. gattii* strains, ranging from 0% to 93.65%, whereas Yan and Xu only found an average of 0.5% leakage across all crosses examined. The mtDNA inheritance seen in *C. gattii* is not only more than in *C. neoformans* but also highly variable. This difference in *C.*

gattii may be caused by a disruption of the mechanisms behind uniparental mitochondrial inheritance that are working in *C. neoformans*.

After fusion of two compatible mating types in *C. neoformans*, the hyphae that will form basidia and finally basidiospores only grow out of the *MATa* pole of the zygote, leaving all progeny with only *MATa* mitochondria. This is likely not the case in *C. gattii* since such a varied mtDNA inheritance pattern is seen. It is more likely that *C. gattii* undergoes more of a sample location dependant mode of mitochondrial inheritance, more like a mix of what is seen in other yeast and in filamentous fungi. In other basidiomycetous yeast, after fusion, the progeny bud from the zygote and have mitochondria from the parent that was closest to where they grew from, or both parents if they budded from the junction zone in the middle of the zygote. In filamentous fungi, the nuclei migrate from their resident homokaryon into the other homokaryon to create heterokaryon with mitochondria persisting only from the resident homokaryon. In *C. gattii* it is possible that after fusion, hyphae grow from anywhere around the zygote, therefore having mitochondria from either parent, or a mix of both. This suggests that mitochondrial inheritance in *C. gattii* is more similar to the other basidiomycetes related to it than it is to *C. neoformans*. This would create progeny with a mitochondrial inheritance similar to what we have observed, but does not explain why some crosses have nearly *MATa* dominant uniparental inheritance and others have nearly *MATalpha* dominant uniparental inheritance, instead of having a consistent pattern regardless of mating partners. The differences in the dominance, or non-dominance, of one mitotype could be attributed to differences in migration speed of the nuclei, similar to what is seen in some fungi with fruiting bodies. This variation in migration speed can be attributed to individual strain variations; with some pairs exhibiting

a mitochondrial inheritance pattern more like *C. neoformans*, with the *MATalpha* nucleus migrating towards the *MATa* nucleus quickly, and others exhibiting a pattern more like a sample location dependant mode, with either the *MATa* nucleus migrating quickly or with both nuclei moving at a similar speed and fusing in the middle, creating heteroplasmic or recombinant genomes.

Lack of heteroplasmy

One overall observation suggested no mtDNA heteroplasmy in any of the progeny in any of the crosses. This does not mean that heteroplasmy did not occur as there is presence of recombinant mtDNA in many progeny, which requires a state of heteroplasmy to occur at some point. Firstly, the threshold at which heteroplasmy could be detected may have been too low for some of the crosses due to low co-dominance of the primers, as indicated by the low detectable threshold ratios for some markers in some crosses in Supplemental Table 2. For some of the markers in some of the crosses, the presence of one *MAT* type's mtDNA could only be detected when present in equal levels as the other *MAT* type's mtDNA. This situation of equal amounts of different *MAT* type's mtDNA is very unlikely in the case of leakage if the initial mechanism at the time of fusion regulating mtDNA inheritance in *C. gattii* is the same as is known in *C. neoformans*. In this mechanism the hyphae from a sexual zygote are produced only from the *MATa* pole of the fusion product (McClelland *et al.* 2004). This barrier to biparental mtDNA inheritance would allow only a small amount of *MATalpha* mtDNA to be present in the mating products. This would cause heteroplasmy to be undetectable in markers that only amplify well in both *MAT* types' mtDNA when the mtDNA is present in equal or near equal quantities from both *MAT* types. Secondly, the methods used to isolate individuals (spore

germination, sub-culturing once then extraction of DNA) may have allowed for any heteroplasmy to be eliminated before genetic analysis had occurred. This suggests that even if there is control of mtDNA inheritance at the time of fusion and hyphal formation, there must also be a post fusion mechanism in place that mediates mtDNA inheritance in *C. gattii*.

Even though it is likely that mating in *C. gattii* starts out differently than in *C. neoformans*, due to presence of recombinant mtDNA types, it is clear that heteroplasmy occurs. Mitochondrial heteroplasmy must be eliminated at some point since it was not found in any of the progeny. The lack of co-dominance of primers in some markers for some crosses is not likely to account for a complete lack of heteroplasmy detection. The elimination of heteroplasmy could be explained by the presence of a post fusion mechanism to regulate mitochondrial inheritance. The post fusion mechanism in *C. neoformans* and *C. gattii* may be similar to that found in corn smut, *Ustilago maydis*. In *U. maydis*, protection of dominant m2 mitochondria by Lga2 prior to fusion is followed by degradation by Rga2 after fusion leading to the persistence of only dominant m2 mitochondria in the progeny of sexual crosses (Fedler *et al.* 2008). In *Cryptococcus neoformans* the *MAT* locus, specifically the master regulatory proteins Sxi1alpha and Sxi2a, regulate mitochondrial inheritance, such that when either or both genes are deleted, the normally dominant uniparental mitochondrial inheritance pattern is disrupted (Yan *et al.* 2004; Yan *et al.* 2007). Sxi1alpha and Sxi2a, in *Cryptococcus sp.*, may co-ordinately regulate products with similar functions as Lga2 and Rga2, in *U. maydis*, in order to regulate mitochondrial inheritance. If this post fusion mechanism is the same in *C. gattii* and *C. neoformans*, yet

the initial growth of hyphae from the zygote is different, the difference in mitochondrial inheritance between these species can be explained.

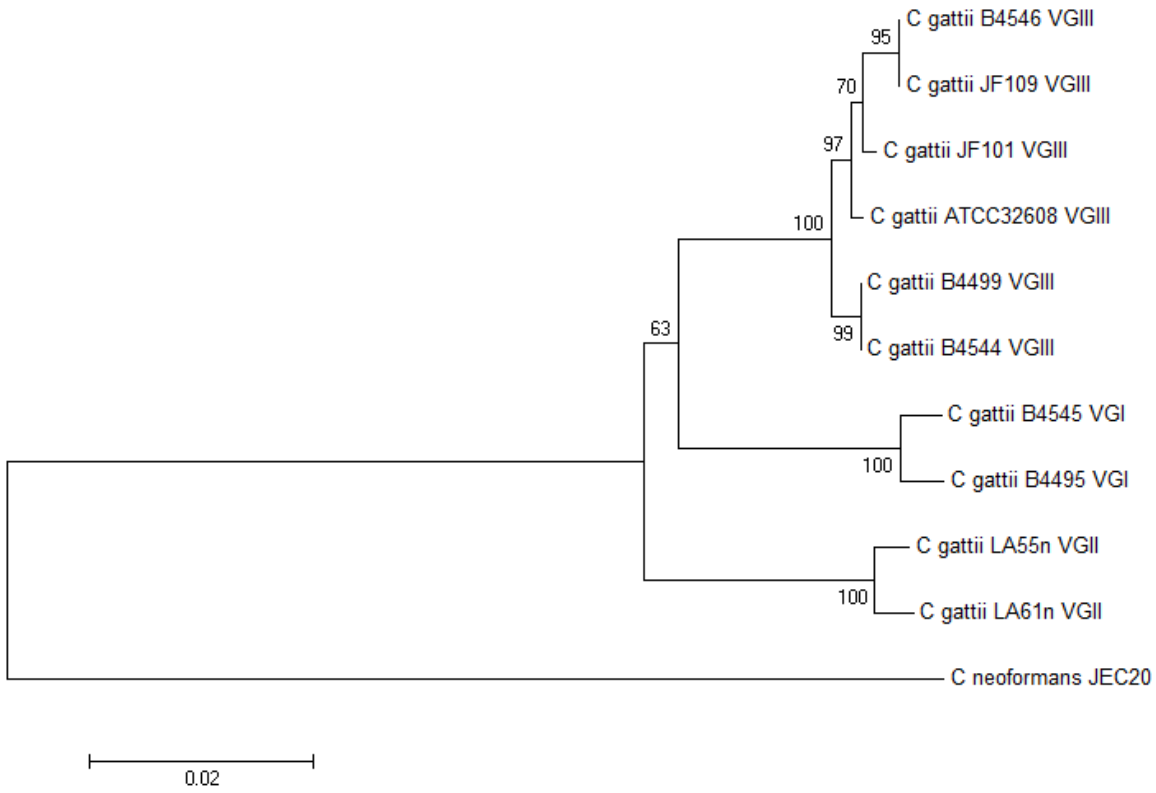

Figure 1. Evolutionary relationships of *C. gattii* strains used
The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the strains used. The percentage in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated. There were a total of 934 positions in the final dataset. Evolutionary analyses were conducted in MEGA5.

Sequencing and Tree Creation

After completing some sequencing and obtaining many sequences from GenBank, a Neighbor-Joining tree was created using MEGA5 (Figure 1). This tree shows expected results, with the strains from the same lineages grouping together, with the expected

relationships. Creating this tree was important to be sure that each strain was truly related to the others as was assumed initially. Obtaining these sequences also allowed for computation of the genetic distances between mating partners (Table 3). The genetic distances between mating partners was then used to analyze the relationship between genetic distance and the amount of mtDNA leakage observed for the cross. This comparison allows us to conclude whether the amount of distance between the parents contributes significantly to the amount of mtDNA leakage observed.

Interestingly, when looking at possible correlations between mtDNA leakage and genetic distance between the parents there seems to be a different pattern happening in intra-varietal crosses when compared to inter-varietal crosses. A Pearson's correlation of the mtDNA leakage and genetic distance between the parents in intra-varietal crosses (1-7) gives a value of -0.74922, which is significant with a p-value of 0.010. This indicates that within intra-varietal crosses, when genetic distance between the two mating partners increases, there is a decrease in mtDNA leakage. Although this goes against our hypothesis based mainly upon animals, it follows logically from the results of inter- and intra-lineage crosses of *C. neoformans.* Across all inter-lineage crosses, there was never any leakage of *MATalpha* mtDNA detected (Xu *et al.* 2000), however the only cross that exhibited *MATalpha* mtDNA leakage was one between isogenic strains, clearly of the same lineage (Yan & Xu 2003). Pearson's correlation tests of the mtDNA leakage and genetic distance between the parents for the inter-varietal crosses (8-14) and of all crosses have no significant results. This is very fascinating to see a significant correlation in intra-varietal crosses, but no correlations in the inter-varietal crosses or overall. One would expect that the same pattern would hold true throughout all types of crosses within a species. This

simply shows that factors other than genetic distance between mating partners control mtDNA inheritance in *C. gattii.*

Overall, the mitochondrial inheritance seen in *C. gattii* is extremely varied, in contrast to the *MATa* dominated uniparental mitochondrial inheritance seen in *C. neoformans.* This diverse inheritance pattern is largely independent of the genetic distance between the two parents, and is not affected by the *crg1* mutation. This different mitochondrial inheritance pattern could be explained by an initial difference between *C. gattii* and *C. neoformans* in the way that fusion and subsequent growth of hyphae occurs.

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APPENDIX

Supplemental Table 1 Mating attempts
This table shows all crosses attempted. A + indicates that there were hyphae produced after one week of incubation (either macroscopically or as seen under the microscope). Where --- is indicated, mating was not successful, even after letting the mating spots incubate for up for 45 days.

MATa Strain			MATalpha Strain		Successful on V8pH5	Successful on V8pH7
VGIIIxVGIII						
B4546	C-VGIII	X	B4499	B-VGIII	+	+
B4546	C-VGIII	X	B4544	B-VGIII	+	+
B4546	C-VGIII	X	JF101	C-VGIII	+	+
ATCC32608	C-VGIII	X	B4499	B-VGIII	+	---
ATCC32608	C-VGIII	X	B4544	B-VGIII	+	+
ATCC32608	C-VGIII	X	JF101	C-VGIII	+	+
JF109	C-VGIII	X	B4499	B-VGIII	+	+
JF109	C-VGIII	X	B4544	B-VGIII	+	+
JF109	C-VGIII	X	JF101	C-VGIII	+	---
VGIIIxVGII						
LA55n	B-VGII	X	JF101	C-VGIII	+	+
LA55n	B-VGII	X	B4544	B-VGIII	+	---
LA55n	B-VGII	X	B4499	B-VGIII	---	---
JF109	C-VGIII	X	LA61n	B-VGII	+	---
JF109	C-VGIII	X	R265	B-VGII	---	---
B4546	C-VGIII	X	LA61n	B-VGII	---	---
ATCC32608	C-VGIII	X	LA61n	B-VGII	---	---
VGIIIxVGI						
B4495	B-VGI	X	B4499	B-VGIII	+	---
B4495	B-VGI	X	JF101	C-VGIII	+	+
B4495	B-VGI	X	B4544	B-VGIII	+	+
B4545	B-VGI	X	B4544	B-VGIII	+	+
B4545	B-VGI	X	B4499	B-VGIII	---	---
B4545	B-VGI	X	JF101	C-VGIII	+	---
B4546	C-VGIII	X	B4492	B-VGI	---	---
ATCC32608	C-VGIII	X	B4492	B-VGI	---	---
JF109	C-VGIII	X	B4492	B-VGI	---	---
VGIIxVGI						
LA55n	B-VGII	X	B4492	B-VGI	---	---
B4495	B-VGI	X	LA61n	B-VGII	---	---
B4545	B-VGI	X	LA61n	B-VGII	---	---

Supplemental Table 1 Continued

MATa Strain			MATalpha Strain		Successful on V8pH5	Successful on V8pH7
VGIIxVGII						
LA55n	B-VGII	X	LA61n	B-VGII	---	---
VGIxVGI						
B4495	B-VGI	X	B4492	B-VGI	---	---
B4545	B-VGI	X	B4492	B-VGI	---	---

Supplemental Table 2 Mitochondrial genotyping restriction enzymes used and fragment properties
Here the enzymes chosen for each cross are shown along with the approximate fragment lengths when applied to the mitochondrial PCR products. Also shown is the threshold ratio at which each mitochondrial type is detectable when present in excess of the other mitochondrial type. This allows for commentary on the detection, or lack thereof, of heteroplasmic mitochondrial DNA in the progeny population.

Cross	ND4						ND2					
	Fragment Lengths*				Detectable Threshold		Fragment Lengths*				Detectable Threshold	
	Enzyme	*MATa* mtDNA	*MATα* mtDNA	Hetero-plasmic	*MATa: MATα*	*MATα: MATa*	Enzyme	*MATa* mtDNA	*MATα* mtDNA	Hetero-plasmic	*MATa: MATα*	*MATα: MATa*
VGIII x VGIII												
1. B4546 (**Ca-VGIII**) x B4499 (**Balpha-VGIII**)	*SacI*	650bp	150bp, 500bp	150bp. 500bp, 650bp	10:1	100:1	N/A	---	---	---	---	---
2. JF109 (**Ca-VGIII**) x B4499 (**Balpha-VGI**)	*SacI*	650bp	150bp, 500bp	150bp, 500bp, 650bp	1000:1	1000:1	*TaqaI*	1150bp	500bp, 650bp	500bp, 650bp, 1150bp	1000:1	1000:1
3. B4546 (**Ca-VGIII**) x B4544 (**Balpha-VGIII**)	*SacI*	650bp	150bp, 500bp	150bp, 500bp, 650bp	10:1	100:1	*TaqaI*	1150bp	500bp, 650bp	500bp, 650bp, 1150bp	100:1	10:1
4. JF109 (**Ca-VGIII**) x B4544 (**Balpha-VGIII**)	*SacI*	650bp	150bp, 500bp	150bp, 500bp, 650bp	1:1	1000:1	*TaqaI*	1150bp	500bp, 650bp	500bp, 650bp, 1150bp	1:1	1000:1
5. ATCC32608 (**Ca-VGI**) x B4499 (**Balpha-VGIII**)	*SacI*	650bp	150bp, 500bp	150bp, 500bp, 650bp	10:1	100:1	*TaqaI*	1150bp	500bp, 650bp	500bp, 650bp, 1150bp	100:1	10:1

Supplemental Table 2 Continued

Cross	ND4						ND2					
	Fragment Lengths*				Detectable Threshold		Fragment Lengths*				Detectable Threshold	
	Enzyme	*MATa* mtDNA	*MATα* mtDNA	Hetero-plasmic	*MATa: MATα*	*MATα: MATa*	Enzyme	*MATa* mtDNA	*MATα* mtDNA	Hetero-plasmic	*MATa: MATα*	*MATα: MATa*
VGIII X VGIII												
6. B4546 (**Ca-VGIII**) x JF101 (**Calpha**)	*N/A*	---	---	---	---	---	*TaqaI*	1150bp	500bp, 650bp	500bp, 650bp, 1150bp	1000:1	1000:1
7. ATCC32608 (**Ca-VGI**) x B4544 (**Balpha-VGIII**)	*SacI*	650bp	150bp, 500bp	150bp, 500bp, 650bp	10:1	100:1	*TaqaI*	1150bp	500bp, 650bp	500bp, 650bp, 1150bp	100:1	100:1
VGII x VGIII												
8. LA55n (**Ba-VGII**) x B4544 (**Balpha-VGIII**)	*SacI*	650bp	325bp	325bp, 650bp	10:1	1000:1	*PvuII*	500bp, 650bp	250bp, 280bp, 310bp	250bp, 280bp, 310bp. 500bp, 650bp	1:1	10:1
9. JF109 (**Ca-VGIII**) x LA61n (**Balpha-VGII**)	*ScaI*	650bp	150bp, 650bp	150bp, 500bp, 650bp	1000:1	1000:1	*TaqaI*	1150bp	50bp, 500bp, 600bp	50bp, 500bp, 600bp, 1150bp	1000:1	1000:1
10. LA55n (**Ba-VGII**) x JF101 (**Calpha-VGIII**)	*ScaI*	650bp	150bp, 650bp	150bp, 500bp, 650bp	100:1	1000:1	*PvuII*	500bp, 650bp	300bp, 550bp	300bp, 500bp, 550bp, 650bp	10:1	10:1

Supplemental Table 2 Continued

Cross	ND4						ND2					
	Fragment Lengths*				Detectable Threshold		Fragment Lengths*				Detectable Threshold	
	Enzyme	*MATa* mtDNA	*MATα* mtDNA	Hetero-plasmic	*MATa: MATα*	*MATα: MATa*	Enzyme	*MATa* mtDNA	*MATα* mtDNA	Hetero-plasmic	*MATa: MATα*	*MATα: MATa*
VGIII x VGI												
11. B4495 (**Ba-VGI**) x B4499 (**Balpha-VGIII**)	*AluI*	250bp, 400bp	50bp, 150bp, 450bp	50bp, 150bp, 250bp, 400bp, 450bp	1000:1	1000:1	*PvuII*	500bp, 650bp	100bp, 500bp, 550bp	100bp, 500bp, 550bp, 650bp	1:1	100:1
12. B4495 (**Ba-VGI**) x B4544 (**Balpha- VGIII**)	*ScaI*	150bp, 500bp	650bp	150bp, 500bp, 650bp	1000:1	10:1	*PvuII* & *HindII*	200bp, 300bp, 650bp	575bp	200bp, 300bp, 575bp, 650bp	10:1	1000:1
13. B4545 (**Ba-VGI**) x B4544 (**Balpha-VGIII**)	*ScaI*	150bp, 500bp	650bp	150bp, 500bp, 650bp	1000:1	100:1	*PvuII*	500bp, 650bp	100bp, 500bp, 550bp	100bp, 500bp, 550bp, 650bp	1:1	100:1
14. B4545 (**Ba-VGI**) x JF101 (**Calpha-VGIII**)	*ND5*	750bp	1250bp	750bp, 1250bp	---	---	*PvuII*	500bp, 650bp	300bp, 550bp	300bp, 500bp, 550bp, 650bp	10:1	10:1

*fragment lengths are approximated from agarose gel electrophoresis results in 1.5% agarose run at 100V for 45min in 1x Tris-Acetic Acid-EDTA buffer.

Supplemental Table 3 Sequences used for constructing phylogenetic tree
Those sequences indicated with "this work" were generated using the protocols outlined. All accession numbers for sequences used are indicated here. --- indicates that there was no sequence available on GenBank.

Strain	CAP1	PLB1	LAC1	GPD1	TEF1
C. neoformans JEC20	AF542530	EU408650	EF211637	---	EF211752
C. gattii B4544	this work	DQ096348	DQ096399	DQ096379	DQ096358
C. gattii B4545	---	DQ096353	DQ096404	DQ096396	DQ096373
C. gattii B4546	this work	AY327616	DQ096405	DQ096383	---
C. gattii B4495	---	EU408660	EF211658	---	EF211773
C. gattii B4499	this work	DQ096348	DQ096399	DQ096379	DQ096358
C. gattii LA55n	AY971994	HM990885	AY973087	HM990744	HM990932
C. gattii LA61n	this work	HM990887	AY973089	HM990746	HM990934
C. gattii ATCC32608	this work	FJ705950	DQ096406	DQ096388	DQ096360
C. gattii JF101	this work	AY327615˜	FJ706051˜	DQ096378˜	---
C. gattii JF109	this work	AY327616*	DQ096405*	DQ096383*	---

*Sequence used from B4546 since JF109 is derived from B4546
˜Sequence used from NIH312 since JF101 is derived from NIH312

Supplemental Table 4A Mitochondrial inheritance and M13 fingerprinting in progeny of cross 1. B4546 x B4499

Progeny #	ND2 Digestion	ND4 digestion with *Sac I*	mtDNA origin [a]	M13 fingerprinting [b]
B4546	**N/A**	**650bp (1)**		**a**
B4499	**N/A**	**150, 500bp (2)**		**alpha**
B4499/B4546	**N/A**	**150, 500, 650bp (3)**		**novel/missing**
1	N/A	1	a	missing
2	N/A	1	a	missing
3	N/A	1	a	missing
4	N/A	3	alpha	novel
5	N/A	1	a	novel
6	N/A	2	alpha	novel
7	N/A	1	a	novel
8	N/A	3	alpha	missing
9	N/A	1	a	novel
10	N/A	1	a	novel
11	N/A	1	a	missing
12	N/A	1	a	missing
13	N/A	1	a	missing
14	N/A	1	a	missing
15	N/A	1	a	novel
16	N/A	1	a	missing
17	N/A	1	a	missing
18	N/A	1	a	novel
19	N/A	1	a	novel
20	N/A	1	a	novel
22	N/A	1	a	missing
23	N/A	1	a	novel
24	N/A	1	a	novel
25	N/A	1	a	missing
26	N/A	1	a	missing
27	N/A	1	a	missing
29	N/A	1	a	novel
30	N/A	1	a	missing
31	N/A	1	a	missing
32	N/A	1	a	novel
33	N/A	1	a	missing
34	N/A	1	a	missing
35	N/A	1	a	novel
36	N/A	1	a	novel
37	N/A	1	a	novel
38	N/A	1	a	novel
39	N/A	1	a	novel
40	N/A	1	a	novel
41	N/A	1	a	missing
42	N/A	1	a	novel

Supplemental Table 4A Continued

Progeny #	ND2 Digestion	ND4 digestion with *Sac I*	mtDNA origin [a]	M13 fingerprinting [b]
43	N/A	1	a	novel
44	N/A	1	a	novel
45	N/A	1	a	novel
46	N/A	1	a	missing
47	N/A	1	a	novel
48	N/A	1	a	missing
49	N/A	2	alpha	missing
50	N/A	1	a	missing
51	N/A	1	a	novel
52	N/A	1	a	missing
53	N/A	1	a	novel
54	N/A	2	alpha	missing
55	N/A	1	a	novel
56	N/A	1	a	novel
57	N/A	1	a	novel
58	N/A	1	a	novel
59	N/A	1	a	missing
60	N/A	1	a	novel
61	N/A	1	a	missing
62	N/A	1	a	missing
63	N/A	1	a	missing
64	N/A	1	a	missing
65	N/A	1	a	novel
66	N/A	1	a	missing
67	N/A	2	alpha	missing
68	N/A	1	a	missing
69	N/A	1	a	missing
70	N/A	1	a	missing
71	N/A	1	a	novel
72	N/A	1	a	missing
73	N/A	1	a	missing
74	N/A	1	a	missing
75	N/A	1	a	missing
76	N/A	1	a	novel
78	N/A	1	a	novel
79	N/A	1	a	missing
80	N/A	1	a	missing
82	N/A	1	a	missing
83	N/A	1	a	missing
85	N/A	1	a	novel
86	N/A	1	a	novel
87	N/A	1	a	novel
88	N/A	3	alpha	novel
89	N/A	1	a	missing

Supplemental Table 4A Continued

Progeny #	ND2 Digestion	ND4 digestion with *Sac I*	mtDNA origin [a]	M13 Fingerprinting [b]
90	N/A	1	a	novel
91	N/A	1	a	missing
92	N/A	1	a	novel
93	N/A	1	a	novel
94	N/A	1	a	missing
95	N/A	2	a	missing
96	N/A	1	a	missing

[a] "a" indicates that the genotyping for ND2 and ND4 had the same pattern as seen in the *MATa* parent while "alpha" indicates the genotyping pattern for ND2 and ND4 is the same as seen in the *MATalpha* parent. "rec" is indicated for any strains that showed ND2 pattern for one parent and ND4 pattern for the other parent.
[b] "a" indicates that the M13 fingerprinting pattern exactly matches that of the *MATa* parent while "alpha" indicates that the fingerprinting pattern exactly matches that of the *MATalpha* parent. "novel" is indicated for strains that have bands that are not present in the parent that the strain has as a mitochondrial donor while "missing" is indicated for those strains that are missing bands shown in its mitochondrial donor parent's M13 fingerprinting pattern.

Supplemental Table 4B Mitochondrial inheritance and M13 fingerprinting of progeny from cross 2. JF109 x B4499

Progeny #	ND2 digestion with *Taqa I*	ND4 digestion with *Sac I*	mtDNA origin[a]	M13 Fingerprinting[b]
B4499 (MATalpha)	**500, 650bp (1)**	**150, 500bp (1)**		**alpha**
JF109 (MATa)	**1150bp (2)**	**650bp (2)**		**a**
B4499/JF109	**500, 650, 1150bp (3)**	**150, 500, 650bp (3)**		**novel/missing**
1	2	2	a	novel
2	2	2	a	novel
3	2	2	a	missing
5	2	2	a	novel
6	2	2	a	novel
7	2	2	a	novel
8	2	2	a	missing
9	2	2	a	novel
10	2	2	a	novel
11	2	2	a	novel
12	2	2	a	novel
13	2	2	a	missing
14	2	2	a	missing
15	2	2	a	missing
16	2	2	a	missing
17	1	1	alpha	missing
18	1	1	alpha	missing
19	1	1	alpha	novel
20	1	1	alpha	novel
21	1	1	alpha	novel
22	1	1	alpha	novel
23	1	1	alpha	missing
24	1	1	alpha	missing
25	1	1	alpha	missing
26	1	1	alpha	missing
27	1	1	alpha	missing
28	1	1	alpha	missing
29	1	1	alpha	novel
30	1	1	alpha	novel
31	1	1	alpha	novel
32	2	2	a	missing
33	2	2	a	novel
34	2	2	a	missing
35	2	2	a	novel
36	2	2	a	missing
37	2	2	a	missing
38	2	2	a	missing
39	2	2	a	novel
40	2	2	a	novel
41	2	2	a	novel

Supplemental Table 4B Continued

Progeny #	ND2 digestion with *Taqa I*	ND4 digestion with *Sac I*	mtDNA origin[a]	M13 fingerprinting[b]
42	2	2	a	missing
43	2	2	a	missing
46	2	2	a	novel
47	2	2	a	missing
48	2	2	a	novel
49	2	2	a	novel
50	2	2	a	novel
51	2	2	a	missing
52	2	2	a	missing
53	2	2	a	missing
54	2	2	a	novel
55	2	2	a	novel
56	2	2	a	novel
57	2	2	a	novel
58	2	2	a	missing
59	2	2	a	novel
60	2	2	a	missing
61	2	1	rec	alpha
62	1	2	rec	missing
63	2	2	a	missing
65	2	1	rec	novel
66	1	1	alpha	missing
67	1	1	alpha	missing
68	1	1	alpha	novel
69	1	1	alpha	novel
70	1	1	alpha	novel
71	1	1	alpha	missing
72	1	1	alpha	novel
73	1	1	alpha	novel
74	1	1	alpha	missing
75	1	1	alpha	novel
76	1	1	alpha	missing
77	1	1	alpha	novel
78	1	1	alpha	missing
79	1	1	alpha	missing
80	1	1	alpha	missing
81	1	1	alpha	novel
82	1	1	alpha	novel
83	1	1	alpha	missing
84	1	1	alpha	novel
85	2	2	a	novel
86	2	2	a	novel
87	2	2	a	missing
88	2	2	a	novel

Supplemental Table 4B Continued

Progeny #	ND2 digestion with *Taqa I*	ND4 digestion with *Sac I*	mtDNA origin[a]	M13 fingerprinting[b]
89	2	2	a	missing
91	2	2	a	novel
92	2	2	a	novel

[a, b] same notes as in Supplemental Table 4A.

Supplemental Table 4C Mitochondrial inheritance and M13 fingerprinting of progeny from cross 3. B4546 x B4544

Progeny #	ND2 Digestion with *Taqa I*	ND4 Digestion with *Sac I*	mtDNA origin[a]	M13 fingerprinting[b]
B4546	**1150bp (1)**	**650bp (1)**		**a**
B4544	**500, 650bp (2)**	**150, 500bp (2)**		**alpha**
B4544/B4546	**500, 650, 1150bp (3)**	**150, 500, 650bp (3)**		**novel/missing**
2	1	1	a	missing
5	1	1	a	novel
6	1	1	a	novel
7	1	1	a	novel
8	1	1	a	novel
10	1	1	a	novel
12	1	1	a	missing
13	1	1	a	novel
14	1	1	a	missing
15	1	1	a	missing
16	1	1	a	novel
17	1	1	a	missing
18	1	1	a	missing
19	1	1	a	novel
20	1	1	a	missing
22	1	1	a	missing
23	1	1	a	missing
24	1	3	rec	novel
25	2	1	rec	missing
26	2	2	alpha	novel
27	1	2	rec	novel
28	1	1	a	novel
29	2	1	rec	novel
30	1	2	rec	novel
31	2	1	rec	novel
32	1	2	rec	novel
34	1	1	a	missing
35	1	1	a	missing
36	1	1	a	missing
37	1	1	a	missing
38	1	1	a	missing
39	1	1	a	missing
40	2	1	rec	novel
41	1	2	rec	novel
42	2	1	rec	missing
43	1	2	rec	novel
45	1	1	a	missing
46	1	1	a	missing
48	1	1	a	novel
50	1	1	a	novel

Supplemental Table 4C Continued

Progeny #	ND2 Digestion with *Taqa I*	ND4 Digestion with *Sac I*	mtDNA origin[a]	M13 fingerprinting[b]
51	1	1	a	missing
52	1	1	a	novel
54	1	1	a	missing
55	1	1	a	novel
56	1	1	a	novel
64	1	1	a	missing
65	1	1	a	missing
66	1	1	a	missing
67	1	1	a	missing
68	1	1	a	novel
69	1	1	a	novel
70	1	1	a	missing
71	1	1	a	missing
75	1	1	a	missing
76	1	1	a	missing
77	1	1	a	novel
78	1	1	a	missing
79	1	1	a	missing
82	1	1	a	missing
85	1	1	a	missing
87	2	1	rec	novel
89	1	2	rec	novel
91	1	1	a	missing
93	1	1	a	missing
94	1	1	a	missing
96	1	1	a	novel
98	1	1	a	missing
100	1	1	a	missing
101	1	1	a	missing
103	1	1	a	missing
104	1	1	a	novel
106	1	1	a	novel
107	1	1	a	missing
108	1	1	a	missing
111	1	1	a	novel
112	1	1	a	missing
114	1	1	a	missing
115	1	1	a	missing
117	1	1	a	novel
118	1	1	a	missing

[a, b] same notes as in Supplemental Table 4A.

Supplemental Table 4D Mitochondrial inheritance and M13 fingerprinting of progeny from cross 4. JF109 x B4544

Progeny #	ND2 digestion with *Taqa I*	ND4 digestion with *Sac I*	mtDNA origin[a]	M13 fingerprinting[b]
B4544	**500bp, 650bp (1)**	**500, 150bp (1)**		**alpha**
JF109	**1150bp (2)**	**650bp (2)**		**a**
B4544/JF109	**500, 650, 1150 (3)**	**150, 500, 650 (3)**		**novel/missing**
1	2	2	a	missing
2	2	2	a	missing
3	2	2	a	missing
4	2	2	a	novel
5	2	2	a	novel
6	2	2	a	novel
7	2	2	a	missing
8	2	2	a	novel
9	2	2	a	alpha
10	2	2	a	novel
11	2	2	a	missing
12	2	2	a	missing
13	2	2	a	novel
14	2	2	a	novel
15	2	2	a	novel
16	2	2	a	missing
17	2	2	a	novel
18	2	2	a	novel
19	2	2	a	novel
20	2	2	a	missing
21	2	2	a	alpha
22	2	2	a	novel
24	2	2	a	missing
25	2	2	a	missing
26	2	2	a	novel
27	2	2	a	novel
28	2	2	a	novel
29	2	2	a	novel
30	2	2	a	missing
31	2	2	a	novel
32	2	2	a	novel
33	2	1	rec	missing
34	2	2	a	novel
35	2	2	a	missing
36	1	1	alpha	novel
37	2	2	a	novel
38	2	2	a	novel
39	2	2	a	novel
40	2	2	a	missing
41	2	2	a	novel

Supplemental Table 4D Continued

Progeny #	ND2 digestion with *Taqa I*	ND4 digestion with *Sac I*	mtDNA origin[a]	M13 fingerprinting[b]
42	2	2	a	novel
43	2	2	a	novel
44	2	2	a	novel
45	2	2	a	novel
46	2	2	a	novel
47	2	2	a	missing
48	2	2	a	missing
49	2	2	a	novel
50	2	2	a	missing
51	2	2	a	novel
52	2	2	a	novel
53	2	2	a	missing
54	2	2	a	missing
55	2	2	a	novel
56	2	2	a	alpha
57	2	2	a	novel
58	2	2	a	novel
59	2	2	a	novel
60	2	2	a	novel
61	2	2	a	missing
62	2	2	a	missing
63	2	2	a	novel
64	2	2	a	novel
66	2	2	a	missing
67	2	2	a	missing
68	2	2	a	novel
69	2	2	a	novel
70	2	2	a	novel
71	2	2	a	missing
72	2	2	a	novel
73	1	1	alpha	novel
74	2	2	a	novel
75	2	2	a	novel
76	2	2	a	novel
77	2	2	a	missing
78	2	2	a	missing
80	2	2	a	missing
81	2	2	a	novel
82	2	2	a	novel
83	2	2	a	missing
84	2	2	a	novel
85	2	2	a	novel
86	2	2	a	novel
87	2	2	a	novel

Supplemental Table 4D Continued

Progeny #	ND2 digestion with *Taqa I*	ND4 digestion with *Sac I*	mtDNA origin[a]	M13 fingerprinting[b]
89	2	2	a	missing
90	2	2	a	novel
91	2	2	a	novel
92	2	2	a	missing

[a],[b] same notes as in Supplemental Table 4A.

Supplemental Table 4E. Mitochondrial inheritance and M13 fingerprinting of progeny from cross 5. ATCC32608 x B4499

Progeny #	ND2 digestion with *Taqa I*	ND4 digestion with *Sac I*	mtDNA origin[a]	M13 fingerprinting[b]
B4499	**500, 650bp (1)**	**150, 500bp (1)**		**alpha**
ATCC32608	**1150bp (2)**	**650bp (2)**		**a**
B4499/ATCC32608	**500, 650, 1150bp (3)**	**150, 500, 650bp (3)**		**missing/novel**
2	2	2	alpha	novel
3	1	1	a	missing
4	1	1	a	missing
5	2	2	alpha	novel
6	2	2	alpha	novel
7	2	2	alpha	novel
8	1	1	a	novel
9	1	1	a	missing
10	2	2	alpha	novel
11	2	2	alpha	novel
12	2	2	alpha	novel
13	2	2	alpha	novel
14	2	2	alpha	novel
15	2	2	alpha	novel
16	2	2	alpha	novel
17	2	2	alpha	novel
18	2	2	alpha	novel
19	2	2	alpha	novel
20	2	2	alpha	novel
21	1	1	a	novel
22	2	2	alpha	novel
23	2	2	alpha	novel
24	2	2	alpha	novel
25	1	1	a	missing
27	2	2	alpha	novel
28	2	2	alpha	novel
29	1	1	a	missing
30	2	2	alpha	novel
31	2	2	alpha	novel
32	2	2	alpha	novel
33	2	2	alpha	novel
34	1	1	a	missing
35	2	2	alpha	novel
36	1	1	a	missing
37	2	2	alpha	novel
38	2	2	alpha	novel
39	2	2	alpha	novel
40	2	2	alpha	novel
41	2	2	alpha	novel
42	2	2	alpha	novel

Supplemental Table 4E Continued

Progeny #	ND2 digestion with *Taqa I*	ND4 digestion with *Sac I*	mtDNA origin[a]	M13 fingerprinting[b]
43	2	2	alpha	novel
44	2	2	alpha	novel
45	2	2	alpha	novel
46	2	2	alpha	novel
47	2	2	alpha	novel
57	2	2	alpha	novel
58	2	2	alpha	novel
59	1	1	a	novel
61	2	2	alpha	novel
62	1	2	rec	novel
63	1	1	a	missing
64	1	1	a	missing
66	1	1	a	missing
67	1	1	a	missing
68	2	2	alpha	novel
69	1	1	a	missing
70	2	2	alpha	novel
71	2	2	alpha	novel
72	2	2	alpha	novel
74	2	2	alpha	novel
77	2	2	alpha	novel
78	2	2	alpha	novel
79	1	1	a	missing
80	2	2	alpha	novel
83	1	1	a	missing
89	2	2	alpha	novel
90	2	2	alpha	novel
91	2	2	alpha	novel
92	2	2	alpha	novel

[a, b] same notes as in Supplemental Table 4A.

Supplemental Table 4F Mitochondrial inheritance and M13 fingerprinting of progeny from cross 6. B4546 x JF109

Progeny #	ND2 digestion with *Taqa I*	ND4 digestion	mtDNA origin[a]	M13 fingerprinting[b]
JF101	**500bp, 700bp (1)**	**N/A**		**alpha**
B4546	**1200bp (2)**	**N/A**		**a**
JF101/B4546	**500, 700, 1200 (3)**	**N/A**		**missing/novel**
1	2	**N/A**	a	missing
2	2	**N/A**	a	novel
3	2	**N/A**	a	missing
4	2	**N/A**	a	novel
5	1	**N/A**	alpha	novel
6	1	**N/A**	alpha	novel
7	1	**N/A**	alpha	novel
8	2	**N/A**	a	novel
9	1	**N/A**	alpha	novel
10	2	**N/A**	a	novel
11	2	**N/A**	a	missing
12	2	**N/A**	a	novel
13	1	**N/A**	alpha	novel
14	1	**N/A**	alpha	novel
15	1	**N/A**	alpha	missing
16	1	**N/A**	alpha	novel
17	2	**N/A**	a	novel
18	2	**N/A**	a	novel
19	2	**N/A**	a	novel
20	1	**N/A**	alpha	novel
21	2	**N/A**	a	novel
22	2	**N/A**	a	novel
23	2	**N/A**	a	novel
24	2	**N/A**	a	missing
25	2	**N/A**	a	novel
26	2	**N/A**	a	missing
27	2	**N/A**	a	novel
28	1	**N/A**	alpha	novel
29	2	**N/A**	a	novel
30	2	**N/A**	a	novel
31	1	**N/A**	alpha	novel
32	2	**N/A**	a	novel
33	2	**N/A**	a	novel
34	2	**N/A**	a	missing
35	1	**N/A**	alpha	missing
36	2	**N/A**	a	novel
37	2	**N/A**	a	novel
38	2	**N/A**	a	novel
39	2	**N/A**	a	missing
40	2	**N/A**	a	novel

Supplemental Table 4F Continued

Progeny #	ND2 digestion with *Taqa I*	ND4 digestion	mtDNA origin[a]	M13 fingerprinting[b]
41	1	**N/A**	alpha	novel
42	2	**N/A**	a	novel
43	2	**N/A**	a	novel
45	2	**N/A**	a	novel
46	2	**N/A**	a	novel
47	2	**N/A**	a	missing
48	1	**N/A**	alpha	novel
49	2	**N/A**	a	novel
50	2	**N/A**	a	novel
51	2	**N/A**	a	novel
52	2	**N/A**	a	missing
53	2	**N/A**	a	novel
54	2	**N/A**	a	novel
55	1	**N/A**	alpha	novel
56	1	**N/A**	alpha	novel
57	2	**N/A**	a	missing
58	2	**N/A**	a	novel
59	1	**N/A**	alpha	novel
60	1	**N/A**	alpha	novel
61	1	**N/A**	alpha	novel
62	1	**N/A**	alpha	novel
63	1	**N/A**	alpha	missing
64	1	**N/A**	alpha	missing
65	1	**N/A**	alpha	novel
66	2	**N/A**	a	novel
67	2	**N/A**	a	novel
68	1	**N/A**	alpha	novel
69	1	**N/A**	alpha	novel
70	2	**N/A**	a	novel
71	2	**N/A**	a	novel
72	1	**N/A**	alpha	novel
73	2	**N/A**	a	novel
74	1	**N/A**	alpha	novel
76	1	**N/A**	alpha	novel
77	1	**N/A**	alpha	novel
78	2	**N/A**	a	novel
79	2	**N/A**	a	novel
80	2	**N/A**	a	missing
81	1	**N/A**	alpha	novel
82	1	**N/A**	alpha	missing
83	2	**N/A**	a	missing
84	2	**N/A**	a	novel
85	2	**N/A**	a	novel
86	1	**N/A**	alpha	novel

Supplemental Table 4F Continued

Progeny #	ND2 digestion with *Taqa I*	ND4 digestion	mtDNA origin[a]	M13 fingerprinting[b]
87	1	**N/A**	alpha	novel
88	1	**N/A**	alpha	missing
89	1	**N/A**	alpha	novel
90	2	**N/A**	a	novel
91	1	**N/A**	alpha	novel

[a] [b] same notes as in Supplemental Table 4A.

Supplemental Table 4G Mitochondrial inheritance and M13 fingerprinting of progeny from cross 7. ATCC32608 x B4544

Progeny #	ND2 digestion with *Taqa I*	ND4 digestion with *Sac I*	mtDNA origin[a]	STE20a	STE12alpha	MAT locus[c]	M13 fingerprinting[b]
B4544	**500bp, 650bp (1)**	**150, 500bp (1)**		**absent (1)**	**present (1)**	**alpha**	**Alpha**
ATCC32608	**1150bp (2)**	**650bp (2)**		**Present (2)**	**absent (2)**	**a**	**A**
B4544/ATCC32608	**500, 650, 1150bp (3)**	**150, 500, 650bp (3)**		**Present (2)**	**present (2)**	**hetero**	**novel/missing**
Isolate # 1	1	1	alpha	1	1	alpha	Novel
3	1	1	alpha	1	1	alpha	Novel
4	1	1	alpha	1	1	alpha	Missing
5	1	1	alpha	1	1	alpha	Missing
6	1	1	alpha	2	2	a	Novel
7	2	2	a	2	2	a	Missing
8	1	1	alpha	1	1	alpha	Missing
9	1	1	alpha	1	1	alpha	Novel
10	1	1	alpha	1	1	alpha	Alpha
11	1	1	alpha	1	1	alpha	Alpha
12	1	1	alpha	1	1	alpha	Alpha
13	1	1	alpha	1	1	alpha	Alpha
14	1	1	alpha	1	1	alpha	Alpha
16	1	1	alpha	1	1	alpha	Alpha
17	1	1	alpha	1	1	alpha	Alpha
18	1	1	alpha	1	1	alpha	Novel
20	1	1	alpha	2	1	hetero	Missing
21	2	2	a	1	2	?	Missing
23	1	1	alpha	1	1	alpha	Alpha
24	1	1	alpha	1	1	alpha	Missing
25	2	2	a	1	1	alpha	Missing
26	1	1	alpha	2	1	hetero	Novel
27	1	1	alpha	1	1	alpha	Missing
28	1	1	alpha	1	1	alpha	Alpha
29	1	1	alpha	1	1	alpha	Novel
30	1	1	alpha	1	1	alpha	Novel
31	1	1	alpha	1	1	alpha	Missing
32	2	2	a	1	2	?	Missing
33	1	1	alpha	2	1	hetero	Missing
35	1	1	alpha	1	1	alpha	Alpha
37	1	1	alpha	1	1	alpha	Alpha
38	1	1	alpha	1	1	alpha	Missing
39	2	2	a	1	1	alpha	Missing
40	1	1	alpha	2	1	hetero	Missing
41	1	1	alpha	1	1	alpha	Missing
42	1	1	alpha	1	1	alpha	Novel
43	1	1	alpha	1	1	alpha	Alpha
44	1	1	alpha	1	1	alpha	Missing
45	1	1	alpha	1	1	alpha	Missing
46	1	1	alpha	1	1	alpha	Alpha

Supplemental Table 4G Continued

Progeny #	ND2 digestion with *Taqa I*	ND4 digestion with *Sac I*	mtDNA origin[a]	STE20a	STE12alpha	MAT locus[c]	M13 fingerprinting[b]
47	1	1	alpha	1	1	alpha	Alpha
49	1	1	alpha	1	1	alpha	Alpha
53	1	1	alpha	1	1	alpha	Novel
54	1	1	alpha	1	1	alpha	Novel
56	1	1	alpha	1	1	alpha	Alpha
57	1	1	alpha	1	1	alpha	Missing
58	1	1	alpha	1	1	alpha	Alpha
59	1	1	alpha	1	1	alpha	Alpha
60	1	1	alpha	1	1	alpha	Alpha
61	1	1	alpha	1	1	alpha	Novel
62	1	1	alpha	1	1	alpha	Novel
63	1	1	alpha	1	1	alpha	Missing
64	2	2	a	2	2	a	Missing
65	1	1	alpha	1	1	alpha	Alpha
68	1	1	alpha	1	1	alpha	Novel
70	1	1	alpha	1	1	alpha	Novel
71	1	1	alpha	1	1	alpha	Novel
73	1	1	alpha	1	1	alpha	Novel
74	1	1	alpha	1	1	alpha	Alpha
76	1	1	alpha	1	1	alpha	Missing
77	1	1	alpha	1	1	alpha	Novel
78	1	1	alpha	1	1	alpha	Novel
80	1	1	alpha	1	1	alpha	Novel
81	1	1	alpha	1	1	alpha	Novel
82	1	1	alpha	1	1	alpha	Missing
83	1	1	alpha	1	1	alpha	Missing
84	1	1	alpha	1	1	alpha	Missing
85	1	1	alpha	1	1	alpha	Alpha
86	1	1	alpha	1	1	alpha	Novel
87	1	1	alpha	1	1	alpha	Novel
88	1	1	alpha	1	1	alpha	Missing
89	1	1	alpha	1	1	alpha	Alpha
90	1	1	alpha	1	1	alpha	Missing
91	1	1	alpha	1	1	alpha	Novel
92	1	1	alpha	1	1	alpha	Novel

[a],[b] same notes as in Supplemental Table 4A.
[c] "a" indicates strains in which the STE20a fragment amplified and the STE12alpha fragment did not, while "alpha" is indicated for strains in which the STE12alpha fragment was amplified and the STE20a fragment was not. "hetero" is indicated for strains in which both STE20a and STE12alpha fragments amplified. "?" is shown for those strains in which neither fragment amplified.

Supplemental Table 4H. Mitochondrial inheritance and M13 fingerprinting of progeny from cross 8. LA55n x B4544

Progeny #	ND2 digestion with *Pvu II*	ND4 digestion with *Sac I*	mtDNA origin[a]	M13 fingerprinting[b]
B4544	**~310bp, ~300bp, ~275bp, (1)**	**~300bp (1)**		**alpha**
LA55n	**~400bp, ~600bp (2)**	**~600bp (2)**		**a**
B4544/LA55n	**~600, ~400bp, ~310bp, ~300bp, ~275bp (3)**	**~300bp, 600bp (3)**		**missing/novel**
Isolate # 1	2	2	a	novel
2	2	2	a	novel
3	2	2	a	novel
4	2	2	a	novel
5	2	2	a	novel
6	2	2	a	novel
7	2	2	a	novel
8	2	2	a	novel
9	2	2	a	novel
10	2	2	a	novel
11	2	2	a	novel
12	2	2	a	missing
13	2	2	a	novel
14	2	2	a	novel
15	2	2	a	novel
16	2	2	a	novel
18	2	2	a	novel
19	2	2	a	novel
20	2	2	a	novel
21	2	2	a	missing
22	2	2	a	missing
23	2	2	a	novel
24	2	2	a	novel
25	2	2	a	novel
26	2	2	a	novel
27	2	2	a	novel
28	2	2	a	novel
29	2	2	a	novel
30	2	2	a	novel
31	2	2	a	novel
32	2	2	a	novel
33	2	2	a	novel
34	2	2	a	novel
35	2	2	a	novel
36	2	2	a	novel
37	2	2	a	missing
38	2	2	a	novel
39	2	2	a	novel
40	2	2	a	novel
41	2	2	a	novel

Supplemental Table 4H Continued

Progeny #	ND2 digestion with *Pvu II*	ND4 digestion with *Sac I*	mtDNA origin[a]	M13 fingerprinting[b]
42	2	2	a	novel
43	2	2	a	novel
45	2	2	a	novel
46	2	2	a	novel
47	2	2	a	novel
48	2	2	a	novel
49	2	2	a	missing
50	2	2	a	novel
51	2	2	a	novel
52	2	2	a	novel
53	2	2	a	missing
54	2	2	a	novel
55	2	2	a	novel
56	2	2	a	novel
57	2	2	a	novel
58	2	2	a	novel
59	2	2	a	novel
60	2	2	a	novel
61	2	2	a	novel
62	2	2	a	novel
63	2	2	a	novel
64	2	2	a	novel
65	2	2	a	novel
66	2	2	a	novel
67	2	2	a	novel
68	2	2	a	novel
69	2	2	a	novel
70	2	2	a	novel
71	2	2	a	novel
72	2	2	a	missing
73	2	2	a	novel
74	2	2	a	novel
75	2	2	a	novel
76	2	2	a	novel
78	2	2	a	novel
79	2	2	a	novel
81	2	2	a	novel
82	2	2	a	novel
83	2	2	a	novel
84	2	2	a	novel
85	2	2	a	novel
86	2	2	a	novel
87	2	2	a	novel
88	2	2	a	novel

Supplemental Table 4H Continued

Progeny #	ND2 digestion with *Pvu II*	ND4 digestion with *Sac I*	mtDNA origin[a]	M13 fingerprinting[b]
89	2	2	a	novel
95	2	2	a	novel
96	2	2	a	novel

[a, b] same notes as in Supplemental Table 4A.

Supplemental Table 4I. Mitochondrial inheritance and M13 fingerprinting of progeny from cross 9. JF109 x LA61n

Progeny #	ND2 digestion with *Taqa I*	ND4 digestion with *Sca I*	mtDNA origin[a]	M13 fingerprinting[b]
JF109	**1150bp (1)**	**650bp (1)**		**a**
LA61n	**50, 500, 600bp (2)**	**150, 500bp (2)**		**alpha**
LA61n/JF101	**50, 500, 650, 1150bp (3)**	**150, 500, 650bp (3)**		**missing/novel**
1	1	1	a	novel
2	1	1	a	novel
3	1	1	a	novel
4	1	1	a	novel
5	1	1	a	novel
6	1	1	a	novel
7	2	2	alpha	novel
8	1	1	a	novel
11	1	1	a	novel
12	2	2	alpha	novel
13	1	1	a	novel
14	1	1	a	missing
15	1	1	a	novel
16	1	1	a	novel
18	1	1	a	novel
19	2	2	alpha	novel
21	1	1	a	novel
23	1	1	a	missing
25	2	2	alpha	novel
26	2	2	alpha	novel
28	1	1	a	novel
30	1	1	a	novel
32	1	1	a	novel
33	1	1	a	novel
36	1	1	a	novel
38	1	1	a	novel
39	1	1	a	novel
41	1	1	a	novel
43	1	1	a	novel
44	1	1	a	novel
45	2	2	alpha	novel
46	1	1	a	novel
48	1	1	a	missing
50	1	1	a	missing
51	2	1	rec	novel
52	2	2	alpha	novel
53	2	2	alpha	novel
54	2	2	alpha	novel
55	2	2	alpha	novel
56	2	2	alpha	novel

Supplemental Table 4I Continued

Progeny #	ND2 digestion with *Taqa I*	ND4 digestion with *Sca I*	mtDNA origin[a]	M13 fingerprinting[b]
57	1	2	rec	novel
58	2	1	rec	novel
60	1	2	rec	novel
62	2	2	alpha	novel
63	2	2	alpha	novel
65	2	2	alpha	novel
66	2	1	rec	missing
67	2	2	alpha	novel
68	2	2	alpha	novel
69	2	2	alpha	novel
70	2	2	alpha	novel
71	2	2	alpha	novel
72	2	2	alpha	novel
73	2	2	alpha	novel
74	2	2	alpha	novel
75	2	2	alpha	novel
76	2	2	alpha	novel
77	2	2	alpha	novel
78	2	2	alpha	novel
79	2	2	alpha	missing
80	2	2	alpha	novel
82	2	2	alpha	novel
84	2	2	alpha	novel
85	2	2	alpha	novel
86	2	2	alpha	novel
87	2	2	alpha	novel
88	1	1	a	novel
89	2	2	alpha	novel
91	1	3	rec	novel
92	2	2	alpha	novel
94	2	2	alpha	novel
95	2	2	alpha	missing
98	2	2	alpha	missing
99	2	2	alpha	novel
100	2	2	alpha	novel
101	2	2	alpha	novel
102	2	2	alpha	novel
103	2	2	alpha	novel
104	2	2	alpha	novel
105	2	2	alpha	novel
106	1	1	a	novel
108	2	2	alpha	novel
109	1	1	a	novel
110	1	1	a	novel

Supplemental Table 4I Continued

Progeny #	ND2 digestion with *Taqa I*	ND4 digestion with *Sca I*	mtDNA origin[a]	M13 fingerprinting[b]
111	2	2	alpha	novel
112	1	1	a	novel
113	1	1	a	novel
114	1	1	a	novel
115	1	1	a	novel

[a, b] same notes as in Supplemental Table 4A.

Supplemental Table 4J. Mitochondrial inheritance and M13 fingerprinting of progeny from cross 10. LA55n x JF101

Progeny #	ND2 digestion with *Pvu II*	ND4 digestion with *Sca I*	mtDNA origin[a]	M13 fingerprinting[b]
LA55n	**500, 650 bp (1)**	**650bp (1)**		**a**
JF101	**300, 550bp (2)**	**150, 500bp (2)**		**alpha**
LA55n/JF101	**300, 500, 550, 650bp (3)**	**150, 500, 650bp (3)**		**missing/novel**
2	1	1	a	novel
3	2	1	rec	novel
4	1	1	a	novel
5	1	1	a	novel
6	1	1	a	novel
7	2	1	rec	novel
8	1	1	a	novel
9	2	1	rec	novel
10	2	1	rec	novel
11	1	2	rec	novel
12	1	1	a	novel
13	1	1	a	novel
14	1	1	a	novel
15	2	1	rec	novel
16	1	1	a	novel
17	1	1	a	missing
18	1	2	rec	missing
19	1	1	a	novel
20	2	2	alpha	novel
22	1	1	a	novel
23	2	1	rec	novel
24	2	2	alpha	novel
25	1	2	rec	novel
26	1	2	rec	missing
27	1	2	rec	novel
29	1	2	rec	novel
30	1	2	rec	novel
31	1	2	rec	novel
32	1	2	rec	novel
33	1	2	rec	novel
35	1	2	rec	novel
36	1	2	rec	novel
37	1	1	a	novel
38	1	1	a	novel
39	1	1	a	missing
40	1	1	a	novel
41	1	1	a	novel
43	2	1	rec	novel
44	1	1	a	novel
45	1	1	a	novel

Supplemental Table 4J Continued

Progeny #	ND2 digestion with *Pvu II*	ND4 digestion with *Sca I*	mtDNA origin[a]	M13 fingerprinting[b]
46	1	1	a	novel
47	1	1	a	novel
48	1	1	a	novel
49	1	1	a	novel
50	1	1	a	novel
51	1	1	a	novel
53	1	1	a	novel
54	1	1	a	novel
55	2	1	rec	novel
57	2	1	rec	novel
58	2	1	rec	novel
59	1	2	rec	novel
60	1	2	rec	missing
61	1	2	rec	novel
62	1	2	rec	novel
63	1	2	rec	novel
64	1	1	a	novel
65	1	1	a	novel
67	1	1	a	novel
68	2	1	rec	novel
69	1	1	a	novel
70	2	1	rec	missing
71	2	1	rec	novel
72	1	1	a	novel
73	1	2	rec	novel
74	1	2	rec	missing
75	1	2	rec	novel
76	1	2	rec	novel
77	1	2	rec	novel
79	1	2	rec	novel
81	1	2	rec	novel
82	1	2	rec	novel
83	1	1	a	novel
84	1	1	a	novel
85	1	1	a	novel
86	1	1	a	novel
87	1	1	a	novel
88	2	2	alpha	novel
89	1	1	a	novel
90	1	2	rec	novel
91	1	2	rec	novel
93	1	2	rec	novel
94	2	1	rec	novel
95	2	1	rec	novel

Supplemental Table 4J Continued

Progeny #	ND2 digestion with *Pvu II*	ND4 digestion with *Sca I*	mtDNA origin[a]	M13 fingerprinting[b]
96	2	1	rec	novel
97	2	1	rec	novel
99	2	1	rec	novel
101	2	2	alpha	novel
102	2	1	rec	novel
103	2	1	rec	missing
104	2	1	rec	novel

[a, b] same notes as in Supplemental Table 4A.

Supplemental Table 4K Mitochondrial inheritance and M13 fingerprinting of progeny from cross 11. B4495 x B4499

Progeny #	ND2 digestion with *Pvu II*	ND4 digestion with *Alu I*	mtDNA origin[a]	M13 fingerprinting[b]
B4499	**~150, ~500 ~600, (1)**	**~300, ~150, ~50 (1)**		**alpha**
B4495	**~500, ~750 (2)**	**~200, ~400 (2)**		**a**
B4499/B4495	**~150, ~500, ~600, ~750 (3)**	**~50, 150, 200, 300, 400 (3)**		**missing/novel**
2	2	2	a	novel
3	2	2	a	novel
4	2	2	a	novel
5	2	2	a	novel
6	2	2	a	missing
7	2	2	a	novel
8	2	2	a	novel
9	2	2	a	novel
10	2	2	a	novel
11	2	2	a	novel
12	2	2	a	novel
13	2	2	a	novel
14	2	2	a	novel
15	2	2	a	novel
16	2	2	a	novel
17	2	2	a	novel
18	2	2	a	novel
19	2	2	a	novel
20	2	2	a	novel
21	2	2	a	novel
22	2	2	a	missing
23	2	2	a	novel
24	2	2	a	novel
25	2	2	a	novel
26	2	2	a	novel
27	2	2	a	missing
28	2	2	a	novel
29	2	2	a	novel
30	2	2	a	novel
31	2	2	a	novel
33	2	2	a	novel
34	2	2	a	novel
35	2	2	a	novel
37	2	2	a	novel
38	2	2	a	novel
42	2	2	a	novel
43	2	2	a	novel
44	2	2	a	novel
45	2	2	a	novel
46	2	2	a	novel

Supplemental Table 4K Continued

Progeny #	ND2 digestion with *Pvu II*	ND4 digestion with *Alu I*	mtDNA origin[a]	M13 fingerprinting[b]
47	2	2	a	missing
48	2	2	a	missing
51	2	2	a	novel
52	2	2	a	novel
53	2	2	a	novel
54	2	2	a	novel
55	2	2	a	novel
57	1	1	alpha	novel
58	1	1	alpha	novel
59	1	1	alpha	novel
60	1	1	alpha	novel
61	1	1	alpha	missing
62	1	1	alpha	novel
63	1	1	alpha	novel
64	1	1	alpha	novel
65	1	2	rec	novel
67	2	2	a	novel
69	2	2	a	novel
71	2	2	a	novel
72	2	2	a	novel
73	2	2	a	novel
74	2	2	a	novel
75	2	2	a	missing
76	2	1	rec	novel
77	1	1	alpha	novel
78	1	1	alpha	novel
80	1	1	alpha	novel
81	1	2	rec	novel
82	1	2	rec	novel
83	2	1	rec	novel
84	1	1	alpha	novel
85	1	1	alpha	novel
86	1	1	alpha	novel
87	1	1	alpha	novel
90	1	1	alpha	novel
91	1	1	alpha	novel
92	1	1	alpha	novel
93	1	1	alpha	novel
94	1	2	rec	novel
95	1	2	rec	novel

[a, b] same notes as in Supplemental Table 4A.

Supplemental Table 4L Mitochondrial inheritance and M13 fingerprinting of progeny from cross 12. B4495 x B4544

Progeny #	ND2 Digestion with Pvu II and Hind II	ND4 digestion with Sca I	mtDNA origin[a]	STE20a	STE12alpha	MAT locus[c]	M13 fingerprinting[b]
B4495	**200, 300, 650bp (1)**	**150, 500bp (1)**		**present (1)**	**absent (1)**	**a**	**A**
B4544	**575bp (2)**	**650bp (2)**		**absent (2)**	**present (2)**	**alpha**	**Alpha**
B4495/B4544	**200, 300, 575, 650bp (3)**	**150, 500, 650bp (3)**		**present (2)**	**present (2)**	**hetero**	**missing/novel**
1	2	2	alpha	2	2	alpha	Novel
2	2	2	alpha	2	2	alpha	Novel
4	2	2	alpha	2	2	alpha	Novel
5	1	1	alpha	1	1	alpha	Novel
7	2	2	alpha	2	2	alpha	Missing
8	2	2	alpha	2	2	alpha	Novel
9	2	2	alpha	2	2	alpha	Novel
10	2	2	alpha	2	2	alpha	Novel
11	2	2	alpha	2	2	alpha	Novel
13	2	2	alpha	2	2	alpha	Alpha
14	2	2	alpha	2	2	alpha	Novel
15	2	2	alpha	2	2	alpha	Alpha
17	1	2	rec	2	2	alpha	Novel
18	2	2	alpha	2	2	alpha	Novel
21	1	2	rec	2	2	alpha	Novel
22	1	2	rec	2	1	?	Novel
23	2	2	alpha	2	2	alpha	Alpha
24	1	2	rec	2	2	alpha	Missing
26	2	2	alpha	2	2	alpha	Alpha
27	1	2	rec	2	2	alpha	Missing
28	1	2	rec	2	2	alpha	Missing
31	2	2	alpha	2	1	?	Novel
32	2	2	alpha	2	2	alpha	Novel
33	2	2	alpha	2	2	alpha	Novel
34	2	2	alpha	2	2	alpha	Novel
40	2	2	alpha	2	2	alpha	Novel
49	1	1	a	1	1	a	Novel
50	1	1	a	1	1	a	Novel
54	1	1	a	1	1	a	Novel
56	2	2	alpha	2	2	alpha	Alpha
73	1	1	a	1	1	a	Novel
79	1	1	a	1	1	a	Novel
80	1	1	a	1	1	a	Novel
83	2	2	alpha	2	2	alpha	Novel
86	1	1	a	1	1	a	Novel
90	1	1	a	1	1	a	Missing
96	1	1	a	1	1	a	Missing
97	1	2	rec	1	2	hetero	Novel
98	1	2	rec	1	1	a	Novel

Supplemental Table 4L Continued

Progeny #	ND2 Digestion with Pvu II and Hind II	ND4 digestion with Sca I	mtDNA origin[a]	STE20a	STE12alpha	MAT locus[c]	M13 fingerprinting[b]
99	2	1	rec	1	2	hetero	Novel
104	1	2	rec	2	1	?	Novel
105	2	1	rec	2	2	alpha	Novel
106	2	2	alpha	2	2	alpha	Novel
108	1	2	rec	2	2	alpha	Novel
109	1	2	rec	2	2	alpha	Novel
110	2	2	alpha	2	2	alpha	Novel
111	2	2	alpha	2	2	alpha	Novel
112	2	2	alpha	2	2	alpha	Novel
113	1	2	rec	2	2	alpha	Missing
114	2	2	alpha	2	2	alpha	Alpha
115	2	2	alpha	2	2	alpha	Novel
116	2	2	alpha	2	2	alpha	Novel
117	1	2	rec	2	2	alpha	Novel
118	2	2	alpha	2	2	alpha	Novel
119	1	2	rec	2	2	alpha	Missing
120	2	2	alpha	2	2	alpha	Novel
121	2	2	alpha	2	2	alpha	Novel
122	2	2	alpha	2	2	alpha	Novel
123	2	2	alpha	2	2	alpha	Novel
125	1	2	rec	1	2	hetero	Novel
127	2	2	alpha	2	2	alpha	Novel
128	1	2	rec	2	2	alpha	Novel
129	1	2	rec	2	2	alpha	Novel
130	1	2	rec	2	2	alpha	Missing
131	1	2	rec	2	2	alpha	Novel
132	1	2	rec	2	2	alpha	Novel
133	1	2	rec	2	2	alpha	Missing
134	1	2	rec	2	2	alpha	Missing
135	1	2	rec	2	2	alpha	Novel
136	2	2	alpha	2	2	alpha	Novel
137	2	2	alpha	2	2	alpha	Novel
139	2	2	alpha	2	2	alpha	Novel
140	2	2	alpha	2	2	alpha	Novel
141	2	2	alpha		2	?	Alpha
142	2	2	alpha	2	2	alpha	Novel
143	2	2	alpha	2	2	alpha	Novel
144	2	2	alpha	2	2	alpha	Novel
147	2	2	alpha	2	2	alpha	Novel
149	2	2	alpha	2	2	alpha	Novel
151	1	2	rec	2	2	alpha	Novel
152	2	2	alpha	2	2	alpha	Alpha

[a, b] same notes as in Supplemental Table 4A.
[c] same notes as in Supplemental Table 4G.

Supplemental Table 4M. Mitochondrial inheritance and M13 fingerprinting of progeny from cross 13. B4545 x B4544

Progeny #	ND2 digestion with *Pvu II*	ND4 digestion with *Sca I*	mtDNA origin[a]	STE20a	STE12alpha	MAT locus[c]	M13 fingerprinting[b]
B4544	**~150, ~500bp, ~600bp (1)**	**~750bp (1)**		**absent (1)**	**present (1)**	**alpha**	**alpha**
B4545	**~500bp. ~750bp (2)**	**~250bp, 500bp (2)**		**Present (2)**	**Absent (2)**	**a**	**a**
B4544/B4545	**~150, ~500bp, ~600bp, ~750bp (3)**	**~250bp, 500bp, 750bp (3)**		**Present (2)**	**Absent (2)**	**hetero**	**missing/novel**
3	2	1	rec	2	2	a	novel
4	2	1	rec	2	2	a	novel
5	1	1	alpha	1	1	alpha	novel
6	2	1	rec	2	1	hetero	novel
7	2	1	rec	2	2	a	novel
9	1	1	alpha	1	1	alpha	novel
10	2	1	rec	2	2	a	novel
13	1	1	alpha	1	1	alpha	novel
14	1	1	alpha	1	1	alpha	novel
15	1	1	alpha	1	1	alpha	novel
16	1	1	alpha	1	1	alpha	novel
17	1	1	alpha	1	1	alpha	alpha
18	1	1	alpha	1	1	alpha	novel
19	1	1	alpha	1	1	alpha	novel
20	1	1	alpha	1	1	alpha	missing
21	1	1	alpha	1	1	alpha	novel
23	1	1	alpha	1	1	alpha	novel
24	1	1	alpha	1	1	alpha	novel
25	1	1	alpha	1	1	alpha	novel
26	1	1	alpha	1	1	alpha	novel
27	2	2	a	2	2	a	missing
29	1	1	alpha	1	1	alpha	alpha
30	1	1	alpha	1	1	alpha	alpha
31	1	1	alpha	1	1	alpha	novel
32	1	1	alpha	1	1	alpha	novel
33	1	1	alpha	1	1	alpha	alpha
35	1	1	alpha	1	1	alpha	missing
36	2	1	rec	2	1	hetero	novel
37	1	1	alpha	1	1	alpha	novel
38	1	1	alpha	1	1	alpha	novel
39	2	2	a	2	2	a	missing
40	1	1	alpha	1	1	alpha	novel
41	1	1	alpha	1	1	alpha	novel
42	1	1	alpha	1	1	alpha	novel
44	1	1	alpha	1	1	alpha	novel
45	1	1	alpha	1	1	alpha	novel
46	1	1	alpha	1	1	alpha	novel
47	1	1	alpha	1	1	alpha	alpha
48	2	1	rec	2	1	hetero	novel
49	1	1	alpha	1	1	alpha	novel

Supplemental Table 4M Continued

Progeny #	ND2 digestion with *Pvu II*	ND4 digestion with *Sca I*	mtDNA origin[a]	STE20a	STE12alpha	MAT locus[c]	M13 fingerprinting[b]
51	1	1	alpha	1	1	alpha	novel
52	1	1	alpha	1	1	alpha	missing
53	1	1	alpha	1	1	alpha	alpha
54	1	1	alpha	1	1	alpha	novel
55	1	1	alpha	1	1	alpha	missing
56	1	1	alpha	1	1	alpha	alpha
57	1	1	alpha	1	1	alpha	novel
58	1	1	alpha	1	1	alpha	alpha
59	1	1	alpha	1	1	alpha	novel
61	1	1	alpha	1	1	alpha	alpha
62	1	1	alpha	1	1	alpha	novel
63	1	1	alpha	1	1	alpha	novel
64	1	1	alpha	1	1	alpha	novel
65	1	1	alpha	1	1	alpha	alpha
66	1	1	alpha	1	1	alpha	novel
69	1	1	alpha	1	1	alpha	novel
71	1	1	alpha	1	1	alpha	alpha
72	1	1	alpha	1	1	alpha	missing
73	1	1	alpha	1	1	alpha	novel
74	1	1	alpha	1	1	alpha	novel
75	1	1	alpha	1	1	alpha	novel
77	2	2	a	2	2	a	novel
78	2	2	a	1	1	alpha	novel
79	1	1	alpha	1	1	alpha	novel
80	1	1	alpha	1	1	alpha	alpha
81	1	1	alpha	1	1	alpha	novel
82	1	1	alpha	1	1	alpha	novel
83	1	1	alpha	1	1	alpha	novel
84	1	1	alpha	1	1	alpha	alpha
85	1	1	alpha	1	1	alpha	novel
87	1	1	alpha	1	1	alpha	novel
88	1	1	alpha	1	1	alpha	novel
89	1	1	alpha	1	1	alpha	alpha
90	1	1	alpha	1	1	alpha	novel
94	1	1	alpha	1	1	alpha	novel
95	1	1	alpha	1	1	alpha	novel
96	1	1	alpha	1	1	alpha	novel

[a],[b] same notes as in Supplemental Table 4A.
[c] same notes as in Supplemental Table 4G.

Supplemental Table 4N Mitochondrial inheritance and M13 fingerprinting of progeny from cross 14. B4545 x JF101

Progeny #	ND2 digestion with *Pvu II*	ND5	mtDNA origin[a]	M13 fingerprinting[b]
JF101	**300, 550bp (1)**	**1250 (1)**		**alpha**
B4545	**500, 650bp (2)**	**750bp (2)**		**a**
JF101/B4545	**300, 500, 550, 650bp (3)**	**750, 1250bp (3)**		**missing/novel**
2	2	2	a	novel
3	2	2	a	novel
4	2	2	a	novel
5	2	2	a	novel
6	2	2	a	novel
7	2	2	a	novel
8	2	2	a	missing
9	2	2	a	novel
10	2	2	a	novel
11	2	2	a	novel
12	2	2	a	novel
13	2	2	a	novel
14	2	2	a	novel
15	2	2	a	novel
16	2	2	a	novel
17	2	2	a	novel
18	2	2	a	missing
19	2	2	a	missing
20	2	2	a	novel
21	2	2	a	novel
22	2	2	a	novel
23	2	2	a	novel
24	2	2	a	novel
25	2	2	a	novel
26	2	2	a	novel
27	2	2	a	novel
28	2	2	a	novel
29	2	2	a	novel
30	2	2	a	novel
31	2	2	a	missing
32	2	2	a	novel
33	2	2	a	novel
34	2	2	a	novel
35	2	2	a	novel
36	2	2	a	missing
37	2	2	a	novel
38	2	2	a	novel
39	1	1	alpha	novel
40	2	2	a	novel
41	2	2	a	novel

Supplemental Table 4N Continued

Progeny #	ND2 digestion with *Pvu II*	ND5	mtDNA origin[a]	M13 fingerprinting[b]
42	2	2	a	novel
43	2	2	a	novel
45	2	2	a	novel
46	2	2	a	novel
47	2	2	a	novel
48	2	2	a	missing
49	2	2	a	novel
50	2	2	a	novel
51	2	2	a	novel
52	1	1	alpha	novel
53	2	2	a	novel
54	2	2	a	novel
55	2	2	a	novel
56	2	2	a	novel
57	2	2	a	novel
58	2	2	a	novel
59	2	2	a	novel
60	2	2	a	novel
61	2	2	a	novel
62	2	2	a	novel
63	2	2	a	novel
64	2	2	a	novel
65	2	2	a	novel
66	2	2	a	missing
67	2	2	a	novel
68	1	1	alpha	novel
69	2	2	a	novel
71	2	2	a	novel
72	2	2	a	novel
73	1	1	alpha	novel
75	2	2	a	novel
76	2	2	a	novel
77	2	2	a	novel
78	2	2	a	novel
79	2	2	a	missing
81	2	2	a	novel
82	2	2	a	novel
83	2	2	a	novel
84	2	2	a	novel
85	2	2	a	novel
86	2	2	a	novel
87	2	2	a	novel
88	2	2	a	novel
89	2	2	a	novel

Supplemental Table 4N Continued

Progeny #	ND2 digestion with *Pvu II*	ND5	mtDNA origin[a]	M13 fingerprinting[b]
90	2	2	a	novel
91	2	2	a	novel
92	2	2	a	novel

[a, b] same notes as in Supplemental Table 4A.

CHAPTER 3:

GENERAL CONCLUSIONS AND PERSPECTIVES

In this thesis I have introduced an overview of mitochondrial inheritance patterns seen across a variety of species, how they are known to be and hypothesized to be controlled, and shown how they have been observed to change after hybrid mating between species (Chapter 1). In Chapter 2, a study was presented that illustrates the difference between mitochondrial inheritance between *C. gattii* and *C. neoformans.* Specifically, it was shown that, (1) leakage of *MATalpha* mitochondria in *C. gattii* is significantly more varied than in haploid x haploid crosses of *C. neoformans*, indicating that there is likely a different method of mitochondrial inheritance occurring in each of these species; (2) *crg1* does not mediate mitochondrial inheritance in a trended fashion in *C. gattii* although it is known to increase mating efficiency and; (3) the amount of genetic difference between the parents of a cross within *C. gattii* does not correlate to the amount of *MATalpha* mitochondrial leakage observed across all types of crosses, although a significant correlation was found in intra-lineage crosses showing that a decrease in genetic distance between the mating partners is related to an increase in *MATalpha* mtDNA leakage.

Future studies could be performed to look at what kind of mitochondrial inheritance is seen in hybrid *C. gattii* x *C. neoformans* matings, further exploring the effects of genetic distance between mating partners upon mitochondrial inheritance. By employing both *MATa* and *MATalpha C. neoformans* strains it would reveal whether the

mechanisms guiding uniparental mitochondrial inheritance in *C. neoformans* are dominant to the mechanisms in *C. gattii* or vice versa. It is possible that if the mechanisms of *C. neoformans* are dominant that either *C. gattii* has lost the function to regulate uniparental mitochondrial inheritance or *C. neoformans* has gained the function to mediate uniparental mitochondrial inheritance. Work could also be done to visualise what is happening after fusion of compatible strains in *C. gattii*, similar to what was done by McClelland *et al.* with *C. neoformans.* This would determine if the difference between these species' mitochondrial inheritance can be attributed to this initial step in mating.

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